# The burden of type 2 diabetes mellitus, dysglycaemia and their co-determinants in the adult population of Malta

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I hereby declare that all material in this research project is my original work.

References to other works are documented.

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Sarah Cuschieri

***To my husband Stephan***… for his love, patience, kindness and encouragement throughout the process of the research study.

***To my parents***… for their support and help throughout my career and for being part of the research fieldwork team.

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# Abstract


## Background

Type 2 diabetes (T2DM) is a growing global epidemic. The International Diabetes Federation reported a T2DM European adult prevalence of 8.8% in 2017. Diabetes has been endemic in the Maltese population for many centuries. However, the last diabetes nationally representative prevalence study conducted in Malta dated back to 1981. Since then the Maltese Islands have experienced a strong immigration flow, bringing along significant genetic, cultural and lifestyle changes. Several attributing factors (genetic and environmental) for T2DM have been reported in the literature. An important contributing risk factor is the overweight-obesity epidemic, which has been reported by 'Eurostat' to be affecting over 50% of the European adult population in 2014. The 2010 European Health Examination pilot survey conducted in Malta reported that the majority of the study population had an elevated body mass index. However, at a population level, the Malta-specific co-determinants of T2DM had never been explored.

## Aims & Objectives

The main aim was to determine the burden of diabetes mellitus, dysglycaemia and their co-determinants within the adult population of Malta. Furthermore, specific objectives included an exploration of the Maltese co-determinants of T2DM including links between different anthropometric, biochemical, and socio-demographic factors as well as between ten specific genetic SNPs and T2DM. This was aimed to provide the required evidence to empower public health efforts to target prevention as well as to develop nation-wide policies and strategies.

## Methodology

The cross-sectional study's target population was adults residing in Malta for at least 6 months aged between 18 and 70 years. The study population was selected from a national registry. A randomized stratified single stage sampling method was conducted to establish the study population. The strata for selection were age, gender and locality. Considering a possible 50% response rate and an expected pre-diabetes prevalence rate of 25% (based on published literature), the PiFace software® was used to estimate the sample size for this study. A sample of 4,000 adults was required. Permissions to conduct this study were granted from the University of Malta research ethics committee, the information and data protection commissioner, the Ministry for health, the chairman of the pathology department, the chief executive officer of Mater Dei Hospital and the laboratory of molecular genetics.

A validated questionnaire and validated tools for health examination measures were utilized based on the European Health Examination Survey guidelines. A health examination hub was set up every weekend at governmental peripheral health clinics across all of the Maltese towns. In order to reduce information bias, a limited number of fieldworkers were enrolled, and trained regularly.

Invitations to the randomly selected participants were sent offering a free health examination, two weeks prior to the examination appointment. Participants gave their informed consent and answered the socio-demographic questionnaire. This was followed by measurements for blood pressure, weight, height, waist and hip circumference. Blood samples for fasting plasma glucose (FPG), lipid profile and a whole blood sample for genetic studies were drawn as the last stage of the examination. An oral glucose tolerance test was offered to those obtaining an impaired fasting plasma glucose (IFG) result. All the

data gathered during the fieldwork was inputted by a single fieldworker to avoid bias. Secure inputting software was used that was programmed to perform data validation while inputting data.

In order to compensate for non-respondents and maintain strata representation, a weighting factor was applied to each individual in the sample using the IBM SPSS software. The weighting data was only used when national representative population analysis was performed. Prevalence rates (T2DM, IFG, overweight-obesity; hypertension and the metabolic syndrome) were established for each category of age and gender. Socio-demographic, anthropometric and biochemical parameters were analysed (descriptive and analytic) and associated links were investigated with T2DM and IFG by using the IBM SPSS software. Non-parametric statistical testing using the Mann-Whitney U test and the Kruskal Wallis test were performed since the data did not follow a normal distribution. Dunn's test was used as a *post-hoc* test following Kruskal Wallis testing. The Chi-squared test was used to identify significance between categorical variables. The Spearman's correlation testing was performed to test for associations between variables. Binary logistic regressions and multiple regression analysis were performed to identify the independent associated risk factors for T2DM and IFG. Using regression analysis and receiver operating curves, a Maltese specific diabetes risk score was established. The cost burden for T2DM and obesity was calculated based on cost per case rates obtained from the scientific literature, after adjusting for gross domestic product (GDP) per capita and for deflation. A 2% compound interest per annum was added on the cost burden obtained for obesity from local data.

A sub-population of the participating study population was randomly selected from within each different metabolic profile category (dysglycaemic, metabolically abnormal and metabolically normal) to undergo case-control genetic analysis. DNA extraction from

whole blood samples gathered during the fieldwork, followed by real time PCR genotyping for ten identified literature based single nucleotide polymorphism (SNPs) was performed. Descriptive and analytic analyses were performed using IBM SPSS software. A case-control design was followed to evaluate this sub-population's biochemical and anthropometric phenotype in relation to the 10 SNPs under study. Multiple regression analysis was performed to identify any associated links between the 10 SNPs and a diagnosis of T2DM.

## Results

The response rate was 47.15% (*n*=1,861). The prevalence of adult T2DM was 10.31% (95% CI: 9.40 - 11.30) among whom 6.31% (CI 95%: 5.59 - 7.11) were previously diagnosed and 4.00% (CI: 3.43 - 4.66) were newly diagnosed diabetes. Both had a male predominance. A proportion of female diabetes presented at an earlier age (30 – 39 years) when compared to their male counterparts. The prevalence of adult IFG was 23.44% (CI 95% 22.14 – 24.78), with a male predominance. A diagnosis of IFG was established as early as the 20 – 29 years group. The prevalence of overweight body mass index (BMI) was 35.65% (95% CI: 33.27 – 37.15) while that of obesity was 34.08% (95% CI: 32.64 – 35.60), both with a male predominance. The female population had a higher normal BMI prevalence than did the male counterpart. The global prevalence of hypertension among the study population was of 31.87% (CI 95%: 30.44 – 33.34), while that of the metabolic syndrome was of 26.30% (CI 95%: 24.95 – 27.69), both with a male predominance.

The socio-economic status (in particular, the education level) of the population was closely linked to the different biochemical and anthropometric parameters. In fact, the diagnosis of

diabetes, IFG and overweight-obese status were all mostly prevalent within the lowest socioeconomic scale. Additionally, certain residential districts appeared to be linked to having an increased diagnosis of diabetes. Furthermore, the Gozo district had a significantly worse metabolic profile than the rest of the Maltese districts.

An increase in adiposity (measured by BMI and waist circumference) within the Maltese population was associated with multiple features. These included an increase in fasting plasma glucose, being newly diagnosed diabetes, having an IFG diagnosis as well as being found to be hypertensive. Additionally, the independent factors associated with having T2DM (when compared to the non-diabetes population) were an increase in fasting plasma glucose (FPG), ageing, increased waist circumference, having a diagnosis of the metabolic syndrome and having a history of hypertension. On the contrary an increase in LDL-C and in the diastolic blood pressure levels appeared to have a negative relationship with T2DM. However, the independent factors associated with having T2DM (when compared to the metabolically healthy population) were an increase in fasting plasma glucose (FPG) level and an increase in triglyceride level, while the female gender had a negative relationship with T2DM. Meanwhile, the independent factors associated with having IFG were an increase in age, in waist circumference and in diastolic blood pressure levels. On the contrary, an increase in triglyceride level and being a female appeared to exhibit a negative influence on IFG.

The Malta-specific diabetes characteristics were an increase in waist circumference (>100cm males; >90cm females), ageing (>55years) and individuals on statin therapy.

The estimated Malta adult global cost burden for diabetes mellitus (combination of newly diagnosed and previously diagnosed) was €29,159,217 (CI 95%: €21,994,676 - €38,919,121) for the year of 2016. The cost burden for obesity was estimated to be €23,732,781 (CI 95%: €21,514,972 - €26,049,204) for the year 2016.

Individuals carrying the *NOTCH2* T/T mutant allele and the *HHEX* C/T mutant allele were associated with having higher FPG levels, especially within the dysglycaemic sub-group. Meanwhile the individuals carrying the mutant T/T allele of the *FABP2* allele were mostly prevalent within the '*metabolically abnormal'* sub-group. Both *FABP2* dominant and recessive models exhibited an associated effect for an increase in triglyceride levels and a decrease in HDL-C levels. The co-dominant and the dominant *FTO* models were associated with a lower risk of having T2DM. Similarly, the recessive *KCNE4* model was associated with a lower risk of having T2DM.

## Discussion

An increase in the adult T2DM prevalence in Malta was observed when comparing to the last national study (1981), especially with regard to the newly diagnosed diabetes. Over the past 35 years, there has also been a shift in the gender predominance of T2DM; from a female to a male predominance. This gender shift is in keeping with that found in studies in other parts of the world, where diabetes now seemingly affects more males than females. This is partly due to the increasing obesity rates affecting the male population. In fact, an increase in the obesity prevalence rate was recorded in this study when compared to previous local studies, especially for the male population. Interestingly, an increase in normal weight prevalence was observed for the female population in contrast to the male

population. Of note, the IFG prevalence was found to be more than twice that of T2DM, which is in keeping with other European countries. The identified Maltese IFG population characteristics were in keeping with the typical phenotypes of the European pre-diabetes population, with the majority being overweight or obese and suffering from dyslipidaemia. An interesting observation was the reduction in hypertension prevalence rates across the years from 1981 to the present study, coinciding with similar findings in both an Italian and a British study.

Health inequalities in the form of abnormal metabolic profiles were observed between the different districts as well as between different sub-populations. The district of Gozo population exhibited a worse metabolic profile than the five districts making up the island of Malta. In fact, an associated risk for an increase in fasting plasma glucose, LDL-C and total cholesterol was evident for those residing in Gozo, even after adjustments for confounders. A number of social, behavioural and cultural characteristics could be the underlying factors contributing to such results. A University of Malta undergraduate thesis in 1988 had reported that the internal migration encountered by Gozitans led to a number of stressors affecting their lives.

The dysglycaemic populations (T2DM and IFG) exhibited abnormal metabolic profiles as well as an independent link with adiposity. The underlying pathophysiology for such characteristics is insulin resistance. This is a well reported vicious cycle whereby the underlying insulin resistance contributes to inflammatory pathways, derangement of fatty acid metabolism, adipose tissue deposition and peripheral vascular resistance. In turn the individual has a predisposition for the development of dysglycaemia, hypertension, increase in adiposity and metabolic syndrome. In fact, underlying insulin resistance and

metabolic abnormalities might have been the result for an anomalous finding in our results, where LDL-C was negatively associated with a diagnosis of T2DM when compared to the non-diabetes population. However, LDL-C had an opposite association with T2DM when the metabolically healthy sub-population was used as the reference group. Hence, this suggests that even if dysglycaemia is not biochemically evident yet, other metabolic abnormalities might still be present that may contribute to health inequalities.

Genetic links between the ten SNPs and the presence of adult T2DM were established, though these were weak, possibly due to the small population sample. However, it appears that individuals carrying the recessive *KCNE4* allele have a lower risk of having T2DM. Individuals carrying the *HHEX* and *NOTCH2* mutant alleles have a higher risk of having dysglycaemia while those with *FABP2* allele have a higher risk of having dyslipidaemia.

A number of limitations were present in this study, including the inevitable inability to consider temporal relationships. A potential selection bias might have occurred through the particular response rates at different age and gender levels. This led to the decision to conduct weighting at an individual level by age, gender and towns in an effort to overcome this for prevalence rates. Socio-demographic, lifestyle, dietary and medical history data was self-reported and carried the risk of human bias or inaccurate recollection of information. In fact, the physical activity and dietary data was strongly suspected to be inaccurate and could not be utilized as originally intended. The utilization of other tools of measure (such as pedometers) for gathering information on physical activity might have been a better choice. A number of unaccounted and unmeasured confounding factors such as environmental, cultural and social, might have been present and had an effect on this study's results. Economic cost estimates were based on reported literature values that were not directly specific to the study's population. More accurate data could have been achieved

if economic data was gathered as part of the questionnaire. Genetic analysis might have had a different outcome if a larger sample population was used.

## Conclusion

Overall this study has demonstrated changes in the Maltese population metabolic profile across time, with a gender shift from a female to a male predominance. The Maltese adult population, when compared to other European countries, had a high prevalence of diabetes mellitus, of overweight and obese individuals and a higher prevalence of hypertension. An increase in visceral adiposity appeared to be centre stage for the Maltese dysregulated metabolic profile.

## Recommendations

Dysglycaemia (T2DM and IFG) and ever higher levels of adiposity (BMI >=25Kgm2 and high waist circumference) are a local as well as a global burden. Although this study was a cross-sectional study and temporal relationships could not be assessed, a number of biochemical and anthropometric associations were evident between these metabolic conditions. Furthermore, it is evident that in this population, these abnormal levels are prevalent from an earlier age than can be seen from the recommended international guidelines for dysglycaemic screening. Hence, it is recommended that a longitudinal cohort study be conducted to follow-up the IFG sub-population in Malta in order to evaluate the subsequent metabolic changes, health outcomes and costs. It is also suggested that policy makers consider the study's findings and the feasibility to implement a dysglycaemic screening protocol for the Maltese population, initiating from a younger

adult age than hitherto considered. A diabetes risk score was established as part of this study which could be implemented in a multi-stage screening process in order to identify the high-risk population that would require further investigations. This diabetes risk score can be easily utilized at the community level, where family doctors can aid in the identification of the high-risk population. Furthermore, the obesity epidemic within Malta appears to be linked with all of the metabolic abnormalities investigated in this study. Hence, it is recommended that further evidence-based action is considered by policy makers and public health officials to tackle this growing epidemic across the social gradient as well as through environmental infrastructures.

A gender effect was clearly observed, where the male population exhibited an increased tendency for metabolic abnormalities, namely T2DM, obesity and hypertension. Such a male gender dominance merits public health action that is gender sensitive, including interventions that reach out to the male population specifically. Age was also observed to be a contributing factor to metabolic abnormalities. This implies the need for policy interventions through the life course that are age sensitive including the encouragement of active ageing to try to reduce the burden caused by weight gain with increasing age.

Geographical variations were established in this study, where the district of Gozo exhibited significantly worse metabolic abnormalities when compared to other districts. Hence, it is recommended that further analysis of this sub-population with a larger cohort is merited along with the consideration of possible region-specific policies to address this health inequality. This finding is also important for community doctors who are responsible for health and wellbeing at an individual level.

A larger population study is recommended to evaluate the cultural and ethnical differences in Malta with regards to dysglycaemic and adiposity prevalence and metabolic

characteristics. Such a study could also enable further genetic analysis of the Maltese population to aid in the identification of the high-risk population at a molecular level. This is especially feasible with the next generation sequencing technology that enables the mapping of entire genomes at a fast and affordable pace.

**Keywords:**

Type 2 diabetes mellitus; Impaired glucose regulation, Obesity; Epidemiology; Malta

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# List of abbreviations

| | |
|---|---|
| ADA | American Diabetes Association |
| *ADRB2* | Adrenoceptor beta 2 |
| ATP | Adenosine triphosphate |
| BMI | Body Mass Index |
| BP | Blood Pressure |
| *CDKAL1* | CDK5 regulatory subunit associated protein 1 like 1 |
| CI | Confidence Intervals |
| CRP | C-reactive protein |
| DALY | Disability-adjusted life year |
| EHES | European Health Examination Survey |
| FABP2 | Fatty acid binding protein 2 |
| FPG | Fasting plasma glucose |
| FFA | Free fatty acids |
| *FTO* | Fat mass and obesity association |
| GDP | Gross domestic product |
| GLP-1 | Glucagon-like peptide-1 |
| HBSC | Health behaviour in school aged children |
| HDL-C | High-density lipoprotein cholesterol |
| *HHEX* | Hematopoietically expressed homeobox |
| HIS | Health Information Survey |
| HOMA-IR | Homeostasis model insulin resistance |
| IDF | International Diabetes Federation |

| | |
|---|---|
| IFG | Impaired fasting glucose |
| IGF-1 | Insulin-like growth factor |
| IGT | Impaired glucose tolerance |
| IL-6 | Interleukin 6 |
| IQR | Interquartile range |
| IR | Insulin resistance |
| *KCNE4* | Potassium voltage-gated channel subfamily E regulatory subunit 4 |
| KDM | Previously known diabetes mellitus |
| LDL-C | Low-density lipoprotein cholesterol |
| MetS | Metabolic Syndrome |
| MONICA | Monitoring trends and determinants in cardiovascular disease |
| NCD | Non-communicable diseases |
| NDM | Newly diagnosed diabetes meiitus |
| OECD | Organization for economic cooperation and development |
| OGTT | Oral glucose tolerance test |
| OR | ODDs Ratio |
| *PPARG* | Peroxisome proliferator activated receptor gamma |
| QUICKI | Quantitative insulin sensitivity check index |
| sdLDL-C | Small dense low-density lipoprotein cholesterol |
| *SLC30A8* | Solute carrier family 30 member 8 |

| | |
|---|---|
| T2DM | Type 2 Diabetes Mellitus |
| TC | Total Cholesterol |
| *TCF7L2* | Transcription factor 7 like 2 |
| TG | Triglycerides |
| TG/HDL | Triglyceride to high-density lipoprotein ratio |
| TNF-α receptor | Tumor necrosis factor alpha receptor |
| VLDL | Very low-density lipoprotein |
| WC | Waist Circumference |
| WHO | World Health Organisation |
| WHR | Waist-Hip Ratio |

# Publications and Conference proceedings

## **Publications** (Appendix E)

- Cuschieri S. Type 2 diabetes – An unresolved disease across centuries contributing to a public health emergency. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2019; 13: 450 – 453. DOI: 10.1016/j.dsx.2018.11.010

- Cuschieri S, Vassallo J, Calleja N, Barbara C, Mamo J. The interaction of dyslipidaemia with glycaemia in an adult population study. Journal of Diabetes & Metabolic disorders 2018. https://doi.org/10/1007/s40200-018-0377-z

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- Cuschieri S, Vassallo J, Calleja N, Pace N, Mamo J. The effect of age, gender, TG/HDL-C ratio and behavioural lifestyles on the metabolic syndrome in the high risk Mediterranean Island of Malta. Diabetes and Metabolic Syndrome Clinical Research and Reviews. 2017; 11S: S321 – S327. DOI: 10.1016/j.dsx.2017.03.009

- Cuschieri S, Vassallo J, Calleja N, Pace N, Abela J, Ali B, Abdullah F, Zahra E, Mamo J. The diabesity health economic crisis – the size of the crisis in a European island state following a cross-sectional study. Archives of Public Health 2016, 74:52 DOI:10.1186/s13690-0164-6

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## Conference proceedings

- Cuschieri S, Vassallo J, Calleja N, Mamo J. (2018) Diabetes mellitus type 2 – the Maltese epidemic. Malta Medical School Conference X. Oral Presentation

- Cuschieri S, Vassallo J, Calleja N, Mamo J. (2018) Hypertension in Malta – A tackled disease? Malta Medical School Conference X. Oral Presentation

- Cuschieri S, Vassallo J, Calleja N, Mamo J. (2018) The contemporary adult Maltese smoker phenotype. Malta Medical School Conference X. Oral Presentation

- Cuschieri S, Vassallo J, Calleja N, Mamo J. (2018) The Maltese high-risk diabetes characteristics. Malta Medical School Conference X. Poster Presentation

- Cuschieri S. (2017) Malta and Gozo: One Archipelago, Two populations? MAPHM Public Health Symposium. Oral Presentation

- Cuschieri S, Abdullah F. Ali B, Bonnici G, Zhang Y, Cini A, Barbara C, Calleja N, Vassallo J, Mamo J. (2015). University of Malta SAHHTEK survey: results from a pilot study. Malta Medical School Conference IX. Oral Presentation

- Cuschieri S, Abela J, Farrugia T, Scicluna M, Bory A, Camilleri R, Bonnici R, Sapiano A, Buhagiar R, Mamo J. (2015) Pilot testing international definitions. Malta Medical School Conference IX. Oral Presentation

- Cuschieri S, Mamo J. (2015). Fasting or Non-Fasting? An insight into Lipid Profile Testing. Malta Medical School Conference IX. Poster Presentation

- Cuschieri S. (2015). Prevalence and determinants of type 2 diabetes and impaired glucose regulation in Malta. Cambridge Diabetes Seminar. Oral presentation

# Chapter one – Introduction

## 1.1 Introduction

A qualitative systematic review was conducted on type 2 diabetes, dysglycaemia and their co-determinants. Primary research peer-reviewed journal articles and secondary data were identified and collected using "PubMed" and "Google Scholar" as the main search databases, then critically appraised while synthesizing the studies. The literature search strategy was to identify the primary sources and accompanying secondary sources covering various aspects of diabetes mellitus including: "*History of diabetes mellitus"; "Classification"; "Physiology and Pathophysiology"; "Risk factors"; "Prevention and Screening"; "Diagnostic criteria and Management"; "Epidemiology and Burden"; "Co-determinates of diabetes" and "Genetics of diabetes"* by first analysing the search list by the article title followed by its abstract and then full text if the article abstract was found to be relevant to the topic. The established 'hierarchy of research evidence' was followed while synthesis the literature, basing the literature review mostly on published meta-analysis, systematic reviews and random control trials up till March 2019, where applicable. Latest published International guidelines were identified and reviewed till the beginning of the year 2019. Literature originating from European countries were given priority although other international studies were also reviewed.

### 1.1.1 History of diabetes mellitus

The initial description of diabetes mellitus dates back to the Egyptian period, where an Egyptian papyrus described the polyuric state in 1550 BC. Hindau physicians Charak

and Sushrut, between 400 and 500 BC, first recognized the sweetness of the diabetes urine. They also noted that this condition was prevalent in those who over indulged in sweet and fatty food, who exhibited a sedentary lifestyle and were overweight. Aretaeus of Cappadocia, in the second century AD, was the first to coin the Greek word “syphon” for “diabetes”. He observed a man’s body was not retaining fluid but rather, the body was used as a channel to release fluid (Tattersall, 2010). In 1775, Matthew Dobson from Liverpool was the pioneer in describing serum hyperglycaemia and the presence of sweet urine in a diabetes patient (Dobson, 1776). The surgeon John Rollo applying the adjective “mellitus”, which is a derivative of a Latin word meaning “honey”. In his book published in 1797, he concluded that sugar was being developed in the stomach from vegetables. He proposed a diet containing animal produce including eggs for breakfast and meat for dinner to treat this condition (Rollo, 1797).

The French chemist Michel Chevreul in 1815 established the fact that glucose was the sugar in the diabetes urine, leading to a change in the diagnostic methods for diabetes. Initially the predominant diabetes diagnostic test was by tasting the urine. However, Trommer in 1841, followed by Moore in 1844 and Fehling in 1848, all proposed chemical testing consisting of reducing agents as a diagnostic test. However, this method was not practical due to the large volume of blood required to perform such a test. The development of the micro-method by physician Ivar Christian Bang and its ability to measure glucose repeatedly led to the development of the glucose tolerance test between 1913 and 1915 (Tattersall, 2010).

Initially it was believed that only plants were capable of synthesizing sugar and that animal metabolism broke down the plant matter into sugar. It was thus perceived that animal blood

only contained sugar post-prandially and this was evident mostly in pathological states such as diabetes. Nevertheless, Claude Bernard challenged this. Between 1846 and 1848, he reported that glucose was constantly present within the blood of normal animals. He also reported that a large starch-like substance was stored in the liver, which he called "glycogen". This could be converted into sugar in the fasted state (Olmsted, 1953). In 1869, Paul Langerhans discovered "islands" of cells within the pancreas parenchyma which, later on in 1893 were named the "islets of Langerhans" by Gustave Laguesse. He suggested that these cells were responsible for pancreatic secretions (Langerhans, 1869; Laguesse, 1893). In the 1900's the pancreatic secretion was named "insulin" by Jean De Meyer (De Meyer, 1904).

In 1921, the orthopaedic surgeon Frederick Banting and the medical student Charles Best (referred in Toronto academic circles as the $B^2$), under the supervision of Professor McLeod made the discovery of injectable insulin as a treatment for diabetes (Banting *et al.*, 1922). Chilled saline extract from the pancreas was injected into dogs that had previously undergone a pancreatectomy. A lowering of plasma glucose levels was observed (Banting *et al.*, 1922). In January 1922 the first human experiment was performed on a 14 years old diabetes boy by using injectable insulin. This led to the reversal of the biological abnormalities and clinical symptoms related to diabetes to normal (Banting *et al.*, 1922). However, not every person with diabetes had the same positive outcome with insulin injections. In fact, both Wilhelm Falta and Harold Himsworth in 1930s proposed that there were patients who were insulin-sensitive and others who were insulin-insensitive (Falta, 1936; Himsworth, 1936).

In 1955, Frederick Sanger reported the amino acid sequence of insulin, while Donald Steiner described the insulin precursor, pro-insulin a few years later in 1967. Meanwhile in 1969 Dorothy Hodgkin reported the three-dimensional structure of the insulin molecule (Brown, Sanger and Kita, 1955; Steiner and Oyer, 1967; Adams *et al.*, 1969). In 1965, Wang Ying-lai synthesized the complete insulin molecule starting from the amino acids (Kung *et al.*, 1966). Pierre Freychet identified the presence of an insulin receptor in 1971 while in 1972 Pedro Cuatrecasas isolated the receptor protein (Freychet, Roth and Neville, 1971; Cuatrecasas, 1972).

In the 1970's there was the development of the "insulin clamp" technique, which measured the hypoglycaemic action of insulin. This led to a multiplex research studies to investigate insulin resistance and its relationship with type 2 diabetes (DeFronzo, Tobin and Andres, 1979).

Over the years, diabetes management became more refined. Genetic engineering led to an evolution in the insulin preparations with the establishment of designer insulin such as fast-acting analogues *lispo* and *aspart* and the peakless basal insulin such as *glargine* and *detemir*. In 1981, John Ireland invented the revolutionary "pen" insulin injection that made the management of serum glucose by insulin more acceptable to patients (Paton *et al.*, 1981). In the early 1940's the oral hypoglycaemic agent sulfonylureas, that proved to be insulin secretagogues, was manufactured (Loubatières, 1946). In 1959 *Phenformin*, the first biguanide was introduced while in 1960 *Metformin* was available in Europe (Tyberghein and Williams, 1957). Recently other oral hypoglycaemic classes were developed such as glitazones and inhibitors of the enzyme dipeptidylpeptidase-4 (DPP-4).

### 1.1.2 Classification of diabetes mellitus

In 1936 Himsworth was the pioneer in suggesting the presence of different types of diabetes. He showed that person with diabetes could be either insulin-sensitive or insulin-resistant (Himsworth, 1936). Over the years, starting from 1965, the World Health Organization (WHO) through a series of Expert Committee meetings attempted to classify the different forms of diabetes (World Health Organization., 1965). At the time, the classification was by age of onset and therefore divided into "Juvenile onset" and "Maturity onset" disease (World Health Organization., 1965). In 1976, the U.S National Diabetes Data Group changed the classification based on the need for insulin as a therapy. Thus, the classification changed to Insulin-dependent (IDDM) and Non-insulin-dependent (NIDDM) diabetes mellitus (National Diabetes Data Group., 1979). This Group further identified other forms of diabetes including NIDDM obese and non-obese; diabetes associated conditions and syndromes; gestational diabetes and individuals with intermediate glucose plasma levels (National Diabetes Data Group., 1979). The American Diabetes Association (ADA) revised the classification of diabetes in 1997 and later on so did the World Health Organization (WHO) in 1999. Table 1.1 illustrates the aetiological classification of glycaemia disorders as agreed by the ADA in 1997 and the WHO in 1999 (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 1997; World Health Organisation Consultation Group., 1999). While, Figure 1.1 demonstrates the changes in the diabetes classifications across time.

| | |
|---|---|
| Type 1 diabetes | |
| | Autoimmune |
| | Idiopathic |
| Type 2 diabetes | |
| Other specific types | |
| | Genetic defects of beta-cell function (MODY) |
| | Genetic defects in insulin action |
| | Disease of the exocrine pancreas |
| | Drug or chemical induced (e.g. Corticosteroids) |
| | Infections (e.g. Congenital Rubella) |
| | Uncommon forms of immune-mediated diabetes |
| | Other genetic syndromes (e.g. Turner syndrome) |
| Gestational diabetes | |

Table 1.1 The consensus between ADA (1997) and WHO (1999) aetiological classification of glycaemia disorders (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 1997; World Health Organisation Consultation Group., 1999).

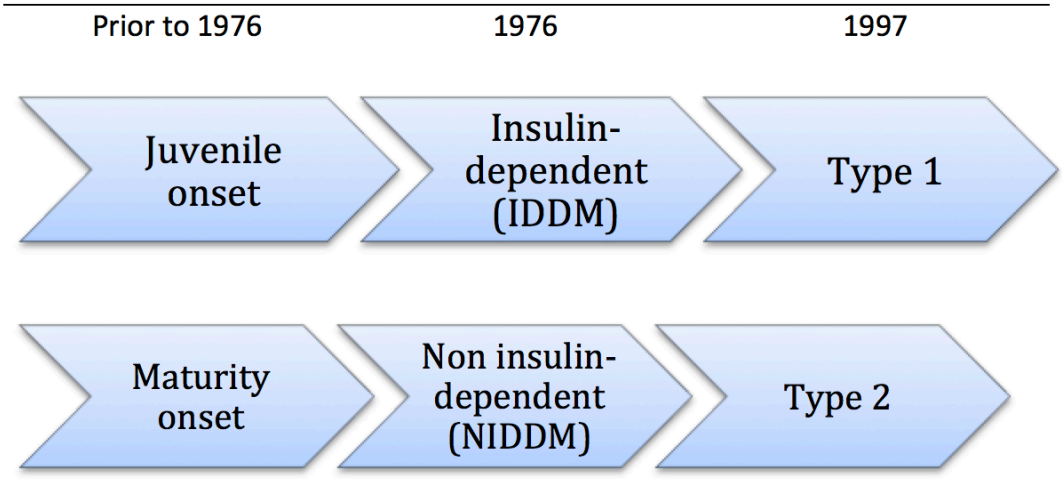



Figure 1.1 Progression of diabetes mellitus classification across the years

#### 1.1.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is characterized by the destruction of the pancreatic beta cell leading to total insulin deficiency. The affected individuals usually have an early onset (< 35 years) with an acute presentation including diabetes ketoacidosis.

##### 1.1.2.1.1 Autoimmune

The commonest (90% of Europids) type of pancreatic beta cell destruction is autoimmune in origin (Alberti, 2010). This is characterized by T-cell mediated destruction of the beta cells leading to exogenous insulin dependence. Autoantibodies including anti-glutamine acid decarboxylase (anti-GAD), anti-insulin and/or islet cell antibodies are usually are usually present prior to diagnosis (Alberti, 2010; Libman IM, LaPorte RE, Libman AM, 2011).

##### 1.1.2.1.2 Idiopathic

This category of type 1 diabetes is most common in non-Europid populations, especially Asians (McLarty *et al.*, 1990). There is loss of beta cell function in the absence of any autoantibodies. These individuals still have episodes of ketoacidosis and require exogenous insulin for survival (Alberti, 2010; Libman IM, LaPorte RE, Libman AM, 2011).

#### 1.1.2.1.3 Neonatal diabetes

This type of diabetes presents with hyperglycaemia in the first 3 – 6 months of life, in the absence of any autoantibodies (Shield *et al.*, 1997). There are two variants: transient and permanent. The transient type is associated with intrauterine growth retardation and is a rare condition (1 in 500,000 births) (von Mühlendah and Herkenhoff, 1995; Sperling, 2006a). This transient type is the commonest form of neonatal diabetes. It has been associated with the inheritance of two chromosome homologs from the father alone (paternal isodisomy) as well as with other imprinting defects on chromosome 6 (Temple *et al.*, 1995; Shield *et al.*, 1997). Those with transient neonatal diabetes are at risk of developing permanent diabetes later on in life (Metz *et al.*, 2002).

The permanent neonatal diabetes cases have been associated with activation of mutations of KCNJ11 (potassium channel subunit Kir6.2) and ABCC8 (sulfonylurea receptor 1) on chromosome 6, as well as with mutations of the insulin promoter factor (IPF-1) on chromosome 7 (Sperling, 2006b). Neonatal diabetes affects the insulin secretion capability of the pancreas.

#### 1.1.2.1.4 Latent autoimmune diabetes of adults (LADA)

This type of diabetes typically presents in middle age with GAD antibodies and will ultimately lead to exogenous insulin dependence (Tuomi *et al.*, 1993). Therefore the characteristics of LADA consist of an overlap between type 1 and type 2 phenotypes (Thai *et al.*, 1997). These individuals were found to be more likely to be non-obese and with lower serum C-peptide levels when compared to type 2 diabetes individuals (Tuomi *et al.*, 1993; Zimmet *et al.*, 1994).

#### 1.1.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes and now constitutes a global epidemic. This is characterized by insulin resistance and relative insulin deficiency. Type 2 diabetes is a multifactorial disease including genetic abnormalities, environmental (sedentary lifestyle and Westernized diet) factors and the presence of obesity. *The pathophysiology, epidemiology, screening techniques and related co-determinants of type 2 diabetes mellitus will be discussed in more detail in subsequent sections*.

#### 1.1.2.3 Other specific types of diabetes

##### 1.1.2.3.1 Maturity-Onset Diabetes of the Youth (MODY)

MODY was first recognized in 1974 by Robert Tattersall (Tattersall, 1974). The MODY family is composed of a group of autosomal-dominant inherited disorders that present with hyperglycaemia at an early age, usually before 25 years with a strong family history of hyperglycaemia. Individuals suffering from any type of MODY are not insulin dependent since this monogenetic diabetes results from beta cell dysfunction rather than insulin resistance (Tattersall, 1974, 1998). The genetic aetiology contributing to MODY could be broadly divided into two main aetiologies namely, a mutation in the gene encoding the glucose sensing enzyme glucokinase (GCK) with a 22% frequency and mutations in several transcription factors (HNF1A, HNF4A, HNF1B, IPF1, NeuroD1) contributing to 66% frequency. Other less frequent (12%) types of MODY can be found, with MODY X being the most prominent (11% frequency) (McCarthy and Hattersley, 2008).

#### 1.1.2.3.1.1 Glucokinase MODY

A functional mutation in glucokinase results in this type of MODY. Individuals are still able to stimulate their beta cells for insulin secretion but with a higher glycaemic set point leading to a mild fasting hyperglycaemia from birth (Byrne *et al.*, 1994). This type of MODY is not usually treated since the hyperglycaemia is mild with preserved regulation of glucose and complications rarely develop (Gill-Carey O SB, Colclough K, Ellard S, 2007).

#### 1.1.2.3.1.2 Transcription factor MODY (HNF1A and HNF4A)

The most common transcription factor mutations are within the hepatic nuclear factors 1A and 4A (HNF1A and HNF4A). These mutations alter insulin secretion from beta cell as well as alter the development and proliferation of the beta cell with eventual cell mortality. It has been observed that these mutations lead to alterations in the GLUT-2 glucose transporter and in mitochondrial enzymes responsible for glucose metabolism (Wang *et al.*, 1998, 2000).

Individuals with HNF1A (MODY 3) mutations present with an elevated HDL-C levels and glycosuria. These individuals have an increased cardiovascular risk when compared to individuals with type 2 diabetes (Isomaa *et al.*, 1998; Pearson *et al.*, 2003; Stride *et al.*, 2005). Individuals with HNF4A (MODY 1) are associated with macrosomia, low HDL-C levels and elevated LDL-C levels (Pearson *et al.*, 2005, 2007).

#### 1.1.2.3.2 Gestational Diabetes Mellitus (GDM)

GDM is a hyperglycaemic state that is detected during pregnancy (Metzger *et al.*, 2007). Screening for GDM is usually undertaken at around 28 weeks of gestation. The risk factors for developing GDM include those with previous GDM, increasing age, obesity, previous macrosomic babies, first line family history of type 2 diabetes mellitus and certain ethnic groups (Chan, Wong and Ho, 2002; Metzger *et al.*, 2007). Following birth, the plasma glucose level goes down to normal but these women are at a higher risk of developing type 2 diabetes mellitus later on in (Lauenborg *et al.*, 2004).

### 1.1.2.4 Novel subgroups of adult onset diabetes

A Scandinavian study explored the possibility of re-classification of adult onset diabetes mellitus following the fact that the current diabetes diagnosis is based on only one metabolite, glucose (Ahlqvist *et al.*, 2018). Considering that diabetes is a heterogeneous disease, a novel classification was proposed based on data-driven cluster analysis of commonly measured parameters while incorporating metabolic, genetic and clinical elements (Ahlqvist *et al.*, 2018). A five-cluster classification was reported as follows:

I. Severe Autoimmune Diabetes (SAID): Early onset disease with relatively low body mass index (BMI), poor metabolic control, insulin deficient and presence of GADA autoantibodies.
II. Severe Insulin-Deficient Diabetes (SIDD): Similar characteristics of cluster 1 (SAID) but without the presence of GADA antibodies
III. Severe Insulin Resistance Diabetes (SIRD): Presence of insulin resistance and high BMI. These individuals had the highest risk of diabetes kidney disease with

persistence microalbuminuria. They were also at higher risk to develop chronic kidney disease.

IV. Mild Obesity Related Diabetes (MOD): Presence of obesity without insulin resistance.

V. Mild Age-Related Diabetes (MARD): Older patients than other clusters with similar characteristics of cluster 4 (MOD) but with modest metabolic derangements.

This is an optional classification for diabetes that can be utilized by physicians, but one needs to keep in mind the shortcomings of this classification. Only Scandinavian populations were investigated in this study. Furthermore, only two antibodies were analysed while eliminating any potential effects from other antibodies. However, it was reported that the combination of variables related to the development of diabetes is a better measurement and classification than the current one metabolite based measure (Ahlqvist *et al.*, 2018).

## 1.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder caused by a combination of genetic and environmental factors that have an effect on the beta cell function and peripheral insulin sensitivity (Hamman, 1992; Gerich, 1998; UK Prospective Diabetes Study (UKPDS) Group, 1998b, 1998a). The genetic factors have a primary effect on the beta cell function. However both genetic factors and acquired factors including sedentary lifestyle and a Westernized diet have an effect on the development of obesity and insulin resistance (Tuomilehto *et al.*, 2001). The global change in lifestyle habits and the

increased consumption of refined sugar have been nicknamed the “Coca-Cola Colonization” (Bruno and Landi, 2011).

The combination of dysfunctional beta cell function and insulin resistance ultimately results in chronic hyperglycaemia with a disturbance in carbohydrate, protein and fat metabolism. The beta cell function is typically reduced by 50% at the time of diagnosis (Holman, 1998).

### 1.2.1 Insulin and plasma glucose control

Insulin is a hormone that is synthesized and stored in the pancreatic beta cell. Insulin is a dipeptide containing A (21 amino acids) and B (30 amino acids) chains linked by disulphide bonds. The A chain has an N-terminal helix that is linked to an anti-parallel C-terminal helix. The B chain has a central helical segment (Dodson and Steiner, 1998).

Insulin is released in response to changes in the extracellular glucose concentration (other non-nutrient regulators also have an effect including islet products, neurotransmitters, gastrointestinal hormones and adipokines). Figure 1.2 illustrates the pancreatic intracellular pathways of (pro)insulin biosynthesis, processing and storage (Jones and Persaud, 2010).

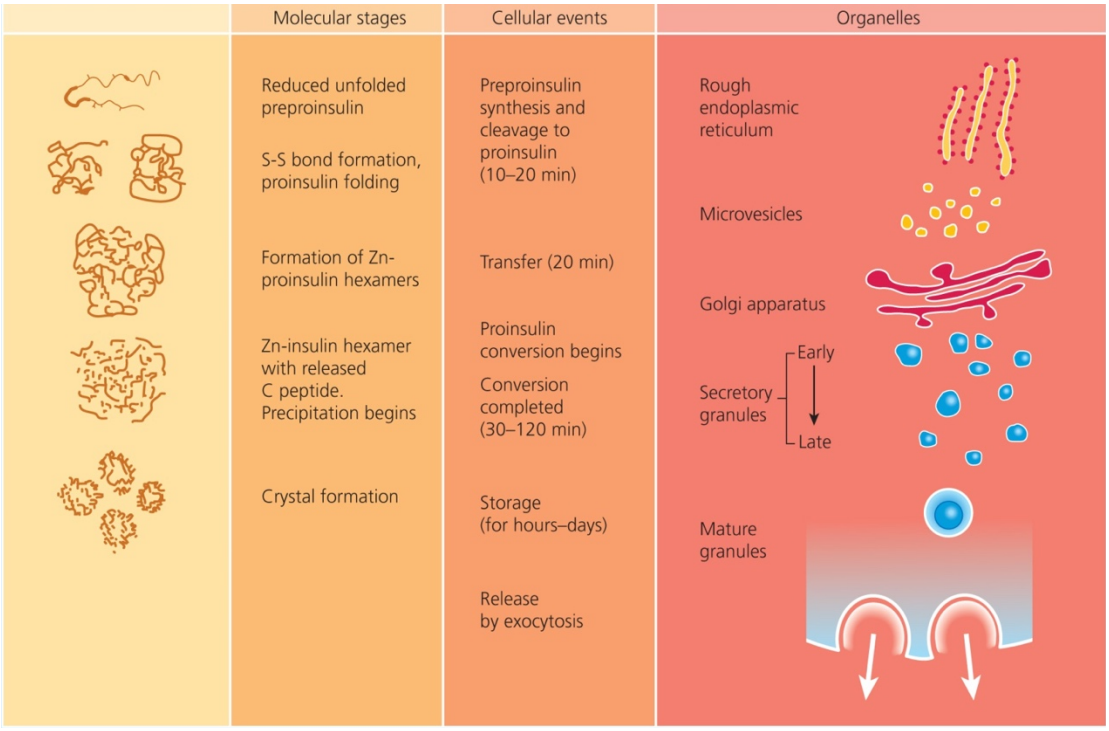



Figure 1.2 Pancreatic intracellular pathways of (pro)insulin biosynthesis, processing and storage (Jones and Presaud, 2010)

Insulin is also responsible for the regulation of carbohydrate, lipid and protein metabolism, as well as for having mitogenic effects which promote cell division and cell growth (Wilcox, 2005). A low-grade insulin concentration is always present even during fasting periods, as seen in Figure 1.3 (Suckale, J; Solimena, 2008).

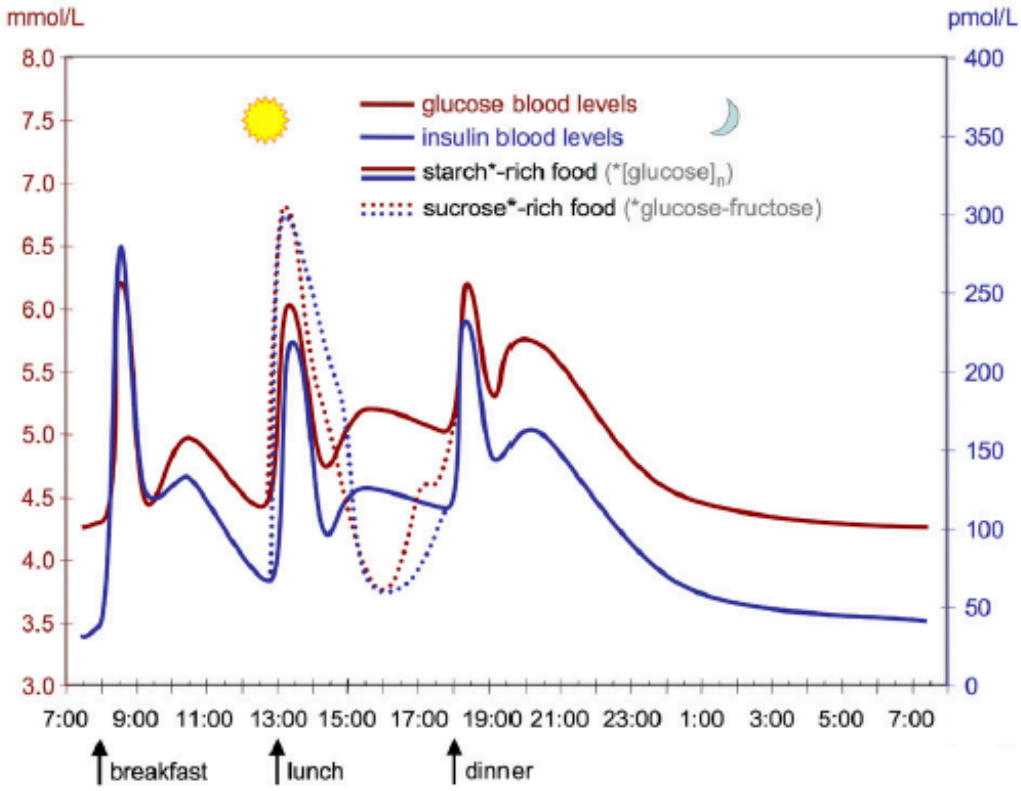



Figure 1.3 Insulin blood concentrations throughout a typical day (Suckale, J; Solimena, 2008)

Extracellular glucose is transported into the beta cell through high capacity glucose transporters (GLUT 1, 2, 3) whilst enabling rapid glucose concentration equilibrium between the extracellular and intracellular environment (De Vos *et al.*, 1995). Once glucose is within the beta cell, it is phosphorylated by glucokinase (a glucose sensor). Glucokinase functions as the glucose sensor of pancreatic beta cells since it is the rate-determining step of the glycolytic pathway resulting in net production of ATP, A high ATP/ADP ratio results in the closure of the beta cell $K_{ATP}$ channels. Subsequently potassium efflux is reduced resulting in depolarization of the beta cell membrane which enhances the influx of calcium ions into the cell through voltage-dependent L-type calcium channels (Cook and Hales, 1984; Miki, Nagashima and Seino, 1999). The increase in cytosolic calcium triggers the exocytosis of insulin granules and initiates an insulin secretory response, as seen in Figure

1.4. This mechanism results in insulin secretion that acts as a regulatory mechanism for the plasma glucose concentration, also known as the first insulin phase (Van Schaftingen, 1994). A glucose concentration above 5mmol/L affects the rate of insulin release (Jones and Presaud, 2010).

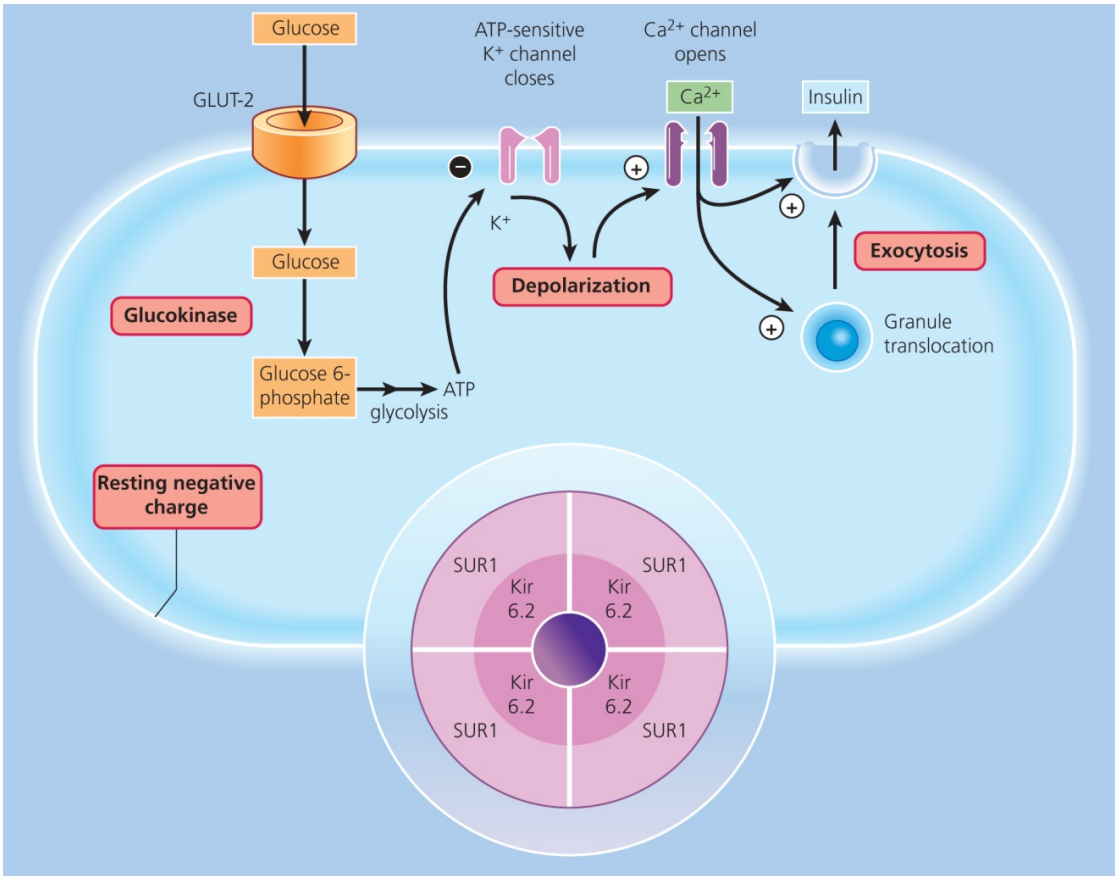



Figure 1.4 Intracellular mechanisms that stimulate insulin secretion (Jones and Presaud, 2010)

The beta cell $K_{ATP}$ channel is a hetero-octamer composed of four potassium channel subunits (Kir6.2) and four sulfonylurea receptor units (SUR1), as seen in Figure 1.4 (Miki, Nagashima and Seino, 1999). Both ATP and the sulfonylurea drugs induce channel closure by binding to Kir6.2 and SUR1 respectively (Aguilar-Bryan *et al.*, 1995). Understandably,

mutations affecting the encoding of Kir6.2 and SUR1 have been reported to be associated with an increased risk of type 2 diabetes (Gloyn *et al.*, 2003).

Insulin release occurs in two phases, as seen in Figure 1.5 (Jones and Presaud, 2010). Phase one consists of an acute increase in insulin release lasting approximately 10 minutes. This is followed by the second phase, which is a slow release of insulin, which typically persists for as long as the glucose level is elevated. Phase one is related to insulin-secretory granules found close to the beta cell membrane. Phase two involves the release of newly synthesized insulin molecules and insulin from storage pools (Jones and Persaud, 2010).

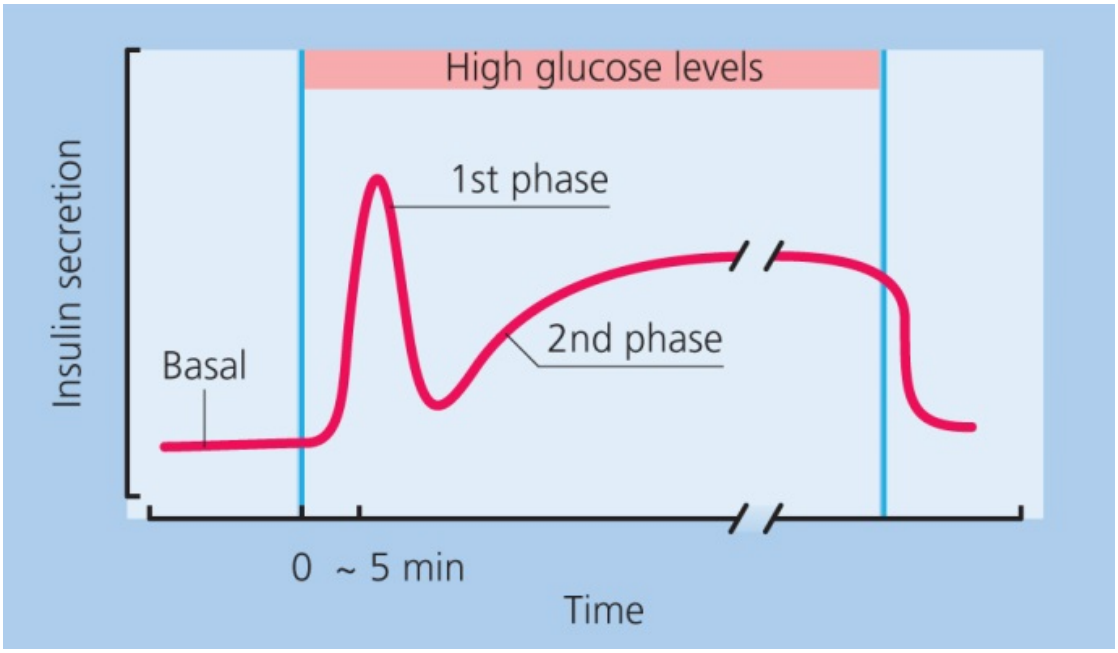



Figure 1.5 Glucose-induced insulin release (Jones and Presaud, 2010)

The secretion of insulin has been reported to follow rapid and long oscillations in order to maintain normal glucose homeostasis (Matthews, 1991). The majority of the secreted insulin follows high frequency bursts every 5 – 15 minutes (Porksen *et al.*, 1997). The

presence of ingested glucose, sulfonylureas and glucagon-like peptide-1 (GLP-1) enhances the amplitude of these secretory pulses (Porksen *et al.*, 1996b, 1996a; Ritzel *et al.*, 2001). On the contrary, somatostatin and insulin-like growth factor (IGF-1) diminish these pulses (Porksen *et al.*, 1996b; Porksen, N; Hussain, M; Bianda, T; Nyholm, B; Christiansen, J; Butler, 1997). In impaired glucose tolerant and type 2 diabetes individuals, there are alterations in these oscillations, resulting in abnormal insulin secretions and glucose homeostasis (Sturis *et al.*, 1992; O'Meara *et al.*, 1993).

Insulin controls the systemic nutrient homeostasis by stimulating the influx of glucose into muscle and fat cells. Insulin also promotes the synthesis of protein and glycogen in muscle and liver. This initiates lipid synthesis and storage within the liver and fat tissue. In a fasting state, the insulin secretion is decreased, and glucagon secretion is promoted along with adipocyte lipolysis for maintenance of glucose homeostasis. All of these are regulated by a negative feedback regulatory mechanism (Randle *et al.*, 1963). Figure 1.6 illustrates the regulation of glucose homeostasis (So *et al.*, 2000).

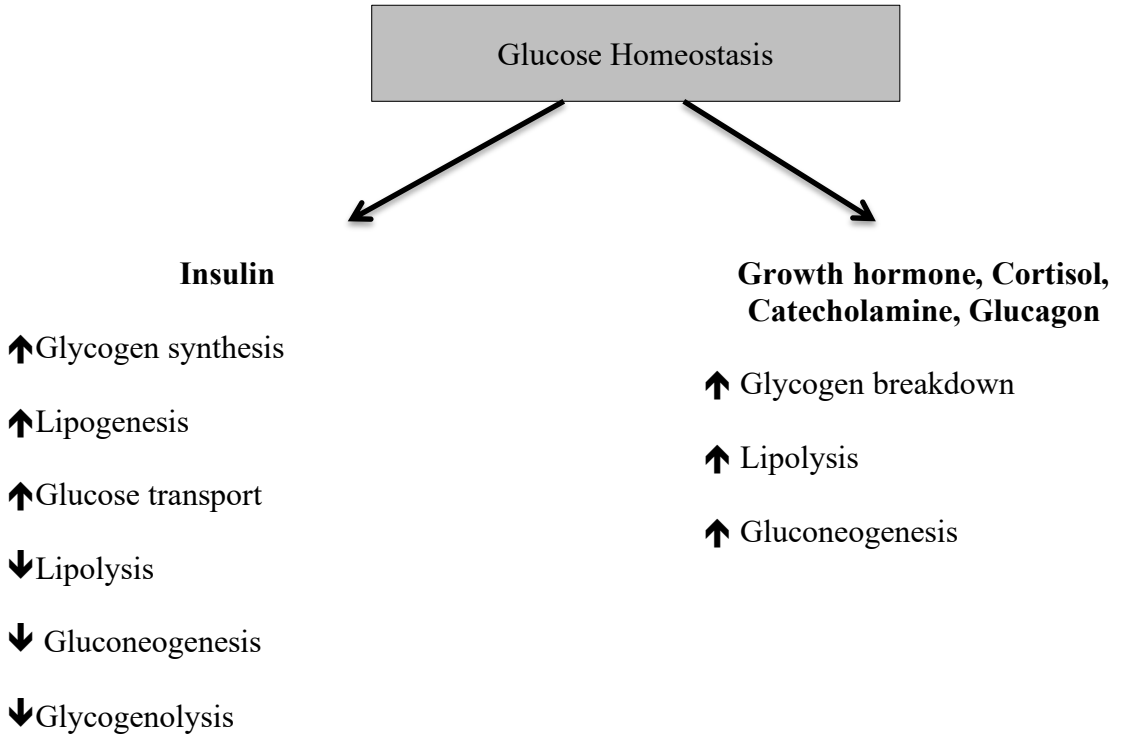



Figure 1.6 Regulation of glucose homeostasis (So *et al.*, 2000)

It has been well established that during our life cycle, insulin sensitivity and resistance fluctuate. In fact, during puberty a transient insulin resistance has been reported (Moran *et al.*, 1999). During the third trimester of pregnancy an element of insulin resistance develops, although those suffering from gestational diabetes were found to have an impaired beta cell function (Buchanan *et al.*, 1990). Increase in age has been recognized as a source of glucose impairment since 1921 (Spence, 1921). This was further supported by other successive research, where it was found that glucose tolerance impairment starts from as early as the third decade (DeFronzo, 1979). On the other hand physical activity was reported to increase insulin sensitivity (Goodyear, and Kahn, 1998).

### 1.2.1.1 Insulin receptor

The circulating insulin stimulates glucose transport into peripheral tissues while inhibiting hepatic gluconeogenesis (So *et al.*, 2000). Insulin also has other cellular functions apart from glucose homeostasis including the regulation of ion and amino acid transport; lipid metabolism; glycogen synthesis, gene transcription and mRNA turnover; DNA synthesis as well as protein synthesis and degradation, as can be seen in Figure 1.7 (Cheatham and Kahn, 1995).

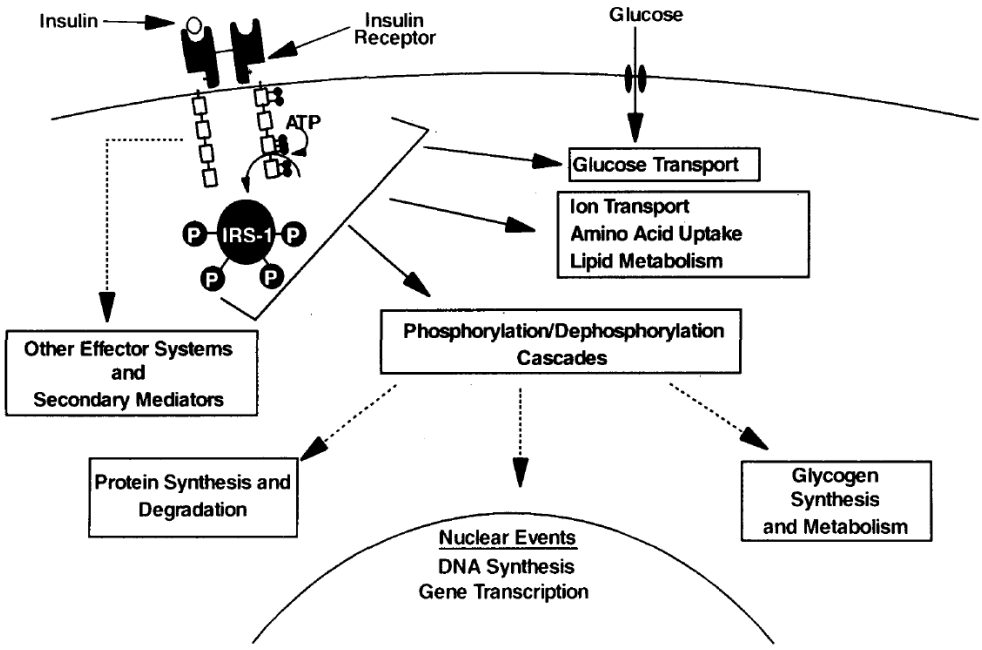



Figure 1.7 Insulin-signalling system affecting different intracellular processes (Cheatham and Kahn, 1995)

The insulin receptor is a hetero-tetramer and composed of two alpha-subunits and two beta-subunits linked together by disulfide bonds, as seen in Figure 1.8 (Cheatham and Kahn, 1995). The alpha-subunit, which is found on the outside of the cell, contains the insulin

binding site, whereas the beta-subunit, which is found spanning the membrane, possesses an insulin-stimulated protein tyrosine kinase (Cheatham and Kahn, 1995).

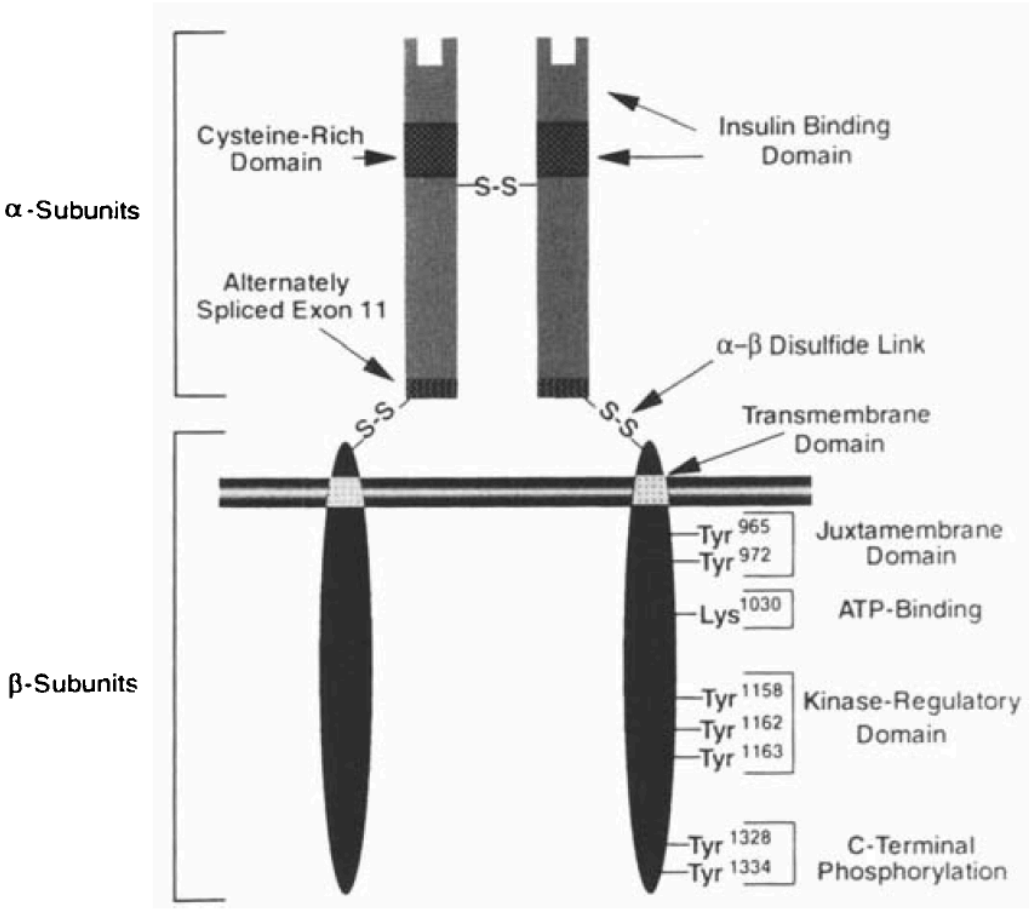



Figure 1.8 Insulin receptor structure and functional domains (Cheatham and Kahn, 1995)

The insulin receptor can be found in all mammalian tissues, although the amount of receptors per tissue varies, with adipose and liver tissues having the highest concentration (Kahn *et al.*, 1981). Conversely, skeletal muscle tends to have a low concentration of insulin receptors but a high concentration of insulin-like growth factor-I (IGF-I) receptors (Caro *et al.*, 1987). The insulin-like growth factor-1 (IGF-1) receptor is similar in structure to the insulin receptor, as are other growth factors and cytokine receptors. In fact, the

insulin-like signalling system is composed of three well-defined ligands including insulin, insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2). All of these ligands can bind and activate the insulin receptor (Ullrich *et al.*, 1985).

On binding of the ligands (insulin, IGF-1, IGF-2) to the alpha-subunits, these subunits move closer together while an ATP molecule binds to the intracellular domain of the beta-subunit. This will activate an autophosphorylation cascade on tyrosine residues (Tornqvist *et al.*, 1987; White *et al.*, 1988). In return, phosphorylation of insulin receptor substrate (IRS) proteins occurs. Both insulin and IGF-I phosphorylate the IRS proteins alike. Once the IRS is stimulated a number of different intracellular pathways could be activated including the protein kinase B and C (which have several functions), $PI_3$ kinase (which stimulates glucose uptake into skeletal muscle and adipose tissue while acting on glycogen synthesis), CAP/CBI/TC10 pathway (glucose transport stimulation) and the Ras-p38 MAPK pathway (cell growth and mitogenesis) (Sun *et al.*, 1991; Tanasijevic *et al.*, 1993; Wu, X; Garvey, 2010). The different insulin signalling pathways are illustrated in Figure 1.9 (Wu and Garvey, 2010).

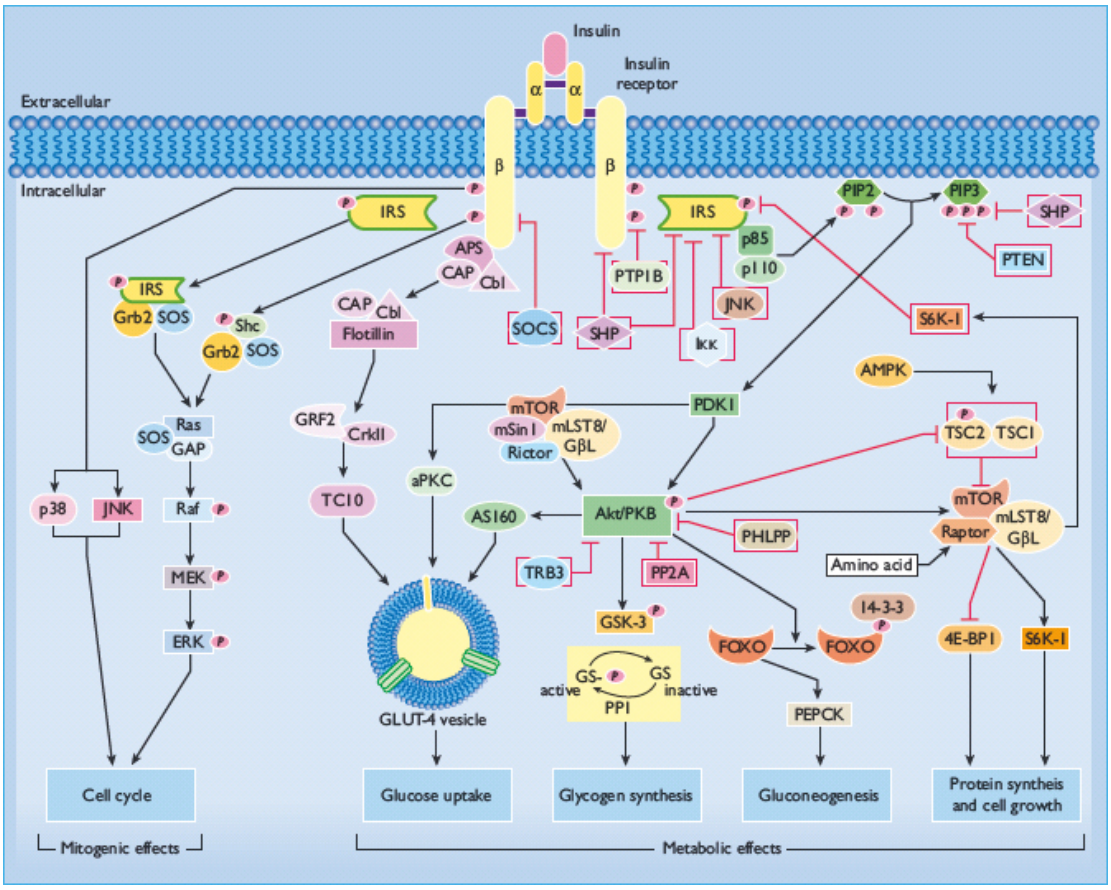



Figure 1.9 Illustration of insulin signalling pathways (Wu and Garvey, 2010)

In insulin resistant Type 2 diabetes individuals, the ability of insulin to stimulate the insulin receptor tyrosine phosphorylation and IRS-1, as well as the IRS-1 associated PI3 kinase activity and GLUT-4 translocation are all abnormal (Guma *et al.*, 1995; Cusi *et al.*, 2000; Miranda *et al.*, 2005).

### 1.2.2 Beta cell function

Impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) individuals were reported to be hyperinsulinaemic and insulin resistant (Lillioja *et al.*, 1988; Warram *et al.*, 1990). Initially, it was thought that insulin resistance occurs first and beta cell

dysfunction is a consequential aftermath (Himsworth and Kerr, 1939). However, more recently it has been established that beta cell dysfunction is the primary genetic defect prior to the development of insulin resistance (Gerich, 1998; van Haeften *et al.*, 2000). This can be seen in acquired insulin resistance states (e.g. obesity) where adaptation of the beta cell function to maintain normal glucose homeostasis has been reported (Weyer *et al.*, 1999; Kahn, 2001). As the tissue insulin sensitivity decreases, the beta cell function increases (sensitivity) to maintain a normal glucose homeostasis (Karam *et al.*, 1974; Bergman, Phillips and Cobelli, 1981; Kahn, 2001; Pratley and Weyer, 2001). In fact, the beta cell function and insulin sensitivity decline progressively in phases as the glucose tolerance shifts from normal to impaired glucose tolerance to eventual diabetes, as illustrated in Figure 1.10.

Furthermore, insulin secretion declines with advancing age. Around 0.7 – 1% less insulin secretion occurs in normal glucose tolerant adults each year (Iozzo *et al.*, 1999; Chiu *et al.*, 2000; Fritsche *et al.*, 2002; Utzschneider *et al.*, 2004; Szoke *et al.*, 2008). However, the rate at which insulin secretion declines is twofold in IGT, while it is approximately 6% less per year in T2DM when compared to normal glucose tolerant adults (UK Prospective Diabetes Study (UKPDS) Group, 1995; Kahn *et al.*, 2006; Szoke *et al.*, 2008). Conversely, the insulin sensitivity decline is dependent on changes in body composition and physical fitness. Therefore the factors contributing to a decline in insulin sensitivity are the same factors contributing to an increase in insulin resistance (Utzschneider *et al.*, 2004). Hence a decline in insulin sensitivity will result in an increase in insulin resistance.

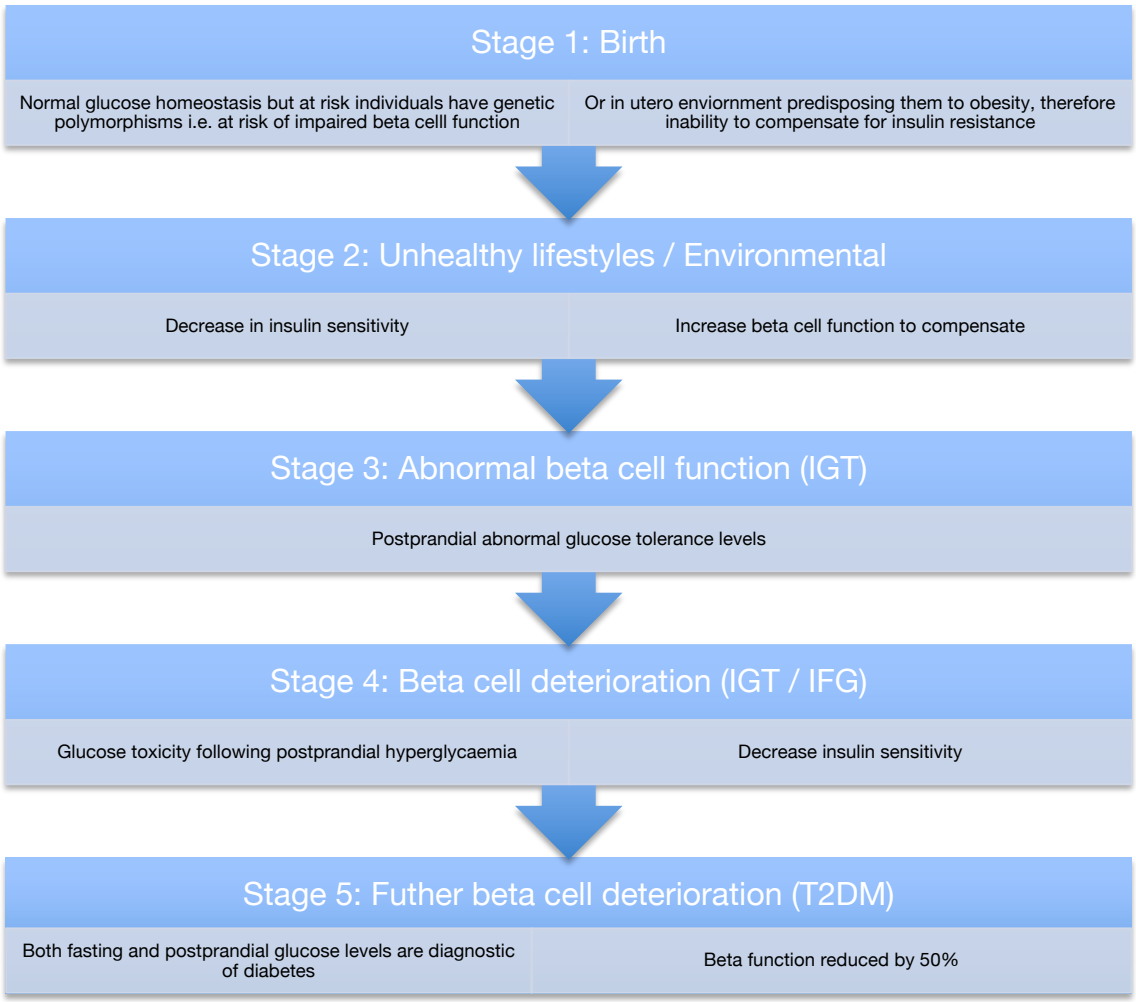



Figure 1.10 Stages of beta cell function and insulin sensitivity from normal glucose tolerance through to type 2 diabetes mellitus (Johnston *et al.*, 1990; Pimenta *et al.*, 1995; UK Prospective Diabetes Study (UKPDS) Group, 1995; Vaag *et al.*, 1995; Ferrannini *et al.*, 2005).

### 1.2.2.1 Beta cell dysfunction

Factors that impair beta cell function can be broadly divided into genetic and acquired. Genetic factors include a number of polymorphisms that target the beta cell function namely (1) peroxisome proliferator-activated receptor-γ (PPAR-γ) that convey susceptibility to free fatty acid adverse effects on insulin release (Stefan *et al.*, 2001); (2) gene encoding calpain-10, which is responsible for the modulation of insulin release as well

as the effect of insulin on muscle and adipose tissue (Horikawa *et al.*, 2000); (3) E23K variant of KIR6.2 gene, which increase the risk of T2DM through its effect on the potassium channels of the beta cells (Gloyn *et al.*, 2003; Florez *et al.*, 2007); (4) variants of the transcription factor 7-like 2 (TCF7L2) gene which have a negative effect on insulin secretion (Florez *et al.*, 2006). There are several acquired / environmental factors that impair beta cell function, as listed in Table 1.2

| Acquired factor | Explanation |
|---|---|
| Malnutrition in utero and early childhood | Inverse relationship between birth weight and at 1 year, with the development of T2DM in adult life. Malnutrition during first few months lead to beta cell damage leading to inability to adapt to over nutrition later on in life (Hales *et al.*, 1991; Hales and Barker, 1992). |
| Glucotoxicity | Both acute and chronic hyperglycaemia accelerate deterioration of beta cell function (Meyer *et al.*, 2002). |
| Lipotoxicity | Prolong elevation of free fatty acid (FFA) in plasma impairs beta cell function. FFA impair insulin gene expression; impair glucose stimulation of insulin secretion; promote beta cell apoptosis (Poitout and Robertson, 2002, 2008). |
| Obesity | Factors released from adipose tissue (elevated levels of FFA, TNF-α, resistin, leptin, adipsin and amylin) can adversely affect beta cell function (Hotamisligil and Spiegelman, 1994; R S Ahima and Flier, 2000; Rexford S. Ahima and Flier, 2000; Steppan *et al.*, 2001). |
| Inadequate incretin effect | Insulin responses to both GLP-1 and GIP are reduced in T2DM (Nauck *et al.*, 1986; Knop *et al.*, 2007). |
| Cytokines | Beta cells express IL-1β in T2DM but not in healthy individuals. High concentrations of IL-1β impairs insulin secretion and enhance apoptosis (Maedler *et al.*, 2002; Donath *et al.*, 2008). |

Table 1.2 Different acquired factors contributing to beta cell dysfunction

It was noted that a decrease in beta cell function does not necessarily lead to the development of diabetes. In fact, when using models to predict the development of diabetes,

beta cell function was only found to be a significant predictor when insulin resistance was included. Hence, the assessment of beta cell function needs to incorporate the degree of insulin resistance. In some individuals, low insulin secretion may be physiologically adequate but the same beta cell function may be inadequate for an insulin-resistant individual (Haffner *et al.*, 1996).

Beta cell function and insulin sensitivity can be assessed by the using the of oral glucose tolerance test (OGTT) and homeostasis model beta (HOMA-B) calculation (National Diabetes Data Group., 1979; Matthews *et al.*, 1985). Insulin sensitivity is assessed during the OGTT by the evaluation of both the suppression of the hepatic glucose production as well as the body's glucose disposal. During an OGTT, insulin sensitivity is inversely proportional to the product of the mean insulin and the mean glucose concentration within the plasma. With increasing insulin resistance in the liver and other peripheral tissues, the mean glucose plasma concentration increases during the OGTT (Matsuda and DeFronzo, 1999).

### 1.2.3 Insulin resistance

Insulin resistance (IR) occurs when there is resistance to the normal physiological actions of pancreatic insulin (Reaven, 1995). This leads to an attenuated biological response following a normal or elevated insulin production. Glucose homeostasis is efficient when the beta cells are insulin sensitive and able to secrete enough insulin to maintain glucose homeostasis. If the beta cell is able to compensate for the insulin resistance by secreting large amounts of insulin, glucose homeostasis can be maintained. However, if

hyperinsulinaemia cannot be sustained (in spite of beta cell adaptation), severe hyperglycaemia will develop in those who are insulin resistant (Reaven, 1988). Furthermore, it was reported that apart from resistance to insulin-stimulated glucose uptake, there is also resistance to insulin suppression of free plasma fatty acids (FFA) (Chen *et al.*, 1987). Hence, this leads to an increased FFA level within the circulation. In turn, this elevated FFA level enhances further the liver oxidation of FFA as well as hepatic gluconeogenesis. Hepatic gluconeogenesis occurs through the ability to generate acetyl-CoA that activates pyruvate carboxylase (a gluconeogenesis enzyme). Additionally, apart from activating hepatic gluconeogenesis, acetyl-CoA reduces the activity of pyruvate dehydrogenase resulting in conversion of glucose to lactate in muscle (Williamson, Browning and Olson, 1968; Randle, 1985).

Various different methods have been used to assess insulin resistance and beta cell function including: Euglycemic hyperinsulinemic clamp; Hyperglycaemic clamp; intravenous glucose tolerance and homeostasis model (HOMA) (DeFronzo, Tobin and Andres, 1979; Matthews *et al.*, 1985; Haffner *et al.*, 1996).

The euglycemic hyperinsulinemic clamp developed by DeFronzo is the gold standard method for evaluating insulin resistance. This evaluates the peripheral insulin sensitivity by inhibiting the hepatic glucose secretion while using a high-insulin injection (DeFronzo, Tobin and Andres, 1979). A radiolabelled glucose is used to quantify the contribution of skeletal muscle and hepatic insulin resistance towards the whole-body insulin-mediated glucose disposal deficiency (DeFronzo, Simonson and Ferrannini, 1982).

A simplified approach to quantify insulin sensitivity was considered through various insulin sensitivity/resistance indices. There are two groups of indices: (1) indices that are calculated by using fasting plasma concentration of insulin, glucose and triglycerides (e.g. HOMA-IR and Quantitative insulin sensitivity check index – QUICKI) (Matthews *et al.*, 1985; Chen *et al.*, 2003) and (2) indices that are calculated by using plasma concentration of insulin and glucose obtained during the 2$^{nd}$ hour of a standard 75g load OGTT (e.g. Matsuda, Belfiore) (Belfiore, Iannello and Volpicelli, 1998; Matsuda and DeFronzo, 1999).

The HOMA was developed in 1985 to quantify the insulin resistance and beta cell function by using fasting glucose and insulin (or C-peptide) concentrations (Matthews *et al.*, 1985). The HOMA-IR has also been validated to assess insulin resistance in children and adolescents and is easy to use in both clinical and epidemiological settings. The HOMA-IR is calculated by multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG), then dividing by the constant 22.5 i.e. HOMA-IR = (FPI x FPG) / 22.5 (Wallace, Levy and Matthews, 2004).

#### 1.2.3.1 Lipoprotein metabolism and insulin resistance

Lack of insulin secretion contributes to overproduction of very low-density lipoprotein (VLDL). This correlates with the amount of fat within the liver as well as with the increasing levels of serum triglycerides in those who are insulin resistant (Adiels *et al.*, 2006, 2007, 2008). The elevated VLDL levels increase the CETP-mediated exchange of cholesterol ester and triglyceride between VLDL and low-density lipoprotein (LDL), leading to an increased density in LDL (Deckelbaum *et al.*, 1984; Zambon *et al.*, 1993). This contributes to the small dense LDL particles evident in T2DM individuals

(Lahdenpera *et al.*, 1996; Gray *et al.*, 1997). These small dense LDL particles are highly atherogenic and provide a link between insulin resistance and cardiovascular disease (Coresh *et al.*, 1993; Austin *et al.*, 1995, 2000; Gardner, Fortmann and Krauss, 1996).

Furthermore, high-density lipoprotein (HDL) levels are reduced as the triglyceride levels increase. The HDL-C particles become enriched with triglycerides and in return are removed from the circulation at an accelerated rate by hepatic lipase (Eisenberg, 1984). Thus, the TG/HDL-C ratio has been described as a better predictive measure of insulin resistance than the isolated presence of abdominal obesity (Reaven, 2002).

#### 1.2.3.2 Insulin resistance and associated conditions

Insulin resistance is associated with a number of conditions, namely inflammatory, metabolic and coagulation abnormalities. Obesity is related to insulin resistance by impairing both insulin stimulated glucose uptake as well by inhibiting the effect of insulin on endogenous glucose production (Kolterman *et al.*, 1981; Bonadonna *et al.*, 1990). The hepatic insulin resistance and liver fat content are related to both waist circumference and body mass index (BMI). In fact, a body weight loss of 8% in obese women was reported to decrease the total body fat content mass by 14% and the liver fat content by 39% (Tiikkainen *et al.*, 2003). There was also a marked improvement in hepatic insulin sensitivity (Tiikkainen *et al.*, 2003).

Individuals suffering from hypertension have been reported to be insulin resistant with hyperinsulinaemic and hyperglycaemic states (Hwang *et al.*, 1987; Reaven, 1988). Insulin resistance is associated with hyperinsulinaemia, glucose intolerance, increase in

triglyceride and decease in HDL-C levels along with an elevation in blood pressure. All of these factors increase the risk for coronary artery disease and it is testament to the fact that insulin resistance plays a role in the aetiology of coronary artery disease (Reaven, 1988).

The presence of insulin resistance does not always lead to the development of diabetes. However, once failure of the beta cells develops, diabetes develops over a period of time (Ferrannini *et al.*, 2004). The presence of the inflammatory cytokines, TNF-α receptor, IL-6, IL-1 and high sensitivity CRP, increase the risk of developing T2DM (Liu *et al.*, 2007). However, it was reported that the combination of IL-1, IL-6 and TNF- α exhibited questionable associations with development of diabetes mellitus (Spranger *et al.*, 2003).

Considering the various effects of insulin resistance, the presence of insulin resistance can be considered as an early marker for both metabolic and cardiovascular disease risks (Cobb *et al.*, 2013).

### 1.2.4 Risk factors for Type 2 Diabetes Mellitus

The risk factors contributing to the development of type 2 diabetes are multiplex with complex interactions among different determinants of health taking course over the life span of an individual. In fact, an ecologic perspective can be considered to determine the various risk factors contributing towards the development of diabetes (Susser and Susser, 1996). Figure 1.11 illustrates a multilevel approach of different risk factors that may contribute towards the development of diabetes over a life course (Kaplan, Everson and Lynch, 2000).

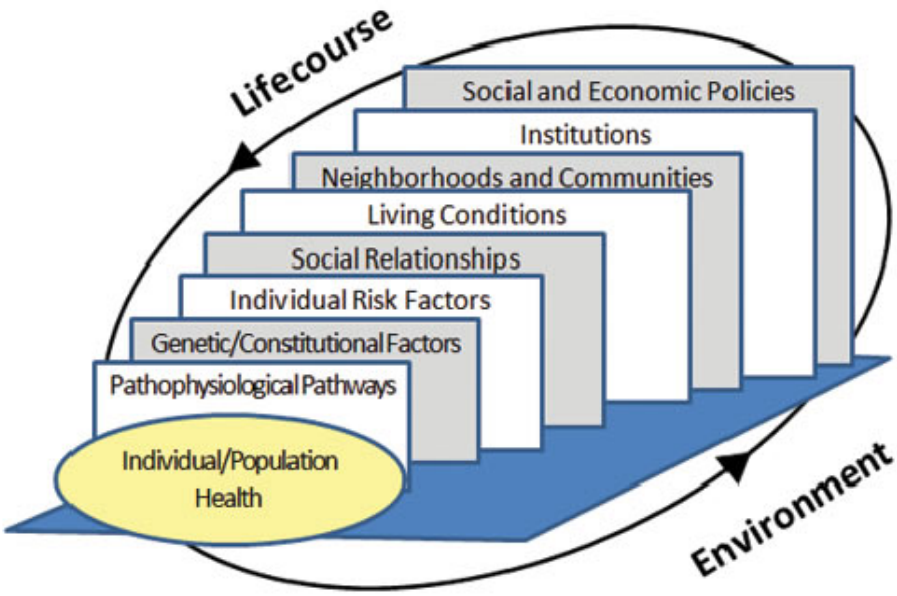



Figure 1.11 Diabetes mellitus ecologic paradigm over the life course (Kaplan, Everson and Lynch, 2000)

Both beta cell dysfunction and the development of insulin resistance contribute to the pathophysiology of T2DM (Lillioja *et al.*, 1993). Type 2 diabetes risk factors can initiate from the intra-uterine environment and early-life growth trajectories. An interaction between genetics (non-modifiable) and environmental (modifiable) factors increase the risk for insulin resistance, beta cell dysfunction and obesity, which ultimately lead to the development of diabetes mellitus (Neel, 1962; Elbein, Wegner and Kahn, 2000; Grant *et al.*, 2006; Kahn, Hull and Utzschneider, 2006; Sladek *et al.*, 2007; Zeggini *et al.*, 2007). Insulin resistance and cardiovascular disease risk factors (commonly associated with adiposity) appear to contribute to the majority of the risk for the development of diabetes (James B Meigs *et al.*, 2006).

### 1.2.4.1 Early-life risk factors

A hyperglycaemic foetal environment predisposes the child to develop insulin resistance, obesity and T2DM later on in life (Beck-Nielsen *et al.*, 2003; Jiang *et al.*, 2013). Children born to females who have diabetes or else developed gestational diabetes, have a predisposition to develop T2DM later on (Boerschmann *et al.*, 2010). In fact, it was reported that these children are seven times more likely to develop impaired glucose tolerance and up to thirty-two times more prone to develop T2DM in their lifetime compared to children born to non-diabetes mothers (Pettitt *et al.*, 1988; Silverman *et al.*, 1991).

Birth weight (either small or large for gestational age) was associated with the development of T2DM later on in life (Hales *et al.*, 1991). The highest risk was reported to be in those who had a small birth weight but became overweight during childhood and/or early adulthood (Ong *et al.*, 2000).

Two hypotheses have been put forward linking birth weight to the development of T2DM later on in life. The *thrifty phenotype hypothesis* suggested that long-term effects of nutritional deprivation in utero lead to biological programming later on in life. Such infants exhibit low birth weight with the risk of developing T2DM later on in life (Hales and Barker, 1992).

McCarthy postulated the *theory of an association* between diabetes and low birth weight due to pleotropic effects of genes influencing both foetal growth and susceptibility to diabetes (McCarthy, 1998). In fact, it was reported that the interaction of the foeto-maternal environment and the foetal genotype set the foundations for lifelong metabolic occurrences.

The genes responsible for the variation in foetal growth, survival and insulin regulation are the same genes that contribute to the development of diabetes susceptibility in adulthood (Hattersley *et al.*, 1998).

### 1.2.4.2 Genetic risk factors

T2DM has strong genetic links. It is a polygenic disease, not obeying the Mendelian mode of inheritance and therefore classified as a complex disease (Collins, Guyer and Charkravarti, 1997). Development of T2DM follows the occurrence of simultaneous common DNA sequence variations in the same individual (Staiger *et al.*, 2009).

The T2DM genetic linkage is further reinforced in comparative studies between mono and dizygotic twins. The concordance rate approached 90% in the former and only 37% in the latter twins (Newman *et al.*, 1987; Beck-Nielsen *et al.*, 2003). A person born to one parent afflicted by T2DM has a 40% lifetime risk of developing the disease himself/herself (Köbberling and Tillil, 1982). This was further evaluated in the Botnia study, where it was determined that those individuals with first-degree relatives suffering from T2DM had increased waist-hip ratio (i.e. abdominal obesity), increased insulin resistance and a decreased resting metabolic rate when compared to individuals with no family history of T2DM, therefore making them more susceptible to develop T2DM (Groop *et al.*, 1996). It was also established that those individuals with a maternal history of T2DM had twice the risk of developing T2DM when compared to those with a paternal history of T2DM (Groop *et al.*, 1996). In fact, the risk of developing T2DM in first-degree relatives was found to be four times higher than the general population average (Köbberling and Tillil, 1982).

An interesting outcome followed two studies conducted in Victoria (Australia) and in Toronto (Canada). Maltese-born individuals, who had emigrated years before, still ran a significantly higher risk of developing T2DM than native-born individuals. These studies support the genetic theory, where genetic factors are responsible for the development of diabetes when compared to environmental factors (Martin *et al.*, 1984; Vuskan *et al*. 1993).

### 1.2.4.3 Environmental risk factors

Lifestyle factors along with age are important determinants which can set off diabetes-susceptible genotypes (McCarthy and Menzel, 2001). In fact, in high-risk populations, lifestyle interventions can reduce the risk of development of T2DM by up to 50% (Tuomilehto *et al.*, 2001; Diabetes Prevention Program Research Group *et al.*, 2009). There are a number of environmental / lifestyle risk factors that contribute to the development of type T2DM, as discussed below and illustrated in Figure 1.12.

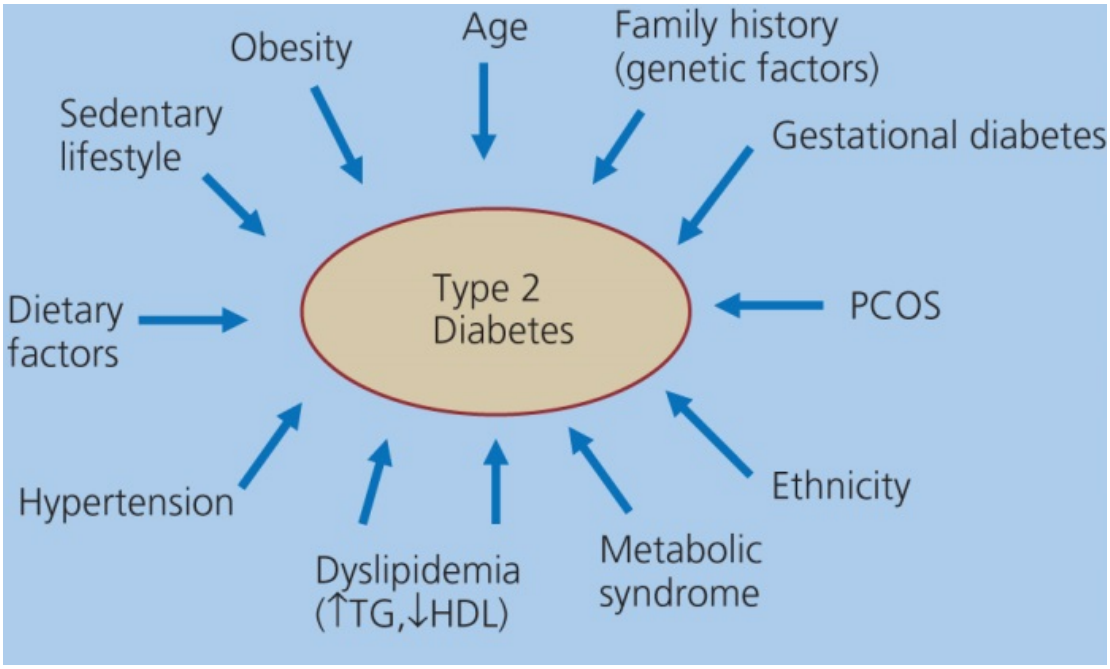



Figure 1.12 Risk factors for the development of type 2 diabetes (Ma and Tong, 2010)

#### 1.2.4.3.1 Obesity

Central obesity and insulin resistance have been reported as predictors of T2DM. In fact, central obesity was reported to be a significant predictive risk factor for T2DM even after adjustment for body fat and insulin resistance (Lillioja *et al.*, 1993). Weight loss leads to T2DM risk reduction irrelevant of whether it was achieved by lifestyle, pharmacological interventions or surgical interventions (Heymsfield *et al.*, 2000; Tuomilehto *et al.*, 2001; Sjöström *et al.*, 2004). However, it must be noted that not every diabetes individual is obese. In fact about 10% of T2DM individuals have normal weight (Staiger *et al.*, 2009).

#### 1.2.4.3.2 Metabolic Syndrome

The metabolic syndrome is composed of a cluster of metabolic abnormalities including obesity (abdominal), insulin resistance, dyslipidaemia and hypertension (Alberti, Zimmet and Shaw, 2006). The metabolic syndrome is an independent predictor for T2DM (Lorenzo *et al.*, 2003).

#### 1.2.4.3.3 Smoking

Smoking may reduce insulin sensitivity, making smoking a recognized risk factor for the development of T2DM (Feskens and Kromhout, 1989; Wannamethee *et al.*, 2001). In fact, an increase in cigarette smoking (therefore increased consumption of tar and nicotine) is associated with an increased risk of T2DM (Meisinger *et al.*, 2006). Considering that smoking is related to other unhealthy lifestyles (diet and sedentary life), it is considered that some studies may have overestimated the association of cigarette smoking per se with T2DM (van Dam, 2003). Of note, the 2015 European smoking

prevalence rate was reported to be 38% for males and 19% for females (World Health Organisation., 2017).

#### 1.2.4.3.4 Dysfunctional sleep

The different sleeping patterns of an individual have been studied. Both short (< 6 hours) and long (> 8 hours) stints of sleep have been associated with increased risk of insulin resistance and T2DM, although gender differences were noted (Spiegel *et al.*, 2005; Tuomilehto, Peltonen, Partinen, Seppä, Saaristo, Korpi-Hyövälti, Oksa, Puolijoki, *et al.*, 2008).

Chronic sleep apnoea was also associated with an increased risk of T2DM in men. This follows the fact that sleep-disorders are associated with obesity (through increased visceral fat deposition) (Tuomilehto, Peltonen, Partinen, Seppä, Saaristo, Korpi-Hyövälti, Oksa, Saltevo, *et al.*, 2008). Obstructive sleep apnoea results in stress hypoxia and increased release of pro-inflammatory cytokine, which may contribute to insulin resistance and T2DM (Alam *et al.*, 2007). Figure 1.13 illustrates the potential mechanisms of sleep loss contributing to T2DM risk.

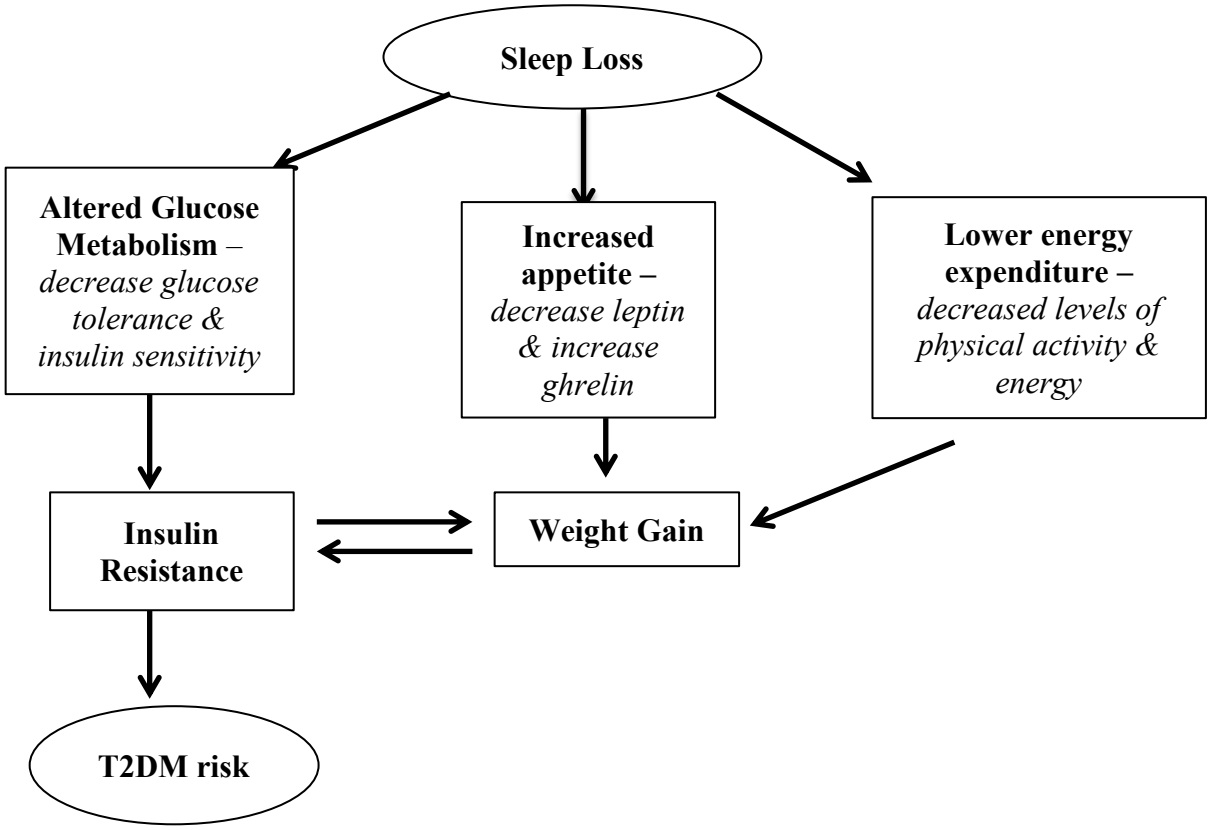



Figure 1.13 Potential mechanisms of sleep loss contributing to T2DM risk (Knutson *et al.*, 2007)

**1.2.4.3.5 Diet**

All types of dietary fat exhibit an adverse effect on insulin sensitivity, particularly saturated fats (Mayer-Davis *et al.*, 1997; Vessby *et al.*, 2001). Prolonged daily consumption of food products with added sugar has been found to increase the T2DM risk. For every 150 calories/person/day of added sugar (equivalent to 1 soda can per day), there is a corresponding 1.1% increased risk of developing T2DM after controlling for poverty, obesity, aging, physical activity and urbanization (Basu *et al.*, 2013). The corporate for this is fructose, which is a component of added sugar and undergoes first pass metabolism within the liver (insulin independent). This initiates fatty acid synthesis through Acetyl-

CoA, resulting in lipid deposition within the liver and eventual development of hepatic insulin resistance through the activation of inflammatory pathways, as seen in Figure 1.14. In return, gluconeogenesis is promoted (through impairment of insulin mediated phosphorylation of Fox01 resulting in hyperglycaemia and the development of T2DM (Bremer, Mietus-Snyder and Lustig, 2012).

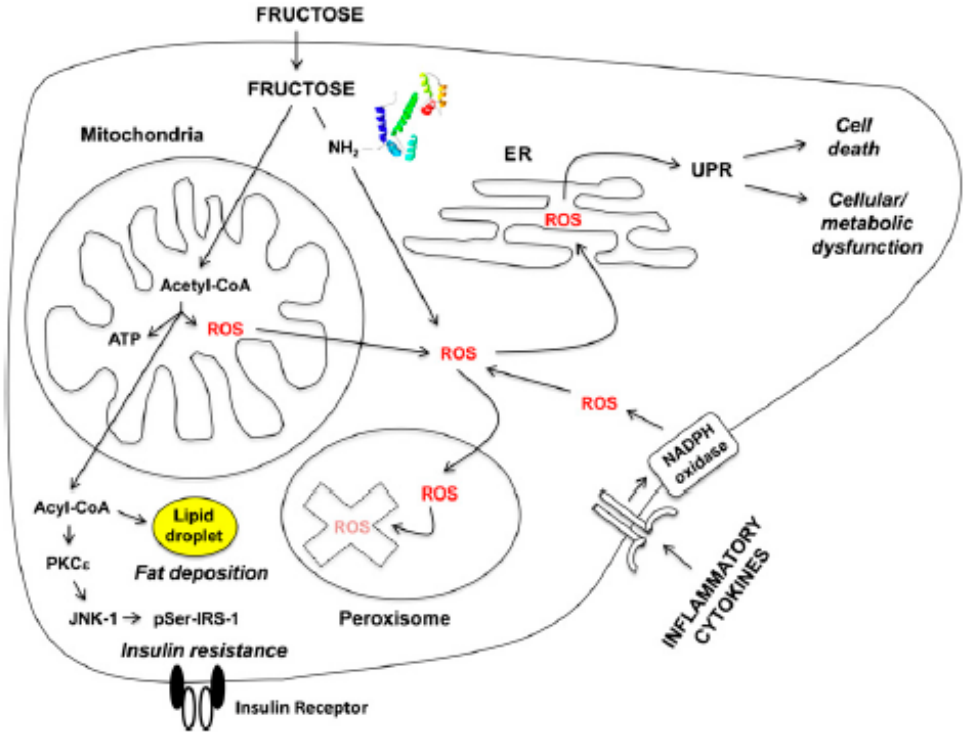



Figure 1.14 Fructose metabolism and consequences (Bremer, Mietus-Snyder and Lustig, 2012)

The biological breakdown of alcohol beverages also leads to a rise in fructose levels (Jordão, Vilela and Cosme, 2015). An approximate 70% of the European adults consume alcohol and hence have a predisposition to hyperglycaemia and development of T2DM (World Health Organization, 2014).

The intake of whole grains and dietary fibre has been associated with higher insulin sensitivity and a reduction in T2DM risk (Meyer *et al.*, 2000; Liese *et al.*, 2003, 2005). The intake of adequate dietary fibre was reported to have a positive effect on the pancreas resulting in adequate insulin secretion relative to insulin resistance (Liese *et al.*, 2005).

The Westernized diet consist of high intake of red meat as well as processed meats, fried foods, sweets and refined grains. All of these have been associated with an increased risk of T2DM. On the other hand, dietary patterns following a Mediterranean inspired diet characterized by high vegetables, fruits, poultry, fish and whole grains have been associated with a reduced risk of T2DM (van Dam, Rimm, *et al.*, 2002; van Dam, Willett, *et al.*, 2002; Fung *et al.*, 2004).

### 1.2.5 Prevention of Type 2 Diabetes Mellitus

Preventive measures against the development or delay of diabetes have been suggested since a number of co-morbid complications are related to T2DM. In fact, it has been reported that a diabetes individual has a 2-fold higher risk of stroke and is 10-times more likely to have a lower limb amputation (Siitonen *et al.*, 1993; Bell, 1994), while diabetes is the leading cause of end stage renal failure (Gross *et al.*, 2005). Furthermore, diabetes decreases the quality of life, increases mortality and health care expenditure (Gong *et al.*, 2011; Li *et al.*, 2014). Therefore, preventive action targeting higher risk populations, especially those with an impaired glucose tolerance (IGT) have been recommended. The annual progression rate from IGT to T2DM was reported to range between 1 to 10% (Jarrett *et al.*, 1979; Sartor *et al.*, 1980; Haffner *et al.*, 1990). The risk factors contributing to this

dyglycaemic progression include age, obesity, hyperinsulinemia and insulin resistance (Saad *et al.*, 1988; Lillioja *et al.*, 1993).

#### 1.2.5.1 Lifestyle management

Sustained weight loss through physical activity is the key contributor to preventing or delaying T2DM (Hamman *et al.*, 2006). This preventive approach (physical activity) incorporated with dietary and weight loss regulation were proved to be effective in a number of lifestyle intervention clinical trials, namely the Malmö feasibility study (1974 - 1985), the Da-Qing IGT and Diabetes Study (1986 – 1992), the Finnish Diabetes Prevention Study (DPS 1993 – 2000) and the US Diabetes Prevention Program (DPP 1996 – 2001) (Eriksson and Lindgärde, 1991; Pan *et al.*, 1997; Tuomilehto *et al.*, 2001; Diabetes Prevention Program (DPP) Research Group, 2002; Lindström *et al.*, 2003; Hamman *et al.*, 2006). Such data originating from these clinical trials is considered to be reliable and superior to any other form of studies and therefore public health officials should base both policies and strategies on these findings.

Moderate exercise of 30 minutes or more per day and up to 150 minutes per week was suggested following these trials (Pan *et al.*, 1997; Tuomilehto *et al.*, 2001; Diabetes Prevention Program (DPP) Research Group, 2002; Lindström *et al.*, 2003). Brisk walking was favoured although other means such as aerobic dance, bicycle, skating and swimming were suggested (Diabetes Prevention Program (DPP) Research Group, 2002). Additionally, moderate and vigorous physical activities have been reported to have a positive effect on insulin sensitivity (Mayer-Davis *et al.*, 1998).

A weight reduction of 5 to 7% from initial body weight was suggested (Diabetes Prevention Program (DPP) Research Group, 2002; Lindström *et al.*, 2003; Dyson *et al.*, 2018). However, this weight loss should be achieved in the first 6 months and then maintained in order to sustain a diabetes risk reduction (Jeffery, Wing and Mayer, 1998). In fact, it was reported that a weight loss of between 3.7 and 6.8 Kg in overweight individuals exhibited a diabetes risk reduction of 33% when compared to those with a stable weight over a period of 2 years (Moore *et al.*, 2000). Recently, the DIRECT trial established that a structured intensive weight management programme, conducted in primary care, was a viable programme to maintain remission of T2DM. It was reported that the majority of the intervention group maintained a 15Kg of weight loss and remained in remission for 24 months (Lean *et al.*, 2019).

Overall, healthy lifestyle interventions exhibited a positive risk reduction in the progression of IGT to T2DM over a follow-up period, as seen in Table 1.3. In addition to lifestyle interventions, other clinical trials have evaluated the effect of Metformin as a diabetes preventive measure.

| **Clinical trial** | **Follow-up period** | **Risk reduction in intervention group versus control group** |
|---|---|---|
| Malmö feasibility study | 6 years | 63% |
| Da-Qing IGT and Diabetes study | 6 years | 38% |
| Diabetes Prevention Study (DPS) | 6 years | 58% |
| Diabetes Prevention Program (DPP) | 3 years | 58% |

Table 1.3 Summary of randomized clinical trials using lifestyle interventions for the prevention of T2DM (Eriksson and Lindgärde, 1991; Pan *et al.*, 1997; Tuomilehto *et al.*, 2001; Diabetes Prevention Program (DPP) Research Group, 2002; Lindström *et al.*, 2003)

The US Diabetes Prevention Program (DPP) reported a more effective T2DM incidence reduction when following a lifestyle intervention (58% reduction) rather than Metformin (31% reduction) as a preventive approach when compared to placebo (Knowler *et al.*, 2002). On the other hand, the Indian Diabetes Prevention Program reported a beneficial outcome against the development of diabetes when IGT individuals followed either a lifestyle modification (28.5% risk reduction) or Metformin therapy (26.4% risk reduction) when compared to the control group. However, no added benefit was established when combining lifestyle modification with metformin therapy (Ramachandran *et al.*, 2006). Clinical trials are at the pinnacle of research designs. Therefore, the weight carried by these should be given the utmost attention. These findings suggest that both preventive measures (lifestyle or metformin) should be implemented in TD2M preventive strategies when targeting high-risk sub-populations (IGT).

A dietary change involving the reduction in saturated fat while increasing dietary fibre has been reported to have a positive reduction in T2DM risk (Lindström *et al.*, 2003; Dyson *et al.*, 2018). Furthermore, a Mediterranean diet supplemented with extra-virgin olive oil was found to have a reducing effect on diabetes incidence within a cardiovascular high-risk population (Salas-Salvadó *et al.*, 2014).

### 1.2.6 Screening for Type 2 Diabetes Mellitus

A screening test is offered to asymptomatic individuals in order to identify the likelihood or unlikelihood to have the disease in question (Morrison, 1998). The screening principles were set out in 1968 by Wilson and Jungner (Wilson and Jungner, 1968). T2DM

fits well the criteria for screening (a serious disease, associated with many complications, shortens expectancy of life, screening tests available, tests relatively cheap with no adverse effects) (Evans *et al.*, 2011). However, different organisations exhibit arguments in favour and against population-based screening. In 2003, following a collaboration meeting between the World Health Organization and the International Diabetes Federation, a report targeting T2DM screening was published (World Health Organisation., 2003). It was reported that at the time, there was no evidence about the benefit of early detection of T2DM through screening. However, it was suggested that if the prevalence of undiagnosed T2DM was high at a population level and significant associated cardiovascular disease risk was evident, then screening would be beneficial (World Health Organisation., 2003). Furthermore, a more recent pan-Europe population-based screening programme study (ADDITION – Europe) investigated the feasibility of undergoing a screening programme for T2DM by using a multi-stage screening process (van den Donk *et al.*, 2011). It was concluded that T2DM population-based screening is challenging but feasible to undertake (van den Donk *et al.*, 2011). Screening for T2DM could be attempted using a screening tool such as a risk assessment questionnaire, biochemical tests or a combination of the two (World Health Organisation., 2003). Different risk scores have been developed specific to the population under study. A well-established risk assessment tool is the *Finnish Risk Score*, which was set out to identify people at risk of T2DM without the need for laboratory tests (Lindström and Tuomilehto, 2003). This validated risk score includes age, BMI, waist circumference, history of anti-hypertensive drug use or high plasma glucose, physical activity and daily consumption of fruit and vegetables. Those obtaining a risk score of more than or equal to 9, had a sensitivity of 77% and a specificity of 66%, with a positive predictive value of 7% (Lindström and Tuomilehto, 2003).

The American Diabetes Association (ADA) has also developed a *Diabetes Risk Test* to assess the individual's risk of developing T2DM. This includes age; gender; history of gestational diabetes; history of immediate family with diabetes; history of hypertension; physical activity and body mass index. If the score is 5 or higher, then the individual is at an increased risk of developing T2DM and should seek medical consultation (American Diabetes Association, 2018b). Furthermore, the ADA provides criteria for screening for diabetes and pre-diabetes in those who are asymptomatic, as illustrated in Table 1.4.

| |
|---|
| 1. Testing considered in overweight or obese adults (≥25Kg/m$^2$) who have one or more of the following risk factors:<br>• First-degree relative with diabetes<br>• High-risk race / ethnicity<br>• History of cardiovascular disease<br>• Hypertension (≥140/90mmHg or on treatment)<br>• HDL-C level <0.90mmol/L and/or Triglyceride level >2.83mmol/L<br>• Women with polycystic ovarian syndrome<br>• Physical inactivity<br>• Associated conditions with insulin resistance (e.g. severe obesity, acanthosis nigricans) |
| 2. Patient with pre-diabetes (IFG, IGT or HbA1C ≥5.7%) should be tested yearly |
| 3. Women with gestational diabetes should have a lifelong testing at least once every 3 years |
| 4. For all other patients, testing should begin at 45 years |
| 5. If normal results, testing should be repeated at a minimum of 3-year intervals |

Table 1.4 Criteria for screening for diabetes or pre-diabetes in asymptomatic adults (American Diabetes Association, 2018b)

If screening for T2DM is undergone, it is important to note that individuals suffering from impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) would also be picked up. These are a high-risk population to develop T2DM, with an annual progression rate ranging between 3% and 13% (Edelstein *et al.*, 1997; Vaccaro *et al.*, 1999; Engberg *et al.*, 2009). Annual screening may be suggested for this sub-population.

### 1.2.7 Diagnostic criteria for Type 2 Diabetes Mellitus

Diagnosis refers to the confirmation of diabetes in individuals who exhibit symptoms or else have a positive screening test (World Health Organisation., 2003). Furthermore, in T2DM, the screening test may also be the diagnostic test such as a fasting plasma glucose (FPG) of more or equal to 7mmol/L, or else part of the initial stage of a diagnostic sequence used to confirm the diagnosis of T2DM in asymptomatic individuals (World Health Organisation., 2003).

The diabetes diagnostic cut-off points are based on data obtained from epidemiological studies investigating complications arising from diabetes. The diagnostic cut-off point is the level that determines the onset of retinopathy, which is the commonest complication encountered (World Health Organization, 2006). In 2006, a consensus was reached between the WHO and the IDF to endorse the cut-off criteria proposed by the ADA for diagnosis of T2DM (World Health Organization, 2006), as shown in Table 1.5 and Figure 1.15.

| |
|---|
| Fasting plasma plasma glucose ≥7mmol/L (no caloric intake for at least 8 hours) |
| *Or* |
| 2$^{nd}$ hour OGTT ≥11.1mmol/L (75g glucose load) |
| *Or* |
| HbA1C ≥6.5% (using a NGSP certified and standardized to DCCT assay) |
| *Or* |
| Individual with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥11.1mmol/L |

Table 1.5 Diagnostic criteria for type 2 diabetes mellitus (American Diabetes Association, 2018b)

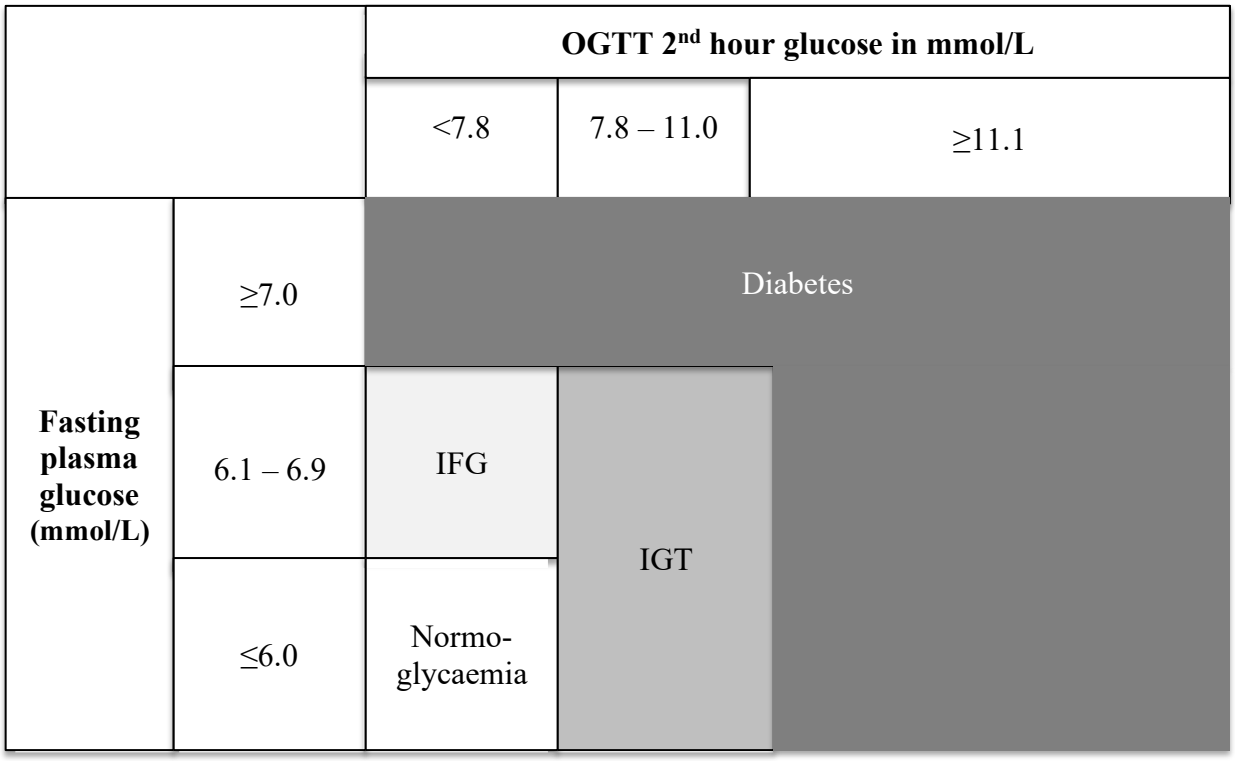



Figure 1.15 Diagnostic criteria for the different glycaemic statuses (World Health Organization, 2006)

The World Health Organization considers the diagnosis of diabetes mellitus and other hyperglycaemic categories through whole blood (venous and capillary) apart from plasma venous blood (World Health Organisation Consultation Group., 1999). However, it has been reported that the variation in red cell volume within whole blood may result in differences in glucose levels. Furthermore, plasma values are believed to be more physiologically representative (Bennett, 2010).

Plasma glucose samples (both fasting plasma glucose and Oral glucose tolerance test) have pre-analytic and analytic variability and are therefore prone to variable results when repeated. It is thus important for analytic precautions to be taken. Plasma should be separated from the cellular component within 60 minutes of bloodletting or else a glycolytic inhibitor tube such as one with sodium fluoride should be used for the plasma glucose sample collection (Sacks *et al.*, 2007). Furthermore, fasting plasma glucose (FPG) undergoes diurnal variation with mean FPG being higher in the morning than in the afternoon. This leads to potentially missing some diabetes cases if FPG samples are taken solely in the afternoon (Troisi, Cowie and Harris, 2000). One needs to also consider the relatively large intra-individual biological variability when interpreting glucose analysis. It was reported that FPG values between 5.8 and 6.9mmol/L should be repeated while an FPG between 5.3-5.7mmol/L should have a follow-up after a short interval (Sacks *et al.*, 2007).

The diagnostic criteria used by an epidemiological study follow that of a clinical setting. However, the diagnosis of T2DM in an epidemiological study is based on a single fasting plasma glucose or oral glucose tolerance test (World Health Organisation., 2003).

#### 1.2.7.1 Fasting plasma glucose (FPG)

Fasting plasma glucose has been considered a good diagnostic test equivalent to an oral glucose tolerance test (OGTT) since 1979 (Rushforth, Miller and Bennett, 1979). Individuals with a FPG of 7mmol/L or higher are considered as suffering from T2DM (World Health Organization, 2006; American Diabetes Association, 2018b). The FPG follows an inverse relationship with the pancreatic beta cell function. As the beta cell function declines and insulin sensitivity decreases, the FPG levels along with insulin

resistance increase to an eventual development of T2DM (Tabák *et al.*, 2009). However, it has been reported that FPG undergoes an abrupt increase 1.5 to 3 years before the diagnosis of diabetes (Tabák *et al.*, 2009). In fact, FPG increases by 0.02 – 0.8mmol/L per year before the diagnosis of diabetes (Ferrannini *et al.*, 2004; Laspa *et al.*, 2007; Tabák *et al.*, 2009).

### 1.2.7.2 Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) was first introduced as a research tool in the 1920's and later adopted as the diagnostic test for diabetes (John, 1922; Meyer and Womack, 1950).

Standardisation of the OGTT procedure was required for comparison of results between different populations and studies. The British Diabetic Association performed the first attempt to standardize OGTT based on 50g of oral glucose in 1964. The recommendations put forward included "*unrestricted diet and physical activity for at least 3 days, 12 hours fasting prior to test, 30 minutes sitting quietly before the test and individuals to remain seated and non-smoking during the two hours duration of the test*" (FitzGerald and Keen 1964). In 1969, the American Diabetes Association (ADA) put forward their recommendations based on 100g oral glucose load (American Diabetes Association., 1969). Later on, standardization was established where it was recommended that a 75g oral glucose load should be used. This load was found to reflect glucose levels associated with the development of complications (Rushforth, Miller and Bennett, 1979). The recommendation to use a 75g oral glucose load was consequently agreed upon by both the WHO and the International Diabetes Federation (IDF) in 2006. The WHO/IDF group retained the 75g OGTT as the "gold standard" for the diagnosis of diabetes mellitus since

fasting plasma glucose levels failed to identify approximately 30% of those with undiagnosed diabetes. Also, the OGTT is the only diagnostic test available for an impaired glucose tolerance state (The DECODE Study Group., 1998; World Health Organisation and International Diabetes Federation, 2006).

The carbohydrate load suppresses the liver glucose output and at the same time enhances the uptake of glucose by the muscle and the liver. In this way, the OGTT increases the secretion of insulin and assesses the insulin sensitivity of the liver and muscle tissues. However both individuals with impaired glucose tolerance as well as those with type 2 diabetes exhibit a reduction in the early (30 minutes) insulin response (Seltzer *et al.*, 1967). This diminishes the suppression of endogenous glucose production after glucose ingestion. The resultant hyperglycaemia acts as a greater stimulus to the beta cell, contributing to a late (2 hours) hyperinsulinaemia (Mitrakou *et al.*, 1992).

The WHO and ADA report the same diagnostic cut-off points for the second hour OGTT, as shown in Table 1.7 (World Health Organization, 2006; American Diabetes Association, 2018b).

| **OGTT value in second hour** | **Glucose status** |
|---|---|
| < 7.8 mmol/L | Normal |
| >= 7.8 – 11.0 mmol/L | Pre-diabetes |
| > = 11.1 mmol/L | Diabetes |

Table 1.6 Second hour OGTT diagnostic cut-off points (World Health Organization, 2006; American Diabetes Association, 2018b)

The OGTT is a laborious and unpleasant test for patients but it is still useful for a variety of reasons including (The DECODE Study Group., 1998; World Health Organization, 2006):

a. 30% of persons with diabetes fail to be diagnosed by a fasting plasma glucose test alone
b. Impaired glucose tolerance (IGT) is only diagnosed by means of an OGTT
c. Asymptomatic patients often require an OGTT to confirm and / or exclude glucose tolerance abnormalities

However, an OGTT is not usually the initial diagnostic test conducted in population studies. A potential reason could be due to potential refusal of participation due to the requirement to drink a glucose load and waiting for two hours for the survey examination to completed (Bartoli, Fra and Carnevale Schianca, 2011).

#### 1.2.7.3 Glycated haemoglobin (HbA1c)

The initial identification of HbA1C was made in 1969, when "unusual" haemoglobin was found to be present in diabetes individuals (Rahbar, Blumenfeld and Ranney, 1969). This test reflects the average plasma glucose over the previous eight to twelve weeks from the time the blood was drawn (Nathan, Turgeon and Regan, 2007). The advantage of the HbA1C over the FPG and the OGTT is the fact that it has less biologic variability and the individual does not need to be fasted (International Expert Committee, 2009). HbA1C also exhibits an equal or almost equal sensitivity and specificity to FPG and

post-load OGTT measurement when considered as a predictor of retinopathy (International Expert Committee, 2009). However, unless standardization between laboratories takes place, the results would be inconsistent (International Expert Committee, 2009). There are certain situations where performing an HbA1C instead of using other diagnostic screening measures for diabetes is inappropriate. It has been reported that in the presence of haemolysis and in haemoglobinopathies the HbA1C test would provide inaccurate results. (Smaldone, 2008; Aggarwal *et al.*, 2013). Haemolysis would result in false negative result since the lifespan of the erythrocyte is shortened and therefore less time for haemoglobin to be glycated. Haemoglobinopathies are more complex depending on the balance of increased haemolysis and altered rate of glycation and hence can give both false negative and false positive results.

An HbA1C of ≥6.5% has been suggested as the cut-off for diagnosing T2DM, although diagnosis should be confirmed by a repeat testing, provided that clinical symptoms and plasma glucose levels ≥11.1 mmol/L are not present. The repeat testing could be another HBA1C or either an FPG or an OGTT. Levels of HbA1C below 6.5% were reported to be indicative of intermediate hyperglycaemia (International Expert Committee, 2009). In fact, it is recommended that an HbA1C level between 6.0 and 6.5% should be considered as at high risk of T2DM and preventive interventions be administered (International Expert Committee, 2009). On the other hand, the ADA suggested that an HbA1C between 5.7 to 6.4% should be considered as a pre-diabetes state (American Diabetes Association, 2018b).

Recently, the American College of Physicians have suggested that T2DM patients should have a target HbA1C level between 7 – 8% following the results of the several trials including the ACCORD and ADVANCE trials (Qaseem *et al.*, 2018). These trials

concluded that intensive diabetes therapy does not lead to any beneficial results in diabetes care (Action to Control Cardiovascular Risk in Diabetes Study Group *et al.*, 2008; The ADVANCE Collaborative Group., 2008).

However, a variety of factors affect the HbA1C levels resulting in misleading results. The most important factors affecting HbA1C include haemoglobinopathies, certain types of anaemias and disorders associated with an acceleration of red cell turnover such as malaria (Roberts *et al.*, 2002; International Expert Committee, 2009).

#### 1.2.7.4 Management of type 2 diabetes mellitus

The key approach for an effective T2DM management is to target the beta cell function as well as peripheral insulin resistance (Salunkhe, VA; Veluthakal, R; Kahn, SE; Thurmond, 2018). Most of the medications found on the market target the beta cell function. Sulfonylureas have long been on the market and have been designed to trigger the $K_{ATP}$/sulfonylurea receptor (SUR) channels to stimulate insulin release. These are effective at enhancing the beta cell function but may lead to beta cell exhaustion and death (Kahn *et al.*, 2006, 2011). The glucagon-like peptide (GLP-1) receptor agonist also enhances insulin release by effecting the action of incretins peptides. This therapy has an effective effect on the promotion of biphasic insulin release. On the other hand, the dipeptidyl peptidases 4 (DPP-4) inhibitors prolong the half-life of incretins and therefore enhance the beta cell function (McIntosh *et al.*, 2005). However, these newer therapies targeting beta cell function provide similar HbA1C profile to sulfonylureas and hence similar glucose control (Nauck *et al.*, 2009).

Nowadays, it is common practice among physicians to prescribe Metformin as the first line medication for T2DM. Metformin reduces the hepatic glucose output and helps in stabilising the circulatory glucose levels. However, over a period of time, additional therapies, including subcutaneous insulin, as the last resort, are required in combination to Metformin in order to establish a controlled glycaemia (Kahn *et al.*, 2006). Recently, a sodium-glucose cotransporter-2 (SGLT2) inhibitor was developed. This medication targets peripheral insulin resistance by inducing glycosuria. In turn, there is a reduction in insulin requirement for glucose clearance within the circulation (Ferrannini *et al.*, 2014).

In recent studies, multitasking factors which enhance the glucose-stimulating insulin secretion (GSIS) as well stimulate insulin-glucose uptake have been studied for T2DM control. There are a number of multitasking factors that have an effect on diabetes human islets such as exocytosis factor syntaxin 4 (STX4) and p21-activated kinase 1 (PAK1), among others. Both of these multitasking factors are deficient in the diabetes islet cells and replenishing these factors are suggested of promoting peripheral insulin sensitivity, protects beta cell function and supports skeletal muscle glucose uptake respectively (Oh *et al.*, 2014; Oh, Miller and Thurmond, 2015; Ahn *et al.*, 2016; Salunkhe, VA; Veluthakal, R; Kahn, SE; Thurmond, 2018). However, further research is required to determine whether these new medications are potential candidates for treatment of T2DM.

### 1.2.8 Impaired hyperglycaemia (Pre-Diabetes)

Pre-diabetes or intermediate hyperglycaemia is a metabolic condition arising from a combination of insulin resistance and primary or secondary pancreatic beta cell

dysfunction (Alberti, 1996; Unwin *et al.*, 2002). Individuals with pre-diabetes have a raised plasma glucose level above the normal glucose baseline but not high enough to be diagnosed with T2DM (Alberti, 1996).

Impaired glucose tolerance (IGT) was first reported in 1979 as a replacement for 'borderline' diabetes that did not appear to have risks for microvascular complications (National Diabetes Data Group., 1979). Later on, impaired fasting glycaemia (IFG) was reported as a non-diabetes fasting hyperglycaemia state (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 1997; World Health Organisation Consultation Group., 1999). Both IFG and IGT are associated with an increased risk for diabetes mellitus as well as for cardiovascular risks including hypertension and dyslipidaemia. Furthermore, both IFG and IGT are often components of the metabolic syndrome, with enhanced cardiovascular risk (Alberti, 1996).

Valensi *et al.* (2005) reported the risk factors contributing to the development of pre-diabetes, as seen in Table 1.8 (Valensi *et al.*, 2005):

| **Risk factors for Pre-diabetes** |
|---|
| > 45 years of age |
| High body mass index, predominantly abdominal obesity |
| Hypertension |
| Sedentary lifestyle |
| Family history of diabetes |
| History of gestational diabetes |
| High birth weight children |

Table 1.7 Risk factors for the development of pre-diabetes (Valensi *et al.*, 2005)

There is a mean gender and phenotype difference between IGT and IFG. However, both dysglycaemic states increase T2DM risk. The majority of individuals with IFG do not have IGT and vice versa. For this reason the terminology of '*isolated IGT'* and '*isolated IFG'* was developed (Unwin *et al.*, 2002). The individuals with isolated IGT (4-6%) and isolated IFG (6-9%) were reported to have lower progression rates to diabetes than those individuals with a combination of IFG and IGT (15 – 19%) (Gerstein *et al.*, 2007). It has been reported that IFG is commoner in men than women across all age groups although male predominance tends to plateau in the middle age. On the other hand, IGT is commoner in women across all age groups except over the age of 60 years (The DECODE Study Group., 1998, 2003; Unwin *et al.*, 2002). However, the risk of developing IGT and IFG is not homogenous and varies depending on the presence of other risk factors namely age, body mass index (BMI) and family history of diabetes (Unwin *et al.*, 2002).

#### 1.2.8.1 Pathophysiology of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT)

Insulin resistance is present in both IFG and IGT, although the site of insulin resistance is different. IGT is associated with peripheral insulin resistance mostly at the level of skeletal muscle with only small changes in liver insulin sensitivity (Unwin *et al.*, 2002; Abdul-Ghani, Tripathy and DeFronzo, 2006; Ferrannini, Gastaldelli and Iozzo, 2011). During an OGTT, IGT individuals have impaired early and late insulin secretion phases (Defronzo, 2009; Ferrannini, Gastaldelli and Iozzo, 2011; Kanat *et al.*, 2012). Individuals with severe IGT would have typically lost approximately 70 – 80% of their beta cell function with an approximate 10% chance of having diabetes retinopathy (DeFronzo and Abdul-Ghani, 2011).

IFG individuals typically exhibit a hepatic insulin resistance with almost normal peripheral insulin resistance at the level of skeletal muscles and with a decreased beta cell sensitivity (Defronzo, 2009; DeFronzo and Abdul-Ghani, 2011; Ferrannini, Gastaldelli and Iozzo, 2011; Kanat *et al.*, 2012). During an OGTT, IFG individuals have severe impairment in early insulin response (1st phase) but their insulin secretion improves during the second phase (Abdul-Ghani, Tripathy and DeFronzo, 2006; Defronzo, 2009; Kanat *et al.*, 2012). The combination of hepatic insulin resistance and impaired early phase insulin secretion results in an excessive hepatic glucose production during fasting accounting for a fasting hyperglycaemia (Nathan *et al.*, 2007).

### 1.2.8.2 Diagnostic criteria for Pre-Diabetes

The diagnostic criteria for pre-diabetes (IFG and IGT) can be seen in Table 1.9. HbA1C has recently been added as a diagnostic test for pre-diabetes. The WHO reported that an individual with an HbA1C between 6.0 and 6.5% is at risk of pre-diabetes while the ADA reported that HbA1C values between 5.7 – 6.4% are at risk of pre-diabetes (International Expert Committee, 2009; American Diabetes Association, 2018b).

| | | **Glucose plasma concentrations (mmol/L)** | |
|---|---|---|---|
| | | **WHO** | **ADA** |
| **IFG** | FPG ***And*** | 6.10 - 6.99 | 5.60 - 6.99 |
| | 2nd hour OGTT | <7.8 | <7.8 |
| **IGT** | 2nd hour OGTT | 7.8 - 11.1 | 7.8 - 11.1 |

Table 1.8 Different diagnostic criteria for pre-diabetes (World Health Organization, 2006; American Diabetes Association, 2018b)

The fasting plasma glucose (FPG) cut-off point is inconsistent between the World Health Organization and the American Diabetes Association. The ADA reported the FPG cut-off point for pre-diabetes to be 5.6mmol/L following a number of studies supporting the fact that the best sensitivity and specificity for FPG as a predictor for future diabetes was lower than 6.1mmol/L (Genuth *et al.*, 2003). This was further supported by the fact that approximately 20% of OGTT diagnosed persons with diabetes have an FPG of less than 6.1mmol/L. This is especially true in females, the elderly and in Asian populations (Shaw, Zimmet and Alberti, 2006). Hence, a reduction in FPG to 5.6mmol/L was predicted to pick up 10% of the at risk population prior to the development of T2DM (Shaw, Zimmet and Alberti, 2006). However, the WHO expressed concerns on the lowering of the FPG level to 5.6mmol/L (World Health Organization, 2006). It was reported that if this cut-off point had to be adopted there would be an increase in IFG prevalence and a greater impact on health systems. The WHO claimed that there was a lack of evidence on the benefit of reducing the adverse outcomes or progression to diabetes among those with an FPG between 5.6 – 6.0mmol/L. Furthermore, individuals with an FPG between 5.6 and 6.0mmol/L exhibit a more favourable cardiovascular risk profile as well as half the risk of developing diabetes when compared to those with a FPG between 6.1 – 6.9mmol/L (World Health Organization, 2006).

The clinical diagnosis of both IFG and IGT should be based on two OGTTs no more than 3 months apart. It is essential that IFG individuals have an OGTT since 5% to 20% of these would have already developed T2DM (Unwin *et al.*, 2002).

### 1.2.8.3 Management of Pre-Diabetes

The primary aim for the management of pre-diabetes is to prevent or delay the onset of T2DM. Lifestyle interventions targeting weight loss, physical activity and dietary choices were found to have a positive outcome in preventing the transition from pre-diabetes to T2DM in a number of randomized controlled trials (Eriksson and Lindgärde, 1991; Pan *et al.*, 1997; Tuomilehto *et al.*, 2001; Lindström *et al.*, 2003; Hamman *et al.*, 2006; Ramachandran *et al.*, 2006; Diabetes Prevention Program Research Group *et al.*, 2009). In fact, a 58% risk reduction in developing T2DM was found in both the Finnish Diabetes Prevention Study and the US Diabetes Prevention Program when following lifestyle interventions (Lindström *et al.*, 2003; Hamman *et al.*, 2006).

The prescription of Metformin as an intervention for the management of pre-diabetes and preventing/delaying T2DM development was found to have a positive outcome. Metformin reduces the fasting glucose through its effect on the hepatic glucose output (Nathan *et al.*, 2009). However, when compared to lifestyle interventions, Metformin was reported to be either similarly effective as lifestyle interventions (Indian Diabetes Prevention Program) or less effective than lifestyle intervention (US Diabetes Prevention Program) (Ramachandran *et al.*, 2006; Diabetes Prevention Program Research Group, 2012). Therefore, since these reports were based on randomised controlled trials that are known to provide the strongest evidence of effectiveness, as already reported previously, pre-diabetes prevention is mostly effective through lifestyle interventions.

Thiazolidinediones work on peroxisome proliferator activator receptor-ϒ (PPARϒ). These act as potent insulin sensitizers in muscle, liver and adipocytes (Spiegelman, 1998; Yki-Järvinen, 2004; DeFronzo, 2010). They also enhance and preserve the beta cell function

(Gastaldelli *et al.*, 2007). It was reported that Troglitazone enhances insulin sensitivity and improves IGT (Nolan *et al.*, 1994; Cavaghan *et al.*, 1997; Knowler *et al.*, 2005). In fact, in the Diabetes Prevention Program, it was reported that those on Troglitazone exhibited a reduction of 23% in IGT conversion to diabetes after a 3 year period when compared to placebo (Knowler *et al.*, 2005). In the DREAM study, rosiglitazone was reported to reduce the conversion of IGT to T2DM by 62%, while pioglitazone was reported to decrease conversion by 72% in the ACT NOW study (Wild *et al.*, 2004; DeFronzo *et al.*, 2011). These studies were random control trials, where such studies are known to provide superior evidence when compared to other types of studies. Therefore, the administering of a thiazolidinedione for the prevention of pre-diabetes is to be considered as an effective intervention and should be implemented in preventive strategies.

Glucagon-like peptide-1 (GLP-1) analogues are potent insulin secretagogues. Liraglutide was reported to prevent the conversion from IGT to T2DM by 84 - 96%, while reducing the incidence of the metabolic syndrome (Astrup *et al.*, 2009).

## 1.2.9 Epidemiology of Type 2 Diabetes Mellitus

### 1.2.9.1 Type 2 diabetes - a non-communicable disease

Non-communicable diseases (NCDs) are defined as non-infectious diseases, which are non-transmissible among people (Kim and Oh, 2013). The four major non-communicable diseases worldwide are cardiovascular disease, cancer, chronic lung disease and diabetes (Hunter and Reddy, 2013; Kim and Oh, 2013; World Health Organization, 2016). These have been associated with four common behavioural risk factors (excessive alcohol consumption, poor diet, tobacco use and lack of physical activity) (Hunter and

Reddy, 2013; World Health Organization, 2016). Behavioural changes at population level such as low levels of physical activity and a poor diet have resulted in an accelerated increase in NCDs prevalence worldwide (Webber *et al.*, 2014). The large-scale availability of processed food with higher fat, salt, sugar and total calorie levels have led to their increased consumption and eventually, to population increases in body weight and waist circumference. These are associated with an increase in urbanization and the state of economic development, which in turn lead to a worsening of the NCD problem (World Health Organization, 1994; Webber *et al.*, 2014).

The four most common NCD's diseases (cardiovascular disease, cancer, chronic lung disease and diabetes) account for about 80% of deaths and the majority of disability worldwide (Lozano *et al.*, 2012; Webber *et al.*, 2014; World Health Organization, 2014). After a lifetime of accumulated risk factors, the elderly population (60+ years) is more prone to develop NCDs. With the world's ever ageing population, people suffering from NCDs are expected to increase each year (Muka *et al.*, 2015). NCDs also have a substantial impact on the socio-economic development, national expenditure and the economic growth of any country (Bloom *et al.* 2011).

#### 1.2.9.2 The 21st century epidemic – Type 2 Diabetes Mellitus

Type 2 diabetes mellitus has been reported to be a global epidemic since the early 2000s (Engelgau *et al.*, 2004). The different factors facilitating this global epidemic include increasing migration, patterns of acculturation, affluence, urbanization and obesity (Misra and Ganda, 2007).

In 2017 it was estimated that 424.9 million adults (20 – 79 years) suffered from T2DM globally. Furthermore, 352.1 million adults (20 - 79 years) suffered from IGT, putting these at risk of developing T2DM (International Diabetes Federation, 2017). Prevalence rate projections for the year 2045 exhibit an increase from 8.8% (2017) to 9.9% (2045) for T2DM and from 7.3% (2017) to 8.3% (2045) for IGT (International Diabetes Federation, 2017). It was estimated that as many as 212.4 million adults have undiagnosed diabetes mellitus globally (International Diabetes Federation, 2017).

In Europe the situation reflects the global epidemic. In fact, it was reported that approximately 58 million adults representing 8.8% of the population aged 20 – 79 years suffered from T2DM in 2017. Furthermore, 22.0 million were unaware of their diabetes and 36 million (5.5%) of adults had IGT. It is predicted that by the year 2045, 66.7 million adults will be living with T2DM in Europe (International Diabetes Federation, 2017). The European region has the second largest healthcare expenditure on diabetes worldwide due to the high T2DM prevalence rate. Additionally, T2DM is attributed 9% of all mortality (in 2017), with 32.9% of these deaths estimated to be occurring in adults under the age of 60 years (International Diabetes Federation, 2017).

Complications of T2DM, namely microvascular (retinopathy, nephropathy and neuropathy) and macrovasular (cardiovascular, cerebrovascular and peripheral vascular diseases), generally contribute to the burden of diabetes. The prevalence of these complications among newly diagnosed persons with diabetes may be as high as 30 – 40% (UK Prospective Diabetes Study, 1990; Kohner *et al.*, 1998). Both microvascular and macrovascular complications lead to an increase in the morbidity and mortality of those affected with the disease. In fact, the age-adjusted mortality rate for diabetes patients is

twice that of non-diabetes (Engelgau *et al.*, 2004; Permutt, Wasson and Cox, 2005; Khalid *et al.*, 2014). Unfortunately, the onset of T2DM is increasingly afflicting younger patients, leading to an earlier onset of complications within the community (Berkowitz, Meigs and Wexler, 2013).

### 1.2.10 Burden of Type 2 Diabetes Mellitus

The progressive increase in global diabetes prevalence rates is contributing to an escalation of the burden in various forms including morbidity, mortality, disability, diminished life expectancy, reduced quality of life, loss of human and social capital along with individual and national financial losses (Ali, M.K., Weber, M.B., Narayan, 2010).

#### 1.2.10.1 Morbidity

The rate of glycaemic control deterioration with time in T2DM contributes to an increasing burden of morbidity. It has been reported that an HbA1C deterioration of 1.4mmol/ml per year occurs on average in cases with T2DM. On the other hand, GADA-positive T2DM has a higher rate of deterioration (average rate of 2.8mmol/mol per year) (Donnelly *et al.*, 2018). The UKPDS had reported a strong association (approximately two fold) between GADA-positive individuals and progression of T2DM (Turner *et al.*, 1997). Furthermore, T2DM individuals diagnosed under the age of 50 years were found to have a faster deterioration rate than those diagnosed at a later age. Those diagnosed above the age of 70 years undergo very limited deterioration (Donnelly *et al.*, 2018).

### 1.2.10.2 Disability

Diabetes has been associated with a 67% increased risk of physical disability (Tabesh *et al.*, 2018). Disability signifies the psychosocial illness or physiological impairment in the ability to perform domestic or occupational tasks and assume social roles. This may inhibit the diabetes individual from the general utility and ability to integrate fully in society (Ali, M.K., Weber, M.B., Narayan, 2010). A large proportion of the disability in society is attributed to the aging population together with the increase in diabetes prevalence. These in turn contribute to disability by affecting both the individual as well as health care system (International Diabetes Federation, 2017). Furthermore, diabetes individuals exhibit a two to three fold-increased risk of physical disability (Wong *et al.*, 2013).

T2DM is associated with disabling diseases such as renal failure, blindness, cardiovascular disease and lower limb amputation (Dolezalova, 1981; Ylitalo, Sowers and Heeringa, 2011; International Diabetes Federation, 2017). An overweight-obese status is a risk factor for T2DM as well as being associated with impaired mobility (Tyrovolas *et al.*, 2015). In fact, it was reported that both obesity and a history of cardiovascular disease contribute to a high proportion of the disability in diabetes individuals (Tabesh *et al.*, 2018).

Diabetes also leads to “hidden” disability. Diabetes adults may exhibit a low work performance due to impaired concentration, urine frequency, decline in cognitive functioning or impaired fine motor skills (Testa and Simonson, 1998; Gregg *et al.*, 2000; Lavigne *et al.*, 2003). Furthermore, diabetes has been associated with higher absenteeism from work as well as with a reduced work efficiency (Lavigne *et al.*, 2003; Tunceli *et al.*, 2007). These factors collectively lead to a psychosocial burden and it has been reported

that symptoms of depression may develop due to these accumulating burdens (McKellar, Humphreys and Piette, 2004; Von Korff *et al.*, 2005).

#### 1.2.10.3 Mortality

Type 2 mellitus is associated with premature mortality that shortens the life expectancy by approximately 7 to 15 years (Morgan, Currie and Peters, 2000; Franco *et al.*, 2007). Cardiovascular disease accounts for 65 to 75% of deaths within the diabetes population in developed countries (Moss, Klein and Klein, 1991; Casiglia *et al.*, 2000). In fact, it was reported that in 2017 diabetes attributed to one death every 8 seconds and accounted for 10.7% of global all-cause mortality among adults (20 to 79 years) (International Diabetes Federation, 2017).

#### 1.2.10.4 Economic burden

The economic burden of diabetes can be broken down into three categories: (1) direct medical costs, (2) indirect costs and (3) intangible costs. The main contributing factor for all of these costs is the development of diabetes complications. The direct medical costs incorporate the medical treatment and care required for the diabetes individual, which includes all outpatient consultations, inpatient care, diagnostic investigations, therapeutic procedures, pharmacotherapy, paramedical care, transportation and rehabilitation. The indirect medical costs include loss of productivity as a consequence of morbidity, disability or premature mortality. Intangible costs refer to the reduced quality of life and the psychosocial impacts (such as stress, emotional problems and depression) that affects a diabetes individual and their families (Ali, M.K., Weber, M.B., Narayan, 2010; Zhang, P., Li, 2011).

### 1.2.10.5 Quality of life

The term *Quality of life* generally incorporate the understanding of how health-related variables affect mental, social and physical functioning as well as the overall feelings of an individual's well-being and satisfaction of life. These include one's job, finance, housing, personal relationships, environment and much more (Polonsky, 2000; Speight, Reaney and Barnard, 2009). The health-related quality of life (HRQOL) is only one aspect that contributes to quality of life (Polonsky, 2000).

Diabetes and HRQOL exhibit a bidirectional relationship. The diagnosis of T2DM may affect negatively the HRQOL, while impaired HRQOL may negatively influence T2DM management (Polonsky, 2002). In fact, in the *Medical Outcome Study*, diabetes individuals reported more physical and social functioning problems when compared to individuals without a chronic condition. However, diabetes individuals were found to function better than those individuals with pulmonary, cardiovascular or gastrointestinal disorders (Stewart *et al.*, 1989).

A number of measuring tools have been created to evaluate the impact of diabetes on the quality of life. The Audit of Diabetes-Dependent Quality of Life (ADDQoL) attempts to evaluate the diabetes specific HRQoL from the patient's perceptive as to how diabetes may be perceived as interfering with their wellbeing (Bradley *et al.*, 1999).

### 1.2.11 Type 2 diabetes in the Maltese Islands

The Maltese islands are situated in the centre of the Mediterranean Sea at the intersection between Europe and North Africa. Many dominant powers have ruled Malta over the years, all of which have left an impact on the population both from a socio-ethnicity diversity aspect as well as through a genetic input (Formosa, Savona-Ventura and Mandy, 2012). In fact, Al-Ashtar A. (2008) as part of his PhD studies reported that there is a similar diabetes and metabolic genetic make-up between the Maltese population and the Libyan population especially for the ADRABbeta2 gene (Arg16Gly) (Al-Ashtar, 2008). However, the IPF gene (missense mutation Cys18Arg) was only associated with Libyan T2DM and not with Maltese T2DM (Al-Ashtar, 2008).

Diabetes mellitus type 2 has been reported to be a health concern since 1886, where it was documented that diabetes had a negative impact on the lives of many Maltese and was responsible for 2.1 per 10,000 population deaths. This mortality impact continued to rise over the years, with a documented highest mortality rate in the world of 26.1 deaths per 10,000 populations by 1955 (Cassar, 1982). It has also been established that the development of diabetes in Malta is significantly associated with both a maternal and paternal diabetes history (Schranz, 1989; Savona-Ventura, Schranz and Chircop, 2003).

The total residents (incorporating both Maltese and non-Maltese) in Malta (for end of 2015) was 434,403 persons (males 217,569) (National Statistics Office., 2017). In 2014, diabetes attributed for 4.8% of the global mortality rate in Malta (Department of health information and research., 2015).

#### 1.2.11.1 Epidemiological studies in Malta

The prevalence of T2DM in Malta was first documented in 1927, where diabetes was estimated to affect 4.5% of the population (at the time). A direct link with obesity was also established (Debono, 1927). Later on in 1964, Professor J.V. Zammit Maemple along with a team from the University of Malta, conducted the first 'pilot' (*n*=5,757) study covering two areas (rural and urban) (Zammit Maemple, 1965). Although the study design was not randomized and not fully representative of the Maltese population at the time, it can be regarded as the first attempt to establish the local prevalence of diabetes. This *pilot* study had various methodological flaws by contemporary standards, but it gave an insight into the considerable diabetes and obesity burden on the Maltese Islands. The measuring tools used were glycosuria and subsequently confirmation with a 50g glucose load OGTT for groups with and without glycosuria. This study reported that the prevalence of T2DM was 19.9% and that 1 out of every 10 inhabitants had unknown diabetes. It also found that 60 out of 100 persons with diabetes were obese (Zammit Maemple, 1965).

In 1981, the World Health Organization (WHO) conducted a representative T2DM prevalence study in Malta (Katona, G, Aganovic, I, Vuskan V, 1983). A randomized stratified sample of the population was obtained from the electoral list, where 2,149 participants took part in the survey. The screening methods used were fasting blood capillary from the ear lobe, a fasting urine sample and a 75g glucose load OGTT. The total prevalence of T2DM found at the time was of 7.7% with 5.9% previously known diabetes and 1.8% newly diagnosed diabetes. It was further reported that 5.6% of the sample population were diagnosed as having impaired glucose tolerance (IGT). It was also documented that obesity was linked to a high caloric intake (Katona, G, Aganovic, I,

Vuskan V, 1983). In 1984, as part of the MONICA study, the cardiovascular situation in Malta was assessed and reported (Cacciottolo, 1989).

The Maltese European Health Interview Survey (HIS) in 2008 (*n*=3,680) used a self-reported questionnaire and established a T2DM prevalence of 8.30% in the population aged 20 to 79 years. In 2010 the Maltese European Health Examination Survey (pilot study) examined a small sample population (*n*=221). However, it was able to report on the increasing problem of diabetes mellitus and obesity in Malta, among others. A prevalence of 10.1% diabetes was established in the studied population aged 20 to 79 years (Directorate for Health Information and Research., 2012). In 2015, the first Diabetes Mellitus National Strategy for Malta was published aiming to plan ways to prevent diabetes by expanding and improving the management of diabetes in Malta (Parliamentary Working Group on Diabetes., 2015).

Over recent years, the Maltese Islands had an influx of different cultural and ethnical populations settling in Malta. Furthermore, Malta experienced a change in lifestyle habits to a more Westernized habit. Considering the environmental, social and behavioural changes within the Maltese population, the epidemic of type 2 diabetes still dominated. In spite of the fact that self-reported health information surveys (HIS) were conducted in 2008 and a health examination pilot study (EHES) was performed in 2010, there has not been any recent (last performed was in the 1980's) Maltese population representative health examination survey to determine the diabetes prevalence or to investigate the determinants contributing to diabetes in Malta. Therefore, it was considered timely to update our evidence base on T2DM and its determinants within the adult population of Malta by means of a representative randomized cross-sectional study in which risk factors could also be accurately quantified (Cuschieri and Mamo, 2014). This would also aid in quantifying the

health services' burden of diabetes and other non-communicable diseases (obesity, hypertension, cardiometabolic) as well as providing an evidence base for future public health policies and interventions (Tolonen *et al.*, 2018).

## 1.3 Co-determinants of Type 2 Diabetes Mellitus

### 1.3.1 Obesity and Overweight

Obesity arises from a chronic imbalance between energy intake and expenditure. The end result is deposition of excess body fat (Alpert, 1990; Galgani and Ravussin, 2008). Obesity is typically defined as a 30% excess body weight compared to the optimal body weight for height. For a man with average height, a 30% excess weight leads to an excessive energy store of 150,000 kcal (kilo calories), or the equivalent of the basic energy requirements for two to three months. In fact, an energy imbalance of just 25kcal per day will result in an obesity state over a period of twenty years (Linde and Jeffery 2010). The accumulation of adipose tissue is accompanied by mild, chronic and systemic inflammation and associated with the development of chronic diseases such as cardiovascular disease and diabetes mellitus (Wyatt, Winters and Dubbert, 2006). These factors contribute to the development of the metabolic syndrome (Alberti, Zimmet and Shaw, 2006). Obesity also contributes to disorders of the locomotor system; many types of cancers; impairs quality of life in the affected individuals as well as reduce the life expectancy (Kopelman, 2000; Prospective Studies Collaboration *et al.*, 2009).

### 1.3.1.2 Pathophysiology of obesity

The pathophysiology of obesity is complex with an interaction between genetic, environmental and psychosocial factors which act through physiological mediators of energy intake and expenditure affecting fat deposition (Shuldiner, 2008; Lean, 2010).

#### 1.3.1.2.1 Genetic predisposition

Development of obesity follows a strong genetic predisposition. It was reported that the BMI of adults that were adopted exhibited no resemblance to the BMI of their adoptive parents. However, on comparing the BMI to their biological parents, a significant correlation was evident (Stunkard *et al.*, 1986).

A number of biological molecules and pathways, including leptin (*Lep*) and its receptor (*Lepr*), melanocortin 4 receptor (*Mc4r*), as well as pro-opiomelanocortin (Pomc), have been associated with changes in body weight through central nervous system pathways (Myers and Leibel, 2000).

Melanocortin-4-receptor functional mutations have been considered to be the most common cause of monogenic obesity in children. These defects affect genes that are responsible for the control of food intake (O'Rahilly and Farooqi, 2008).

The *fat mass associated protein* (FTO) is a gene found on chromosome 16 and it's (FTO) non-coding variants have exhibited a significantly high association with obesity risk (Frayling *et al.*, 2007; Scuteri *et al.*, 2007). It was reported that the FTO locus might have

an effect on body weight by regulating thermogenesis, appetite, adipocyte browning and epigenetic mechanisms (Yang *et al.*, 2012; Milagro, Moreno-Aliaga and Martinez, 2016).

#### 1.3.1.2.2 Ectopic fat

Ectopic fat deposition is associated with the development of obesity through pathological growth of adipocytes (Després and Lemieux, 2006; Rosen and Spiegelman, 2014; Shulman, 2014; González-Muniesa *et al.*, 2016). These have a profound effect on the cardio-metabolic risk profile, insulin sensitivity and dyslipideamia of the individual (Shulman, 2014). Adipose tissues may undergo hyperplasia and act as a 'metabolic sink' while protecting lean muscle. The 'healthiest metabolic sink' is the gluteal-femoral fat depot (Karpe and Pinnick, 2015). Conversely, when the subcutaneous adipose tissue is unable to expand through hyperplasia, on reaching the saturation limit, the adipocyte would either rupture resulting in macrophage invasion or else result in an increase in pro-inflammatory adipokines and a decrease in anti-inflammatory adipokines. Furthermore, the triglycerides would then be stored in undesired sites such as the heart, kidney, liver and pancreas (Ouchi *et al.*, 2011; de Heredia, Gómez-Martínez and Marcos, 2012). Consequently, an atherogenic, diabetogenic and inflammatory environment would develop, as illustrated in Figure 1.16 and Figure 1.17.

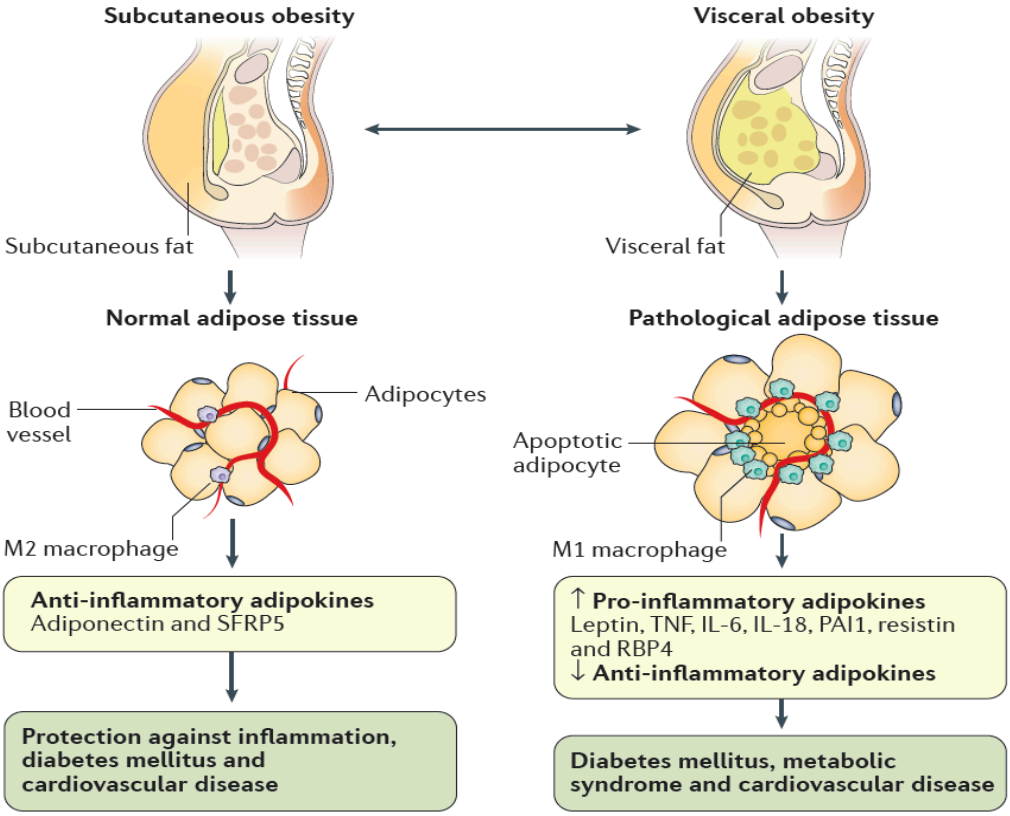



Figure 1.16 Pathological changes in adipose tissues (González-Muniesa *et al.*, 2017)

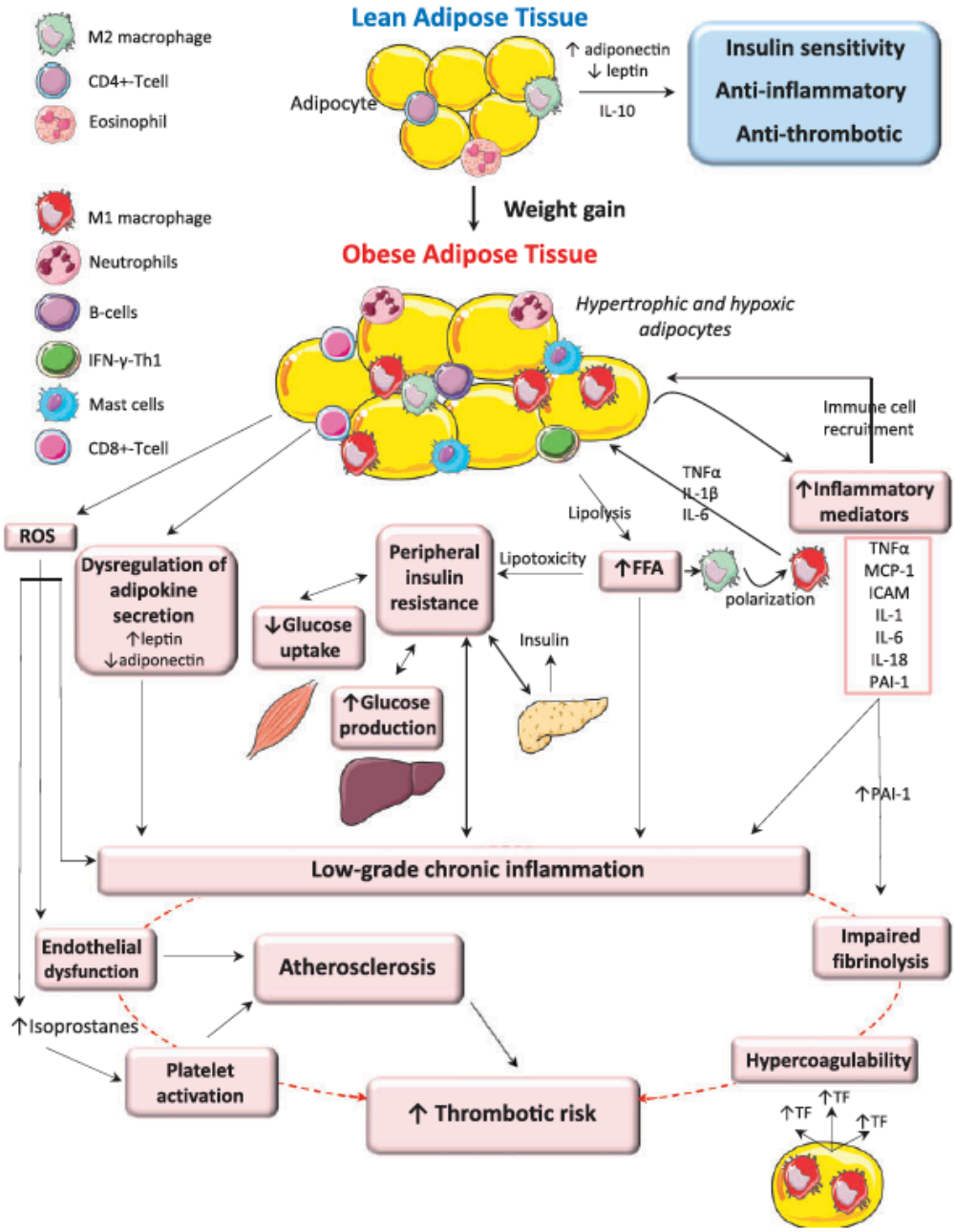



Figure 1.17 Pathophysiological consequences of obesity (Rocca *et al.*, 2018)

'Metaflammation' was introduced as a description for the obesity-induced inflammation. This is a chronic low-grade inflammation that is initiated by excessive nutrition within metabolic cells along with circulating inflammatory markers (including C-reactive protein, Interleukin-1, Interleukin-6, plasminogen activator inhibitor-1 and tumour necrosis factor-

alpha) as illustrated in Figure 1.18 (Trayhurn, Wang and Wood, 2008; Gregor and Hotamisligil, 2011).

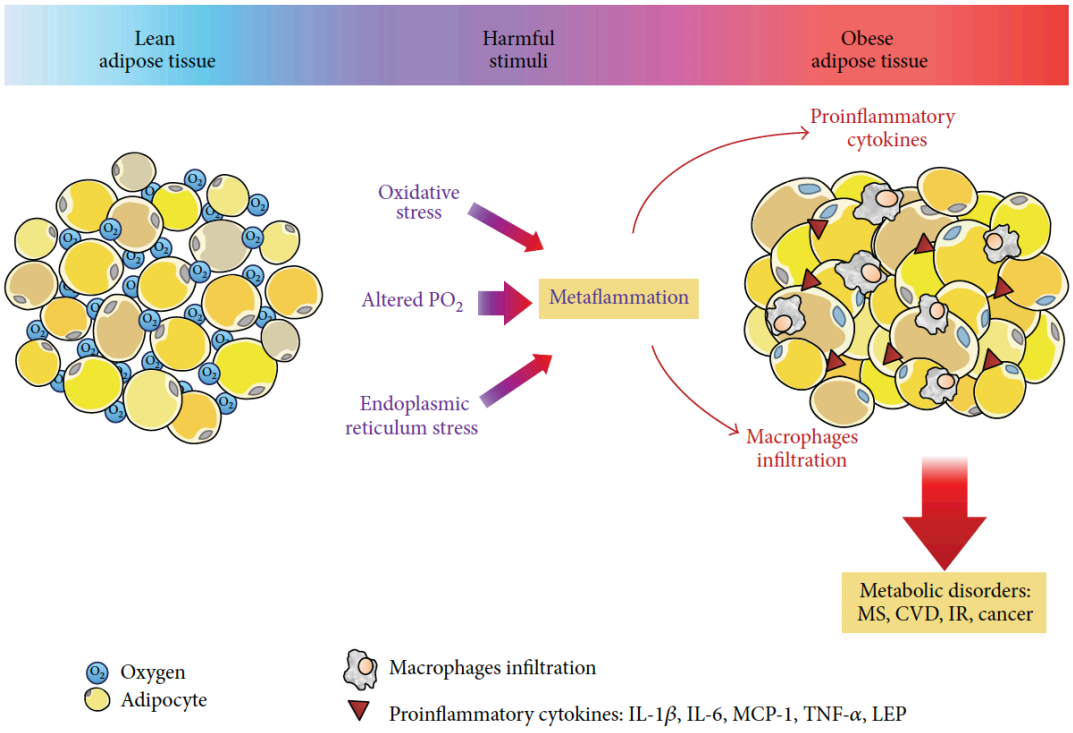



Figure 1.18 Effect of obesity on adipose tissue (González-Muniesa *et al.*, 2016)

Meta-inflammation has also a role in muscle protein metabolism. Adipose deposition within skeletal muscle lead to accumulation of lipid metabolites and glucose utilisation dysfunction with eventual obesity-induced insulin resistance, as illustrated in Figure 1.19 (Shulman, 2014; Bischoff *et al.*, 2017). This affects muscle protein turnover with possibility of sarcopenic obesity in advancing age, where sarcopenia is defined as a reduction on muscle mass with a diminished muscle function (Cruz-Jentoft *et al.*, 2010; Prado *et al.*, 2012).

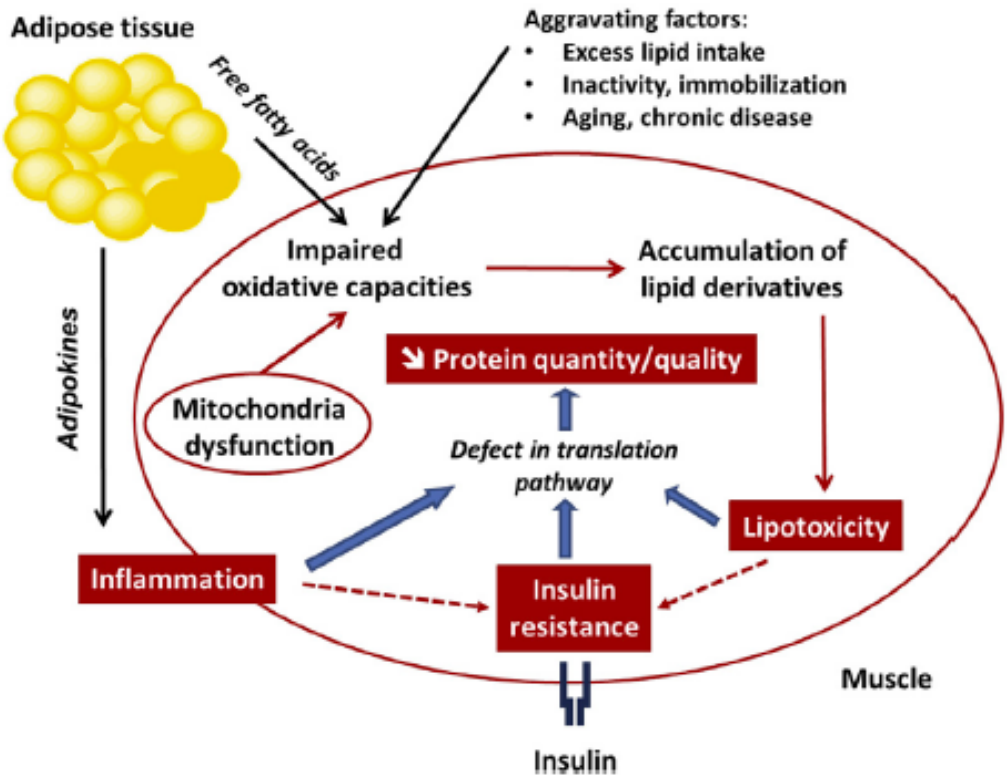



Figure 1.19 Obesity-related insulin resistance (Bischoff *et al.*, 2017)

#### 1.3.1.2.3 Metabolic imprinting

The prenatal and neonatal periods contribute to metabolic imprinting at the genomic and epigenomic levels, with possible permanent effect on future health and disease risk (Hanley *et al.*, 2010; Eriksson, 2016). Maternal gestational weight gain especially during early pregnancy can affect the prenatal period contributing to an increased risk towards the development of overweight children. However, both over nutrition and under nutrition can trigger pathways that can lead to obesity later on in life (Dearden and Ozanne, 2015; Lin *et al.*, 2017).

#### 1.3.1.2.4 Environmental factors

The obesity epidemic is mostly attributed to an increase in body fat deposition as a result of reduced physical activity and unhealthy dietary habits. The *neoclassical theory* tries to explain this by blaming modern technological advancements such as motorized transport, television and virtual gaming. These advances have led to a reduction in physical activity while promoting a sedentary lifestyle (Philipson and Posner, 2003).

The *behavioural theory*, on the other hand puts the onus on dietary changes. There has been a gradual but progressive increase in the consumption of high-calorie, fatty, salty and sugary food, due to the ease of availability and affordable price. All this leads to an overall weight gain (Jones *et al.*, 2007; Bolhuis *et al.*, 2015).

The dietary habit has changed over the years with an increase in portion size and consumption of food both from home and from fast-food restaurants (Nielsen, 2003). Both of which promote weight gain and obesity (Ello-Martin, Ledikwe and Rolls, 2005).

Furthermore, the socioeconomic status has an effect on the development of obesity. In fact, it was reported that low socioeconomic status individuals tend to consume low cost energy-dense food rather than healthy diets based on lean meat, fish, fruit and vegetables, which tend to be more expensive (Drewnowski and Specter, 2004).

### 1.3.1.3 Measurements of adiposity

#### 1.3.1.3.1 Body mass index (BMI)

There are various tools utilized for body weight measurements. The body mass index (BMI) is a useful tool in obesity and has been the method of choice in defining excess body adiposity. It is calculated using the weight (in kilos) divided by the square of the height (in meters) (BMI = $Kg/m^2$). The WHO established a classification of obesity based on BMI classification, as seen in Table 1.10 (World Health Organization, 2000).

| **Classification** | **$Kg/m^2$** |
|---|---|
| Underweight | <18.50 |
| Normal weight | 18.50 – 24.90 |
| Overweight | 25.00 – 29.99 |
| Obesity | |
| Grade I | 30.00 – 34.99 |
| Grade II | 35.00 – 39.99 |
| Grade III | ≥40 |

Table 1.9 Body mass index classification (World Health Organization, 2000)

A limitation of the BMI is that it does not take into consideration the body fat distribution. Hence, it does not differentiate between body weight due to muscle bulk and fat (Burkhauser and Cawley, 2008).

#### 1.3.1.3.2 Waist Circumference and Waist-Hip Ratio

Both the waist circumference and the waist-hip ratio (WHR) are measurements for central obesity (Molarius and Seidell, 1998; Goh *et al.*, 2014). Waist circumference

measurement is defined as the abdominal circumference measurement midway between the lowest ribs and the iliac crest (World Health Organisation Consultation Group., 1999; World Health Organization., 2008). The WHR is measured by dividing the waist circumference by the hip circumference. This has long been reported to be more accurate than skinfold thickness measurement as it provides an index for both intra-abdominal and subcutaneous adipose tissue (Bjorntorp, 1987). The waist circumference and waist-hip ratio are the ideal measurements when considering visceral and abdominal obesity (Kok, Seidell and Meinders, 2004). However, the waist circumference only assesses visceral adiposity and is not capable of distinguishing between subcutaneous and visceral fat masses (Pouliot *et al.*, 1994).

There have been various recommendations for waist circumference cut-off points depending on the ethnicity of the study population, as seen in Table 1.10 (Misra, Wasir and Vikram, 2005; Alberti, Zimmet and Shaw, 2007).

| **Ethnicity** | **Gender** | **Waist circumference (cm)** |
|---|---|---|
| Europids | Men | >=94 |
| | Women | >=80 |
| South Asians | Men | >=90 |
| | Women | >=80 |
| Chinese | Men | >=90 |
| | Women | >=80 |
| Japanese | Men | >=90 |
| | Women | >=80 |

Table. 1.10 Different ethnic cut-off points for waist circumference

It was reported that central obesity could be defined as a waist circumference equal or above 94cm in men and 80cm in women (Okosun *et al.*, 1998; World Health Organization, 2008b). Furthermore, if the waist circumference was above 102cm for men and 88cm for women, these stood at a higher risk for health problems (Lean, Han and Morrison, 1995).

The Waist-Hip Ratio (WHR) has been used successfully as a tool for the measurement of visceral adiposity. The cut off values are 0.90 for men and 0.85 for women (World Health Organization, 2008b). The WHR tool was reported to determine impaired glucose tolerance in young age groups as well as determine high-risk diabetes individuals (Łopatyński, Mardarowicz and Szcześniak, 2003).

#### 1.3.1.3.3 Body mass index and waist circumference predictive ability

There have been a number of controversial study results, which place adiposity measurement as the best predictor for health outcomes. Evidence was found that both general obesity measures (BMI) and abdominal adiposity measures (waist circumference) were associated with increased cardiovascular risk factors and incidence of cardiovascular diseases (Huxley *et al.*, 2010). Similarly, both measures were associated independently to T2DM (Qiao and Nyamdorj, 2010). Conversely, another study reported that waist circumference is a better measure for obesity and prediction of health risk when compared to BMI (Ford, Mokdad and Giles, 2003). Meanwhile Stevens *et al*. reported that waist circumference exhibited better discriminatory performance for diabetes when compared to BMI (Stevens *et al.*, 2001). Furthermore, it was reported that waist circumference could act as a single risk factor for all-cause mortality (Seidell, 2010).

The different cut-off values for both BMI and waist circumference in relation to associated health risks are illustrated in Table 1.11.

| Definition | Body mass index | Disease risk in relation to normal weight & differing waist circumference | |
|---|---|---|---|
| | | **Men <102 cm<br>Women <88cm** | **Men >102cm<br>Women > 88cm** |
| **Underweight** | <18.5 | | |
| **Normal** | 18.5 – 24.9 | | |
| **Overweight** | 25.0 – 29.9 | Increased | High |
| **Obesity** | 30.0 – 34.9<br>35.0 – 39.9 | High<br>Very high | Very high<br>Very high |
| **Extreme Obesity** | >40.0 | Extremely high | Extremely high |

Table 1.11 Distribution of BMI and waist circumference cut-off points in relation to the diagnosis of overweight or obesity and their associated risks (National Institute of Health, 2000).

### 1.3.1.4 Obesity and Type 2 Diabetes Mellitus

It is assumed that an obese individual develops T2DM in the presence of a genetic failure affecting the pancreas. Consequently, failure to compensate for insulin resistance (a characteristic consequence of obesity) occurs with T2DM being the end result. This is however not always the case. In fact, it was reported that only 30 – 40% of severe obese (BMI $\geq$40Kg/m$^2$) individuals will develop T2DM throughout life (Polonsky, Sturis and Bell, 1996).

Furthermore, diabetes risk is dependent on the BMI of the individual. As from the upper normal range of BMI (23.0 – 24.9 Kg/m$^2$) a four-to five-fold increased risk of developing diabetes was observed over a 14-year period, when compared to the reference group (BMI

<22Kg/m$^2$). As the BMI increased (29.0 – 30.9 Kg/m$^2$), the risk of diabetes was reported to be 27.6-fold higher than the reference group (Colditz *et al.*, 1995).

**1.3.1.4.1 Lipids and insulin resistance**

In obese individuals there is an increased supply of non-esterified fatty acids originating from the expanded adipose tissue depots. The fatty acids compete with glucose utilization within muscle, resulting in rate reduction of glucose oxidation and consequently an increase in glucose concentration occurs. Concomitantly glycerol is released from adipose tissue and reutilized for hepatic glucose production, resulting in an imbalance in glucose metabolism. Furthermore, the elevated free fatty acids directly impair insulin action through the activation of IRS-1 and IRS-2. This may cause impairment of phosphoinositol-3 kinase activation as well as other downstream elements (Kahn, Hull and Utzschneider, 2006).

Adipocytes secrete a number of hormones such as adiponectin that protects against insulin resistance as well as inflammatory cytokines that contribute to insulin resistance. Adiponectin is less abundant in T2DM and obese individuals when compared to healthy individuals and was reported to be inversely correlated with insulin resistance (Arita *et al.*, 1999). Furthermore, a genetic mutation in one locus of the adiponectin gene contributes to a higher genetic susceptibility for T2DM (Comuzzie *et al.*, 2001).

Inflammatory markers, such as TNF-α, IL-6 and CRP are positively associated with obesity. An increase in cytokines TNF-α and IL-1β may induce insulin resistance within adipocytes. Consequently an increase in lipolysis and free fatty acids levels result that will

act as regulators of insulin sensitivity (Pradhan *et al.*, 2001; Dandona, Aljada and Bandyopadhyay, 2004; Mayer-Davis, EJ; Dabelea, D; Lawrence, JM; Meigs, JB; Teff, 2011).

#### 1.3.1.4.2 Lipids and beta cell function

Obesity is characterized by an increase in insulin secretion and a decrease in hepatic insulin clearance. In otherwise healthy obese individuals, there can be a hypertrophy of beta cells equivalent to 50% of the original state. Insulin release and insulin sensitivity are normally reciprocally related, but failure of this feedback system leads to a progressive decline in beta cell function with development of T2DM. In addition, long chain fatty acids may exert a stimulatory effect on pancreatic beta cells for insulin release through the generation of fatty acyl CoA and activation of protein kinase C (Kahn, Hull and Utzschneider, 2006). A chronic exposure of the beta cells to excessive fatty acids is associated with marked impairment of glucose-stimulated insulin secretion and a decline in insulin biosynthesis (Carpentier *et al.*, 1999).

#### 1.3.1.4.3 Body fat distribution

Abdominal fat exhibit an increased risk of developing T2DM and other cardiovascular complications (Krotkiewski *et al.*, 1983). The intra-abdominal fat cells are more ‘lipid active’ than subcutaneous adipocytes, with greater accumulation of macrophages and lymphocytes contributing to a greater pro-inflammatory activity. Visceral adiposity also exhibits higher metabolic activity with fatty acids directly depositing in the liver and consequently enhancing hepatic insulin resistance (Hauner, 2010).

#### 1.3.1.4.4 Cancers attributed to diabetes and obesity

Both T2DM and obesity have been associated with the development of various cancers (Arnold *et al.*, 2015; Tsilidis *et al.*, 2015). On combining the presence of both obesity and T2DM, the cancer incidence was reported to be 4.5% out of all cancers developed over a 10-year period. Liver cancer and colon cancer were the most common cancers affecting men that were attributed to the presence of both diabetes and high BMI. Breast and endometrial cancers were the most common form of cancers affecting women that were attributed to the presence of both diabetes and high BMI (Pearson-Stuttard *et al.*, 2018). The underlying link between T2DM, high BMI and cancer was proposed to be the presence of hyperinsulinaemia, hyperglycaemia, chronic inflammation as well as the deregulation of sex hormone activity (Giovannucci *et al.*, 2010). It has been reported that insulin itself could be oncogenic (Nead *et al.*, 2015). In fact, hyperinsulinaemic individuals irrespective of their BMI were found to be at increased risk of colon and breast cancers (Nead *et al.*, 2015; Murphy *et al.*, 2016).

### 1.3.1.5 Obesity Epidemiology

Obesity is a global epidemic, with over 1.9 billon adults (+18 years) estimated to be overweight, among whom 650 million estimated to be obese in 2016 (World Health Organization, 2018b). It was reported that a 5 unit increase in BMI leads to a 30% increased mortality rate, as well as an increased chronic kidney disease by 60% and diabetes mellitus by 120% (Prospective Studies Collaboration *et al.*, 2009). Furthermore, over the past few decades, the growing ageing population has been linked to the global obesity epidemic (Doak *et al.*, 2012). The obesity epidemic has also affected children with an approximate

41 million children under the age of 5 years estimated to be either overweight or obese (World Health Organization., 2016).

The prevalence of obesity is influenced by socio-cultural factors including socio-economic position (SEP) (Crawford and Jeffery, 2005). This epidemic impacts on health care cost, employment opportunities, social status and educational achievement (Allison, Zannolli and Narayan, 1999; Must *et al.*, 1999; Hruby and Hu, 2015). In 2017, the World Obesity Federation declared obesity as a chronic, relapsing and progressive disease that requires immediate action (Bray *et al.*, 2017).

#### 1.3.1.5.1 Obesity situation in Malta

In Malta, the overweight-obese prevalence rates rose over time. The WHO MONICA Project carried out in 1984 (*n*=3,217) reported that 59% of males and 45% of females within the Maltese population aged 25 to 34 years were overweight or obese. The obesity rates increased in the 55 to 64 years age group, where 77% of males and 85% females were obese (Cacciottolo, 1990). In 2008, the self-reported Health Interview Survey (HIS) (*n*=3,680) found 22% of the sample population aged 15+ years as being obese, while 36% were overweight. These figures ranked Malta as having the highest male obesity and the third highest female obesity in Europe (at the time) (Directorate for Health Information and Research., 2008). The Maltese European Health Examination Survey (EHES) conducted in 2010 was a pilot study. It reported that in the sample population (*n*=221) aged 19+ years, 29.80% were obese and 47.20% were overweight (Directorate for Health Information and Research., 2012). The latest Eurostat data (2014) reports that Malta has the highest European obesity rates for both men (28.10%) and women (23.90%), for the

population 18 years and over (European Commission, 2014). A similar picture could be visualized for children, where in the 2013 – 2014 health-behaviour in school aged children (HBSC) study, Malta ranked first in terms of obesity in children aged 15, 13 and 11 years within Europe (World Health Organization., 2016).

#### 1.3.1.6 Obesity as a health burden

Obesity is an economical and public health burden. It leads to medico-psychosocial conditions (e.g. tiredness, arthritis, sleep apnoea, hypertension, infertility, low self-esteem, depression etc.). It is also associated with other health problems such as insulin resistance, dyslipidaemia and T2DM (Organization, 2000). Furthermore, obesity has been associated with cardiovascular disease including hypertension. Increase in weight as well as achieving an obese status have been reported to be causative factors for the development of hypertension (Must *et al.*, 1999).

Recommendations have been set out to target this obesogenic environment epidemic. Various actions have been suggested and implemented in different countries such as price policy and manipulation, food labelling, public and school's education, and mass media contribution among others. However, the obesity epidemic is still raging.

##### 1.3.1.6.1 Obesity and healthcare expenditure

The global economic impact of obesity for 2014 was estimated to be US $2.0 trillion or equivalent to 2.8% of the global gross domestic product (GDP) (Dobbs *et al.*, 2014). Obesity healthcare expenditure is related to both direct medical expenses and indirect costs including lost workdays, lower work productivity, mortality and permanent disability

(Shrestha *et al.*, 2016). The BMI levels are directly proportional to direct and indirect costs, although the latter have a higher cost effect (Dee *et al.*, 2014).

### 1.3.2 Cardiovascular disease and Type 2 Diabetes Mellitus

Individuals with T2DM are associated with an increased risk (two to four fold) of developing coronary heart disease (Kannel and McGee, 1979b). Insulin resistance is the predisposing contributor for the development of both T2DM and cardiovascular disease (CVD) (Lebovitz, 2006). The presence of insulin resistance does not necessarily lead to the initial development of T2DM followed by CVD, but CVD may develop without frank hyperglycaemia, as shown in Figure 1.20 (Reaven, 2012).

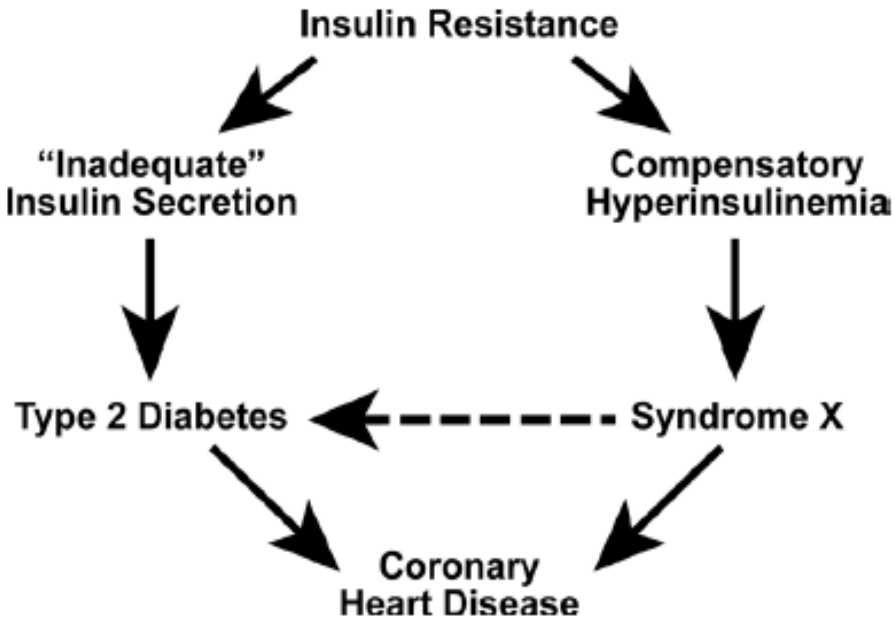



Figure 1.20 Link between insulin resistance and coronary heart disease (Reaven, 2012)

The factors that contribute to the development of coronary heart disease in the presence of insulin resistance are listed in Table 1.12.

| Abnormalities associated with insulin resistance |
|---|
| Dysglycaemia |
| Elevated blood pressure |
| Dyslipideamia |
| Inflammation |
| Endothelial dysfunction |
| Pro-coagulant state |
| Hyperuricemia |
| Enhanced sympathetic nervous system activity |
| Increased renal tubular sodium reabsorption |

Table 1.12 Factors associated with insulin resistance that may contribute to coronary heart disease (Reaven, 2012)

The plasma level of cholesterol has been reported to be a strong predictor for cardiovascular events in both diabetes individuals and individuals with coronary heart disease (Rosengren *et al.*, 1989; Pekkanen *et al.*, 1990).

Diabetes individuals without a prior myocardial infarction history were reported to exhibit the same cardiovascular risk as non-diabetes individuals with a prior myocardial infarction history (Haffner *et al.*, 1998). One needs to consider that a number of study limitations were evident in this study (Haffner *et al.*, 1998). Of note, the study examined only Caucasian Finnish population with fasting plasma glucose cut-off points inconsistent with international standards (World Health Organisation and International Diabetes Federation, 2006; American Diabetes Association, 2018b). Furthermore, the studied diabetes subjects were already on diabetes medication. This may be interpreted as these diabetes individuals had a more severe disease than community-based people with diabetes that may have variable degree of disease severity. In fact, Kengne *et al*. concluded this fact in 2010 after evaluating the Framingham and UKPDS cardiovascular risk equation as part of the ADVANCE study (Kengne *et al.*, 2010). However, a local Maltese study, also reported that

individuals suffering from diabetes and sustaining a myocardial infarction had a long term all-cause and cardiovascular mortality risk (Gruppetta, Calleja and Fava, 2010). In fact, diabetes individuals sustaining a coronary heart disease event such as acute myocardial infarction were found to be at a higher mortality risk than non-diabetes individuals (Smith, Marcus and Serokman, 1984).

The female gender tends to exhibit a protective effect against cardiovascular events prior to menopause due to the presence of high oestrogen levels. The cardiovascular protective effect may be through a direct effect on endothelial cell function, glucose metabolism and haemostatic system (Grady *et al.*, 1992). After menopause, this cardio-protective effect is lost, where the lipid metabolism changes to an atherogenic form with an increase in LDL-C and total cholesterol levels and a decrease in HDL-C levels (Matthews *et al.*, 1989; Bonithon-Kopp *et al.*, 1990). However, women tend to lose the cardiovascular protective ability on developing of T2DM (Brezina and Padmos, 1994). In fact, diabetes women were found to exhibit a significantly higher coronary heart disease risk than diabetes men (Yusuf *et al.*, 2004; Huxley, Barzi and Woodward, 2006). However, the underlying reason as to the pathophysiology for this phenomenon is still unclear. Different hypotheses have been brought forward including: more severe endothelial dysfunction; more severe elevations in blood pressure and in circulating lipids; greater tendency for poor glycaemic control; increased development of central obesity; higher rates of depression as well as a lower socioeconomic status (Koerbel and Korytkowski, 2003; Huxley, Barzi and Woodward, 2006; Legato *et al.*, 2006).

### 1.3.2.1 Epidemiology of cardiovascular disease

Cardiovascular disease (CVD) contributes to more than 4 million deaths per year in Europe. This accounts for 45% of all deaths with coronary heart disease (1.8 million) and cerebrovascular disease (1.0 million) being the most common types of CVD related deaths. The female population exhibits higher CVD mortality rate accounting for 49% of all deaths in females while the male population CVD mortality rate accounts for 40% of all deaths in men (Townsend *et al.*, 2016).

The average CVD prevalence rate across the European countries was reported to be 9.2%, with five countries reporting a higher (>10%) male prevalence rate (Poland – 14.7%; Finland – 13.0%; Netherlands – 12.4%; Germany – 11.6% and Austria – 10.4%) and four countries reporting a higher (>10%) female prevalence rate (Poland – 20.2%; Germany – 14.1%; Slovenia – 13.6% and Finland – 10.9%) (Townsend *et al.*, 2016).

### 1.3.2.2 Hypertension and Type 2 Diabetes Mellitus

Hypertension is a chronic elevation of systemic arterial pressure above a set threshold (Giles *et al.*, 2009). The progression of prehypertension to full-blown hypertension can occur over a period of 4 years especially in the elderly (Zhang *et al.*, 2006). Such progression is associated with both functional and structural cardiac and vascular abnormalities (Mayet and Hughes, 2003). A number of risk factors have been attributed for the development of hypertension, as seen in Table 1.13. Insulin resistance is associated with high blood pressure and a number of possible mechanisms have been proposed as seen in Figure 1.21.

| Modifiable risk factors | Non-modifiable risk factors |
|---|---|
| Active and passive smoking | Chronic kidney disease |
| Diabetes mellitus | Family history |
| Dyslipidaemia / hypercholesterolemia | Increasing age |
| Overweight / obesity | Low socioeconomic status |
| Physically inactive | Male sex |
| Unhealthy diet | Psychosocial stress |
| Obstructive sleep apnoea | |

Table 1.13 Risk factors for the development of hypertension (Whelton *et al.*, 2017)

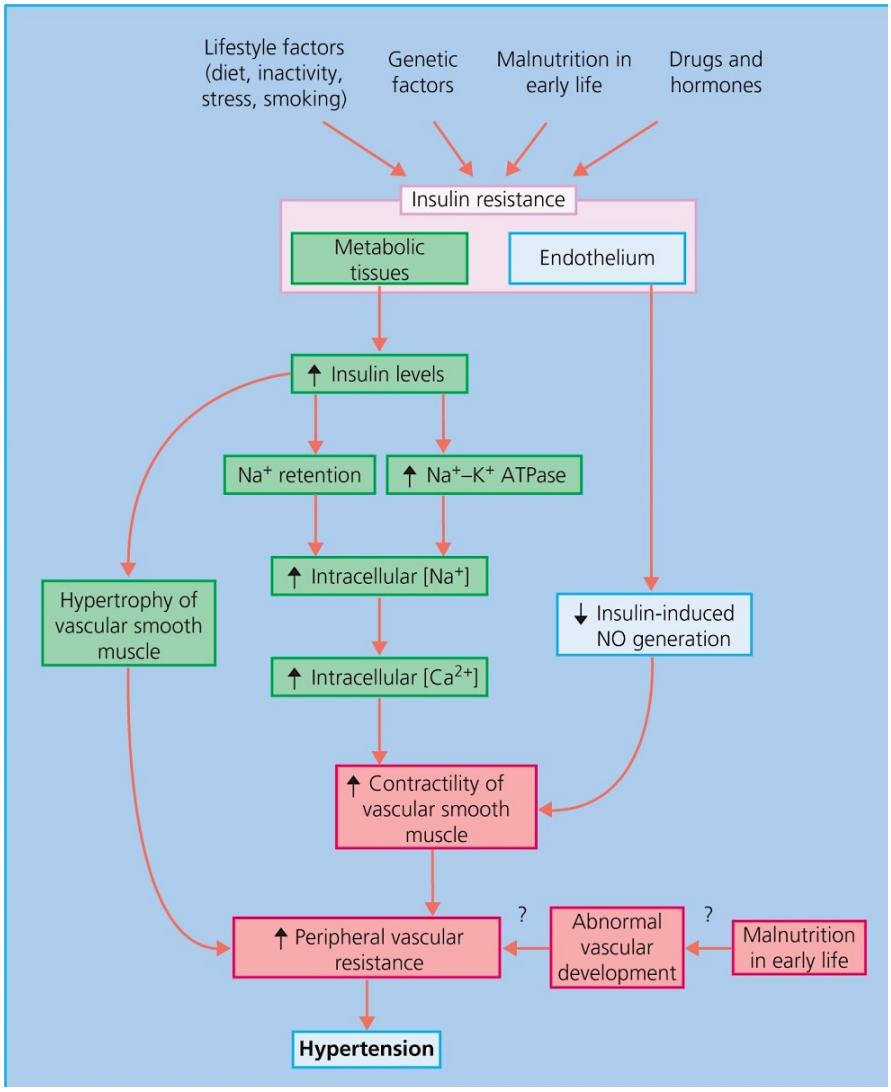



Figure 1.21 Possible mechanisms of hypertension in an insulin resistant environment (Nilsson, 2010)

Hypertension is an independent risk factor for the development of atherogenesis. It is also associated with both impaired glucose tolerance and T2DM (Kylin, 1923; Kannel and McGee, 1979a; Salomaa *et al.*, 1991). The hypertension atherogenesis is augmented with the presence of T2DM, where individuals stand a higher risks for the development of coronary heart disease, stroke and peripheral vascular disease (Stamler *et al.*, 1993; The Hypertension in Diabetes Study Group, 1993; Adler *et al.*, 2000). There are also higher risks for microvascular complications, namely nephropathy, end-stage renal failure and retinopathy (The Hypertension in Diabetes Study Group, 1993; UK Prospective Diabetes Study Group, 1998; Adler *et al.*, 2000; Do *et al.*, 2015). The mortality rate also increases with the combination of hypertension and T2DM (The Hypertension in Diabetes Study Group, 1993).

A number of randomized controlled trials have been conducted to investigate the effect of hypertension control on cardiovascular events. The *Hypertension Optimal Treatment* (HOT) trail (1992 – 1997) concluded that intensive blood pressure control would result in lower cardiovascular events. This also elucidated the beneficial effect of lowering the diastolic blood pressure (Hansson *et al.*, 1998). Similarly, the SPRINT trial evaluated the effect of intensive blood pressure treatment in non-diabetes individuals with a systolic blood pressure target of less than 120mmHg. Lower mortality rates and cardiovascular events were reported (The SPRINT Research Group., 2015). However, in diabetes individuals, achieving a systolic blood pressure target of less than 120mmHg resulted in a 'J curve' phenomenon with adverse outcomes (Mancia and Grassi, 2014).

The *Appropriate Blood Pressure Control in Diabetes* (ABCD) trail evaluated the effect of intensive against moderate blood pressure control on the incidence and progression of

T2DM complications. It was reported that initiation of anti-hypertensive medication in T2DM individuals would stabilize the renal function with a reduction in all-cause mortality when intensive blood pressure control is maintained (Estacio *et al.*, 2000). While in the *Action to Control Cardiovascular Risk in Diabetes* (ACCORD –BP) trial, intensive glycaemia or blood pressure control alone exhibited improved cardiovascular outcome, although no additional benefits were reported when combining the two (Margolis *et al.*, 2014). However, when near normal glycaemic control was established in the ACCORD trial, an association with higher risk of all-cause cardiovascular mortality was established (Margolis *et al.*, 2014). The ADVANCE trial evaluated the effect of anti-hypertensive medication on the risk of both macrovascular and microvascular complications. It was reported that systolic blood pressure should be targeted to the lower end of 130-139mmHg for diabetes individuals (Patel *et al.*, 2007). Furthermore, the ADVANCE trial evaluated the effect of standard glucose control against intensive glucose control and the risk of developing both macrovascular and microvascular complications. Gliclazide-modified release plus other drugs (as required) were utilized with the aim to achieve HbA1C of 6.5% or less. The intensive glucose control group achieved a 10% relative reduction risk against the development of major macrovascular and microvascular events (The ADVANCE Collaborative Group., 2008).

Considering that clinical trials are the pinnacle of research designs, data obtained from these studies should be given importance by both physicians and public health officials when formulating preventive strategies. It is therefore suggestive that intensive blood pressure control should be implemented in both diabetes and non-diabetes hypertensive populations to reduce cardiovascular events, related complications and mortality.

#### 1.3.2.2.1 Screening for hypertension

A number of trials brought forward the recommendation that T2DM individuals should have their blood pressure controlled at not higher than 130/80mmHg. Anti-hypertensive medication should be initiated in order to maintain blood pressure below the 130/80mmHg target (Whelton *et al.*, 2017).

On the other hand Table 1.14 illustrates the blood pressure classification for the general adult population according to the latest guidelines (Whelton *et al.*, 2017).

| **Blood pressure category** | **Systolic blood pressure** | | **Diastolic blood pressure** |
|---|---|---|---|
| Normal | <120 mmHg | And | <80mmHg |
| Elevated | 120 – 129mmHg | And | <80mmHg |
| Hypertension | | | |
| Stage 1 | 130 – 139mmHg | Or | 80 – 89mmHg |
| Stage 2 | ≥140mmHg | Or | ≥90mmHg |

Table 1.14 Classification of blood pressure in adults (Whelton *et al.*, 2017)

#### 1.3.2.2.2 Epidemiology of hypertension

The global hypertension prevalence is on the rise. This has been attributed to the increasing prevalence of contributing factors such as obesity, unhealthy diet and sedentary lifestyle along with increased longevity (Yusuf *et al.*, 2001). The WHO adult (18+ years) hypertension prevalence estimate for 2015 was 23.20% (CI 95%: 21.00 – 25.40). The male population (27.20% CI 95%: 23.80 – 30.70) exhibits a higher prevalence of hypertension than the female population (19.10 CI 95%: 16.30 – 22.00) (World Health Organization., 2017).

The Maltese islands are no exception, with the first national representative health examination survey targeting blood pressure in adults (25 – 64 years) being conducted in 1984 as part of the 'Monitoring trends and determinants in cardiovascular disease' (MONICA) study (World Health Organization., 1988; Cacciottolo, 1989). The prevalence of hypertension was reported to be 26% in the 1984 study (Cacciottolo, 1989). The self-reported hypertension prevalence rate (15+ years) established during the Maltese 2008 Health Information Survey (HIS) was of 23.70% (Directorate for Health Information and Research., 2008). Meanwhile the prevalence established from the 2010 Maltese European health examination survey pilot study (EHES) was of 41.80% for the Maltese population (15 + years) (Directorate for Health Information and Research., 2012). However, the WHO Global Health Observatory reported the adult (18+ years) prevalence of the hypertension to be 21.20% for 2014 in Malta (World Health Organization., 2017).

#### 1.3.2.2.3 Blood pressure measurement at population level

Blood pressure measurements are required to accurately establish the burden of hypertension at a population level. However, accuracy is imperative since small changes in the mean population pressure values are important from the public health point of view (Rose, GA; Khaw, LT; Marmot, 2008). A number of environmental factors have been reported to have an effect on the behaviour of the subject being examined, on the examiner and on the blood pressure measurement outcome. These factors include temperature of the examination room and disturbances (example: telephone ringing, traffic noise). Activities affecting the subject's blood pressure reading include heavy meals or drinking of coffee, tea, alcohol, or smoking, or full bladder as well as strenuous physical exercise (Campbell *et al.*, 1994; Handler, 2009). The blood pressure protocol followed by the examination team

was also reported to affect the blood pressure readings. In fact, the resting time of the subjects prior to measurements, the arm (right or left) used for the measurement, posture of the subject (sitting or supine), the support and position of the arm, back and feet as well as placement of the cuff (over clothing or onto bare arm) all affect the blood pressure outcome (Jamieson *et al.*, 1990; Campbell *et al.*, 1994; Netea *et al.*, 1998, 1999; Peters, Binder and Campbell, 1999; Keele-Smith and Price-Daniel, 2001; Handler, 2009).

The use of validated blood pressure device is adamant, with the device having been validated against either the British Hypertension Society protocol, or the International protocol or the Association for the Advancement of Medical Instrumentation (AAMI) protocol (O'Brien *et al.*, 1990, 2010; American National Standards Institute, 2009). The type of device used, whether aneroid sphygmomanometer or oscillometric device does not make a difference at a population level measurement, provided validation of the device is available as well as calibration. The use of the correct cuff size is also essential for reliable outcomes (O'Brien *et al.*, 2003).

When undergoing the auscultation method, the side of the stethoscope used may have an effect on the outcome. The examiner is also subject to errors when utilising this method. Systemic errors may arise if the examiner does not hear well enough or else has subdued reactions to cues (auditory and visual) or misinterpretation of Korotkoff sounds (Beevers, Lip and O'Brien, 2001). Terminal digital preferences can also lead to errors (Hessel, 1986; Hla, Vokaty and Feussner, 1986).

Standardization of blood pressure measurements across countries through a protocol will enhance accuracy, reproducible and ability to compare results (Tolonen *et al.*, 2015).

### 1.3.3 Dyslipidaemia and Type 2 Diabetes Mellitus

Individuals suffering from T2DM can exhibit a varied spectrum of dyslipidaemia characteristics (Hachem and Mooradian, 2006). However, the most common phenotype attributed to insulin resistance and insulin deficiency is the presence of high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C) and high small dense lipoprotein low-density lipoprotein cholesterol (sdLDL-C) levels (Ginsberg, 1991).

The mechanism attributing to the diabetes dyslipidaemia is initiated through the presence of insulin resistance, as seen in Figure 1.22. Insulin resistance increases lipolysis within adipocytes, which in turn accelerates the secretion of free fatty acids from adipocytes. These are then transported into the liver (Ginsberg, 1996). In the presence of adequate glycogen stores within the liver, the free fatty acids promote the production of triglycerides. In return the secretion of both hepatic very-low-density lipoprotein (VLDL) cholesterol and apolipoprotein B (ApoB) is stimulated (Frayn, 2001). This lipid production correlates to hepatic fat accumulation (Adiels *et al.*, 2007). The elevated levels of triglycerides and VLDL lead to a decrease in HDL-C levels and an increase in sdLDL-C concentrations.

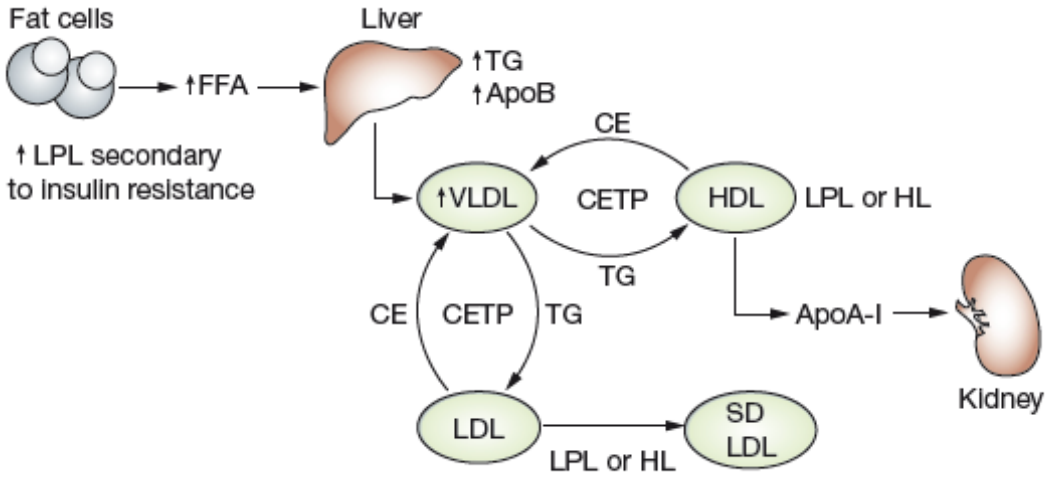



Figure 1.22 Development of diabetes dyslipidaemia (Mooradian, 2009)

The VLDL is enriched by triglycerides and leads to the activation of the cholesteryl ester transfer protein (CETP). The CETP facilitates the exchange between HDL transported cholesteryl ester (CE) and triglycerides, resulting in triglyceride-rich cholesterol depleted HDL particle (Ginsberg, 1996). Consequently, a cholesterol-rich VLDL remnant particle develops. Hepatic lipase or lipoprotein lipase hydrolyse the triglyceride-rich HDL leading to ApoA-I dissociation within the liver (Ginsberg, 1996). ApoA-I undergoes filtration by the renal glomeruli and degradation within the renal tubular cells (Ginsberg, 1996; Mooradian, Haas and Wong, 2004).

Similarly, an exchange occurs for the LDL-transported cholesteryl ester which is transformed into triglyceride rich LDL particle. Hepatic lipase (HL) or lipoprotein lipase (LPL) hydrolysis the triglyceride-rich LDL, resulting in lipid-depleted small dense LDL participle (Ginsberg, 1996).

The increased levels of free fatty acids in the circulation modulate the cascade that links insulin receptors with glucose transporters with an eventual impairment of the pancreatic beta cell function. Fatty acids are also modulators of inflammation. The production of hypertriglyceridemia may induce subclinical inflammation, which in turn leads to beta cell dysfunction and insulin resistance (Kraegen and Cooney, 2008; Rachek, 2014).

#### 1.3.3.1 LDL-B phenotype

The LDL-B phenotype is composed of high plasma sdLDL-C, high plasma triglyceride and low plasma HDL-C levels (Austin *et al.*, 1990; Feingold *et al.*, 1992; Ginsberg, 1996). This is a common phenotype in T2DM individuals (two-fold frequency) in contrast to the LDL-A phenotype, which is decreased by 41% in T2DM (Feingold *et al.*, 1992). The LDL-B phenotype frequency has also been reported to increase with age (possibly influenced by a common allele at a single genetic locus) (Austin and Krauss, 1986). A genetic variation in the LDL receptor locus or a variation in the vicinity of chromosome 19, may be contributing towards the metabolic alterations in the atherogenic lipoprotein phenotype accounting for the LDL-B pattern. This may be the predisposing factor for familial coronary artery disease risk within the general population (Nishina *et al.*, 1992). However, LDL-B phenotype frequency decreases with exercise induced weight loss due to a decrease in sdLDL-C concentration (Williams *et al.*, 1989).

The plasma triglyceride levels are directly correlated with glycaemic control (Lopes-Virella *et al.*, 1981; Pfeifer *et al.*, 1983). As insulin deficiency sets in, triglyceride clearance decreases with an increase in triglyceride-rich lipoprotein synthesis (Reaven and Greenfield, 1981; Gibbons, 1989). However, the sdLDL-C levels were reported not to have

any relation with improved glycaemic control (Feingold *et al.*, 1992). Therefore a normolipidaemic profile may be present in T2DM individuals with an underlying LDL-B phenotype (Feingold *et al.*, 1992).

The LDL-B phenotype is associated with higher risks of coronary heart disease due to the atherogenic characteristics of sdLDL-C. Various potential mechanisms contributing to the atherogenicity of sdLDL-C have been put forward. An enhanced inflow of sdLDL-C into the arterial wall has been proposed, along with an increased binding affinity to the arterial wall proteoglycans (Camejo, G; Hurt-Camejo, E; Bondjers, 1990; Anber *et al.*, 1996; Björnheden *et al.*, 1996). It was also postulated that sdLDL-C exhibits a decreased binding affinity to the LDL receptor along with an increased susceptibility to oxidative modification (Chait *et al.*, 1993; Galeano *et al.*, 1994). Furthermore, sdLDL-C has been associated with an increased risk of developing ischemic heart disease (Lamarche *et al.*, 1997). The *Diabetes Atherosclerosis Intervention Study* (DAIS) reported that T2DM individuals with high levels of sdLDL-C on fenofibrate, as compared to placebo, exhibited an anti-atherogenic benefit. In fact, those on fenofibrate showcased an elevation in LDL particle size with a decrease in sdLDL-C and apoB levels (Vakkilainen, 2003).

#### 1.3.3.2 Lipid profile measurement

There is a lack of clear consensus in the literature as to the correct period of fasting prior to lipid testing (Cuschieri, S; Mamo, 2015). A 12-hour fast is usually recommended prior to a lipid test, although total cholesterol and HDL-C levels can be reliably measured in nonfasting states (National Cholesterol Education Program (NCEP), 2002; Soh, J; Josekutty, J; Hussan, 2011). In a non-fasting state, scoring a total cholesterol ≥5.17mmol/L

or an HDL-C <1.03mmol/L would promote a follow-up fasting lipid profile testing (National Cholesterol Education Program (NCEP), 2002).

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) recommended a fasting of at least 9 hours before a lipid specimen is taken. It was reported that such a fasting period should equate to only minor and clinically insignificant errors in the lipid profile measurements (National Cholesterol Education Program (NCEP), 2002).

The triglyceride, LDL-C and HDL-C levels are all affected by changes in posture at the time of sampling. It is recommended that the patient undergoes blood letting always at the same position to prevent changes in the lipid profile measurements (Soh, J; Josekutty, J; Hussan, 2011). The NCEP recommended that the individual is in the sitting position for at least 5 minutes before blood sampling takes place to prevent hemoconcentration (National Cholesterol Education Program (NCEP), 2002). It is further recommended to limit veno-occulsion using a tourniquet for to less than two mintues, since prolonged venous occlusion can lead to hemoconcentration and an increase in cholesterol level by 10 to 15% (Soh, J; Josekutty, J; Hussan, 2011).

The NCEP ATPIII classifies the lipid profile (LDL-C, triglycerides, HDL-C and total cholesterol) ranging from optimal to very high states for the general population (non-diabetes) as seen in Table 1.15 (National Cholesterol Education Program (NCEP), 2002).

| **LDL Cholesterol (mmol/L)** | |
|---|---|
| <2.59 | Optimal |
| 2.59 – 3.35 | Above optimal |
| 3.36 – 4.15 | Borderline high |
| 4.16 – 4.89 | High |
| ≥4.91 | Very high |
| **Triglycerides (mmol/L)** | |
| <1.69 | Optimal |
| 1.69 – 2.25 | Borderline |
| 2.26 – 5.63 | High |
| ≥5.65 | Very high |
| **HDL Cholesterol (mmol/L)** | |
| <1.03 | Low |
| ≥1.55 | High |
| **Total Cholesterol (mmol/L)** | |
| <5.17 | Desirable |
| 5.17 – 6.20 | Borderline high |
| ≥6.21 | High |

Table 1.15 ATPIII classification of the lipid profile (mmol/L) for the general population (National Cholesterol Education Program (NCEP), 2002)

The American Diabetes Association further classifies the HDL-C levels according to gender, where HDL-C values for males <1.03mmol/L and females <1.29mmol/L are considered as low (American Diabetes Association, 2018a).

#### 1.3.3.3 Management of dyslipidaemia in Type 2 Diabetes Mellitus

Lifestyle changes (physical activity and dietary modifications) should be the initial management plan for T2DM individuals along with blood-glucose control. Both interventions have been associated with an improved dyslipidaemia profile (National Cholesterol Education Program (NCEP), 2002; Taskinen, 2002; Haffner and American Diabetes Association, 2004; Bantle *et al.*, 2008). Lowering the plasma glucose levels

improves the dyslipidaemia by either enhancing the insulin action or else providing extraneous insulin. Conversely, good glycaemic control will improve dyslipideamia but not eliminate it (Mooradian, 2009; Parhofer, 2015).

The lipid phenotype and genetic factors of an individual all play a role in the lipid profile changes attributed to dietary modification (Krauss, 2005). Physical activity has been reported to improve insulin sensitivity independent of weight loss, while increasing HDL-C levels (Williams, 2004). However, the magnitude of increase in HDL-C levels is dependent on genetic factors affecting the CETP and endothelial lipase (Wilund *et al.*, 2002; Halverstadt *et al.*, 2003). Pharmacological interventions should be considered, if optimal lipid levels are not achieved after 6 – 12 weeks of intensive lifestyle changes along with dietary supplements such as omega-3 fatty acids (Bantle *et al.*, 2008). The LDL-C goal for diabetes individuals with additional cardiovascular risk factors is of <1.80mmol/L, while the goal for all other diabetes individuals is of <2.30mmol/L (Catapano *et al.*, 2016).

A number of clinical trials have been conducted to evaluate the effect of pharmacological intervention on the health outcomes. The *Heart Protection Study* (HPS) evaluated the effect of simvastatin (40mg daily) on cardiovascular outcomes as opposed to a placebo. It was reported that lower LDL-C levels were evident along with a reduction in major vascular events in those with previously known coronary disease as well as those with previous cerebrovascular disease or peripheral arterial disease or diabetes (Heart Protection Study Collaborative Group, 2002). The *Collaborative Atorvastatin Diabetes Study* (CARDS) followed a similar protocol but 10mg of daily atorvastatin was prescribed to known diabetes individuals without a previous cardiovascular history. Those on atorvastatin as opposed to placebo exhibited a risk reduction for major cardiovascular events as well as for strokes.

The all-cause mortality was also reduced. These results put forward the benefit of simvastatin as a primary preventive measure against cardiovascular disease in diabetes individuals (Colhoun *et al.*, 2004). The *Diabetes and Combined Lipid Therapy Regimen* (DIACOR) study evaluated the effect of combination therapy (simvastatin plus fenofibrate) on the improvement of cardiovascular risk and reduction in sdLDL-C, VLDL levels while increasing HDL-C levels. This combination was found to have superior ability than monotherapy in reducing cardiovascular risk (May *et al.*, 2008). Similarly, the *HDL-Atherosclerosis Treatment Study* showed that a combination of simvastatin and nicotinic acid had a positive effect against the progression of atherosclerosis and its subsequent events (Zhao *et al.*, 2004). The various clinical trials mentioned above, which are superior research designs, concluded that diabetes individuals should not only have glycaemic control but also be started on dual lipid lowering therapy. Such regimen was proved to have positive cardiovascular and cerebrovascular outcome. Therefore, it is suggested that strategies targeting diabetes individuals consider such a dual regimen even if initially it may increase the financial burden on the health system, but it will be cost-effective in the long run.

Another pharmacological lipid lowering therapy that could be considered in persons with diabetes with high LDL-C is ‘Ezetimibe’. According to the IMPROVE-IT trial, this drug was shown to reduce the LDL-C levels by selectively inhibiting the absorption of intestinal cholesterol. This trial reported that ‘Ezetimibe’ significantly reduced the major cardiovascular risks in high-risk individuals with already established cardiovascular disease and low LDL-C (Cannon *et al.*, 2015). Another therapeutic therapy that can be used in atherosclerotic at-risk individuals with hypercholesterolemia is proprotein convertase subtilisin/kexin type 9 (PCSK9). The PCSK9 inhibitors binds to the low-density lipoprotein

receptor (LDLR) leading to LDL-C degradation in the lysosome while inhibiting LDLR from recirculating to the cell membrane, hence lowering the LDL-C levels (Wang and Liu, 2018).

#### 1.3.3.3.1 Statins and Type 2 Diabetes Mellitus

Statins are pharmacological medications that block the conversion of 2-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) to mevalonic acid and therefore limiting cholesterol synthesis (Wierzbicki, Poston and Ferro, 2003). In turn, lower hepatic cholesterol levels result, leading to an increase in low-density lipoprotein (LDL) receptor expression in hepatocytes. This leads to an enhanced LDL-particle clearance from the blood (Chogtu, Magazine and Bairy, 2015). Statins also reduce the production of plasma LDL-C as well as increase the catabolism of apoB100 (Sirtori, 2014).

The predominant action of statins is decreasing LDL-C levels by as much as 28 – 42% and reducing serum total cholesterol by 20 – 31%. However, they also act on reducing the triglyceride levels whilst increasing the high-density lipoprotein (Bonetti *et al.*, 2003; Wierzbicki, Poston and Ferro, 2003). Triglyceride levels are reduced in proportion to the statin effect on apolipoprotein B and LDL-C reduction (Wierzbicki *et al.*, 2000). The intra-hepatocyte lipid pool is reduced by statins. This in turn leads to fewer, smaller very-low-density-lipoprotein (VLDL) particles containing less triglycerides and cholesterol (Cardozo *et al.*, 2002). The HDL-C levels are enhanced to a small degree by the intake of statins, although this effect is drug specific. Statins have a more pronounced effect on individuals with low HDL-C levels when compared to those with elevated levels (Wierzbicki *et al.*, 2000).

Statins are prescribed for primary and secondary prevention of cardiovascular disease since they bring about a significant decrease in cardiovascular morbidity and mortality in those with hypercholesterolemia (Scandinavian Simvastatin Survival Study Group, 1994; Taylor *et al.*, 2013).

Apart from the lipid lowering properties, statins exhibit other pleiotropic effects and benefits. In fact, statins possess anti-inflammatory properties, inhibit vascular smooth muscle cell proliferation as well as inhibit platelet function and improve vascular endothelial function (Wierzbicki, Poston and Ferro, 2003). These properties aid in the prevention of atherosclerotic plaque formation, limit atherosclerosis and thrombus formation (Bonetti *et al.*, 2003; Wierzbicki, Poston and Ferro, 2003).

Diabetes individuals exhibit a linear relationship between cholesterol levels and cardiovascular disease (Stamler *et al.*, 1993). Diabetes dyslipidaemia is a common comorbidity in diabetes individuals. Hence, statins appear to be beneficial medications for diabetes individuals due to their effect on lowering LDL-C levels and to a lesser degree also other lipoproteins (Brunzell *et al.*, 2008). It has been suggested that statins should be initiated in diabetes individuals on the basis of prevention of cardiovascular complications rather than elevated LDL-C levels (Eldor and Raz, 2009). In fact, statins were found to significantly reduce stroke incidence by 48% and acute coronary events by 36% (Kotseva *et al.*, 2009).

The JUPITER trail (Justification for the Use of Statins in Primary Prevention: An Intervention Trail Evaluating Rosuvastatin) reported that high dose rosuvastatin exhibited a higher risk for developing T2DM when compared to those on placebo (Ridker *et al.*,

2008). In fact, it has been suggested that statins actually cause a rise in serum fasting glucose levels as well as glycated haemoglobin (HbA1C) (Ridker *et al.*, 2008). This T2DM risk was found to be dose-dependent (Sattar *et al.*, 2010). The mechanism underlying this finding could be due to statin-induced insulin resistance leading to the inhibition of isoprenoid biosynthesis (Nakata *et al.*, 2006). This will decrease insulin-mediated cellular glucose uptake with a possibility of promoting glucose intolerance (Kohli *et al.*, 2015). Figure 1.23 illustrates the possible underlying mechanisms contributing to statin induced T2DM.

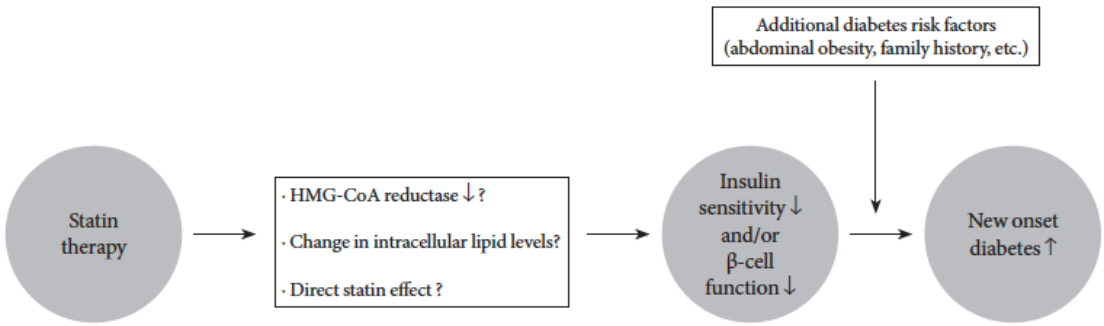



Figure 1.23 Possible underlying pathophysiology of statin induced T2DM (Parhofer, 2015)

Other proposed mechanisms for statin induced diabetes include a decrease in glucose transporter 4 (GLUT4) expression, blockage of calcium channels within beta cells, decreased levels of coenzyme Q10 and diminished cholesterol uptake in the pancreatic cells (Backes *et al.*, 2016). This finding was further evaluated by different control trials where it was suggested that statins increase diabetes risk in already susceptible pre-diabetes individuals rather than those without susceptibility (Kohli *et al.*, 2015). Development of diabetes was also more pronounced in individuals with the metabolic syndrome, obese (BMI $\geq$30Kg/m$^2$) or HbA1C >6% (Ridker *et al.*, 2012). Therefore the proposed statin-diabetes-induced mechanism is not statin-specific but rather an acceleration of the typical

glycemic deterioration (Crandall *et al.*, 2017) However, on comparing the risk of new onset diabetes (3%) to the beneficial effects (54% lower risk of myocardial infarction, 30% lower risk for stroke and 20% lower mortality risk), it was suggested that statins should still be prescribed with caution (Ridker *et al.*, 2008; Bhatia and Byrne, 2010).

Recently, it was reported that pitavastatin has a neutral or positive effect on glucose metabolism along with no increased risk of new-onset diabetes. Therefore it was put forward that pitavastatin can be prescribed to patients with diabetes or at risk of diabetes (Barrios and Escobar, 2016).

#### 1.3.3.4 Epidemiology of dyslipideamia

Both the Framingham Heart Study and UK Prospective Diabetes Study (UKPDS) found that when comparing diabetes against non-diabetes men and women, there was an increased prevalence of hypertriglyceridemia and low HDL cholesterol within the diabetes cohort. Meanwhile, both studies found that the LDL cholesterol and the total cholesterol levels did not differ between the diabetes and the non-diabetes patients. Of note, the UKPDS did find that diabetes women had markedly higher LDL cholesterol than their normoglycaemic counterparts (Kannel, 1985; UK Prospective Diabetes Study (UKPDS), 1997).

The prevalence of hypercholesterolaemia is not affected by the presence of diabetes mellitus, but serum cholesterol levels do have a negative effect on mortality. The mortality from coronary heart disease is exponentially increased as the level of serum cholesterol increases (Mooradian, 2009).

The WHO reported that in 2008 the European continent (53.70% CI 95%: 48.10 – 58.80) had the highest adult (45+ years) total cholesterol (>5mmol/L) prevalence rate (World Health Organization., 2017). Meanwhile, the total cholesterol (>5mmol/L) prevalence rate for the Maltese adult (45+ years) population for 2008 was of 59% (CI 95%: 42.40 – 73.60), with a male predominance (60.70% CI 95%: 37.50 – 80.20) (World Health Organization., 2017).

On comparing local Maltese health examination studies conducted in 1984 and 2010, a decline in cholesterol prevalence rate was observed (Cacciottolo, 1990; Directorate for Health Information and Research., 2012). This was markedly noted for the female population, where in the 1984 MONICA study, 49.10% of the female population was reported to have high cholesterol whereas in the 2010 EHES the rate declined to 14.70% (Cacciottolo, 1990; Directorate for Health Information and Research., 2012).

### 1.3.4 Metabolic Syndrome

The metabolic syndrome (MetS) is a complex condition that is made up of a constellation of metabolic changes and signs. Its development is attributed to the presence of both genetic and environmental factors (Povel *et al.*, 2011; Turkovic *et al.*, 2012; Liu *et al.*, 2015). Environmental changes contributing to MetS are urbanized living, sedentary lifestyle and high-calorie food diet (Miranda *et al.*, 2005).

### 1.3.4.1 Pathophysiology of the metabolic syndrome

Multifactorial contributors have been reported to be the underlying pathophysiology of metabolic syndrome. The most common associated factors are insulin resistance and central obesity (Reaven, 1988). Similar to T2DM, in the MetS the insulin receptor activation pathway PI 3-kinase is blocked leading to the inhibition of GLUT4 transporter translocation to the cell membrane and resulting in a hyperglycaemic environment (Cusi *et al.*, 2000).

Dysfunctional energy storage is also considered as a fundamental mediator for the development of MetS. Insulin resistance occurs as the consequence of abnormal processing and storage of triglycerides and fatty acids when adipocytes are saturated. Consequently, the excess triglycerides accumulate in the liver and muscle leading to insulin resistance. This process has been referred to as the "*overflow hypothesis*". In fact, one of the features of MetS is the elevation of triglyceride levels. Insulin resistance continues to enhance the elevation of triglyceride levels by impairing lipoprotein lipase, which is the enzyme responsible for clearing triglycerides within the liver. Meanwhile, hepatic lipase, the enzyme responsible for HDL-C clearance within the liver is enhanced by the presence of insulin resistance. Consequently, a decline in HDL-C levels results. This is a key feature of the MetS (Singh, Gupta and Khajuria, 2015). Additionally, an increase in visceral adiposity arises. The waist circumference and the waist-hip ratios are measures of visceral adiposity and correlate with both insulin resistance and other metabolic syndrome features (Miranda *et al.*, 2005).

The different components (high triglycerides, low HDL-C, high fasting plasma glucose, high blood pressure and abdominal obesity) making up the metabolic syndrome have been

associated with elevated levels of C-reactive protein (CRP) (Ridker *et al.*, 2003). The CRP marker has been also correlated with other factors that contribute to the metabolic syndrome namely fasting insulin, microalbuminuria and impaired fibrinolysis (Festa *et al.*, 2002; Stehouwer *et al.*, 2002). Furthermore, high CRP levels have been shown to be a predictor for metabolic syndrome, apart from being an independent predictor for the development of diabetes, atherothrombotic and cardiovascular diseases (Pickup and Crook, 1998; Pradhan *et al.*, 2001; Ridker *et al.*, 2002; Sattar *et al.*, 2003).

Other contributory mechanisms leading to the development of MetS include: disorders of the hypothalamic-pituitary-adrenal axis; altered glucocorticoid hormone action; chronic activation of the immune system; chronic stress and contributions of cytokines, hormones and other molecular adipocyte products (Eckel, Grundy and Zimmet, 2005).

#### 1.3.4.2 Definition of the metabolic syndrome

Over the years, the cluster of obesity, hyperlipidaemia, hyperglycaemia and hypertension have been described as “Syndrome X” or “Insulin resistance syndrome” (Reaven, 1988; Moller and Kaufman, 2005). Later on, the term “Metabolic Syndrome” was adopted and the clinical features established as seen in Table 1.15 (DeFronzo and Ferrannini, 1991; Alberti and Zimmet, 1998; National Cholesterol Education Program (NCEP), 2002; Kahn *et al.*, 2005; Alberti, Zimmet and Shaw, 2006; Simmons *et al.*, 2010). A definition consensus was reached in 2004 between the International Diabetes Federation (IDF), the World Health Organization (WHO) and the National Cholesterol Education Program – Third Adult Treatment Panel (ATP III) (Alberti, Zimmet and Shaw, 2006). The MetS was defined as the presence of central abdominal obesity along with any two additional features, as seen in Table 1.16 (*IDF consensus column*). As part of the consensus,

individuals with known hypertension and on treatment as well as those with known diabetes mellitus and on treatment were also considered as a contributing factor/s towards the identification of the metabolic syndrome (Alberti, Zimmet and Shaw, 2006).

| Metabolic Parameter | ATPIII | WHO | IDF consensus |
|---|---|---|---|
| Male waist circumference (cm) | >102 | >102 | >94 |
| Female waist circumference (cm) | >88 | >88 | >80 |
| FPG (mmol/L) | >6.1 | >6.1 | >5.5 |
| Blood pressure (mmHg) | >130/85 | >140/90 | >130/85 |
| Triglycerides (mmol/L) | >1.69 | >1.69 | >1.69 |
| HDL-C (mmol/L) | | | |
| Men | <1.03 | <0.91 | <1.03 |
| Women | <1.29 | <1.01 | <1.29 |

Table 1.16 Metabolic syndrome clinical features (Alberti and Zimmet, 1998; National Cholesterol Education Program (NCEP), 2002; Alberti, Zimmet and Shaw, 2006)

### 1.3.4.3 Epidemiology of the metabolic syndrome

MetS has become a public health concern due to its increasing global prevalence especially following the obesity epidemic (Grundy, 2008). In fact, it was noted the prevalence of MetS was 60% among obese, 22% among overweight and 5% among normal weight individuals (Park *et al.*, 2003).

The worldwide prevalence of MetS varies depending on the ethnicity, gender and age of the population (Kolovou *et al.*, 2007). The prevalence of MetS varies between different countries, with Nepal having the highest worldwide prevalence (61.70%) and France

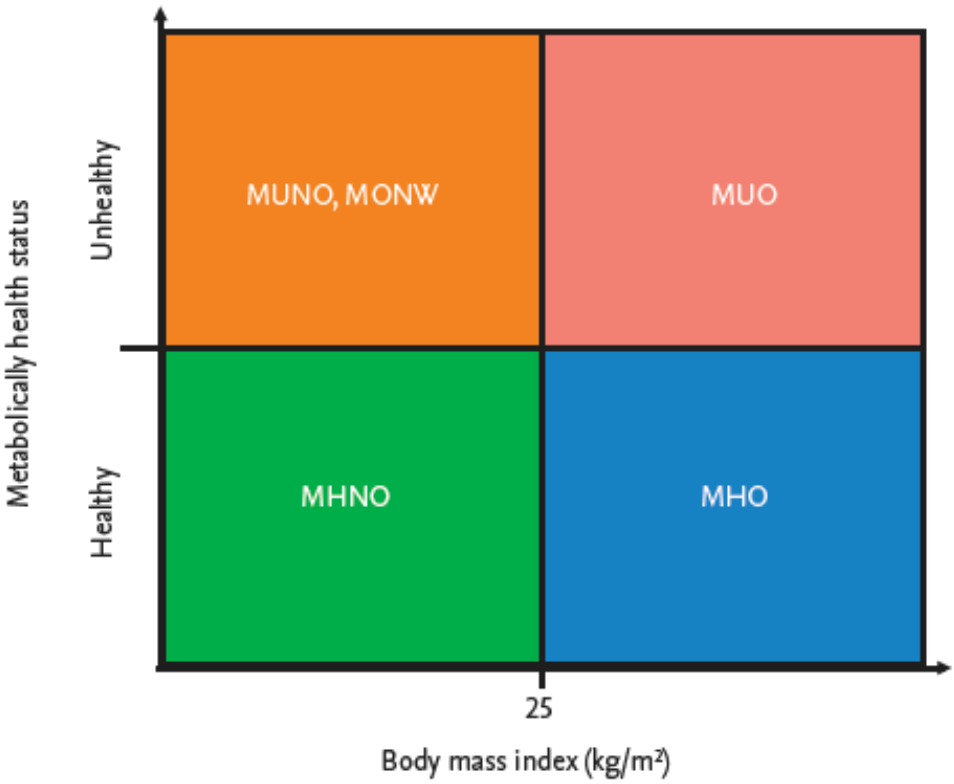
having the lowest prevalence (21.10%) (Maharjan *et al.*, 2013; Vernay *et al.*, 2013). In the Mediterranean region, MetS affects about one-fourth to one-fifth of the population (Anagnostis, 2012).

A European study reported a significant association between gender and increase in age with the development of MetS (Vishram *et al.*, 2014). This phenomenon has been most clearly delineated in women. The declining female sex hormones following menopause influence the metabolic risks, resulting in altered lipid profiles and central fat accumulation. All these predispose the female population to MetS (Carr, 2003; Schubert *et al.*, 2006). Gender exhibits a strong relationship to the development of MetS especially in European women (Ford *et al.*, 2008).

Furthermore, individuals falling within the MetS category have a threefold higher risk for the development of cardiovascular disease (O'Neill and O'Driscoll, 2015). The WHO therefore concluded that the "*metabolic syndrome is a pre-morbid condition rather than a clinical diagnosis*" (Simmons *et al.*, 2010).

### 1.3.5 Metabolic health statuses

Over the years, it was reported that the metabolic health status and the body fat status of an individuals could be classified into different categories, as seen in Figure 1.24 by Jung *et al*. (2017).




*MUNO – metabolically unhealthy non-obese; MONW – metabolically unhealthy normal weight; MUO – metabolically unhealthy obese; MHNO – metabolically healthy non-obesity; MHO – metabolically healthy obesity*

Figure 1.24 Classification of metabolic health statuses on the basis of metabolic health and body mass index (Jung, Lee and Song, 2017)

### 1.3.5.1 Metabolically Healthy Obese (MHO)

Metabolically Healthy Obese (MHO) are individuals who are obese (BMI ≥30Kg/m$^2$) but have a normal metabolic profile (Mathew, Farr and Mantzoros, 2016). These individuals do not have insulin resistance; type 2 diabetes; hypertension or dyslipidaemia (Blüher, 2014). Additionally, these individuals have less ectopic and visceral fat than other obese phenotypes (Samocha-Bonet *et al.*, 2014). The underlying

pathophysiology is complex with an interplay between environmental, behavioural and genetic factors, as seen in Figure 1.25 (Blüher, 2010).

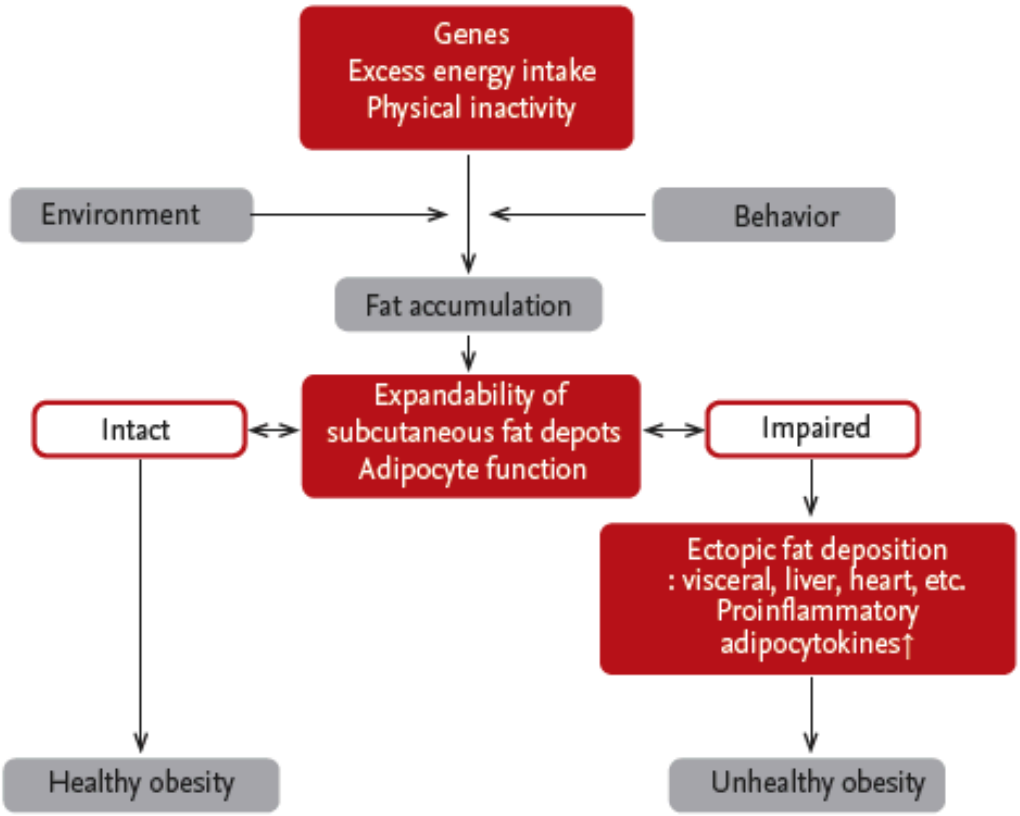



Figure 1.25 Pathophysiological model for the distinction between "healthy" and "unhealthy" obesity (Jung, Lee and Song, 2017)

The EPIC-CVD pan-European cohort study concluded that obese individuals who are metabolically healthy (defined as no metabolic syndrome features) are still at a higher risk of coronary heart disease and T2DM when compared to normal weight individuals (Lassale *et al.*, 2018). Similarly, Caleyachetty *et al.* reported that MHO is not benign but contribute to an increased risk for cerebrovascular disease, heart failure, peripheral vascular disease as well as coronary heart disease (Caleyachetty *et al.*, 2017). Furthermore, it was reported

that an approximate 50% of the MHO individuals within 10 years eventually shift to a metabolically unhealthy phenotype (Schröder *et al.*, 2014).

#### 1.3.5.2 Metabolically Obese Normal Weight (MONW)

Metabolically obese normal weight (MONW) individuals have a normal BMI (<25Kg/m$^2$) but demonstrate metabolic abnormalities, which are typical of obese individuals (Ruderman, Schneider and Berchtold, 1981; Mathew, Farr and Mantzoros, 2016). The prevalence of this occurrence is between 3 to 28% of the population (St-Onge, Janssen and Heymsfield, 2004).

These individuals exhibit insulin resistance, increased central adiposity, elevated triglyceride levels, decreased high-density lipoprotein cholesterol (HDL-C), hypertension and impaired fasting glucose (James B. Meigs *et al.*, 2006). No definite consensus has been reached as to the exact cut-offs for this condition. MONW individuals can be misled by having a normal BMI, while in actual fact have an increased waist circumference and high body fat (Shea *et al.*, 2012). Furthermore, these individuals may be accounting for the higher T2DM prevalence rate as well as other cardiovascular disorders in people with a BMI between 20-27Kg/m$^2$ (Ruderman *et al.*, 1998).

### 1.3.6 Depression and Type 2 Diabetes Mellitus

Major depression is a global health burden and estimated to be the second leading global disease burden by 2020, following ischemic heart disease (Reddy, 2010). Depression is a co-morbidity for a number of chronic medical conditions including diabetes mellitus (Brown *et al.*, 2005). Individuals diagnosed with diabetes are prone to psychological

vulnerability following the change in their medical status. In fact, depression affects 20 to 25% of diabetes mellitus patients (Bot *et al.*, 2012). Those already suffering from major depressive disorder have a two-fold increased risk of developing a new-onset myocardial infarction when they are further diagnosed with diabetes (Scherrer *et al.*, 2011).

There appears to be a bidirectional relationship between diabetes and depression (Chen *et al.*, 2013). Individuals suffering from an early onset depression have been shown to have a two-fold increased risk of developing T2DM (Kawakami *et al.*, 1999). In fact, individuals with a history of depression or who are on antidepressant medication have a substantially higher risk of developing T2DM. This is especially so in the setting of obesity and a positive family history for diabetes (Rubin *et al.*, 2008; de Groot *et al.*, 2016). Diabetes individuals suffering from depression, experience a decreased quality of life, increased medical morbidity and mortality with higher medical costs (Katon *et al.*, 2004, 2005, 2006; Lustman and Clouse, 2005; Naicker *et al.*, 2017). Thus, it was recommended that diabetes individuals as well as the high-risk pre-diabetes individuals should have regular routine screening for depressive symptoms, more so if these individuals have an overweight/obese profile (American Diabetes Association., 2018).

Both major and minor depressive disorders have been associated with individuals having high BMI as well as with smokers (Glassman *et al.*, 1990; Katon *et al.*, 2004). Consequently, those who smoke are more prone to develop diabetes mellitus since smoking has been associated with insulin resistance (Chiolero *et al.*, 2008).

#### 1.3.6.1 Diabetes distress

Diabetes-related distress (DD) is a common occurrence in diabetes individuals as well as in their family members (Fisher *et al.*, 2012; Nicolucci *et al.*, 2013). DD is a negative psychological reaction resulting from the individual's emotional distress brought about by fear and worry in managing the condition and any related complications (Fisher *et al.*, 2012). High levels of DD are linked to lower self-efficacy, poorer dietary behaviours, non-adherence to medication and higher HbA1C (Aikens, 2012; Fisher *et al.*, 2013).

#### 1.3.6.2 Depression Assessment

Depression can be assessed and quantified using the validated 'Patient Health Questionnaire' (PHQ-9) questionnaire. The PHQ-9 is a multipurpose tool of measure that is used for screening, diagnosing, monitoring and measuring the severity of depression. On completion of the 9-question questionnaire, a score is calculated which determines the severity of the depression. Using this score, a high sensitivity (73%) and specificity (98%) was established in diagnosing major depression (Spitzer, Kroenke and Williams, 1999). PHQ-9 scores of 5, 10, 15 and 20 represent mild, moderate, moderately severe and severe depression respectively (Kroenke, Spitzer and Williams, 2001).

### 1.3.7 Genetics and Type 2 Diabetes Mellitus

Type 2 diabetes is a heterogeneous disease caused by a complex interaction between both genetic and environmental factors, as seen in Figure 1.26 (So *et al.*, 2000; Bonadonna, 2004; Stumvoll, Goldstein and van Haeften, 2005; Doria, Patti and Kahn, 2008). Individuals inheriting the genetic risk do not necessarily develop the disease phenotype,

unless exposed to particular environmental cues. Both genetic and environmental (alone and in combination) factors influence different traits that contribute to the diabetes phenotype (beta cell mass, insulin action, insulin secretion, fat distribution and obesity) (So *et al.*, 2000; Bonadonna, 2004; Stumvoll, Goldstein and van Haeften, 2005).

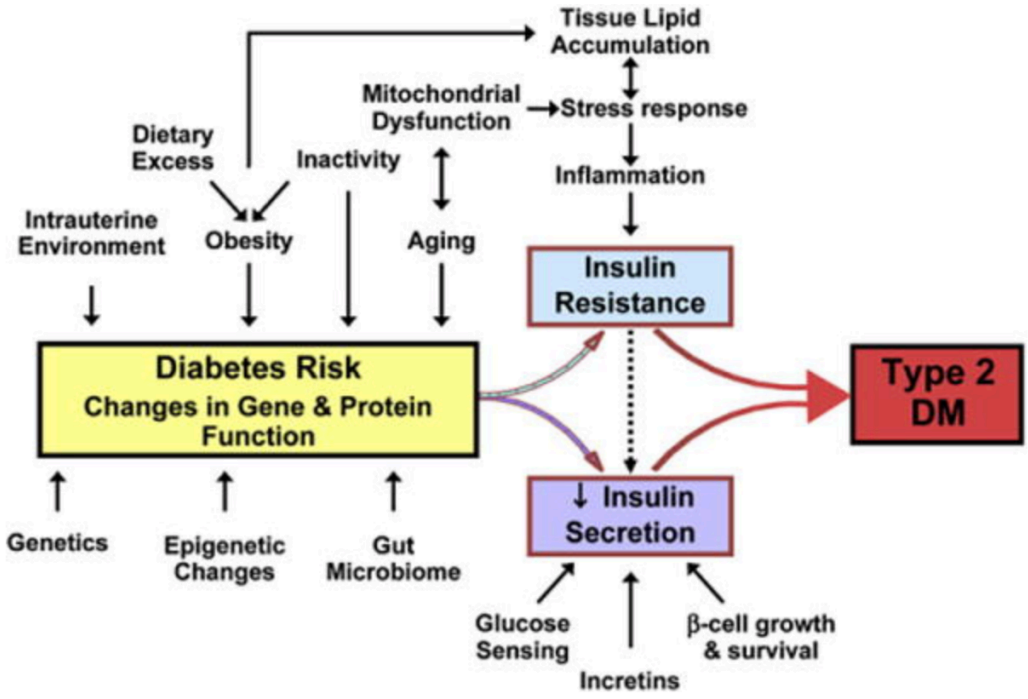



Figure 1.26 Complex pathogenesis of T2DM (Doria, Patti and Kahn, 2008)

T2DM is multigenic, where multiple genetic defects may exist among subgroups of diabetes populations. A genetic susceptibility is further enhanced by environmental factors driven by sedentary lifestyles and unhealthy dietary change (Doria, Patti and Kahn, 2008). In fact, physical activity was reported to reduce insulin resistance, while high carbohydrate and fat diets with poor fibre content aggravate insulin resistance (Riccardi *et al.*, 1984). Obesity is a risk factor for T2DM development and has been reported to be under genetic

influence as well. Due to the association between T2DM and obesity, it has been suggested that both conditions share susceptibility genes (Carmelli, Cardon and Fabsitz, 1994).

Understanding the genetics of T2DM along with obesity genetics, may lead to a better perspective of the pathogenesis contributing to T2DM. This may provide new information that will aid clinical diagnosis. It will also help public health specialists in burden prevention as well as developing predictive screening. Such understanding may also contribute to new drug therapy, particularly targeted pharmacotherapy with possible individual optimized treatment.

#### 1.3.7.1 Heritability of T2DM – an interaction between genes and environment

Heritability plays an important role in the development of T2DM, with an estimated 20 – 80% of T2DM being acquired in such manner (Poulsen *et al.*, 1999; Meigs, Cupples and Wilson, 2000). A poor nutritional prenatal environment and subsequently low birth weight has been associated with insulin resistance and T2DM in adulthood (Hales *et al.*, 1991; Lithell *et al.*, 1996). It was further proposed that such poor foetal nutrition results in metabolic adaptation and "programming" leading to the development of metabolic syndrome and cardiovascular disease apart from T2DM (Barker *et al.*, 1993; Hattersley and Tooke, 1999; Savona-Ventura, C; Zammit, K; Vella, 2007).

Heritability can present in clusters within families and first-degree relatives when compared to the general population, with a 3.5 fold increased risk of developing diabetes (Gloyn and McCarthy, 2001; Weires *et al.*, 2007). Individuals born to a single diabetes parent, have a 40% lifetime risk of developing T2DM, while having both parents suffering

from T2DM increases the risk to 70% (Tillil and Köbberling, 1987). The diabetes genetic link is further reinforced by twin studies that show a higher concordance for the disease in monozygotic twins (70%) when compared to dizygotic twins (10%) (Barnett *et al.*, 1981; Newman *et al.*, 1987).

Despite the importance of the genetic element for the development of T2DM, environmental factors must play a role in accelerating the disease in those having a genetic predisposition. In fact, a number of observations have been reported where an increase in diabetes prevalence was evident when ethnic groups migrate from a less developed regions to a more urbanized regions and gradually adopt the Westernized lifestyle and dietary habits (van Tilburg *et al.*, 2001).

Furthermore, epigenetics plays a role in the development of T2DM. This involves the hereditary ability to alter gene functions without a change in the nucleotide sequences. A number of mechanisms may occur such as DNA-methylation, histone acetylation and non-coding RNA, which are responsible for the regulation of gene expression and may be altered following environmental cues (Skinner, 2011). Maternal environment may influence the infant metabolic risk due to epigenetic changes rather than due to inherited variation in the DNA sequence (Seki *et al.*, 2012). The *Pedersen hypothesis* covers this phenomenon, where maternal hyperglycaemia contributes to foetal hyperglycaemia and hypertrophy of the pancreas islet cells of the foetus due to insulin-hypersecretion. This contributes to macrosomia and increased risk of development of T2DM later on in life (Pedersen, 1967).

### 1.3.7.2 Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are variations found at single bases within the DNA sequence of a genome. The exchange of single base pairs is the most common sequence variation in the human genome accounting for 90% of variations (Staiger *et al.*, 2009).

SNPs are the basis of complex diseases' genetic variations. The genetics of common complex diseases are elucidated by means of SNPs by using genome-wide association studies, candidate gene case-control association studies as well as genome-wide linkage analyses (Shen, Abdullah and Wang, 2009). Identifying the SNPs responsible for the development of these complex diseases leads to earlier diagnosis, prevention and treatment of the disease (Suh and Vijg, 2005; Wang, Luhm and Lei, 2007).

SNPs may come into two main forms: (1) synonymous – where mutations do not change the polypeptide sequence and (2) non-synonymous - where mutations result in different polypeptide sequence (Peters *et al.*, 2010).

The method of undergoing SNP genotyping depends on the number of SNPs and DNA samples needing to be genotyped. When it comes to study a small number of SNPs in a large population, the TaqMan® assay has been found to be the most cost-effective, time-efficient and accurate technology (Shen, Abdullah and Wang, 2009). In fact, this has been the method used for the current study.

### 1.3.7.3 Candidate gene studies

There are two main approaches for identifying susceptibility genes in T2DM, namely: (1) the use of candidate gene studies and (2) the genome wide scan approach. Candidate gene studies are based on known or presumed biological function of selective putative genes that are related to the disease under study (Gloyn and McCarthy, 2001). This requires prior knowledge of the disease being investigated including the role of the gene within the disease pathophysiology (*priori hypothesis*) (Kwon and Goate, 2000). Candidate gene association studies consider the statistical efficiency of the biological understanding of the phenotype and the association analysis of the complex disease under study (Tabor, Risch and Myers, 2002). One can compare this gene study to a traditional epidemiological approach in order to identify the cause of the disease. The association between the gene and the disease is investigated by means of case-control studies. This kind of study has been found to be clinically relevant apart from acting as a potential disease diagnostic tool for genetic disorders (Peters *et al.*, 2010). In diabetes studies, the genes encoding for insulin signalling proteins and glucose homeostasis are excellent candidates for such candidate gene studies. Candidate gene studies usually comprise of a random unrelated T2DM cohort with a matched control cohort. Such an approach enables the identification of possible polymorphic alleles that occur at a significantly higher frequency within the T2DM cohort than in the control cohort, leading to the establishment of an association between T2DM and the allelic marker (van Tilburg *et al.*, 2001). These associations can be influenced by selection bias, recall bias, misclassification and confounding factors (Tabor, Risch and Myers, 2002). However, an observational association has been reported to be a crucial initial step in the understanding of the disease aetiology and a potential causal pathway to understand the genetic determinants of complex diseases (Tabor, Risch and Myers, 2002).

Candidate gene analyses depend on the selection of SNPs, which can range from a dozen to thousands unlike in genome-wide association studies (GWAS) where more than 100,000 SNPs are genotyped. Candidate gene analysis utilizes custom arrays of SNPs, which enable the researcher to select the target SNPs to be investigated. These analyses are also performed when budget constrains are present as opposed to GWAS (Peters *et al.*, 2010).

Preconditions and limitations of candidate gene studies are several. The biology of the disease being investigated needs to be known. False positive results may arise due to confounding factors following population stratification. This follows the fact that non-random mating or population subdivision leads to variations in marker allele frequencies among population segments as a result of founder effects or genetic drift (Slatkin, 1991). Population stratification is therefore referring to differences in allele frequencies between the case and control following systemic differences in the ancestry rather than the association between genes and the disease (Freedman *et al.*, 2004). In order to compensate for this, the transmission disequilibrium test (TDT) could be utilized to look at the genotypes of the parents of the affected subjects. Unfortunately this method is not suitable for T2DM, as this disease usually presents in old age, with a greater likelihood of parents having passed away (van Tilburg *et al.*, 2001). Furthermore, candidate gene studies are unable to identify entirely new T2DM genes or pathways, for which case genome wide association studies are required.

A candidate gene association study was found to be appropriate to answer the research question of the current study. Ten SNPs were identified through a systematic literature review, including local research studies. The SNPs chosen were previously reported to have

had an effect on inflammation, on insulin secretion, on insulin sensitivity, glucagon levels and on obesity pathophysiology.

#### 1.3.7.4 Effect on insulin secretion

##### 1.3.7.4.1 Transcription factor 7 like 2 (*TCF7L2*)

The *TCF7L2* is expressed in various human tissues, including subcutaneous adipose tissue, pancreatic beta cells, liver, brain as well as the omentum (Vcelak *et al*. 2012). *TCF7L2* is found on chromosome 10 (10q25.3) and forms part of a high mobility group with a role in the Wnt signalling pathway (Grant *et al.*, 2006). The Wnt signalling pathway is responsible for at least four branches (1) β-catenin pathway; (2) the planar cell polarity pathway; (3) the Wnt/$Ca^{2+}$ pathway and (4) the pathway that regulates spindle orientation and asymmetric cell division, as seen in Figure 1.27.

Therefore Wnt forms part of stem cell amplification, differentiation, migration, organogenesis and the development of the body plan (Liu Z, 2010). Furthermore, the Wnt signalling pathway forms part of the development and growth regulatory mechanism of the cell, which is mediated by the secretion of glycoproteins. Therefore *TCF7L2* forms part of the regulation of the pro-glycogen gene transcription and the production of glucagon-like peptide – 1 (GLP-1) (Yi, Brubaker and Jin, 2005).

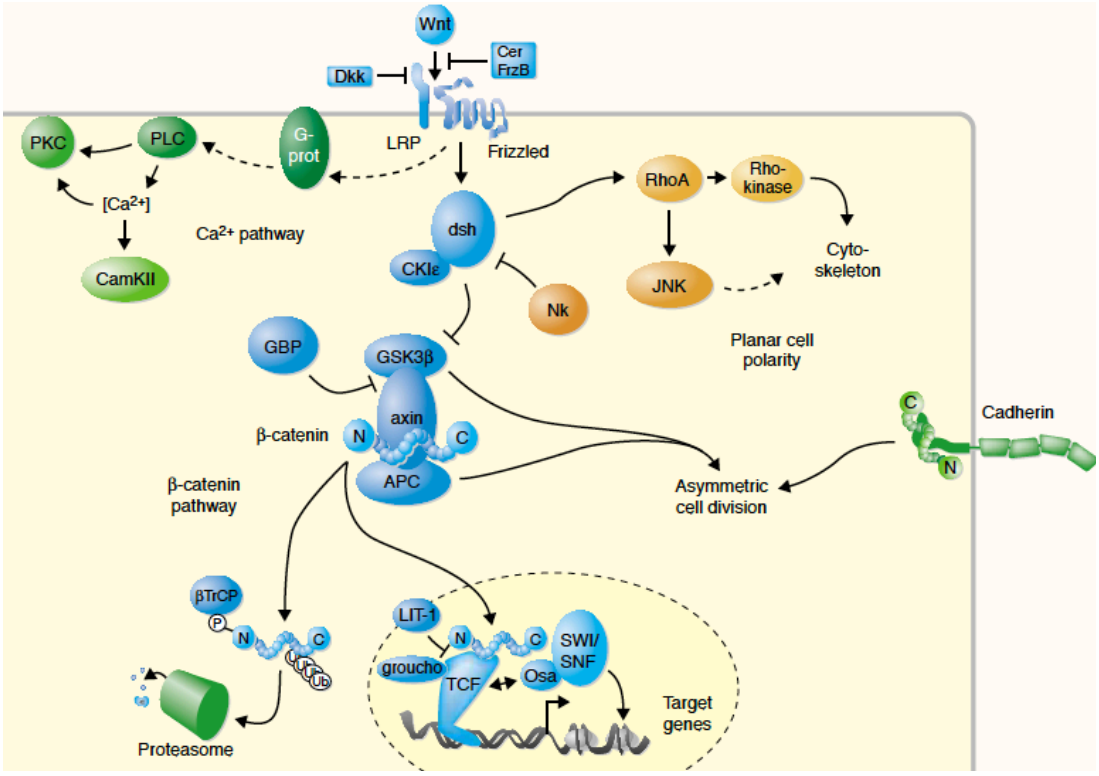



Figure 1.27 Summary of the diversification of the Wnt signaling pathway (Huelsken and Birchmeier, 2001)

*TCF7L2* is responsible for the β- cell proliferation and the β- cell survival, since the deletion of *TCF7L2* in human islet cells reduces insulin secretion and increases apoptosis (Shu *et al.*, 2008). However, the over expression of *TCF7L2* protects the β- cells from glucose and cytokine-induced apoptosis (Shu *et al.*, 2008). *TCF7L2* is also responsible for the glucose and incretin stimulated insulin secretion (Shu *et al.*, 2008). *TCF7L2* polymorphisms affect the production of the incretin hormone GLP-1 leading to an increased risk of developing diabetes (Grant *et al.*, 2006). The risk allele affects the whole-body insulin sensitivity with a reduction in incretin-stimulated insulin secretion and pro-insulin conversion. It also leads to the reduction of liver insulin sensitivity (Staiger *et al.*, 2009).

The DeCODE Genetics study identified a strong association in the Icelandic population between the intron variants of the *TCF7L2* gene with T2DM risk, through a linkage signal on chromosome 10q (Grant *et al.*, 2006). The same strong linkage signal was present in various ethnic backgrounds including Europeans, West Africans, Mexican Americans, Indian and Japanese populations (Duggirala *et al.*, 1999; Cauchi and Froguel, 2008). The association has been established between the common microsatellite (DG10S478) and the T allele of *TCF7L2* variants within intron 3 of the gene (Florez, Hirschhorn and Altshuler, 2003; Helgason *et al.*, 2007; Jin and Liu, 2008). The mostly commonly linked T2DM associated variant was the T allele rs7903146, which exhibited an estimated risk of 1.46 (Cauchi *et al.*, 2007; Helgason *et al.*, 2007). Meanwhile the T allele (CT/TT) carriers exhibit a significant elevation of the *TCF7L2* mRNA expression in the pancreatic islets. This was associated with impaired insulin secretion and incretin effects (Lyssenko and Laakso, 2013). Following the strong association between the T allele rs7903146 variant with T2DM, it was recommended that such SNP should be included in any association study (Grant *et al.*, 2006). In fact, this SNP was studied in this current study.

At individual level, a carrier of a single *TCF7L2* risk allele may have a 40 – 60% increased diabetes risk (Cauchi and Froguel, 2008). However different ethnic groups exhibit different rs7903146 T allele frequency that contributes to variable impact on the respective populations. In fact in lean diabetes European populations the rs7903146 T allele frequency was estimated to be 10 – 25%, while in Eastern Asian the population attributing risk fraction was 18.70% (Chang *et al.*, 2007).

The rs7903146 T allele was associated with lower BMI in T2DM individuals and with higher risk for T2DM in non-obese subjects (Cauchi and Froguel, 2008). This finding is

further enhanced by the fact that T allele carriers exhibit a greater mean insulin sensitivity that is correlated with concomitant lower mean BMI and waist circumference (Florez *et al.*, 2006). However, rs7903146 is not a risk factor for obesity in European populations but the effect on T2DM risk was modulated by adiposity (Cauchi *et al.*, 2008). This relationship between *TCF7L2* and adiposity may arise from a better beta cell compensation in obese individuals (Watanabe *et al.*, 2007).

#### 1.3.7.5 Effect on glucose-stimulated insulin secretion

##### 1.3.7.5.1 CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*)

The CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) has been reported to be a susceptible gene for T2DM, body mass index and adipocyte differentiation (Scott *et al.*, 2007; Steinthorsdottir *et al.*, 2007; Winkler *et al.*, 2010; Okada *et al.*, 2012; Take *et al.*, 2017). This gene is found on chromosome 6 (6p22.2) and encodes 65-kDa proteins. It forms part of the methylthiotransferase family (Stančáková *et al.*, 2008). *CDKAL1* is expressed in various organs including pancreatic islet cells, adipose tissue, liver, muscle and brain (Wei *et al.*, 2011; Okamura *et al.*, 2012).

*CDKAL1* controls the first phase insulin exocytosis within β- cells through the facilitation of ATP generation, responsiveness of the potassium (ATP) channel and the subsequent activity of the calcium ion ($Ca^{2+}$) channels (Ohara-Imaizumi *et al.*, 2010). It was reported that *CDKAL1* is essential for accurate translation of codons for lysine in proinsulin within β- cells. Individuals carrying the mutant C-risk allele (rs7754840) showed an associated impairment within the first phase of insulin, hence impaired insulin secretion occurs with a subsequent increased risk of T2DM (Stančáková *et al.*, 2008). Furthermore, a deficiency

of *CDKAL1* leads to aberrant proinsulin synthesis with an increased endoplasmic reticulum stress and therefore resulting in an impaired glucose metabolism (Wei *et al.*, 2011; Brambillasca *et al.*, 2012). In fact, the homozygotes (CC allele) exhibited an approximately 20% reduction in insulin response when compare to heterozygotes (CG) and non-carriers (GG). Carrying the risk variant was associated with 20% risk of T2DM within individuals of European ancestry (OR: 1.20 CI 95%: 1.13 – 1.27 $p$=7.7 x$10^{-9}$) (Steinthorsdottir *et al.*, 2007).

Genetic control of childhood weight has been established through mechanisms that are associated with insulin release and action. In fact, *CDKAL1* was found to influence the homeostasis of body mass when excessive foetal growth (large for gestational age) was present. In such cases, *CDKAL1* was associated with reduction in body mass index and weight in children (Winkler *et al.*, 2010). Hence, deficiency of *CDKAL1* may lead to increase in body weight in susceptible individuals.

*CDKAL1* is also associated with anti-adipogenic action and suppression of *PPARγ* expression. It functions through a negative feedback loop in order to prevent excess lipid accumulation within the adipocytes (Take *et al.*, 2017). *CDKAL1* is expressed in large quantities during the early stages of adipocyte differentiation through the activation of the Wnt signalling pathway (Christodoulides *et al.*, 2009; Take *et al.*, 2017). An increase in β-catenin and of the active unphosphorylated β-catenin in the nucleus occurs in the presence of *CDKAL1*. Hindrance of the Wnt signalling pathway or *CDKAL1* deficiency result in lipid saturated adipocytes and over activation of *PPARγ* (Take *et al.*, 2017). In summary, a manipulation of *CDKAL1* activity may be appropriate for the treatment of T2DM and obesity.

#### 1.3.7.5.2 Hematopoeitically expressed homeobox (*HHEX*)

*HHEX* is found at the chromosome 10q23.33 locus and has a role in the development (hematopoietically expressed homeobox, *HHEX*) and function (insulin degrading enzyme IDE) of the pancreas (Farris *et al.*, 2003; Bort *et al.*, 2004). The hematopoietically expressed homeobox (*HHEX*) gene, is a member of the homeobox gene family and has been reported to be an essential regulator of embryogenesis and hematopoietic progenitor development (Paz *et al.*, 2010). Furthermore, the *HHEX* gene is involved in the encoding of a transcription factor of the Wnt signalling pathway, which is the fundamental pathway for cell growth and development of the pancreas (Morgutti *et al.*, 2001; Foley and Mercola, 2005; Smith, 2006). It was reported that the *HHEX-IDE* (rs1111875) variant was associated with insulin secretion response following glucose load (Grarup *et al.*, 2007). Therefore, a *HHEX* deficiency may affect the β- cell function with a predisposition to T2DM.

The *HHEX* polymorphism rs1111875 has been associated with increased T2DM susceptibility across different multi-ethnic groups (van Vliet-Ostaptchouk *et al.*, 2008; Y. Wang *et al.*, 2011; Li *et al.*, 2012). This may be explained by the '*phenomenon of canalization*', which relates to the developmental compensation for disruptive environmental or genetic forces (Sheehan *et al.*, 2008). Both the heterozygous (C/T) and the homozygous (C/C) were associated with increased susceptibility for T2DM when compared to non-carriers (T/T) (van Vliet-Ostaptchouk *et al.*, 2008). The odds association for the rs1111875-C allele in pooled Caucasians studies was reported to be 1.14 (CI 95%: 1.07 – 1.22 $p$=<0.01) (Li *et al.*, 2012).

More recently *HHEX* has been found to be a prerequisite of the pancreatic δ-cell differentiation. In a *HHEX* deficient subject there was a decrease in somatostatin levels with a disrupted paracrine inhibition of insulin release from the β- cells. This suggests that *HHEX* is a transcriptional regulator specifically required for the paracrine control of the δ-islet cells. Therefore a distribution in this gene contributes to type 2 diabetes mellitus (Zhang *et al.*, 2014).

*HHEX* rs1111875 variants (T/C or C/C) have also been related to colorectal cancer in diabetes Chinese population. Furthermore, those carrying the heterozygous and homozygous alleles were also found to be susceptible to develop colorectal cancer independent of their diabetes status (Sun *et al.*, 2016).

#### 1.3.7.5.3 Solute carrier family 30 member 8 (*SLC30A8*)

The *SLC30A8* is a zinc transporter 8 (ZnT8) and forms part of the zinc transporter family. It is coded for by soluble carrier family 30 members 8 gene on chromosome 8q24.11 (Chimienti, Favier and Seve, 2005). The *SLC30A8* is predominantly expressed in the insulin producing pancreatic beta cells. It is responsible for the zinc transport from the cytoplasm into the insulin secretary vesicle (Chimienti *et al.*, 2004; Sladek *et al.*, 2007; Staiger *et al.*, 2009). Insulin is stored within this vesicle as a solid hexamer (crystallization) whilst bound to two zinc ions before it is secreted (Emdin *et al.*, 1980; Gold and Grodsky, 1984; Lemaire *et al.*, 2009). Zinc has an important role in insulin trafficking (synthesis, storage and secretion) (Chimienti *et al.*, 2004). A co-secretion of insulin and zinc from the granules affects the neighbouring paracrine and autocrine islets of Langerhans (Kim *et al.*, 2000; Ishihara *et al.*, 2003; Prost *et al.*, 2004).

Variations within *SLC30A8* may influence insulin stability and insulin trafficking due to zinc accumulation within the insulin granules (Xiang *et al.*, 2008). The C allele SCL30A8 variant (rs13266634) is a risk allele for T2DM as it is associated with a reduction in pancreatic β- cell function (Scott *et al.*, 2007; Sladek *et al.*, 2007; Staiger *et al.*, 2007, 2009; Steinthorsdottir *et al.*, 2007; Zeggini *et al.*, 2007). Presence of the C risk allele variant is related to a decreased response of glucose-stimulated secretion and a reduction in pro-insulin conversion (Staiger *et al.*, 2009).

Low peripheral insulin levels were reported in homozygous carriers (C/C) during early phase intravenous glucose tolerance test (Boesgaard *et al.*, 2008). In fact, a link was established between *SCL30A8* and hepatic insulin clearance (Tamaki *et al.*, 2013). Normally, insulin is secreted from the islets of Langerhans into the portal vein where it is transported into the liver, where about half of the insulin is cleared, while the reminder enters the systemic circulation (Eaton, Allen and Schade, 1983). This clearance rate is hindered by 20% in the postprandial state (Caumo, Florea and Luzi, 2007). The co-secreted zinc with insulin (in a ZnT8-dependent manner) acts as an endogenous switch that regulates pre- and post-meal hepatic insulin clearance. Therefore the co-released zinc causes a reduction in hepatic insulin degradation in order to optimize the peripheral insulin levels (Tamaki *et al.*, 2013). In the *SLC30A8* mutation there might be persistent insulin secretion from the β- cells following deregulation of insulin clearance by the liver and hence increased risk of T2DM (Tamaki *et al.*, 2013).

### 1.3.7.6 Effect on insulin sensitivity

#### 1.3.7.6.1 Peroxisome proliferator-activated receptor gamma (*PPARγ*)

Peroxisome proliferator-activated receptors (PPARs) proteins form part of the ligand-activated transcription factors of nuclear hormone receptor superfamily (Michalik and Wahli, 2006). The major role of the PPAR family involves the homeostasis of energy and metabolic function. In particular, the PPARs mediate the gene expressions involving adipogenesis, inflammation, lipid metabolism and maintenance of metabolic haemostasis. There are three subclasses of PPARs namely: α; β/δ and γ, all exhibiting different functions in energy metabolism regulation (Wang *et al.*, 2014).

The *PPAR-γ* protein is found on chromosome 3 (3p25) with 9 exons and two isoforms that utilize distinct promoters and 5'exons (Sanghera *et al.*, 2008; Abbas *et al.*, 2013). The isoform *PPARγ-1* is found abundantly in adipose tissue, large intestine and hematopoietic cells as well as in lesser quantities within the kidney, liver, pancreas, muscles and small intestine. The isoform *PPARγ-2* has an additional 30 amino acid residue stretch at the *N*-terminal end leading to higher transcriptional activity than *PPARγ-1. PPARγ-2* is only found within white and brown adipose tissue (Auboeuf *et al.*, 1997; Vidal-Puig *et al.*, 1997; Medina-Gomez *et al.*, 2007).

The ligands that attach to *PPAR-γ* include endogenous fatty acids, prostanoids and synthetic thiazolidinedione agonists (Lehmann *et al.*, 1995; Cho and Momose, 2008). On binding of the ligands, the *PPAR-γ* forms a heterodimer with retinoid X receptor (RXR) that in combination will bind to peroxisome proliferator response elements (PPREs) within the

promoter region of the respective target genes as seen in Figure 1.28 (Gearing *et al.*, 1993; Lemberger, Desvergne and Wahli, 1996; Yu and Reddy, 2007).

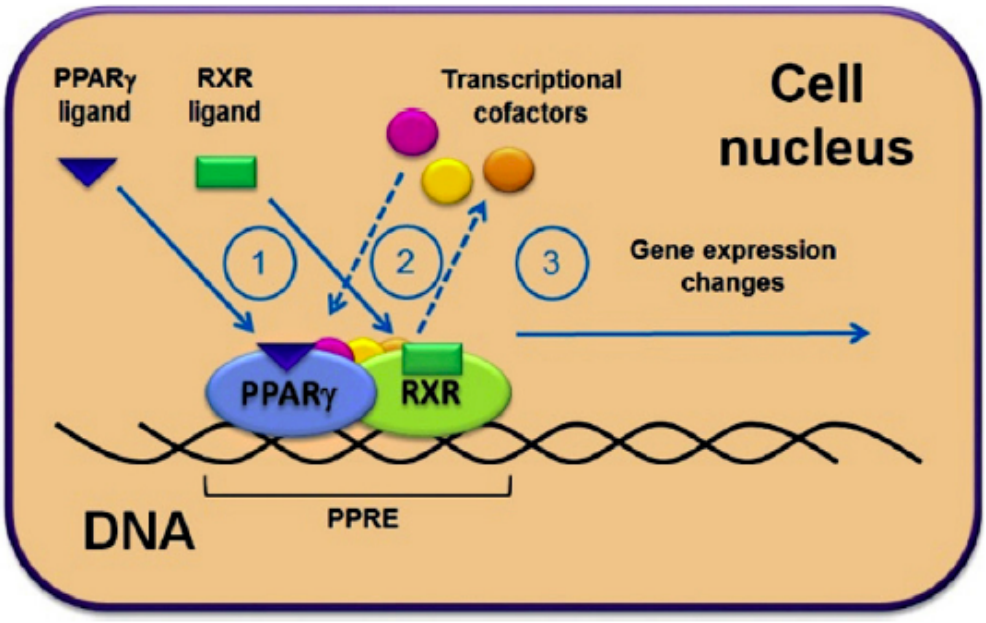



Figure 1.28 *PPAR-γ* transcription activation (Wang *et al.*, 2014)

*PPAR-γ* regulates adipocyte differentiation (pre-adipocytes to adipocytes), activates genes involved in adipocyte lipid storage as well as plays a role in: lipid metabolism; in glucose homeostasis; inflammation in immune cells and in cell proliferation (Na and Surh, 2003; Tontonoz and Spiegelman, 2008; Christodoulides and Vidal-Puig, 2010). Furthermore, *PPAR-γ* is responsible for the expression control that effects adipose tissue secretion factors which in turn influence insulin sensitivity positively (e.g. adiponectin and leptin) and negatively (e.g. resistin and tumour necrosis factor-α) (Heikkinen, Auwerx and Argmann, 2007). The levels of free fatty acids (FFAs) within the circulation correlate with insulin sensitivity (Boden *et al.*, 1994; Roden *et al.*, 1996). Therefore, reducing the levels of circulating FFAs would reverse insulin resistance. *PPAR-γ* ligands regulate the gene

expression and promote the influx of FFAs into the adipose tissue through the increased expression of cell-surface molecules including fatty acid transport protein (FATP) and CD36, as seen in Figure 1.28 (Frohnert, Hui and Bernlohr, 1999; Teboul *et al.*, 2001). Additionally *PPAR-γ* ligands increase the adipocyte numbers as well as indirectly enhance triglyceride synthesis, as seen in Figure 1.29 (Tontonoz *et al.*, 1995; Tordjman *et al.*, 2003).

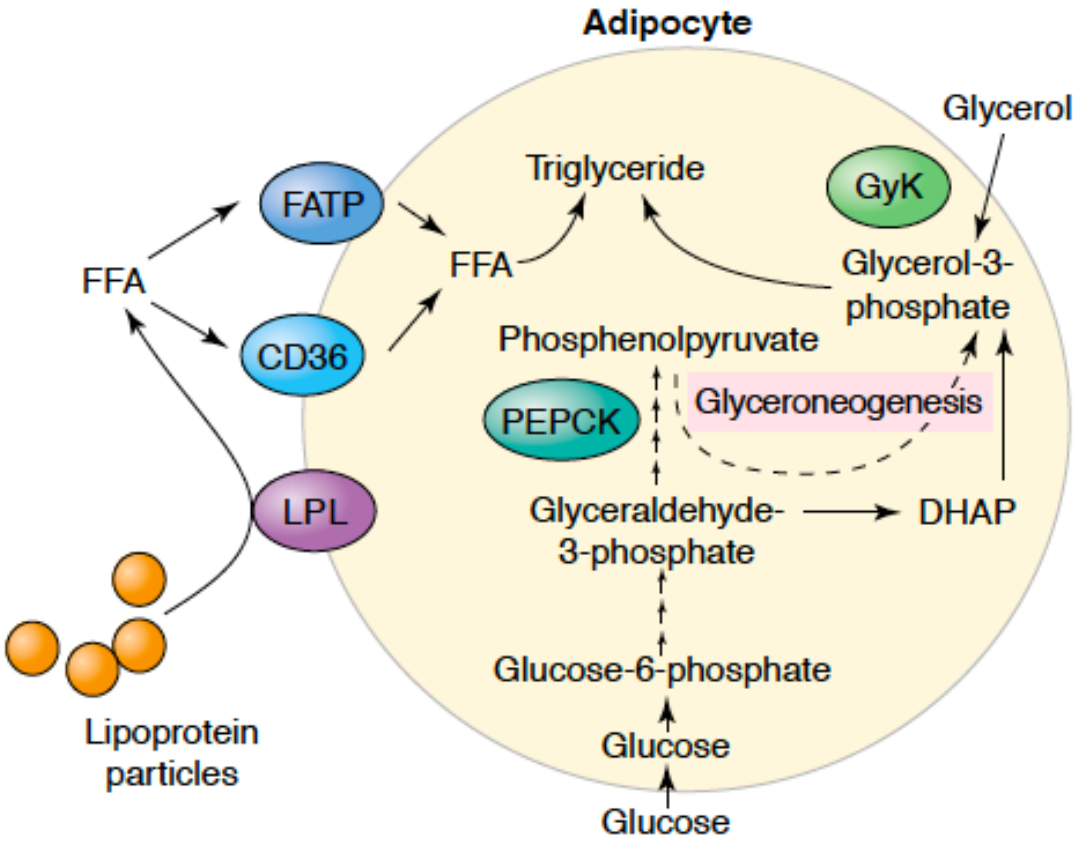



Figure 1.29 The effect of *PPAR-γ* ligands on fatty acid metabolism within adipocytes (Rangwala and Lazar, 2004)

*PPAR-γ* is also responsible for the modulation of genes involved in glucose homeostasis, such as the up regulation of glucose transporter type 4 (Glut4) and c-CBL-associated

protein (CAP) expression (Picard and Auwerx, 2002; Heikkinen, Auwerx and Argmann, 2007).

Obesity leads to the deregulation of *PPAR-γ* along with an increase in adipocytes. Consequently, there is an increase in resistin and TNF-α along with a decrease in adiponectin. The increased FFAs within the circulation contribute to insulin resistance through its accumulation within the liver and muscles, as seen in Figure 1.30.

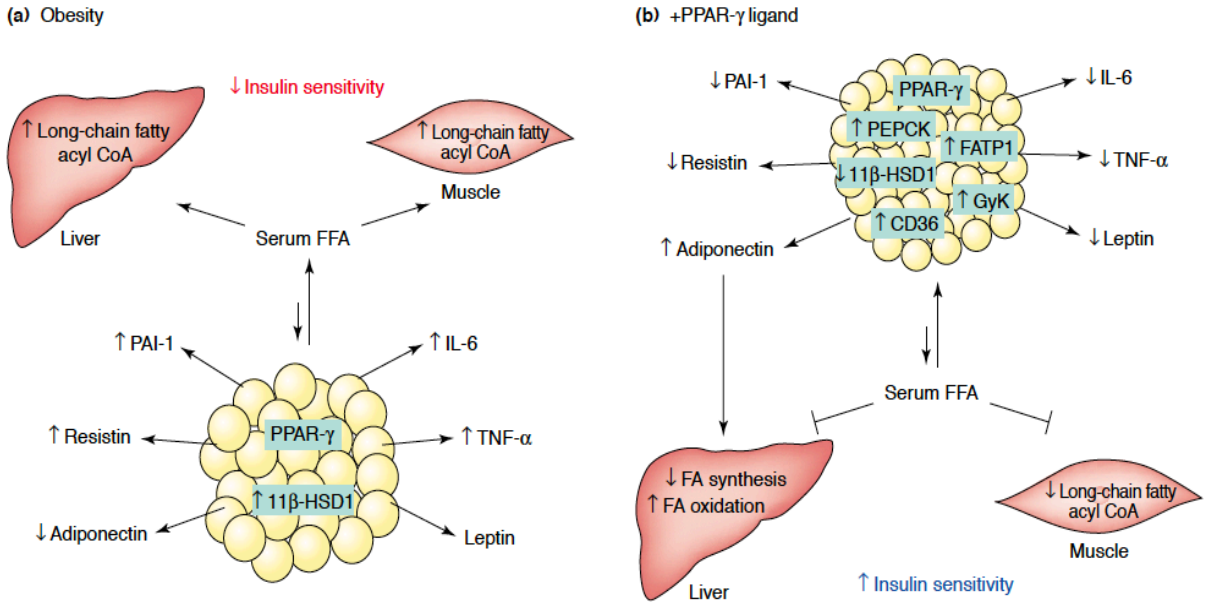



Figure 1.30 Mechanism of adipocyte regulation by PPAR-γ (Rangwala and Lazar, 2004)

The commonest polymorphism in the isomer *PPAR γ -2* gene (rs1801282) consists of a substitution of a proline at position 12 to an alanine (Pro12Ala), i.e. from a C allele to a G allele (Yen *et al.*, 1997; Yates *et al.*, 2015). This is the commonest missense polymorphism in Caucasians within the *PPAR-γ2* gene, with its presence detected in up to 25% of cases (Stumvoll and Häring, 2002; Yates *et al.*, 2015).

The alanine variant in normoglycaemic individuals exhibits an increase in insulin sensitivity when compared to those without the allele (Deeb *et al.*, 1998; Hara *et al.*, 2000). This Ala isoform may contribute to less effective *PPAR-γ* target gene stimulation leading to a lower adipose tissue accumulation, which in turn may improve insulin sensitivity (Deeb *et al.*, 1998). Furthermore, those with the Ala/Ala genotype had significantly lower BMI, lower insulin levels, higher HDL-C and lower triglyceride levels when compared to Pro/Pro and Pro/Ala genotype (Deeb *et al.*, 1998). Hence, the Ala variant in *PPAR-γ* is associated with a reduced T2DM risk (Mori *et al.*, 2001). However, in T2DM individuals carrying the Ala variant exhibited higher HbA1C, lower insulin secretion and higher serum total cholesterol levels. Consequently T2DM individuals with the Ala variant are more likely to experience a more severe form of the disease (Mori *et al.*, 2001).

#### 1.3.7.7 Other functions

##### 1.3.7.7.1 Fat mass and obesity association (*FTO*)

The *FTO* gene is located on chromosome 16 (16q12.2), encoding a 502-amino acid protein with a molecular mass of 58kD (Dina *et al.*, 2007). It is mostly expressed in the brain (particularly in the hypothalamic nuclei region which is responsible for energy balance), pancreatic islets and liver (Staiger *et al.*, 2009).

A strong association between the *FTO* variant and risk of T2DM was reported although this association was mediated through body mass index (BMI) (Frayling *et al.*, 2007; Scuteri *et al.*, 2007). In fact, the *FTO* polymorphism predisposes to an increased weight gain (high BMI) and subsequently increases the risk of developing diabetes (Frayling *et al.*,

2007). Around 16% of adults who are homozygous for this risk allele (A/A) weigh about 3 kilos more with a 1.67 fold increased in odds of obesity when compared to non-carriers of the risk allele (T/T) (Frayling *et al.*, 2007). Obesity appears to be mediated through energy intake, eating behaviour and appetite regulation. In fact the A-allele of the *FTO* variant has been associated with increased energy intake especially fatty foods with aberrant food responsiveness and satiety (Wardle *et al.*, 2008, 2009; Tanofsky-Kraff *et al.*, 2009). A Danish study reported that carrying an *FTO* risk allele does not imply that the individual will definitely develop obesity especially if physically active. In fact, homozygous individuals for the *FTO* risk allele were found to be two BMI units heavier if they were physically inactive when compare to physical active homozygous individuals for the same allele variant. The latter had the same BMI as non-carriers for the *FTO* risk allele (Andreasen *et al.*, 2008).

The A-allele rs9939609 variant has been associated with higher weight, waist circumference and subcutaneous mass with no height difference. Those children with this variant are associated with BMI changes and the presence of obesity by the age of 7 years. These changes persist into the pre-pubertal period and beyond puberty (Frayling *et al.*, 2007). Therefore it comes to no surprise that the variant is associated with increased cardiovascular risk as well as inflammatory markers including hsCRP (Lappalainen *et al.*, 2011). This phenomenon has been speculated to be due to the *FTO* expression within the hypothalamus, where it may have an effect on the sympathetic vasomotor tone modulation regulation leading to the development of hypertension (Pausova *et al.*, 2009).

This variant has been found to sustain the highest genotyping success rate (100%) with a risk allele frequency of 39% within the adult population exhibiting the A allele (Frayling *et al.*, 2007).

#### 1.3.7.7.2 *NOTCH2*

*NOTCH* signalling pathways have various roles including cell-fate determination, stem cell maintenance, immune system activation and angiogenesis (Guruharsha, Kankel and Artavanis-Tsakonas, 2012; Bray, 2016). Four *NOTCH* receptors (*NOTCH1* – *NOTCH4*) and at least five *NOTCH* ligands are present in humans. The *NOTCH* proteins are single-pass type I membrane proteins consisting of an intracellular domain and an extracellular domain (Isidor *et al.*, 2011). When *NOTCH* ligands activate the *NOTCH* receptors, there is ligand-dependent activation of a series of cleavage events leading to the *NOTCH* intracellular domain entering the nucleus. This leads to the binding and activation of transcription factor Rbp-Jk as well as downstream expression of *Hairy enhancer of split* (Hes) and *Hes-related* (*Hey*) family, which are *NOTCH* target genes (Valenti *et al.*, 2013).

*NOTCH 2* is located on chromosome 1 (1p13-p11) and expressed in various tissues including the pancreas, liver and the heart with a risk allele frequency of 10% in Europeans (Larsson *et al.*, 1994; Staiger *et al.*, 2009). During pancreatic organogenesis, the *NOTCH2* receptor is expressed in embryonic ductal cells of branching pancreatic buds. This may act as a source of exocrine and endocrine stem cells (Lammert, Brown and Melton, 2000). The variant rs10923931 has been associated with a lower particle concentration of the IDL, VLDL and LDL lipid subclasses while exhibiting higher insulin sensitivity (Stančáková *et al.*, 2011). Individuals with this variant have been found to exhibit a lower pre-diabetes risk

(Zyriax *et al.*, 2013). This gene allele effects are not well known, and further research is merited.

#### 1.3.7.7.3 Fatty acid binding protein 2 (*FABP2*)

The *FABP2* gene found on chromosome 4 (4q28-q31) encodes the intestinal fatty acid binding protein (IFABP) and is a family member of the intracellular lipid binding proteins (Baier *et al.*, 1995; Hertzel and Bernlohr, 2000; Furuhashi and Hotamisligil, 2008; Qiu *et al.*, 2014). This is expressed only within the columnar absorptive small intestine enterocytes and is responsible for absorption and intracellular transport of dietary fatty acids (Abbas *et al.*, 2013). The IFABP contains a single ligand-binding site with a high affinity for both saturated and unsaturated long-chain fatty acids (Lowe *et al.*, 1987). The *FABP2* transports hydrophobic fatty acids from the plasma membrane through aqueous cytosol to the endoplasmic reticulum, where the fatty acids are esterified to form triglycerides (Figure 1.31). These triglycerides are transported into the circulation as chylomicrons (Weiss *et al.*, 2002).

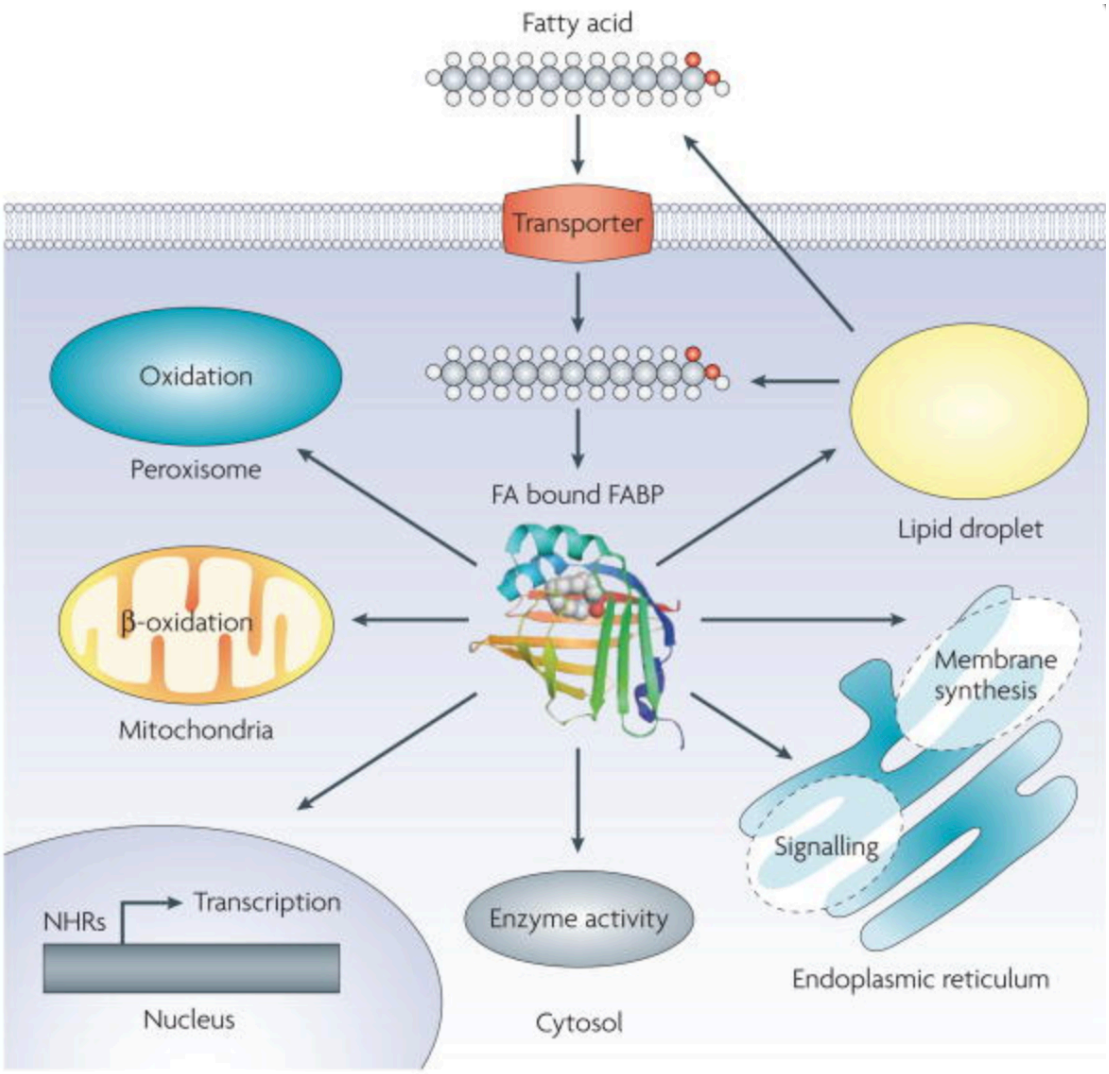



Figure 1.31 Summarises the role of *FABP2* within the enterocyte (Furuhashi and Hotamisligil, 2008)

A transition from G to A (rs1799883) within exon 2 of codon 54 results in a substitution of amino acids from Alanine (Ala) to Thymine (Thr) (Baier *et al.*, 1995). This results in an increased binding affinity of long chain fatty acids as well as enhanced triglyceride secretion (Baier, Bogardus and Sacchettini, 1996; Qiu *et al.*, 2014). In fact, homozygous Thr54-carriers exhibited increased postprandial concentrations of 14-18 carbon fatty acids as well as exhibited a two-fold higher affinity for long-chain fatty acids absorption when compared to Ala54 allele (Agren *et al.*, 2001). The increased fatty acid uptake and

conversation to triglycerides leads to an increased peripheral tissue concentration of triglycerides. This results in an increased fat oxidation rate within the peripheral tissues such as muscles while inhibiting glucose uptake leading to insulin resistance (Felley *et al.*, 1989; Kelley *et al.*, 1993; Baier *et al.*, 1995). Consequently the individuals with Thr54 allele were reported to have a hyperinsulinemic state two hours postprandial as well as after an oral glucose tolerance test (Baier *et al.*, 1995). Hence, the polymorphism (Ala54Thr) is associated with higher insulin resistance, dyslipidaemia and increased risk of type 2 diabetes mellitus (Liu *et al.*, 2015). The polymorphism has also been associated with obesity through elevated production of TNF-α (Albala *et al.*, 2004).

#### 1.3.7.7.4 Adrenoceptor-beta 2 (*ADRB2*)

Catecholamines play an important role in the regulation of energy expenditure as well as regulation of adipose tissue lipolysis, non-esterified fatty acid distribution, lipoprotein metabolism, glucose homeostasis, vascular tone and blood pressure regulation (Lafontan and Berlan, 1993). There are three adrenoceptors ($\beta_1$, $\beta_2$, $\beta_3$) with a role in stimulating human fat cells (Enocksson *et al.*, 1995; Barbe *et al.*, 1996).

Adrenoceptor-beta2 is a G-protein-coupled stimulatory receptor encoded by intronless gene on chromosome 5q31-q32 (Kobilka *et al.*, 1987). This receptor is involved in lipid mobilization and impairment of this receptor leads to obesity (Jalba, Rhoads and Demissie, 2008). In fact, genetic variability in the $\beta_2$-adrenergic receptor (*ADRB2*) may have an important role in energy expenditure; obesity and lipolytic function in adipose tissue (Large *et al.*, 1997). Two substitution mutations, one at codon 16 (arginine for glycine - Arg16Gly – rs1042713) and one at codon 27 (glutamic acid for glutamine - Gln27Glu – rs1042714)

have been reported. Both of which are located in the extracellular amino-terminal region of the *ADRB2* receptor and result in altering the cellular trafficking and desensitization of this receptor (Green *et al.*, 1994). The pathophysiological basis of these polymorphisms was attributed to blunted lipolysis and fat oxidation with development or maintenance of adipose stores. This contributed to an association with insulin resistance, obesity and hypertension (Blaak *et al.*, 1994; Ishiyama-Shigemoto *et al.*, 1999; Meirhaeghe *et al.*, 1999; Bengtsson *et al.*, 2001; Iwamoto *et al.*, 2001). In fact, diabetes individuals with the Arg16Gly allele were found to have a higher risk of developing hypertension, while Gln27Glu individuals were associated with hypertriglyceridemia independent of obesity (Bengtsson *et al.*, 2001; Iwamoto *et al.*, 2001).

#### 1.3.7.7.5 Potassium voltage-gated channel subfamily E regulatory subunit 4 (*KCNE4*)

The *KCNE* family consists of small non-conducting single transmembrane domain proteins that modulate certain voltage-gated potassium channels (Takumi, Ohkubo and Nakanishi, 1988). These function as accessory β-subunits (Grunnet *et al.*, 2002).

The *KCNE4* allele is found on chromosome 2 (2q36.1) and its variant is rs1440072. *KCNE4* is a voltage-gated potassium channel, part of the Isk-related family, representing the most complex class of functional and structural voltage-gated ion channels. Their diverse functions include regulating neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume (NCBI Resouces., 2018). *KCNE4* (C-allele) loci was associated with both waist circumference and BMI (Croteau-Chonka *et al.*, 2011). Furthermore *KCNE4* was reported to have an inhibitory effect on *KCNQ1* (potassium-voltage channel, subfamily Q, member

1), where *KCNQ1* has been associated with T2DM in both European and Asian populations (Grunnet *et al.*, 2002; Unoki *et al.*, 2008; Yasuda *et al.*, 2008).

# Aim

To determine the burden impact of type 2 diabetes mellitus and dysglycaemia at a population level while exploring the links between diabetes and recognized risk factors.

## Objectives

- To determine the prevalence rates of diabetes mellitus, impaired fasting plasma glucose, overweight, obesity, hypertension and metabolic syndrome within the adult population of Malta
- To determine the socio-demographic characteristics of: the whole survey population; the diabetes mellitus population; the impaired fasting glucose; population; the overweight-obese population and the metabolic syndrome population
- To determine the lifestyle habits (smoking, alcohol, physical activity) of the whole population, the diabetes mellitus population; the impaired fasting glucose population; the overweight-obese population and the metabolic syndrome population
- To determine the anthropometric and biochemical parameters characteristics of the whole population, the diabetes mellitus population; the impaired fasting glucose population; the overweight-obese population and the metabolic syndrome population
- To determine the relationships between anthropometric (blood pressure, body mass index and waist circumference) and biochemical (FPG and lipid profile) parameters to age and gender

- To determine the relationships between anthropometric and biochemical parameters to socio-demographic characteristics (district, education and employment status)
- To determine the relationships between anthropometric and biochemical parameters to lifestyle habits (smoking, alcohol, physical activity)
- To determine the relationships between anthropometric and biochemical parameters to the diabetes mellitus population; the impaired fasting glucose; population and the overweight-obese population
- To determine which anthropometric and biochemical parameter/s is/are determinant/s for the diabetes mellitus population; the impaired fasting glucose population; and the overweight-obese population
- To formulate a non-invasive predictive diabetes risk model for easy utilisation in the Maltese Primary Health Care
- To establish the Malta-specific epidemiological risk factors contributing to type 2 diabetes
- To establish the economic burden of type 2 diabetes and of obesity in Malta for the year the survey was conducted
- To estimate the projected total population prevalence of type 2 diabetes and obesity in Malta for the year 2050
- To estimate the economic burden of diabetes and obesity for the year 2050
- To acquire basic laboratory techniques in molecular genetics
- To explore genotype-phenotype associations in a sub-set of the study cohort that was selected for genetic analysis
- To explore the effect of the selected ten single nucleotide polymorphisms from candidate genes on risk of type 2 diabetes mellitus

# Chapter two – Material and Methods

## 2.1 Protocol

The research title, aims and protocol were formulated with the help of various local experts in different fields including epidemiology, medical statistics, endocrinology, pathology and genetics, taking into consideration the available local and international literature. It was a long consultative process between all parties involved in order to finalise the study structure and protocols.

## 2.2 Definition of the population

A population is a well-defined collection of individuals known to have similar characteristics.

### 2.2.1 Target population

The target population included all adults residing in Malta for at least 6 months aged between 18 and 70 years. This included both genders and those living anywhere within the islands of Malta and Gozo (which comprise the inhabited islands of Malta) irrespective of locality, education and occupation.

### 2.2.2 Study population

The study population consisted of all individuals listed on the Identification cards and Passports national register of Malta between 18 to 70 years of age living anywhere in Malta or Gozo (> 6 months).

### 2.2.3 Inclusion and Exclusion criteria

The survey was targeted at the adult population in Malta. For this reason, 18 years was taken as the lower age limit (onset of legal adulthood in Malta). Even though it has been established that type 2 diabetes is being diagnosed at an early age in various countries, the prevalence rate would still be relatively low in early adulthood when compared to the older population (Alberti *et al.*, 2004). The upper age limit was taken to be 70 years due to the fact that older adults may either exhibit long-standing diabetes or have incident disease (Kirkman *et al.*, 2012). Approximately 20% of those older than 65 years are known to suffer from diabetes and studying even older age groups might skew the true prevalence of diabetes (Mooradian, AD; McLaughlin, S; Boyer, CC; Winter, 1999). Diabetes studies recruiting participants aged 70+ years old found that diabetes diagnosis and control showed no significant increase in cardiovascular benefits. Alarmingly, some studies showed a higher mortality rate in those undergoing intensive glucose control (Kirkman *et al.*, 2012). However, excluding individuals beyond 70 years would lead to underestimation of the total burden of diabetes, obesity and their comorbidities. The economic burden would also be affected. Alas, since this was an epidemiological study with the primary objective to establish evidence-based data for prevention purpose and on pre-diabetes, it was considered as appropriate to set the upper cut-off point at 70 years.

All residents in Malta for over 6 months with a Maltese Identification Card including immigrants, tourists, illiterate and those living within residential homes and institutions were included, irrespective of their country of origin. However, people living abroad temporarily or permanently, pregnant women, those who were too sick to attend, were excluded.

The study was divided into stages. The first stage consisted of the data collection phase through a health examination and interview survey, while the second and third stages were the study of a sub-groups of the total population. The second phase only included subjects who had a fasting plasma glucose level between 5.60 – 6.99mmol/L in the first stage of the study. The third stage of the study consisted of DNA extraction and genotyping for ten SNPs on a sub-group of the examined population depending on their measured glycaemic status, as discussed below.

### 2.2.4 Sample population

A weighted, randomized, stratified sample was selected from the National Identification and Passports Registry in Malta in order to be representative of the Maltese resident population. The weighting criteria for sample selection depended on age (between 18 to 70 years), gender and locality. Considering that Malta is a small country, a single-stage sampling method was utilized since this type of sampling is able to cover the entire population without major logistic difficulties.

In view of the expected rate of pre-diabetes as per the American Diabetes Association (ADA), the rate of pre-diabetes in Malta was estimated at 25% of the population (Centers for Disease Control, 2014). The U.S estimate was used to estimate the required Maltese

sample population in view of the close proximity of the reported U.S diabetes burden to the reported Maltese diabetes burden (Directorate for Health Information and Research., 2012; Centers for Disease Control, 2014). The pre-diabetes rate in Malta could possibly be higher than that observed in the US given indications by previous monitoring of the prevalence of diabetes (International Diabetes Federation, 2015). Since one primary aim of the study was to identify the subpopulation of pre-diabetes, it was assumed that the size of the population sample would have to be around four times the number of pre-diabetes required, based on the US estimate. Should the pre-diabetes prevalence rate in Malta be higher than 25%, it would be to the study's advantage. Based on local health examination studies, a response rate of around 50% was predicted (Directorate for Health Information and Research., 2012). Therefore, ultimately the size of the population sample drawn from the population register had to be eight times the required sample size of pre-diabetes. Since the study was designed to explore associations with a large number of factors, for the purpose of sample size estimation, a positive reply for any of the factors under study of around 50% was assumed. Using the PiFace software® with a maximum allowed confidence interval of +/- 5%, a sample of 384 participants suffering from pre-diabetes was suggested (Kim and Seo, 2013; Centers for Disease Control, 2014). Therefore, a minimum sample of 3,072 had to be extracted from the population. In order to allow for any decrease in response rate since the Malta 2010 European Health Examination Survey, and for any individuals who might have passed away or emigrated during the fieldwork period, a sample of 4,000 was chosen (Directorate for Health Information and Research., 2012).

The choice of a nationally representative sample drawn from the population register would allow the study to draw conclusions at a population level, and also allow the study of specific subgroups like the diabetes and pre-diabetes subgroups.

## 2.3 Definition of a case

### 2.3.1 Definition of fasting plasma glucose (FPG) test

Individuals fasting for at least 9 hours prior to having their plasma glucose measured were considered as undergoing a fasting plasma glucose (FPG) test. The ADA (American Diabetes Association, 2018b) recommends that individuals should fast at least 8 hours prior to an FPG test. In the study, 9 hours fasting was specified. The rationale was that a lipid profile was simultaneously performed and this requires a minimum of 9 hours fasting (Sundvall *et al.*, 2008).

### 2.3.2 Definition of impaired fasting plasma glucose (IFG)

The World Health Organization (WHO) defines impaired fasting plasma glucose (IFG) as an FPG concentration of ≥ 6.1mmol/l but < 7mmol/l, without impaired glucose tolerance (IGT) (World Health Organization, 2006). The ADA defines IFG as FPG levels between 5.60mmol/l and 6.99mmol/l without the presence of IGT (American Diabetes Association, 2018b).

In the study, IFG was defined as FPG levels between 5.60mmol/l to 6.99mmol/l without the presence of IGT. Those participants falling within the IFG criteria were eligible to undergo an oral glucose tolerance test (OGTT) and thus enter stage 2 of the study.

### 2.3.3 Definition of impaired glucose tolerance (IGT)

The WHO and ADA define IGT as an FPG concentration of < 7mmol/l and a plasma glucose concentration of ≥7.8mmol/l but <11.1mmol/l after a 2-hour 75g oral glucose tolerance test (OGTT) (Table 2.1) (World Health Organization, 2006; American Diabetes Association, 2018b).

| 2nd hour OGTT value | Glucose tolerance |
|---|---|
| < 7.8mmol/L | Normal |
| ≥ 7.8 – < 11.1mmol/L | Impaired glucose tolerance (IGT) |
| ≥ 11.1mmol/L | Diabetes |

Table 2.1 WHO interpretations of an OGTT 2nd hour result (World Health Organization, 2006).

### 2.3.4 Definition of pre-diabetes

Pre-diabetes is a metabolic state where the body is not processing plasma glucose efficiently but full-blown diabetes has yet not set in (Valensi *et al.*, 2005).

In the study, a pre- diabetes state (at a population level) was considered as being present in those with IFG (FPG 5.60 – 6.99mmol/L).

### 2.3.5 Definition of diabetes

Participants reporting a previous diagnosis of diabetes mellitus or else on diabetes treatment were labelled as *previously known diabetes*. In the study, subjects with an FPG measurement ≥ 7mmol/L were also considered as having type 2 diabetes mellitus (labelled as *newly diagnosed diabetes*) (World Health Organization., 2013). This definition is widely used in health examination surveys due to logistics and feasibility (NCD Risk Factor

Collaboration (NCD-RisC), 2016). Subjects undergoing OGTT and obtaining a 2-hour glucose level of 11.1mmol/L or above were additionally also diagnosed as *newly diagnosed diabetes* (World Health Organization, 2006; American Diabetes Association, 2018b).

### 2.3.6 Definition of overweight and obese

The most widely used body composition tool is the body mass index (BMI) although this was found to have some limitations. The body mass index (BMI) was calculated by measuring the weight in kilograms and dividing this by the height in meters squared ($Kg/m^2$). A BMI of < $24.99Kg/m^2$ was labelled as normal; 25 – $29.99Kg/m^2$ as overweight and ≥ $30Kg/m^2$ as obese (World Health Organization, 2000).

Other tools for body composition measurements are the waist circumference and the waist-hip ratio (WHR - waist circumference divided by the hip circumference). The cut-off values for these vary by gender and ethnicity. The European male lower limit cut off point for waist circumference is 94cm and for the WHR is 0.95, while the European female lower limit cut off point for waist circumference is 80cm and that for WHR is 0.80 (Okosun *et al.*, 1998; World Health Organization, 2008b). Furthermore, the waist-index was measured by dividing the waist circumference by 94cm for males and 80cm for females. Following a local study finding, the cut-off point for an elevated waist-index was taken as 1.115 for both females and males (Magri, Fava and Galea, 2016).

### 2.3.7 Definition of hyperlipidaemia

The lipid profile consists of a number of different lipid tests. These are commonly the low-density lipoprotein (LDL-C), the high-density lipoprotein (HDL-C), the serum triglycerides, the total cholesterol and the non-HDL.

An elevated LDL-C level was considered as ≥ 3mmol/l, while the total cholesterol lower limit cut off point was of ≥ 5mmol/L (Hockley and Gemmill, 2006). In the case of diabetes participants, the LDL-C cut off point was ≥ 2.59mmol/L (Solano and Goldberg, 2006). The triglyceride level was considered as elevated when it was ≥ 1.69mmol/L and the non-HDL level was elevated at ≥ 3.36mmol/L (Solano and Goldberg, 2006). The HDL-C levels were considered as abnormally low at ≤ 1.03mmol/L for males and ≤ 1.29mmol/L for females (Solano and Goldberg, 2006).

### 2.3.8 Definition of hypertension

Hypertension was considered as present in those who gave a previous history of hypertension or who were already on anti-hypertensive medications. Those participants that were on certain drugs such as angiotensin-converting-enzyme inhibitor (ACEi) or on angiotensin II receptor blockers (ARBs) or on diuretics but did not claim to be aware of suffering from hypertension, they were labelled as having cardiovascular disease rather than hypertension.

Newly diagnosed hypertension (for non-diabetes) was diagnosed when the systolic blood pressure was ≥ 140mmHg or the diastolic blood pressure was ≥ 90mmHg (Whelton *et al.*, 2017; American Diabetes Association, 2018b).

The blood pressure cut-off points for diabetes mellitus population are more stringent. A lower cut off point has been shown to be beneficial. The systolic blood pressure cut-off point taken was 130mmHg while the diastolic blood pressure cut-off point taken was of 80mmHg for the diabetes mellitus population under study (Whelton *et al.*, 2017; American Diabetes Association, 2018b).

### 2.3.9 Definition of the Metabolic Syndrome (MetS)

The definition used for the metabolic syndrome (MetS) followed that of the International Diabetes Federation Consensus (Alberti, Zimmet and Shaw, 2006). Metabolic syndrome was defined as present if participants exhibited an increased waist circumference (male ≥ 94cm, female ≥ 80cm) and the presence of any two of the following features:

- Triglycerides ≥ 1.7mmol/L

  *or*

- HDL-C < 1.03mmol/L in male, < 1.29mmol/L in female;

  *or*

- High blood pressure (Systolic blood pressure ≥ 130mmHg or Diastolic blood pressure ≥ 85mmHg or on anti-hypertensive treatment);

  *or*

- FPG ≥ 5.6mmol/L *or* previously T2DM *or* on diabetes treatment.

### 2.3.10 Definition of the Metabolically Healthy Obese (MHO)

Metabolically Healthy Obese (MHO) individuals were defined as those who were obese (≥ 30Kg/$m^2$ BMI) but had a normal metabolic profile (Mathew, Farr and Mantzoros, 2016).

### 2.3.11 Definition of the Metabolically Obese Normal Weight (MONW)

Metabolically obese normal weight (MONW) individuals had a normal BMI (< 25Kg/$m^2$) but demonstrated metabolic abnormalities, which are typical of obese individuals (Ruderman, Schneider and Berchtold, 1981; Mathew, Farr and Mantzoros, 2016).

### 2.3.12 Definition of gender

Gender was defined as the person's concept of self as being male and masculine or else as being female and feminine, based on physical characteristics (U.S. National Library of Medicine, 2016).

### 2.3.13 Definition of districts

The random population sample for this study was scattered over 68 different localities across Malta and Gozo. Following the Eurostat system of Local Administrative Units (LAUs) these localities were grouped into six districts, as shown in Table 2.2 (National Statistics Office., 2017). These six districts were used in all association and statistical analyses performed throughout this study.

| District | Localities |
|---|---|
| Southern Harbour | Cospicua; Fgura; Floriana; Ħal Luqa; Ħaż-Żabbar; Kalkara; Marsa; Paola; Santa Luċija; Senglea; Ħal Tarxien; Valletta; Vittoriosa; Xgħajra. |
| Northern Harbour | Birkirkara; Gżira; Ħal Qormi; Ħamrun; Msida; Pembroke; San Ġwann; Santa Venera; St Julian's; Swieqi; Ta' Xbiex; Tal-Pietà; Tas-Sliema. |
| South Eastern | Birżebbuġa; Gudja; Ħal Għaxaq; Ħal Kirkop; Ħal Safi; Marsaskala; Marsaxlokk; Mqabba; Qrendi; Żejtun; Żurrieq. |
| Western | Ħad-Dingli; Ħal Balzan; Ħal Lija; Ħ'Attard; Ħaż-Żebbuġ; Iklin; Mdina; Mtarfa; Rabat; Siġġiewi. |
| Northern | Ħal Għargħur; Mellieħa; Mġarr; Mosta; Naxxar; St Paul's Bay. |
| Gozo | Fontana; Għajnsielem; Għarb; Għasri; Munxar; Nadur; Qala; San Lawrenz; Ta' Kerċem; Ta' Sannat; Victoria; Xagħra; Xewkija; Żebbuġ. |

Table 2.2 Demonstrate the grouped localities within Malta and Gozo in six districts (National Statistics Office., 2017)

## 2.4 Permissions

The Research Ethics Committee of the Faculty of Medicine and Surgery at the University of Malta and the University Research Ethics Committee (UREC) together with the Information and Data Protection Commissioner gave their permission for this study (Appendix A).

The Ministry for Energy & Health, through the Parliamentary Secretary and the Department of Primary Health Care, gave their logistical support. They allowed the use of government locality premises for data collection and the use of the state's hospital biochemistry laboratory against payment at overtime rates. The Chairman of the Pathology and the Head of the Clinical Biochemical laboratory granted permission to use the laboratory for routine blood tests.

Permission was also granted to use the Laboratory of Molecular Genetics of the University of Malta (Appendix A). The Mater Dei Hospital Chief Executive Officer (CEO) and the hospital data protection office granted permission to use the hospital's blood results software (iSoft) (Appendix A).

## 2.5 Recruitment of fieldworkers

Interviewers and phlebotomists (responsible for bloodletting and health examination measurements) were recruited following adverts in local newspapers. The ability to communicate in both the Maltese and English languages was considered as essential. Training sessions using all tools of measurements were organized quarterly in order to have uniform execution and validity of the information, thereby reducing information error throughout the whole data collection period.

## 2.6 Tools of measurements

Various tools of measurement were used throughout the period of study. Consent forms and invitation letters were formulated (with back translation from English to Maltese back to English) both in Maltese and in English (Appendix B).

### 2.6.1 Questionnaire

A questionnaire covering demographic data, physical activity, smoking and alcohol habits, typical weekly dietary intake, depressive disorder symptoms, as well as medical, drug and family histories was developed using validated sources (Appendix B). Table 2.3 illustrates all the different sections incorporated in the questionnaire.

| |
|---|
| **Demographic data** including Gender, Age, Locality, Education Level and Occupation (Eurostat, 2015) |
| **Physical activity** including leisure & work (Meriwether *et al.*, 2006) |
| **Smoking** habits, history and exposure to passive smoking (Eurostat, 2015) |
| **Alcohol** intake and frequency (Bjerregaard and Becker, 2013) |
| **Typical weekly diet**, including refined sugar items, red meat, whole grain (Toft *et al.*, 2007) |
| **Medical history**, including already diagnosed with diabetes mellitus type 1 or 2, history of pre-diabetes or gestational diabetes (in females), the presence of cardiovascular disease, cerebrovascular disease, thyroid disorders, polycystic ovarian syndrome (PCOS in female), non-alcoholic fatty liver disease (NAFLD), depression, obesity, eye/kidney/peripheral vascular disease/s (Tolonen, 2013) |
| **Family history** including having 1st degree relative with diabetes mellitus type 1 and type 2, maternal gestational diabetes and parents with diabetes mellitus (Walter *et al.*, 2013) |
| **Drug history** including statins, anti-hypertensive, thyroid medication, diabetes medication (for diabetes or polycystic ovarian syndrome) and anti-depression medications (Tolonen, 2013) |
| Presence of **depression** symptoms (Kroenke, Spitzer and Williams, 2001) |

Table 2.3 Validated questionnaire sections and their validated source

### 2.6.2 Blood pressure

An aneroid sphygmomanometer was used since mercury sphygmomanometers have been removed from clinical practice (O'Brien *et al.*, 2003). The aneroid sphygmomanometer used was a "Welch Allyn® aneroid sphygmomanometer model 7670-04", which had been validated against a mercury sphygmomanometer and given a "pass" by the Association for the Advancement of Medical Instrumentation as well as a grade B for systolic pressure and grade A for diastolic blood pressure recordings by the British Hypertension Society (Saxena, Saxena and Gupta, no date; Ma *et al.*, 2009). Regular calibration every 6 months was done in accordance to the WHO regulations (World Health Organization, 2005). The detailed step-by-step blood pressure measurement protocol followed during the survey can be found in Appendix C.

### 2.6.3 Electronic physical body weight scale with height rod

A calibrated certified ADAM® MDW-250L physical digital scale with height rod was used to measure the body weight in kilograms and the height in centimetres. Calibration was done before each physical examination session. The detailed step-by-step weight and height measurement protocol that was followed during the survey can be found in Appendix C.

### 2.6. Measuring tape

A stretch-resistant tape that provided a constant 100g of tension through the use of a special indicator buckle was used as recommended by the WHO STEPwise Approach to Surveillance to measure the waist and hip circumference (World Health Organization., 2008).

## 2.7 Health examination survey

The health examination survey was entitled “SAHHTEK” study.

### 2.7.1 Pilot study

A randomized sample of 50 participants was selected for a pilot study that was conducted over the first weekend in November 2014 prior to the actual health examination survey fieldwork. This was performed in order to ‘test run’ the survey’s protocol as well as to identify the fieldworker’s strengths while ensuring accuracy in the actual fieldwork. A number of lessons were learnt from the pilot study that helped iron out some imperfections for smoother running later on during the actual fieldwork as listed in Table 2.4. The results

obtained during the pilot study were not incorporated as part of the actual health examination survey results.

| Pilot study lessons learnt |
|---|
| Holding health examinations in the participant's home town led to a good response rate |
| Flexible appointment dates and times led to a good response rate |
| Early appointments (07:00am to 07:30am) were appreciated by participants aged 50 years and over. Later appointments (08:30 to 09:00) were appreciated by the younger participants |
| Carrying out an interview, followed by health examination measurements and finishing with blood collection was accepted by participants |
| Measuring of waist and hip circumference just after measuring weight and height was found most appropriate and acceptable by participants |
| A constant explanation of the whole process prior to and during the survey made the experience more enjoyable for the participants |
| An individual explanation to each participant of the measured parameters at the end of the health examination was appreciated by participants |

Table 2.4 Summary of the lessons learnt from the pilot study

### 2.7.2 Health examination survey data collection

The health examination hub (where an interview, bloodletting and other measurements were carried out) was set up every weekend in state-run peripheral town health clinics (*Bereg*) found at each locality and/or state health centre clinics scattered throughout each district throughout the Maltese Islands. Each weekend a different town was visited between November 2014 and November 2015. The health examination was not performed between the months of June and August 2014. Summer time is associated with holiday periods and mass movement into summerhouses. This would have carried a negative impact on the response rate.

Participants were selected randomly from each Maltese town (*as described previously*). They were recruited by means of a postal invitation letter. This contained an explanation of the aim of the study, the benefits of attending, as well as a given appointment (date and time). This was organised two weeks prior to each visit. Participants were also given the options to change their appointment to a more suitable date and time if it was required or to refuse to attend. Each participant was asked to fast for at least 9 hours while abstaining from smoking or physical activity for at least one hour before the appointed time. A copy of the invitation letter is found in Appendix B.

Participants attending the health examination survey followed a standardised sequence in order to maintain the validity and reproducibility of the data. On arrival, written informed consent was obtained and each participant was given a unique code number. A trained interviewer conducted the interview and filled in the validated questionnaire. This was followed by a health examination including the measurement of blood pressure, height, weight, waist and hip circumference. Bloodletting for FPG and lipid profile samples along with a whole blood sample for DNA extraction followed. All blood bottles were labelled with the participant's unique code and then put on ice in a cooler bag. The detailed sequence followed during the health examination can be found in Appendix C.

Caution was taken to eliminate any pre-analytic variability during the blood sample collection, transportation, processing and storage of the samples. Preservative tubes were used to avoid haemolysis or glycolysis. The tubes were temporary stored on ice then transported to the Mater Dei Hospital's accredited laboratory within 2 hours of bloodletting. The last participants' appointment was set to 09:30am in order to ensure delivery of the blood samples within the 2-hour time frame. This was feasible due to the

short distances between each town and the laboratory, which is situated in a central location. The tubes for FPG and lipid profile sampling were handed over to the laboratory while the third venous blood sample, taken in an EDTA tube, was stored at -20$^{o}$C at the University of Malta allocated freezers for later use in genotyping analysis.

This process was repeated throughout the whole duration of the study. The same group of phlebotomists and trained interviewers took the blood samples and the other anthropometric measurements in order to maintain validity and reproductively. For blood pressure measurements, the actual blood pressure readings were used for the purposes of this study, avoiding rounding up to the nearest "0" or "5". The protocols used are found in Appendix C.

The fieldwork process and protocols were externally audited following a site visit by the European Health Examination Survey coordinators, Prof. Hanna Tolonen and Prof. Paivikki Koponen.

### 2.7.3 Blood tests

The FPG and the lipid profile testing were performed at the Mater Dei Hospital biochemistry laboratory. Automated and regularly internally and externally quality controlled COBAS INTEGRA® analysers were used to perform these tests.

The FPG sample was collected using a fluoride containing tube in order to minimize glycolysis. The FPG measurement result was obtained by following a hexokinase and glucose oxidase enzyme reactions.

A serum clot activator tube was used for the collection of the lipid profile test. Using an aliquot serum sample, various lipid measurements results were obtained as follows: (1) high-density lipoprotein (HDL-C) by following the clearance method; (2) triglyceride by following an enzymatic colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenozone reaction; (3) total cholesterol by a cholesterol oxidase enzymatic reaction while (4) the low-density lipoprotein (LDL) result was measured by following the Friedwald formula calculation. Of note, none of the participants achieved a triglyceride level beyond 4.5mmol/L. All blood results were reported in millimoles per litre (mmol/L).

### 2.7.4 Oral glucose tolerance test data collection

Those participants scoring FPG between 5.60 and 6.99mmol/L and not previously diagnosed with diabetes mellitus or on oral hypoglycaemic agents, fell under the category of 'undetermined glucose' status in accordance to the ADA classification. These warranted oral glucose tolerance testing (American Diabetes Association, 2018b). The principle investigator contacted those participants with fasting plasma glucose between 5.60 – 6.99mmol/L by a telephone call and offered the gold standard oral glucose tolerance test (OGTT) in order to establish their diabetes status. The process of the test was explained in detail. If the participant consented to have this test, an appointment was set up with the principle coordinator. Participants were contacted not more than two weeks prior to the OGTT sessions in order to prevent any dietary pattern alteration. The OGTT sessions were conducted within the Pathology Department in Mater Dei Hospital.

The protocol followed is found in Appendix C. In summary, a 75g of glucose was dissolved in 300ml of water and participants were asked to consume it within 5 minutes. The

laboratory processed all blood samples in an expedited manner. The results of the glucose blood measurements taken during the OGTT (0 hour and 2nd hour) were noted and inputted in a spreadsheet by the principle investigator. The OGTT results were interpreted according to the WHO (2006) and ADA (2016) criteria as illustrated below:

| OGTT value | Glucose tolerance |
|---|---|
| < 7.8mmol/L | Normal |
| ≥ 7.8 – < 11.1mmol/L | Impaired glucose tolerance (IGT) |
| ≥ 11.1mmol/L | Diabetes |

Table 2.5 Oral glucose tolerance test interpretation (World Health Organization, 2006; American Diabetes Association, 2018b)

This was performed in order to pick out any new persons with diabetes missed by utilizing the FPG (more than 7mmol/L) criteria alone (The DECODE Study Group., 1998; World Health Organization, 2006). The FPG cut-off point of 5.60mmol/l has been confirmed an ideal for prediction of future diabetes (Herman, 2007).

### 2.7.5 Data inputting

A single fieldworker inputted all the questionnaires and health examination measurements data in order to avoid bias. Data inputting was performed using secure form software replicating the questionnaire form used during the survey including inputting of the accompanying participant's measurements taken. The software used was programmed

to perform data validation to ensure data quality such as, but not limited to, setting upper and lower limits for the different parameters measured and age. The same software was used to input the blood testing results of each participant. The inputted data could be transferred into a spreadsheet program to allow for later data analysis. Data analysis was carried out using IBM SPSS (Statistical Package for the Social Science) version 21.

Cross-referencing the participants' hard-copy questionnaires to the online version was performed to ensure the accuracy of the data stored. At the end of each weekend, a random number of questionnaires were chosen and cross-referenced to the online version to assess the validity of the process.

## 2.8 Health examination survey data analysis

### 2.8.1 Test for normality

The Kolmogorov-Smirnov test for normality was performed to assess which data followed a normal distribution as seen in Table 2.6. Non-parametric statistical testing (Mann-Whitney U and Kruskal Wallis tests) was used in view of a non-normal data distribution.

**Tests of Normality**

| | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| FBG (mmol/L) | .241 | 1856 | .000 | .554 | 1856 | .000 |
| LDL (mmol/L) | .036 | 1859 | .000 | .990 | 1859 | .000 |
| HDL (mmol/L) | .065 | 1859 | .000 | .949 | 1859 | .000 |
| Triglycerides (mmol/L) | .143 | 1857 | .000 | .771 | 1857 | .000 |
| Total Cholesterol (mmol/L) | .035 | 1859 | .000 | .978 | 1859 | .000 |
| Age | .077 | 1861 | .000 | .964 | 1861 | .000 |
| BMI (Kg/m2) | .067 | 1861 | .000 | .939 | 1861 | .000 |
| Systolic Bp (mmHg) | .075 | 1861 | .000 | .831 | 1861 | .000 |
| Diastolic Bp (mmol/L) | .071 | 1861 | .000 | .959 | 1861 | .000 |
| Waist Circumference (cm) | .023 | 1861 | .023 | .987 | 1861 | .000 |
| WHR | .472 | 1861 | .000 | .020 | 1861 | .000 |

Table 2.6 Kolmogorov Smirnov test

### 2.8.2 Representativeness

In public health research it is impossible to obtain survey data that is totally representative of the whole population, even if the study population is a representative random sample (Karvanen *et al.*, 2019). Hence, in order to compensate for the non-respondents and maintain the national level representation, using the SPSS software, a weighting factor was applied to each individual in the sample. The method used considered the respondents and non-respondents for each town by age and gender. Weights were derived by multiplying each individual (by age and gender) by the ratio of the total sample size to the total population size within each town. Ultimately, each participant was representative of approximately 1% of the population of each Maltese town. The weighed data was used when national representative population analyses were performed. However,

it should be appreciated that such method does not fully compensate for the non-responders such as for health literacy among other factors.

### 2.8.3 Calculation of prevalence rates

Prevalence is a statistical concept that refers to the number of cases of a disease that are present in a particular population at a given time.

From the survey data, a number of different national representative prevalence rates (type 2 diabetes mellitus; overweight; obesity; pre-diabetes; hypertension; smoking; alcohol consumption; educational levels etc.) were worked out using the adjusted (weighted) population and following the algorithm below:

$$\frac{\text{Cases with the condition}}{\text{Total adjusted population}}$$

### 2.8.4 Descriptive analysis

Descriptive analysis using IBM SPSS vs. 21 was performed to establish counts and percentages for the different parameters under study including gender, age, body weight, blood pressure, smoking habits, alcohol consumption, physical activity, education level and family history of cardiovascular disease and diabetes mellitus for the general population. The same analysis was performed for each subgroup including the type 2 diabetes mellitus, IFG, overweight-obese and MetS subgroups.

Confidence intervals were calculated using 'GraphPad Software' (https://www.graphpad.com/quickcalcs/confInterval1/) . The calculator considered the values to be independently and randomly sampled from a population whose values follow a Gaussian distribution. The confidence interval is centred in equal distances from the standard error. However, since this study's population did not follow a normal distribution, this was not always the case.

The median with its corresponding interquartile range (IQR) of the fasting plasma glucose (FPG) and the lipid profile (LDL-C, HDL-C, Triglycerides, Total Cholesterol) were analysed for the general population, as well as for each subgroup (type 2 diabetes mellitus, IFG, overweight-obese and MetS).

The lipid ratio of triglyceride to HDL-C (TG/HDL-C) was calculated. A TG/HDL-C ratio < 2 was considered as optimal; 2 – 3.99 as at risk of insulin resistance and coronary heart disease while > 4 as at very high risk (Amin *et al.*, 2016). The population under study was categorized using these different cut-off points.

### 2.8.5 Statistical analysis

All data was analysed using IBM SPSS version 21. Prevalence data analyses utilised an adjusted sample population (weighted by age, gender and locality), in order to establish national representative analyses. While analytic analyses for links, relationships and modelling were performed on the crude unadjusted sample population. Statistical modelling was performed in order to establish relationships within this study. Generalized linear models (GLMs) are associated with normal, binomial, multinomial and gamma

distributions and hence found to be appropriate for this study. In fact GLMs are flexible generation of ordinary linear regression that allows for the response variables that have error distribution models other than a normal distribution (Nelder and Wedderburn, 1972).

**Statistical significance testing for continuous parameters:**

Non-parametric statistical testing using the Mann-Whitney U test and the Kruskal Wallis test were performed since the data did not follow a normal distribution. Dunn's test was used as a *post-hoc* test following Kruskal Wallis testing in order to specify which sample pairs are statistically significant.

**Statistical significance testing for categorical parameters:**

The Chi-squared test was used to identify significance between categorical variables.

**Statistical significance testing for correlations:**

The Spearman's correlation testing was performed to test for associations between variables.

**Testing for significant associations and modelling:**

Binary logistic regressions were performed to identify associations between biochemical and anthropometric parameters for a number of diseases/conditions including type 2 diabetes mellitus, impaired fasting plasma glucose, obesity and overweight populations.

Multiple logistic regressions through generalized linear models were performed to evaluate for the effect of potential confounding factor/s on the predictors' relationship to the dependent variable.

**Confounding factors:**

Confounding factors considered in this study included residing district, occupation and education levels along with lifestyle characteristics (smoking, alcohol habit, physical activity).

The Maltese towns were distributed into districts by following the classification of the national statistics office (National Statistics Office., 2017).

The highest education levels distribution followed the European Health Interview Survey - HIS categories (Eurostat, 2015). In fact, the education level was divided into no-formal education; primary education; unfinished secondary school; finished secondary school; tertiary (sixth form level); university and post-graduate education levels.

The occupation status were divided according to whether participants were employed, unemployed, students, retired or performed domestic tasks as their main job (Eurostat, 2015).

Lifestyle data included smoking, alcohol and physical activity. Those that currently smoked (irrelevant to the number of cigarettes consumed daily) were labelled as "smokers". Those that consumed any alcohol beverage during a week were labelled as "consumed alcohol". Physical activity was assessed according to whether a participant performed any physical

activity in a week (none, walk more than 10 minutes, moderate activity, vigorous activity) (Meriwether *et al.*, 2006).

#### 2.8.5.1 Anthropometric and biochemical analytic analysis

Relationships between the body mass index (BMI) and glucose impairment (impaired fasting plasma glucose and diabetes mellitus) status for the total study population (crude unadjusted data) were sought taking into consideration known confounders including age, gender, residing locality, occupation and education levels along with lifestyle characteristics (smoking, alcohol habit, physical activity). These relationships were also explored using different anthropometric and biochemical parameters.

Multi-variant regression analyses were preformed using generalized linear models to establish links between anthropometric or biochemical parameters and the development of diabetes mellitus status; impaired fasting plasma glucose and overweight-obesity status. At the same time establish possible confounders.

#### 2.8.5.2 Diabetes risk score

This study's diabetes risk score was formulated by analyzing various non-invasive measurements as well as a medical history, all of which are easily obtainable during a routine appointment at primary health care clinics. The scope to formulate a Maltese-specific diabetes risk score was to identify the Maltese specific diabetes characteristics and to aid the primary care physicians in their clinic consultations to identify potential diabetes at risk factor individuals.

General linear model (GLM) analyses through multiple binary regressions were performed to establish which consultation variables were significant independent predictors for type 2 diabetes mellitus.

The consultation variables considered were the following:

- Gender
- Age
- Systolic and diastolic blood pressure
- Waist circumference
- Body mass index (BMI)
- History of
    - smoking (in years)
    - coronary heart disease
    - myocardial infarction
    - hypertension
    - dyslipidaemia
    - depression
    - statin intake
    - physical activity

Once significant independent predictors for T2DM were identified, Receiver Operating Characteristic (ROC) curves were created for the continuous predictors. The ROC was used to further establish which of the predictors (identified by GLM analysis) were truly predictive for T2DM.

The significant continuous predictors obtained from the ROC curves were further analyzed to identify the optimal cut-off points for the Maltese population. The optimal cut-off points were identified through the predictor's sensitivity and specificity obtained from the ROC curves (Hajian-Tilaki, 2013). The optimal cut-off points were identified as the sensitivity and specificity closest to the left-hand (0,1) corner of the ROC plane.

The continuous variables were then transformed into categorical variables using the established cut-off points. ROC curves were formulated once again utilizing only the significant predictive contributors (in categorical format) to determine whether all the contributors found previously remained significant. After this, the significant predictors were incremental combined (in descending order according to area under the curve).

The significant predictors and their associated *area under the curve* (AUC) were used to identify the best predictor combination. The predictive diabetes risk score model with the highest AUC utilizing the least number of significant predictors (in order to maintain simplicity) was obtained.

The established best predictor combination was re-assessed while using the combined cohorts of persons without diabetes along with those with newly diagnosed diabetes. This was done in order to establish the sensitivity and the specificity of the risk score when the previously known diabetes cohort was excluded.

#### 2.8.5.2.1 Maltese-specific epidemiological risk factors

The identified Malta-specific diabetes characteristic and their corresponding cut-off points (age, waist circumference (male and female) and systolic blood pressure) were used to analyse the whole SAHHTEK population. The general population and the diabetes population were divided according to the specific cut-off points (age ≥ 55 years; waist circumference male ≥ 100cm; waist circumference female ≥ 90cm; systolic BP ≥ 125mmHg), which were established from the optimal sensitivity and specificity of the ROC. Prevalence rates of each predictive risk factor were calculated for both the general and the diabetes populations.

The diabetes risk score was also implemented to the SAHHTEK non-diabetes population. For every predictive factor/s combination/s, the total population at risk of diabetes was calculated according to gender.

#### 2.8.5.2.2 Comparison with FINDRISC score

The FINDRISC score is a well-established and validated predictive risk score for type 2 diabetes mellitus. This was modelled on a representative sample of the Finnish population through the use of easily obtainable variables during primary health care consultation. The variables considered by the FINDRISC score were age, BMI, waist circumference, history of antihypertensive drug treatment and high blood glucose, physical activity and daily consumption of fruits, berries or vegetables (Lindström and Tuomilehto, 2003). On summation of the different variables a total risk score is developed that estimates the risk prediction to develop type 2 diabetes within 10 years as follows: Low risk (<7);

Slightly elevated risk (7 – 11); Moderate risk (12 – 14); High risk (15 – 20) and Very high risk (>20) (Lindström and Tuomilehto, 2003).

Considering that this risk score has been based on easily obtainable variables, similar to the current study's score and has been validated over the course of 10 years follow-up, it was considered appropriate to compare the Maltese specific risk predictive equation to the FINDRISC equation.

The different variable categories reported by the FINDRISC were incorporated to the SAHHTEK study population as single entities as well as in combination. ROC curve was formulated using all the different significant categories as well as in combination to assess for its predictive ability within the SAHHTEK study population (excluding the previously known diabetes cohort). The ROC and the AUC achieved through the FINDRISC equation were compared to the SAHHTEK equation.

### 2.8.6 Economic burden estimation

The established prevalence rates of the *newly diagnosed persons with diabetes* and for the *previously known persons with diabetes* were considered individually to calculate the estimated cost burden for diabetes in Malta. The cost burden for Malta was calculated based on cost per case rates obtained from the scientific literature for each diabetes subgroup. The cost burden for the newly diagnosed diabetes population was based on estimates performed by Zhang Y. *et al* (2009) in the USA after adjusting for the gross domestic product (GDP) per capita and by adjusting for deflation between the USA and Malta (Zhang *et al.*, 2009). However, this does not fully adjust for differences between

countries such as cost of medicines and different pay structures. The cost of a newly diagnosed diabetes was calculated to be €1,052 per person per year. The cost burden for the *previously known diabetes population* (€1,887 per person annually) was obtained from published International Diabetes Federation (IDF) data on Malta (International Diabetes Federation, 2016). The obese population cost burden was based on the literature from the Department of Health Information and Research (Calleja, N; Gauci, 2009). A 2% compound interest per annum was performed on the cost burden obtained for obesity.

A bottom-up prevalence-based design was performed to estimate the projections for both the prevalence and cost burden of diabetes and obesity rates for the year 2050. The data from the 1981 (WHO) survey and the 2014 – 2015 (SAHHTEK) survey were considered for this design (Katona, G, Aganovic, I, Vuskan V, 1983). For purposes of comparative and statistical analysis, only the subgroup of adults between 25 and 64 years of age were utilised from SAHHTEK. It was also hypothesised that no other factors distorted linear projections for both diseases. The estimation of the affected population and their economic burden was based on the EUROSTAT 2050 population projections for Malta (Eurostat European Commission, 2016).

## 2.8.7 Genetic analysis

### 2.8.7.1 Cohort selection for genetic analysis

The genetic profiling of diabetes is still not totally understood with continuous research being conducted globally (Morris, 2014). Over recent years research has been carried out on diabetes mellitus and gestational diabetes within the Maltese Islands (Al-Ashtar, 2008; Pace, 2013; Craus, 2016). However, these local studies were not based on

nationally representative population samples of the Maltese population, but were based on hand-picked convivence population samples such as 'individuals with known diabetes with complications' and 'gestational diabetes mothers'. This is in opposite to the current study where the genetic cohort is arising from a stratified population sample. The current study set out to identify the diabetes profile within the Maltese adult population. The SAHHTEK population sample was divided into different sub-populations in order to perform case-control studies and compare genetic analyses.

The crude unadjusted study population (*n*=1,861) was sub-categorised into four groups. One sub-group consisted of a combination of *previously known* and *newly diagnosed diabetes* (*n*=224). The second group consisted of all pre-diabetes (*n*=136). The third group consisted of healthy individuals i.e. had normal FPG (<5.60mmol/L), normal lipid profile (LDL-C <3mmol/L; HDL-C ≥1.03mmol/L for males and ≥1.29mmol/L for females; Triglycerides <1.69mmol/L; Total cholesterol <5mmol/L), normal BMI (<25Kg/m$^2$), normal waist circumference (males <90cm and females <80cm), normal blood pressure measurement (systolic <130mmHg, diastolic <90mmHg) and did not self-report any daily medication or past medical history. These were considered as the "Metabolically Healthy cohort" (*n*=140). The remaining sub-population was normoglycaemic but had at least one metabolic abnormality and was considered as the "Metabolically abnormal cohort" (*n*=1,361). None of the *metabolically abnormal* cohort individual were on oral hypoglycaemic agents.

The four cohorts were age stratified by 5-year age groups. The distribution of the population across all cohorts by 5-year age group stratification was evaluated. The age groups between 33 and 62 years were considered for this study in view of the relatively homogeneous

distribution of the population counts across the different cohorts. Individuals below the age of 33 and above the age of 62 years were excluded.

In order to maximize the yield of genetic data from these patients, the whole diabetes mellitus cohort within the selected age group bracket, was considered for genetic analysis, together with all the pre-diabetes and healthy sub-category population. The 'metabolically abnormal' sub-category included an exceptionally larger number of participants than the rest of the sub-categories. In order to study a representative sample of this subgroup (which was comparable to the other subgroups) one in every ten participants was randomly selected for each age group for genetic analysis by using the SPSS software, as seen in Figure 2.1. Gender stratification was also applied in order to create a virtually equal gender distribution among the different age groups of the 'metabolically abnormal' subgroup. Of note, there was a significant gender mismatch especially in the 'Metabolically healthy' subgroup, as seen in Figure 2.2.

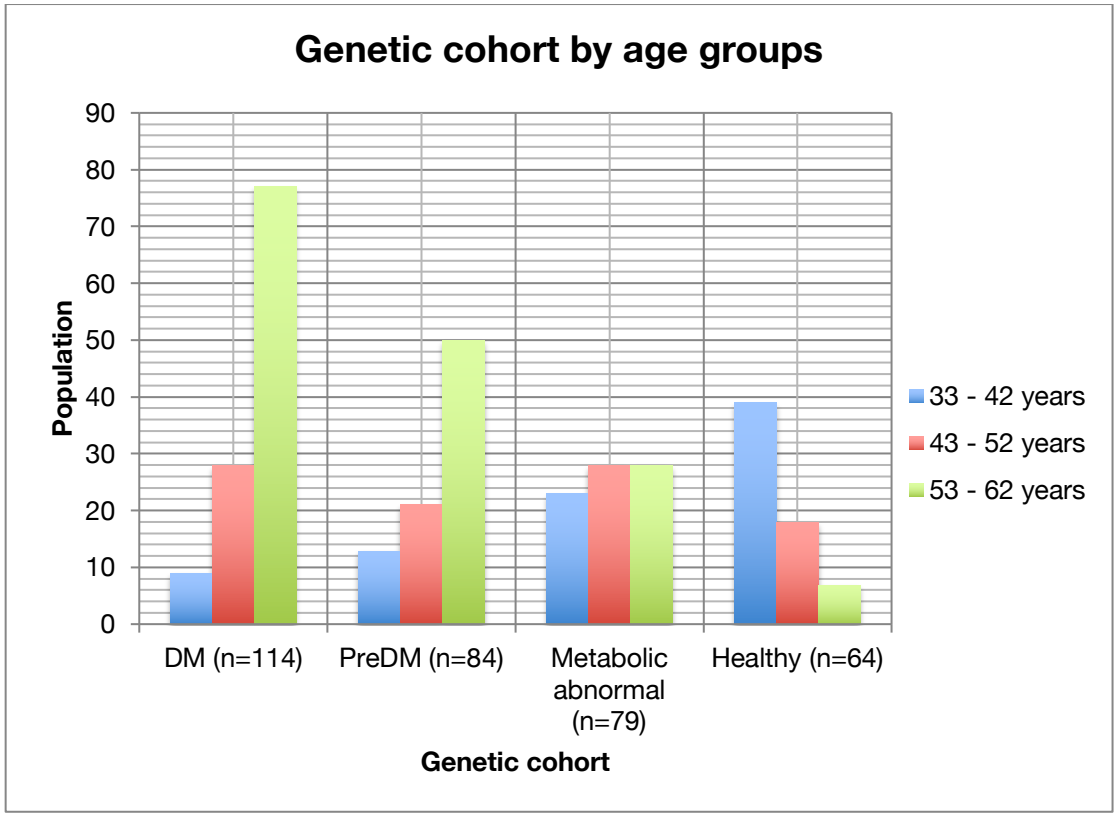



Figure 2.1 Distribution of the genetic cohort by age groups

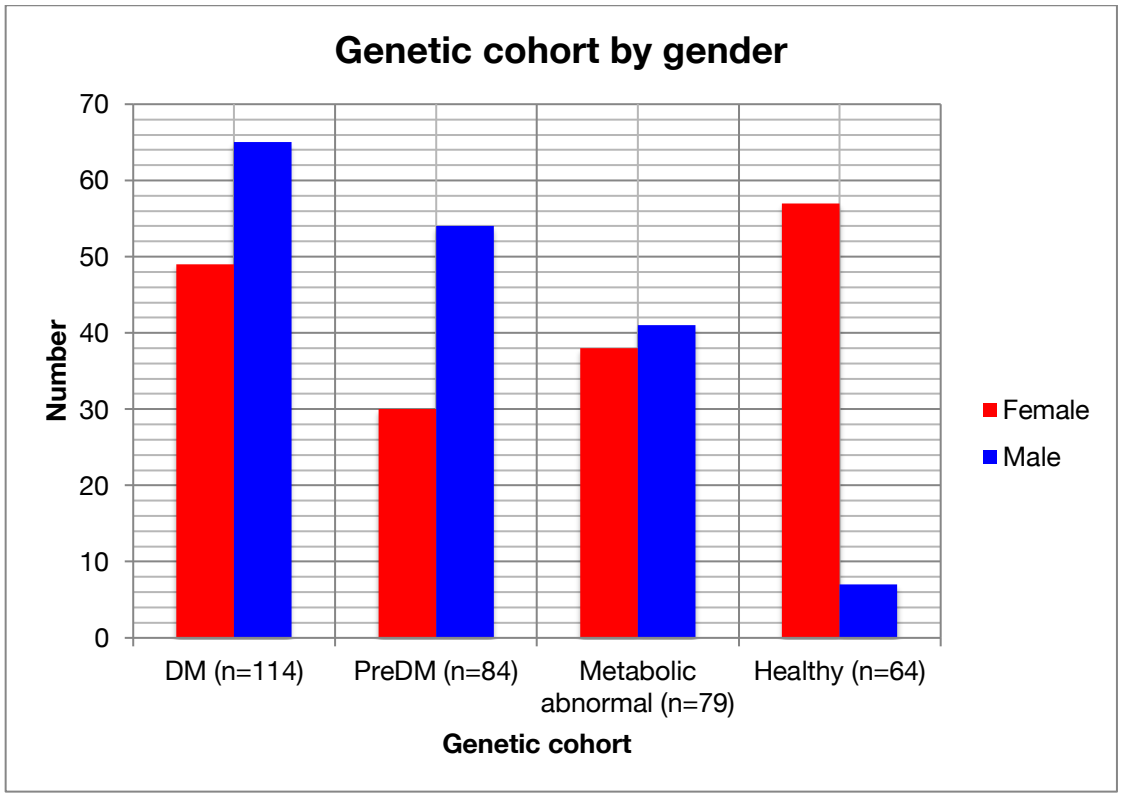



Figure 2.2 Distribution of the genetic cohort by gender

Two comparisons groups were created in order to investigate the biochemical and anthropometric phenotype of the subgroups under study.

(1) For ease of comparisons, the sub-categories of diabetes mellitus and pre-diabetes mellitus were combined together as a 'dysglycaemic' subgroup and considered as the '*Case*' cohort while the 'metabolically healthy' subgroup was considered as the 'Control' cohort.

(2) The 'metabolically abnormal' subgroup although exhibiting normoglycaemia may still have an underlying element of insulin resistance and could represent an intermediate cardio-metabolic state. For this reason, this subgroup was considered as another 'Case' cohort and compared to the 'metabolically healthy' subgroup as the 'Control' group (Nyenwe and Dagogo-Jack, 2011; Grundy, 2012).

**2.8.7.2 DNA extraction**

DNA from peripheral blood was extracted using a QIAamp® Blood Mini Kits (QIAGEN, Hilden, Germany) according to manufacturer protocols. Briefly, 20µl of proteinase K was pipetted into the bottom of a 1.5ml microcentrifuge tube, to which 200µl sample of the participant's whole blood and 200µl of buffer AL was added to the sample followed by pulse-vortex mixing for 15 seconds. The sample was incubated in a heating block at 56ºC for 10 minutes, followed by a brief centrifugation. 200µl of ethanol (96-100%) was added to the sample and mixed again by pulse-vortexing for 15 seconds and centrifuged briefly. The mixture was transferred to QIAamp Mini spin column and centrifuged at 8000 rpm in an Eppendorf® Centrifuge for 1 minute. The QIAamp Mini spin

column was placed in a new clean 2ml collection tube and 500ul of buffer AW1 was added and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini spin column was then placed in a clean 2ml collection tube and 500µl of buffer AW2 was added and centrifuged at 14,000 rpm for 3 minutes. The spin column was placed in a clean 1.5ml microcentrifuge tube and 200µl of Buffer AE was added. Incubation at room temperature for 5 minutes was performed followed by centrifugation at 8,000 rpm for 1 minute. The end filtrate containing DNA was refrigerated at temporary 4$^{o}$C until genotyping was performed.

#### 2.8.7.3 DNA quantification using UV spectrophotometry

The NanoDrop UV Spectrophotometer (Thermo Fisher Scientific) was used to quantify the extracted genomic DNA and check its purity. The system employs a shorter path length compared to traditional cuvette spectrophotometers, which result in a broad range of nucleic acid concentration measurements, and essentially eliminates the need to perform dilutions. The protocol followed for the microvolume nucleic acid quantification can be found in Appendix C.

#### 2.8.7.4 Genotyping by flurogenic 5'nuclease assay (TaqMan®)

Flurogenic 5' nuclease assays were used to genotype the ten single nucleotide variants selected for this study, using an Applied Biosystems 7300 kinetic real time PCR instrument. This assay (TaqMan®) was chosen following the fact that this particular assay was found to be the most time-efficient and accurate technology when a small number of SNPs is being investigated (Shen, Abdullah and Wang, 2009). This versatile PCR based system permits rapid SNP genotyping in real-time during thermal cycling by monitoring the generation of fluorescence signals from within closed reaction tubes. This assay system

dispenses the need for post PCR manipulation and gel electrophoresis, and also has the ability to generate quantitative data that allows determination of target copy numbers.

In a 5'nuclease allelic discrimination assay, two TaqMan® probes are used, one for each allele in a two-allele system. The probes are designed to anneal specifically to the polymorphism being genotyped and are flanked by the forward and reverse primers. Each probe consists of an oligonucleotide with a 5′-reporter dye and a 3′-quencher dye. VIC ® is covalently linked to the 5′ end of probe 1 for the detection of Allele 1. FAM (6-carboxyfluorescein) is covalently linked to the 5′ end of probe 2 for the detection of Allele 2. Each of the reporters is quenched by TAMRA (6-carboxy-N, N, N′, N′-tetramethylrhodamine) attached via a linker arm located at the 3′ end of each probe. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster-type energy transfer (Cardullo *et al.*, 1988).

During PCR, forward and reverse primers hybridize to a specific sequence of the target DNA. The TaqMan® probe hybridizes to a target sequence within the PCR product. The DNA polymerase used in this assay (AmpliTaq Gold® DNA Polymerase) has a 5' nuclease activity that cleaves the TaqMan probe. The 5' nuclease activity results in separation of the reporter and quencher dye, with increased fluorescence of the reporter. The 3' end of the probe is blocked to prevent extension of the probe during PCR (Applied Biosystems Life technologies, 2010).

This process occurs in every cycle and does not interfere with the exponential accumulation of product. The separation of the reporter dyes from the quencher dye results in increase in

fluorescence for each of the FAM and VIC reporters. The increase in fluorescence is measured and is a direct consequence of target amplification during PCR. Both primer and probe must hybridize to their targets for amplification and cleavage to occur. The fluorescence signals are generated only if the target sequences for the probes are amplified during PCR. Because of these requirements, interference from non-specific products is avoided.

The selected polymorphisms were genotyped using a commercially available TaqMan® assay from Applied Biosystems. The primer and probe sequences were diluted in 1x TE buffer and stored at -20$^0$C in the dark. All the TaqMan probes were stored in same conditions. The assay was performed on a 96-well clear optical reaction plate.

Each 25 µL reaction contained:

- 20 ng of genomic DNA (2 µL of DNA at 100 ng/µL in 9.2 µL sterile water)
- 12.5 µL of TaqMan® Univesal MasterMix
- 1.25 µL of the dilute primer and probe sequences

Two non-template control (NTC) wells were included in each plate. These contained DNase-free water instead of the genomic DNA sample and were used to orient the VIC-dye and/or FAM-dye clusters to an origin, as well as to enable the detection of DNA contamination. After adding the DNA to the reagents, the plate was mixed thoroughly and then centrifuged to avoid stratification and air bubbles in the wells. The plate was covered with a MicroAmp™ 96-well optical reaction plate cover and loaded into the thermal cycler.

PCR was performed on an Applied Biosystems 7300 Real-Time PCR system using the following protocol:

- $95^0$C for 10 minutes (activation of AmpliTaq Gold® DNA Polymerase)
- 40 cycles of denaturing at $92^0$C for 15 seconds and annealing/extension at $60^0$C for 1 minute.

Prior to the initiation of thermal cycling, a pre-read run was performed to enable detection and correction for background dye fluorescence. After PCR amplification, an allelic discrimination endpoint plate read was performed using the Applied Biosystems Sequence Detection Software. This uses the fluorescence measurement made during the plate read to plot the florescence (Rn) values based on the signals from each well, thereby indicating which alleles are in each sample. Based on the probe sequences, the correlation between fluorescence signals and genotype is given by:

- VIC-dye fluorescence only: homozygosity for wild-type allele
- FAM-dye fluorescence only: homozygosity for allele variant allele
- Both VIC-dye and FAM-dye fluorescence: heterozygote.

The same Real Time PCR assay protocol (as discussed above) was followed for all the different assays in accordance to the guideline provided by 'Life technologies™'.

Figure 2.3 provides a summary of the TaqMan® genotyping assay process while Figure 2.4 shows a typical software output after manual allele calling for the *CDKAL1* polymorphism.

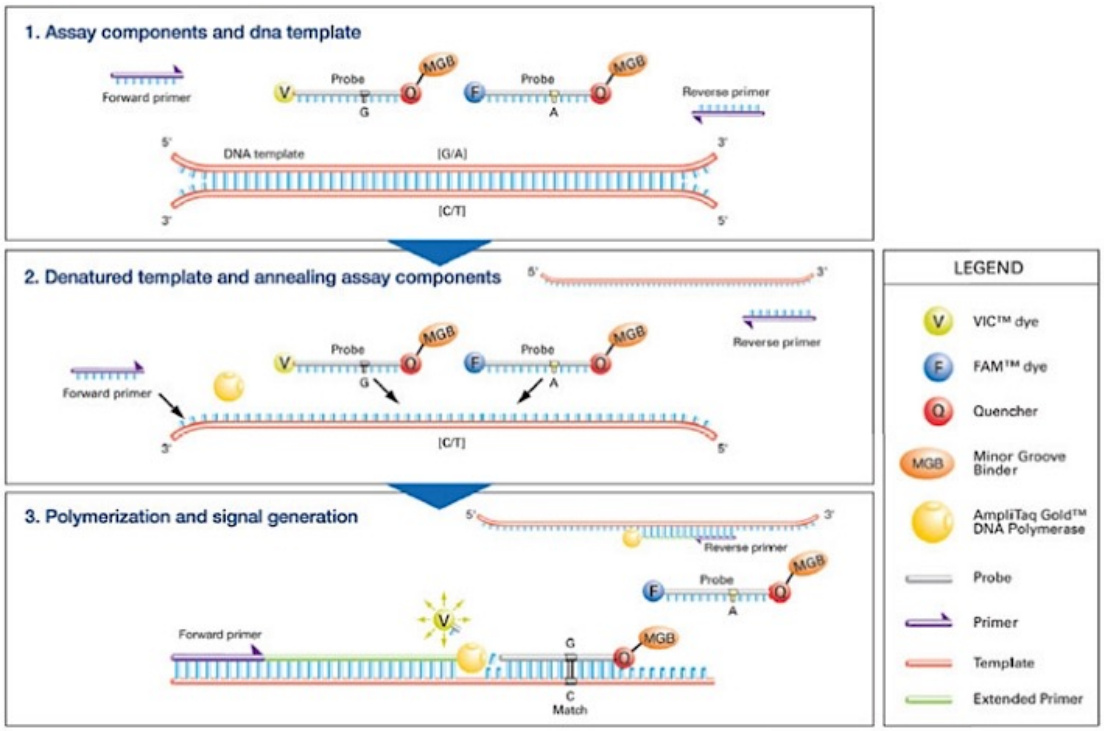



Figure 2.3 TaqMan® genotyping assay process

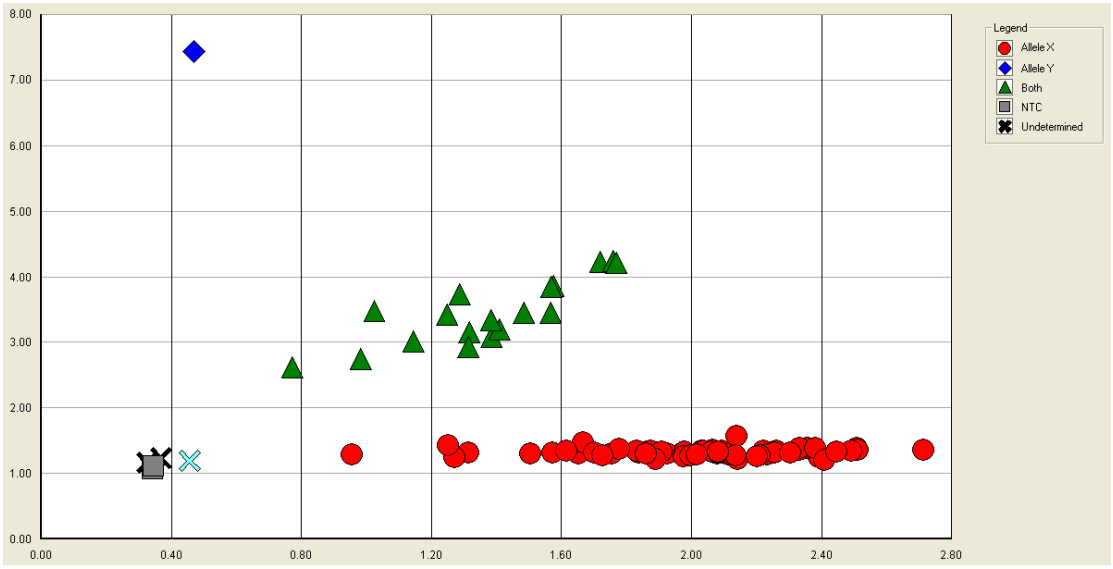



Figure 2.4 *CDKAL1* allele TaqMan software output

#### 2.8.7.5 Genotyping by Sanger Sequencing

The genotype of rs1801282 polymorphism in *PPRAy* was confirmed by Sanger sequencing of PCR amplicons. Briefly, PCR primers were designed to amplify a short sequence surrounding the polymorphism of interest. The primers were amplificated by means of PCR followed by agarose gel electrophoresis in order to analyse for the presence or absence of the variant in question. The detailed protocol for the Sanger sequencing can be found in Appendix C.

#### 2.8.7.6 Single nucleotide polymorphism (SNPs) selection

The genetic analysis for this study followed a replication of established loci after reviewing published literature especially literature covering genome-wide association studies (GWAS). Furthermore, previous work published locally from the Laboratory of Molecular Genetics at the University of Malta were reviewed (Grant *et al.*, 2006; Zeggini *et al.*, 2007; Pace, 2013; Craus, 2016). Due to human resources and financial constrains only ten SNPs were chosen. However, the selection was based on known or presumed role of the risk alleles in diabetes and obesity pathophysiology. This constitutes further to the aim of the study in understanding the burden of diabetes, dysglycaemia and their co-determinants. Therefore, variants affecting the different aspects of the insulin physiology and variants associated with obesity were considered as seen in Table 2.7.

| Gene Symbol | Cytogenetic Location | Gene name | SNP | Effect |
|---|---|---|---|---|
| *FTO* | 16q12.2 | Fat mass and obesity association | rs9939609 | Intronic |
| *CDKAL1* | 6p22.2 | CDK5 regulatory subunit associated protein 1 like 1 | rs7754840 | Intronic |
| *SLC30A8* | 8q24.11 | Solute carrier family 30 member 8 | rs13266634 | Missense |
| *HHEX* | 10q23.33 | Hematopoietically expressed homeobox | rs1111875 | Intergenic |
| *PPARG* | 3p25 | Peroxisome proliferator activated receptor gamma | rs1801282 | Missense |
| *TCF7L2* | 10q25.3 | Transcription factor 7 like 2 | rs7903146 | Intronic |
| *FABP2* | 4q28-q31 | Fatty acid binding protein 2 | rs1799883 | Missense |
| *NOTCH2* | 1p13-p11 | Notch 2 | rs10923931 | Intronic |
| *ADRB2* | 5q31–q32 | Adrenoceptor beta 2 | rs1042713 | Missense |
| *KCNE4* | 2q36.1 | Potassium voltage-gated channel subfamily E regulatory subunit 4 | rs1440072 | Intronic |

Table 2.7 SNPs analysed by gene symbol, cytogenetic location and gene name

#### 2.8.7.7 Genetic statistical analysis

Statistical Package for the Social Science (SPSS) version 21.0 was utilized to calculate all chi square analyses and *p*-values, together with the calculation of risk ratios and log risk ratios.

The Odds Ratio and tests for deviation of Hardy-Weinberg equilibrium were calculated by an online software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Odds ratios enable a comparison of the probability of a certain event for two groups. An odds ratio of one implies that the event is equally likely in both groups. An odds ratio greater than one implies that the event is more likely in the first group, while an odds ratio less than one implies that the event is less likely in the first group.

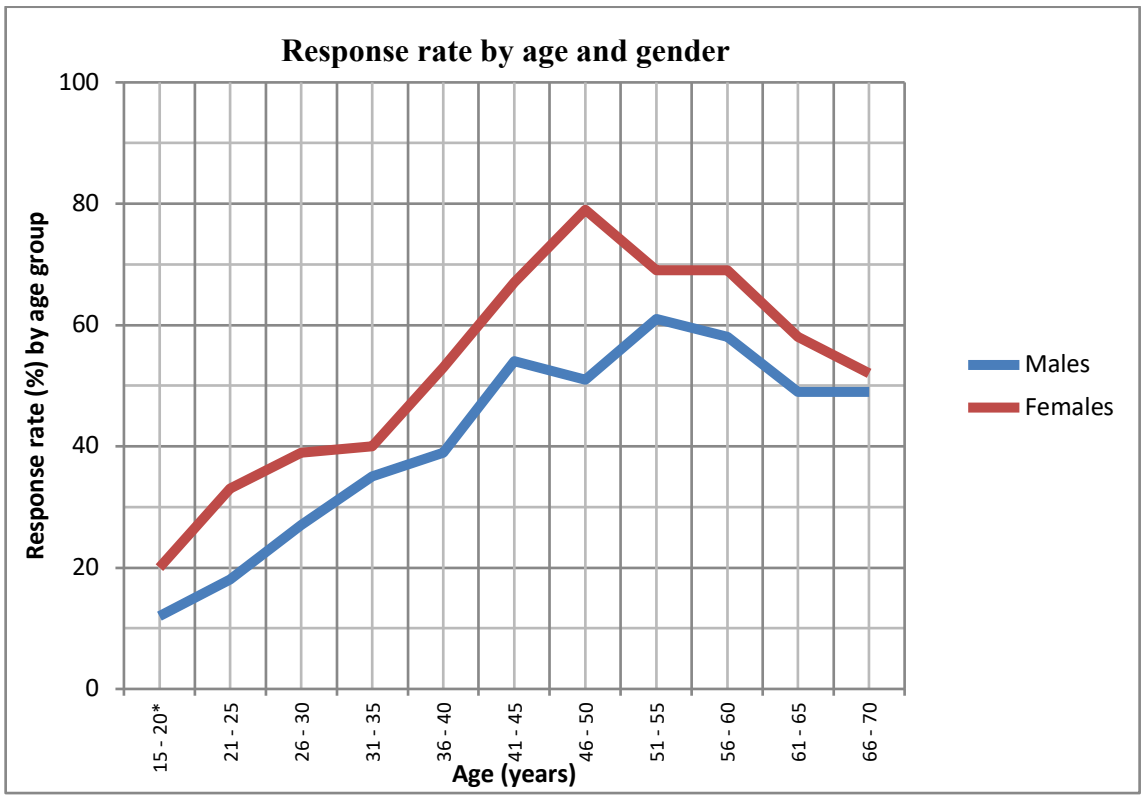
Generalised linear models were used to investigate the associations between each of the ten genetic variants and their corresponding clinical and biochemical parameters in the study sub-population. This statistical modelling technique enables the characterization of the effect size of each allele on the phenotypic characteristics of the study cohort. Each SNP was analysed in relation to the clinical and biochemical parameters separately using co-dominant, dominant and recessive genetic models. For the co-dominant model, the wild type [WT] of each genotype was considered as the reference. The dominant model followed the sequence: A/A [WT] vs. (a/A [HT] + a/a [MT]), where WT represented the wild type allele, HT the heterozygous allele and MT the mutant risk allele. The recessive model followed the sequence: (A/A [WT] + a/A [HT]) vs. a/a [MT]. Stepwise backward generalized linear models (GLM) were used to investigate the association/s between the ten SNPs and a diagnosis of type 2 diabetes mellitus following co-dominant, dominant and recessive models, while adjusting for possible confounding factors.

# Chapter three – Results

## 3.1 Descriptive analysis

### 3.1.1 Response rate

A total of 3,947 adults (1,997 male and 1,950 female) were invited to participate in the health examination survey held between November 2014 and November 2015. Of these, 1,861 adults (836 male and 1,025 female) participated, giving a response rate of 47.15% ($p$=<0.01). The population under study was age stratified and the response rate calculated, as seen in Figure 3.1.




**Age from 18 years*

Figure 3.1 Response rate by age and gender

The distribution of the crude population by age (single years) and gender can be found in Appendix D (Table 1). The response rate by town and gender for all the towns in the Maltese Islands (Table 2) is also found in Appendix D. The response rate varied across the ages and gender, as seen in Figure 3.1. This exposed the need to carry out statistical weighting in order to establish national representativeness when it came to establishing prevalence rates (described in Section 2.8.2). The distribution of the adjusted participants by age (single years) and gender (Table 3) is found in Appendix D.

### 3.1.2 Response rate for oral glucose tolerance test (OGTT)

Of the attending participants (*n*=1,861), a total of 451 had an FPG between 5.60 – 6.99mmol/L and an OGTT was subsequently offered to them as per established study protocol. The OGTT invitation was accepted by 47.23% (CI 95%: 42.66 – 51.84; *n*=213) of the eligible participants, with a male predominance (60.09% CI 95%: 53.39 – 66.44; *n*=128).

#### 3.1.2.1 Oral glucose tolerance test (OGTT) cohort

On comparing the IFG population who attended the OGTT to the IFG population who declined the invitation for the test, no statistically significant difference was present between both cohorts. This non-significance held true when comparing the age groups of both cohorts ($p$=0.06). The median age of those attending the OGTT was significantly similar to those who declined the invitation ($p$=0.47) even on comparing the median age by gender (female $p$=0.73; male $p$=0.39). No educational level difference was evident between the attendees and the non-attendees ($p$=0.92). This held true even on comparing the education level by gender (female $p$=0.62, male $p$=0.72) and age groups (20-29 years

*p*=0.36; 30-39 years *p*=0.15; 40-49 years *p*=0.35; 50-59 years *p*=0.77; 60 – 69 years *p*=0.96) respectively.

*The following section presents the descriptive analysis for this thesis. A statistical adjusted population (weighting methodology described in Section 2.8.2) will be used for this section, in order to establish national representativeness when establishing prevalence rates (Appendix D - Table 3). The crude unadjusted population (Appendix D - Table 1) will be used when presenting the analytic analysis for this thesis (Section 3.2).*

### 3.1.3 Age-gender profiles

A total adjusted population of 3,947 (male *n*=1,998; female *n*=1,949) was included in the study. On adjusting the data from the female dominant responders to its weighted composition, the data regains its male predominance, a reflection of the study and target general population of Malta. The adjusted population by age and gender (Table 3) is found in Appendix D.

The median age of the population was 45 years (IQR 26 years) with no significant difference in median age between the male (44 years IQR: 26) and the female population (45 years IQR 27 years; *p*=0.21).

### 3.1.4 Prevalence rates

#### 3.1.4.1 Prevalence rate of type 2 diabetes mellitus (T2DM)

Of the total adjusted study population (*n*=3,947), 407 participants suffered from type 2 diabetes mellitus (T2DM), resulting in a total diabetes prevalence of 10.31% (CI 95%: 9.40 - 11.30). This included those previously diagnosed (6.31%, CI 95%: 5.59 - 7.11) (*n*=249) as well as the newly diagnosed (4.00% CI 95%: 3.43 - 4.66) (*n*=158) diabetes (Table 3.1 and Table 3.2).

| **Previously diagnosed diabetes** | **Number** | **%** | **95% CI** |
|---|---|---|---|
| Total | 249 | 6.31 | 5.59 - 7.11 |
| Male | 168 | 4.26 | 3.67 – 4.93 |
| Female | 81 | 2.05 | 1.65 – 2.55 |
| **Newly diagnosed diabetes** | **Number** | **%** | **95% CI** |
| Total | 158 | 4.00 | 3.43 - 4.66 |
| Male | 103 | 2.61 | 2.15 – 3.16 |
| Female | 55 | 1.39 | 1.07 – 1.81 |
| **Total diabetes** | **Number** | **%** | **95% CI** |
| Total | 407 | 10.31 | 9.40 - 11.30 |
| Male | 271 | 13.56 | 12.13 - 15.14 |
| Female | 136 | 6.98 | 5.93 - 8.20 |

Table 3.1 Previously diagnosed diabetes, newly diagnosed diabetes and total diabetes population prevalence

| Gender | Previously diagnosed diabetes (%) | Newly diagnosed diabetes (%) |
|---|---|---|
| Male (*n*=1,998) | 168 (8.41% CI 7.27 – 9.71) | 103 (5.16% CI 4.26 – 6.22) |
| Female (*n*=1,949) | 81 (4.16% CI 3.35 – 5.14) | 55 (2.82% CI 2.17 – 3.66) |

*%: percentage of total population by gender; CI: confidence interval 95%*

Table 3.2 Previously diagnosed diabetes; newly diagnosed diabetes and total diabetes population prevalence by gender.

The newly diagnosed T2DM subgroup (*n*=158) was further subdivided by the method of diagnosis.

i. There was a total of 135 (3.42%) participants (Female *n*=46; Male *n*=89) who had an FPG ≥7mmol/L and were therefore immediately diagnosed as diabetes following the health examination.
ii. Among the OGTT responders, a total of 23 (5.18%) participants were diagnosed with new onset diabetes. Table 3.3 illustrates the adjusted OGTT population final diagnosis after undergoing the test.

| 2nd Hour Glucose Measurement | Diagnosis | Gender | |
|---|---|---|---|
| | | Male (%) | Female (%) |
| < 7.8 mmol/L | Normal | 237 (83.16) | 127 (79.87) |
| 7.8 – 11.0 mmol/L | IGT | 34 (11.93) | 23 (14.47) |
| > 11.1 mmol/L | Diabetes | 14 (4.91) | 9 (5.66) |
| **Total** | | **285** | **159** |

***IGT – Impaired Glucose Tolerance***

Table 3.3 Distribution of OGTT diagnosis by gender and 2nd hour plasma glucose results among the adjusted OGTT population

### 3.1.4.2 Prevalence rate of impaired fasting glucose (IFG)

The adjusted population prevalence of impaired fasting plasma glucose (excluding all diabetes i.e. previously known diabetes; newly diagnosed diabetes following health examination and those with a new diagnosis of diabetes following an OGTT) was of 23.44% (CI 95% 22.14 – 24.78; *n*=925), with 60.97% (CI 95%: 57.79 – 64.07) of these being male.

### 3.1.4.3 Prevalence rate of the overweight-obese

Amongst the adjusted population (*n*=3,947), 35.65% (95% CI: 33.27 – 37.15) were found to be overweight while 34.08% (95% CI: 32.64 – 35.60) were found to be obese, with only 30.27% (95% CI: 28.84 – 31.70) having a normal body mass index. Thus, 69.73% (95% CI: 68.32 – 71.18) of the total adult population (18-70 years old) suffered from an abnormally high body mass index (BMI).

As expected, males (overweight 39.40% CI 95%: 37.27 – 41.50; obese 36.84% CI 95%: 34.75 – 38.98) and females (overweight 31.81% CI 95%: 29.78 – 33.91 and obese 31.25% CI 95%: 29.23 - 33.34) were significantly different from each other by BMI distribution (overweight *p*=<0.01; obese *p*=<0.01). Additionally, as can be summarized from Figure 3.2, the female population exhibited a significantly higher prevalence of normal weight individuals (36.90% 95% CI: 33.83 – 39.11, *p*=<0.01) than did males.

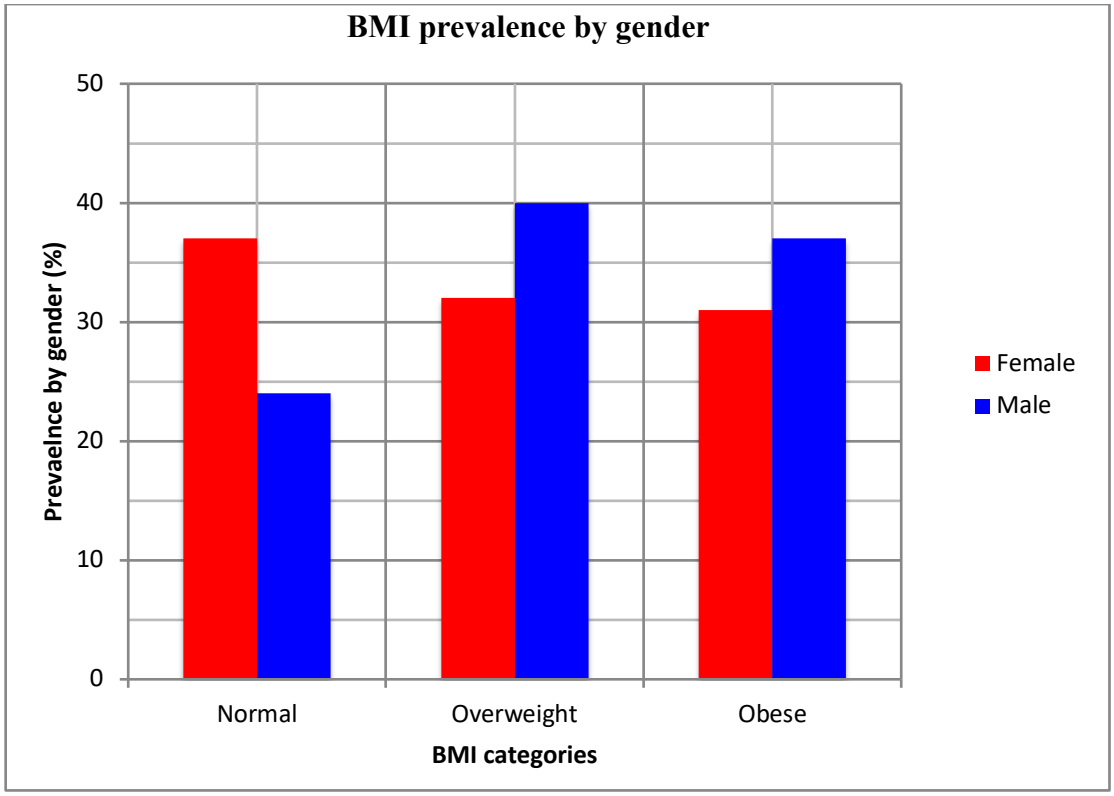



Figure 3.2 Distribution of BMI categories prevalence by gender

### 3.1.4.4 Prevalence rate of the Metabolic Syndrome (MetS)

The MetS was evident in 26.30% (CI 95%: 24.95 – 27.69, *n*=1,038) of the total SAHHTEK adjusted population (*n*=3,947). The male population had a significantly higher overall prevalence of MetS (31.63%, CI 95%: 29.63 – 33.70) than did the female population (20.83%, CI 95%: 19.09 – 22.69) (*p*=<0.01).

#### 3.1.4.5 Prevalence rate of the Metabolically Healthy but Obese (MHO) population

The MHO prevalence rate was 3.37% (CI 95%: 2.85 – 3.98), with an almost equal gender distribution (Male *n*=66; Female *n*=67). No statistically significant difference (*p*=0.82) between genders was evident.

#### 3.1.4.6 Prevalence rate of the Metabolically Obese Normal Weight (MONW)

The prevalence rate of MONW within the SAHHTEK population was 7.75% (CI 95%: 6.96 – 8.63) with no statistical difference between the male and female populations (*p*=0.35).

### 3.1.5 Prevalence by age and gender

*The study population covered an age range between 18 and 70 years. On age stratification this age range could not be divided into 5 years or 10 years. Therefore, for ease of description, for comparisons and graphical representation, the age groups were stratified into 10-years, while omitting the 18, 19 and 70 years just for representation. However, prevalence rates were always worked out using the whole adjusted population (n=3,947).*

#### 3.1.5.1 Type 2 diabetes mellitus by age-gender profile

An expected increase in the prevalence of diabetes by age for both genders was observed as seen in Figure 3.3. There were significant relationship between age and diabetes (*p*=<0.01) and between gender and diabetes (*p*=<0.01) respectively. Females

exhibited the onset of T2DM at an earlier age when compared to their male counterparts. Females also had significantly higher diabetes prevalence in the 60 – 69 years group than males ($p$=<0.01).

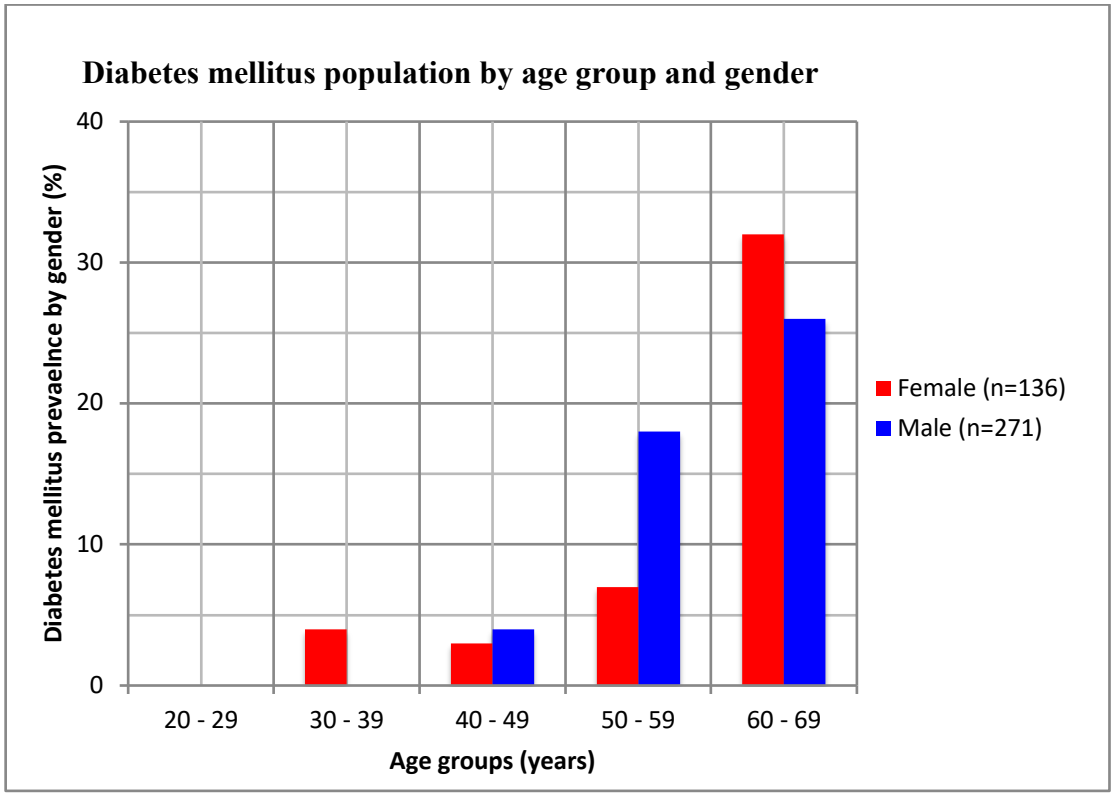



Figure 3.3 Total diabetes population prevalence, by age groups and gender

As expected, the prevalence of previously known T2DM and newly diagnosed T2DM increased with older age groups, as seen in Figure 3.4 and Figure 3.5. Of note, the newly diagnosed T2DM females were diagnosed at an earlier age (30 - 39 years) compared to the male counterpart, as seen in Figure 3.5. Additionally, the male diabetes population (both previously and new) between the ages of 40 and 59 years exhibited a higher prevalence rate than their counterparts. The opposite was true for the 60 to 69 years age group, as seen in Figure 3.4 and Figure 3.5, where the female T2DM had a higher prevalence.

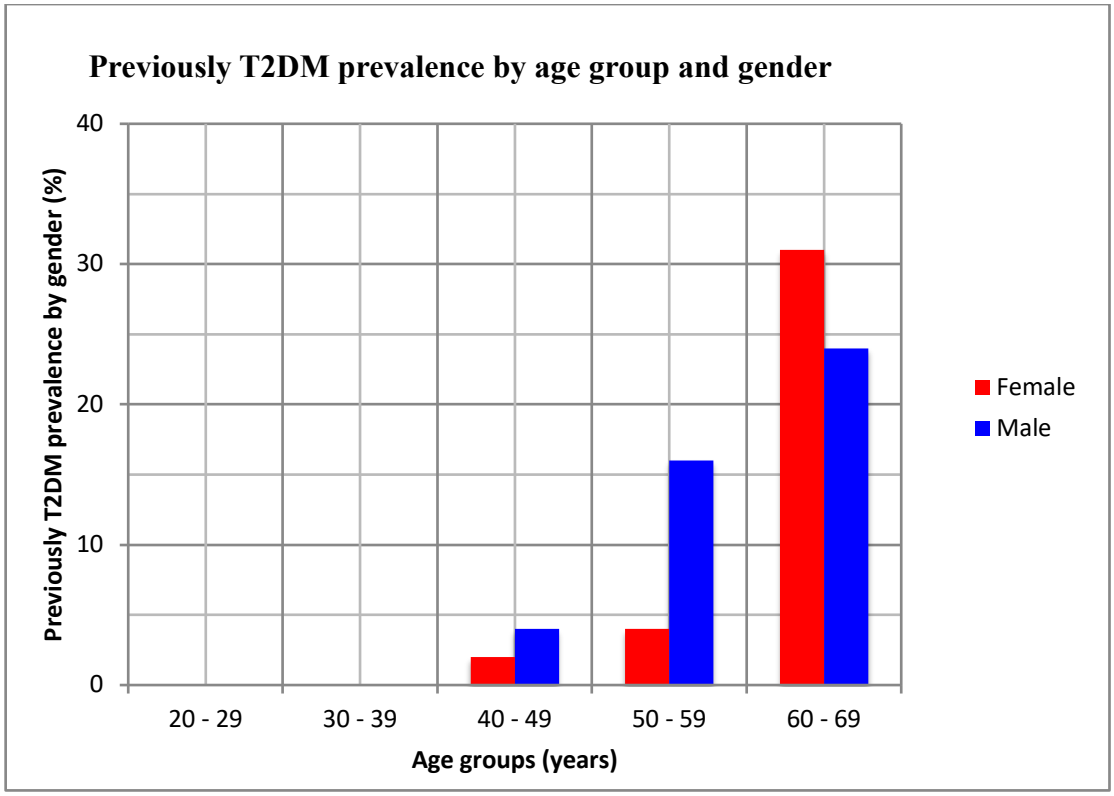



Figure 3.4 Prevalence of previously known T2DM by age groups and gender

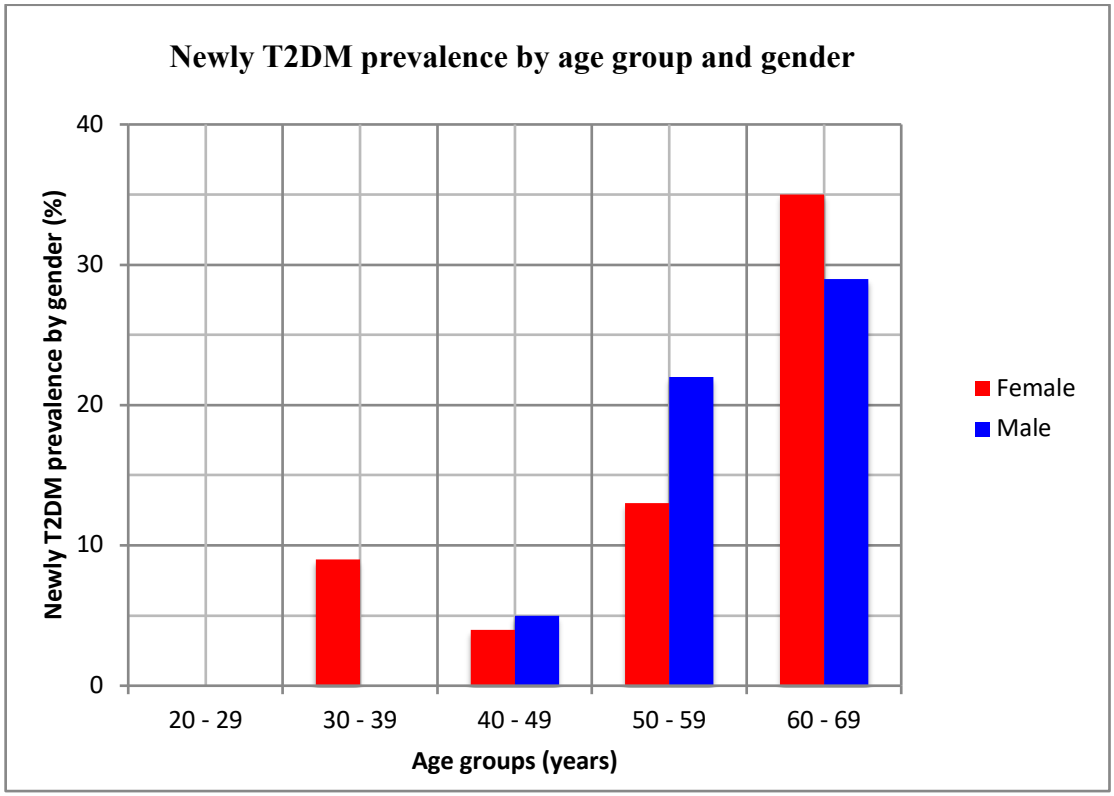



Figure 3.5 Prevalence of newly diagnosed T2DM by age groups and gender

### 3.1.5.2 Impaired fasting glucose by age-gender profile

A significant positive relationship existed between IFG and age as well as between IFG and gender respectively ($p$=<0.01 respectively).

Males were overall mostly affected by IFG (60.86% CI 95%: 57.68 – 63.96). However, on 10-year stratification, this male predominance was mostly marked only in the 30-39-year age band (Figure 3.6).

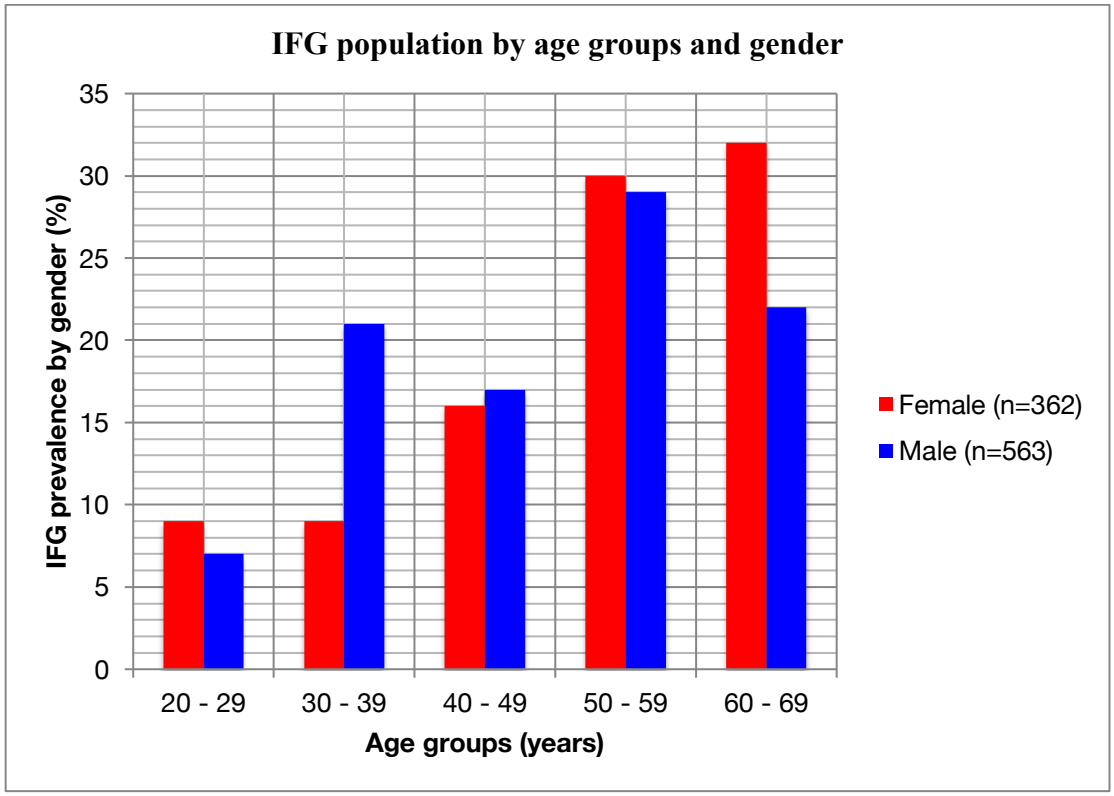



Figure 3.6 Distribution of IFG population, by age groups and gender

### 3.1.5.3 Body mass index (BMI) by age-gender profile

The normal weight category decreased as age increased. The overweight and obese prevalence rates were higher with increasing age, with both categories peaking between 60 and 69 years (Figure 3.7). There were significant statistical differences between the distribution of obesity and overweight across age groups ($p$=<0.01 respectively).

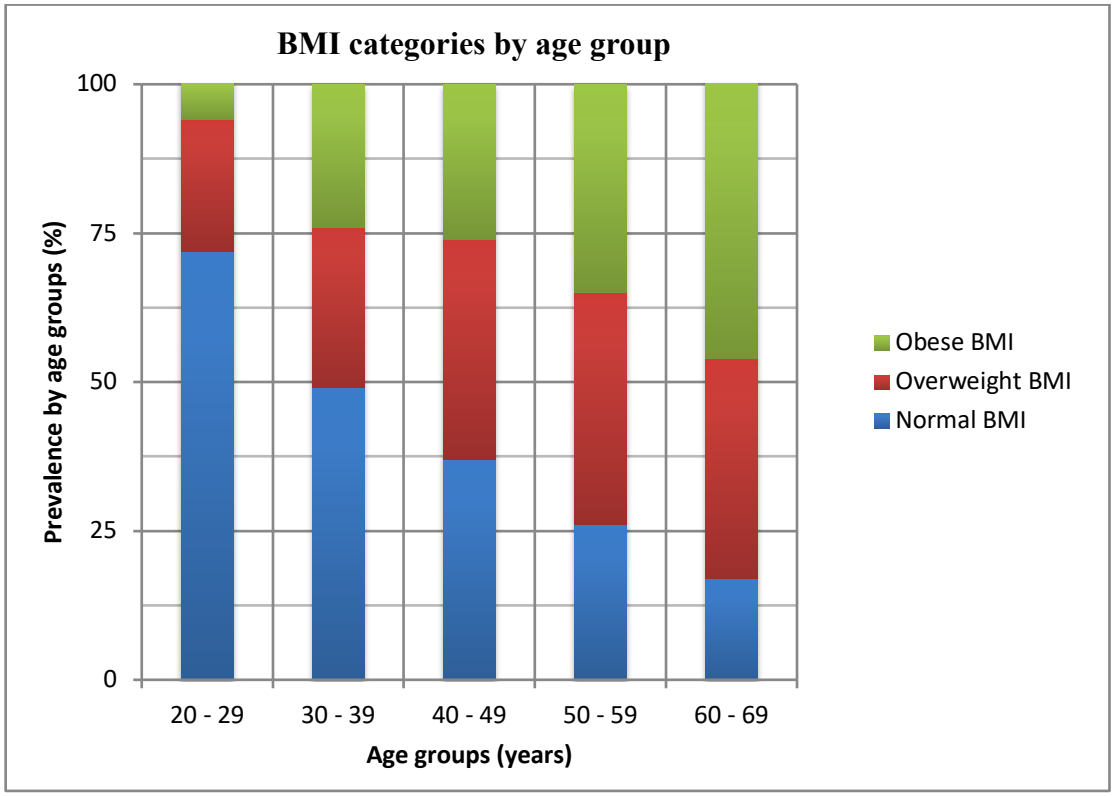



Figure 3.7 Prevalence of BMI categories, by age groups

The female and male populations exhibited similar BMI trends by age as seen in Figure 3.8. However, the female population between 20 – 29 years did not include anyone in the obese status (unlike the males). An overall higher proportion of normal BMI females were evident when compared to the male population.

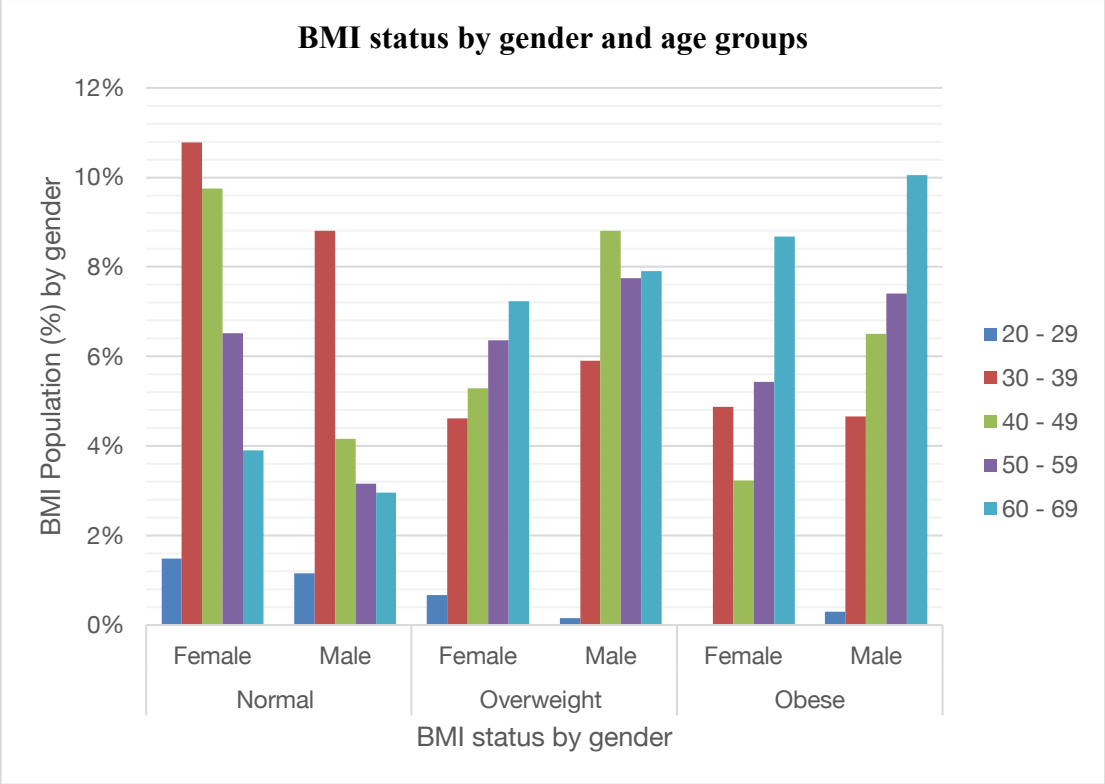



Figure 3.8 Distribution of BMI, by age groups and gender

### 3.1.5.4 Metabolic syndrome by age-gender profile

On age stratification (10 year) of the MetS population, the prevalence escalated with increasing age in both genders as seen in Figure 3.9 (with the exception of the female 40 – 49-year group). Females exhibited higher prevalence rates than males, at both extremes of the age spectrum (20 – 39 years and 60 – 69 years), while the male population had higher MetS prevalence rates within the remaining age groups ($p$=<0.01). A significant correlation was present between gender and age ($p$=0.01).

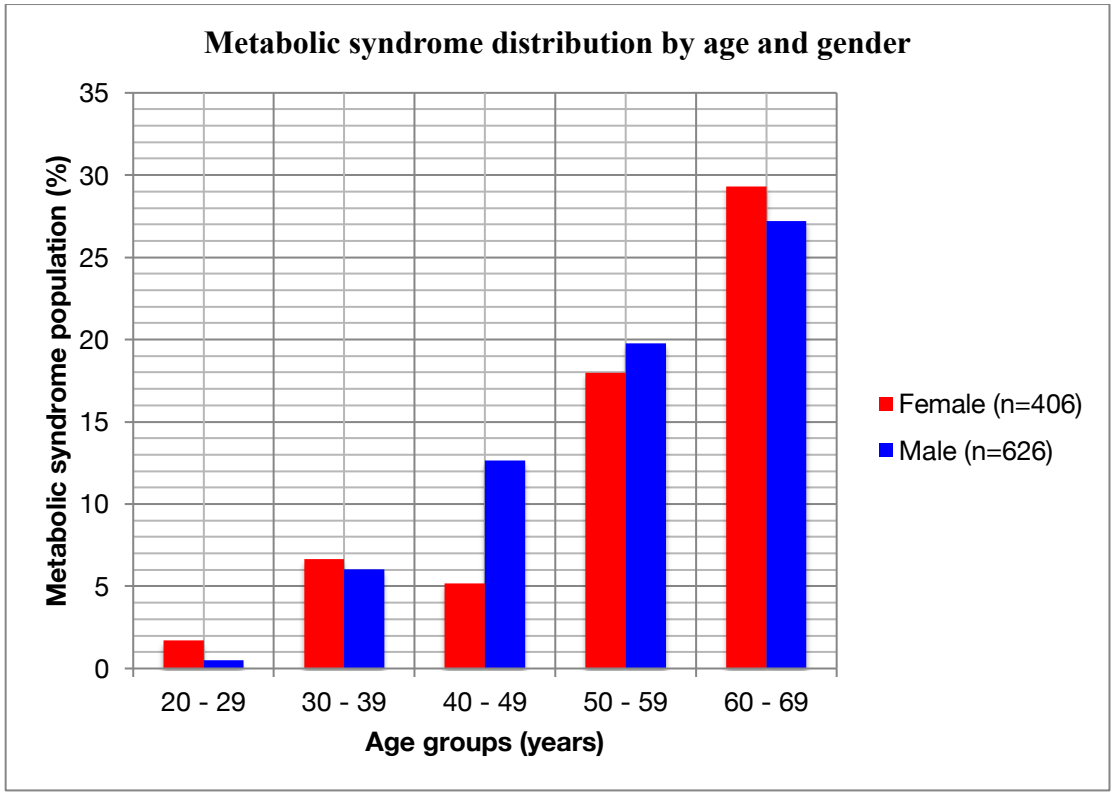



Figure 3.9 Distribution of MetS prevalence rates by age groups and gender

## 3.1.6 Demographic characteristics

### 3.1.6.1 Districts

In keeping with the general population in the Maltese Islands, the study population mostly resided in the Northern Harbour area (Ħal Qormi, Birkirkara, Gżira, Ħamrun, Msida, Pembroke, Tal-Pietà, St Julian's, San Ġwann, Santa Venera, Tas-Sliema, Swieqi, Ta' Xbiex), which is a highly urbanized region. The population distribution across the different regions is illustrated in Figure 3.10. Equal gender distribution was exhibited across all the different districts. In fact, no difference was found between gender and districts ($p$=0.85).

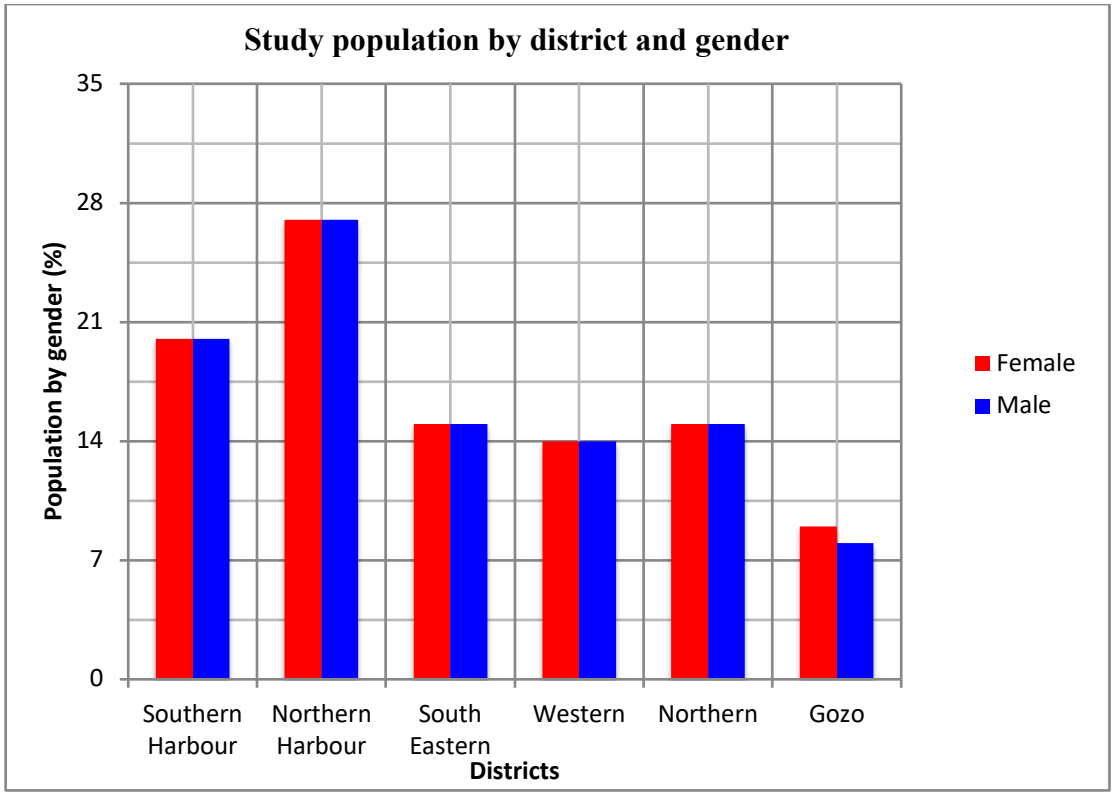



Figure 3.10 Districts distribution of the study population, by gender

The median age distribution across the six districts ranged between 42 to 47 years as seen in Figure 3.11, with a statistically significant difference ($p$=<0.01) between districts.

On pairwise comparison of the median age between each district, significant age difference was limited to five comparisons. The Southern Harbour population was significantly younger than the Northern Harbour population ($p$=0.01), while the Northern Harbour population was significantly older than the Southeastern population ($p$=<0.01), the Western population ($p$=0.02) and Northern population ($p$=<0.01). On the other hand, the Northern population was younger than the Gozo population ($p$=0.02).

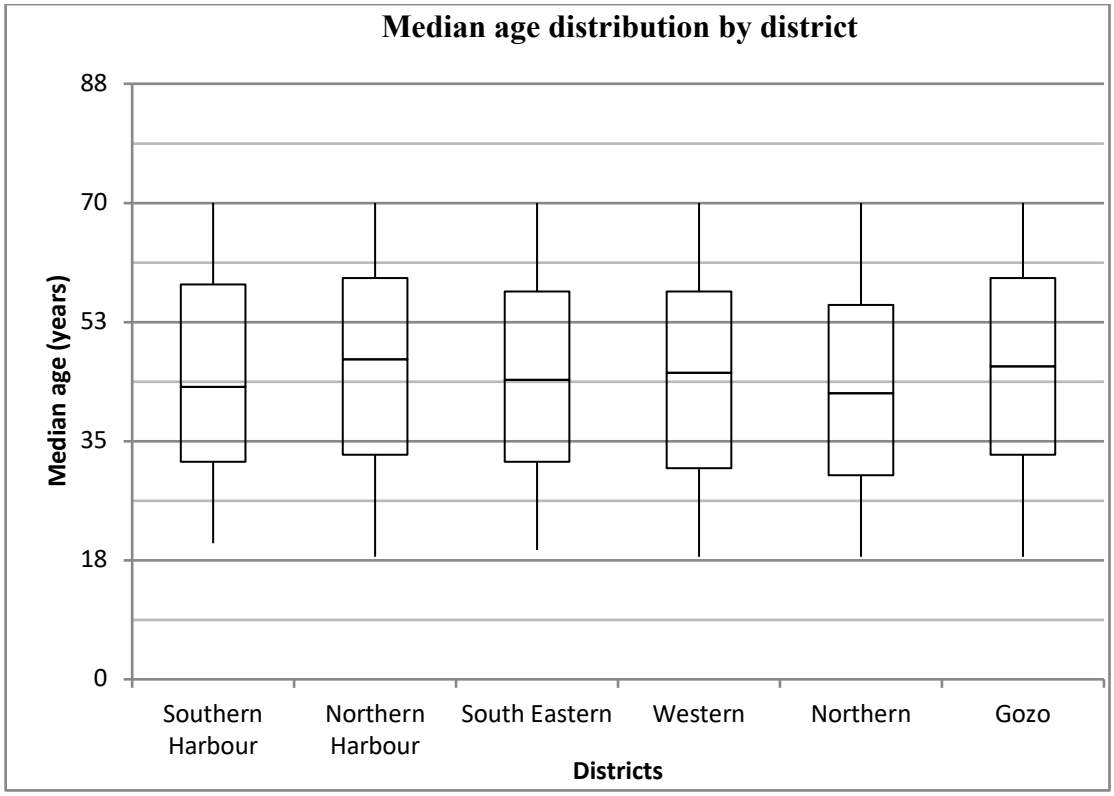



Figure 3.11 Distribution of median age and IQR, by localities

On further analysing the districts by median age and gender, only the female population exhibited a statistically significant difference in the median age between the different districts (Female $p$=<0.01; Male $p$=0.08).

### 3.1.6.1.1 Districts by socio-demographic profile

The reported education levels and the employment status contributed to the socio-demographic profile of the SAHHTEK study. The highest education level was also used as a marker for socioeconomic status.

The education level was found to be significantly different across the six districts ($p$=<0.01) as seen in Table 3.4.

| | Districts | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Southern Harbour (*n*=800)** | **Northern Harbour (*n*=1,070)** | **South Eastern (*n*=602)** | **Western (*n*=546)** | **Northern (*n*=587)** | **Gozo (*n*=342)** | **Total Number** |
| No formal education | 2 (0.25%) | 4 (0.37%) | 0 | 6 (1.10%) | 4 (0.68%) | 0 | 16 |
| Primary | 84 (10.50%) | 110 (10.28%) | 50 (8.31%) | 37 (6.13%) | 36 (6.13%) | 45 (13.16%) | 362 |
| Did not finish secondary | 69 (8.63%) | 64 (5.98%) | 51 (8.47%) | 29 (5.31%) | 25 (4.26%) | 19 (5.56%) | 257 |
| Finished secondary | 336 (42.00%) | 418 (39.07%) | 235 (39.04%) | 225 (41.21%) | 210 (35.78%) | 107 (31.29%) | 1531 |
| Tertiary | 175 (21.88%) | 179 (16.73%) | 140 (23.26%) | 106 (19.41%) | 137 (23.34%) | 55 (16.08%) | 792 |
| University | 111 (12.88%) | 224 (20.93%) | 100 (16.61%) | 105 (19.23%) | 138 (23.51%) | 100 (29.24%) | 778 |
| Post-graduate | 23 (2.88%) | 71 (6.64%) | 26 (4.32%) | 38 (6.96%) | 37 (6.30%) | 16 (4.68%) | 211 |

% - Education level by each district

$X^2$ Districts by Educational levels $p$=<0.01

Table 3.4 Distribution of highest education level, by district

Of note, the Western district exhibited the highest population proportion with no formal education and with post-graduate education.

The majority of the population reported to be employed (63.95% CI 95%: 62.44 – 65.43), out of which 26.78% (CI 95%: 25.09 – 28.54%) lived in the Northern Harbour, as seen in Table 3.5 The majority of the unemployed (30.56% CI 95%: 21.08 – 42.00) lived in the Southern Harbour.

| | **Employed (*n*=2,524)** | **Unemployed (*n*=72)** | **Student (*n*=147)** | **Retired (*n*=518)** | **Domestic tasks (*n*=689)** | **Total Number** |
|---|---|---|---|---|---|---|
| Southern Harbour | 506 (20.05%) | 22 (30.56%) | 6 (4.08%) | 120 (23.17%) | 147 (21.34%) | 800 |
| Northern Harbour | 676 (26.78%) | 12 (16.67%) | 35 (23.81%) | 145 (27.99%) | 204 (29.61%) | 1070 |
| South Eastern | 387 (15.33%) | 6 (8.33%) | 37 (25.17%) | 78 (15.06%) | 94 (13.64%) | 602 |
| Western | 349 (13.83%) | 18 (25.00%) | 26 (17.69%) | 66 (12.74%) | 87 (12.63%) | 546 |
| Northern | 400 (15.85%) | 7 (9.72%) | 22 (14.97%) | 66 (12.74%) | 92 (13.35%) | 587 |
| Gozo | 206 (8.16%) | 7 (9.72%) | 21 (14.29%) | 43 (8.30%) | 65 (9.43%) | 342 |

% - Population by employment status
$X^2$ - Districts by Employment $p$=<0.01

Table 3.5 Distribution of employment status, by district

#### 3.1.6.1.2 Districts by self-reported medical history

Self-reported medical histories exhibited statistically significant differences between different regions (except for hypothyroid disease), as seen in Table 3.5. The commonest medical history was that of hypertension. The South Eastern district population reported the highest percentage proportion suffering from this condition. The population of Gozo self-reported a higher prevalence of coronary heart disease, myocardial infarction and dyslipidaemia when compared to the Maltese districts.

| Self-reported Medical History | Southern Harbour (n=800) | Northern Harbour (n=1,070) | South Eastern (n=602) | Western (n=546) | Northern (n=587) | Gozo (n=342) | Total Number | $X^{2*}$ |
|---|---|---|---|---|---|---|---|---|
| Coronary Heart Disease | 26 (3.25%) | 35 (3.27%) | 10 (1.66%) | 12 (2.20%) | 11 (1.87%) | 19 (5.56%) | 113 | **<0.01** |
| Myocardial infarction | 17 (2.13%) | 21 (1.96%) | 8 (1.33%) | 4 (0.73%) | 13 (2.21%) | 19 (5.56%) | 82 | **<0.01** |
| Hypertension | 172 (21.50%) | 259 (24.21%) | 163 (27.08%) | 141 (25.82%) | 118 (20.10%) | 91 (26.61%) | 944 | **0.02** |
| Hypothyroid disease | 186 (0.75%) | 82 (7.66%) | 58 (9.63%) | 48 (8.79%) | 44 (7.50%) | 28 (8.19%) | 346 | 0.19 |
| Dyslipidemia | 146 (18.25%) | 241 (22.52%) | 122 (20.27%) | 129 (23.63%) | 111(18.91%) | 102 (29.82%) | 851 | **<0.01** |

% - Self-reported medical history by each district

* $X^2$ –Chi-square test between reported medical history and districts

Table 3.5 Distribution of self-reported medical histories by district

### 3.1.6.1.3 Districts by fasting plasma glucose regulation

The median fasting plasma glucose (FPG) was within normal range (<5.6mmol/L) for all localities except for Gozo. Gozo exhibited a median FPG within the impaired fasting plasma glucose range (5.60 – 6.99 mmol/L) according to the ADA criteria (American Diabetes Association, 2018b). Figure 3.12 illustrates a box and whisker plot of the median FPG across the different districts.

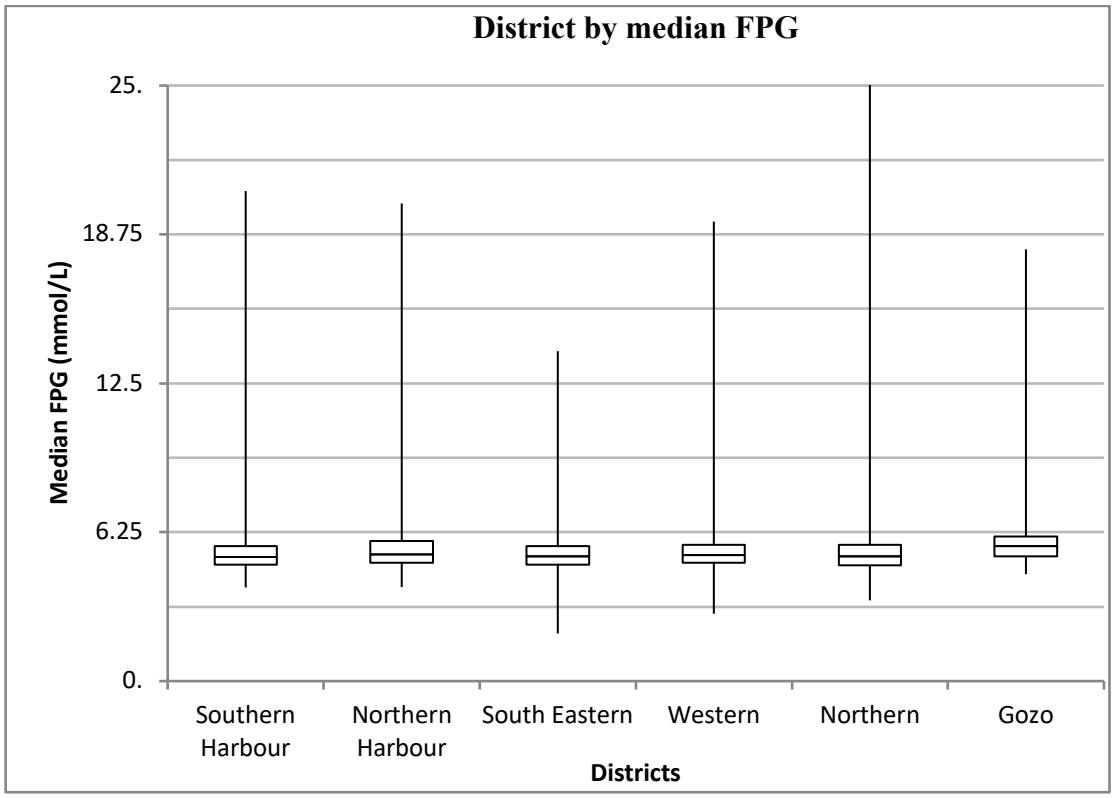



Figure 3.12 Distribution of median FPG and IQR, by district

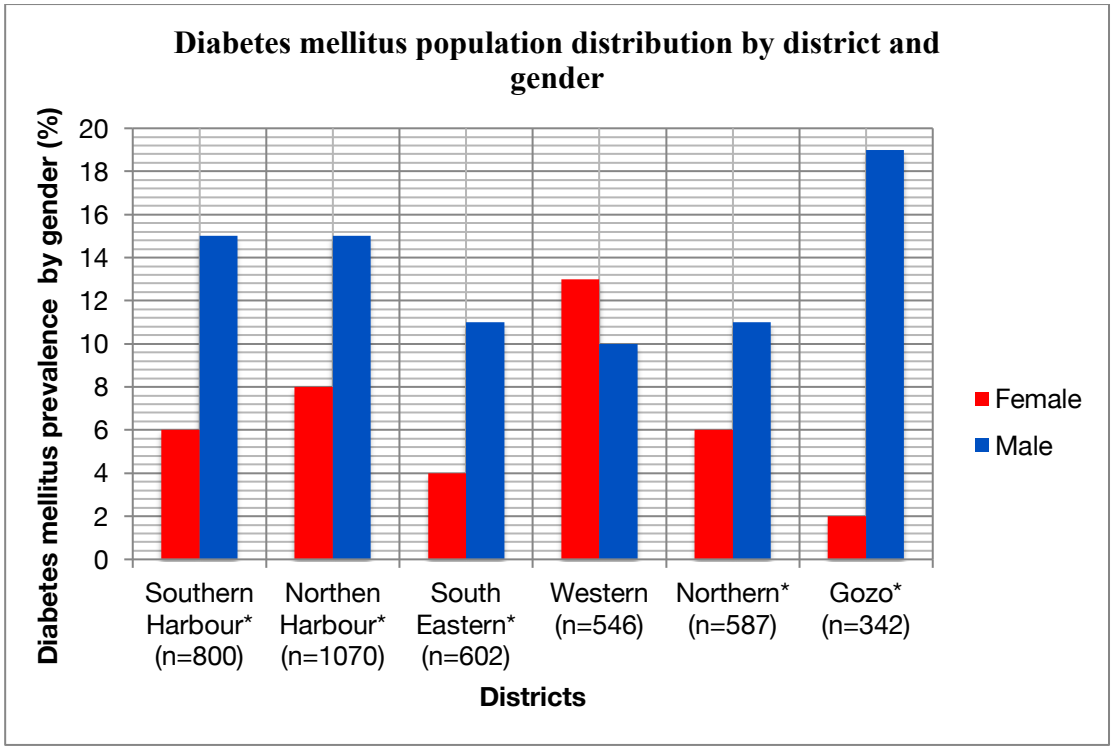
A significant difference between the median FPG and the different districts was evident ($p$=<0.01). On pairwise comparison of the different localities' median FPG, it was found that Gozo had a significantly higher median FPG value then the rest of Malta

districts ($p$=<0.01 respectively). On gender stratification, both the males and the females residing in Gozo (only) exhibited a significant higher median FPG. The Gozitan males had significantly higher median FPG than those residing in South Eastern, South Harbour, Western districts ($p$=<0.01 respectively) and Northern district ($p$=0.01). The Gozitan females had significantly higher median FPG than those residing in South Eastern, South Harbour, Western districts ($p$=0.01 respectively) and Northern district ($p$=<0.01).

#### 3.1.6.1.4 Districts by Type 2 diabetes mellitus status

The diabetes subgroup (previously known and newly diagnosed) followed the general population distribution trend, where the majority of the diabetes population resided in the Northern Harbour area. All the different districts exhibited statistically significant ($p$=<0.05) male diabetes predominance except for the Western district (Mdina, Ħaż-Żebbuġ, Siġġiewi, Ħ'Attard, Ħal Balzan, Ħad-Dingli, Iklin, Ħal Lija, Rabat, Mtarfa), where diabetes was mostly predominant amongst females. This is illustrated in Figure 3.13 (where significance is marked with an * within the figure). However, the Western district exhibited no statistically significant difference between the diabetes females and males ($p$=0.34). Furthermore, no relationship was observed between the districts and the diagnosis of diabetes ($p$=0.35).

The measured median FPG within the diabetes mellitus population exhibited a statistically significant difference between the districts ($p$=0.03) as visualised in Figure 3.14. The diabetes mellitus population in Gozo exhibited a higher male proportion apart from an overall higher median FPG.




*$p$-value <0.05

Figure 3.13 Distribution of the diabetes mellitus population, by district

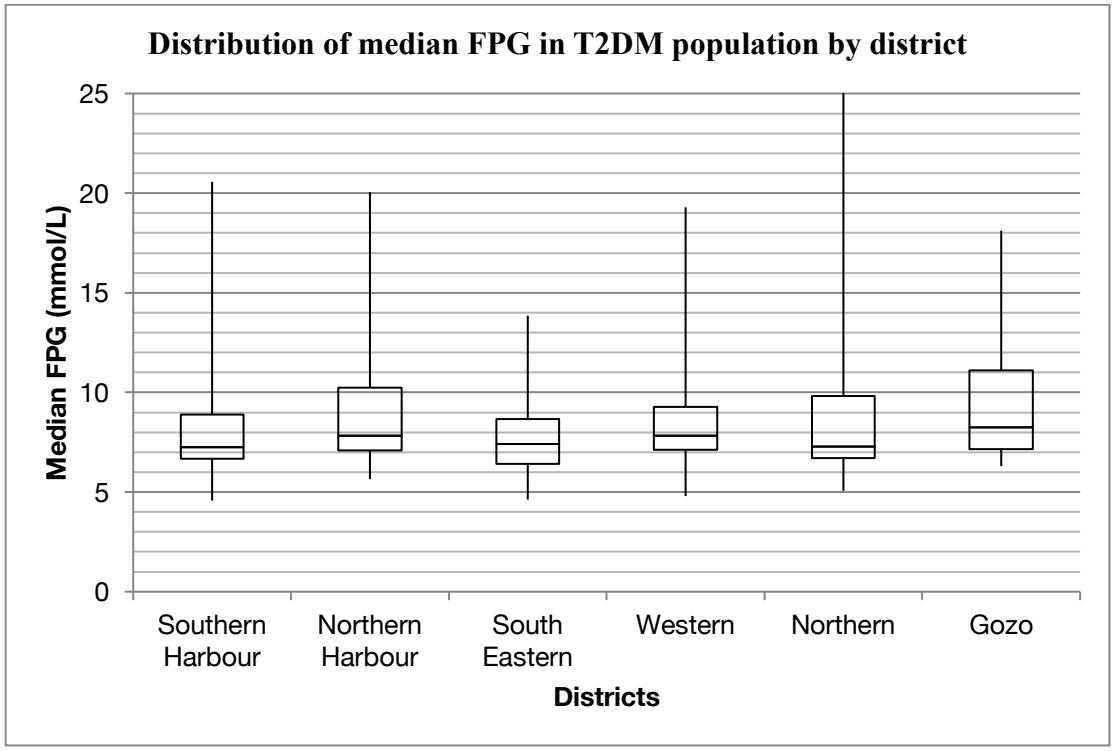
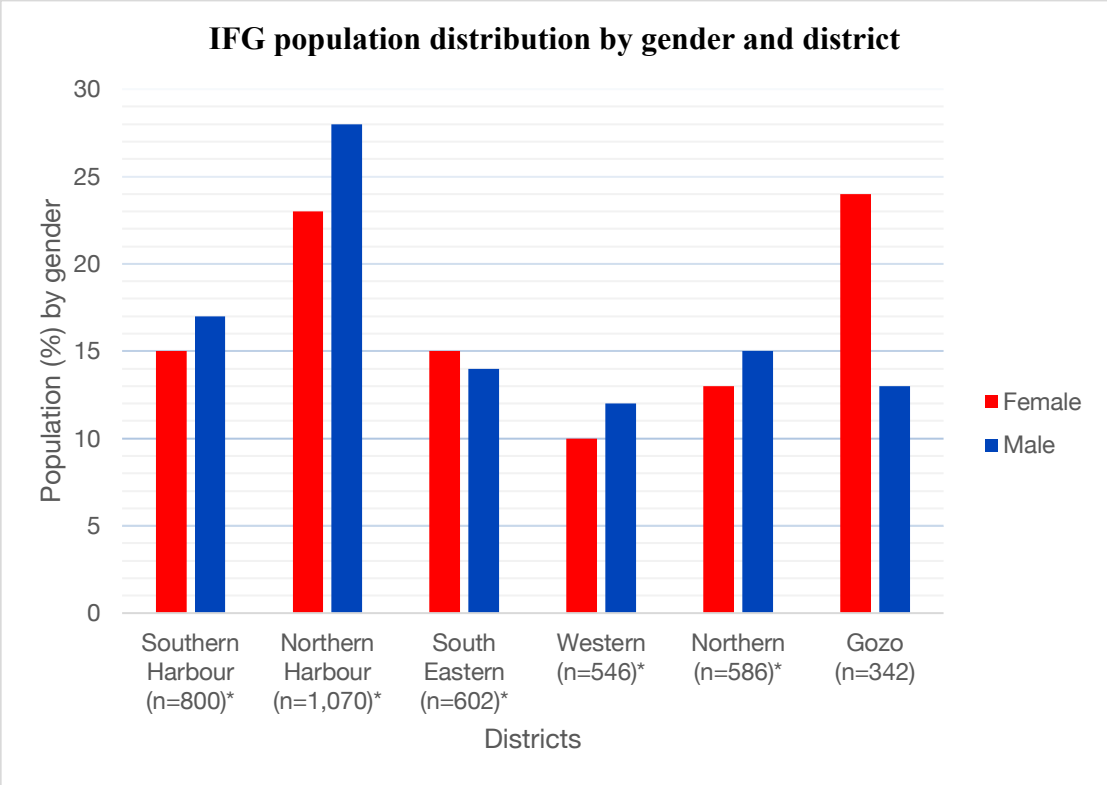



Figure 3.14 Distribution of median FPG and IQR within the diabetes mellitus (T2DM) population, by district

### 3.1.6.1.5 Districts by impaired fasting plasma glucose status

The impaired fasting plasma glucose (IFG) subgroup, similar to the T2DM subgroup, resided mostly in the Northern Harbour area ($p$=<0.01). The IFG subgroup exhibited a male predominance across all the districts in Malta but not in Gozo, as seen in Figure 3.15. However, no significant difference was evident between the male and female IFG population living in Gozo ($p$=0.34), in contrast to the male and female IFG population living in Malta ($p$=<0.05). A significant relationship was evident between the districts and the IFG population ($p$=<0.01).




* $p$-value <0.05

Figure 3.15 Distribution of the IFG population proportion, by the different district and gender

The measured FPG (median) within the IFG population exhibited no differences between districts ($p$=0.41) as illustrated in Figure 3.16.

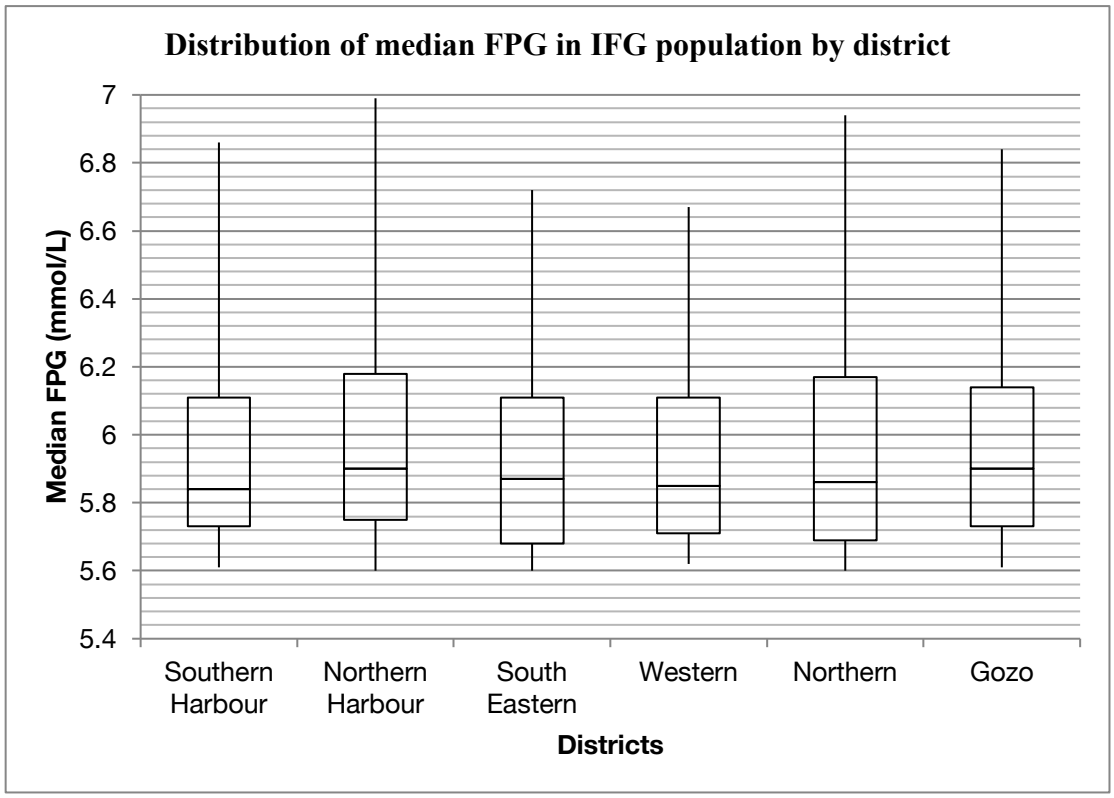
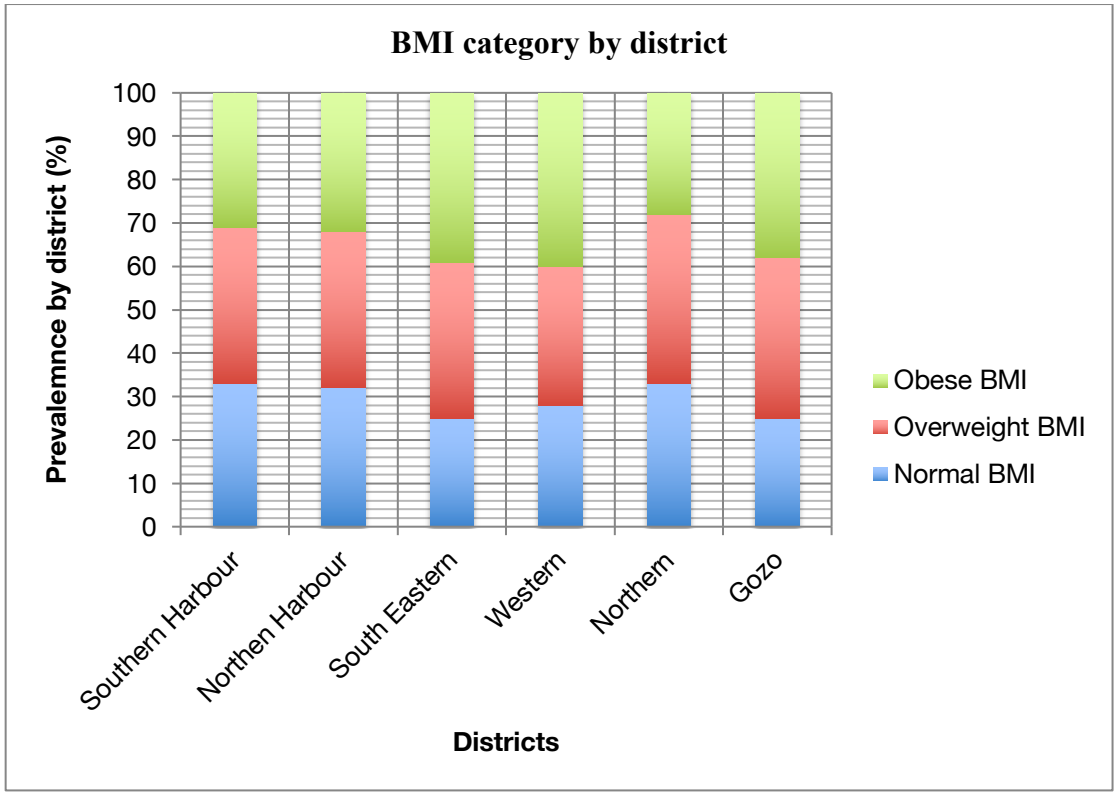



Figure 3.16 Distribution of median FPG and IQR within the impaired fasting glucose population by district

### 3.1.6.1.6 Districts by body mass index status

The Gozo district had the highest overweight-obesity rate (74.85% CI 95%: 69.99 – 79.17) when compared to the other districts, as seen in Figure 3.17. On stratification by gender, the normal BMI status was female predominant across all districts ($p$=<0.01) except for Gozo and South Eastern districts, as seen in Figure 3.18. Otherwise, the overweight-obese categories were similar across both genders and districts ($p$=0.08, $p$=0.18 respectively).




*Chi-square p*=<0.01

Figure 3.17 Distribution of BMI status by district

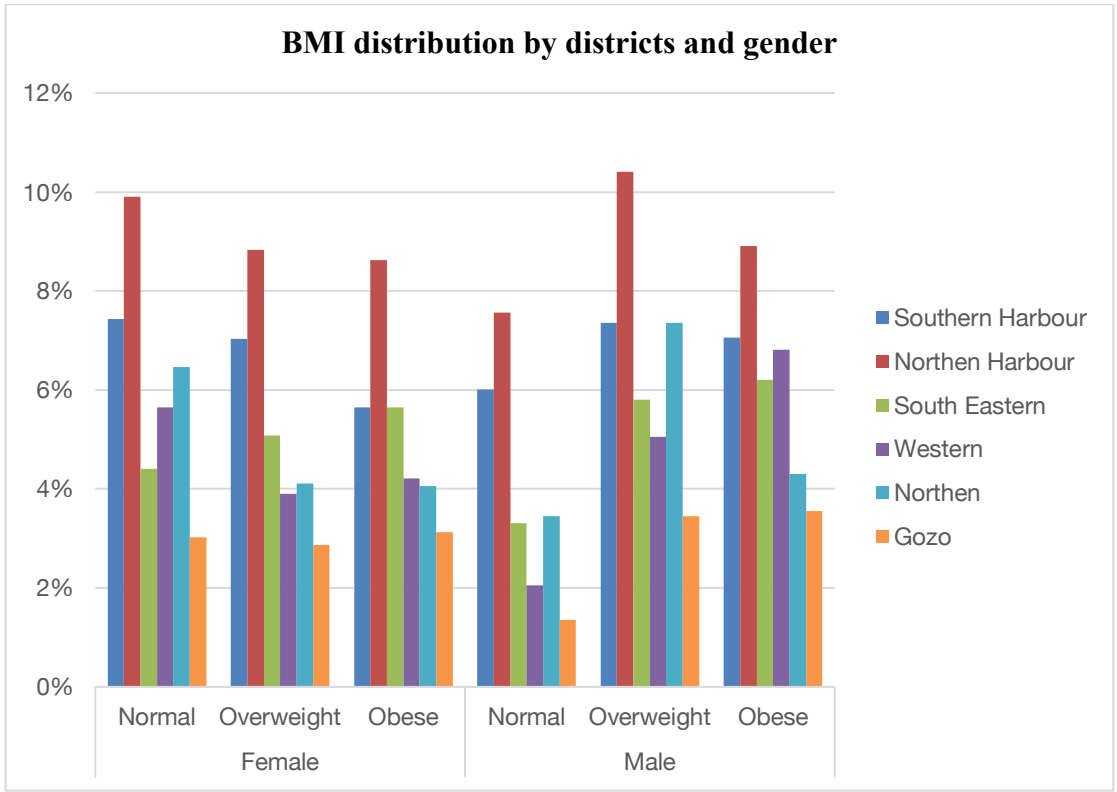



Figure 3.18 Distribution of BMI status by district and gender

Following from the above statement, the Gozo district exhibited the highest median BMI (28.8Kg/m$^2$ IQR: 6.7). Figure 3.19 illustrates a box and whisker plot of the median BMI across the different localities. On pairwise comparison (Dunn's test), the Southern Harbour had a statistically significant lower median BMI than the Western and the Southern Eastern districts ($p$=0.04 and $p$=<0.01 respectively). While the Southern Eastern district had a significantly higher median BMI than did the Northern and the Northern Harbour districts ($p$=0.02 and $p$=0.01 respectively).

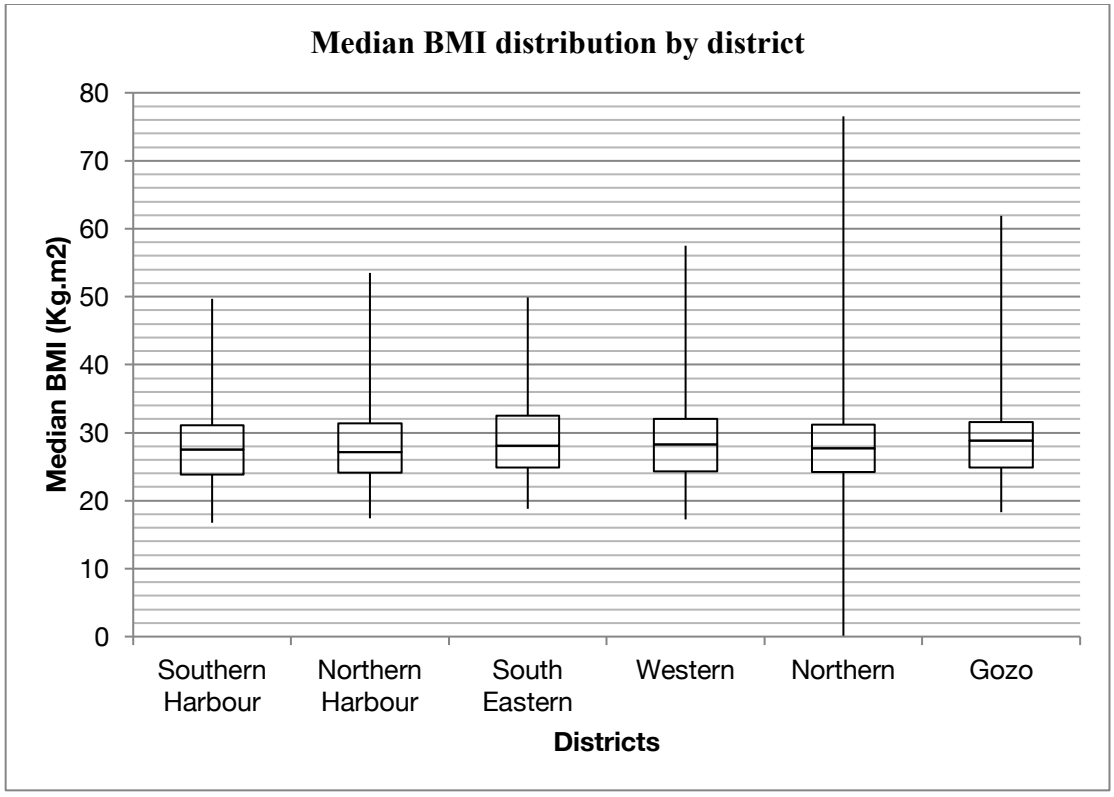



Figure 3.19 Distribution of median BMI and IQR, by district

### 3.1.6.1.7 Districts by metabolic syndrome status

The highest prevalence rate of MetS was found within the Gozo district ($p$=<0.01), with a male predominance, as seen in Figure 3.20.

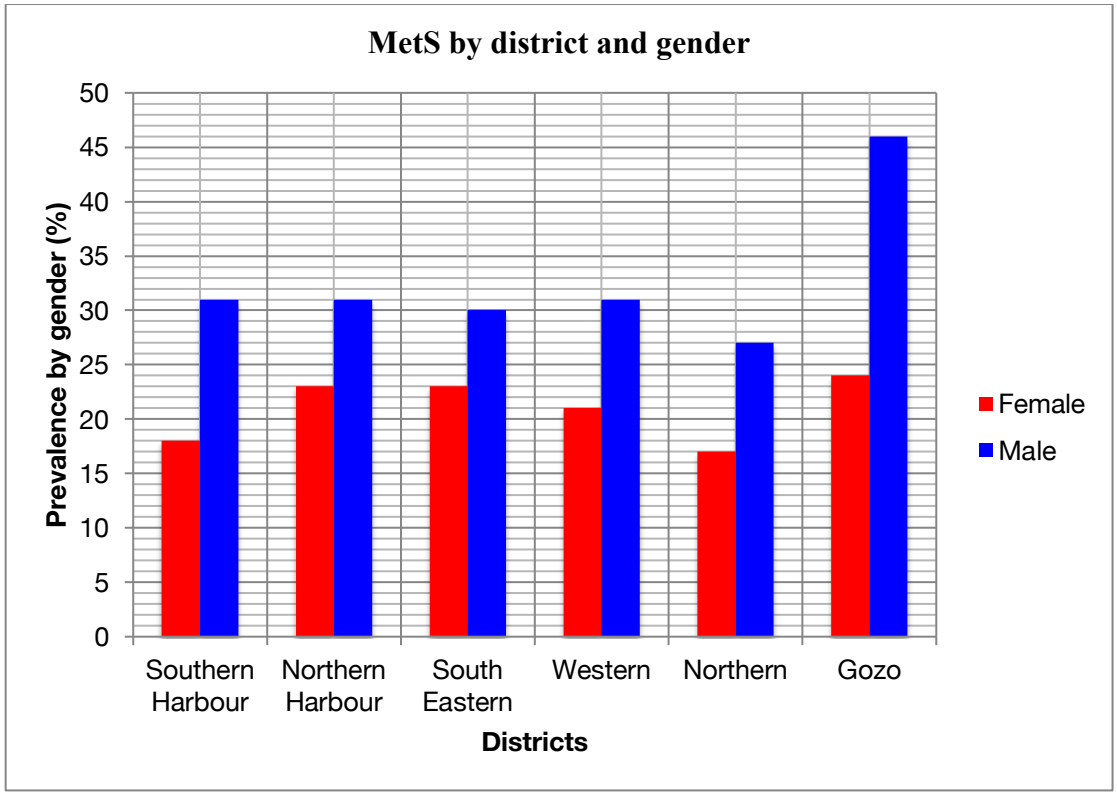
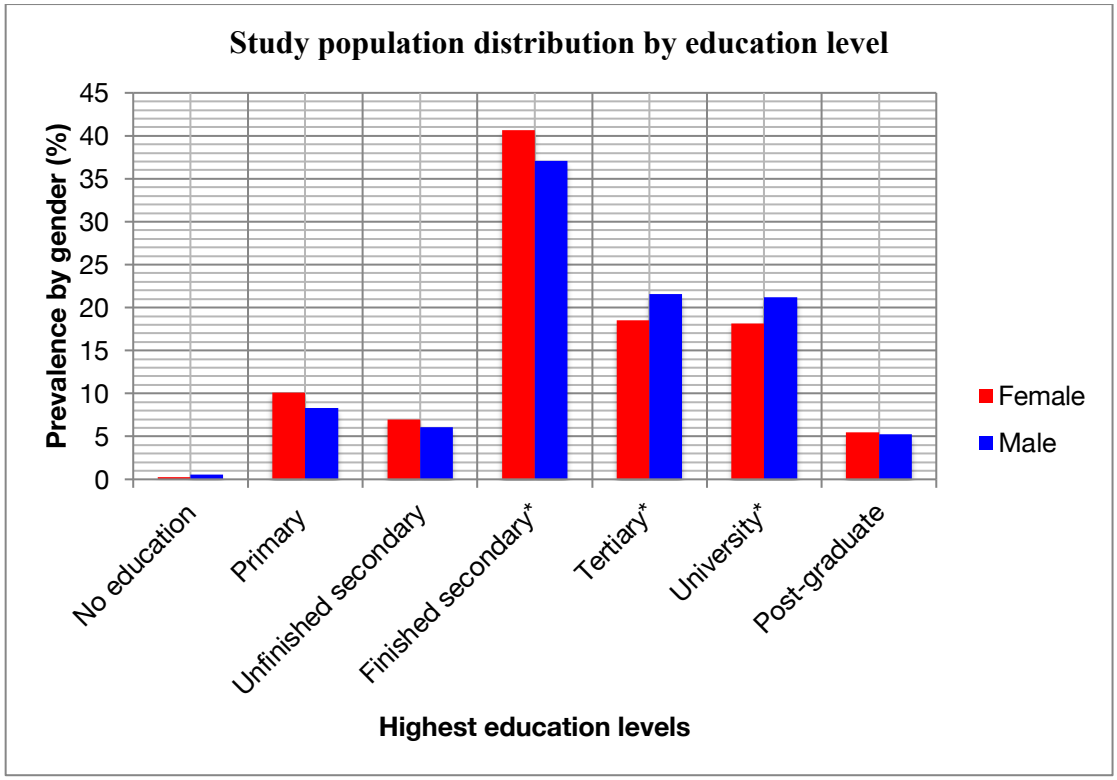



Figure 3.20 Distribution of MetS by districts and gender

### 3.1.6.2 Education and employment status

The highest education level for the majority of the study population was up to secondary school. Education distribution by gender appeared to be homogenous with some small significant gender differences ($p$=<0.05) as illustrated in Figure 3.21. The younger aged (18 – 49 years) participants had generally higher education levels when compared to older participants (>50 years). In fact, a significant positive difference was present between age and education level ($p$=<0.01). As demonstrated in Figure 3.22, those reporting their highest education up to: no formal education, primary level or unfinished secondary education exhibited the oldest median age when compared to higher education levels ($p$=<0.01).




*Significantly different by gender $p$=<0.05 respectively

Figure 3.21 Distribution of highest education level by gender

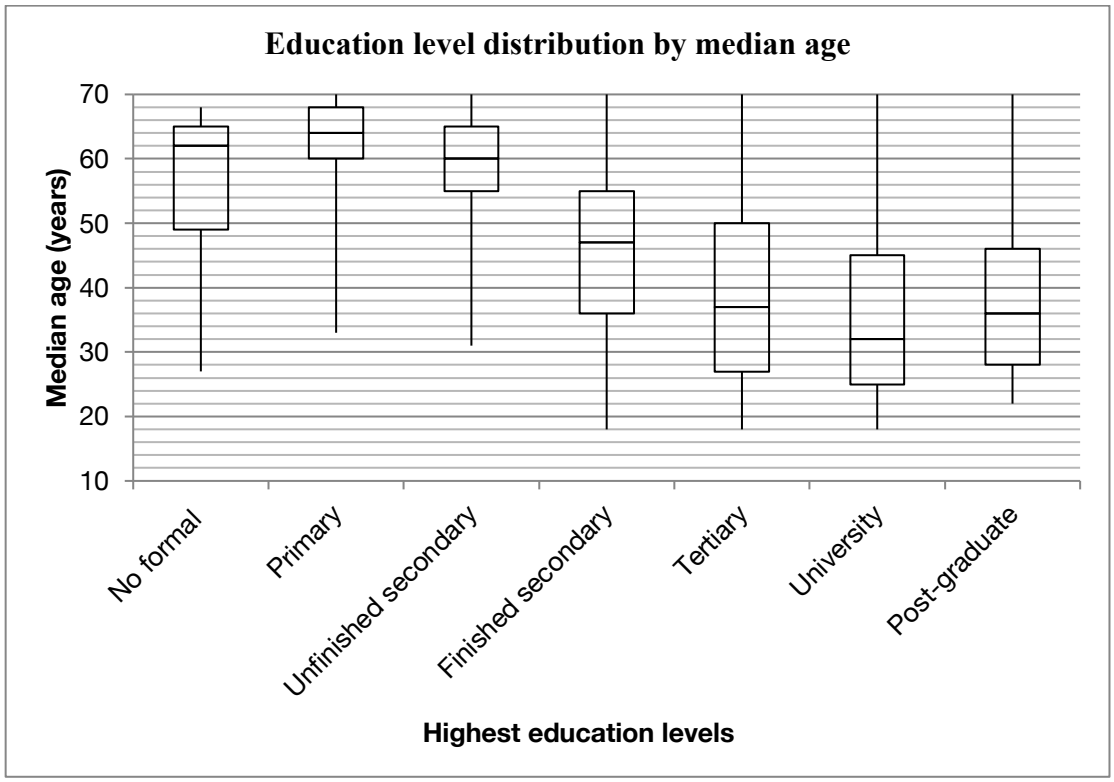



Figure 3.22 Distribution of the highest education level obtained within the study population, by median age (in years) and IQR

The majority of the sample population reported to be employed (63.95% CI 95%: 62.44 – 65.43). A male predominance was evident across all the different employments except for domestic task as the primary job, as seen in Figure 3.23.


Figure 3.23 Distribution of the employment status by gender

A significant inverse relationship was evident between the level of education and the employment status ($p$=<0.01). Those reporting no formal education or education up to primary school were mostly retired. The distribution of the education level by the employment status can be seen in Figure 3.24

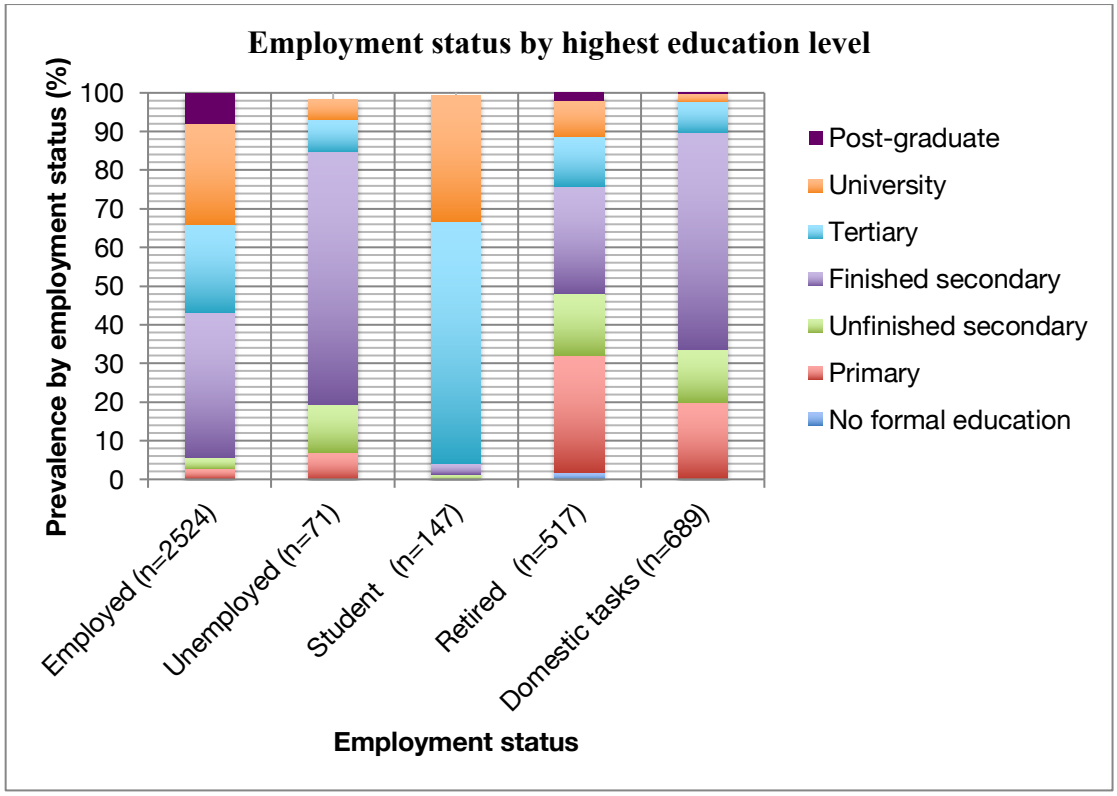



Figure 3.24 Distribution of the employment status by highest education level

The female population exhibited a significantly ($p$=<0.01) higher proportion of those finishing secondary school, also reporting domestic tasks as being their primary employment status. This was in contrast to males who reported to be mostly employed when having an education level below or at the level of secondary school. However, the majority of the male and female population with an education level at tertiary level (sixth form) to post-graduate level reported to be employed.

Interestingly, the female population exhibited an inverse relationship between employment and age, where the younger the female, the more likely they were employed. As demonstrated in Figure 3.25, females between 30 – 39 years dominated within the employed category irrespective of educational level reported at finished secondary school or above. Similar trends were evident for those females between the

age of 40 and 59 years. Noteworthy, those achieving a post-graduate education rarely reported performing domestic tasks as their primary job.

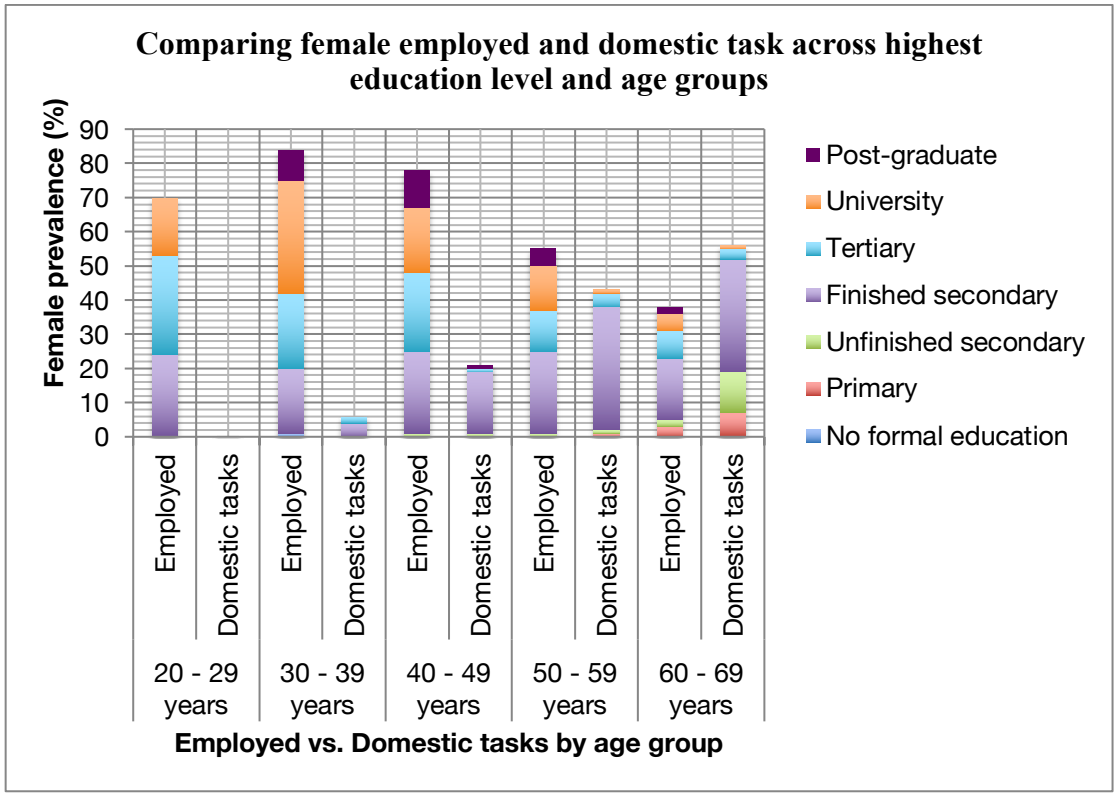



Figure 3.25 Comparisons within the female population between those employed and those with a domestic work status by education levels and age groups

### 3.1.6.2.1 Education and employment status by type 2 diabetes mellitus

The large proportion of the diabetes population (37.59% CI 95%: 33.02 – 42.39%) exhibited the highest education level up till secondary school, in keeping with the whole population. The same highest education distribution was evident on gender stratification of the T2DM population ($p$=0.34) as seen in Figure 3.26.

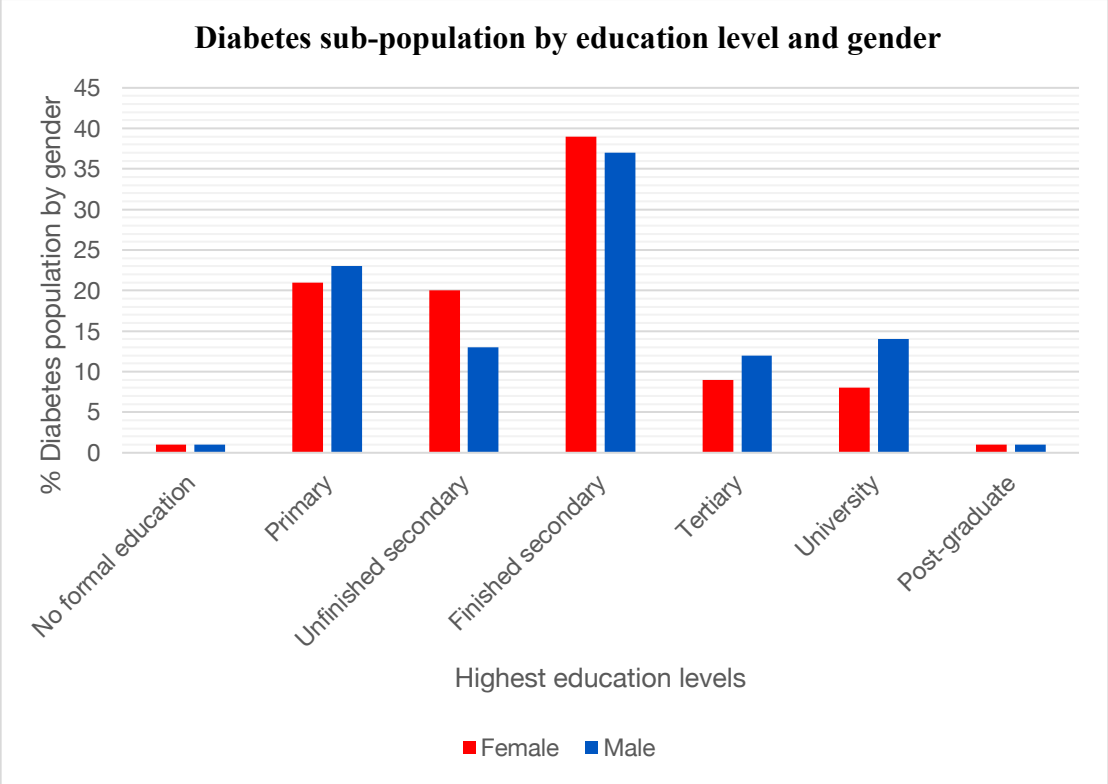



Figure 3.26 Distribution of the diabetes population by education level and gender

On analyzing the median FPG levels within the diabetes mellitus subgroup in accordance to the education levels, it was found that the highest median FPG (14.26 mmol/L IQR: 12.19) was present among those with no formal education ($p$=0.02). Similarly, on gender stratification, the diabetes females were found to exhibit the highest medial FPG levels (19.29mmol/L IQR: 10.20) within those reporting no formal education ($p$<0.01). Conversely, no significant differences were established between the median FPG of the male diabetes sub-group across the different education levels.

A large proportion of the diabetes population (41.52% CI 95%: 36.84 – 46.37) reported to be employed with a male predominance, as seen in Figure 3.27. The majority of the

female diabetes population performed domestic tasks as their primary job, as seen in Figure 3.27.

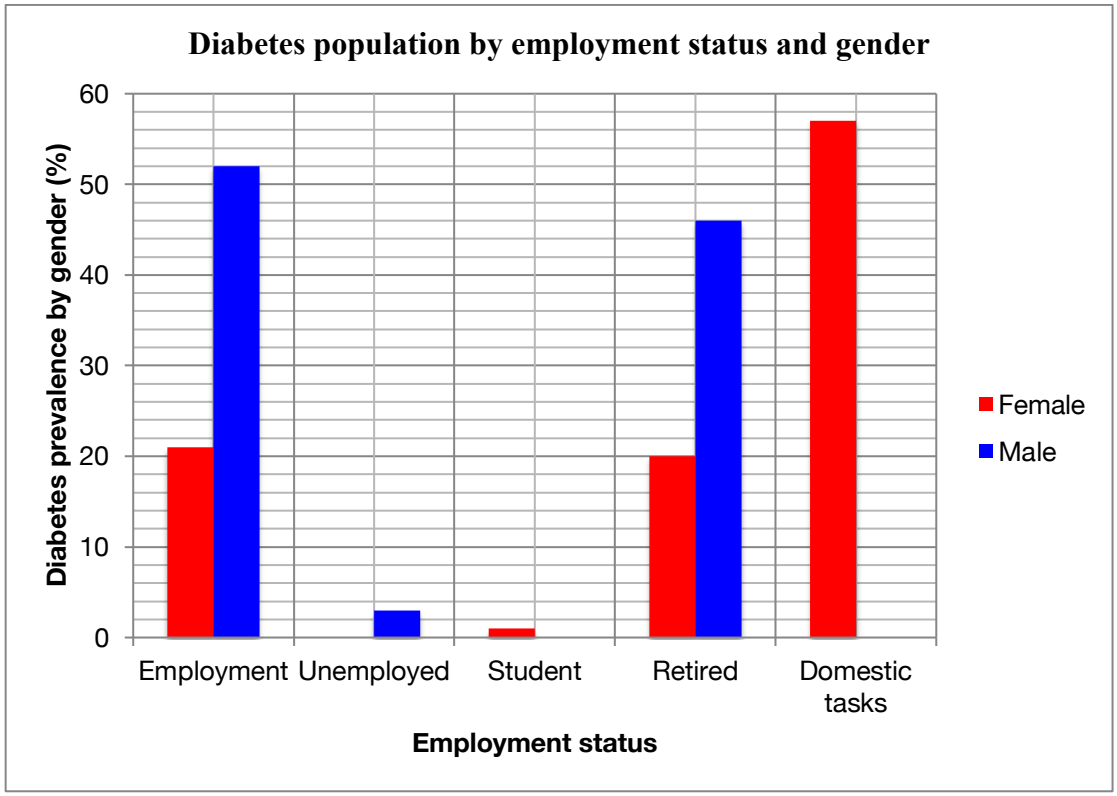



Figure 3.27 Distribution of the diabetes population employment status by gender

### 3.1.6.2.2 Education and employment status by impaired fasting glucose

The education level distribution within the IFG population was in keeping with that of the whole population, where the majority (43.24% CI 95%: 40.08 – 46.46) had an education level up till secondary school. This held true on gender stratification, as seen in Figure 3.28, with significant difference between gender ($p$=0.02).

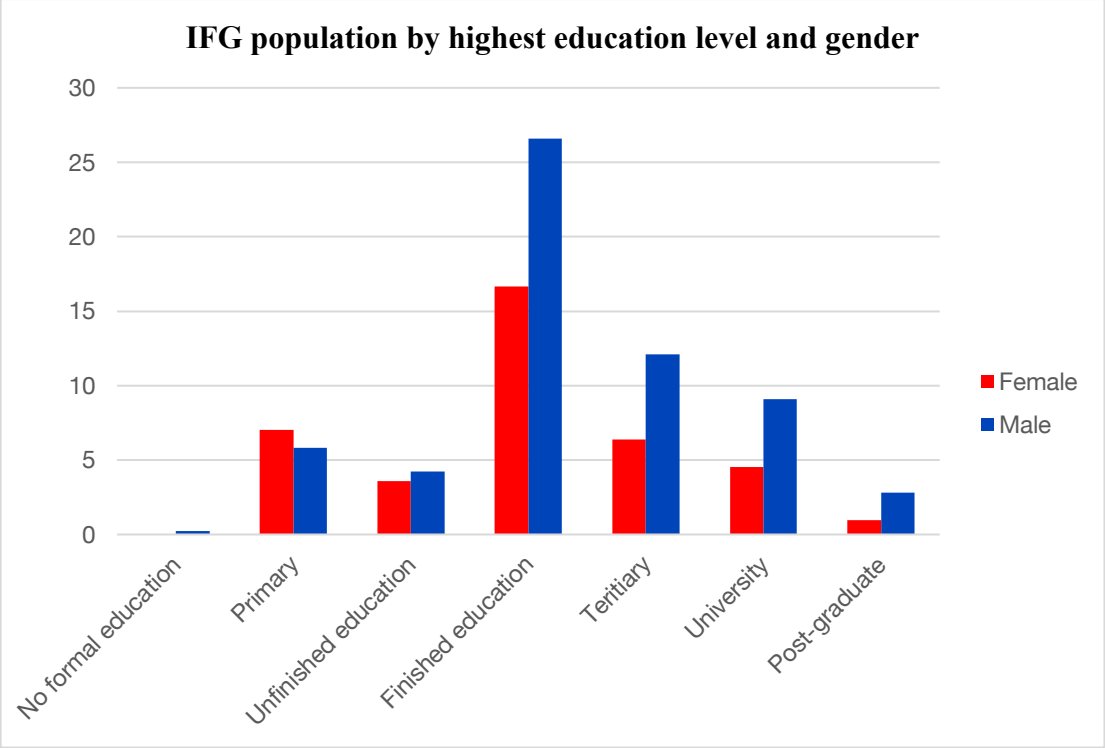



Figure 3.28 Distribution of IFG population by highest education level and gender

The majority of the IFG population reported to be employed (60.76% CI 95%: 57.57 – 63.85) with a male predominance (74.42% CI 95%: 70.66 – 77.86) ($p$=<0.01), as seen in Figure 3.29. The majority of the female IFG population reported to have domestic tasks as their primary job (44.04% CI 95%: 39.01 – 49.20), as seen in Figure 3.29.

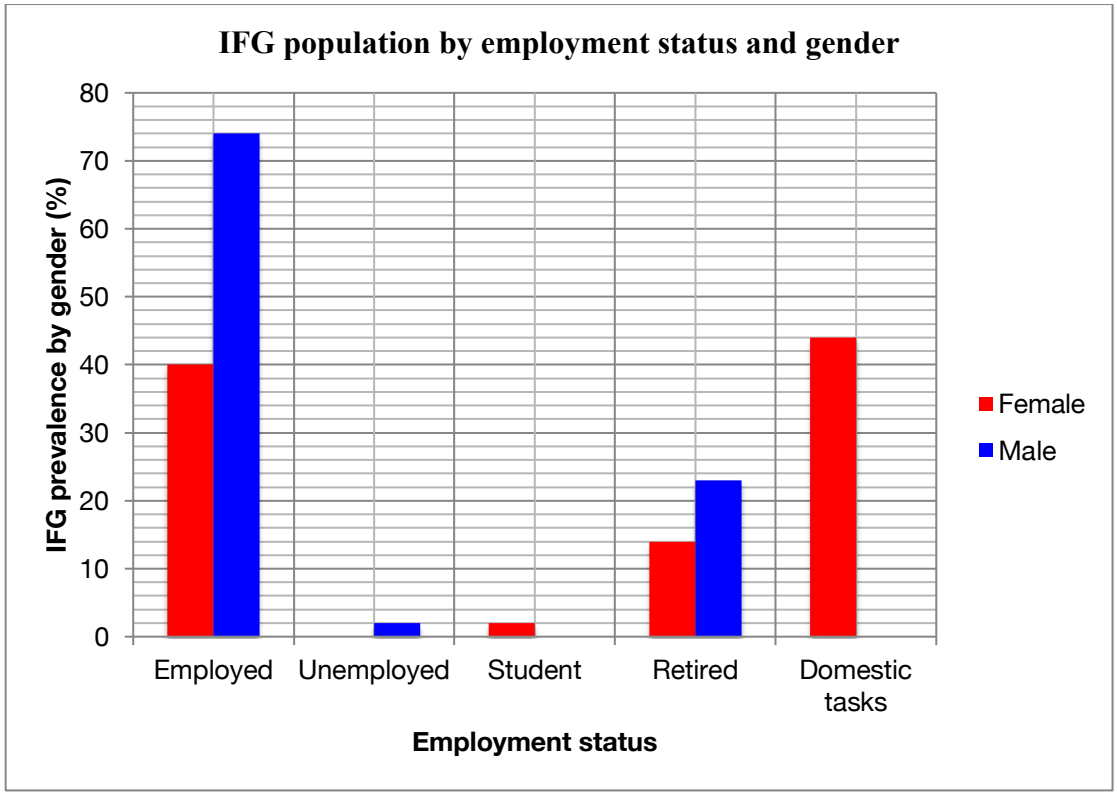



Figure 3.29 Distribution of the IFG population employment status by gender

### 3.1.6.2.3 Education and employment status by body mass index status

As the population education level advanced beyond secondary school, a larger proportion of the population was found to have a normal body weight rather than an overweight-obese BMI status. This was reflected in a significant negative relationship between body type and education level in years (R=-0.25 $p$=<0.01). A similar relationship was present on gender stratification of BMI status by highest education levels, as seen in Figure 3.30

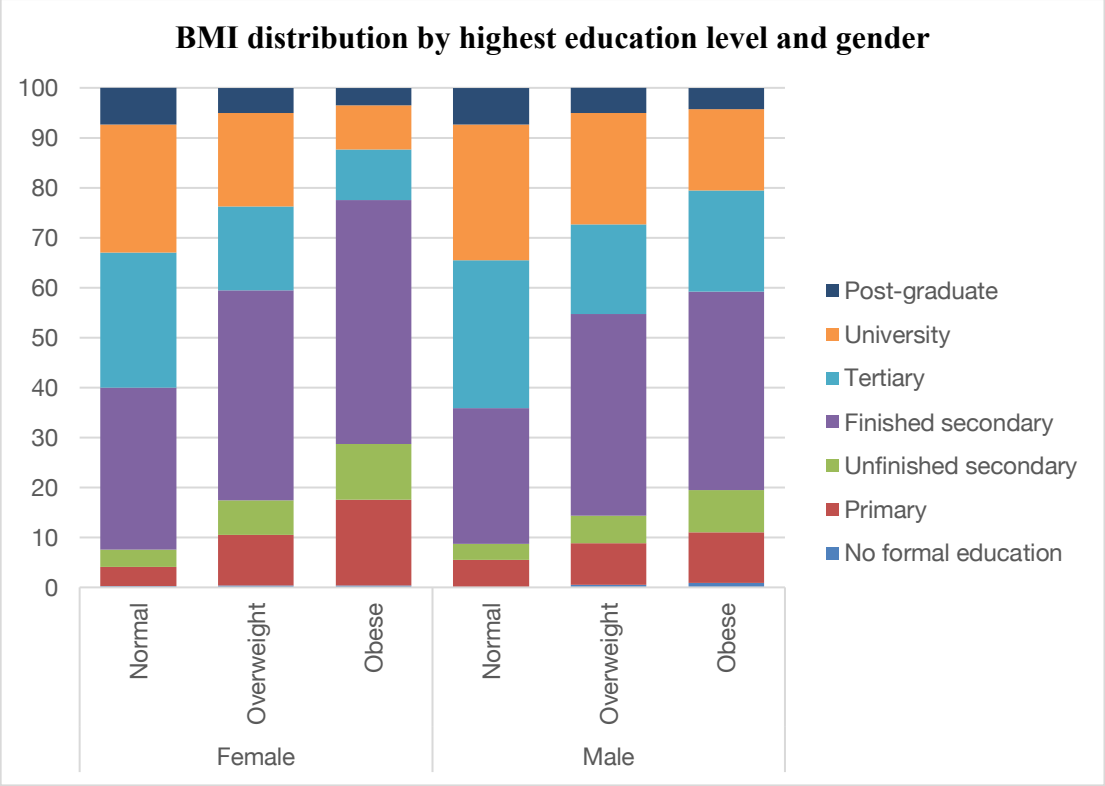



Figure 3.30 Distribution of education level by BMI category

Generally, the population BMI (median) decreased as the education level increased ($p$=<0.01) as seen in Figure 3.31. On pairwise comparison between education level and median BMI, those reporting a post-graduate education had a significantly lower median BMI (25.80Kg/m$^2$) than those reporting education till primary school (BMI 29.90 Kg/m$^2$), unfinished secondary school (BMI 30.10 Kg/m$^2$) and finished secondary school (BMI 28.42 Kg/m$^2$), ($p$=<0.01 respectively). Similarly, those reporting education till university level (BMI 26.30 Kg/m$^2$) or till sixth form (tertiary, BMI 26.30 Kg/m$^2$) had a statistically significantly lower median BMI than those reporting education till primary, unfinished secondary and finished secondary levels ($p$=<0.01 respectively). Those participants reporting education level till secondary school level had a significantly lower median BMI than those reporting education till primary level ($p$=<0.01) or an unfinished secondary education level ($p$=<0.01).

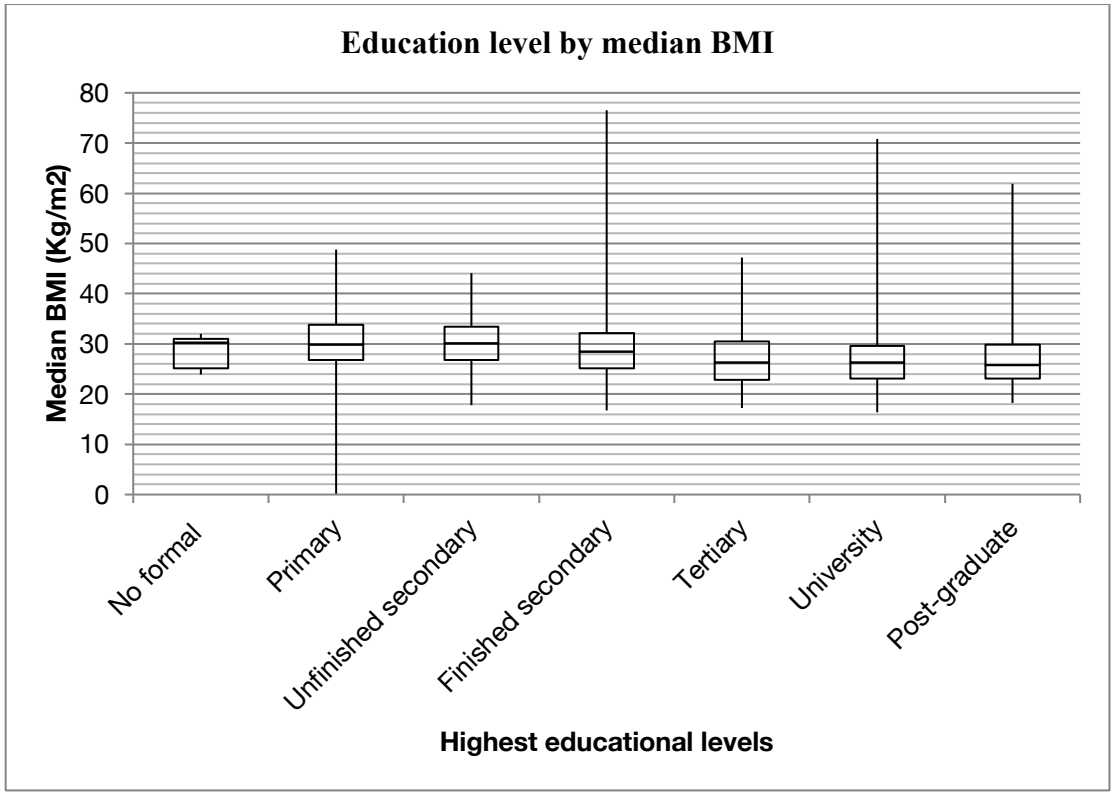



Figure 3.31 Distribution of median BMI by highest education level

The employment status was categorized by BMI status, as seen in Figure 3.32. Of note, the majority of the retired sub-group (83.17% CI 95%: 79.70 – 86.16) and the domestic tasks sub-group (78.37% CI 95%: 75.14 – 81.29) were either overweight-obese. Thus, a significant relationship between BMI (median) status and employment status was found ($p$=<0.01).

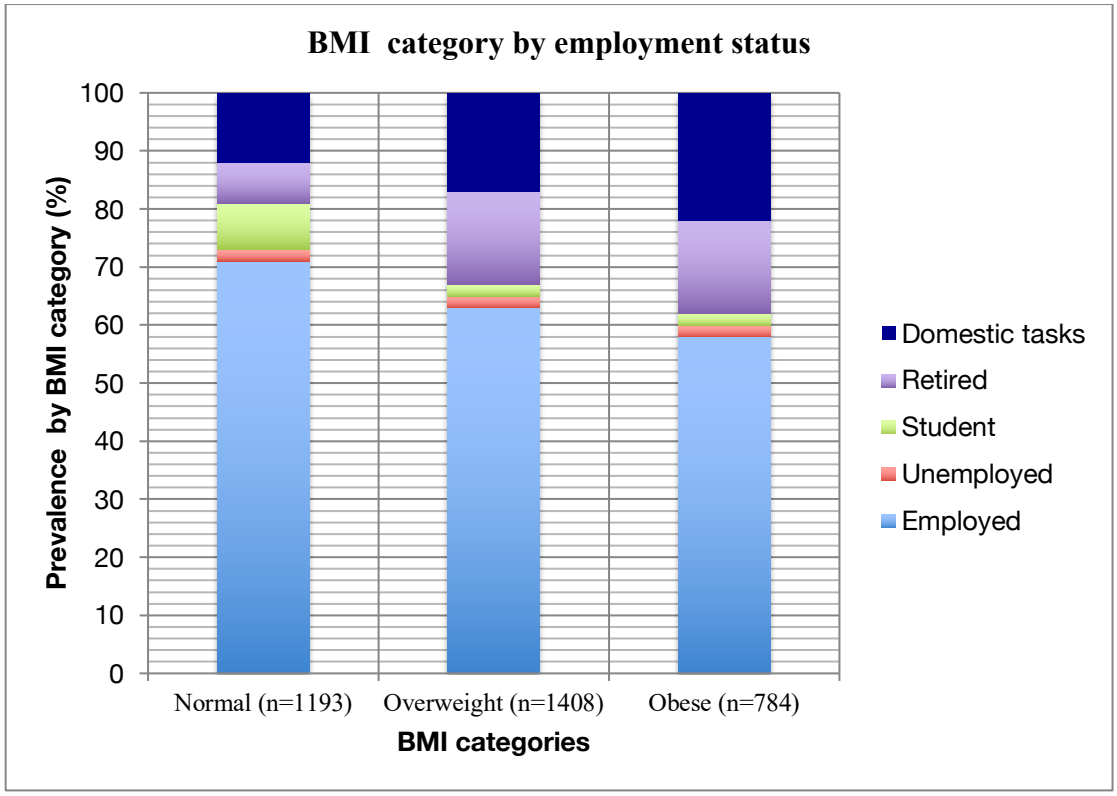



Figure 3.32 Distribution of BMI category by employment status

The median BMI was within the normal range (< 25Kg/m$^2$) only in the students' sub-group. The remaining employment status median BMI fell within the overweight range (25 – 29.99 Kg/m$^2$), with the highest median BMI exhibited by those undergoing domestic tasks as their primary job. There was a significant relationship between median BMI and employment status ($p$=0.01). Figure 3.33 illustrates the distribution of the median BMI across the different employment status. On gender stratification, the female population appeared to have a lower median BMI across the different employment status levels when compared to the male population. However, only the median BMI of the employed and student status showed significant differences between the female and male populations, as seen in Table 3.6.

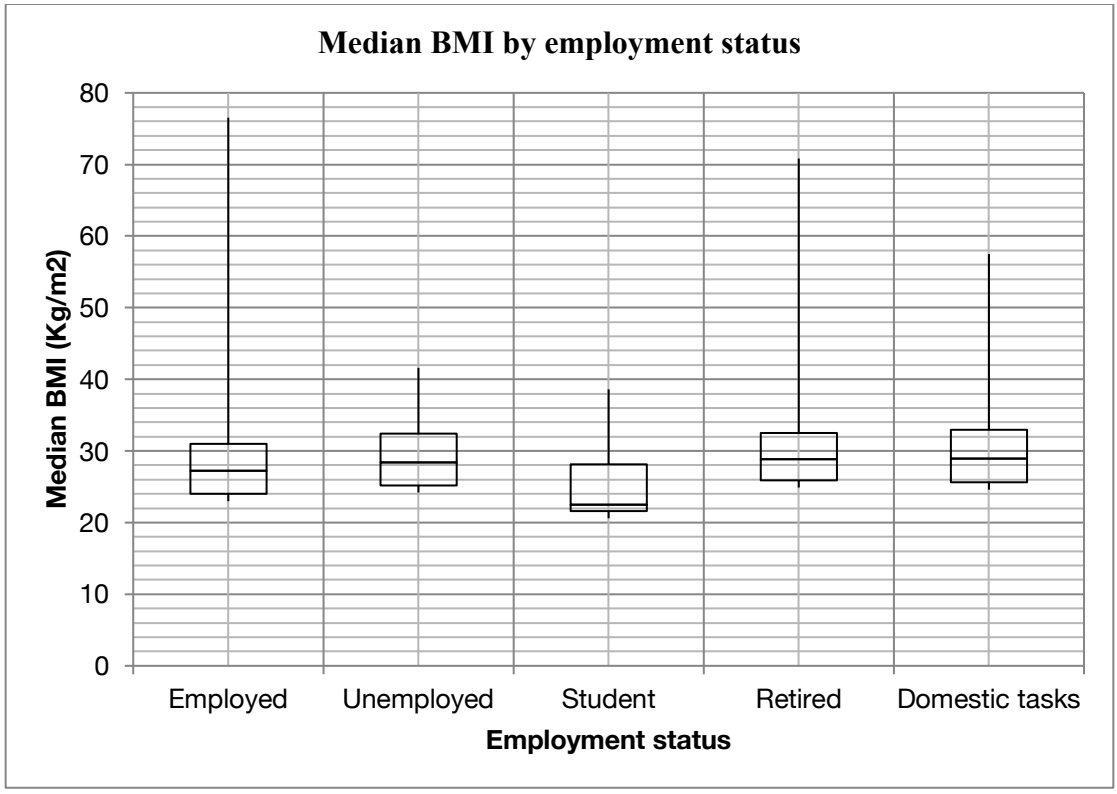



Figure 3.33 Distribution of the median BMI by employment status

| | **Female (*n*=1,949)** | **Male (*n*=1,998)** | |
|---|---|---|---|
| | **BMI Median (IQR)** | **BMI Median (IQR)** | ***p*-value*** |
| Employed | 25.30 (7.26) | 28.30 (6.60) | **<0.01** |
| Unemployed | 26.78 (14.60) | 29.10 (5.87) | 0.34 |
| Student | 21.63 (5.80) | 24.00 (7.80) | **<0.01** |
| Retired | 29.23 (8.16) | 28.58 (6.17) | 0.70 |
| Domestic tasks | 28.91 (7.46) | 28.50 (4.02) | 0.53 |
| *Mann-Whitney U test | | | |
| IQR= Interquartile range | | | |

Table 3.6 Comparisons between employment status by the median BMI and gender

### 3.1.6.2.4 Education and employment status by metabolic syndrome

The education level distribution within the metabolic syndrome population followed similar trends between the male and female populations as seen in Figure 3.34. However, there were significant differences between the male and female population ($p$=<0.01). The commonest education level of the MetS population was education level up to secondary school, similar to the non-MetS population. Above this education level, MetS declined gradually.

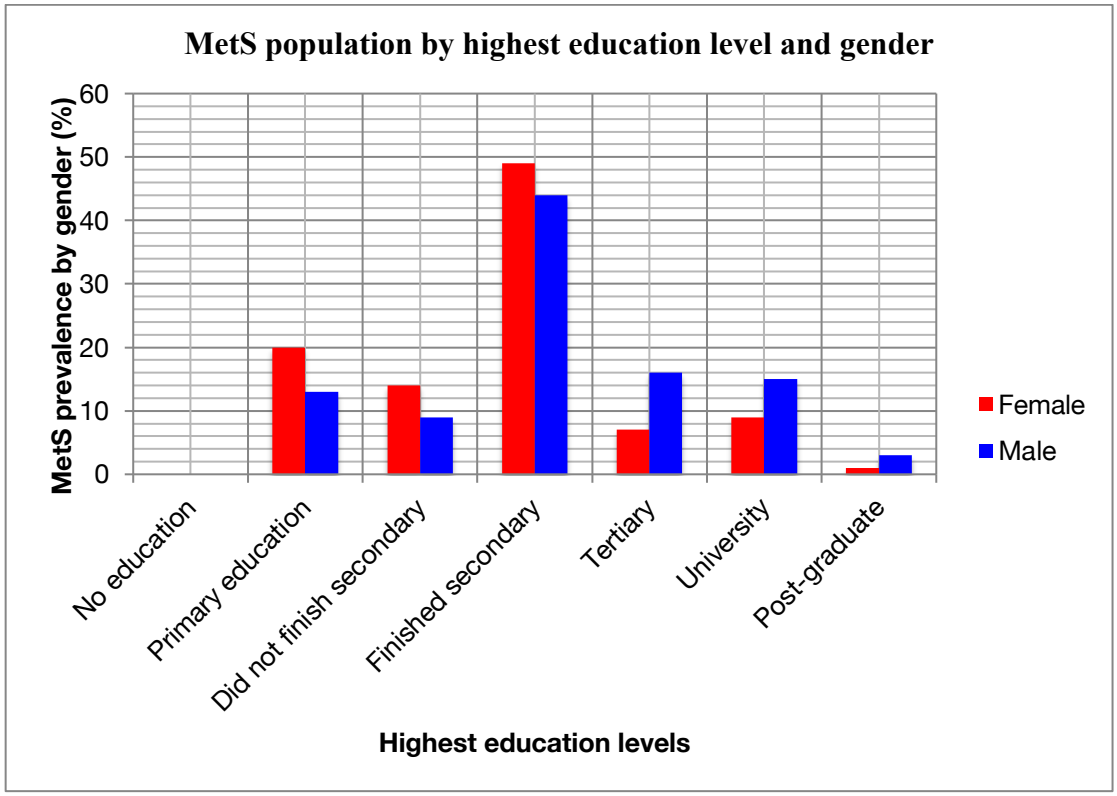



Figure 3.34 Distribution of education level within the metabolic syndrome population, by gender

The majority of the male MetS population reported being employed, while the majority of the female MetS population reported domestic tasks as their primary job, as seen in

Figure 3.35. A significant difference was present between gender and employment status ($p$=<0.01).

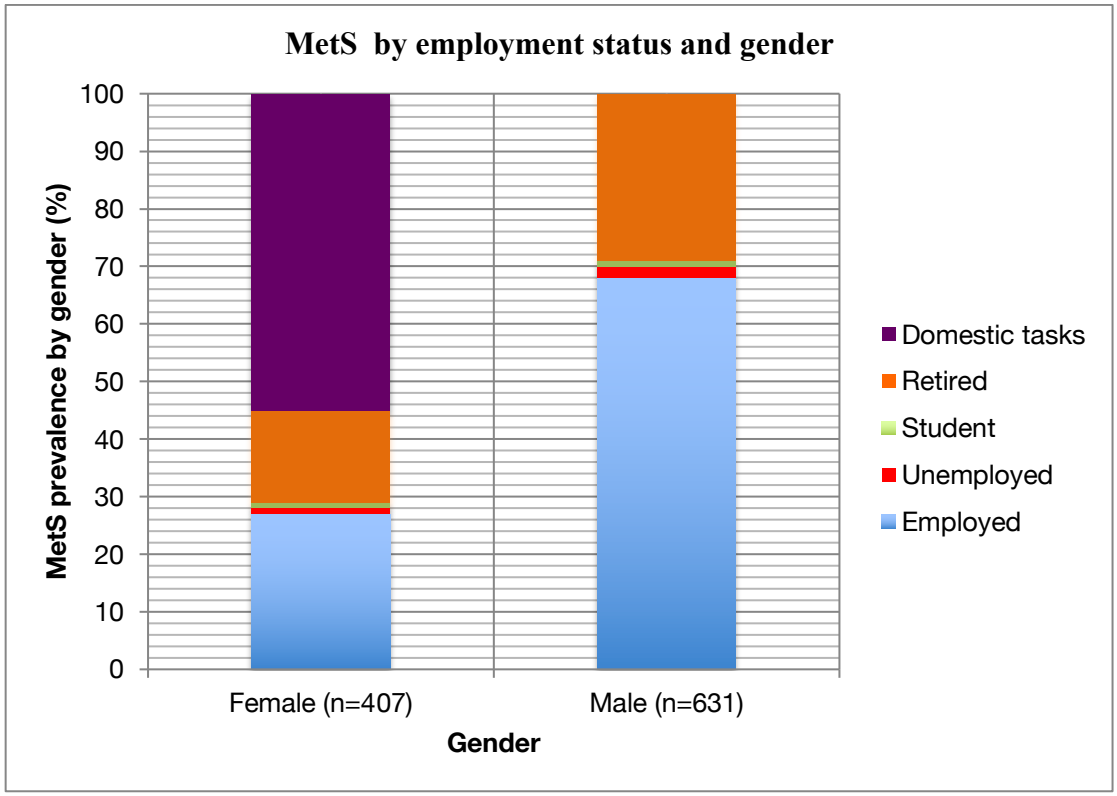



Figure 3.35 Distribution of MetS population by employment status and gender

## 3.1.7 Lifestyle characteristics

### 3.1.7.1 Smoking habits

Of the total adjusted study population ($n$=3,947), 75.70% ($n$=2988; CI 95%: 74.34 – 77.01) were non-smokers with a female majority (53.01% CI 95%: 51.22 – 54.79). A significant difference was apparent by gender and smoking habit ($p$=<0.01).

The study's smoking population included participants reporting to smoke daily or occasionally.

Tobacco smokers (daily and occasional smokers) accounted for 24.22% of the total SAHHTEK population (CI 95%: 22.91 – 25.58), with a median age of 41 years (IQR: 24 years). Males contributed to the majority of the smoking population (62.13%, CI 95%: 59.01 – 65.15%). The distribution of smoking habits by gender among the adult Malta population is illustrated in Figure 3.36.

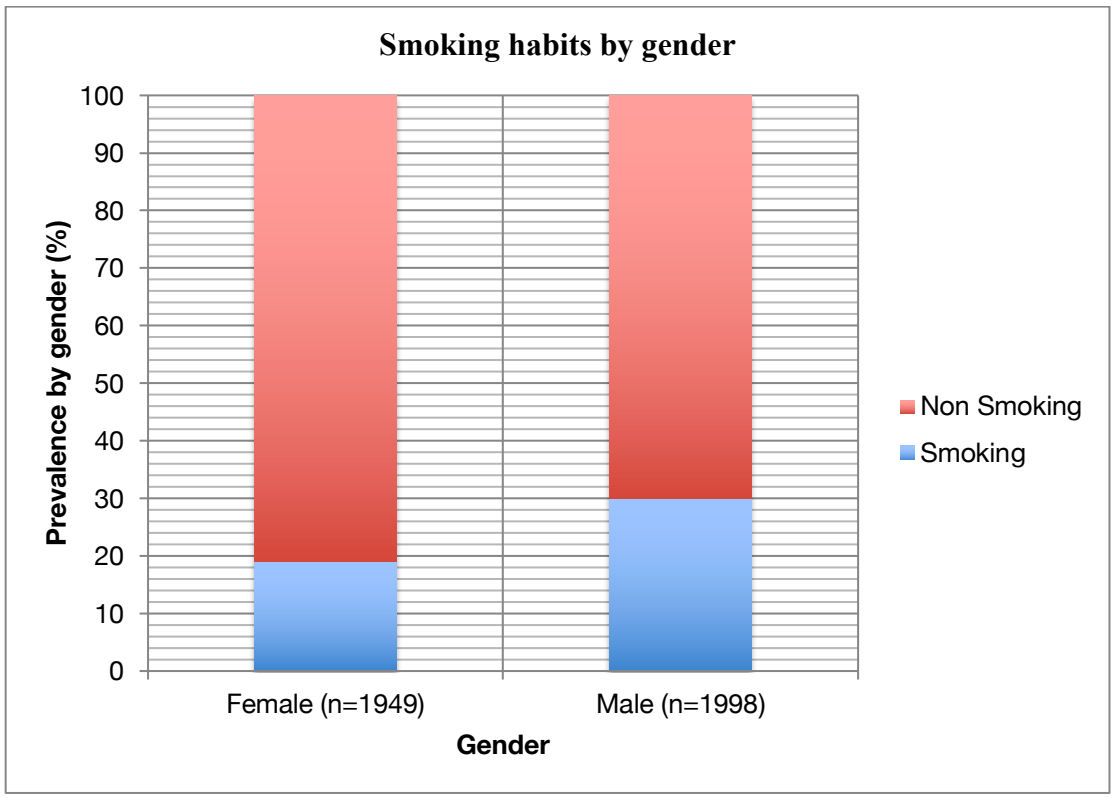



Figure 3.36 Distribution of smoking habits, by gender

Non-smoking was significantly commoner in the general population across all age groups than smoking as seen in Table 3.7. Males between the ages of 30 to 39 years exhibited the highest frequency of smoking out of all the age groups ($p$=0.03).

| | Smoking (*n*=816) | | Non-smoking (*n*=2,310) | | | | |
|---|---|---|---|---|---|---|---|
| **Age groups** | **Female (*n*=319)** | **Male (*n*=497)** | **Female (*n*=1,216)** | **Male (*n*=1,094)** | ***p*-value*** | ***p*-value**** | ***p*-value***** |
| 20 - 29 | 8 (2.51%) | 13 (2.62%) | 34 (2.80%) | 20 (1.83%) | **0.05** | 0.78 | 0.31 |
| 30 - 39 | 76 (23.82%) | 138 (27.77%) | 318 (26.15%) | 248 (22.67%) | **<0.01** | 0.40 | **0.03** |
| 40 - 49 | 85 (26.65%) | 124 (24.95%) | 271 (22.29%) | 265 (24.22%) | **0.02** | 0.10 | 0.75 |
| 50 - 59 | 70 (21.94%) | 104 (20.93%) | 287 (23.60%) | 262 (23.95%) | **0.01** | 0.53 | 0.18 |
| 60 - 69 | 80 (25.08%) | 118 (23.74%) | 306 (25.16%) | 299 (27.33%) | **0.01** | 0.09 | 0.13 |

*Chi square test: Smoking vs. Non-smoking by age group

**Chi square test: Smoking females vs. Non-smoking females by age group

***Chi squared test: Smoking males vs. Non-smoking males by age group

Table 3.7 Distribution of the smoking habits, by age and gender

The highest prevalence of smoking was reported among those with highest education up till secondary school level. The next highest prevalence was among those reporting no formal education as seen in Figure 3.37. A significant difference was found between smoking habits and education levels ($p$=<0.01).

The smoking population was mostly predominated by those reporting to be unemployed, followed by those reporting to be employed, as seen in Figure 3.38. A significant difference existed between smoking habits and employment status ($p$=<0.01).

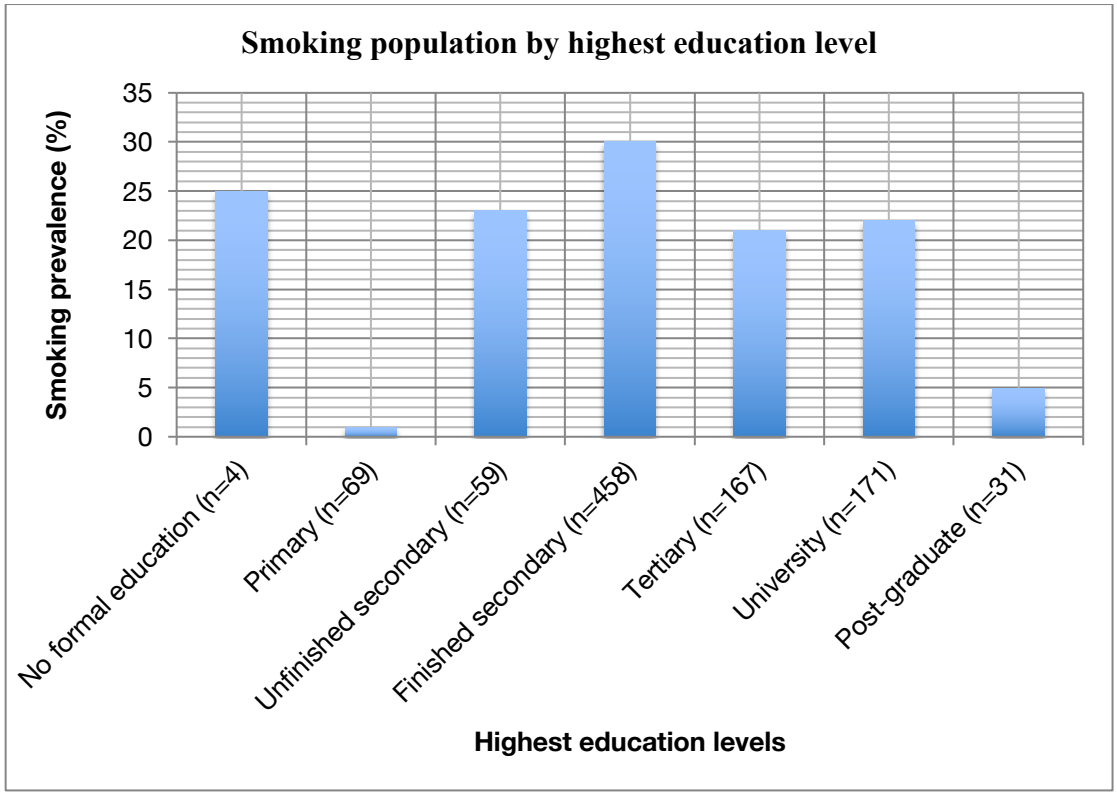



Figure 3.37 Prevalence of smoking within each different education level

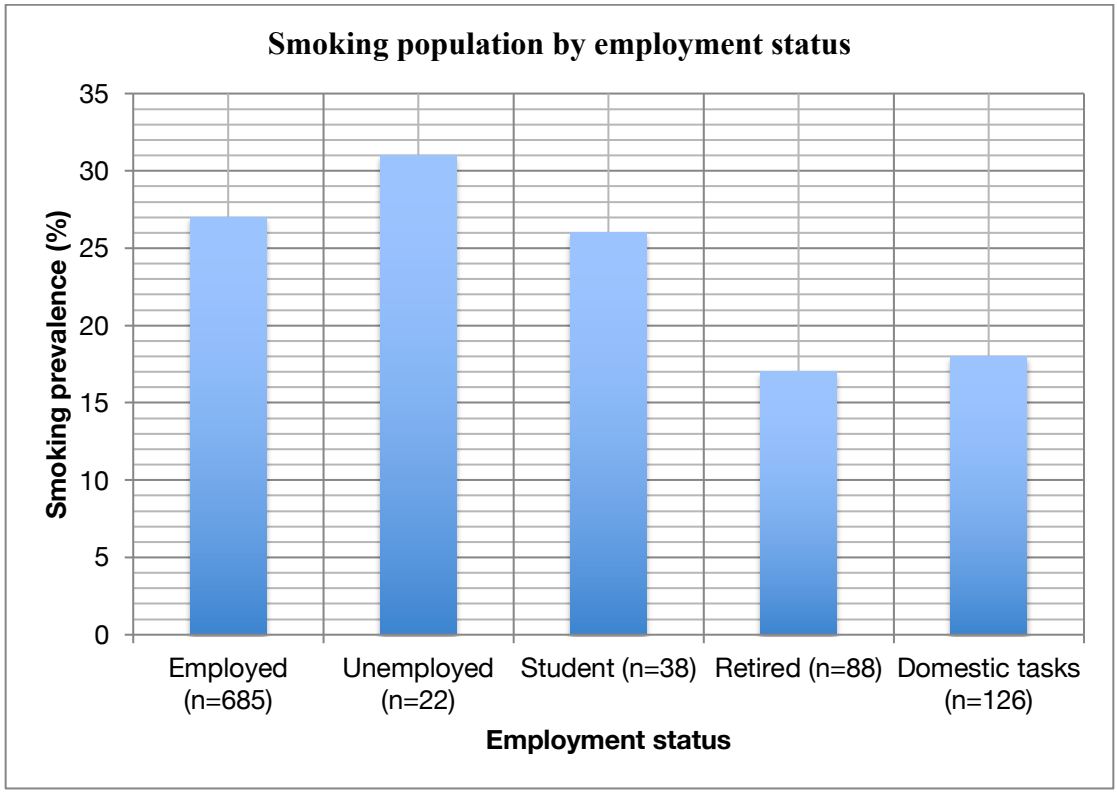



Figure 3.38 Prevalence of smoking within each different employment status

#### 3.1.7.1.1 Smoking habits by glucose regulation

Both the smoking and the non-smoking populations had a median FPG within normal limits, although the smoking population had a significantly lower FPG level (median 5.21mmol/L IQR: 0.87) than did the non-smoking population (5.33mol/L IQR: 0.80; $p$=<0.01). The female smokers exhibited a significantly lower FPG level (median 5.04mmol/L IQR: 0.72) than their male counterparts (5.35mmol/L IQR: 0.89; $p$=<0.01).

#### 3.1.7.1.2 Smoking habits by type 2 diabetes mellitus

The majority of the diabetes population was non-smokers (75.92% CI: 71.53 – 79.83), with a male preponderance. This is in keeping with the non-diabetes population, where the majority was also predominantly non-smoking (75.74% CI 95%: 73.30 – 77.13). However, in the non-diabetes population, there was a female majority that reported having non-smoking habit unlike within the diabetes population. Despite the fact that both populations were predominately non-smokers, a significant difference was exhibited between both populations ($p$=<0.01), with the diabetes non-smoking population being significantly older (median 62 years, IQR: 12) than the non-diabetes non-smoking population (median 44 years IQR: 26; $p$=<0.01). The prevalence of smoking within the previously known diabetes population was of 20.56% (CI 95% 15.98 – 26.05), while that of newly diagnosed diabetes was of 29.11% (CI 95% 22.58 – 36.64; $p$=0.05), with a male predominance (Previously - 72.55% CI 95%: 58.96 – 82.98; Newly – 84.78% CI95%: 71.46 0 92.74).

Only 23.63% of the diabetes population (CI 95%: 20.71 – 29.03) reported to be smokers with a median age of 60 years (IQR 15) and a male majority. However, no significant difference in median age was present between the smokers and non-smokers within the diabetes population ($p$=0.98).

On the other hand, the non-diabetes population exhibited a similar smoking prevalence of 23.26% (CI 95%: 22.87 – 25.70) to the diabetes population. Significantly more non-diabetes males reported to smoke ($p$=<0.01).

No relationship was found between smoking habit and a diagnosis of diabetes ($p$=0.92).

An analysis of the serum fasting plasma glucose control within the smoking and non-smoking diabetes populations was performed. The smoking diabetes population appeared to have a (non-significant) higher median FPG (8.13 IQR: 4.01) when compared to the non-smoking diabetes population (median 7.74 IQR: 2.32), ($p$=0.15).

#### 3.1.7.1.3 Smoking habits by impaired fasting glucose

The majority of the IFG population also reported to be non-smokers (79.93% CI 95% 77.20 – 82.41), as was the case with the general population. However, there were a significantly higher proportion of non-smokers in the IFG population when compared to the general population ($p$=<0.01). A positive relationship was present between IFG and smoking habit ($p$=<0.01) although smoking was not found to be associated with having IFG ($p$=0.98).

In the IFG population, non-smoking was a habit predominated by males as contrasted by the situation within the general population where females were the predominant non-smokers ($p$=<0.01). Meanwhile, within the smoking population, males were predominant in both the IFG and the general population. The IFG smoker population was significantly younger (median 51 years IQR: 22) and more male dominant ($p$=<0.01) when compared to the non-smoking IFG population (median 54 years IQR: 22; $p$=<0.01).

#### 3.1.7.1.4 Smoking habits by body mass index status

There was a strong and significant relationship between smoking and BMI statuses ($p$=<0.01 respectively). The median BMI in smokers was significantly lower (26.03 Kg/m$^2$ IQR: 3.23) than that BMI 27.10 Kg/m$^2$ (IQR: 7.83) of the non-smokers ($p$=<0.01). Female smokers had a significantly lower BMI (median 26.30Kg/m$^2$ IQR: 8.20) than their male counterpart (median 27.82 Kg/m$^2$ IQR: 7.02; $p$=<0.01).

The smoking population was almost equally of normal body weight (34.86% CI 95%: 31.91 – 37.94) or overweight (35.07% CI 95%: 32.12 – 38.15) BMI status, while 30.06% (CI 95%: 27.24 – 33.04) of smokers were obese.

#### 3.1.7.1.5 Smoking habits by metabolic syndrome

The MetS population, similarly to the non-MetS population, was predominantly non-smoking (75.24% CI 95%: 72.52 – 77.77) and by males (43.64% CI 95% 40.65 – 46.68). Of the smoking MetS sub-population ($n$=257), 69.65% (CI 95%: 63.77 – 74.95)

were males ($p$=<0.01). No significant relationship was found between a diagnosis of MetS and smoking ($p$=0.92).

### 3.1.7.2 Alcohol consumption

The reported alcohol consumption data was categorized into "alcohol habit" for all those who claimed to consume any type and frequency of alcoholic beverage and "no alcohol habit" for those that reported no alcohol consumption in the last 12 months.

The majority of the population reported to consume alcohol (53.51% CI 95%: 51.95 – 55.06) with a significantly male preponderance (61.55% CI 95%: 59.46 – 63.61) ($p$=<0.01). This corresponds to 65.07% (CI 95%: 62.95 – 67.13) of the total male population. The majority of both male and female populations reported to drink *"1 – 5 glasses of beer / wine / liquor"* in every typical occasion with a tendency towards wine. Figure 3.39 shows the frequency of alcohol consumption by gender.

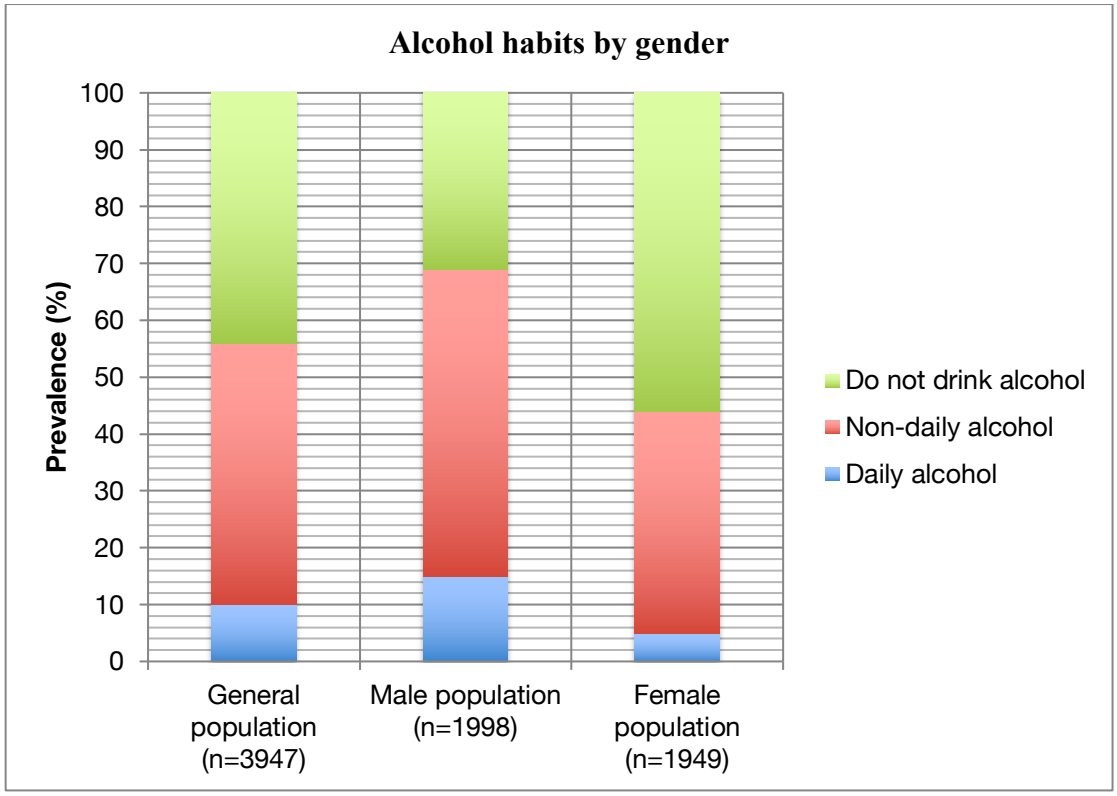



Figure 3.39 Distribution of alcohol consumption habit, by gender

A 10-year age group stratification of alcohol consumption habit was performed. The 30 to 39 years age group showed the highest alcohol consumption habit, while the 60 to 69 years age group had the highest proportion of non-alcohol consumers. A correlation was found between alcohol consumption frequency and age (R=0.15 $p$=<0.01). Non-alcoholics were significantly older (median: 48 years IQR: 26) than the alcohol consumption population (median: 42 years IQR: 26; $p$=<0.01) and more female. The female population that did consume alcohol (median: 41 years IQR: 27) was significantly younger than their male (median: 43 years IQR: 26) counterparts ($p$=0.03).

Alcohol consumption habit prevalence was highest within the post-graduate education level followed by those reporting no formal education and university education levels, as seen in Figure 3.40.

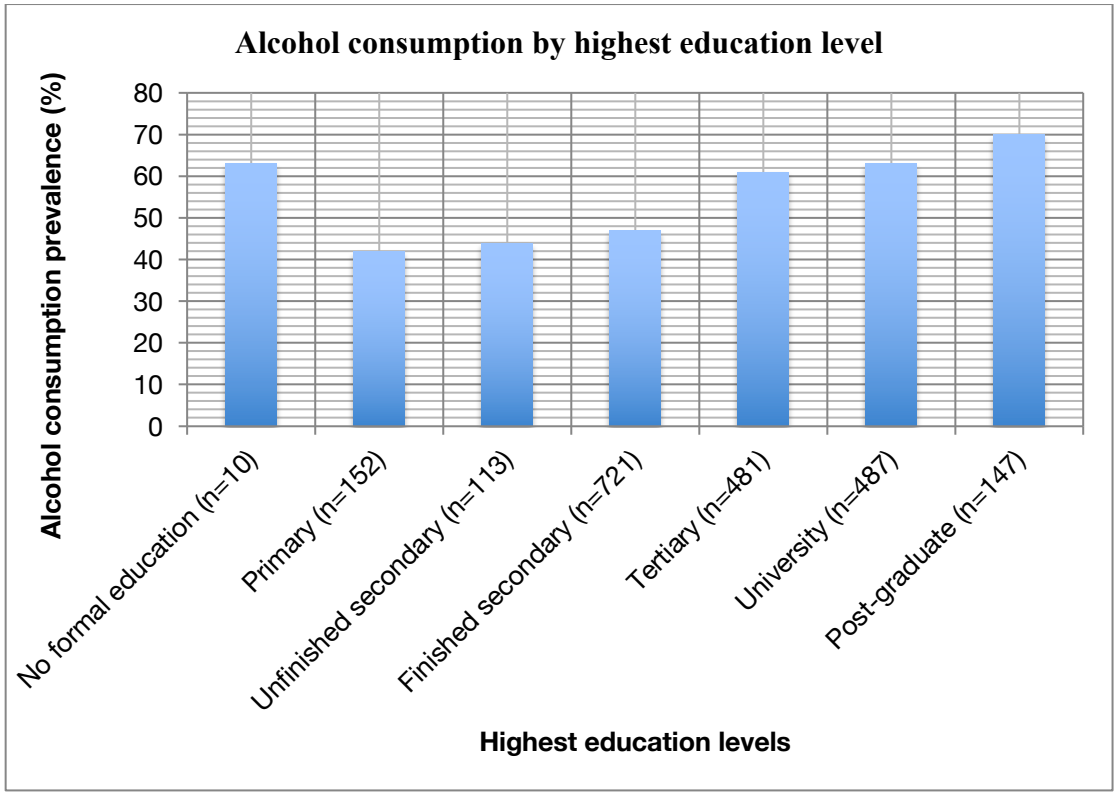



Figure 3.40 Distribution of prevalence of alcohol consumption habits within each education level

A significant difference was evident between alcohol consumption and education ($p$=<0.01). However, no significant difference was evident when comparing alcohol consumption by education and gender ($p$=0.41).

Of interest was the fact that students reported the highest alcohol consummation frequency as seen in Figure 3.41.


Figure 3.41 Distribution of alcohol habits prevalence within each employment status

#### 3.1.7.2.1 Alcohol consumption by glucose regulation

Alcohol consumers (median 5.32mmol/L IQR: 0.83) had no difference in median FPG than teetotalers (median 5.29mmol/L IQR: 0.8; $p$=0.08).

#### 3.1.7.2.2 Alcohol consumption by Type 2 diabetes mellitus

The diabetes mellitus population reported a significantly higher proportion with no alcohol habit (51.60% CI 95%: 46.75 – 56.41; $p$=0.03), which is in contrast to the non-diabetes population. In fact, no significant relationship was present between alcohol intake (in days per week) and diabetes mellitus ($p$=0.99). However, the male

diabetes population exhibited higher alcohol consumption habits (79.80% CI 95%: 73.63 – 84.83; *p*=<0.01) when compared to their female counterparts.

The diabetes population who drank alcohol had a significantly lower median BMI (30.27 Kg/m$^2$ IQR: 7.1 *p*=0.02) but a higher median FPG (7.83mmol/L IQR: 3.21; *p*=0.02) when compared to persons with diabetes who didn't drink alcohol. These persons with diabetes who did drink alcohol were significantly younger (median 60 years IQR: 15; *p*=<0.01) than teetotaling persons with diabetes.

#### 3.1.7.2.3 Alcohol consumption by impaired fasting plasma glucose

The IFG population exhibited a majority reporting an alcohol consumption habit (56.86% CI 95%: 53.65 – 60.02), with a male preponderance (71.86% CI 95%: 67.86 – 75.54) as with the consumption trend of the general population. In fact, a significantly positive relationship (*p*=<0.01) was found between having IFG and alcohol intake (in days per week).

The median age between those who consumed alcohol and those who do not (*p*=0.87) among the IFG population was not different. However, the IFG (median 52 years IQR: 23) alcohol consuming population, exhibited a higher median age when compared to the non-IFG (median 39 years IQR: 25 *p*=<0.01) alcohol consuming population.

The IFG population reporting alcohol consumption habits had a statistically significant lower median BMI (28.79Kg/m$^2$ IQR: 5.70) when compared to those who did not report

any alcohol consumption habits (median 29.88Kg/$m^2$ IQR: 8.33; $p$=<0.01). However, both median BMI's fell within the overweight status.

#### 3.1.7.2.4 Alcohol consumption by body mass index status

The alcohol consuming population (median 27 Kg/$m^2$ IQR: 6.72) exhibited a significantly lower BMI than those reporting no alcohol habit (median 28.50 Kg/$m^2$ IQR: 7.63 $p$=<0.01). The commonest BMI within the alcohol consuming population was that of overweight (36.70% CI 95%: 34.67 – 38.77), followed by normal body weight (33.38% CI 95%: 31.40 – 35.42) and then obese (29.92% CI 95% 28.01 – 31.91). This is in contrast to the non-alcohol consuming population where the commonest BMI was obese (38.89% CI 95%: 36.68 – 41.14), followed by overweight BMI (34.48% CI 95%: 32.34 – 36.68) then by normal weight BMI (26.63% CI 95%: 24.66 – 28.70).

An interesting observation is that the population reporting an alcohol habit exhibited a gender difference between the BMI statuses ($p$=<0.01). In fact, alcohol-consuming males had the highest proportion with an overweight status followed by obese and normal weight statuses. On the other hand, alcohol-consuming females were mostly of normal weight status, followed by overweight and then obese.

#### 3.1.7.2.5 Alcohol consumption by metabolic syndrome

The male MetS population showed significantly higher weekly alcohol consumption levels than did females ($p$=<0.01). The same gender difference holds true for the non-MetS population.

### 3.1.7.3 Physical activity

Physical activity data was gathered using a validated questionnaire that included questions covering frequency, duration, intensity and type of physical activity performed (Meriwether *et al.* 2006, European Commission EUROSTAT 2008).

The majority of the population reported to perform moderate levels of physical activity (59.99% CI 95%: 58.45 – 61.51) with no significant differences between males and females ($p$=0.32). Table 3.8 illustrates the distribution levels of reported physical activity within the general population. No significant difference was present between physical activity and gender ($p$=0.39).

| | **Female [*n*=1,949] (%)** | **Male [*n*=1,998] (%)** |
|---|---|---|
| No activity | 208 (11) | 180 (9) |
| Walk | 363 (19) | 388 (19) |
| Moderate activity | 1158 (59) | 1212 (61) |
| Vigorous activity | 220 (11) | 218 (11) |

Table 3.8 Distribution of physical activity in the general population, by gender

#### 3.1.7.3.1 Physical activity by type 2 diabetes mellitus

The commonest reported physical activity level within the diabetes population was that of moderate activity. However, no significant difference between the males and females was evident (Table 3.9).

| | Type 2 diabetes mellitus | | |
|---|---|---|---|
| | **Female [*n*=136] (%)** | **Male [*n*=271] (%)** | **Chi square *p*-value** |
| No activity | 16 (12) | 18 (7) | 0.17 |
| Walk | 15 (11) | 45 (17) | |
| Moderate activity | 87 (64) | 177 (65) | |
| Vigorous activity | 18 (13) | 31 (11) | |

Table 3.9 Distribution of the diabetes mellitus population by physical activity

#### 3.1.7.3.2 Physical activity by impaired fasting plasma glucose

Moderate physical activity was the commonest activity reported within the IFG population (57.95% CI 95%: 54.74 – 61.09), as can be seen in Table 3.10. No significant difference was established between the different categories of physical activity and impaired fasting plasma glucose ($p$=0.10).

| | IFG | | Chi square *p*-value |
|---|---|---|---|
| | **Female [*n*=362] (%)** | **Male [*n*=563] (%)** | |
| No activity | 43 (12) | 54 (10) | **0.01** |
| Walk | 62 (17) | 132 (23) | |
| Moderate activity | 208 (57) | 328 (57) | |
| Vigorous activity | 49 (14) | 49 (9) | |

Table 3.10 Distribution of the IFG population, by physical activity

### 3.1.7.3.3 Physical activity by body mass index

Moderate physical activity was predominantly reported among both the overweight and the obese populations as seen in Tables 3.11.

| | **Overweight BMI [1408] (%)** | **Obesity BMI [1345] (%)** | *p*-value1 | *p*-value2 |
|---|---|---|---|---|
| No activity | 147 (10) | 122 (9) | 0.15 | **0.03** |
| Walk | 269 (19) | 290 (22) | | |
| Moderate activity | 856 (61) | 782 (58) | | |
| Vigorous activity | 136 (10) | 151 (11) | | |

***p*-value1:** Chi square overweight vs. non-overweight

***p*-value2:** Chi square obese vs. non-obese

Table 3.11 Distribution of the overweight and obese populations, by physical activity

Overweight males reported higher proportions in all the different physical activity categories compared to the overweight females though no significant difference were present ($p$=0.85). The same distribution was evident for obese males when compared to obese females ($p$=0.29). In fact, no relationship was found between physical activity and overweight ($p$=0.10) or obese categories ($p$=0.63).

#### 3.1.7.3.4 Physical activity by metabolic syndrome

The great majority of MetS population (both the male and female), as with the non-MetS population, followed a declared sedentary lifestyle (52.31% CI 95%: 49.27 – 55.34). The different physical activity levels did not have a relationship with a diagnosis of MetS (Low level $p$=0.83, Moderate level $p$=0.77, High level $p$=0.47).

### 3.1.8 Anthropometric characteristics

The anthropometric characteristics that were measured during the health examination survey included blood pressure, waist and hip circumference, as well as weight and height. Tables 3.12 – 3.16 illustrate the median anthropometric characteristics in the general population, the T2DM population, the IFG population and the overweight-obese populations, with comparisons between males and females.

| | Female (*n*=1,949) | | Male (*n*=1,998) | | |
|---|---|---|---|---|---|
| **Anthropometric characteristics** | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| BMI (Kg/m$^2$) | 26.82 | 7.99 | 28.31 | 6.50 | **<0.01** |
| Systolic Blood Pressure (mmHg) | 117.00 | 20.00 | 122.00 | 18.00 | **<0.01** |
| Diastolic Blood Pressure (mmHg) | 72.00 | 13.00 | 78.00 | 12.00 | **<0.01** |
| Body Weight (Kg) | 67.20 | 19.30 | 82.00 | 20.30 | **<0.01** |
| Waist Circumference (cm) | 83.00 | 21.00 | 96.00 | 17.50 | **<0.01** |
| WHR | 0.86 | 0.08 | 0.96 | 0.08 | **<0.01** |
| * Mann-Whitney U test | | | | | |

Table 3.12 Distribution of the median anthropometric characteristics within the general population, by gender

| | Diabetes mellitus population | | | | |
|---|---|---|---|---|---|
| | Female (*n*=136) | | Male (*n*=271) | | |
| **Anthropometric characteristics** | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| BMI (Kg/m$^2$) | 31.90 | 8.42 | 30.40 | 6.60 | **<0.01** |
| Systolic Blood Pressure (mmHg) | 130.00 | 22.00 | 130.00 | 20.00 | 0.64 |
| Diastolic Blood Pressure (mmHg) | 75.00 | 10.00 | 78.00 | 12.00 | 0.22 |
| Body Weight (Kg) | 78.20 | 22.80 | 85.00 | 20.20 | **<0.01** |
| Waist Circumference (cm) | 99.90 | 20.00 | 103.00 | 16.80 | **<0.01** |
| WHR | 0.90 | 0.08 | 0.98 | 0.07 | **<0.01** |

* Mann-Whitney U test

Table 3.13 Distribution of the median anthropometric characteristics within the diabetes population, by gender

| | IFG population | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=362)** | | **Male (*n*=563)** | | |
| **Anthropometric characteristics** | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| BMI (Kg/m$^2$) | 29.30 | 8.22 | 29.10 | 5.70 | 0.68 |
| Systolic Blood Pressure (mmHg) | 123.00 | 20.00 | 124.00 | 16.00 | 0.30 |
| Diastolic Blood Pressure (mmHg) | 76.00 | 10.00 | 80.00 | 13.00 | **<0.01** |
| Body Weight (Kg) | 71.50 | 19.00 | 84.00 | 18.30 | **<0.01** |
| Waist Circumference (cm) | 92.00 | 20.60 | 99.50 | 15.50 | **<0.01** |
| WHR | 0.87 | 0.08 | 0.97 | 0.07 | **<0.01** |

* Mann-Whitney U test

Table 3.14 Distribution of the median anthropometric characteristics within the impaired fasting glucose (IFG) population, by gender

| | Overweight BMI | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=620)** | | **Male (*n*=788)** | | |
| **Anthropometric characteristics** | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| BMI (Kg/m$^2$) | 27.33 | 2.50 | 27.58 | 2.35 | 0.37 |
| Systolic Blood Pressure (mmHg) | 119.00 | 17.00 | 122.00 | 17.00 | **<0.01** |
| Diastolic Blood Pressure (mmHg) | 73.00 | 13.00 | 77.00 | 11.00 | **<0.01** |
| Body Weight (Kg) | 67.80 | 8.10 | 79.30 | 11.50 | **<0.01** |
| Waist Circumference (cm) | 84.00 | 10.00 | 94.00 | 8.00 | **<0.01** |
| WHR | 0.87 | 0.09 | 0.96 | 0.06 | **<0.01** |

* Mann-Whitney U test

Table 3.15 Distribution of the median anthropometric characteristics within the overweight population, by gender

| | Obese BMI | | | | |
|---|---|---|---|---|---|
| | Female (*n*=609) | | Male (*n*=736) | | |
| **Anthropometric characteristics** | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| BMI (Kg/m$^2$) | 33.54 | 6.10 | 33.20 | 4.90 | 0.06 |
| Systolic Blood Pressure (mmHg) | 124.00 | 21.00 | 125.00 | 16.00 | 0.06 |
| Diastolic Blood Pressure (mmHg) | 78.00 | 12.00 | 80.00 | 12.00 | **<0.01** |
| Body Weight (Kg) | 83.80 | 15.00 | 96.40 | 16.30 | **<0.01** |
| Waist Circumference (cm) | 99.50 | 16.00 | 108.00 | 13.00 | **<0.01** |
| WHR | 0.88 | 0.09 | 0.98 | 0.07 | **<0.01** |

* Mann-Whitney U test

Table 3.16 Distribution of the median anthropometric characteristics within the obese population, by gender

### 3.1.8.1 Blood pressure profile

#### 3.1.8.1.1 Self-reported hypertension prevalence rate

The self-reported prevalence of hypertension within the study population was 22.17% (CI 95%: 20.90 – 23.49). The male self-reported prevalence was 24.97% (CI 95%: 23.13 – 26.92), although only 83.77% of those (males) reporting to have hypertension also reported to be on anti-hypertension medication. The female self-reported prevalence was 19.29% (CI 95%: 17.60 – 21.10), although only 88.03% of the reported hypertensive (females) also reported to be on medication (*p*=<0.01).

#### 3.1.8.1.2 Examination hypertension prevalence rate

At the time of the study, 3.22% of participants (CI 95%: 2.71 – 3.82) were found to have either an elevated systolic blood pressure (>=140mmHg) or an elevated diastolic blood pressure (>=90mmHg).

The male population exhibited a statistically significant higher prevalence of elevated blood pressure (2.05% CI 95%: 1.65 – 2.55) when compared to the female population (1.17% CI 95%: 0.87 – 1.55) ($p$=<0.01). These hypertensive individuals included all those who exhibited an elevated blood pressure measured during the health examination, irrespective of whether they were newly diagnosed or known hypertensive.

#### 3.1.8.1.3 Global hypertension prevalence rate

The total prevalence of hypertension within the study population was of 31.87% (CI 95%: 30.44 – 33.34) among whom 57.95% (CI 95%: 54.01 – 59.42) were males, as seen in Table 3.17. The total (global) hypertension population included those self-reported to be hypertensive, those on treatment and the newly diagnosed hypertensive during the health examination survey.

Hypertensive males accounted for 36.49% (CI 95%: 34.40 – 38.62) of the total male population ($n$=1,998), while hypertensive females accounted for 27.14% (CI 95%: 25.21 – 29.16) of the total female population ($n$=1,949), as seen in Table 3.17.

| Blood pressure characteristics | Male (%) [n=1998] | Female (%) [n=1949] | Total (%) [n=3947] |
|---|---|---|---|
| **Normotensive**[#] | 1,269 (63.51) | 1,420 (72.86) | 2,689 (68.13) |
| **Global hypertension** | 729 (36.49) | 529 (27.14) | 1,258 (31.87) |
| ➢ **Unaware *(newly hypertensive)*** | 36 (4.94) * | 15 (2.84) * | 51 (4.05) * |
| ➢ **Aware *(self-reported hypertensive)*** ** | 693 (95.06) * | 514 (97.16) * | 1,207 (95.95) * |
| ⇒ Untreated | 139 (19.07) * | 110 (20.79) * | 249 (19.79) * |
| → Uncontrolled | 13 (1.78) * | 16 (3.02) * | 29 (2.31) * |
| → Controlled | 126 (17.28) * | 94 (17.77) * | 220 (17.49) * |
| ⇒ Treated | 390 (53.50) * | 306 (57.84) * | 696 (17.63) * |
| → Uncontrolled | 32 (4.39) * | 15 (2.84) * | 47 (3.74) * |
| → Controlled | 358 (49.11) * | 291 (55.01) * | 649 (16.44) * |
| **Total uncontrolled blood pressure** | 81 (4.05) | 46 (2.36) | 127 (3.22) |
| **Total controlled blood pressure** | 484 (24.22) | 385 (19.75) | 869 (22.02) |

[#]*Normotensive* – Population did not self-report being hypertensive or on treatment or had an elevated blood pressure during examination
* The percentage is the proportion out of the global hypertension sub-population, accordingly
** Aware (*self-reported hypertension*) includes those who self-reported a history of hypertension or were on treatment

Table 3.17 Demonstrate the hypertension characteristics by gender

### 3.1.8.1.4 Blood pressure by age and gender

An exponential curve relationship was exhibited between increasing age groups and hypertension, as seen in Figure 3.42.

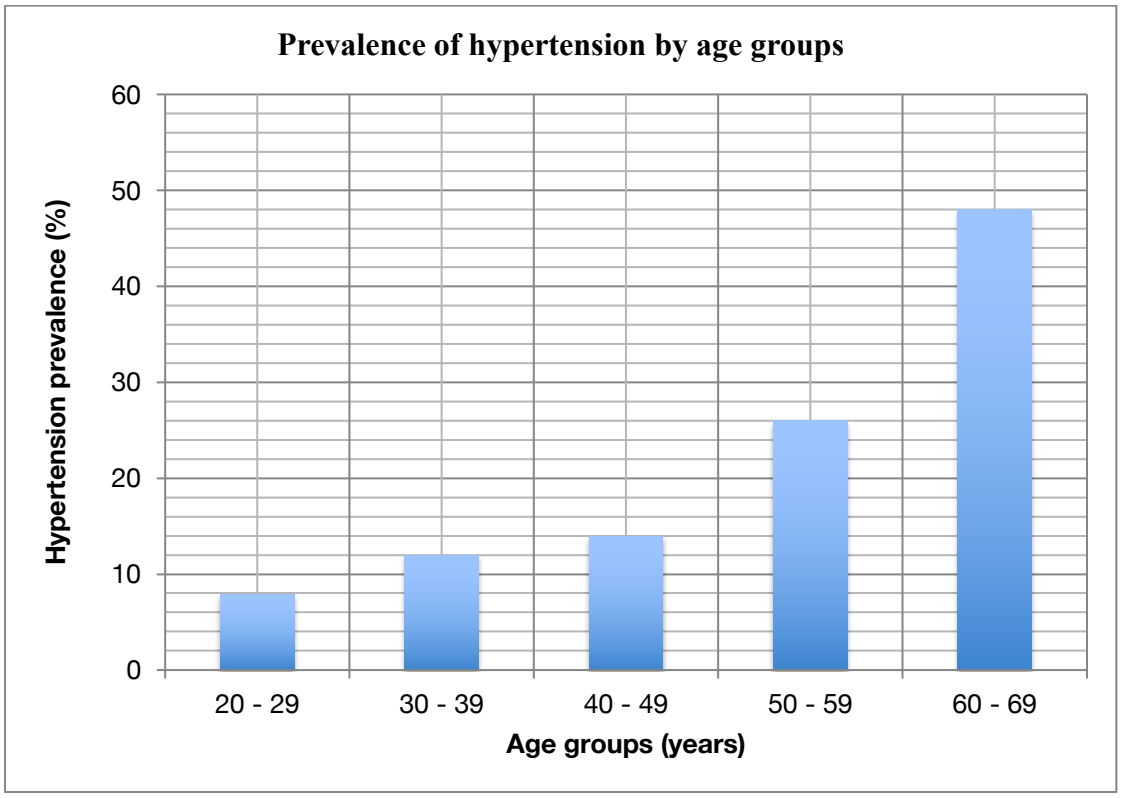



Figure 3.42 Distribution of the prevalence of hypertension within the total population, by age groups

The same general prevalence trend was present for the male population in contrast to the female population, as seen in Figure 3.43.

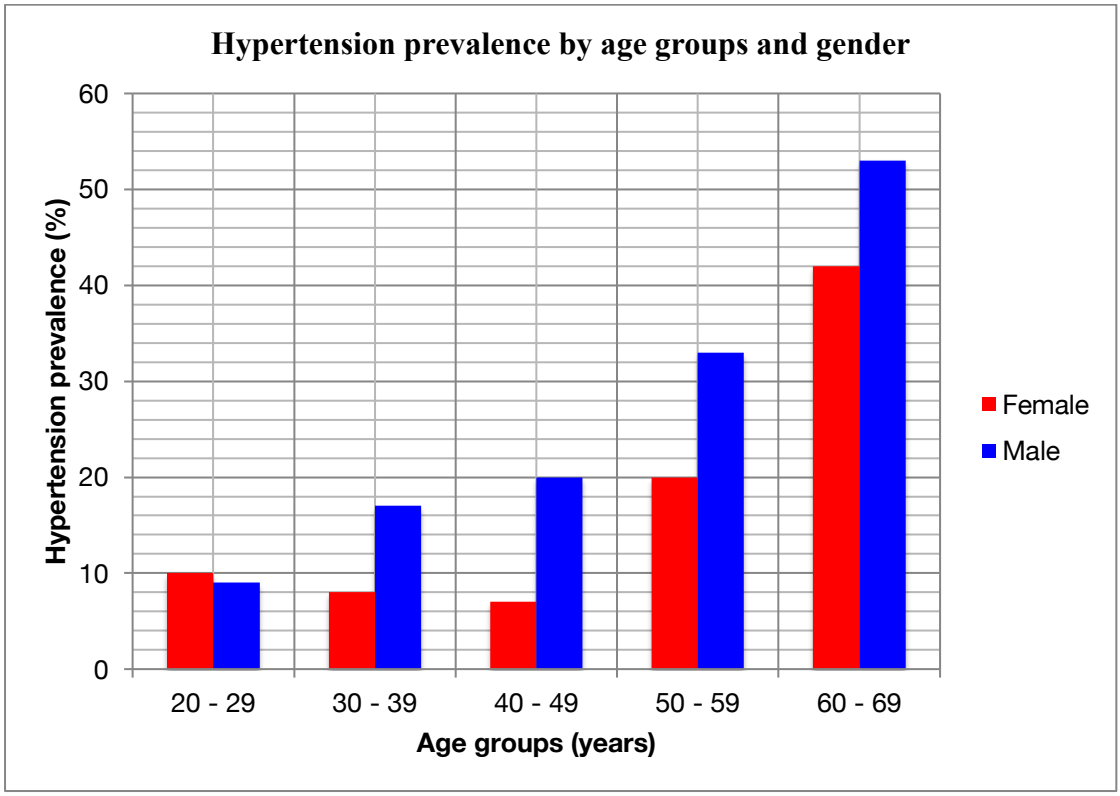



Figure 3.43 Distribution of hypertension prevalence, by gender and age groups

As expected, the hypertensive population was significantly older (median 57 years IQR: 17) than the normotensive population (median 39 years IQR: 23; $p$=<0.01).

### 3.1.8.1.5 Blood pressure by socio-demographic

The socio-demographic comparisons between the global hypertensive population and the normotensive population can be seen in Table 3.18.

Of note, the hypertension prevalence was higher in those (both male and female) reporting their highest education level up till secondary school, as seen in Table 3.18. The hypertension prevalence diminished as the education level advanced. However, the individuals reporting highest educational level up till university level exhibited a higher prevalence rate ($p$=<0.01) of hypertension than those reporting education till tertiary level (sixth form which is a lesser educational level than university).

#### 3.1.8.1.6 Blood pressure by lifestyle characteristics

The majority of the hypertensive population was non-smoker with a male predominance, as seen in Table 3.19.

The hypertensive population reporting an alcohol habit was almost at a par to the hypertensive population reporting no alcohol, as seen in Table 3.19.

The majority of the hypertensive population reported a moderate physical activity per week, as seen in Table 3.19.

| | | | | | Hypertensive | | |
|---|---|---|---|---|---|---|---|
| | | **Normotensive [*n*=2,689] (%)** | **Global Hypertensive [*n*=1,258] (%)** | ***p-value**** | **Male [*n*=729] (%)** | **Female [*n*=529] (%)** | ***p-value**** |
| Locality | Southern Harbour | 585 (21.76) | 215 (17.09) | **<0.01** | 124 (17.01) | 91 (17.20) | **<0.01** |
| | Northern Harbour | 731 (27.18) | 340 (27.03) | | 195 (26.75) | 145 (27.41) | |
| | South Eastern | 382 (14.21) | 220 (17.49) | | 123 (16.87) | 97 (18.34) | |
| | Western | 360 (13.39) | 186 (14.79) | | 109 (14.95) | 77 (14.56) | |
| | Northern | 423 (15.73) | 163 (12.96) | | 91 (12.48) | 72 (13.61) | |
| | Gozo | 208 (7.73) | 134 (10.65) | | 87 (11.93) | 47 (8.88) | |
| Highest education | No formal education | 8 (0.30) | 9 (0.72) | **<0.01** | 7 (0.96) | 2 (0.38) | **<0.01** |
| | Primary education | 160 (5.95) | 201 (15.98) | | 82 (11.25) | 119 (22.50) | |
| | Unfinished secondary education | 105 (3.90) | 152 (12.08) | | 72 (9.88) | 80 (15.12) | |
| | Finished secondary education | 1,033 (38.42) | 497 (39.51) | | 284 (38.96) | 213 (40.26) | |
| | Tertiary education | 615 (22.87) | 178 (14.15) | | 129 (17.70) | 49 (9.26) | |
| | University level | 588 (21.87) | 190 (15.10) | | 136 (18.66) | 54 (10.21) | |
| | Post-graduate education | 180 (6.69) | 31 (2.46) | | 20 (2.75) | 11 (2.08) | |
| Employment status | Employed | 1,898 (70.58) | 625 (49.68) | **<0.01** | 473 (64.88) | 152 (28.73) | **<0.01** |
| | Unemployed | 58 (2.16) | 14 (1.11) | | 8 (1.10) | 6 (0.19) | |
| | Student | 125 (4.65) | 21 (1.69) | | 17 (2.33) | 3 (0.19) | |
| | Retired | 196 (7.29) | 322 (25.60) | | 229 (31.41) | 93 (17.58) | |
| | Domestic tasks | 412 (15.32) | 276 (21.94) | | 2 (0.27) | 275 (51.98) | |

*Chi square

Table 3.18 Distribution of hypertensive and normotensive population by socio-demographic characteristics

| | | | | | Hypertensive | | |
|---|---|---|---|---|---|---|---|
| | | **Normotensive [*n*=2689] (%)** | **Global Hypertensive [*n*=1258] (%)** | ***p-value**** | **Male [*n*=729] (%)** | **Female [*n*=529] (%)** | ***p-value**** |
| Daily Cigarettes | No cigarettes | 2,172 (80.77) | 1,082 (86.01) | **<0.01** | 608 (83.40) | 474 (89.60) | **<0.01** |
| | Light smoker (2 - 9 cigarettes) | 121 (4.50) | 31 (2.46) | | 17 (2.33) | 14 (2.65) | |
| | Moderate smoker (10 - 20 cigarettes) | 316 (11.75) | 114 (9.06) | | 77 (10.56) | 37 (6.99) | |
| | Heavy smoker (>20 cigarettes) | 80 (2.98) | 31 (2.46) | | 27 (3.70) | 4 (0.76) | |
| Alcohol Consumption | Alcohol consumption | 1486 (55.26) | 625 (49.68) | **<0.01** | 458 (62.83) | 167 (31.57) | **<0.01** |
| | No alcohol consumption | 1203 (44.74) | 633 (50.32) | | 271 (37.17) | 362 (68.43) | |
| Physical Activity | No activity | 290 (10.78) | 98 (7.79) | **0.02** | 45 (6.17) | 53 (10.02) | 0.08 |
| | Walk | 522 (19.41) | 230 (18.28) | | 138 (18.93) | 91 (17.20) | |
| | Moderate activity | 1585 (58.94) | 783 (62.24) | | 458 (62.83) | 326 (61.63) | |
| | Vigorous activity | 292 (10.86) | 147 (11.69) | | 88 (12.07) | 59 (11.15) | |

*Chi square

Table 3.19 Distribution of hypertensive and normotensive population by lifestyle characteristics

### 3.1.8.1.7 Blood pressure by type 2 diabetes

Global hypertension was defined as either having a systolic blood pressure ≥130mmHg or a diastolic blood pressure ≥80mmHg or a history of hypertension or on hypertension medication for the diabetes population (as described in Section 2.3.8). The majority of persons with diabetes suffered from global hypertension (69.53% CI 95%: 64.89 – 73.81), among which 63.25% were males (CI 95%: 57.49 – 68.66), as seen in Table 3.20. The hypertension prevalence differences between the male diabetes population and the female counterpart by age groups can be seen in Figure 3.44. Blood pressure (both systolic and diastolic) in the T2DM population was positively correlated with age (R=0.64 $p$=0.02; R=0.75 $p$=<0.01 respectively).

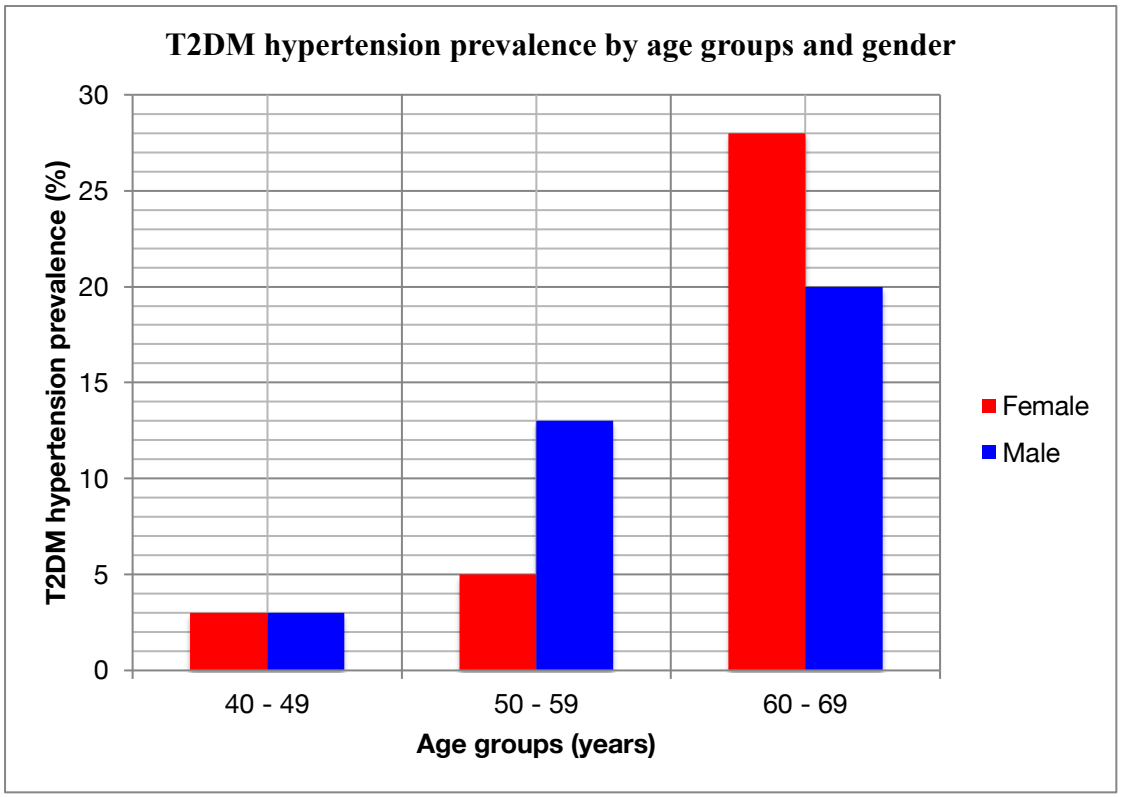



Figure 3.44 Distribution of global hypertension prevalence within the diabetes mellitus population, by age groups and gender

| T2DM Blood pressure characteristics | Male (%) [*n*=271] | Female (%) [*n*=136] | Total (%) [*n*=407] |
|---|---|---|---|
| ▪ **Normotensive** [#] | 92 (33.95%) | 32 (23.53%) | 124 (30.47%) |
| ▪ **Global Hypertensive** | 179 (66.05%) | 104 (76.47%) | 283 (69.53%) |
| ➢ Unaware (newly hypertensive) | 22 (12.29) * | 9 (8.65) * | 31 (10.95) * |
| ➢ Aware (known hypertensive) ** | 157 (87.71) * | 95 (91.35) *] | 252 (89.05) * |
| ⇒ Untreated | 8 (4.47) * | 3 (2.88) * | 11 (3.89) * |
| → *Uncontrolled* | 4 (2.23) * | 3 (2.88) * | 7 (2.47) * |
| → *Controlled* | 4 (2.23) * | 0 | 4 (1.41) * |
| ⇒ Treated | 149 (83.24) * | 92 (88.46) * | 241 (85.16) * |
| → *Uncontrolled* | 105 (58.66) * | 42 (30.88) * | 147 (51.94) * |
| → *Controlled* | 44 (24.58) * | 50 (48.08) * | 94 (33.22) * |
| **Total uncontrolled blood pressure** | 131 (48.34) | 54 (39.71) | 185 (45.45) |
| **Total controlled blood pressure** | 48 (17.71) | 50 (48.08) | 98 (24.08) |

[#]*Normotensive* – Population did not self-report being hypertensive or on treatment and exhibited a normal blood pressure measurement during examination
* The percentage is showing the proportion of the male / female / total T2DM out of the global hypertension sub-population respectively
** Aware (*known hypertension*) includes those who self-reported a history of hypertension or were on treatment

Table 3.20 Demonstrate the T2DM population blood pressure characteristics, by gender

Among the diabetes population, the median systolic blood pressure was 130mmHg (IQR: 21), while the median diastolic blood pressure was 77mmHg (IQR: 11). Although both of these values fell within the normal ranges, these were found to be statistically higher than among the non-diabetes population (systolic 120mmHg IQR: 18, diastolic 75mmHg IQR: 11 $p$=<0.01 respectively). On comparing the median blood pressure value among male with diabetes (systolic 130mmHg IQR: 20; diastolic 78mmHg IQR: 12) to those among male non-diabetes (systolic 121mmHg IQR: 17; diastolic 78mmHg IQR: 13), a statistically higher systolic blood pressure was found within the male with diabetes ($p$=<0.01). There was, however, no significant difference for the diastolic blood pressure ($p$=0.71). On comparing the female populations (diabetes vs. non-diabetes), a significantly higher systolic (130mmHg IQR: 22) and diastolic blood pressure (75mmHG IQR: 10) was evident in the diabetes female population ($p$=<0.01 respectively).

#### 3.1.8.1.8 Blood pressure by impaired fasting plasma glucose

Among the IFG population, 45.41% (CI 95%: 42.22 – 48.63) were found to suffer from hypertension (global), as seen in Table 3.21. Blood pressure (both high systolic and diastolic) in the IFG population was positively related with age groups and gender ($p$=<0.01 respectively). The IFG hypertensive population ($n$=420) contributed to 33.39% (CI 95%: 30.83 – 36.04) of the total hypertensive population ($n$=1,258).

| IFG Blood pressure characteristics | Male (%) [*n*=563] | Female (%) [*n*=362] | Total (%) [*n*=925] |
|---|---|---|---|
| ▪ **Normotensive**[#] | 316 (56.13) | 189 (52.21) | 505 (54.59) |
| ▪ **Global Hypertensive** | 247 (43.87) | 173 (47.79) | 420 (45.41) |
| ➢ Unaware (newly hypertensive) | 107 (43.32) * | 63 (36.42) * | 170 (40.48) * |
| ➢ Aware (known hypertensive) ** | 140 (56.68) * | 110 (63.58) * | 250 (59.52) * |
| ⇒ Untreated | 7 (1.24) * | 6 (3.47) * | 13 (3.10) * |
| → *Uncontrolled* | 2 (0.81) * | 2 (1.16) * | 4 (0.95) * |
| → *Controlled* | 5 (2.02) * | 4 (2.31) * | 9 (2.14) * |
| ⇒ Treated | 133 (53.85) * | 104 (60.12) * | 237 (56.43) * |
| → *Uncontrolled* | 14 (5.67) * | 4 (2.31) * | 18 (4.29) * |
| → *Controlled* | 119 (48.18) * | 100 (57.80) * | 219 (52.14) * |
| **Total uncontrolled blood pressure** | 123 (21.85) | 69 (19.06) | 192 (20.76) |
| **Total controlled blood pressure** | 124 (22.02) | 104 (28.73) | 228 (24.65) |

[#]*Normotensive* – Population did not self-report being hypertensive or on treatment and exhibited a normal blood pressure measurement during examination
* The percentage is showing the proportion of the male / female / total T2DM out of the global hypertension sub-population respectively
** Aware (*known hypertension*) includes those who self-reported a history of hypertension or were on treatment

Table 3.21 Demonstrate the IFG population blood pressure characteristics, by gender

Both the systolic and the diastolic hypertensive IFG populations exhibited a higher (systolic/diastolic) blood pressure than the general population with hypertension (systolic $p$=<0.01; diastolic $p$=<0.01 respectively).

On age stratification, most of the 60 to 69-year-old IFG males were hypertensive, as seen in Figure 3.45.

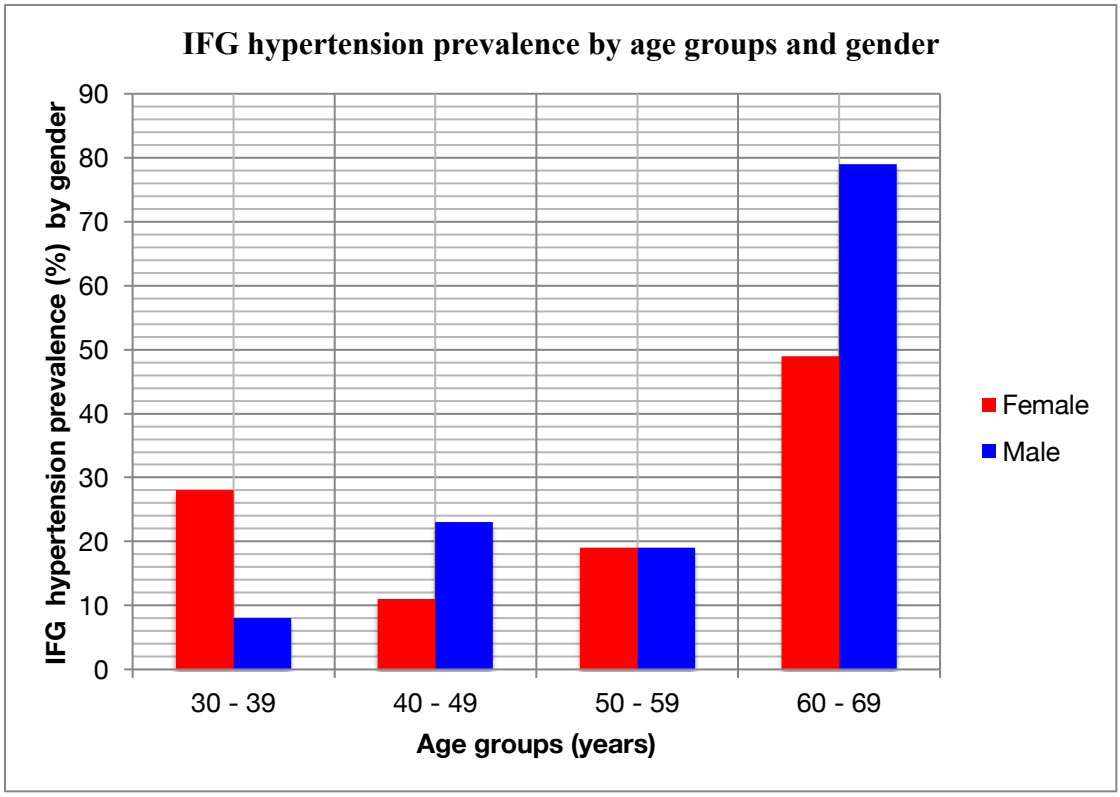



Figure 3.45 Distribution of the IFG hypertensive prevalence, by age groups and gender

The median systolic blood pressure for the IFG males and IFG females was similar (males 133mmHg IQR: 15 and females 131mmHg IQR: 21; $p$=0.30). However, the IFG

population's systolic blood pressure (median) was significantly higher than the systolic blood pressure of the non-IFG population (median male 112mmHg IQR: 18 and female 114mmHg IQR: 20; $p$=<0.01).

The median IFG diastolic blood pressure was 80mmHg (IQR: 13) for the male population and 76mmHg (IQR: 10) for the female population. On comparing the median IFG population diastolic blood pressure to the median non-IFG population diastolic blood pressure, both the male and female IFG populations exhibited a significantly higher diastolic blood pressure than the non-IFG population (male 77mmHg IQR: 11; female 71mmHg IQR: 14; $p$=<0.01).

**3.1.8.1.9 Blood pressure by body mass index**

The hypertensive population was mostly obese (51.99% CI 95%: 49.22 – 54.74), followed by overweight (36.65% CI 95%: 34.05 – 39.35) then normal weight (11.37% CI 95%: 9.73 – 13.24). The male hypertensive population exhibited a far greater preponderance for obesity (57.95% CI 95%: 54.13 – 61.68; $p$=0.36) and for overweight (60.74% CI 95% 56.21 – 65.09; $p$=0.13) when compared to their female counterparts.

Systolic pre-hypertension (120 – 139mmHg) was the leading systolic blood pressure status in the overweight (45.60% CI 95%: 43.01 – 48.21) and in the obese population (50.89% CI 95%: 48.22 – 53.56). This is in contrast to the whole population, where the majority had a normal (<120mmHg) systolic blood pressure ($p$=<0.05). In fact, a significant relationship was evident between having a high systolic blood pressure and having a high BMI

($p$=<0.01). The normal weight population more often had a normal systolic blood pressure in the majority of cases (68.09% CI 95%: 65.39 – 70.67).

A normal diastolic blood pressure (≤89.99 mmHg) was the leading blood pressure in all the different BMI sub-populations. A positive relationship was found between diastolic blood pressure and BMI in both females and males (Female R=0.36, Male R = 0.31 $p$=<0.01 respectively).

### 3.1.8.2 Body mass index profile

#### 3.1.8.2.1 Body mass index by type 2 diabetes mellitus

An obese profile was the norm in the diabetes population (Figure 3.46). This contrasts with the non-diabetes population, which was predominantly overweight. Similarly, both the previously known (56.22% CI 95%: 50.01 – 62.25) and the newly diagnosed (55.70% CI 95%: 47.91 – 63.22) diabetes sub-groups were predominately obese.

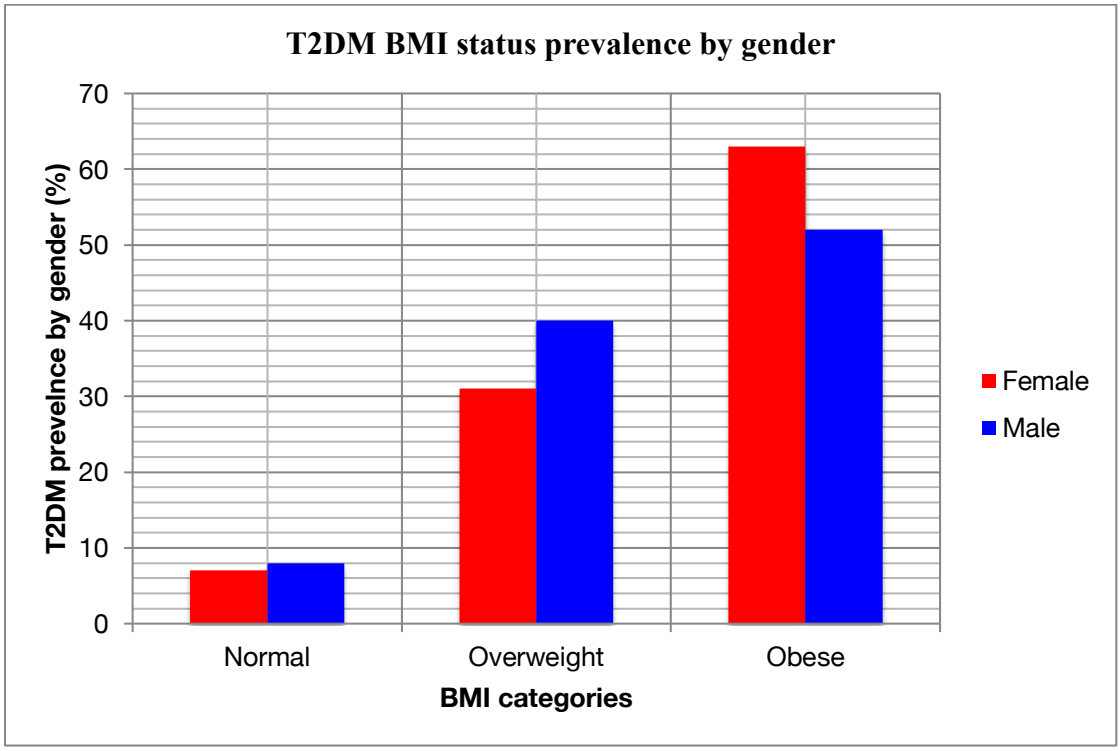



Figure 3.46 Distribution of BMI in the diabetes population by gender

The median BMI across all age groups could be seen in Table 3.22.

| Age groups (years) | Median BMI (IQR) |
|---|---|
| 30 - 39 | 25.81 |
| 40 - 49 | 31.60 ±12.30 |
| 50 - 59 | 29.56 ± 9.24 |
| 60 - 69 | 32.50 ± 7.43 |

Table 3.22 Distribution of the diabetes population median BMI by age groups

Both males and females in the diabetes populations exhibited an elevated median BMI across all age groups when compared to non-diabetes in the study population. The only significant BMI (median) difference between genders was found within the 40 – 49 years age group (Table 3.23) among whom the males had a substantially lower median BMI.

| | **T2DM Females (*n*=136)** | **T2DM Males (*n*=271)** | |
|---|---|---|---|
| **Age groups in years** | **Median BMI (IQR)** | **Median BMI (IQR)** | ***p*-value*** |
| 30 - 39 | 25.81 | N/A | |
| 40 - 49 | 38.65 (0.10) | 26.30 (5.30) | **0.02** |
| 50 - 59 | 29.56 (12.31) | 29.70 (4.60) | 0.80 |
| 60 - 69 | 34.60 (8.25) | 32.50 (5.79) | 0.08 |

* Mann-Whitney U test
IQR – Interquartile range

Table 3.23 Distribution of the diabetes population median BMI by age groups and gender

### 3.1.8.2.2 Body mass index by impaired fasting plasma glucose

The IFG population exhibited a 43.88% (CI 95%: 40.68 – 47.13) obesity rate making most of the IFG individuals obese. This was also in contrast to the situation in the general population, among whom most individuals were overweight ($p$=0.04).

The male IFG population was almost equally overweight and obese ($p$=0.14). The female IFG population exhibited a wider BMI variance (Figure 3.47), with most being obese ($p$=<0.01).

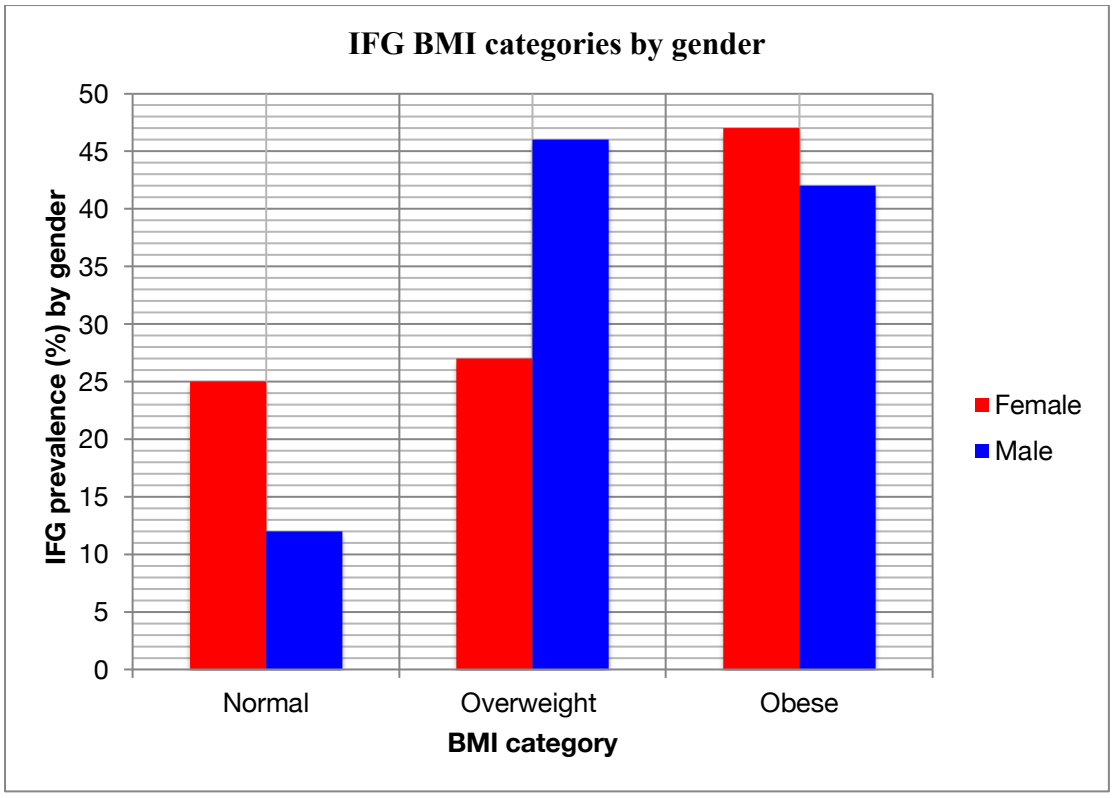



Figure 3.47 Distribution of the IFG population by BMI and gender

### 3.1.9 Biochemical characteristics

The biochemical variables that were measured during the health examination survey included the fasting plasma glucose (FPG) and lipid profile (LDL-C, triglycerides, HDL-C and total cholesterol). Tables 3.24 to 3.29 illustrate the median biochemical characteristics of the whole study population, the diabetes population, the IFG population, the overweight/obese population and the metabolic syndrome population with comparisons between the males and females respectively.

| | **Female (*n*=1,949)** | | **Male (*n*=1,998)** | | |
|---|---|---|---|---|---|
| | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| FPG (mmol/L) | 5.16 | 0.77 | 5.44 | 0.81 | **<0.01** |
| LDL-C (mmol/L) | 2.91 | 1.29 | 3.12 | 1.28 | **<0.01** |
| HDL-C (mmol/L) | 1.65 | 0.57 | 1.31 | 0.44 | **<0.01** |
| Triglycerides (mmol/L) | 0.80 | 0.59 | 0.99 | 0.8 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.01 | 1.47 | 5.02 | 1.41 | 0.10 |

* Mann-Whitney U test

Table 3.24 Distribution of median biochemical profile in the general population, by gender

The male population had an overall significantly worse biochemical profile than the female population.

| Diabetes Mellitus Population | | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=136)** | | **Male (*n*=271)** | | |
| | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| FPG (mmol/L) | 7.33 | 1.52 | 7.99 | 3.74 | **0.02** |
| LDL-C (mmol/L) | 2.74 | 1.24 | 2.96 | 1.25 | 0.32 |
| HDL-C (mmol/L) | 1.50 | 0.44 | 1.12 | 0.48 | **<0.01** |
| Triglycerides (mmol/L) | 1.21 | 0.77 | 1.47 | 1.10 | **<0.01** |
| Total Cholesterol (mmol/L) | 4.97 | 1.50 | 4.90 | 1.32 | 0.32 |

* Mann-Whitney U test

Table 3.25 Median biochemical profile in the diabetes mellitus population, by gender

The median FPG for both the male and female diabetes populations was above 7mmol/L, exposing an inadequate control of their diabetes mellitus, although significant difference between both genders was evident. The male diabetes population exhibited a worse biochemical profile than their female counterparts.

| IFG Population | | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=362)** | | **Male (*n*=563)** | | |
| | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| FPG (mmol/L) | 5.86 | 0.39 | 5.89 | 0.46 | 0.07 |
| LDL-C (mmol/L) | 3.22 | 1.28 | 3.43 | 1.15 | **0.02** |
| HDL-C (mmol/L) | 1.61 | 0.58 | 1.29 | 0.41 | **<0.01** |
| Triglycerides (mmol/L) | 0.96 | 0.74 | 1.11 | 0.75 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.34 | 1.49 | 5.29 | 1.40 | 0.06 |

* Mann-Whitney U test

Table 3.26 Median biochemical profile in the IFG population, by gender

The IFG female population exhibited a better lipid profile than their male counterparts (except for total cholesterol, although not significant).

| | **Overweight BMI** | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=620)** | | **Male (*n*=788)** | | |
| | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| FPG (mmol/L) | 5.19 | 0.68 | 5.52 | 0.76 | **<0.01** |
| LDL-C (mmol/L) | 3.05 | 1.29 | 3.25 | 1.32 | **<0.01** |
| HDL-C (mmol/L) | 1.61 | 0.53 | 1.34 | 0.46 | **<0.01** |
| Triglycerides (mmol/L) | 0.82 | 0.60 | 0.98 | 0.73 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.18 | 1.46 | 5.19 | 1.45 | 0.97 |

* Mann-Whitney U test

Table 3.27 Median biochemical profile in the overweight population, by gender

The overweight female population exhibited a better biochemical profile than their male counterparts.

| | **Obese BMI** | | | | |
|---|---|---|---|---|---|
| | **Female (*n*=609)** | | **Male (*n*=736)** | | |
| | **Median** | **IQR** | **Median** | **IQR** | ***p*-value*** |
| FPG (mmol/L) | 5.45 | 0.98 | 5.60 | 1.13 | **<0.01** |
| LDL-C (mmol/L) | 3.28 | 1.20 | 3.25 | 1.08 | 0.25 |
| HDL-C (mmol/L) | 1.49 | 0.46 | 1.19 | 0.38 | **<0.01** |
| Triglycerides (mmol/L) | 1.02 | 0.73 | 1.33 | 0.92 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.32 | 1.52 | 5.15 | 1.27 | **<0.01** |

* Mann-Whitney U test

Table 3.28 Median biochemical profile in the obese population, by gender

The female obese population had an overall, better biochemical profile than their male counterparts with the exception of the total cholesterol level.

The metabolic syndrome (MetS) is a condition constituted by the presence of a high waist circumference along with the presence of two or more biochemical abnormalities. Table 3.29 demonstrates the median biochemical parameters within the MetS population and the non-MetS population by gender.

| | **Metabolic Syndrome** | | |
|---|---|---|---|
| | **Female** | **Male** | **Mann-Whitney U** |
| | Median ±IQR | Median ±IQR | *p*-value |
| FPG (mmol/L) | 5.79 ±0.93 | 6.01 ±1.34 | **<0.01** |
| LDL-C (mmol/L) | 3.32 ±1.34 | 3.29 ±1.23 | 0.34 |
| HDL-C (mmol/L) | 1.29 ±0.51 | 1.08 ±0.43 | **<0.01** |
| Triglycerides (mmol/L) | 1.43 ±0.99 | 1.71 ± 1.04 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.38 ±1.66 | 5.25 ±1.54 | **<0.01** |
| | **Non-Metabolic Syndrome** | | |
| | **Female** | **Male** | **Mann-Whitney U** |
| | Median ±IQR | Median ±IQR | *p*-value |
| FPG (mmol/L) | 5.05 ±0.56 | 5.27 ±0.55 | **<0.01** |
| LDL-C (mmol/L) | 2.81 ±2.38 | 3.04 ±2.19 | **<0.01** |
| HDL-C (mmol/L) | 1.72 ±1.06 | 1.39 ±0.76 | **<0.01** |
| Triglycerides (mmol/L) | 0.72 ±0.99 | 0.87 ±1.18 | **<0.01** |
| Total Cholesterol (mmol/L) | 4.95 ±2.52 | 4.87 ±2.22 | 0.12 |

Table 3.29 Distribution of the median biochemical parameters within the metabolic syndrome and the non-metabolic syndrome populations, by gender

Table 3.30 compares the biochemical profiles of the MetS to the non-MetS populations.

| | **MetS** | **Non-MetS** | **Mann-Whitney U** |
|---|---|---|---|
| | **Median ±IQR** | **Median ±IQR** | ***p*-value** |
| FPG (mmol/L) | 5.94 ±1.13 | 5.17 ±0.59 | **<0.01** |
| LDL-C (mmol/L) | 3.32 ±1.25 | 2.91 ±2.28 | **<0.01** |
| HLD-C (mmol/L) | 1.17 ±0.45 | 1.55 ±1.06 | **<0.01** |
| Triglycerides (mmol/L) | 1.56 ±1.05 | 0.79 ±1.05 | **<0.01** |
| Total Cholesterol (mmol/L) | 5.29 ±1.60 | 4.92 ±2.45 | **<0.01** |

Table 3.30 Comparison of the median biochemical parameters between the metabolic syndrome and the non-metabolic syndrome populations

### 3.1.9.1 NCEP ATPIII lipid classification

The lipid profile obtained during the health examination survey was classified according to the NCETP ATPIII classification, as seen in Table 3.31 (National Cholesterol Education Program (NCEP), 2002).

The majority of the general population exhibited above optimal (2.59 – 3.35mmol/L) LDL-C levels, optimal (<=1.68mmol/L) triglyceride levels, normal HDL-C levels (1.04 – 1.54mmol/L) and desirable (<5.17mmol/L) total cholesterol levels.

Similar distributions were evident for the IFG, overweight and obese populations, as seen in Table 3.31. The diabetes population exhibited a marginal higher proportion of the population with optimal LDL-C levels, as seen in Table 3.31.

| NCEP ATP III Lipid Classification in mmol/L | | General Population | | Diabetes Population | | IFG Population | | Overweight Population | | Obese Population | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n*=3,947 | % | *n*=407 | % | *n*=925 | % | *n*=1,408 | % | *n*=1,345 | % |
| **LDL-C** | < 2.59 Optimal | 1169 | 29.62% | 144 | 35.38% | 162 | 17.51% | 348 | 24.72% | 254 | 18.88% |
| | 2.59 - 3.35 Above optimal | 1373 | 34.79% | 142 | 34.89% | 310 | 33.51% | 478 | 33.95% | 478 | 35.54% |
| | 3.36 - 4.15 Borderline high | 875 | 22.17% | 82 | 20.15% | 271 | 29.30% | 343 | 24.36% | 390 | 29.00% |
| | 4.16 - 4.90 High | 382 | 9.68% | 22 | 5.41% | 134 | 14.49% | 173 | 12.29% | 157 | 11.67% |
| | 4.91+ Very high | 145 | 3.67% | 17 | 4.18% | 48 | 5.19% | 66 | 4.69% | 66 | 4.91% |
| **Triglyceride** | <= 1.68 Optimal | 3347 | 84.80% | 262 | 64.37% | 760 | 82.16% | 1186 | 84.23% | 1014 | 75.39% |
| | 1.69 - 2.25 Borderline | 359 | 9.10% | 83 | 20.39% | 94 | 10.16% | 135 | 9.59% | 193 | 14.35% |
| | 2.26 - 5.64 High | 230 | 5.83% | 62 | 15.23% | 69 | 7.46% | 87 | 6.18% | 129 | 9.59% |
| | 5.65+ Very high | 11 | 0.28% | 0 | 0.00% | 2 | 0.22% | 0 | 0.00% | 9 | 0.67% |

| NCEP ATP III Lipid Classification in mmol/L | | General Population | | Diabetes Population | | IFG Population | | Overweight Population | | Obese Population | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n*=3,947 | % | *n*=407 | % | *n*=925 | % | *n*=1,408 | % | *n*=1,345 | % |
| HDL-C | <= 1.03 Low | 464 | 11.76% | 122 | 29.98% | 131 | 14.16% | 151 | 10.72% | 263 | 19.55% |
| | 1.04 - 1.54 Normal | 1824 | 46.21% | 178 | 43.73% | 480 | 51.89% | 700 | 49.72% | 732 | 54.42% |
| | 1.55+ High | 1659 | 42.03% | 107 | 26.29% | 314 | 33.95% | 557 | 39.56% | 350 | 26.02% |
| Total Cholesterol | < 5.17 Desirable | 2207 | 55.92% | 241 | 59.21% | 404 | 43.68% | 689 | 48.93% | 648 | 48.18% |
| | 5.17 - 6.20 Borderline high | 1142 | 28.93% | 119 | 29.24% | 314 | 33.95% | 475 | 33.74% | 437 | 32.49% |
| | 6.21+ High | 598 | 15.15% | 47 | 11.55% | 207 | 22.38% | 244 | 17.33% | 260 | 19.33% |

Table 3.31 Distribution of the NCEP ATP III classification by the general, diabetes, IFG, overweight and obese populations

### 3.1.9.2 Lipid profile by diabetes mellitus population

The majority of the diabetes mellitus population exhibited normal levels (<2.59mmol/L) of LDL-C (55.77% CI 95%: 50.92 – 60.52), triglycerides (64.86% CI 95%: 60.11 – 69.35), and total cholesterol (52.09% CI 95%: 47.24 – 56.90), among whom the male population predominated (LDL-C: 64.78% CI 95%: 58.34 – 70.68; Triglyceride: 59.85% CI 95%: 53.83 – 65.58, Total Cholesterol: 66.98 CI 95%: 60.39 – 72.97). A predominantly high HDL-C (64.86% CI 95%: 60.11 – 69.35) was present in both the male (62.50% CI 95%: 56.52 – 68.12) and female diabetes (72.79% CI 95%: 64.74 – 79.60) populations.

Of note, the female population exhibited a generally higher (median) lipid profile level with increasing age. A plateau was observed after the age of 50 - 59 years with the exception of HDL-C (Figure 3.48). Conversely, the male diabetes population appeared to exhibit a progressively decline linear relationship between the lipid variables and age (Figure 3.49).

No significant relationship was present between age and dyslipidaemia was evident ($p$=0.08) within the diabetes population.

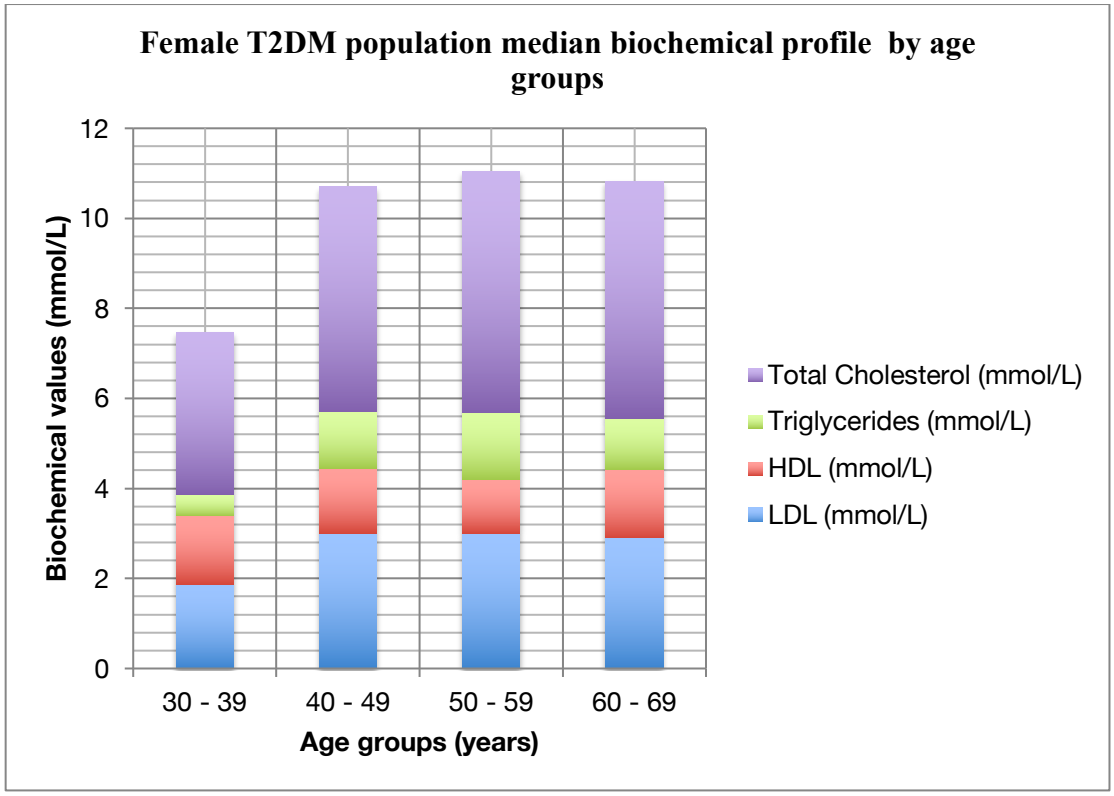



Figure 3.48 Distribution of the median lipid profiles, by age groups among the female diabetes population

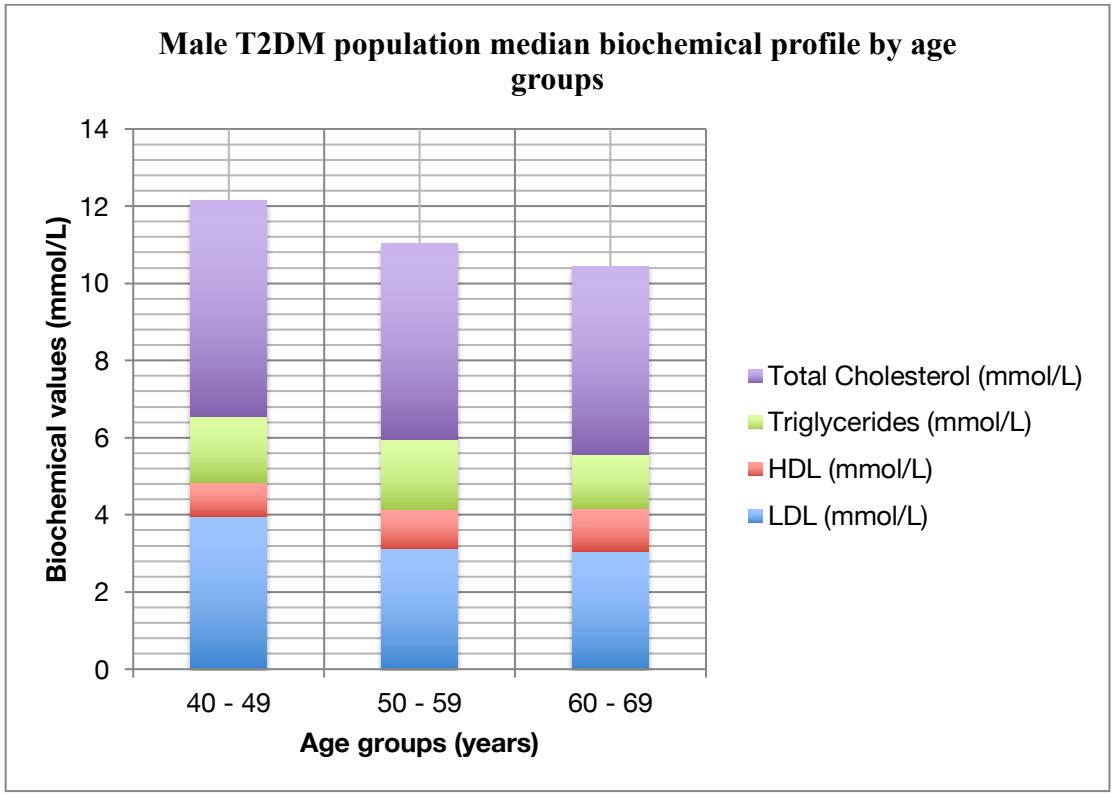



Figure 3.49 Distribution of the median lipid profiles, by age groups among the male diabetes population

### 3.1.9.3 Lipid profile by impaired fasting glucose population

Approximately two thirds of the IFG population had an elevated LDL-C level (66.49% CI 95%: 63.38 – 69.46) as well as an elevated total cholesterol level (63.46% CI 95%: 60.31 – 66.50), among whom a male predominance was exhibited (LDL-C: 69.98% CI 95%: 66.07 – 73.62; total cholesterol: 58.63% CI 95%: 54.63 – 62.51).

The elevated LDL-C (≥2.59mmol/L) proportion within the IFG population was found to be significantly higher than that found in the general population ($p$=<0.01). Both populations exhibited a male predominance throughout all age groups ($p$=<0.01) except for the IFG 50 to 59-year age group.

The total cholesterol was statistically higher in the IFG population (even after age and gender stratification) as compared with the situation in the general population ($p$=<0.01). The age group with the highest IFG population proportion exhibiting an elevated cholesterol level was the 50 to 59-year age group for females and the 20 – 29 years age group for males. In contrast, among the general population, it was the 60 to 69-year age group (for both the males and females) that exhibited the highest total cholesterol levels ($p$=<0.01 respectively).

On the contrary, a normal triglyceride (TG) and a high HDL-C levels dominated within the IFG population (TG: 82.38% CI 95%: 79.79 – 84.70; HDL-C: 79.89% CI 95%: 77.18 – 82.35), with a male preponderance (TG: 59.84% CI 95%: 56.32 – 63.27; HDL-C: 61.84% CI 95%: 58.28 – 65.27).

The IFG population had significantly higher elevated triglyceride prevalence (≥1.69mmol/L) when compared to the general population ($p$=0.01), even though the majority of the IFG population had normal triglyceride levels. Both the IFG and total study populations exhibited a male predominance with triglyceride elevation ($p$=0.91).

The IFG population had a preponderance of individuals with an elevated HDL-C. However, the proportion exhibiting a low HDL-C was found to be similar to those with a low HDL-C in the general population ($p$=0.41). As age increased, so too did the proportion among the IFG population with a low HDL-C. The IFG male population with the highest low HDL-C proportion was within the 40 to 49 years age group while for the IFG female population was within the 50 – 59-year age group.

#### 3.1.9.4 Dyslipidaemia prevalence

The prevalence of *uncontrolled dyslipidaemi*a (high LDL-C + high TG (triglycerides) + Low HDL-C) at the point of the study was of 7.75% (CI 95%: 6.69 – 8.63). The *uncontrolled dyslipidaemi*a population was composed of naïve dyslipidaemic individuals ($n$=275) and individuals reported to be on statins *(*$n$=485) yet still with uncontrolled dyslipidaemia during the examination ($n$=31). Thus, the 7.75% of prevalent current dyslipidemia consisted of a proportion with naïve dyslipidaemia - 6.97% (CI 95%: 6.21 – 7.81) and a proportion of those with known dyslipidaemia, on statins and yet uncontrolled - 0.79% (CI 95%: 0.55 – 1.12). The naïve dyslipidaemia sub-population was predominantly male (76.73% CI 95%: 71.37 – 81.35) aged between 30 and 70 years.

Participants on statins may have been taking these due to either a known dyslipidaemic status or else had been started as a preventive measure following certain conditions such as cardiovascular disease or at onset of diabetes mellitus.

The *global dyslipidaemia* population contributed to 19.26% (CI 95%: 18.05 – 20.52) of the total general population. This *global dyslipidaemia* population consisted of the combination of individuals that were already on statin treatment (*n*=485) in addition to naïve cases (*n*=275).

*Global dyslipidaemia* was found to be present in 46.59% (CI 95%: 40.49 – 52.69) of the known diabetes sub-population.

### 3.1.9.5 Dyslipidaemic profile by body mass index status

The dyslipidaemic profile (LDL-C, HDL-C, triglycerides, total cholesterol) showed a gradual increase in median values as the BMI category shifted from normal weight to overweight and to obese BMI. While, the HDL-C exhibited a corresponding decrease in median values as seen in Figures 3.50 to 3.53.

All the biochemical profile median values were found to be significantly different between the normal, overweight and obese populations ($p$=<0.01 respectively).

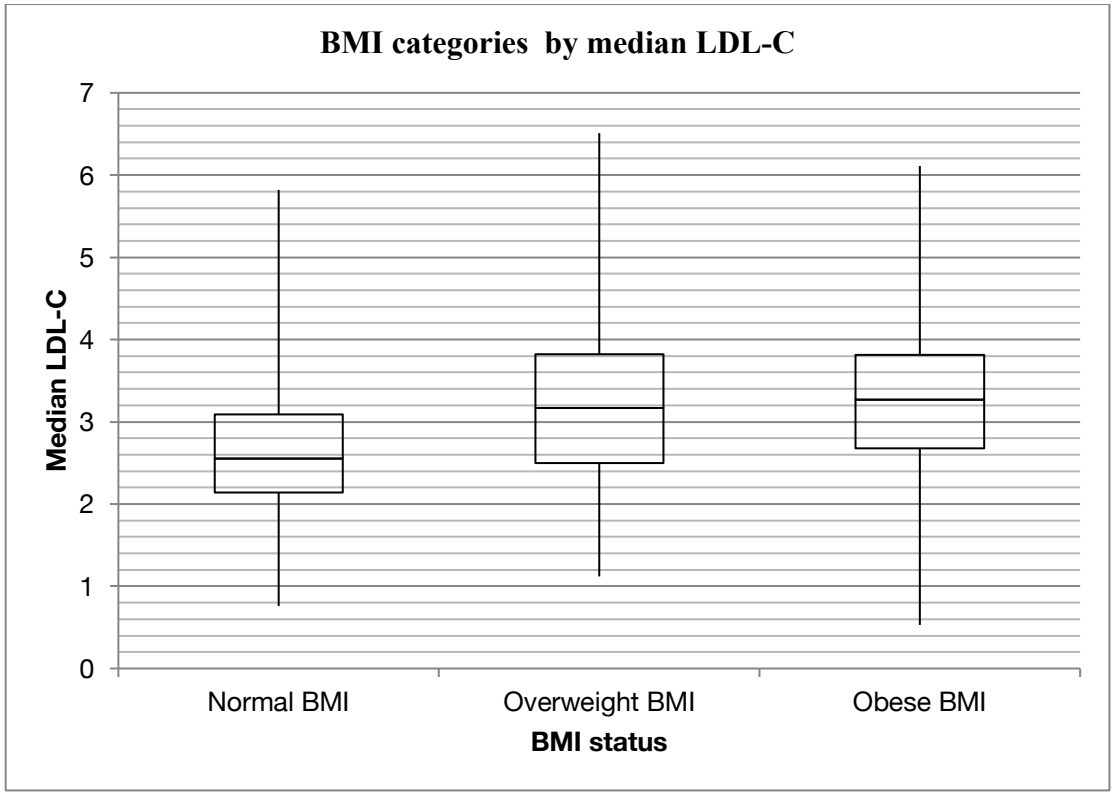



Figure 3.50 Distribution of median LDL-C by BMI categories

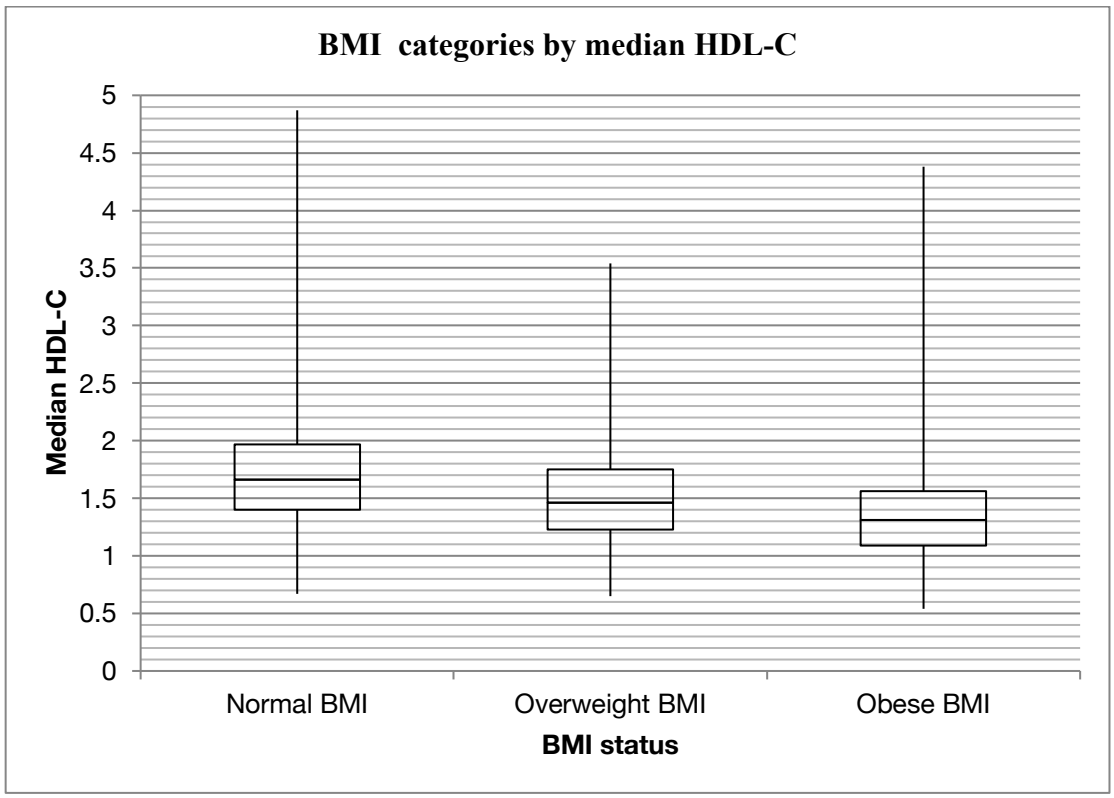



Figure 3.51 Distribution of median HDL-C by BMI categories

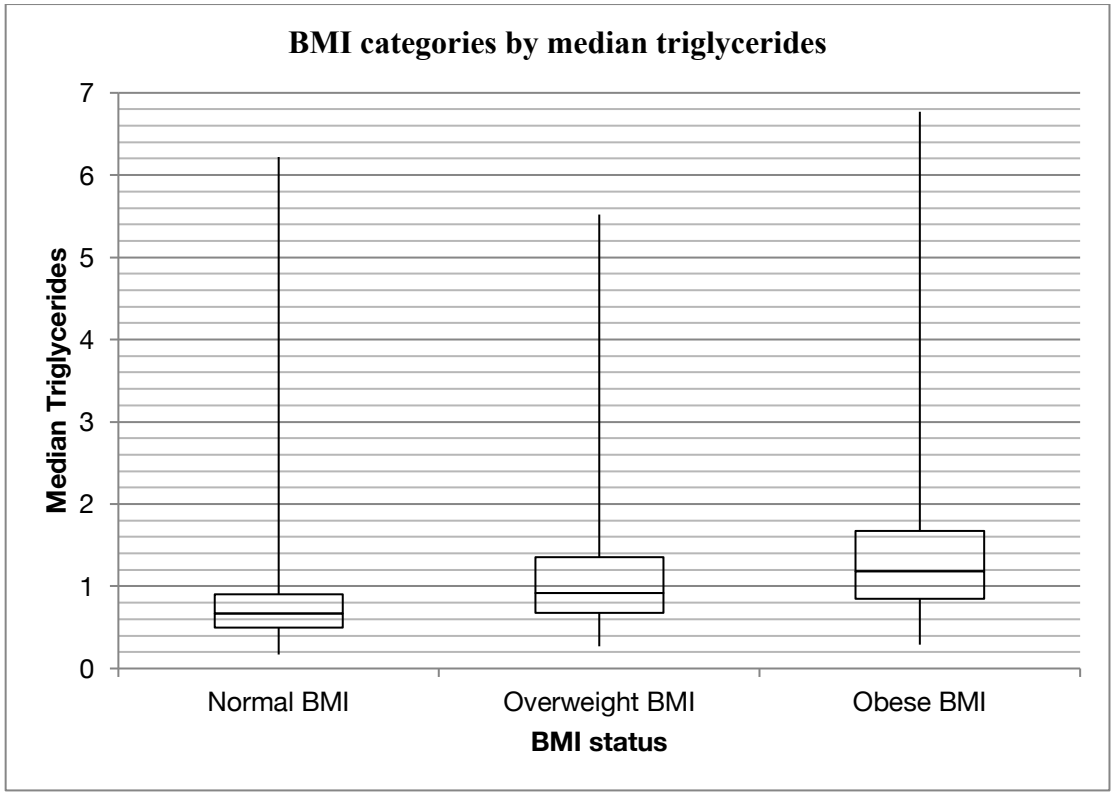



Figure 3.52 Distribution of median triglycerides by BMI categories

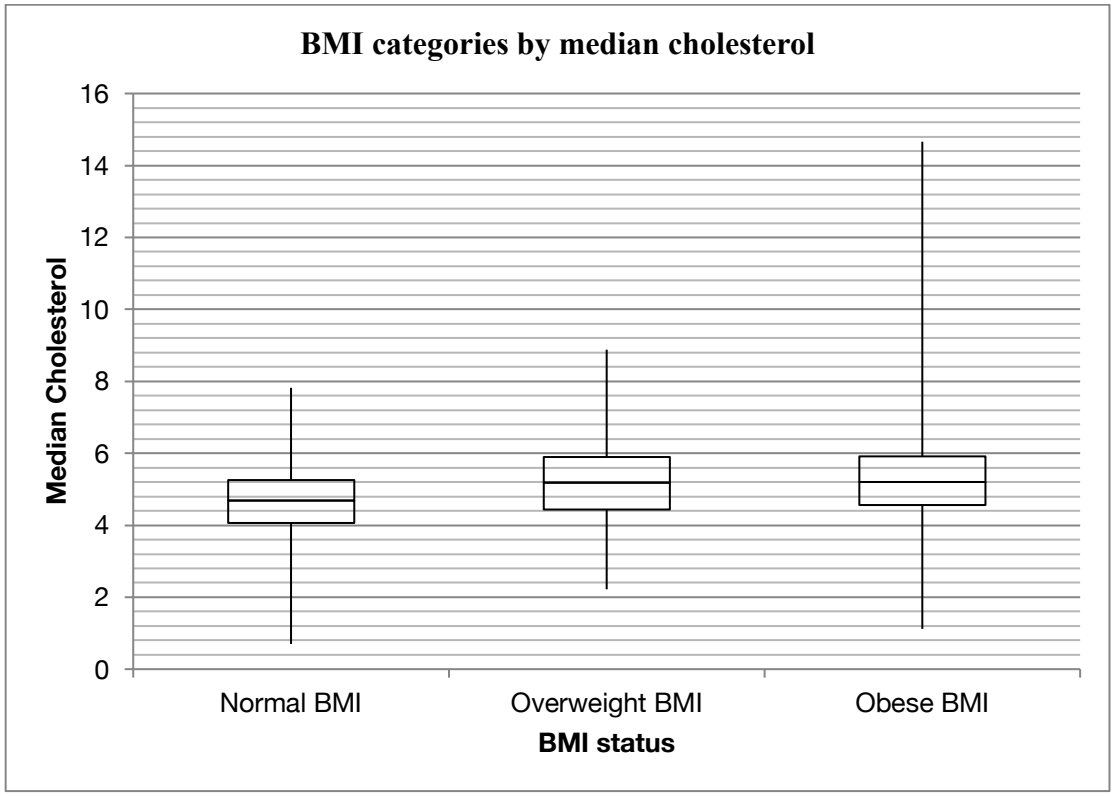



Figure 3.53 Distribution of median total cholesterol by BMI categories

## 3.1.10 Dysglycaemic Population characteristics

### 3.1.10.1 Diabetes population characteristics

The diabetes study population (adjusted) was composed of *previously known* diabetes (*n*=249) and *newly diagnosed* diabetes (*n*=158), with a male predominance in both sub-populations. The *previously known* diabetes sub-population was significantly older (median age 64 years;) than the *newly diagnosed* diabetes sub-population (median age 58 years) (*p*=<0.01).

The majority of the *previously known* diabetes population reported to be on oral hypoglycaemic agents (89.56% CI 95%: 85.09 – 92.82). However, the health examination fasting plasma glucose measurement exhibited that 71.75% (CI 95%: 65.50 – 77.26) of the *previously known* diabetes population on oral hypoglycaemic agents had an elevated FPG above or equal to 7mmol/L. Table 3.32 demonstrates the diabetes study population characteristics.

A first-degree family history of type 2 diabetes mellitus was reported by 46.19% (CI 95%: 41.41 – 51.05) of the diabetes population (Males: 45.02% CI 95%: 39.21 – 50.97; Females: 48.53% CI 95%: 40.29 – 56.85; *p*=0.53). Whilst 2.46% (CI 95%: 1.28 – 4.52) reported a first-degree family history of type 1 diabetes mellitus. Of female with diabetes, 12.50% (CI 95%: 7.86 – 19.20) recalled a first-degree family history of gestational diabetes. Table 3.33 demonstrates the reported first-degree family history of dyglycaemic conditions within the diabetes study population.

| Diabetes characteristics | Male [*n*=271] (%) * | Female [*n*=136] (%) ** | Total [*n*=407] (%) *** |
|---|---|---|---|
| **Diabetes (*global*)** | | | |
| Unaware (newly diabetes) | 103 (38.00%) | 55 (40.44%) | 158 (38.82%) |
| Aware (known diabetes) | 168 (62.00%) | 81 (59.56%) | 249 (61.18%) |
| *Untreated* | 19 (7%) | 7 (5.18%) | 26 (6.39%) |
| Uncontrolled (FPG >=7mmol/L) | 6 (2.21%) | 7 (5.18%) | 13 (3.19%) |
| Controlled (FPG <7mmol/L) | 13 (4.80%) | 0 | 13 (3.19%) |
| *Treated* | 149 (55%) | 74 (54.4%1) | 223 (54.79%) |
| Uncontrolled (FPG >=7mmol/L) | 104 (38.38%) | 56 (41.18%) | 160 (39.31%) |
| Controlled (FPG <7mmol/L) | 45 (16.61%) | 18 (13.24%) | 63 (15.48%) |
| **Total with Uncontrolled FPG (>=7mmol/L)** | 213 (78.60%) | 118 (86.76%) | 331 (81.32%) |
| **Total with Controlled FPG (<7mmol/L)** | 58 (21.40%) | 18 (13.24%) | 76 (18.67%) |

* Percentage expressed as proportion of all diabetes males

** Percentage expressed as proportion of all diabetes females

*** Percentage expressed as proportion of all diabetes

Table 3.32 Knowledge, treatment and control of the adult diabetes study population

| Diabetes characteristics | Male (%)* [*n*=271] | Female (%)** [*n*=136] | Total (%)*** [*n*=407] |
|---|---|---|---|
| **Diabetes (*global*)** | | | |
| **Unaware *(newly diabetes)*** | 103 (38.00) | 55 (40.44) | 158 (38.82) |
| Family History of T2DM | 45 (16.61) | 26 (19.12) | 71 (17.44) |
| Family History of maternal GDM | 4 (1.48) | 7 (5.15) | 11 (2.70) |
| Family History of T1DM | 5 (1.85) | 0 | 5 (1.23) |
| **Aware *(known diabetes)*** | 168 (62.00) | 81 (59.56) | 249 (61.18) |
| Family History of T2DM | 77 (28.41) | 40 (29.41) | 117 (28.75) |
| Family History of maternal GDM | 9 (3.32) | 10 (7.35) | 19 (4.67) |
| Family History of T1DM | 0 | 5 (3.69) | 5 (1.23) |

T2DM - type 2 diabetes mellitus

GDM - gestational diabetes mellitus

T1DM - type 1 diabetes mellitus

*Percentage expressed as proportion of all diabetes males

** Percentage expressed as proportion of all diabetes females

*** Percentage expressed as proportion of all diabetes

Table 3.33 Self-reported first-degree family history in the known and unknown adult diabetes population

The *previously known* diabetes sub-population reported a higher proportion of co-morbidities than the *newly diagnosed* diabetes sub-population, as illustrated in Table 3.34.

In both sub-populations (*previously known* diabetes and *newly diagnosed* diabetes), the males reported higher cardiovascular co-morbidities, while the females reported higher prevalence of hypothyrodism.

| Diabetes characteristics | Male (%)* [*n*=271] | Female (%)** [*n*=136] | Total (%)*** [*n*=407] |
|---|---|---|---|
| **Diabetes (*global*)** | | | |
| **Unaware *(newly diabetes)*** | 103 (38.00) | 55 (40.44) | 158 (38.82) |
| History of myocardial infarction | 7 (2.58) | 0 | 7 (1.72) |
| History of coronary heart disease | 4 (1.48) | 4 (2.94) | 8 (1.97) |
| History of stroke | 2 (0.74) | 0 | 2 (0.49) |
| History of hypothyroidism | 4 (1.48) | 13 (9.56) | 17 (4.18) |
| History of gestational diabetes | 0 | 8 (5.88) | 8 (1.97) |
| **Aware *(known diabetes)*** | 168 (62.00) | 81 (59.56) | 249 (61.18) |
| History of myocardial infarction | 18 (6.64) | 4 (2.94) | 22 (5.41) |
| History of coronary heart disease | 21 (7.75) | 8 (5.88) | 29 (7.13) |
| History of stroke | 2 (0.74) | 1 (0.74) | 3 (0.74) |
| History of hypothyroidism | 4 (1.48) | 16 (11.76) | 20 (4.91) |
| History of gestational diabetes | 0 | 16 (11.76) | 16 (3.93) |

* Percentage expressed as proportion of all diabetes males

** Percentage expressed as proportion of all diabetes females

*** Percentage expressed as proportion of all diabetes

Table 3.34 Self-reported co-morbidities in the known and unknown adult diabetes populations

### 3.1.10.2 Impaired fasting plasma glucose characteristics

A first-degree family history of type 2 diabetes mellitus was reported by 46.16% (CI 95%: 42.97 – 49.38) of the IFG population (Males: 41.03% CI 95%: 37.04 – 45.14; Females: 54.14% CI 95%: 48.99 – 59.21; $p$=<0.01). Among the female IFG population 9.67% (CI 95%: 7.01 – 13.18) recalled a first-degree family history of gestational diabetes. Table 3.35 shows the reported first-degree family history of dyglycaemic conditions within the IFG study population.

The commonest self-reported medical co-morbidity was hypothyroidism, with a female predominance followed by gestational diabetes (only in females). Table 3.35 illustrates the different reported medical co-morbidities by the IFG population

| IFG characteristics | Male (%)* [*n*= 563] | Female (%)** [*n*= 362] | Total (%)*** [*n*= 925] |
|---|---|---|---|
| **Family history** | | | |
| Family History of T2DM | 231 (41.03) | 196 (54.14) | 427 (46.16) |
| Family History of maternal GDM | 19 (3.37) | 35 (9.67) | 54 (5.84) |
| Family History of T1DM | 27 (4.80) | 15 (4.14) | 42 (4.54) |
| **Medical history** | | | |
| History of myocardial infarction | 21 (3.73) | 7 (1.93) | 28 (3.03) |
| History of coronary heart disease | 23 (4.09) | 6 (1.66) | 29 (3.14) |
| History of stroke | 4 (0.71) | 3 (0.83) | 7 (0.76) |
| History of hypothyroidism | 29 (5.15) | 48 (13.26) | 77 (8.32) |
| History of gestational diabetes | 0 | 45 (12.43) | 45 (4.86) |

* Percentage expressed as proportion of all IFG males
** Percentage expressed as proportion of all IFG females
*** Percentage expressed as proportion of all IFG

Table 3.35 Self-reported family history and co-morbidities in the IFG

### 3.1.11 Metabolic syndrome population characteristics

The metabolic syndrome population had an elevated waist circumference (MetS median 102cm ±16 IQR, non-MetS median 86.10 ± 19.70 IQR) with the male median waist circumference being significantly higher than that of females (median male – 104cm ±13 IQR, median female – 96cm ±19 IQR, $p$=<0.01).

Comparisons between the MetS population characteristics and the non-MetS population are illustrated in Tables 3.36 to 3.37.

The metabolic syndrome components with the highest prevalence rates were: the presence of an elevated FPG (77.07% CI 95%: 74.41 – 79.53) and that of an elevated systolic blood pressure (73.70% CI 95%: 70.94 – 76.29).

Table 3.38A and Table 3.38B demonstrate the distribution of the median values of the MetS components, by gender. A significant gender difference was exhibited within all MetS components in the Maltese population (Table 3.38A and 3.38B).

A positive relationship was evident between MetS and all the different MetS components ($p$=<0.01 respectively).

| | Metabolic Syndrome Population | | Non-Metabolic Syndrome Population | | |
|---|---|---|---|---|---|
| | Total (*n*=1,038) | | Total (*n*=2,909) | | |
| | **Median ±IQR** | ***n* (%)** | **Median ±IQR** | ***n* (%)** | ***p*-value** |
| **HDL Cholesterol (mmol/L)** | 1.17 ±0.45 | | 1.61 ±0.52 | | **<0.01[d]** |
| Normal / High | | 537 (51.73%) | | 2725 (93.67%) | **<0.01[e]** |
| Low[a] | | 501 (48.27%) | | 184 (6.33%) | |
| **Triglycerides (mmol/L)** | 1.56 ±1.06 | | 0.79 ±0.50 | | **<0.01[d]** |
| Normal | | 557 (53.66%) | | 2792 (95.32%) | **<0.01[e]** |
| High[b] | | 481 (46.34%) | | 117 (4.02%) | |
| | | | | | |
| **Systolic Blood pressure (mmHg)** | 131 ±17 | | 116 ±16 | | **<0.01[d]** |
| Normal[c1] | | 273 (26.30%) | | 2326 (79.96%) | **<0.01[e]** |
| Normal[c2] | | *120* | | 197 (6.77%) | |
| High[c3] | | *645* | | 386 (13.27%) | |
| MetS - Systolic | | 765 (73.70%) | | | |

a - Low HDL is defined as HDL<1.03mmol/L (male), HDL < 1.29mmol/L (female)

b - High Triglycerides is defined as triglycerides >1.70mmol/L

c1 - Normal systolic blood pressure <130mmHg and not on anti-hypertensive

c2 - Normal systolic blood pressure <130mmHg and on anti-hypertensive

c3 - High Systolic blood pressure is defined as >=130mmHg

MetS - Systolic: combination of C2 and C3 make up a component of MetS

d – Mann-Whitney U comparing median of MetS vs. non-MetS per category

e - Chi squared test comparing MetS vs. non-MetS with and without abnormality

Table 3.36 Comparison of the MetS components (HDL, Triglycerides and systolic blood pressure) within the MetS population and the non-metabolic syndrome population

| | Metabolic Syndrome Population | | Non-Metabolic Syndrome Population | | |
|---|---|---|---|---|---|
| | **Total (*n*=1038)** | | **Total (*n*=2909)** | | |
| | Median ±IQR | *n* (%) | Median ±IQR | *n* (%) | *p*-value |
| **Diastolic Blood pressure (mmHg)** | 80 ±12 | | 73 ±12 | | **<0.01[f]** |
| Normal[d2] | | 393 (37.86%) | | 2413 (82.95%) | **<0.01[g]** |
| High[d] | | *295* | | 247 (8.49%) | |
| MetS - Diastolic | | *350* | | 249 (8.56%) | |
| | | 645 (62.14%) | | | |
| **Fasting plasma glucose (mmol/L)** | 5.94 ±1.14 | | 5.17 ±0.59 | | **<0.01[f]** |
| Normal[e1] | | 238 (22.93%) | | 439 (15.09%) | **<0.01[g]** |
| Normal[e2] | | *8* | | 102 (3.51%) | |
| High[e3] | | *792* | | 2368 (81.40%) | |
| MetS - FPG | | 800 (77.07%) | | | |

d1 - Normal diastolic blood pressure <85mmHg and not on anti-hypertensive

d2 - Normal diastolic blood pressure <85mmHg and on anti-hypertensive

d3 - High Diastolic blood pressure is defined as >=85mmHg

MetS - Diastolic: combination of D2 and D3 make up a component of MetS

e1 - Fasting plasma glucose <5.6mmol/L and No history of diabetes

e2 - Fasting plasma glucose < 5.6mmol/L and a history of diabetes

e3 - High Fasting plasma glucose is defined as >=5.6mmol/L

MetS - FPG: combination of e2 and e3 make up a component of MetS

f – Mann-Whitney U comparing median of MetS vs. non-MetS per category

g - Chi squared test comparing MetS vs. non-MetS with and without abnormality

Table 3.37 Comparison of the MetS components (Diastolic blood pressure, FPG) within the MetS population and the non-metabolic syndrome population

| | MetS whole population | | | | | |
|---|---|---|---|---|---|---|
| | **Total (*n*=1,038)** | | **Male (*n*=632)** | | **Female (*n*=406)** | |
| | **Median ±IQR** | ***n* (%)** | **Median ±IQR** | ***n* (%)** | **Median ±IQR** | ***n* (%)** | ***p*-value** |
| **HDL Cholesterol (mmol/L)** | 1.17 ±0.45 | | 1.08 ±0.43 | | 1.29 ±0.51 | | **<0.01[f]** |
| Normal | | 537 (51.73%) | | 340 (53.80%) | | 197 (48.52%) | **<0.01[g]** |
| Low[a] | | 501 (48.27%) | | 292 (46.20%) | | 209 (51.48%) | |
| | | | | | | | |
| **Triglycerides (mmol/L)** | 1.56 ±1.06 | | 1.71 ±1.04 | | 1.43 ±0.99 | | **<0.01[f]** |
| Normal | | 557 (53.66%) | | 310 (49.05%) | | 247 (60.84%) | **<0.01[g]** |
| High[b] | | 481 (46.34%) | | 322 (50.95%) | | 159 (39.16%) | |
| | | | | | | | |
| **Systolic Blood pressure (mmHg)** | 131 ±17 | | 131 ±17 | | 132 ±17 | | 0.40[f] |
| Normal[c1] | | 273 (26.30%) | | 184 (29.11%) | | 89 (21.92%) | **0.01[g]** |
| Normal[c2] | | *120* | | *76* | | *44* | |
| High[c3] | | *645* | | *372* | | *273* | |
| MetS - Systolic | | 765 (73.70%) | | 448 (70.89% | | 317 (78.08%) | |

a - Low HDL is defined as HDL<1.03mmol/L (male), HDL < 1.29mmol/L (female)
b - High Triglycerides is defined as triglycerides >1.70mmol/L
c1 - Normal systolic blood pressure <130mmHg and not on anti-hypertensive
c2 - Normal systolic blood pressure <130mmHg and on anti-hypertensive
c3 - High Systolic blood pressure is defined as >=130mmHg
MetS - Systolic: combination of C2 and C3 make up a component of MetS
f – Mann-Whitney U Test comparing means of males vs. females per category
g - Chi squared test comparing males vs. females with and without abnormality

Table 3.38A Comparison of metabolic syndrome components (HDL, triglycerides & systolic blood pressure), by gender in the MetS population

| MetS whole population | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Total (*n*=*1038*)** | | **Male (*n*=632)** | | **Female (*n*=406)** | | |
| | **Median ±IQR** | ***n* (%)** | **Median ±IQR** | ***n* (%)** | **Median ±IQR** | ***n* (%)** | ***p*-value** |
| **Diastolic Blood pressure (mmHg)** | 80 ±12 | | 81 ±11 | | 79 ±13 | | **<0.01[f]** |
| Normal[d1] | | 393 (37.86%) | | 224 (35.44%) | | 169 (41.63%) | **0.05[g]** |
| Normal[d2] | | *295* | | *164* | | *131* | |
| High[d] | | *350* | | *244* | | *106* | |
| MetS - Diastolic | | 645 (62.14%) | | 408 (64.56%) | | 237 (58.37% | |
| | | | | | | | |
| **Fasting plasma glucose (mmol/L)** | 5.94 ±1.14 | | 6.01 ±1.34 | | 5.79 ±0.93 | | **<0.01[f]** |
| Normal[e1] | | 238 (22.93%) | | 125 (19.78%) | | 113 (27.83% | **<0.01[g]** |
| Normal[e2] | | *8* | | *5* | | *3* | |
| High[e3] | | *792* | | *502* | | *290* | |
| MetS - FPG | | 800 (77.07%) | | 507 (80.22%) | | 293 (72.17%) | |

d1 - Normal diastolic blood pressure <85mmHg and not on anti-hypertensive

d2 - Normal diastolic blood pressure <85mmHg and on anti-hypertensive

d3 - High Diastolic blood pressure is defined as >=85mmHg

MetS - Diastolic: combination of D2 and D3 make up a component of MetS

e1 - Fasting plasma glucose <5.6mmol/L and No history of diabetes

e2 - Fasting plasma glucose < 5.6mmol/L and a history of diabetes

e3 - High Fasting plasma glucose is defined as >=5.6mmol/L

MetS - FPG: combination of e2 and e3 make up a component of MetS

f – Mann-Whitney U test comparing means of males vs. females per category

g - Chi squared test comparing males vs. females with and without abnormality

Table 3.38B Comparison of metabolic syndrome components (Diastolic blood pressure & Fasting plasma glucose), by gender in the MetS population

A previous history of hypertension was reported in 49.13% (CI 95%: 46.10 - 52.17) of the MetS population. Among the MetS population, females (53.20% CI 95%: 48.34 – 58.00) reported to suffer from hypertension more frequently than did their male counterparts (46.52% CI 95%: 42.66 – 50.42). This resulted in a significant difference in hypertension frequency between the two genders ($p$=0.04). A positive relationship between MetS diagnosis and a medical history of hypertension was evident ($p$=<0.01).

#### 3.1.11.1 Metabolic syndrome by body weight

On analyzing the body mass index (BMI) within our study MetS population, the median BMI for both females (32.56 Kg/m$^2$ ±6.7 IQR) and male (31.95 Kg/m$^2$ ±4.80 IQR) populations were within the 'obese' category. No statistical difference was evident for median BMI between both genders ($p$=0.09). On age standardization, the MetS female population between 20 and 39 years had a median BMI within the 'overweight' category whilst from 40 years onwards, female median BMI was within the 'obese' category. On the other hand, the entire age spectrum of the male population had a median BMI within the 'obese' category. A statistical difference was evident between the median BMI values of both genders by age ($p$=0.05).

#### 3.1.11.2 Metabolic syndrome by family history

The majority of the MetS population, as with the non-MetS population ($p$=0.09), did not report a family history of cardiovascular disease (76.49% CI 95%: 72.82 – 78.05). A family history of diabetes mellitus was significantly higher in the male MetS population (43.51% CI 95%: 39.70 - 47.41) when compared to female MetS population. These are illustrated in Table 3.39. On the contrary to the MetS population, the non-MetS females predominantly had a

family history of diabetes mellitus ($p$=<0.01). Both cardiovascular disease and diabetes mellitus family history independently showed no significant association with having MetS ($p$=0.10 and $p$=0.06 respectively). A family history of diabetes mellitus was, however, found to show an association with having MetS after adjusting for gender (OR: 0.86 CI 95% 0.75 – 0.99, $p$=0.04) and after adjusting for both gender and age (OR: 0.86 CI 95%: 0.74 – 0.99, $p$=0.05).

| | MetS population | | | Chi squared |
|---|---|---|---|---|
| | Total ($n$=1,038) | Male ($n$=632) | Female ($n$=406) | $p$-value |
| **Family History of Cardiovascular Disease** | | | | |
| No | 784 (76.49%) | 486 (77.00%) | 308 (75.90%) | 0.67 |
| Yes | 243 (23.41%) | 145 (23.00%) | 98 (24.10%) | |
| | | | | |
| **Family History of Diabetes Mellitus** | | | | |
| No | 543 (52.31%) | 356 (56.40%) | 187 (46.20%) | **<0.01** |
| Yes | 494 (47.59%) | 275 (43.60%) | 219 (53.80%) | |

Table 3.39 Comparison of reported family histories within the metabolic syndrome population

### 3.1.11.3 Metabolic syndrome risk prediction

The lipoprotein ratio of triglycerides to high-density lipoprotein (TG/HDL-C) has been found to be a marker for insulin resistance; LDL-C; atherosclerosis and coronary artery disease. Insulin resistance forms part of the pathophysiology of MetS. Meanwhile, individuals with MetS are at a higher risk of developing coronary heart disease. It may be deduced therefore

that the TG/HDL-C ratio may be considered as a predictor of MetS. A TG/HDL-C ratio above 4 has been considered as '*at higher risk'* for insulin resistance and coronary artery disease. The '*optimal'* ratio should be below 2 (Janiszewska, Kubica and Odrowąż-sypniewska, 2015).

The majority of the population with the MetS exhibited an optimal TG/HDL-C ratio (89.60% CI 95%: 87.58 – 91.31). A small proportion of the male MetS population exhibited a TG/HDL-C ratio above 4 and was considered at high risk for insulin resistance (3.48%, CI 95: 2.28 – 5.24). In contrast, the non-metabolic syndrome (non-MetS) population exhibited a higher proportion of males (as well as females) with a TG/HDL-C ratio above 4, as seen in Table 3.40.

| | **MetS** | |
|---|---|---|
| **TG/HDL-C ratio Risk Ratio** | **Female** | **Male** |
| <= 2.00 (Optimal) | 371 | 559 |
| 2.01 - 3.99 (At risk) | 35 | 51 |
| >=4 (High risk) | 0 | 22 |

| | **Non-MetS** | |
|---|---|---|
| **TG/HDL-C ratio Risk Ratio** | **Female** | **Male** |
| <= 2.00 (Optimal) | 1453 | 1215 |
| 2.01 - 3.99 (At risk) | 72 | 114 |
| >=4 (High risk) | 18 | 27 |

Table 3.40 Distribution of TG/HDL-C risk ratio within the MetS and non-MetS populations, by gender

The median TG/HDL-C ratio values for the metabolic syndrome population (0.68 ±0.77) was statistically higher ($p$=<0.01) than that of the non-metabolic syndrome population (0.61 ±1.95). In this study, the TG/HDL-C ratio was found to exhibit a significant positive association with having the metabolic syndrome ($p$=<0.01). The association remained significant, even after adjustment for age and gender (OR: 1.10, CI95%: 1.01 – 1.19, $p$=<0.01).

### 3.1.11.4 Metabolic syndrome and type 2 diabetes mellitus

A diagnosis of diabetes mellitus compromises one of the components of the metabolic syndrome. In the SAHHTEK population there were 16.76% (CI 95%: 14.61 – 19.16) of participants with a previous diagnosis of diabetes who also had MetS. Of these, males were in the majority (70.11% CI 95%: 62.93 – 76.44) to a significant extent ($p$=0.01). Following the SAHHTEK health examination, 11.37% (CI 95%: 9.57 – 13.45) with a diagnosis of MetS were also newly diagnosed diabetes. A male majority was exhibited in this subgroup (71.19% CI 95%: 62.42 – 78.61), which was significantly higher than that of the female subpopulation ($p$=0.01). Overall, diabetes mellitus was evident in 28.13% (CI 95%: 25.48 – 30.94) of those with a diagnosis of the metabolic syndrome.

On 10-year age stratification, diabetes individuals with MetS were found with a male majority across all age groups above 40 years (Table 3.41). The 50 to 59 years age group exhibited the widest significant discrepancy between male MetS diabetes compared to females MetS diabetes ($p$=0.01).

| Age Groups | MetS T2DM Female ($n$=43) | MetS T2DM Male ($n$=102) | $p$-value |
|---|---|---|---|
| 40 - 49 | 4 (9.30%) | 10 (9.80%) | 0.44 |
| 50 - 59 | 10 (23.26%) | 40 (39.22%) | **0.01** |
| 60 - 69 | 29 (67.44%) | 52 (50.98%) | 0.24 |

MetS – Metabolic syndrome; DM – Diabetes Mellitus

Table 3.41 Distribution of the diabetes population with MetS, by age groups

### 3.1.11.5 Metabolic syndrome and impaired fasting glucose

Elevated FPG is one of the components of MetS. IFG was defined as a fasting plasma glucose ≥5.60mmol/L and ≤6.99mmol/L. A total of 498 participants (47.98% CI 95%: 44.95 – 51.02) with MetS had a diagnosis of impaired fasting plasma glucose (IFG).

The male MetS population (60.44% CI 95%: 56.08 – 64.64) had a statistically higher diagnosis of IFG than did the female MetS population ($p$=0.01).

The IFG MetS population age range was between 30 to 70 years, whereas IFG within the non-MetS population was found from above the age of 20 years. On 10-year age stratification, a male MetS predominance was evident in all the MetS age groups, as seen in Table 3.42. This was also present in the non-MetS population. The male MetS population between the ages of 30 to 39 years was statistically more numerous (four-fold) as compared to the female MetS population. Within the MetS population, the male to female predominance diminished with increasing age. Only the 30 to 39 years age group and the 50 – 59 years age group exhibited a significant difference between both genders, as seen in Table 3.42.

**IFG MetS population by age**

| Age groups | Female (*n*=109) | Male (*n*=207) | *p*-value |
|---|---|---|---|
| 30 - 39 | 5 (4.59%) | 22 (10.63%) | **<0.01** |
| 40 - 49 | 12 (11.01%) | 41 (19.81%) | 0.53 |
| 50 - 59 | 23 (21.10%) | 55 (26.57%) | **<0.01** |
| 60 - 69 | 69 (63.30%) | 89 (43.00%) | 0.86 |

Table 3.42 Distribution of the IFG population with MetS, by age groups

### 3.1.11.6 Comparison between IFG and T2DM with metabolic syndrome

Fasting plasma glucose above 5.6mmol/L or a frank diagnosis of diabetes mellitus forms one of the MetS diagnostic components.

The median waist circumference of the MetS diabetes population (male 107 ±27.6cm; female 100.5 ±23cm IQR) was statistically higher than that of the MetS IFG population ($p$=<0.01). On comparing the remaining MetS components between the two dysglycaemic categories, it was noted that the median values for the diabetes population were statistically higher than those in the MetS IFG population. An exception was evident in the diastolic blood pressure median value, where the MetS IFG population, had a significantly higher value than did the MetS diabetes population. Table 3.43 and Table 3.44 compares the MetS components between the IFG and diabetes mellitus MetS population.

| | IFG MetS population | | Diabetes MetS population | | |
|---|---|---|---|---|---|
| | **Total (*n*=498)** | | **Total (*n*=292)** | | |
| | Median ±IQR | *n* (%) | Median ±IQR | *n* (%) | *p*-value |
| **HDL-C Cholesterol (mmol/L)** | 1.25 ±0.89 | | 1.1 ±1.11 | | **<0.01[f]** |
| Normal / High | | 318 (63.86%) | | 241 (82.53%) | **<0.01[g]** |
| Low[a] | | 180 (36.14%) | | 51 (17.47%) | |
| | | | | | |
| **Triglycerides (mmol/L)** | 1.35 ±2.34 | | 1.67 ±2.58 | | **<0.01[f]** |
| Normal | | 337 (67.67%) | | 154 (52.74%) | **<0.01[g]** |
| High[b] | | 161 (32.33%) | | 138 (47.26%) | |
| | | | | | |
| **Systolic Blood pressure (mmHg)** | 131 ±34 | | 133 ±38 | | **<0.01[f]** |
| Normal[c1] | | 114 (22.89%) | | 45 (15.41%) | **0.01[g]** |
| Normal[c2] | | *60* | | *47* | |
| High[c3] | | *324* | | *200* | |
| MetS - Systolic | | 384 (77.11%) | | 247 (84.59%) | |

a - Low HDL-C is defined as HDL-C<1.03mmol/L (male), HDL-C < 1.29mmol/L (female)
b - High Triglycerides is defined as triglycerides >1.70mmol/L
c1 - Normal systolic blood pressure <130mmHg and not on anti-hypertensive
c2 - Normal systolic blood pressure <130mmHg and on anti-hypertensive
c3 - High Systolic blood pressure is defined as >=130mmHg
MetS - Systolic: combination of C2 and C3 make up a component of MetS
f - Mann-Whitney U comparing median of IFG vs. T2DM per category
g - Chi squared test comparing IFG vs. T2DM with and without abnormality

Table 3.43 Distribution of MetS components (HDL-C, Triglyceride and Systolic blood pressure) within the IFG and diabetes mellitus metabolic syndrome population

| | IFG population | | Diabetes population | | |
|---|---|---|---|---|---|
| | **Total (*n*=498)** | | **Total (*n*=292)** | | |
| | Median ±IQR | *n* (%) | Median ±IQR | *n* (%) | *p*-value |
| **Diastolic Blood pressure (mmHg)** | 81 ±10 | | 79 ±24 | | **<0.01[f]** |
| Normal[d1] | | 199 (39.96%) | | 86 (29.45%) | **<0.01[g]** |
| Normal[d2] | | *121* | | *140* | |
| High[d] | | *178* | | *66* | |
| MetS - Diastolic | | 299 (60.04%) | | 206 (70.55%) | |
| | | | | | |
| **Fasting plasma glucose (mmol/L)** | 5.92 ±0.99 | | 7.83 ±8.6 | | **<0.01[f]** |
| Normal[e1] | | 0 | | 0 | |
| Normal[e2] | | *0* | | *8* | |
| High[e3] | | *498* | | *284* | |
| MetS - FPG | | 498 (100%) | | 292 (100%) | |

d1 - Normal diastolic blood pressure <85mmHg and not on anti-hypertensive
d2 - Normal diastolic blood pressure <85mmHg and on anti-hypertensive
d3 - High Diastolic blood pressure is defined as >=85mmHg
MetS - Diastolic: combination of D2 and D3 make up a component of MetS
e1 - Fasting plasma glucose <5.6mmol/L and No history of diabetes
e2 - Fasting plasma glucose < 5.6mmol/L and a history of diabetes
e3 - High Fasting plasma glucose is defined as >=5.6mmol/L
MetS - FPG: combination of e2 and e3 make up a component of MetS
f - Mann-Whitney U comparing median of IFG vs. T2DM per category
g - Chi squared test comparing IFG vs. T2DM with and without abnormality

Table 3.44 Distribution of MetS components (Diastolic blood pressure and FPG) within the IFG and diabetes mellitus metabolic syndrome population

### 3.1.12 Depression

The self-reported (*n*=412) prevalence of current depression was 10.44% (CI 95%: 9.66 – 11.27), among whom 61.65% (CI 95%: 56.87 – 66.22) were female. The gender difference was statistically significant (*p*=<0.01). Among those reporting to suffer from depressive illness, only 7.77% (CI 95%: 5.53 – 10.79) were on current medication. It was noteworthy that 6.70% (*n*=237; CI 95%: 5.92 – 7.58) of the study population who did not self-report suffering from depression, reported to be on anti-depressive medication. Those suffering from depression were significantly older (mean 50 ±14 SD) than the others (mean 44 ±15 SD) (*p*=<0.01).

#### 3.1.12.1 Depression by type 2 diabetes mellitus

Diabetes mellitus type 2 was present among 14.32% (CI 95%: 11.25 – 18.05) of the population with depression (self-reported *n*=59). Out of which, the majority (71.19% CI 95%: 58.55 – 81.23) were previously known diabetes (*n*=42).

The male diabetes population exhibited a significant majority (55.15%, CI 95%: 42.45 – 67.25; *p*=0.05) of those suffering from depression. On age stratification, it was observed that among the male population a diagnosis of diabetes and self-reported depression coexisted from the age of 50 years whereas among the female population's diabetes and self-reported depression first coexisted after the age of 60 years (Table 3.45).

| | Age groups in years | Diabetes Mellitus | | |
|---|---|---|---|---|
| | | **No Depression (*n*=349)** | **Depression (*n*=58)** | ***p*-value*** |
| Male | 30 - 39 | | | |
| | 40 - 49 | 10 | 0 | |
| | 50 - 59 | 45 | 5 | |
| | 60 - 69 | 63 | 7 | 0.73** |
| | 70 | 121 | 20 | 0.71*** |
| Female | 30 - 39 | 5 | 0 | |
| | 40 - 49 | 4 | 0 | |
| | 50 - 59 | 10 | 0 | |
| | 60 - 69 | 38 | 7 | |
| | 70 | 53 | 19 | |

*Chi-square test - depression by gender and age groups
**Male vs. Female 60 – 69 years
*** Male vs. Female 70 years

Table 3.45 Distribution of diabetes mellitus population, by self-reported depression diagnosis and age groups

On comparing the demographic characteristics of the diabetes individuals according to the presence or absence of depression (self-reported), a significant difference was evident between the two populations with regard to employment and educational levels (Table 3.46). The largest group making up the *diabetes individuals with a history of depression* comprised of participants who were retired and had an educational level only up to primary school.

| Demographic characteristics | | Diabetes Mellitus | | |
|---|---|---|---|---|
| | | **No Depression (*n*=349)** | **Depression (*n*=58)** | ***p*-value*** |
| Districts | Southern Harbour | 73 (20.92%) | 12 (20.69%) | 0.82 |
| | Northern Harbour | 105 (30.09%) | 19 (32.76%) | |
| | South Eastern | 43 (12.32%) | 4 (6.90%) | |
| | Western | 53 (15.19%)) | 11 (18.97%) | |
| | Northern | 43 (12.32%) | 8 (13.79%) | |
| | Gozo | 32 (9.17%) | 4 (6.90%) | |
| | | | | |
| Employment Status | Employed | 161 (46.13%) | 7 (12.07%) | **<0.01** |
| | Unemployed | 4 (1.15%) | 2 (3.45%) | |
| | Pupil | 2 (0.58%) | 0 | |
| | Retired | 119 (34.10%) | 32 (55.17%) | |
| | Full fill domestic tasks | 62 (17.77%) | 17 (29.31%) | |
| | Caring for parents | 1 (0.29%) | 0 | |
| | | | | |
| Education | No education | 4 (1.15%) | 2 (3.45%) | **0.01** |
| | Primary education | 69 (19.77%) | 21 (36.21%) | |
| | Unfinished secondary | 49 (14.04%) | 12 (20.69%) | |
| | Finished secondary | 136 (38.97%) | 15 (25.86%) | |
| | Tertiary | 38 (10.89%) | 6 (10.34%) | |
| | University | 47 (13.47%) | 2 (3.45%) | |
| | Post-graduate | 6 (1.72%) | 0 | |

* Chi square test

Table 3.46 Distribution of demographic characteristics in the diabetes mellitus population, by the presence or absence of self-reported history of depression

Individuals suffering from diabetes mellitus and a history of depression (self-reported) did not exhibit any statistical difference in lifestyle and risk behavioural characteristics from those persons with diabetes without depression as seen in Table 3.47.

| Behavioural characteristics | | Diabetes Mellitus | | |
|---|---|---|---|---|
| | | **No Depression (*n*=349)** | **Depression (*n*=58)** | ***p*-value*** |
| Smoke at present | Not at all | 259 (74.21%) | 50 (86.21%) | 0.06 |
| | Yes, occasionally | 28 (8.02%) | 0 | |
| | Yes, daily | 62 (17.77%) | 8 (13.79%) | |
| | | | | |
| Alcohol habits | Alcohol consumption | 170 (48.71%) | 27 (46.55%) | 0.76 |
| | No alcohol consumption | 179 (51.29%) | 32 (55.17%) | |
| | | | | |
| Physical activity | No activity | 31 (8.88%) | 3 (5.17%) | 0.16 |
| | Walking | 56 (16.05%) | 4 (6.90%) | |
| | Low Activity | 221 (63.32%) | 43 (74.14%) | |
| | Moderate Activity | 40 (11.46%) | 8 (13.79%) | |
| | High Activity | 1 (0.29%) | 0 | |

*Chi square test

Table 3.47 Distribution of behavioural characteristics within the diabetes mellitus population, by the presence or absence of self-reported history of depression

Interestingly, the diabetes population that reported to suffer from depression had a significant higher median FPG than the persons with diabetes without depression ($p$=0.04). However, on comparing the median FPG between the previously known diabetes and newly diagnosed diabetes with a history of depression, no significant difference in FPG was present ($p$=0.69).

## 3.2 Analytic analysis

This section (3.2) describes the analytic results performed on the unadjusted population data (*n*=1,861) to determine the relationships between anthropometric and biochemical parameters. In particular, links between: (1) socio-demographic parameters; (2) lifestyle parameters; (3) type 2 diabetes mellitus (T2DM) and impaired fasting glucose (IFG) as well as with (4) body mass index (BMI) are explored.

The unadjusted population was subdivided into four sub-groups according to the dysglycaemic status (diabetes mellitus *n*=219 and IFG *n*=460) and metabolic status (metabolically abnormal *n*=1,056 and metabolically healthy *n*=126) of individuals.

The metabolically abnormal sub-group was defined as those participants having a normal fasting plasma glucose (FPG) with at least one other abnormal parameter such as an anthropometric measure (BMI, waist circumference or blood pressure) or lipid profile (LDL-C, HDL-C, total cholesterol or triglycerides). The metabolically abnormal sub-group was considered a priori as having possible higher underlying risk for insulin resistance and therefore at risk of developing the dysglycaemic state later on in life (Nyenwe and Dagogo-Jack, 2011; Grundy, 2012).

The metabolically healthy subgroup was defined as those participants with a normal FPG, normal anthropometric parameters and normal lipid profile. The diabetes (T2DM) sub-population was compared to the metabolically healthy subgroup when analysing for links between the different anthropometric and biochemical parameters. By comparing to the metabolically healthy sub-group, the presence of any underlying metabolic or insulin resistance within the reference group, that might affect the linkage analyses, was excluded.

### 3.2.1 Relationship between anthropometry, age and gender

This section considers the unadjusted total population (*n*=1,861) to determine the relationships between the different anthropometric parameters (BMI, waist circumference and blood pressure) with age and gender.

#### 3.2.1.1 Body mass index (BMI) by age and gender

Age exhibited a positive correlation with BMI (R = 0.23, *p*=<0.01) with the median age being significantly different across the different BMI categories, as seen in Figure 3.54.

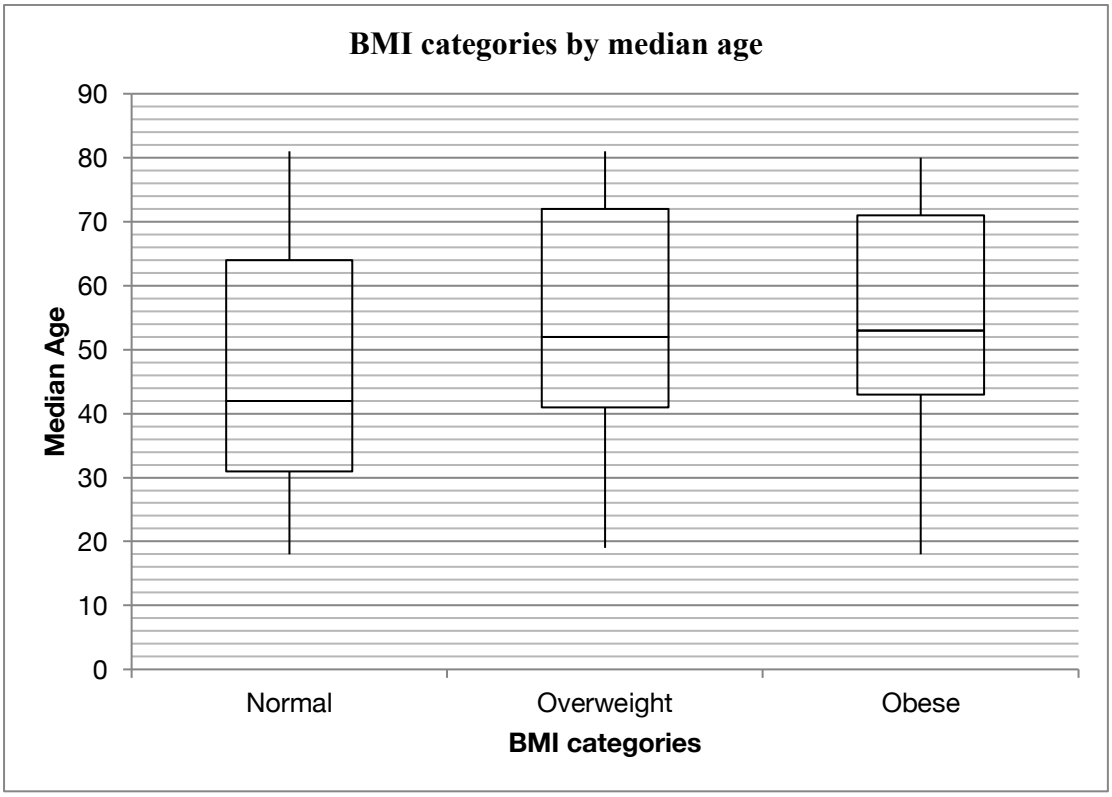



Figure 3.54 Distribution of BMI categories, by median age and IQR

On 10-year age stratification, a steady rise in median BMI could be seen as age groups progressed, as seen in Figure 3.55.

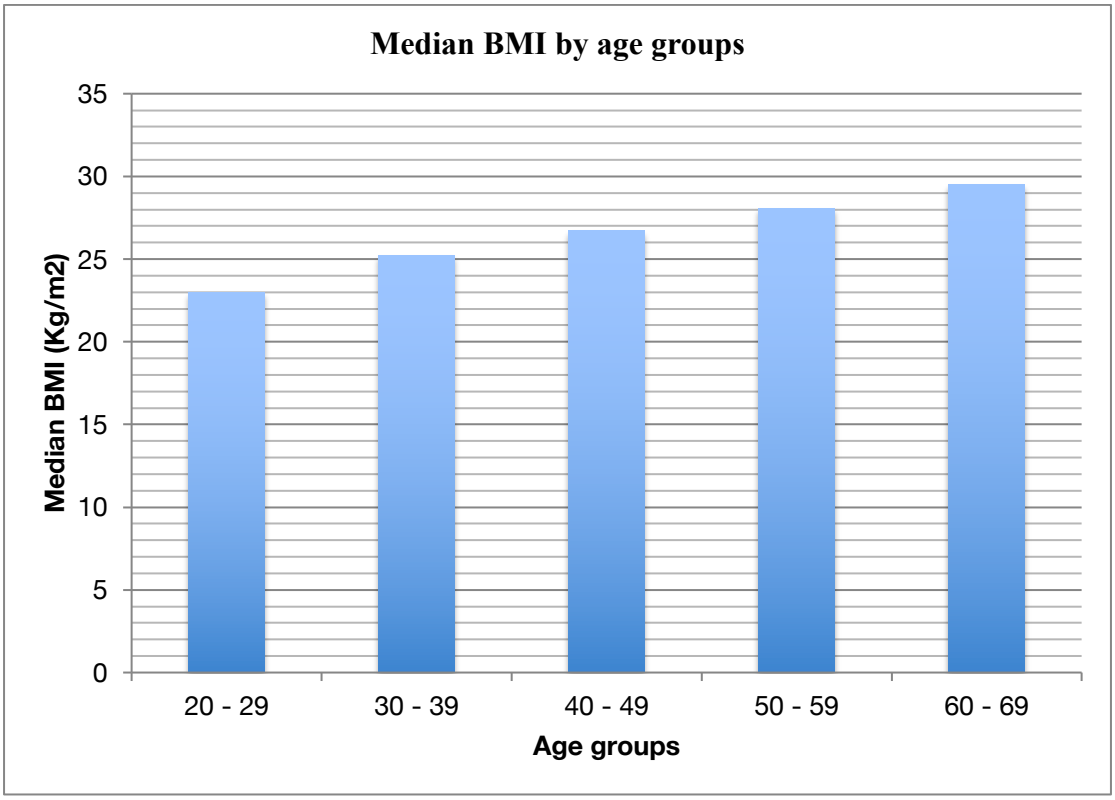



Figure 3.55 Distribution of median BMI, by age groups

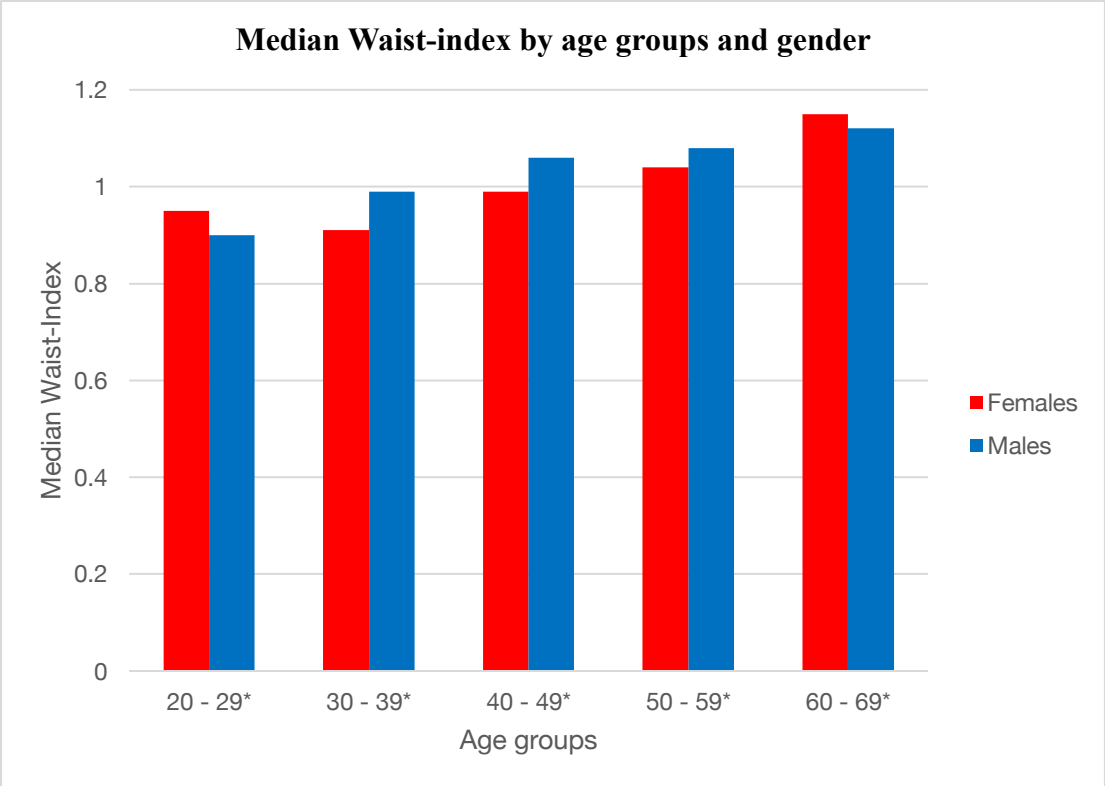
The majority of the population exhibited a median BMI within the overweight category (25.00 – 29.99Kg/m$^2$) with the exception of the 20 – 29 years age group that had a normal median BMI. On gender stratification, both the male population ($p$=<0.01) and the female populations ($p$=<0.01) exhibited a significant relationship between age and median BMI.

With an increase in age (per year) there was a positive association with having the status of being overweight (OR: 1.01 CI 95%: 1.01 – 1.02, $p$=<0.01). The associated risk remained significant on adjusting for gender, suggesting that gender was not a cofounding

factor in this relationship. Similarly, for every increase in age (per year) a positive association for having an obese status was evident (OR: 1.02 CI 95%: 1.02 – 1.03, $p$=<0.01) and this remained significant on adjusting for gender.

### 3.2.1.2 Waist circumference by age and gender

The median waist circumference of the male sample population was found to be statistically higher than that in the female population ($p$=<0.01). The median waist-index for the males was 1.23 (IQR: 0.2) while that for females was 1.06 (IQR: 0.25). As the 10-year age groups increased, the median waist-index for both the male and female populations increased gradually, as seen in Figure 3.56.




*Mann-Whitney U test: male vs. female $p$-value <0.05

Figure 3.56 Distribution of the median waist-index, by age groups and gender

Within the unadjusted population, an elevated waist index was negatively associated across most age groups, as seen in Table 3.48.

| Elevated waist-index as outcome ( >=1.115) | | | |
|---|---|---|---|
| **Age groups (in years)** | **Odds ratio** | **95% Confidence interval** | ***p*-value** |
| 18 - 19 | 0.23 | 0.05 - 0.99 | **0.05** |
| 20 - 29 | 0.40 | 0.01 - 0.17 | **<0.01** |
| 30 - 39 | 0.15 | 0.10 - 0.21 | **<0.01** |
| 40 - 49 | 0.29 | 0.21 - 0.39 | **<0.01** |
| 50 - 59 | 0.44 | 0.33 - 0.59 | **<0.01** |
| 60 - 69 | 0.88 | 0.66 - 1.17 | 0.36 |
| 70 | Reference | | |

Table 3.48 Association between age group and elevated waist-index

### 3.2.1.3 Blood pressure by age and gender

Age had positive correlations with systolic blood pressure (R= 0.43, *p*=<0.01) and with diastolic blood pressure (R= 0.21, *p*=<0.01). As expected, gradual increases in both the median systolic and diastolic blood pressure levels were evident as age increased, as seen in Figure 3.57. Significant median differences between both systolic (*p*=<0.01) and diastolic (*p*=<0.01) blood pressures and the different age groups were evident.

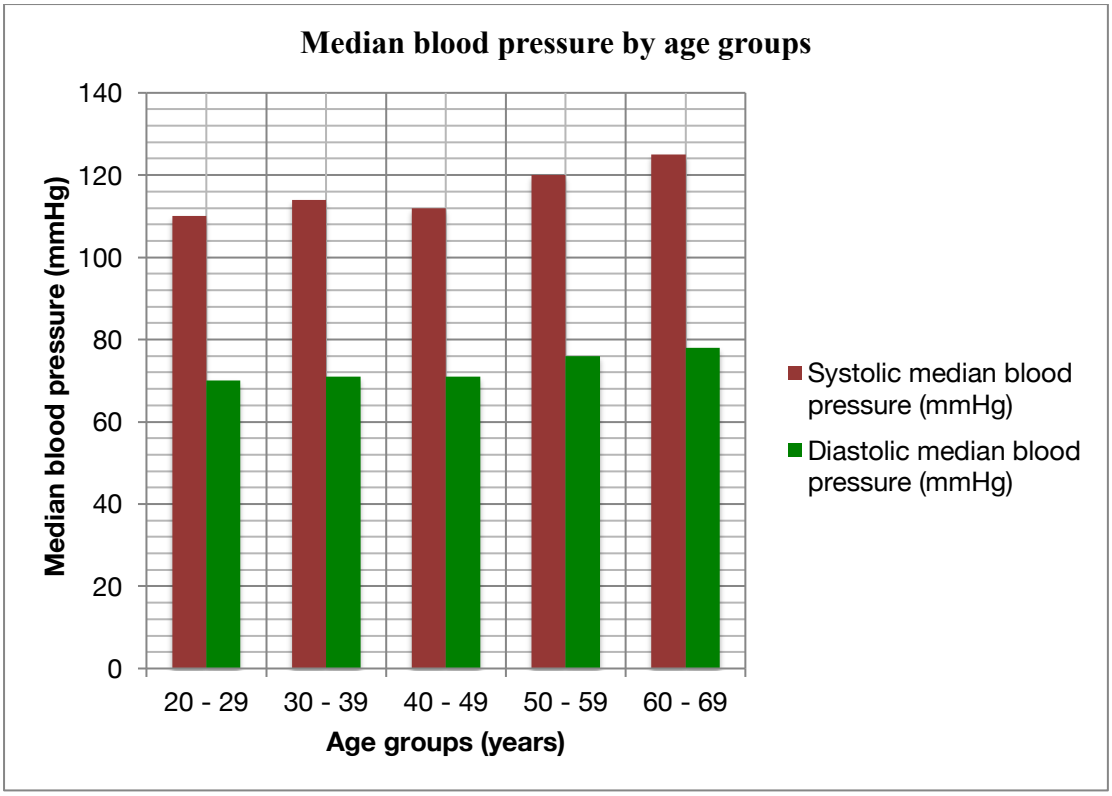



Figure 3.57. Distribution of the median systolic and diastolic blood pressures, by 10-year age group

The female population exhibited significantly lower median systolic and diastolic blood pressure levels then did their male counterparts for every age group, as seen in Table 3.49.

There was a positive association between a unit increase in age (per year) and having hypertension (elevated systolic or elevated diastolic blood pressure), (OR: 1.08, CI 95%: 1.07 – 1.09, $p$=<0.01), even after adjusting for gender.

| **Age groups in years** | **Median Systolic Blood pressure (mmHg)** | | *p*-value* |
|---|---|---|---|
| | **Female (*n*=1,025)** | **Male (*n*=836)** | |
| 20 - 29 | 105 ±10 | 120 ±15 | **0.04** |
| 30 - 39 | 110 ±17 | 120 ±13 | **<0.01** |
| 40 - 49 | 108 ±18 | 119 ±14 | **<0.01** |
| 50 - 59 | 115 ±16 | 121 ±15 | **<0.01** |
| 60 - 69 | 123 ±17 | 128 ±18 | **0.03** |

| **Age groups in years** | **Median Diastolic Blood pressure (mmHg)** | | *p*-value* |
|---|---|---|---|
| | **Female (*n*=1,025)** | **Male (*n*=836)** | |
| 20 - 29 | 64 ±10 | 80 ±7 | **0.03** |
| 30 - 39 | 70 ±13 | 75 ±10 | **<0.01** |
| 40 - 49 | 69 ±12 | 75 ±11 | **<0.01** |
| 50 - 59 | 72 ±12 | 79 ±15 | **<0.01** |
| 60 - 69 | 76 ±11 | 80 ±13 | **<0.01** |

* Mann-Whitney U test

Table 3.49 Distribution of the median systolic and diastolic blood pressure, by age group and gender

On analysing for the relationship between age group (10 year) and having hypertension, only the oldest age (70 years) exhibited a positive association when compared to the youngest age group, as seen in Table 3.50. This held true even after adjusting for gender.

| Hypertension - dependent variable | | | |
|---|---|---|---|
| **Age groups in years** | **Odds ratio** | **95% Confidence interval** | ***p*-value** |
| 18 - 19 | Reference | | |
| 20 - 29 | 0.70 | 0.06 - 8.97 | 0.78 |
| 30 - 39 | 0.94 | 0.11 - 8.01 | 0.96 |
| 40 - 49 | 0.97 | 0.12 - 8.08 | 0.96 |
| 50 – 59 | 2.44 | 0.30 - 20.07 | 0.41 |
| 60 - 69 | 6.39 | 0.78 - 52.36 | 0.08 |
| 70 | 11.26 | 1.37 - 92.37 | **0.02** |
| | | | |
| 18 – 19 | Reference | | |
| 20 – 29 | 0.73 | 0.06 - 9.50 | 0.81 |
| 30 – 39 | 1.01 | 0.12 - 8.61 | 0.10 |
| 40 - 49 | 0.99 | 0.12 - 8.36 | 0.99 |
| 50 - 59 | 2.57 | 0.31 - 21.27 | 0.38 |
| 60 - 69 | 6.66 | 0.81 - 54.99 | 0.08 |
| 70 | 11.91 | 1.44 - 98.55 | **0.02** |
| Female gender | Reference | | |
| Male gender | 1.74 | 1.41 - 2.14 | **<0.01** |

Table 3.50 Association analysis between age groups and elevated systolic and diastolic blood pressure

### 3.2.2 Relationship between anthropometry and socio-demographic profiles

This section considers the crude unadjusted sample population (*n*=1,861) to determine the relationships between the different anthropometric parameters (BMI, waist circumference and blood pressure) and socio-demographic profiles (districts, education and employment status).

### 3.2.2.1 Body mass index and socio-demographic profile

#### 3.2.2.1.1 Body mass index by districts

No relationship was present between BMI status and the different districts ($p$=0.06). The median BMI did not differ between the different districts ($p$=0.20), even when stratified by gender (male $p$=0.09 and females $p$=0.40).

#### 3.2.2.1.2 Body mass index and education level

The median BMI decreased as the educational level increased ($p$=<0.01). This BMI (median) relationship with education was also present on gender stratification as seen in Table 3.51. It was noteworthy that all median BMI values across the age and gender groups were found to be above the normal (>25Kg/m$^2$).

| Highest education level | BMI (Kg/m2) | | | | |
|---|---|---|---|---|---|
| | Female ($n$=1,025) | | Male ($n$=836) | | |
| | Median | IQR | Median | IQR | $p$-value* |
| No formal education | 26.57 | 7.20 | 30.80 | 2.76 | 0.42 |
| Primary | 30.20 | 6.88 | 29.25 | 7.60 | 0.10 |
| Unfinished secondary | 29.94 | 8.10 | 30.38 | 5.46 | 0.95 |
| Finished secondary | 28.16 | 7.80 | 29.00 | 5.78 | **0.05** |
| Tertiary | 25.10 | 6.80 | 28.25 | 7.03 | **<0.01** |
| University | 25.60 | 7.00 | 28.20 | 5.30 | **<0.01** |
| Post-graduate | 25.00 | 7.56 | 28.10 | 5.99 | **0.01** |

*Mann Whitney U test

Table 3.51 Distribution of the crude unadjusted study population median BMI (Kg/m$^2$) by education level and gender

The female population exhibited a lower BMI (median) when compared to the male population. This held for all educational levels except for those completing primary school education only (not significant). The significance varied between the different education levels as seen in Table 3.51. In fact, a significantly lower BMI status (within the borderline overweight BMI range) was evident for the female population with a high educational attainment (tertiary, university and postgraduate) when compared to their male counterparts (Table 3.51).

On analysing the different BMI categories (normal, overweight, obese) separately by education levels, a significant difference was evident between individuals who were obese ($p$=<0.01), overweight ($p$=<0.01) and normal ($p$=<0.01) and their education level. On gender stratification, the obese subgroup exhibited significant differences between the male and female populations and their education levels ($p$=<0.01). No statistical difference was exhibited between the male and female populations with a normal weight ($p$=0.35) or who were overweight BMI ($p$=0.50) across the different education levels.

On comparing those with all other educational levels to those with post-graduate education (reference category), no formal education (OR: 3.90 CI 95%: 1.01 – 14.99, $p$=0.05), primary education (OR: 2.55 CI 95%: 1.56 – 4.83, $p$=<0.01), unfinished secondary education (OR: 2.75 CI 95%: 1.56 – 4.83, $p$=<0.01) and completed secondary education (OR: 1.66, CI 95%: 1.02 – 2.69, $p$=0.04) all exhibited a significantly strong link with being obese. Following age and gender adjustment; primary education (OR: 2.03 CI 95%: 1.15 – 3.58, $p$=<0.01) and unfinished secondary school (OR: 2.28 CI 95%: 1.27 – 4.10, $p$=<0.01) remained positively associated with being obese.

#### 3.2.2.1.3 Body mass index by employment status

The median BMI for all the different occupational categories were within the overweight range except for students ($p$=<0.01), as seen in Figure 3.58.

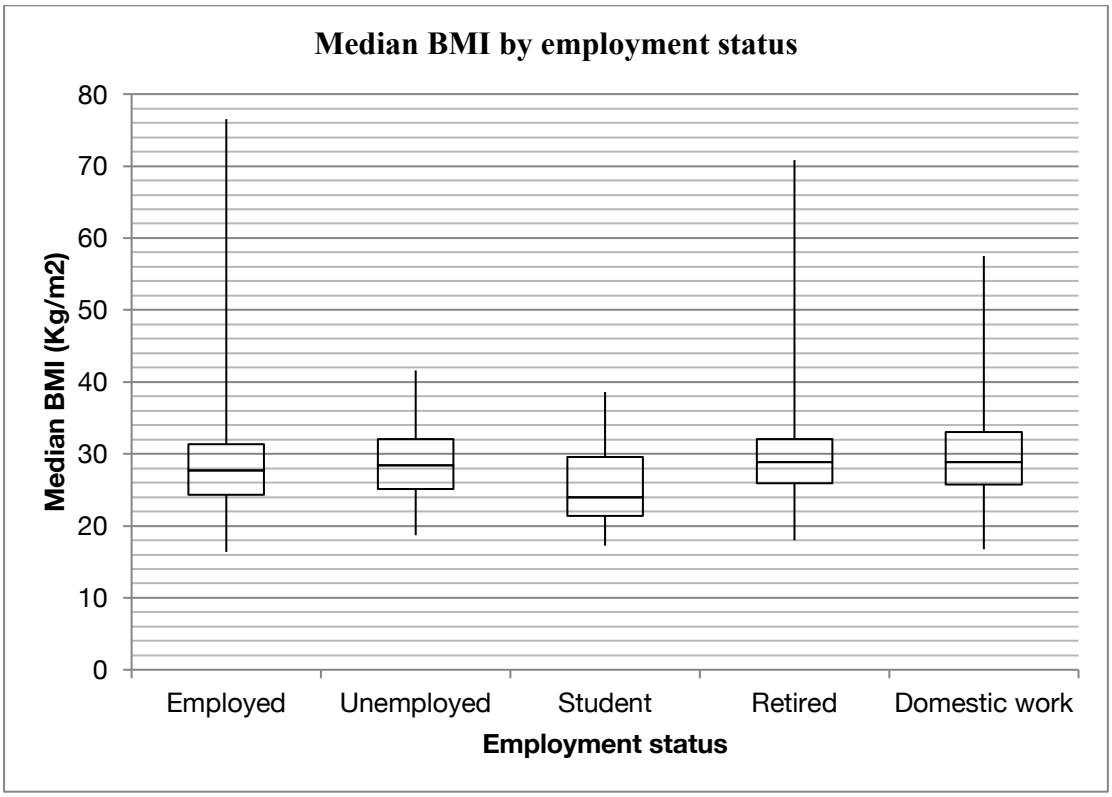



Figure 3.58 Demonstrates employment status, by median BMI (Kg/m2) and IQR

When compared to domestic work (reference category), both employed, and student status exhibited a negative association with having obesity (Table 3.52). After adjusting for both gender and age, only the employed status remained negatively associated with being obese BMI, as seen in Table 3.52.

No significance associations were present between employment status and overweight BMI after adjusting for age and gender.

| Obesity as dependent variable | | | |
|---|---|---|---|
| Variable | Odds ratio | 95% Confidence interval | *p*-value |
| Employed (*n*=1095) | 0.65 | 0.52 - 0.82 | **<0.01** |
| Unemployed (*n*=36) | 0.73 | 0.36 - 1.48 | 0.39 |
| Student (*n*=35) | 0.32 | 0.14 - 0.76 | **<0.01** |
| Retired (*n*=282) | 0.87 | 0.64 - 1.18 | 0.36 |
| Domestic work (*n*=413) | Reference | | |
| | | | |
| Employed | 0.58 | 0.44 - 0.78 | **<0.01** |
| Unemployed | 0.60 | 0.29 - 1.24 | 0.17 |
| Student | 0.49 | 0.20 - 1.21 | 0.12 |
| Retired | 0.47 | 0.33 - 0.67 | 0.10 |
| Domestic work | Reference | | |
| Female gender | 0.57 | 0.45 - 0.71 | **<0.01** |
| Male gender | Reference | | |
| Age | 1.02 | 1.01 - 1.03 | **<0.01** |

Table 3.52 Link between employment status and obesity

### 3.2.2.2 Waist circumference and socio-demographic profile

#### 3.2.2.2.1 Waist circumference by districts

The waist circumference did not exhibit any relationships with districts ($p$=0.55), nor was there any difference between the median waist circumference and the different districts ($p$=0.30).

#### 3.2.2.2.2 Waist circumference by highest education levels

As with BMI, the median waist circumference exhibited a general decline as the educational level increased ($p$=<0.01) as seen in Figure 3.59.

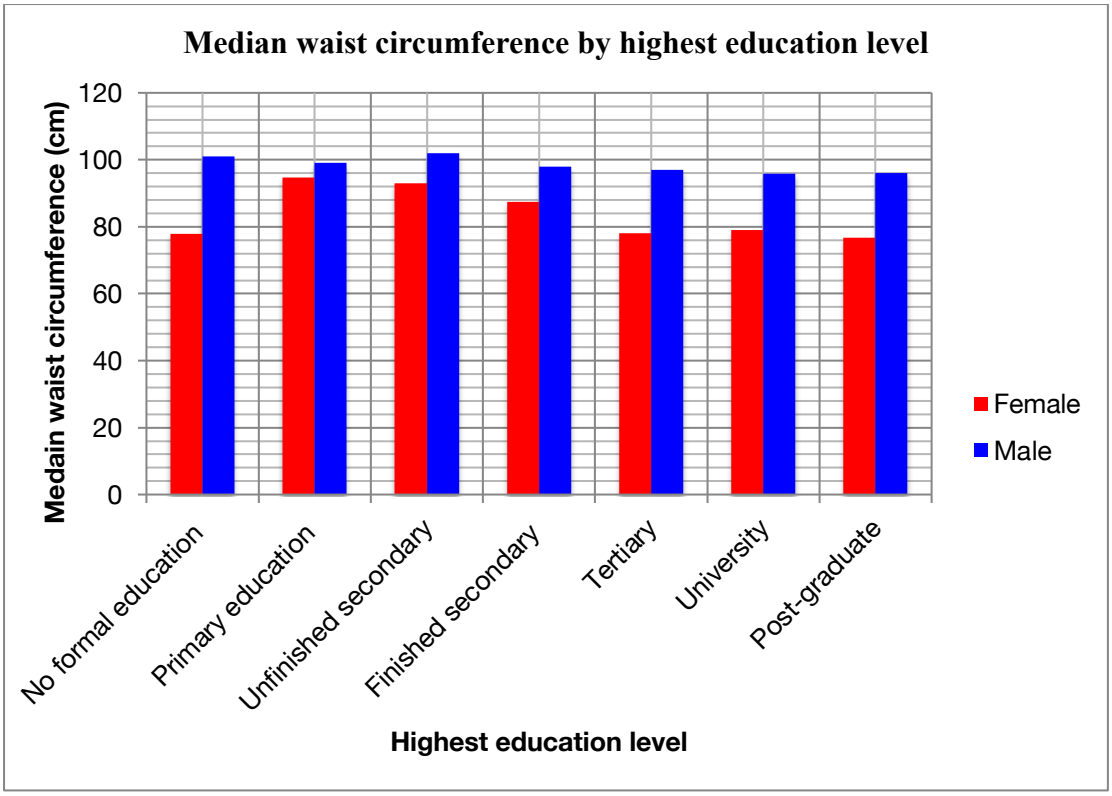



Figure 3.59 Distribution of median waist circumference by highest education level

In fact, a negative correlation was present between waist circumference and educational levels in years (R= -0.22, *p*=<0.01).

The male population exhibited no statistical association between education levels and waist circumference (*p*=0.13) after adjusting for age. The female population on the other hand exhibited a positive link between (low) educational level and elevated waist circumference after adjusting for age (Primary education OR: 3.07 CI 95%: 1.43 – 6.59, *p*=<0.01; Finished secondary education OR: 1.76 CI 95%: 1.09 – 2.85, *p*=0.02).

### 3.2.2.2.3 Waist circumference by employment status

The median waist circumference was statistically different across the different employment categories ($p$=<0.01 respectively), as seen in Figure 3.60.

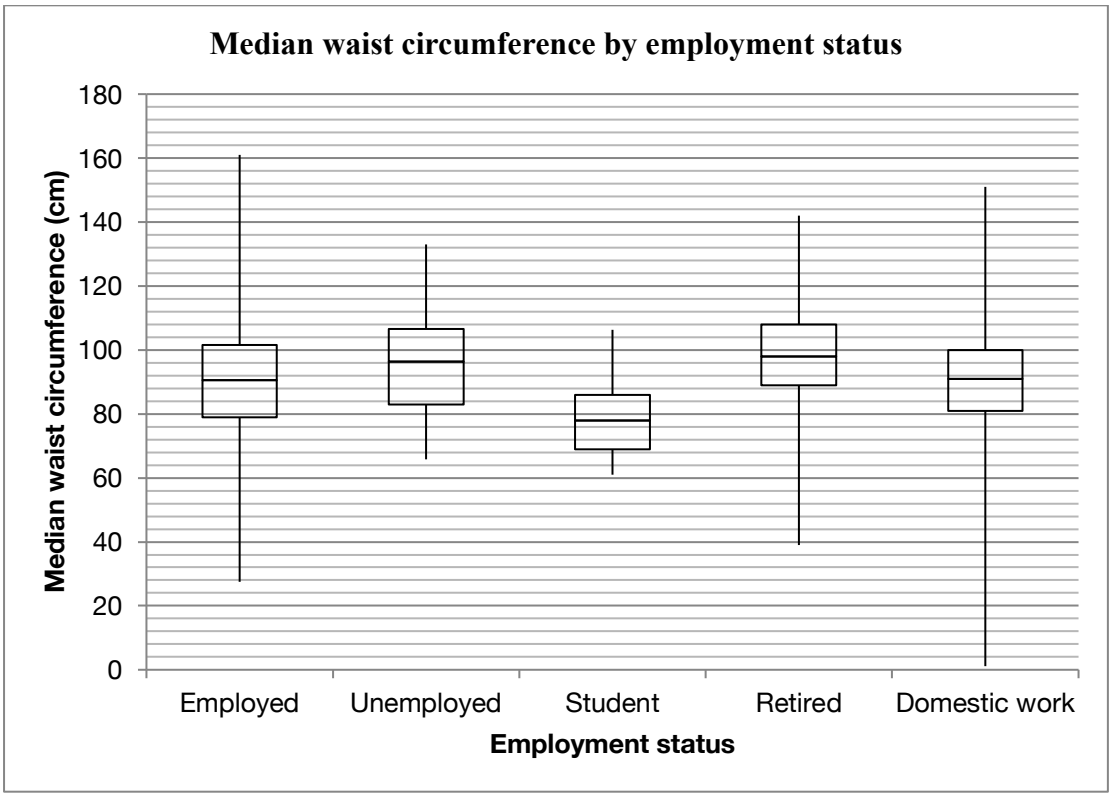



Figure 3.60 Distribution of the median waist circumference by employment status

The student sub-group had the lowest median circumference when compared to all the other occupations ($p$=<0.01). The retired sub-group had a higher median waist circumference than did the employed and the domestic sub-groups ($p$=<0.01 respectively).

On comparing to domestic work (reference category), the male population was found to have a positive association of having an elevated waist circumference if they were retired (OR: 2.51 CI 95%: 1.81 – 3.49, $p$=<0.01) while a negative association of having an elevated

waist circumference if they were students (OR: 0.18 CI 95%: 0.07 – 0.45, *p*=<0.01). After adjusting for age, only the employed (OR: 1.81 CI 95%: 01.40 – 2.35, *p*=<0.01) and the unemployed (OR: 2.33 CI 95%: 1.11 – 4.91, *p*=<0.01) males were found to have a positive association of having an elevated waist circumference.

Similarly, the female population (when compared to domestic work reference category) was found to have a positive association of having an elevated waist circumference in the retired category (OR: 4.14 CI 95%: 2.46 – 6.95, *p*=<0.01), while student females exhibited a negative association of having an elevated waist circumference (OR: 0.25 CI 95%: 0.13 – 0.51, *p*=<0.01). After adjustment for age, only the employment status (OR: 1.61 CI 95%: 1.19 – 2.18, *p*=<0.01) and the retired status (OR: 2.04 CI 95% 1.19 – 3.51, *p*=0.01) were found to exhibit a significant association of having an elevated waist circumference among females.

#### 3.2.2.3 Blood pressure and socio-demographic profile

##### 3.2.2.3.1 Blood pressure by districts

There were statistical differences between the median systolic blood pressure (*p*=0.02) and diastolic blood pressure (*p*=0.01) across the different districts. However, on pairwise comparisons, the Gozo district exhibited a significantly higher systolic blood pressure when compared to the Western district (*p*=0.02), the Southern Harbour (*p*=0.02) and to the Northern district (*p*=0.03). Similarly, the median diastolic blood pressure in Gozo was significantly higher than it was in the Southern Harbour district (*p*=<0.01).

The inhabitants of Gozo, Western and South Eastern districts (when compared to South Harbour district as reference category) showed a positive link to having an elevated blood pressure (Gozo – OR: 1.51 CI 95%: 1.02 – 2.24, *p*=0.04; Western – OR: 1.38 CI 95%: 1 – 1.89, *p*=0.05; South Eastern – OR: 1.46 CI 95%: 1.06 – 2.00, *p*=0.02). After adjusting for age and gender, the inhabitants of Gozo district (OR: 1.60 CI 95%: 1.03 – 2.48, *p*=0.04); the inhabitants of Western district (OR: 1.50 CI 95%: 1.05 – 2.14, *p*=0.02) and the inhabitants of the South Eastern (OR: 1.74 CI 95%: 1.22 – 2.47, *p*= <0.01) remained significantly associated with having an elevated blood pressure. These associations remained significant for all three districts on adjusting for age, gender, education level, employment status, smoking and alcohol habits (Gozo – OR: 1.56 CI 95%: 1.00 – 2.44, *p*=0.05; Western – OR: 1.51 CI 95%: 1.05 – 2.17, *p*=0.03; South Eastern – OR: 1.71 CI 95%: 1.20 – 2.44, *p*= <0.01). However, on adjusting further for BMI, only the Western district remained positively associated with having an elevated blood pressure (OR: 1.64, CI 95%: 1.14 – 2.35, *p*=0.01).

#### 3.2.2.3.2 Blood pressure by education level

The median blood pressure (systolic and diastolic) showed an inverse relationship with education level as two of the lowest educational attainment categories (finished only primary and unfinished secondary education) had the highest blood pressure measurements (p=<0.05). Those with post-graduate education, but also, those with no formal education had significantly the lowest blood pressure as seen in Figure 3.61 (*p*=<0.01 median systolic vs. education level, *p*=<0.01 median diastolic vs. education level).

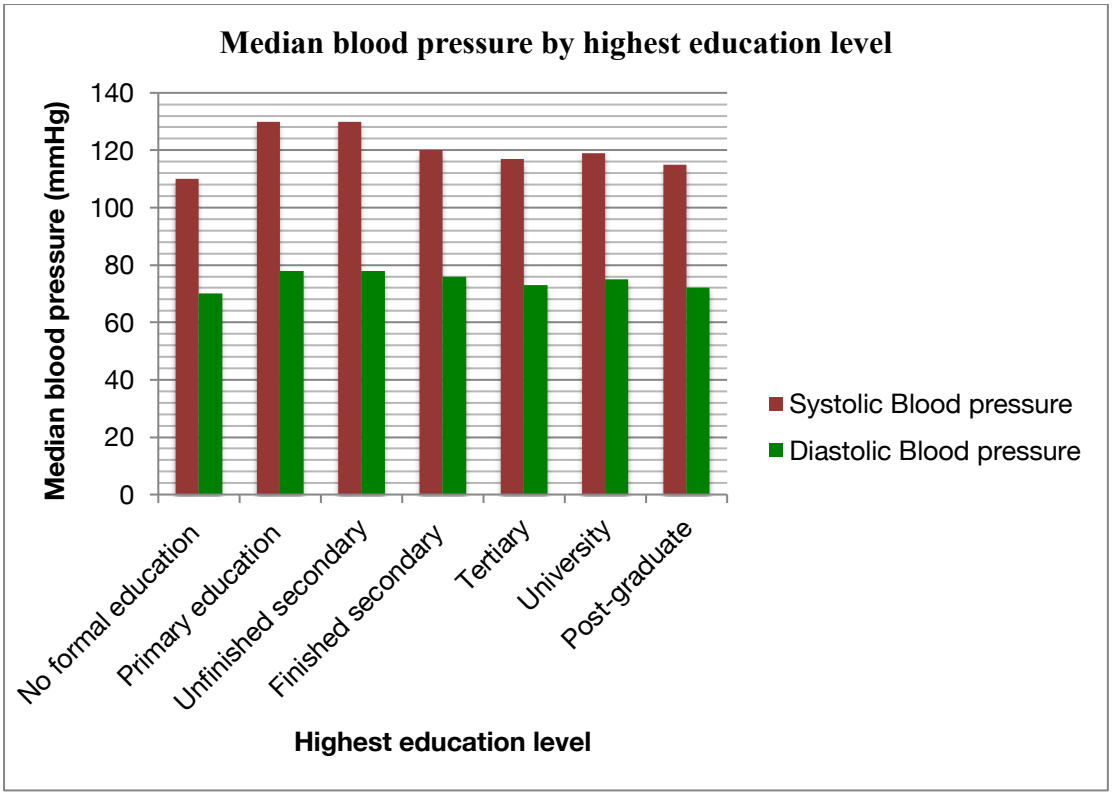



Figure 3.61 Distribution of the median systolic and diastolic blood pressure, by education levels

Negative correlations were also present between both systolic (R= -0.17, *p*=<0.01) and diastolic (R= -0.08, *p*=<0.01) blood pressures with education levels.

The relationship between education and blood pressure is entirely confounded by age and gender except for the category of those '*not finishing their secondary education*' (OR: 2.14 CI 95%: 1.09 – 4.18, *p*=0.03). The other educational attainment levels did not exhibit any association with blood pressure.

#### 3.2.2.3.3 Blood pressure by employment status

The retired subgroup had the highest median systolic blood pressure ($p$=<0.01) while the unemployed subgroup had the highest median diastolic blood pressure ($p$=<0.01).

On comparing to domestic work (reference category), the employed exhibited a negative association with having an elevated blood pressure (OR: 0.61 CI 95%: 0.48 – 0.78, $p$=<0.01), although, after adjusting for gender and age, this association was lost ($p$=0.32). Similarly, students exhibited a negative association with having an elevated blood pressure (OR: 0.32 CI 95%: 0.13 – 0.79, $p$=0.01) but the association was lost after adjustment for gender and age ($p$=0.08). Retired status exhibited a positive associated risk for having an elevated blood pressure (OR: 2.53 CI 95%: 1.86 – 3.46, $p$=<0.01). However, once again the effect was lost after adjustment for gender and age ($p$=0.22).

### 3.2.3 Relationship between anthropometry and lifestyle profiles

This section considers the crude unadjusted sample population ($n$=1,861) to determine the relationships between the different anthropometric parameters (BMI, waist circumference and blood pressure) and lifestyle profiles (smoking habit, alcohol habit and physical activity).

#### 3.2.3.1 Body mass index and smoking habit

No relationship was present between median BMI and smoking habit ($p$=0.22) as well as between overweight status and smoking habit, and between obese status and smoking habit ($p$=0.54, $p$=0.42 respectively).

#### 3.2.3.2 Body mass index and alcohol habit

A negative correlation was present between the BMI and alcohol intake frequency (in days per week) (R: -0.08, $p$=<0.01) although no significant correlation was present between BMI and the quantity alcohol intake ($p$=0.81).

On univariant analysis, those reporting alcohol consumption showed a negative association with having an obese BMI status (OR: 0.66 CI 95%: 0.58 – 0.75, $p$=<0.01) although no significant association was evident for the overweight BMI status ($p$=0.10). On multi-variant analyses, those reporting alcohol consumption persistently showed a negative association with having an obese status, even after adjustment for age, gender, smoking, physical activity, education level and employment status (OR: 0.71 CI 95%: 0.62 – 0.82, $p$=<0.01).

#### 3.2.3.3 Body mass index and physical activity

No relationships were present between physical activity and median BMI, overweight status and obese status ($p$=0.92, $p$=0.42, $p$=0.91 respectively).

#### 3.2.3.4 Waist circumference and smoking habit

A negative correlation was present between waist circumference and smoking habit (R= -0.05, $p$=0.04). However, on gender stratification, smoking habit did not exhibit any correlation with waist circumference (males $p$=0.11; females $p$=0.21).

#### 3.2.3.5 Waist circumference and alcohol habit

No correlation was present between waist circumference and alcohol habit ($p$=0.25).

#### 3.2.3.6 Waist circumference and physical activity

No relationship was present between median waist circumference and physical activity ($p$=0.83).

#### 3.2.3.7 Blood pressure and smoking habit

A negative correlation was present between diastolic blood pressure and smoking habit (R= -0.05, $p$=0.03), although no correlation was evident between systolic blood pressure and smoking habit. However, smoking was not linked with hypertension ($p$=0.75), even after adjusting for gender and age ($p$=0.78).

#### 3.2.3.8 Blood pressure and alcohol habit

No relationships were present between median systolic and median diastolic blood pressures and alcohol consumption ($p$=0.54; $p$=0.64).

#### 3.2.3.9 Blood pressure and physical activity

No relationships were present between median systolic and median diastolic blood pressures and physical activity ($p$=0.68; $p$=0.79).

### 3.2.4 Relationship between body mass index, waist circumference and blood pressure

An increase in body mass index (per Kg/m$^2$) was positively associated with having an elevated blood pressure (OR: 1.11, CI 95%: 1.09 – 1.13, $p$=<0.01). This association remained significant after adjusting for age, gender, lifestyle habits (smoking, alcohol), occupation, residing district and education level (OR: 1.10, CI 95%: 1.08 – 1.21, $p$=<0.01).

An increase in waist circumference (per cm) showed a positive association with having an elevated blood pressure (OR: 1.06 CI 95%: 1.05 – 1.06, $p$=<0.01). On adjusting for age, gender, lifestyle habits (smoking, alcohol), employment status, residing district and education level, the association between increase in waist circumference and blood pressure remained significant (OR: 1.04 CI 95%: 1.03 – 1.05, $p$=<0.01).

A significant association was present between increased blood pressure (per mmHg) and having an obese BMI (OR: 2.79 CI 95%: 2.29 – 3.40, $p$=<0.01). On adjusting for age, gender, lifestyle habits (smoking, alcohol), occupation, residing district and education level, the association between increase in blood pressure and obese BMI remained significant (OR: 2.46 CI 95%: 1.98 – 3.08, $p$=<0.01). However, there was no significant association between blood pressure and an overweight BMI status ($p$=0.87).

A significant association was also present between an increase in blood pressure (per mmHg) and having an elevated waist circumference. An increase in blood pressure (per mmHg) showed positive association with having an elevation in waist circumference (OR: 1.20 CI 95%: 1.14 – 1.25, $p$=<0.01). On adjusting for age, gender, lifestyle habits (smoking, alcohol), employment status, residing district and education level, the association between increase in blood pressure and waist circumference remained significant (OR: 1.08, CI 95%: 1.04 – 1.13, $p$=<0.01).

### 3.2.5 Relationship between body mass index and waist circumference

The median waist circumference exhibited a gradual increase as the BMI progressed from normal status to overweight status and to obese status, as seen in Figure 3.62.

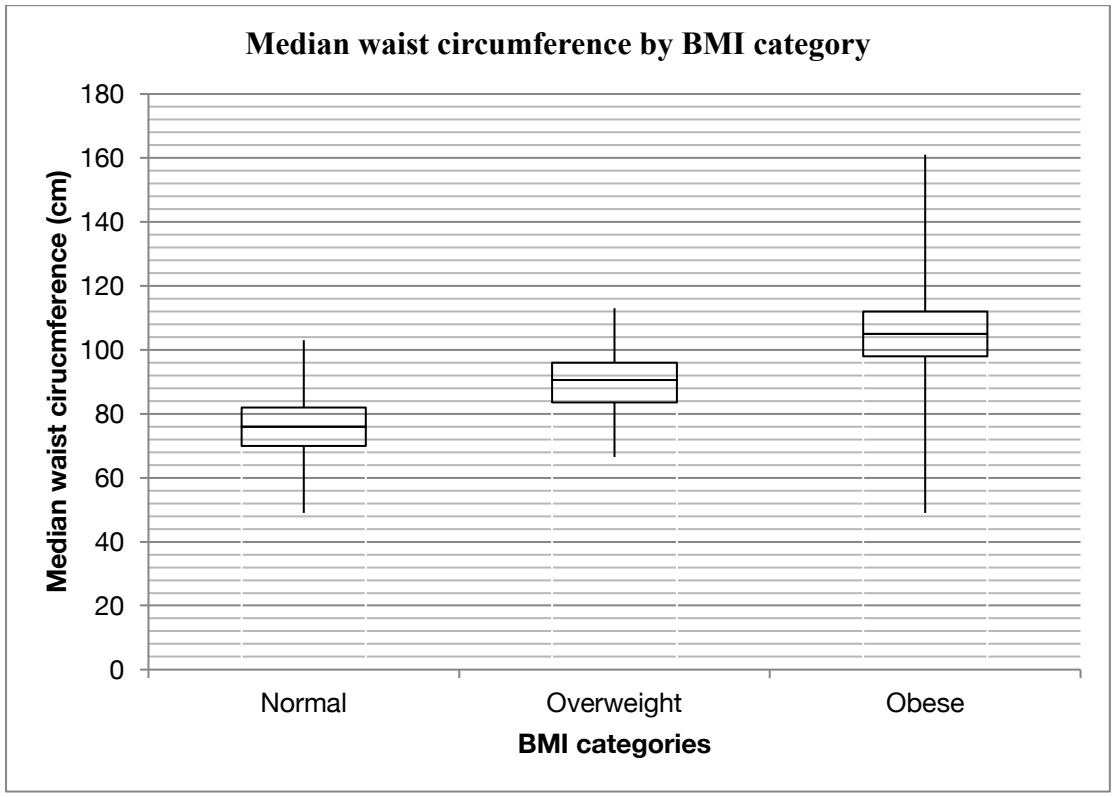



Figure 3.62 Distribution of the median waist circumference by BMI categories

The distribution of BMI categories with waist circumference, by gender, is present in Table 3.53 and Table 3.54

| | **Waist Circumference Male** | | *p*-value* |
|---|---|---|---|
| **BMI Categories (Kg/m$^2$)** | <= 93.99cm | >= 94cm | |
| Normal (<=24.99) | 131 | 23 | **<0.01** |
| Overweight (25 – 29.99) | 75 | 268 | 0.09 |
| Obese (>=30) | 2 | 337 | **<0.01** |

Gender = Male (*n*=836)
*Chi-square: normal vs. high waist circumference by BMI category

Table 3.53 Distribution of BMI categories by waist circumference for males

| | **Waist Circumference Female** | | *p*-value* |
|---|---|---|---|
| **BMI Categories (Kg/m$^2$)** | <= 79.99cm | >=80cm | |
| Normal (<=24.99) | 283 | 58 | **<0.01** |
| Overweight (25 – 29.99) | 78 | 266 | **<0.01** |
| Obese (>=30) | 7 | 333 | **<0.01** |

Gender = Female (*n*=1025)
* Chi-square: normal vs. high waist circumference by BMI category

Table 3.54 Distribution of BMI categories by waist circumference for females

Among the male population, 9.21% (CI 95%: 7.42 – 11.37) were classified as overweight-obese by BMI category but had a normal waist circumference. Conversely, 2.75% (CI 95%: 1.82 – 4.11) were classified as having normal BMI but had an elevated waist circumference. Thus, despite the obvious link between BMI and waist circumference, significant differences were present between the BMI categories and their corresponding waist circumference categories within the male population.

Similarly, among the female population, 8.29% (CI 95%: 6.75 – 10.15) were classified as overweight-obese but fell within the normal waist circumference range, while 5.66% (CI 95%: 4.39 – 7.26) had a normal BMI but elevated waist circumference. Significant differences were present between all the BMI categories and their corresponding waist circumference within the female population as was evident among the male population.

A positive correlation was found between the study population BMI and waist circumference (R=0.74 *p*=<0.01).

### 3.2.5.1 Body mass index vs. waist circumference by glucose regulation

The comparisons between BMI categories and waist circumference were further analysed with regards to glucose regulation. This was done in order to assess whether the discrepancies between the different body weight tools of measurements were related to underlying dysglycaemia. Table 3.55 to Table 3.58 illustrate the BMI categories by waist circumference, gender and glucose regulation (diabetes mellitus and IFG).

| **BMI categories (Kg/m2)** | **Waist circumference (cm) – Male (*n*=836)** | | | | | |
|---|---|---|---|---|---|---|
| | **Non-diabetes** | | **Diabetes** | | | |
| | <=93.99 | >=94 | <=93.99 | >=94 | *p*-value1 | *p*-value2 |
| Normal (<=24.99) | 139 | 4 | 10 | 1 | 0.21 | 1.00 |
| Overweight (25 – 29.99) | 133 | 158 | 17 | 35 | 0.12 | 0.22 |
| Obese (>=30) | 8 | 256 | 0 | 75 | 0.37 | 0.22 |

*p*-value1: waist circumference <=93.99cm non-diabetes vs. diabetes
*p*-value2: waist circumference >=94cm non-diabetes vs. diabetes

Table 3.55 Distribution of BMI categories by waist circumference for males, by diabetes mellitus status

| BMI categories (Kg/m2) | Waist circumference (cm) Female (*n*=1,025) | | | | | |
|---|---|---|---|---|---|---|
| | Non-diabetes | | Diabetes | | | |
| | <=79.99 | >=80 | <=79.99 | >=80 | *p*-value1 | *p*-value2 |
| Normal (<=24.99) | 279 | 57 | 4 | 1 | 0.07 | **0.02** |
| Overweight (25 – 29.99) | 75 | 246 | 3 | 20 | 0.25 | **0.02** |
| Obese (>30) | 6 | 281 | 1 | 52 | **0.03** | **<0.01** |

*p*-value1: waist circumference <=79.99cm non-diabetes vs. diabetes
*p*-value2: waist circumference >=80cm non-diabetes vs. diabetes

Table 3.56 Distribution of BMI categories by waist circumference for females, by diabetes mellitus status

| BMI categories (Kg/m2) | Waist circumference (cm) Male (*n*=836) | | | | | |
|---|---|---|---|---|---|---|
| | Non-IFG | | IFG | | | |
| | <=93.99 | >=94 | <=93.99 | >=94 | *p*-value1 | *p*-value2 |
| Normal (<=24.99) | 104 | 17 | 27 | 6 | 0.06 | 0.47 |
| Overweight (25 – 29.99) | 50 | 175 | 25 | 93 | **0.04** | 0.42 |
| Obese (>=30) | 2 | 229 | 0 | 108 | 0.41 | 0.60 |

*p*-value1: waist circumference <=93.99cm non-IFG vs. IFG
*p*-value2: waist circumference >=94cm non-IFG vs. IFG

Table 3.57 Distribution of BMI categories by. waist circumference for males, and the presence or absence of impaired fasting glucose (IFG)

| BMI categories (Kg/m2) | Waist circumference (cm) Female (*n*=1,025) | | | | | |
|---|---|---|---|---|---|---|
| | Non-IFG | | IFG | | | |
| | <=79.99 | >=80 | <=79.99 | >=80 | *p*-value1 | *p*-value2 |
| Normal (<=24.99) | 248 | 48 | 35 | 10 | 0.88 | 0.22 |
| Overweight (25 – 29.99) | 68 | 214 | 10 | 52 | 0.86 | **0.04** |
| Obese (>=30) | 7 | 239 | 0 | 94 | 0.32 | **0.01** |

*p*-value1: waist circumference <=79.99cm non-IFG vs. IFG
*p*-value2: waist circumference >=80cm non-IFG vs. IFG

Table 3.58 Distribution of BMI categories by waist circumference for females, and the presence impaired fasting glucose (IFG).

On comparing the BMI categories and waist circumference measurements within the different glucose regulatory subgroups (IFG and diabetes), the female population, unlike the male population, appeared to exhibit significant differences between BMI and waist circumference measurements. In fact, females had a significantly higher proportion of the overweight-obese BMI status with associated elevated waist circumference status in the normoglycaemic sub-groups when compared to the diabetes sub-group. This relationship was also present when comparing the IFG to the non-IFG female population. The same was not found when comparing the male IFG to the non-IFG populations.

### 3.2.6 Relationship between body mass index and glucose regulation

This section considers the crude unadjusted diabetes population (*n*=219) and IFG population (*n*= 460) to determine any relationships with body mass index (BMI).

#### 3.2.6.1 Relationship between body mass index and type 2 diabetes mellitus population

The crude unadjusted diabetes mellitus population had a significantly higher median BMI (31.10Kg/$m^2$ IQR: 6.92) than did the non-diabetes population (*p*=<0.01).

For every one unit increase in BMI (per Kg/$m^2$), the odds of being an individual with diabetes rather than without increases by 9% (OR: 1.09 CI 95: % 1.07 – 1.11, *p*=<0.01). This risk remained present after adjusting for gender and age (OR: 1.09 CI 95%: 1.06 – 1.12, *p*=<0.01). The association between diabetes and BMI remained significant after

adjusting for age, gender, lifestyle (smoking and alcohol habits), education level, employment status and residing districts (OR: 1.09 CI 95%: 1.06 – 1.12, $p$=<0.01).

Similarly, for every one unit increase in BMI (per Kg/m$^2$), the odds of being an individual with diabetes rather than being metabolically normal increases by 145% (2-fold) (OR: 2.45 CI 95%: 1.51 – 2.52, $p$=<0.01). This risk remained present after adjusting for gender and age (OR: 1.95 CI 95%: 1.06 – 1.12, $p$=<0.01). The association between diabetes and BMI remained significant after adjusting for age, gender, lifestyle (smoking and alcohol habits), education level, employment status and residing districts (OR: 2.69 CI 95%: 1.65 – 4.40, $p$=<0.01).

### 3.2.6.2 Relationship between body mass index and impaired fasting glucose population

The crude unadjusted IFG population had a significantly higher median BMI (29.15Kg/m$^2$ IQR: 6.45) than did the non-IFG population ($p$=<0.01).

For every unit increase in BMI (per Kg/m$^2$), the odds of being IFG rather than not increases by 4% (OR: 1.04 CI 95%: 1.03 – 1.88 $p$=<0.01). After adjusting for age and gender, this associated risk remained significant (OR: 1.32 CI 95%: 1.06 – 1.65, $p$=0.01). On further adjusting for lifestyle (smoking and alcohol habits), education level, employment status, residing district, apart from age and gender, the association between having an increase in BMI and having IFG remained equally significant (OR: 1.31 CI 95%: 1.04 – 1.06, $p$=<0.01).

### 3.2.7 Relationship between blood pressure and glucose regulation

This section considers the crude unadjusted diabetes population (*n*=219) and IFG population (*n*= 460) to determine any relationships between glucose regulation and blood pressure.

#### 3.2.7.1 Relationship between blood pressure and type 2 diabetes mellitus population

The systolic blood pressure (median 130mmHg IQR: 21) and the diastolic blood pressure (median 78 mmHg IQR: 12) were found to be significantly higher within the crude unadjusted diabetes mellitus than in non- diabetes (*p*=<0.01 respectively).

For every one unit increase in blood pressure (per mmHg), the odds of being an individual with diabetes rather than without increases by six-fold (OR: 6.67 CI 95%: 4.84 – 9.19, *p*=<0.01). The risk remained significant even after adjustment for gender and age, although the association was thereby reduced (OR: 3.47 CI 95%: 2.46 – 4.88, *p*=<0.01). The association between an increase in blood pressure and diabetes mellitus remained significant even after adjusting for lifestyle (smoking and alcohol habits), education level, employment status and residing district, apart from age and gender (OR: 3.46 CI 95%: 2.45 – 4.90, *p*=<0.01).

For every one unit increase in blood pressure (per mmHg), the odds of being an individual with diabetes rather than being metabolically healthy increases by 119% (two-fold) while adjusting for age, gender, lifestyle, education level, employment status and residing district (OR: 2.19 CI 95%: 2.13 – 2.27, *p*=<0.01).

#### 3.2.7.2 Relationship between blood pressure and impaired fasting glucose population

The systolic blood pressure (median 125mmHg IQR: 17) and the diastolic blood pressure (median 79mmHg IQR: 13) were found to be significantly higher within the crude unadjusted IFG population than in the non-IFG population ($p$=<0.01 respectively).

For every one unit increase in blood pressure (per mmHg), the odds of being an individual with IFG rather than without increases by 109% (two-fold) (OR: 2.09, CI 95%: 1.68 – 2.59, $p$=<0.01). This association remained after adjustment for gender and age, although the association was thereby attenuated (OR: 1.48 CI 95%: 1.17 – 1.88, $p$=<0.01). The relationship between blood pressure and IFG remained significant on further adjusting for lifestyle factors (smoking and alcohol habits), educational level, employment status and residing district (OR: 1.49 CI 95%: 1.17 – 1.90, $p$=<0.01).

### 3.2.8 Relationship between glucose regulatory sub-groups and anthropometry

The median values of the available anthropometric parameters (BMI, waist circumference, WHR, systolic and diastolic blood pressure) were compared between the different glucose regulatory subgroups, by gender as seen in Table 3.59.

The median BMI for both males and females were within the overweight category for the normoglycaemia (NGR) and IFG populations, while in diabetes (newly and previously diagnosed) subgroups median values were within the obese BMI range for both genders. The female median waist circumference and median WHR were found to be statistically lower than in males throughout all the subgroups, as seen in Table 3.59.

| | | | | | | Mann-Whitney U test | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **NGR (*n*=1,182)** | **IFG (*n*=460)** | **NDM (*n*=85)** | **KDM (*n*=134)** | ***p*-value1** | ***p*-value2** | ***p*-value3** | ***p*-value4** |
| Median BMI (Kg/m$^2$) | F | 26.96 ±5.83 | 29.50 ±8.3 | 33.51 ±6.53 | 32.12 ±5.60 | **<0.01** | **0.0001** | 0.10 | 0.06 |
| | M | 27.70 ±5.03 | 29.10 ±6.04 | 31.63 ±7.06 | 30.92 ±4.34 | | | | |
| Median WC (cm) | F | 82.34 ±14.74 | 92.14 ±20.20 | 98.72 ±19.66 | 98.04 ±15.38 | **<0.01** | **<0.01** | **0.05** | **<0.01** |
| | M | 92 ±15.47 | 100 ±16 | 103.94 ±14.11 | 104.25 ±11.57 | | | | |
| Median WHR (cm) | F | 0.86 ±0.09 | 0.88 ±0.09 | 0.94 ±0.10 | 0.89 ±0.06 | **0.02** | **<0.01** | **<0.01** | **<0.01** |
| | M | 1.08 ±3.55 | 0.97 ±0.07 | 0.98 ±0.05 | 0.99 ±0.05 | | | | |
| Median Systolic BP (mmHg) | F | 115 ±21 | 125 ±16 | 134 ±19 | 130 ±15 | **<0.01** | **<0.01** | 0.52 | 0.55 |
| | M | 120 ±13 | 124 ±13.30 | 132 ±18.82 | 131 ±16 | | | | |
| Median Diastolic BP (mmHg) | F | 72 ±10 | 76 ±9 | 79 ±11 | 75 ±9 | **<0.01** | 0.11 | 0.75 | 0.40 |
| | M | 76 ±12 | 79 ±9.50 | 78 ±6 | 76 ±10 | | | | |

| | |
|---|---|
| *p*-value1 | NGR male vs. female |
| *p*-value2 | IFG male vs. female |
| *p*-value3 | NDM male vs. female |
| *p*-value4 | KDM male vs. female |

NGR- normoglycaemia; IFG- impaired fasting glucose; NDM- newly diagnosed diabetes; KDM – known diabetes

Table 3.59 Statistical comparisons between the different glucose regulatory subgroups, by anthropometric profiling and gender

Both the male and female populations exhibited median systolic and diastolic blood pressures within normal ranges throughout all the glucose regulatory subgroups. Comparisons were performed by gender for anthropometric measurements according to the glucose regulation subgroups, as seen in Table 3.60.

The male and female populations exhibited statistical differences between all medians of the anthropometric variables and within each of the glucose regulatory subgroup, except when comparing the NDM to the KDM subgroups. In this case (NDM vs. KDM), the male population exhibited a significantly higher diastolic blood pressure in the NDM group when compared to the KDM population. Meanwhile within the female population (NDM vs. KDM), only the median WHR and diastolic blood pressure measurements were found to be significantly different as seen in Table 3.60.

| | **Female (*n*=1,025)** Kruskal-Wallis test | | | | | |
|---|---|---|---|---|---|---|
| | *p*-value 1 | *p*-value 2 | *p*-value 3 | *p*-value 4 | *p*-value 5 | *p*-value 6 |
| Median BMI (Kg/m$^2$) | 0.37 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median WC (cm) | 0.10 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median WHR | **0.01** | **<0.01** | **<0.01** | 0.23 | **<0.01** | **<0.01** |
| Median Systolic BP (mmHg) | 0.21 | **<0.01** | **<0.01** | **0.01** | **<0.01** | **<0.01** |
| Median Diastolic BP (mmHg) | **0.03** | **<0.01** | **<0.01** | 0.17 | **<0.01** | **<0.01** |

| | **Male (*n*=836)** Kruskal-Wallis test | | | | | |
|---|---|---|---|---|---|---|
| | *p*-value1 | *p*-value2 | *p*-value3 | *p*-value4 | *p*-value 5 | *p*-value 6 |
| Median BMI (Kg/m$^2$) | 0.76 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median WC (cm) | 0.73 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median WHR | 0.18 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median Systolic BP (mmHg) | 0.27 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median Diastolic BP (mmHg) | **0.03** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |

*p*-value 1: NDM vs KDM
*p*-value 2: NDM vs IFG
*p*-value 3: NDM vs NGR
*p*-value 4: KDM vs IFG
*p*-value 5: KDM vs NGR
*p*-value 6: IFG vs NGR

NGR- normoglycaemia; IFG- impaired fasting glucose; NDM- newly diagnosed diabetes; KDM – known diabetes

Table 3.60 Statistical comparisons between the different glucose regulatory subgroups, by anthropometric profiling for each gender

### 3.2.8.1 Type 2 diabetes associations with anthropometric profile

Further analyses were performed to establish which of the anthropometric parameters have an independent association with having diabetes mellitus while adjusting for socio-demographic confounding factors including age, gender, education, locality, employment status and on anti-hypertension treatment.

All anthropometric parameters were found to have an independent association with having diabetes mellitus (combination of previously diabetes and newly diagnosed diabetes) when compared to the non-diabetes sub-population, as seen in Table 3.61, where all significant factors were listed.

**T2DM as dependent variable**

| Independent risk factors | Odds ratio | CI 95% | *p*-value |
|---|---|---|---|
| Body mass index (BMI) | 1.04 | 1.00 - 1.09 | **0.05** |
| Systolic blood pressure | 1.02 | 1.01 - 1.03 | **<0.01** |
| Diastolic blood pressure | 0.97 | 0.95 - 0.99 | **<0.01** |
| Waist circumference | 1.02 | 1.00 - 1.04 | **0.05** |
| Age | 1.07 | 1.05 - 1.10 | **<0.01** |
| On blood pressure treatment* | 2.85 | 2.02 - 4.01 | **<0.01** |
| Female** | 0.41 | 0.23 - 0.66 | **<0.01** |

*Not taking antihypertensive medication as the reference category
** Male gender as the reference category

Table 3.61 Independent association analyses between anthropometric parameters and having diabetes mellitus (global)

However, when T2DM sub-population was compared to the metabolically healthy sub-population, only BMI (OR: 2.33 CI 95%: 1.03 – 5.31 $p$=0.04) and waist circumference (OR: 1.40 CI 95%: 1.01 – 1.94 $p$=0.05) were significant independent factors associated with T2DM

after adjusting for confounders (age, gender, education, locality, employment status and on anti-hypertension treatment).

These relationships were further evaluated by analysing for the independent associations with anthropometric parameters in: (1) previously diagnosed and (2) newly diagnosed diabetes sub-populations, as seen in Table 3.62 and Table 3.63.

**Previously diagnosed T2DM as dependent variable*****

| Independent risk factors | Odds ratio | CI 95% | *p*-value |
|---|---|---|---|
| Systolic blood pressure | 1.02 | 1.01 - 1.03 | **<0.01** |
| Diastolic blood pressure | 0.96 | 0.94 - 0.98 | **<0.01** |
| Waist circumference | 1.03 | 1.01 - 1.06 | **<0.01** |
| Age | 1.08 | 1.04 - 1.10 | **<0.01** |
| On blood pressure treatment* | 3.92 | 2.54 - 6.04 | **<0.01** |
| Female** | 0.45 | 0.25 - 0.81 | **<0.01** |

*Not taking antihypertensive medication as the reference category
** Male gender as the reference category
*** Compared to non-T2DM sub-population

Table 3.62 Independent association analysis between anthropometric parameters and having previously diagnosed diabetes mellitus, compared to non-diabetes population

However, all significant anthropometric relationships were lost when previously diagnosed T2DM were compared to metabolically healthy sub-population.

**Newly diagnosed T2DM as dependent variable**

| Independent risk factors | Odds ratio | CI 95% | *p*-value |
|---|---|---|---|
| BMI | 1.08 | 1.03 - 1.13 | **<0.01** |
| Systolic blood pressure | 1.02 | 1.01 - 1.03 | **<0.01** |
| Age | 1.06 | 1.03 - 1.09 | **<0.01** |
| Female* | 0.44 | 0.23 - 0.84 | **0.01** |

* Male gender as the reference category

Table 3.63 Independent association analysis between anthropometric parameters and having newly diagnosed diabetes mellitus compared to non-diabetes population

On comparing the newly diagnosed T2DM with the metabolically healthy sub-population, BMI remained a significant independent factor associated with newly diagnosed T2DM (OR: 1.08 CI 95%: 1.01 – 1.16 *p*=0.04).

#### 3.2.8.2 Impaired fasting glucose associations with anthropometric profile

Analyses were performed to establish which of the anthropometric parameters have an independent association with having IFG while adjusting for socio-demographic confounding factors including age, gender, education, locality, employment status and on anti-hypertension treatment.

Only an increase in diastolic blood pressure was found to have an independent association with IFG (OR: 1.02 CI 95%: 1.00 – 1.03 *p*=0.02) on adjusting for the confounding factors.

### 3.2.9 Relationship between the biochemical parameters, age and gender

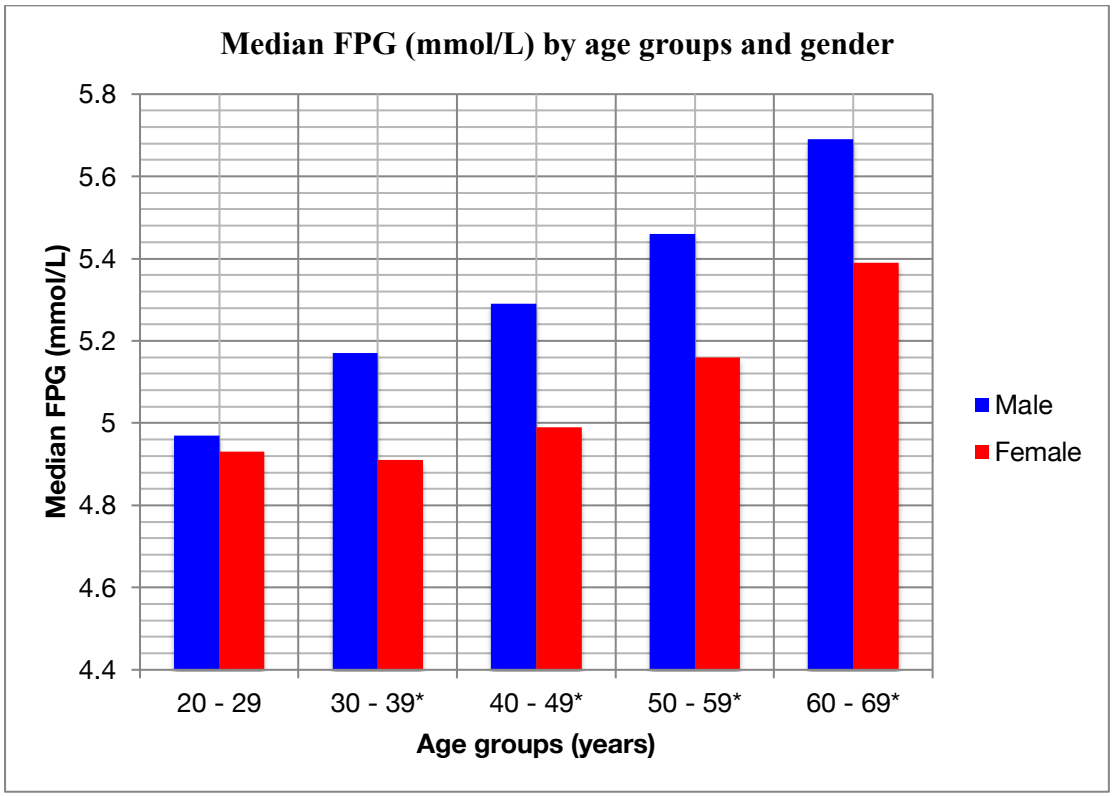
This section considers the crude unadjusted sample population (*n*=1,861) to determine the relationships between the different biochemical parameters (FPG, LDL-C, HDL-C, Triglycerides and Total Cholesterol), age and gender

#### 3.2.9.1 Fasting plasma glucose (FPG) by age and gender

The median FPG increased with increasing age for both males and females, as seen in Figure 3.63. The male population exhibited a significantly higher median FPG (5.54mmol/L IQR: 0.92) than the female population (5.20mmol/L IQR: 0.77, *p*=<0.01). A positive

correlation was present between age and FPG (R= 0.40, *p*=<0.01), which is consistent with the increase in median FPG as the age groups progressed as seen in Figure 3.63.

Only the male 60 to 69-year age group had a median FPG within the IFG category (≥5.6mmol/L to ≤6.9mmol/L), while for all the other age groups, the median FPG was within the normoglycaemic category (<5.6mmol/L).



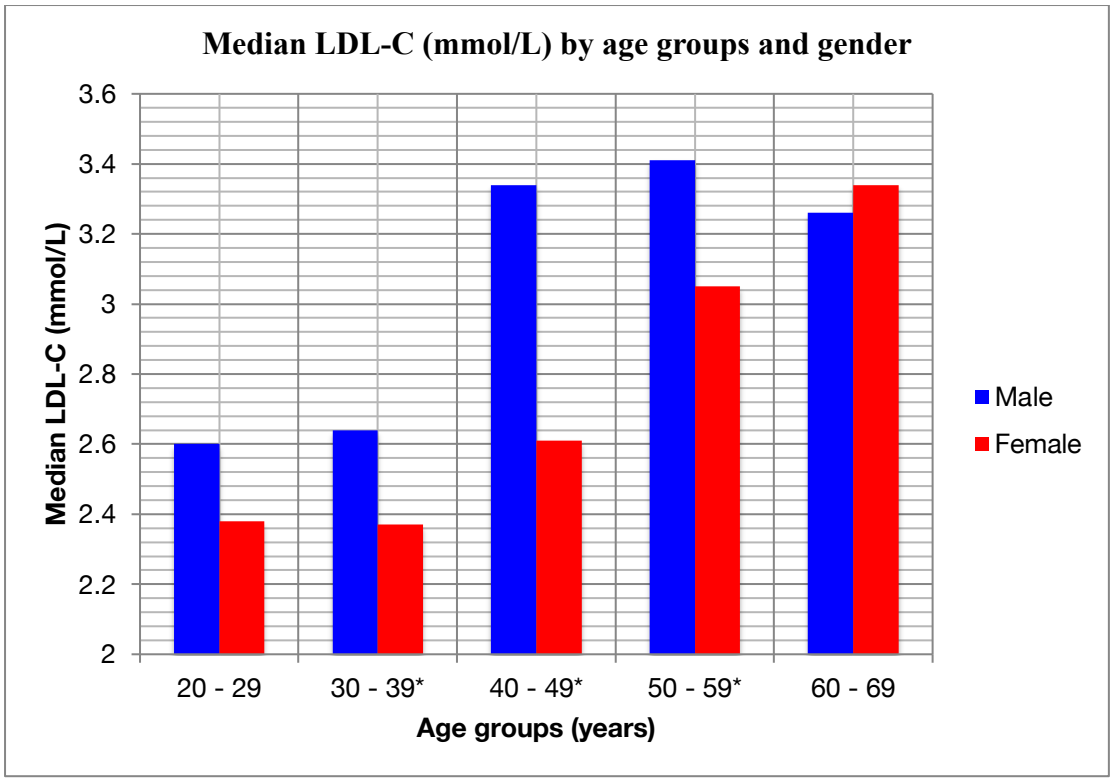

* Mann-Whitney U test *p*=<0.05

Figure 3.63 Median FPG, by age groups and gender

An increase in age (per year) was positively associated with having an elevation in FPG (OR: 1.03 CI 95%: 1.03 – 1.04, *p*=<0.01). On comparing to the male gender, the female gender exhibited a negative association for FPG elevation (OR: 0.55 CI 95%: 0.8 – 0.64, *p*<0.01) after adjusting for age (*p*=<0.01).

### 3.2.9.2 LDL-C by age and gender

The median LDL-C levels exhibited similar links with increasing age and againas seen in Figure 3.64.




* Mann-Whitney U test $p$=<0.01

Figure 3.64 Median LDL-C, by age groups and gender

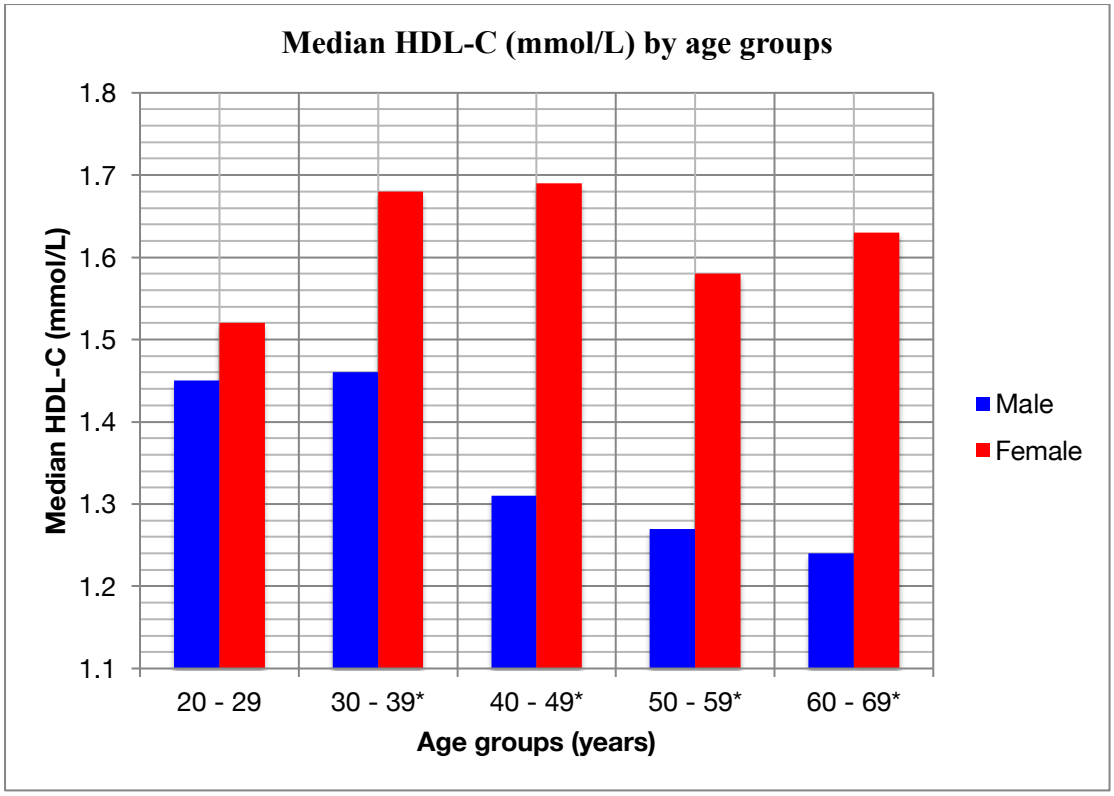
The female population had lower median LDL-C levels than the male population up until 59 years of age ($p$=<0.01 respectively), after which the female median LDL-C levels were found to be higher than those of males. A significant difference in the median LDL-C level between both genders was evident from the 30 to 59 years age groups only ($p$=<0.01 respectively). In fact, a positive correlation was present between LDL-C and age (R=0.17, $p$=<0.01).

The male population exhibited a significantly higher median LDL-C (3.20mmol/L IQR: 1.2) than the female population (2.99mmol/L IQR: 1.28, $p$=<0.01).

An increase in age (per year) was positively associated with having an elevation in LDL-C levels (OR: 1.01 CI 95%: 1.01 – 1.02, $p$=<0.01). On comparing to the male gender, the female gender exhibited a negative association for the elevation in LDL-C (OR: 0.86 CI 95%: 0.80 – 0.94, $p$=<0.01) after adjusting for age ($p$=<0.01).

#### 3.2.9.3 HDL-C by age and gender

The distribution of the median HDL-C across age groups varied between both genders as seen in Figure 3.65, with a significant difference throughout all age groups ($p$=<0.01) except between 20 and 29 years. The female population exhibited significantly higher median HDL-C levels across all age groups ($p$=<0.01 respectively) except for the 20-29-year category when compared to the male population.



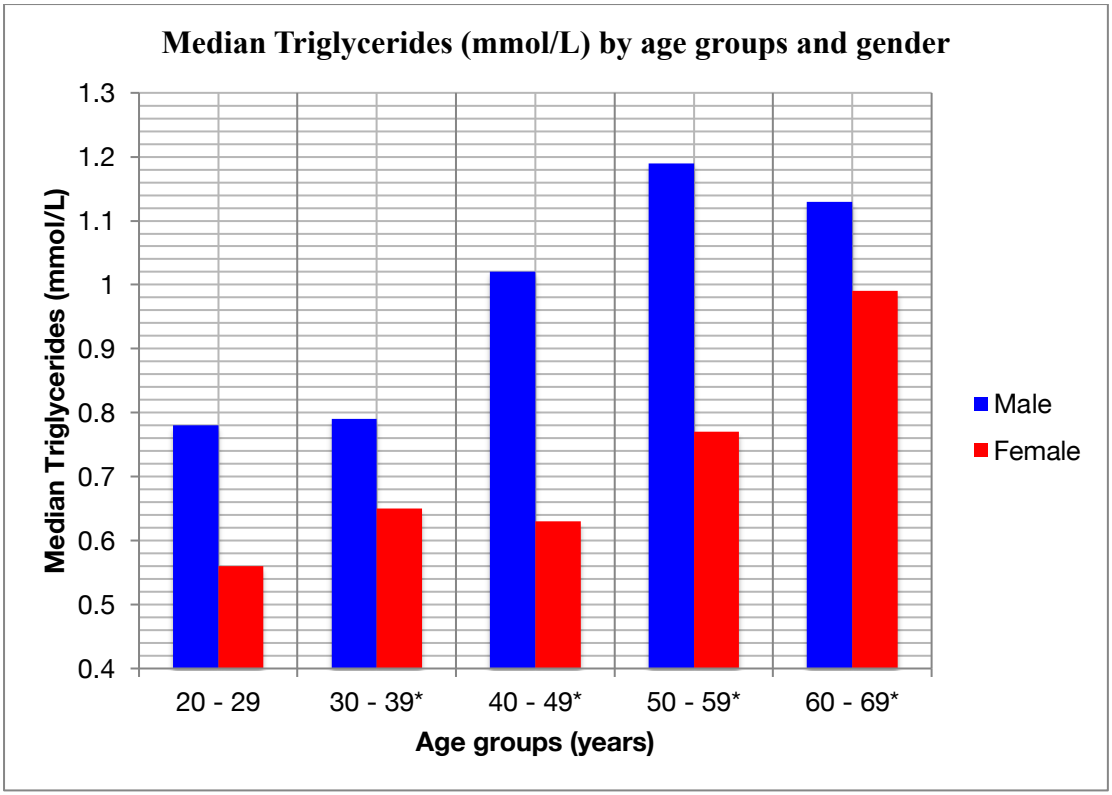

* Mann-Whitney U test $p$=<0.01

Figure 3.65 Median HDL-C, by age groups and gender

A negative correlation was present between HDL-C levels and age (R= -0.44, $p$=<0.01). An increase in age (per year) exhibited a negative association with the elevation of HDL-C levels (OR: 0.10 CI 95%: 0.99 – 1.00, $p$=0.02). Female gender exhibited a positive association with the elevation of HDL-C levels (OR: 1.46 CI 95%: 1.41 – 1.52, $p$=<0.01). However, significance was lost on adjusting for age ($p$=0.08).

### 3.2.9.4 Triglycerides by age and gender

The median triglyceride levels rose as the age groups increased, with some exceptions, as seen in Figure 3.66. In fact, a positive correlation was present between triglycerides and age (R=0.28, $p$=<0.01). The male population (median 1.10 IQR: 0.82) had a significantly higher median triglyceride level than did the female population (median 0.84 IQR: 0.60; $p$=<0.01).



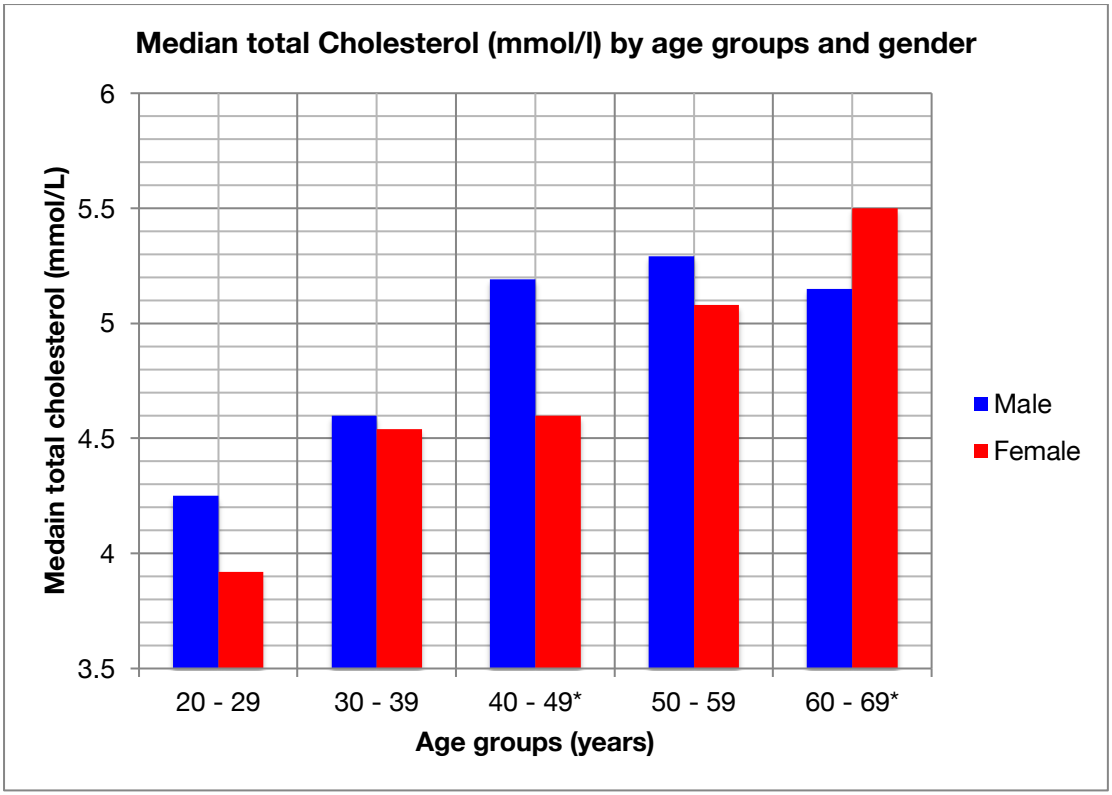

* Mann-Whitney U test $p$=<0.05

Figure 3.66 Median triglyceride levels, by age groups and gender

An increase in age (per year) had a positive association with an elevation in triglycerides levels (OR: 1.01 CI 95%: 1.01 – 1.01, $p$=<0.01). The female gender exhibited a negative association with an elevation in triglyceride levels (OR: 0.72 CI 95%: 0.67 – 0.77, $p$=<0.01) when compared to the male gender and adjusted for age ($p$=<0.01).

### 3.2.9.5 Total cholesterol by age and gender

A progressive increase in total cholesterol levels with advancing age group was evident, with some exceptions, as seen in Figure 3.67. In fact, a positive correlation was present between total cholesterol and age (R=0.20, $p$=<0.01).




* Mann-Whitney U test $p$=<0.05

Figure 3.67 Median total cholesterol levels, by age groups and gender

The male population median total cholesterol levels were not found to be significantly different from their female counterparts ($p$=0.48). On comparing the total cholesterol (median) of both genders by age groups, significant differences were evident only in the 40 to 49 years and 60 – 69 years age groups ($p$=<0.01 respectively).

An increase in age (per year) resulted in a positive association with an elevation in total cholesterol level (OR: 1.02 CI 95%: 1.01 – 1.02, $p$=<0.01). This association remained the same on adjusting for gender (OR: 1.02 CI 95%: 1.01 – 1.02, $p$=<0.01).

### 3.2.10 Relationship between the biochemical parameters and socio-demographic profiles

This section considers the crude unadjusted sample population (*n*=1,861) to determine the relationships between the different biochemical parameters (FPG, LDL-C, HDL-C, Triglycerides and Total Cholesterol) and the socio-demographic profiles (district, highest education and employment status).

#### 3.2.10.1 FPG by socio-demographic profile

##### 3.2.10.1.1 FPG by districts

The median FPG levels were all within the normoglycaemic range across all the districts in Malta. However, the Gozo district exhibited a statistically higher median FPG level (IFG range) when compared to all other Maltese districts (*p*=<0.01), as seen in Figure 3.68.

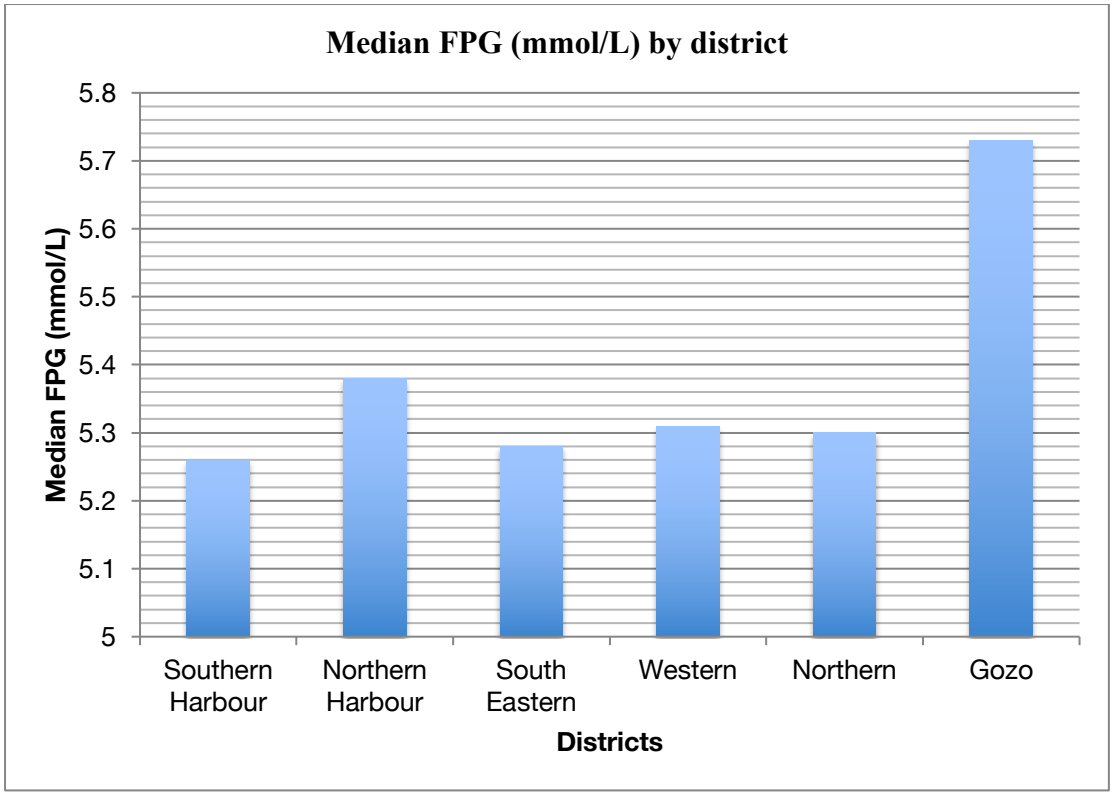



Figure 3.68 Median FPG, by districts

Considering the Southern Harbour district as the reference category, the district of Gozo exhibited a positive association with having an elevated FPG (OR: 1.67, CI 95%: 1.22 – 2.30 *p*=<0.01). The significant association remained present on adjusting for age, gender, BMI, highest education level, employment status, smoking habit and alcohol habit (OR: 1.63, CI 95%: 1.21 – 2.20, *p*=<0.01).

#### 3.2.10.1.2 FPG by education level

The group which reported an education level up till 'Primary school' exhibited the highest median FPG levels, as seen in Figure 3.69. These individuals (education till primary school) were found to exhibit a significantly higher median FPG when compared to all other

educational levels ($p$=<0.01 respectively). In fact, a negative correlation between education level and FPG levels was evident (R= -0.22, $p$=<0.01).

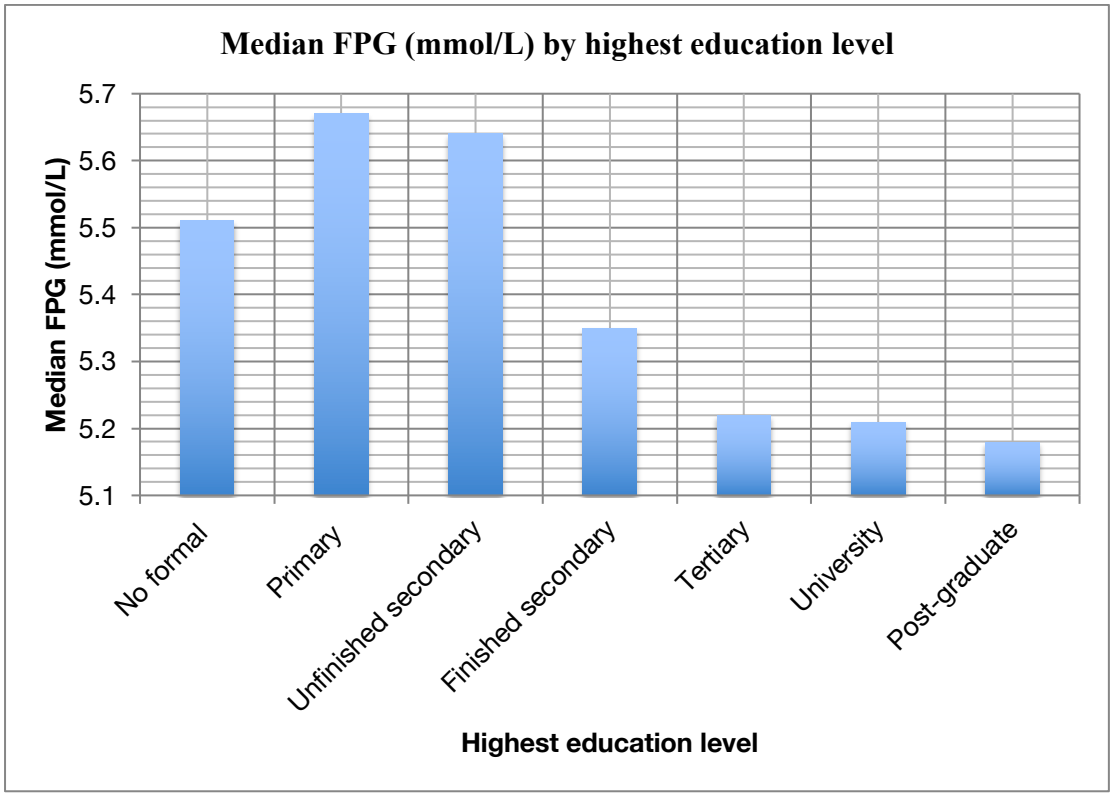



Figure 3.69 Median FPG (mmol/L) by highest educational levels

With post-graduate education as the reference category, having no formal education (OR: 12.57 CI 95%: 4.29 – 36.83, $p$=<0.01); primary level (OR: 2.69 CI 95%: 1.78 – 4.06, $p$=<0.01); unfinished secondary levels (OR: 2.35 CI 95%: 1.52 – 3.62, $p$=<0.01) and finishing secondary education level (OR: 1.52 CI 95%: 1.06 – 2.17, $p$=0.02), all exhibited a positive association with having an elevation in the FPG level. The other educational levels did not exhibit any significant associations with FPG. On adjusting for age, gender, BMI, employment status, smoking and alcohol habits, only no formal education (OR: 6.93 CI 95% 2.46 – 19.56, $p$=<0.01) and primary education (OR: 1.53 CI 95%: 1.00 – 2.34, $p$=0.05) remained significantly associated with an elevation in FPG.

### 3.2.10.1.3 FPG by employment status

The majority of the populations in different employment had a median FPG level within the normoglycaemic range. This held true except for those retired, as seen in Figure 3.70.

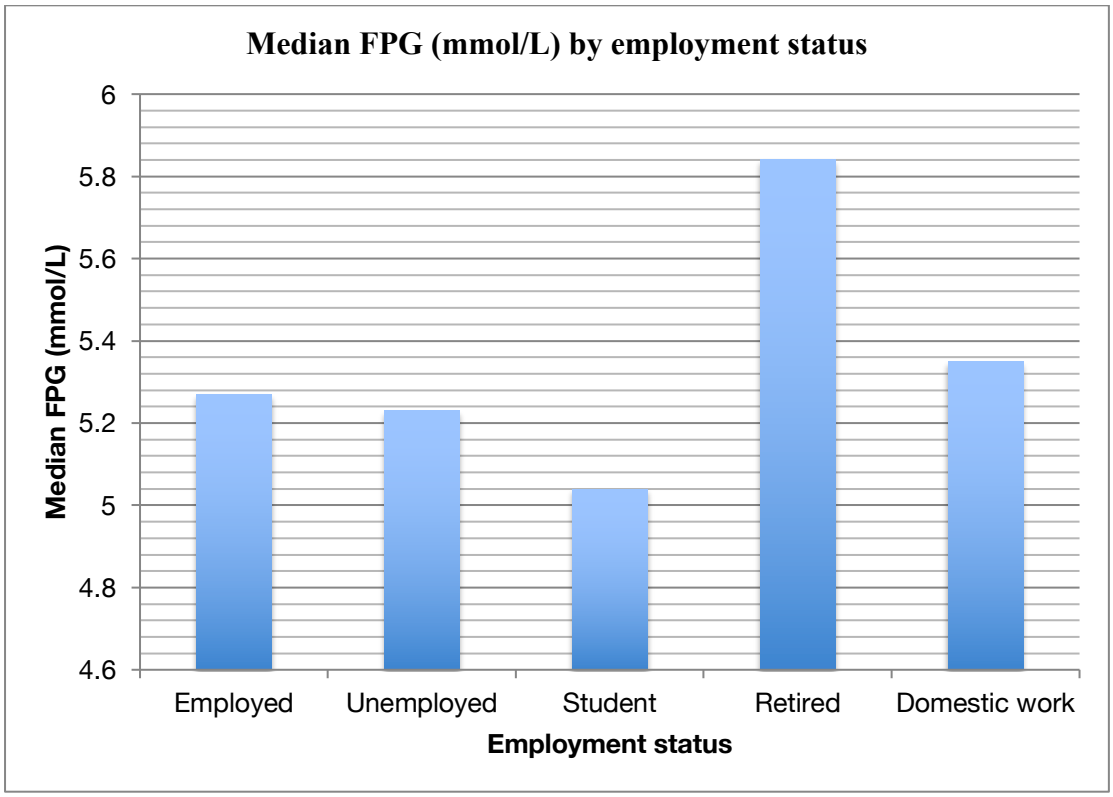



Figure 3.70 Median FPG, by employment status

In fact, a significant difference was exhibited between the retired status and all the other occupations ($p$=<0.01 respectively). Of note, the student median FPG levels were found to be significantly lower than for any other employment status ($p$=<0.01).

With 'domestic work' status as the reference category, only the 'retired' status exhibited significant positive association with having a rise in FPG (OR: 2.19 CI 95%: 1.71 – 2.82, $p$=<0.01). On adjusting for age and gender, the significance was lost.

### 3.2.10.2 LDL-C by socio-demographic profile

#### 3.2.10.2.1 LDL-C by districts

The study population analysed by districts exhibited an elevated median LDL-C (>2.59mmol/L) as seen in Figure 3.71. The Gozo population exhibited the highest median LDL-C levels (significant) when compared to all other Malta districts (Southern Harbour $p$=<0.01; Western $p$=<0.01; South Eastern $p$=<0.01; Northern $p$=<0.01; Northern Harbour $p$=0.03).

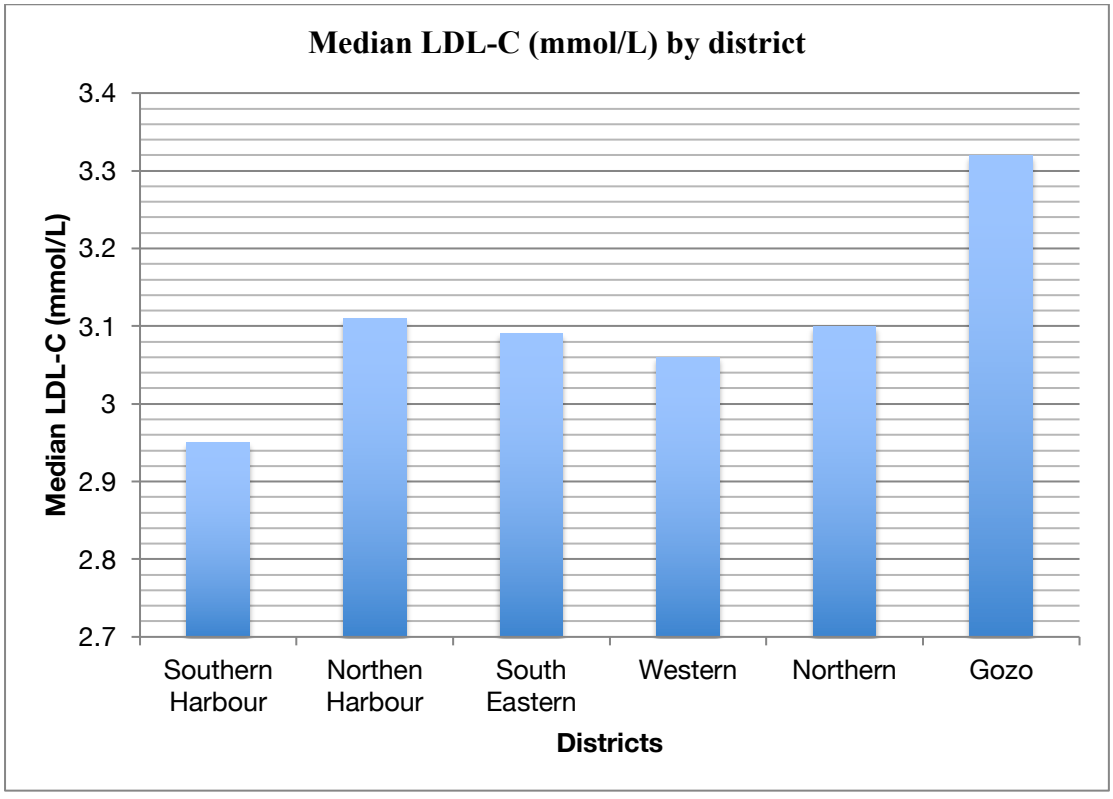



Figure 3.71 Median LDL-C, by districts

Considering the Southern Harbour as the reference category, the district of Gozo exhibited a positive association with having an elevated LDL-C level (OR: 1.53, CI 95%: 1.28 – 1.82, $p$=<0.01). The Northern Harbour district also exhibited a positive association with an elevated LDL-C level (OR: 1.15, CI 95%: 1.02 -1.31, $p$=0.02). This significant association remained present only for the Gozo district on adjusting for age, gender, BMI, highest education level, employment status, smoking habit and alcohol habit (Gozo: OR: 1.55, CI 95%: 1.29 – 1.81, $p$=<0.01).

#### 3.2.10.2.2 LDL-C by highest education level

The median LDL-C levels across the different education levels were all above the normal range as seen in Figure 3.72. A significant difference was present between each education level and the median LDL-C levels ($p$=<0.01 respectively). The median LDL-C level exhibited an elevation from ‘no formal education’ level and peaking at ‘unfinished secondary education’ level. There was a steady decrease in median LDL-C levels as the education level progressed thereafter to post-graduate education (Figure 3.72). In fact, a negative correlation was present between LDL-C levels and education levels.

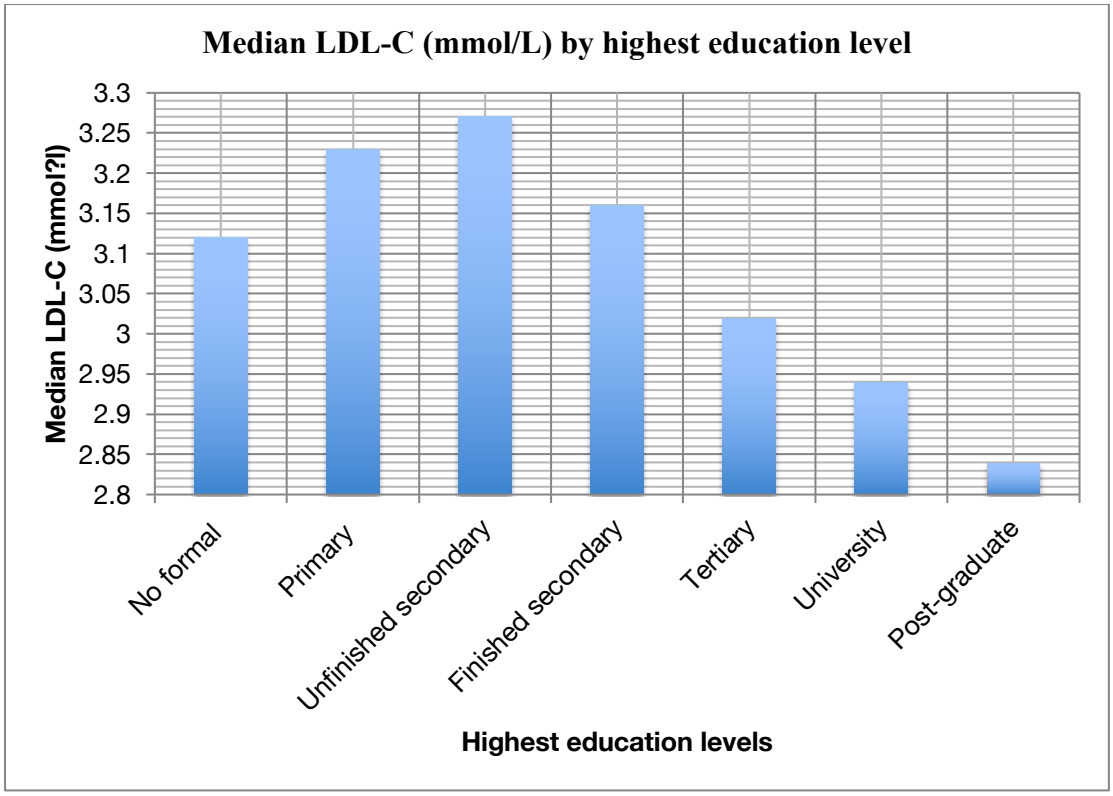



Figure 3.72 Median LDL-C, by highest education level

On comparing to post-graduate education (reference point), primary education (38%), unfinished secondary (38.20%) and finished secondary (30.80%) exhibited a significant positive association with an elevated LDL-C level (Primary OR: 1.38 CI 95%: 1.09 – 1.75 *p*=<0.01; unfinished secondary OR: 1.38 CI 95%: 1.08 – 1.77 *p*=0.01; finished secondary OR: 1.31 CI 95%: 1.06 – 1.61 *p*=0.01). However, significance was lost after adjustment for age, gender, BMI, employment status, smoking habit and alcohol habit.

### 3.2.10.2.3 LDL-C by employment status

The median LDL-C levels were above the normal range in all employment categories except for student status, as seen in Figure 3.73. In fact, a student status exhibited a significantly lower median LDL-C level compared to the employed (*p*=<0.01), retired (*p*=<0.01), domestic

status ($p$=<0.01) and the unemployed ($p$=<0.01). No significant correlation was exhibited between LDL-C level and employment status ($p$=0.09).

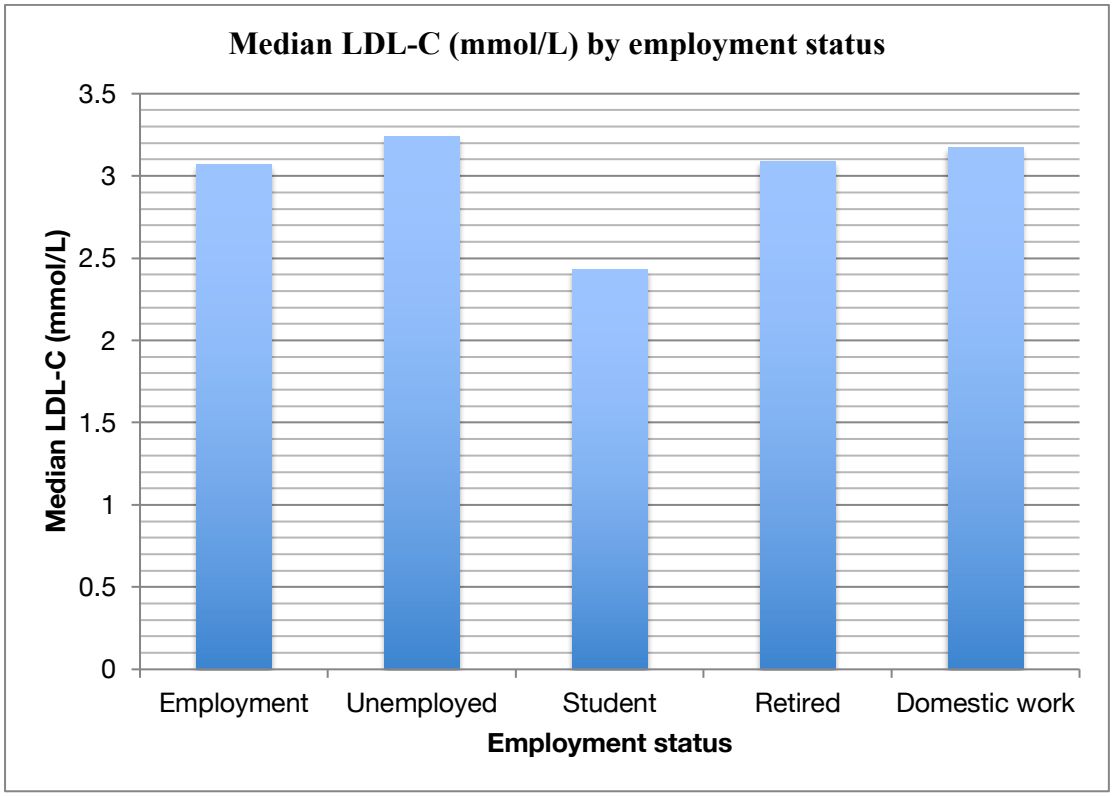



Figure 3.73 Median LDL-C, by employment status

A negative association existed between an elevation in LDL-C level and the 'employed' status and 'student' status (employed OR: 0.89 CI 95%: 0.80 – 0.99, $p$=0.03; students OR: 0.49 CI 95%: 0.35 – 0.68 $p$=<0.01), when 'domestic work' was considered as the reference employment status. Significance was lost on age and gender adjustment.

### 3.2.10.3 HDL-C by socio-demographic profile

#### 3.2.10.3.1 HDL-C by districts

All districts exhibited median HDL-C levels above the lower limit ($p$=0.10), as seen in Figure 3.74, although HDL-C levels varied between districts. The Northern Harbour district had the highest median HDL-C level. However, no significant relationship or association effect was present between districts and HDL-C.

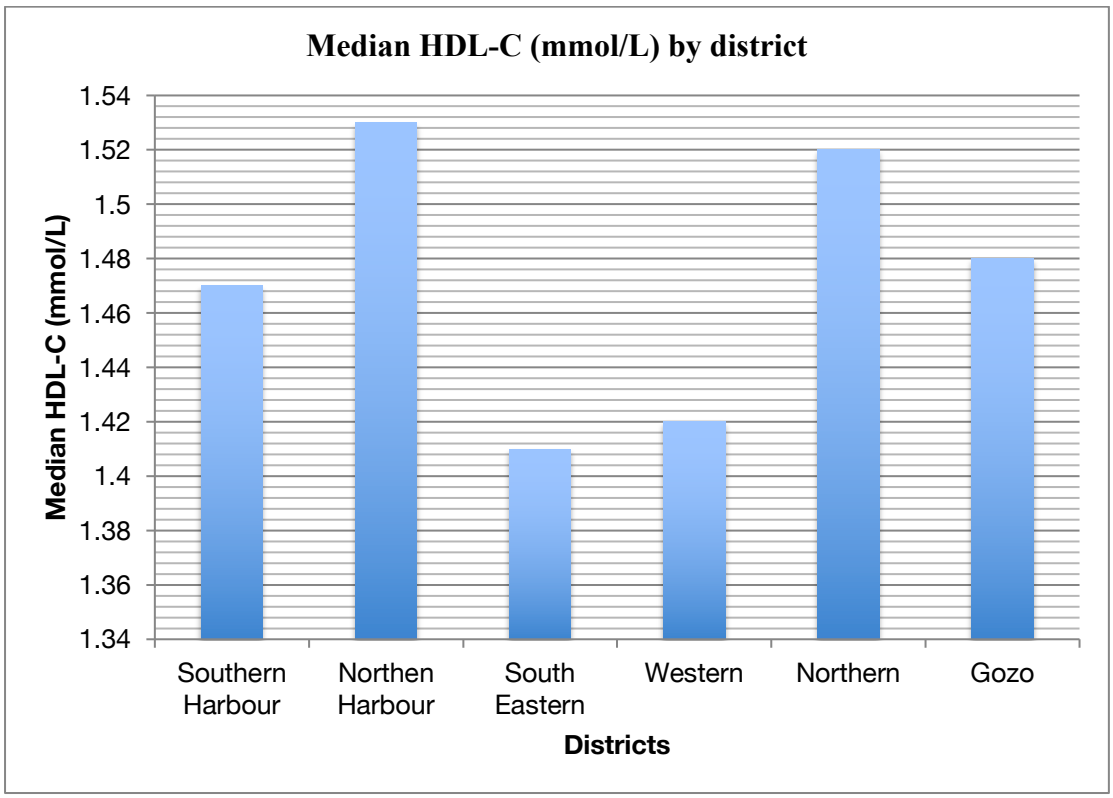



Figure 3.74 Median HDL-C, by districts

### 3.2.10.3.2 HDL-C by highest education level

Increased median HDL-C levels were present as education levels increased (Figure 3.75). A positive correlation was exhibited between HDL-C levels and education level (R=0.09, *p*=<0.01). However, all the education levels (except tertiary and university levels) exhibited a negative association with elevation in HDL-C levels when post-graduate education was considered as the reference category (No formal education OR: 0.68 CI 95%: 0.50 – 0.91 *p*=0.01; Primary education OR: 0.87 CI 95%: 0.78 – 0.97 *p*=0.02; Unfinished secondary education OR: 0.86 CI 95%: 0.76 – 0.97 *p*=0.01; Finished secondary education OR: 0.87 CI 95%: 0.78-0.96 *p*=<0.01). Significance was lost on age and gender adjustment.

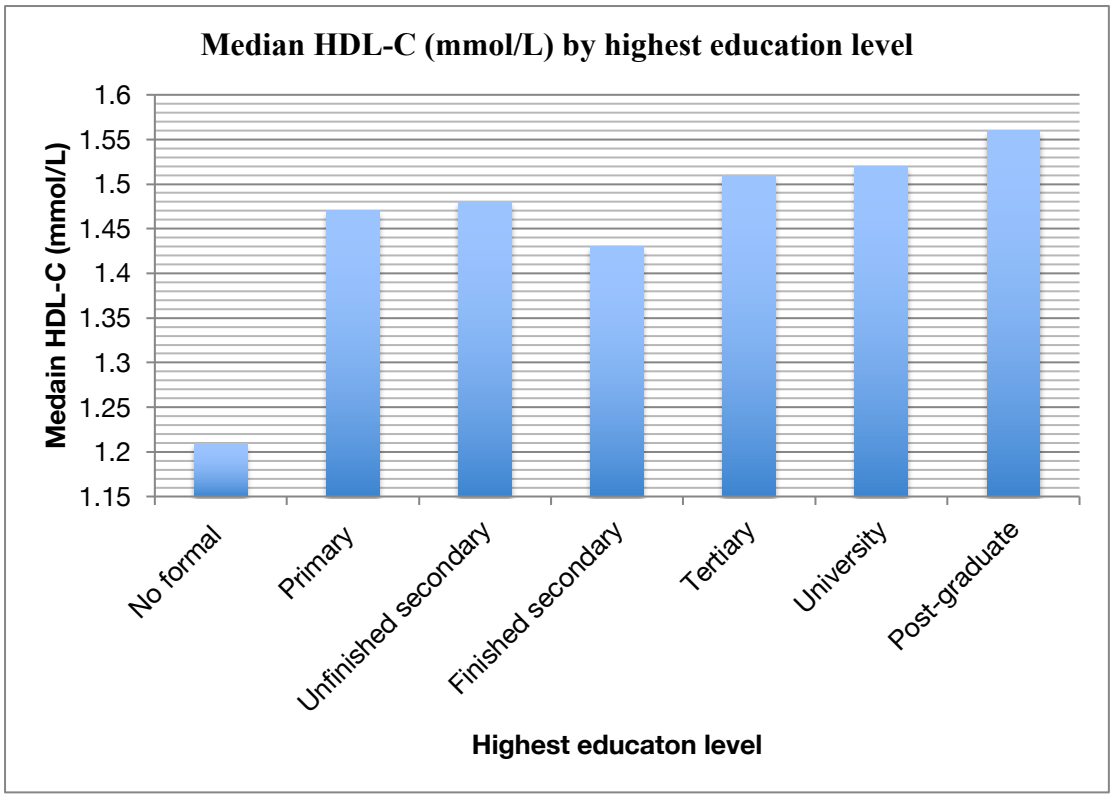



Figure 3.75 Median HDL-C, by highest education level

### 3.2.10.3.3 HDL-C by employment status

Irrespective of employment status, the median HDL-C levels were all above the lower normal level ($p$=<0.01) with the student status having the highest HDL-C level, as seen in Figure 3.76. On considering 'domestic work' as the reference category, the 'employed', 'unemployed' and 'retired' employments all exhibited a negative association with the elevation of HDL-C levels (Employed OR: 0.89 CI 95%: 0.84 – 0.93 $p$=<0.01; Unemployed OR: 0.80 CI 95%: 0.69 – 0.93 $p$=<0.01; Retired OR: 0.83 CI 95%: 0.76 – 0.89 $p$=<0.01). However, significance was lost on age and gender adjustment.

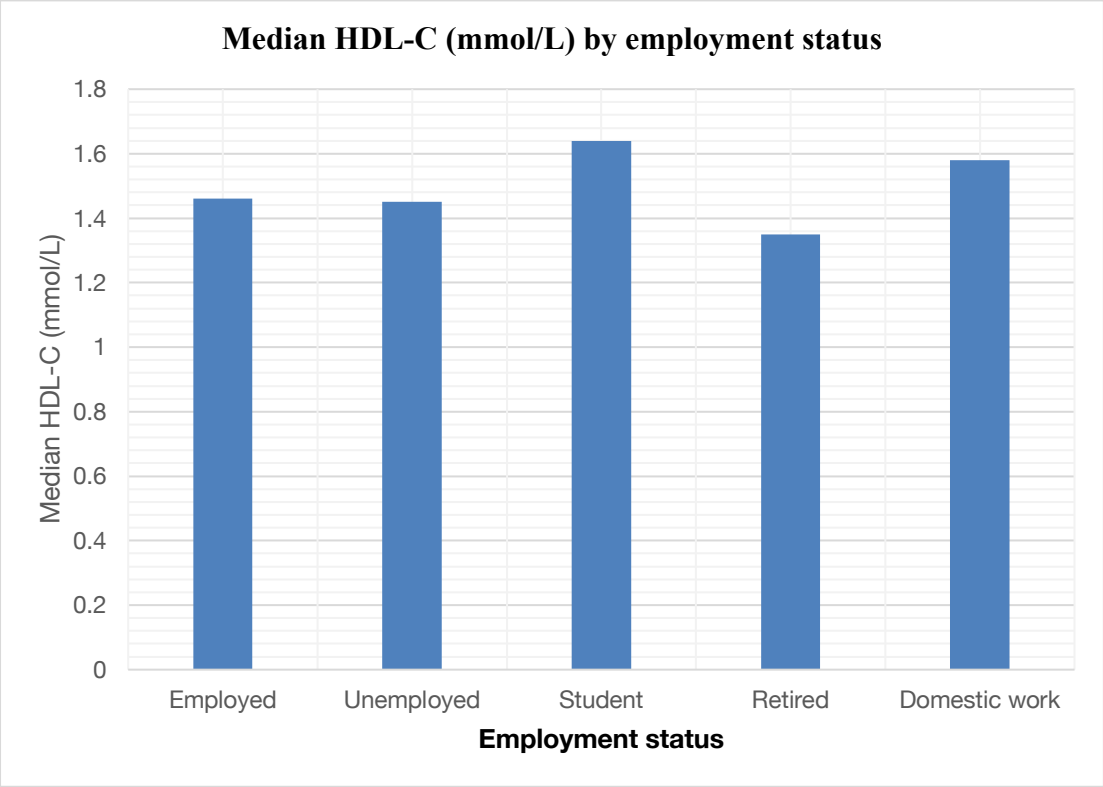



Figure 3.76 Median HDL-C, by employment status

### 3.2.10.4 Triglycerides by socio-demographic profile

#### 3.2.10.4.1 Triglycerides by districts

The median triglyceride levels across all districts were within the normal range (<1.69mmol/L) and exhibited no significant difference between the different districts. No associations were present between triglycerides and the different districts.

#### 3.2.10.4.2 Triglycerides by highest education levels

The median triglyceride levels exhibited a decline in level with an increase in education level ($p$=<0.01) as seen in Figure 3.77. In fact, a clear negative correlation was present between triglyceride and education level (R= -0.18, $p$=<0.01).

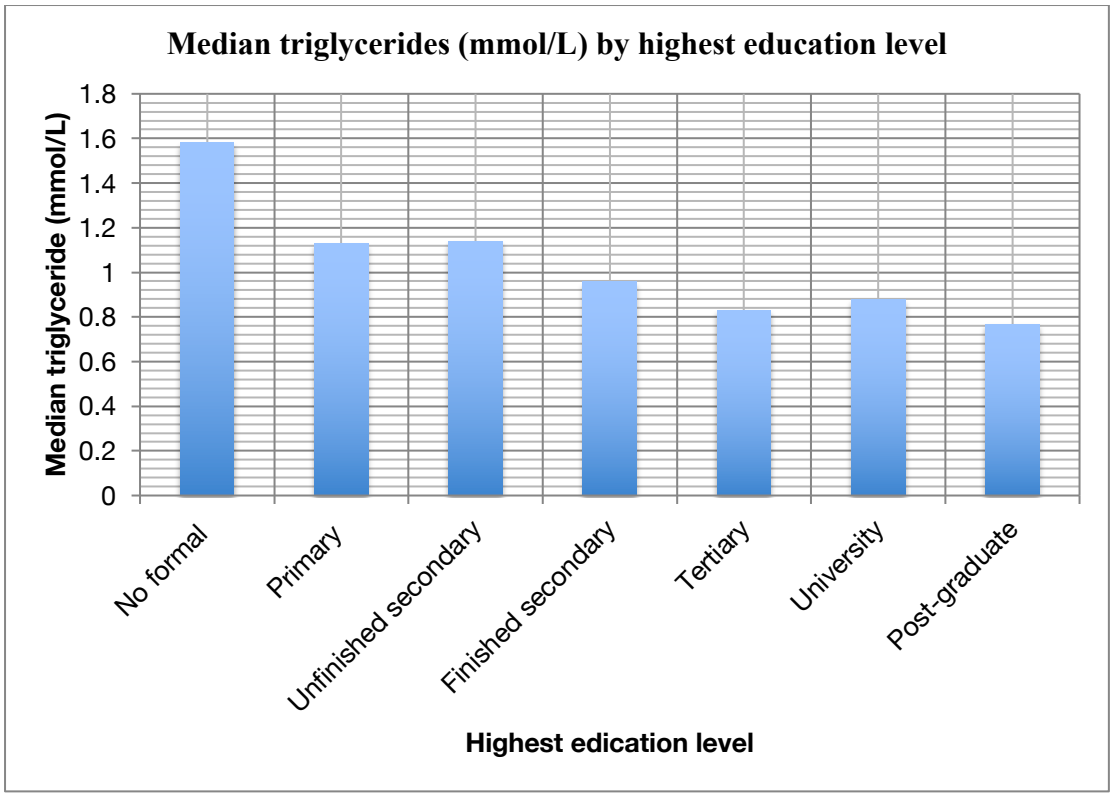



Figure 3.77 Median triglyceride, by highest education level

On comparing to post-graduate education (reference category), all other education levels (except for tertiary) exhibited a positive association with an elevation in triglyceride levels as seen in Table 3.64. However, once again significance was lost on age and gender adjustment.

| Triglyceride level as dependent variable | | | |
|---|---|---|---|
| **Educational level** | **Odds ratio** | **CI 95%** | ***p*-value** |
| No formal | 2.22 | 1.35 - 3.67 | **<0.01** |
| Primary | 1.55 | 1.28 - 1.88 | **<0.01** |
| Unfinished secondary | 1.62 | 1.32 - 1.98 | **<0.01** |
| Finished secondary | 1.37 | 1.16 - 1.62 | **<0.01** |
| Tertiary | 1.15 | 0.96 - 1.37 | 0.14 |
| University | 1.24 | 1.04 - 1.49 | **0.02** |
| Post-graduate | Reference | | |

Table 3.64 Association analysis between the different education levels and triglyceride level

### 3.2.10.4.3 Triglycerides by employment status

The median triglyceride levels were all within the normal range irrelevant of employment status. However, the median triglyceride level for the 'retired' (1.15mmol/L IQR: 0.77) was found to be significantly higher than that of 'students' (0.73mmol/L IQR: 0.39, $p$=<0.01), of those 'employed' (0.90mmol/L IQR: 0.75, $p$=<0.01) and of 'domestic workers' (0.92mmol/L IQR: 0.67, $p$=<0.01).

On comparing with domestic work status (reference category), only the retired status exhibited a significant positive association with elevation in triglyceride levels (OR: 1.31 CI 95%: 1.17 – 1.48 $p$=<0.01). This association was lost after adjusting for age.

### 3.2.10.5 Total cholesterol by socio-demographic profile

#### 3.2.10.5.1 Total cholesterol by districts

All districts exhibited a median total cholesterol level above the normal range (>5mmol/L), with Gozo having the highest median total cholesterol level, as seen in Figure 3.78. In fact, the median total cholesterol level for Gozo was significantly higher than that for the Southern harbour ($p$=<0.01), the Western ($p$=<0.01) and South Eastern ($p$=0.03) districts.

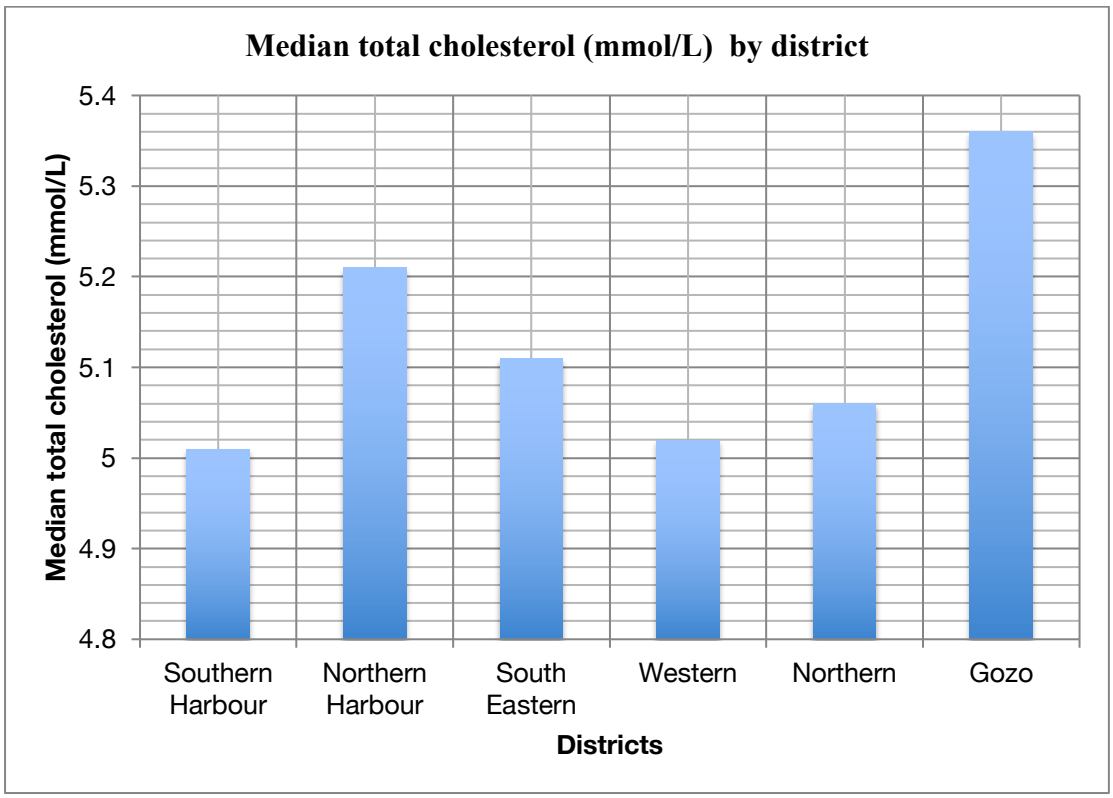



Figure 3.78 Mean total cholesterol, by districts

On comparing to Southern Harbour district (reference category), the districts of Gozo and the Northern Harbour had a higher risk of having an elevation of total cholesterol (Gozo – OR: 1.55, CI 95%: 1.27 – 1.90, $p$=<0.01; Northern Harbour – OR: 1.22, CI 95%: 1.06 – 1.41, $p$=0.01). The risks remained significant when adjusting for age, gender, BMI, highest education

levels, employment status, smoking and alcohol habits (Gozo – OR: 1.55 CI 95%: 1.27 – 1.88, *p*=<0.01; Northern Harbour – OR: 1.17, CI 95%: 1.02 – 1.35, *p*=0.03).

#### 3.2.10.5.2 Total cholesterol by highest education level

The median cholesterol levels were highest among those reporting a 'primary' education or 'unfinished secondary' education (Figure 3.79). In fact, a negative correlation was present between total cholesterol and education levels (R= -0.11, *p*=<0.01).

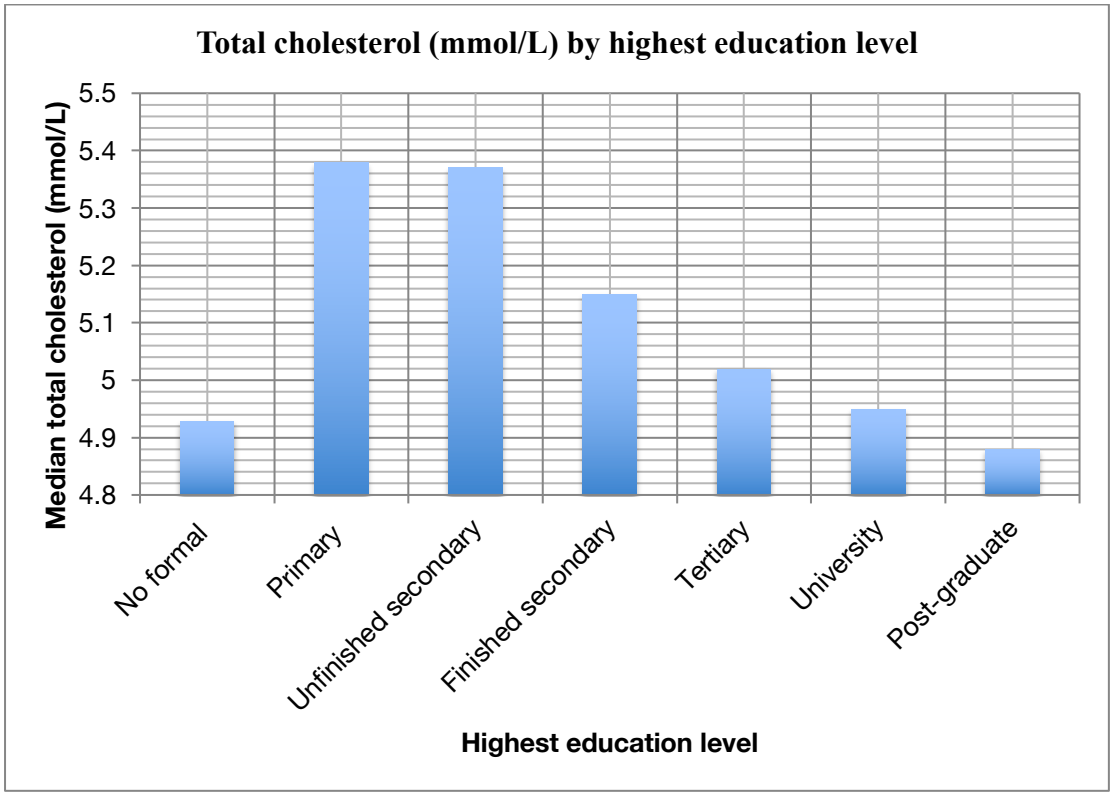



Figure 3.79 Median total cholesterol, by highest education level

On comparing to 'post-graduate education' as the reference category, primary education (OR: 1.40 CI 95%: 1.08 – 1.83 *p*=0.01), unfinished education (OR: 1.38 CI 95%: 1.04 – 1.81 *p*=0.02) and finished secondary education (OR: 1.30 CI 95%: 1.03 – 1.63 *p*=0.03) levels exhibited a

positive risk of having an elevation in total cholesterol level. Significance was lost on age and gender adjustment.

### 3.2.10.5.3 Total cholesterol by employment status

Students had the lowest median total cholesterol levels when compared to all other employment status (employed $p$=0.01, retired $p$=0.01, unemployed $p$=0.01, domestic work $p$=<0.01). Being employed was also linked to a lower median total cholesterol level when compared to domestic work ($p$=<0.01).

While comparing to domestic work (as a reference category), other employment categories except those unemployed exhibited a negative association with a raised total cholesterol level, as seen in Table 3.65. Only the retired category remained negatively associated with a raised total cholesterol level after adjusting for age and gender, as seen in Table 3.65.

**Total cholesterol level as dependent variable**

| Employment status | Odds ratio | CI 95% | *p*-value |
|---|---|---|---|
| Employed | 0.79 | 0.70 - 0.89 | **<0.01** |
| Unemployed | 1.19 | 0.83 - 1.71 | 0.34 |
| Student | 0.45 | 0.31 - 0.64 | **<0.01** |
| Retired | 0.80 | 0.68 - 0.94 | **<0.01** |
| Domestic work | Reference | | |
| | | | |
| Employed | 0.98 | 0.85 - 1.13 | 0.81 |
| Unemployed | 1.38 | 0.96 - 1.97 | 0.08 |
| Student | 0.82 | 0.56. 1.20 | 0.35 |
| Retired | 0.66 | 0.55 - 1.20 | **<0.01** |
| Domestic work | Reference | | |
| Age | 1.02 | 1.02 - 1.02 | **<0.01** |
| Gender (F) | 1.02 | 0.92 - 1.14 | 0.68 |

Table 3.65 Association analysis between employment status and total cholesterol level

### 3.2.11 Relationship between the biochemical parameters and lifestyle

This section explored the relationships between the different biochemical parameters (FPG, LDL-C, HDL-C, Triglycerides and Total Cholesterol) and lifestyle parameters (smoking habit, alcohol habit and physical activity) in the crude, unadjusted study population (*n*=1,861).

#### 3.2.11.1 Fasting plasma glucose by smoking habit

There was no difference in FPG levels between smokers and non-smokers (*p*=0.90).

#### 3.2.11.2 Fasting plasma glucose by alcohol habit

Median FPG levels were similar among those that consumed alcohol and those that did not (*p*=0.95).

#### 3.2.11.3 Fasting plasma glucose by physical activity

There was no significant link between FPG and physical activity (*p*=0.57).

#### 3.2.11.4 LDL-C by smoking habit

There was no significant difference between LDL-C levels among the different smoking categories (*p*=0.66).

#### 3.2.11.5 LDL-C by alcohol habit

There was no significant link between LDL-C levels and alcohol habits (*p*=0.79).

#### 3.2.11.6 LDL-C by physical activity

There was no significant link between LDL-C levels and physical activity ($p$=0.53).

#### 3.2.11.7 HDL-C by smoking habit

There was no significant link between HDL-C levels and smoking habits ($p$=0.58).

#### 3.2.11.8 HDL-C by alcohol habit

A significant difference was found between the median HDL-C levels of those that consume alcohol (median 1.50mmol/L IQR: 0.53) and those that do not (median 1.45 IQR: 0.59 $p$=0.02). For every increase in one alcohol unit, there was a unit increase in the chance of having a higher HDL-C level (OR: 1.05 CI 95%: 1.01 – 1.09 $p$=0.03). However, significance was lost on adjustment for age, gender and smoking habit.

#### 3.2.11.9 HDL-C by physical activity

There was no link between the median HDL-C levels and the different physical activities ($p$=0.47).

#### 3.2.11.10 Triglyceride by smoking habit

There was no link between the median triglyceride levels and smoking status ($p$=0.30).

#### 3.2.11.11 Triglyceride by alcohol habit

There was no significant difference between the median triglycerides levels of those that consume alcohol and those that do not ($p$=0.22).

#### 3.2.11.12 Triglyceride by physical activity

There was no significant link between the median triglyceride levels and the different physical activity levels ($p$=0.10).

#### 3.2.11.13 Total cholesterol by smoking habit

There was no significant difference between the median total cholesterol levels among smokers and non-smokers ($p$=0.83).

#### 3.2.11.14 Total cholesterol by alcohol habit

There was no significant difference between the median total cholesterol levels among those that consumed alcohol and those that did not ($p$=0.92).

#### 3.2.11.15 Total cholesterol by physical activity

There was no significant link between the median total cholesterol levels and the different physical activity levels ($p$=0.57).

### 3.2.12 Relationship between biochemical parameters and body mass index

This section considers the relationships between the different biochemical parameters (FPG, LDL-C, HDL-C, Triglycerides and Total Cholesterol) and body mass index in the crude unadjusted sample population (*n*=1,861).

#### 3.2.12.1 Fasting plasma glucose by body mass index

A significant steady increase in median FPG level was exhibited as BMI status increased from normal (<25Kg/m$^2$) to overweight (25.00 – 29.99 Kg/m$^2$) to obese (>=30 Kg/m$^2$) (*p*=<0.01), as seen in Figure 3.80. On pairwise comparison, a significant difference was present between each BMI status and median FPG (*p*=<0.01 respectively).

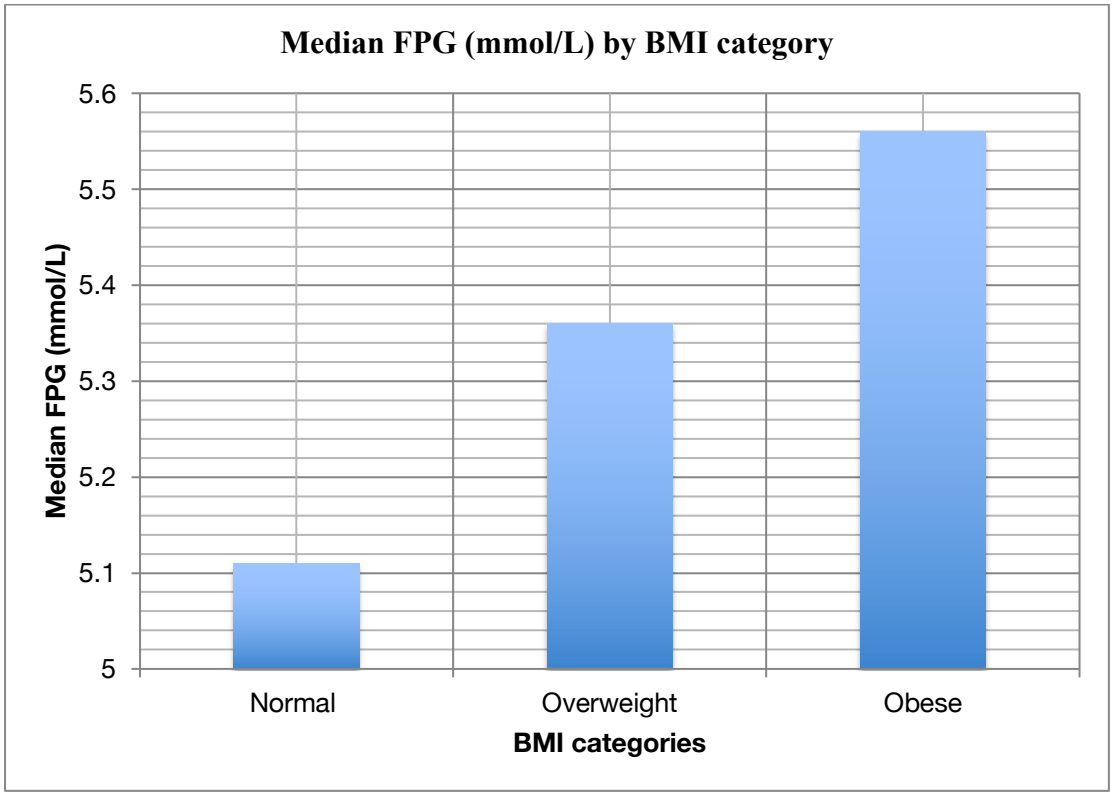



Figure 3.80 Median FPG, by BMI categories

A positive correlation was present between FPG and BMI (R=0.29, *p*=<0.01). For every unit increase in BMI (per Kg/$m^2$) there was a positive increased risk of having an elevated FPG level (OR: 1.05 CI 95%: 1.04 – 1.07, *p*=<0.01). This association remained significant on adjusting for age and gender (OR: 1.04 CI95%: 1.02 – 1.05, p=<0.01).

Similarly, for every unit increase in BMI (per Kg/$m^2$) above 30Kg/$m^2$, there was an increased risk of having an elevated FPG level (OR: 1.69 CI 95%: 1.45 – 1.98, *p*=<0.01). The association remained significant after adjustment by gender and age (OR: 1.44, CI 95%: 1.24 – 1.68, *p*=<0.01). However, within the overweight BMI category, there was no link between unit BMI and FPG (*p*=0.97).

**3.2.12.2 LDL-C by body mass index**

A significant increase in median LDL-C level was found as BMI status increased from normal (<25Kg/$m^2$) to overweight (25.00 – 29.99 Kg/$m^2$) to obese status (>=30 Kg/$m^2$) (*p*=<0.01), as seen in Figure 3.81.

On pairwise comparison, the normal BMI status had significantly lower median LDL-C levels than overweight and obese status (*p*=<0.01 respectively), although the median LDL-C levels between overweight and obese BMI status were not significantly different (*p*=0.44).

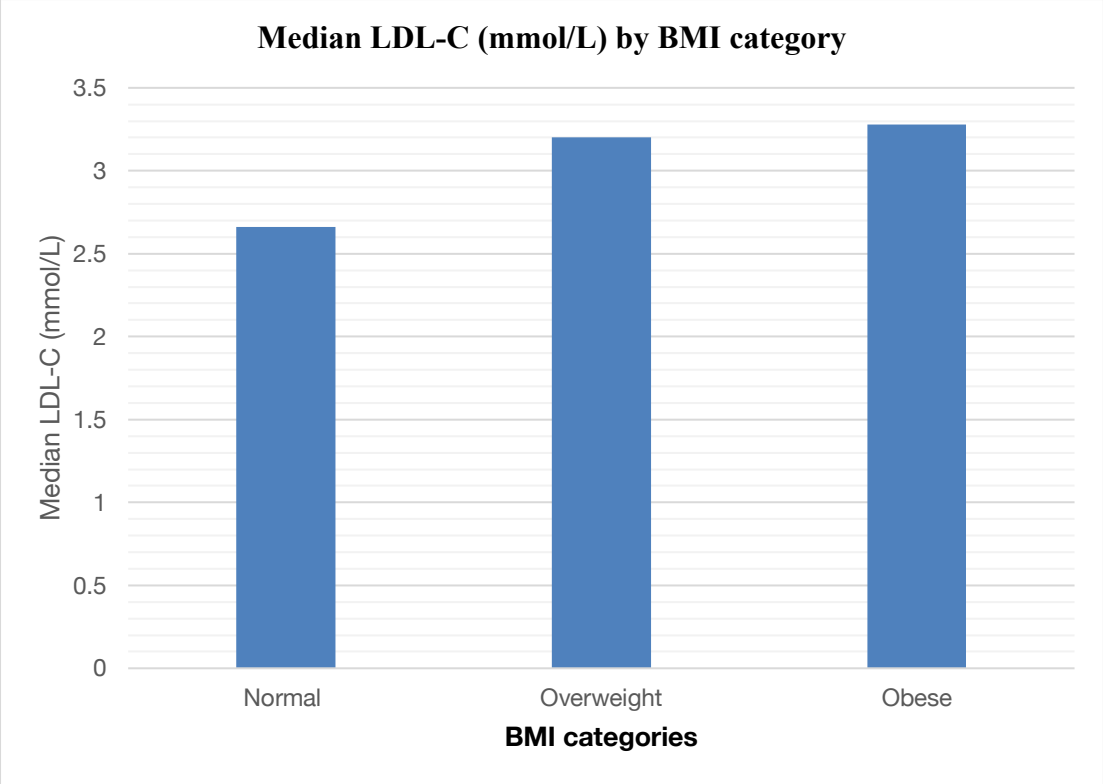



Figure 3.81 Median LDL-C, by BMI categories

A positive correlation was found between LDL-C and BMI (R=0.23, *p*=<0.01). For every unit increase in BMI (per Kg/m$^2$) there was a positive increased risk of having an elevated LDL-C level (OR: 1.19 CI 95%: 1.09 – 1.29, *p*=<0.01). The link remained significant after adjusting for age and gender (OR: 1.02, CI 95%: 1.02 – 1.03).

An increase in obese BMI status (per Kg/m$^2$ above 30Kg/m$^2$) was associated with an increased risk of having a raised LDL-C level. The association remained significant after adjusting for age and gender (OR: 1.24, CI 95%: 1.34 – 1.35, *p*=<0.01).

A unit increase in overweight BMI status (per Kg/m$^2$ above 25Kg/m$^2$) was positively linked with having a raised LDL-C level (OR: 1.19 CI 95%: 1.09 – 1.29, *p*=<0.01). The association

remained significant after adjusting for age and gender (OR: 1.14, CI 95%: 1.04 – 1.24, $p$=<0.01).

### 3.2.12.3 HDL-C by body mass index

A significant and steady decrease in median HDL-C was exhibited as the BMI status progressed from normal (<25Kg/m$^2$) to overweight (25.00 – 29.99 Kg/m$^2$) to obese (>=30 Kg/m$^2$) ($p$=<0.01), as seen in Figure 3.82. On pairwise comparison, a significant difference was present between each BMI status and median HDL-C ($p$=<0.01 respectively). In fact, a negative correlation was present between BMI and HDL-C (R= -0.36, $p$=<0.01).

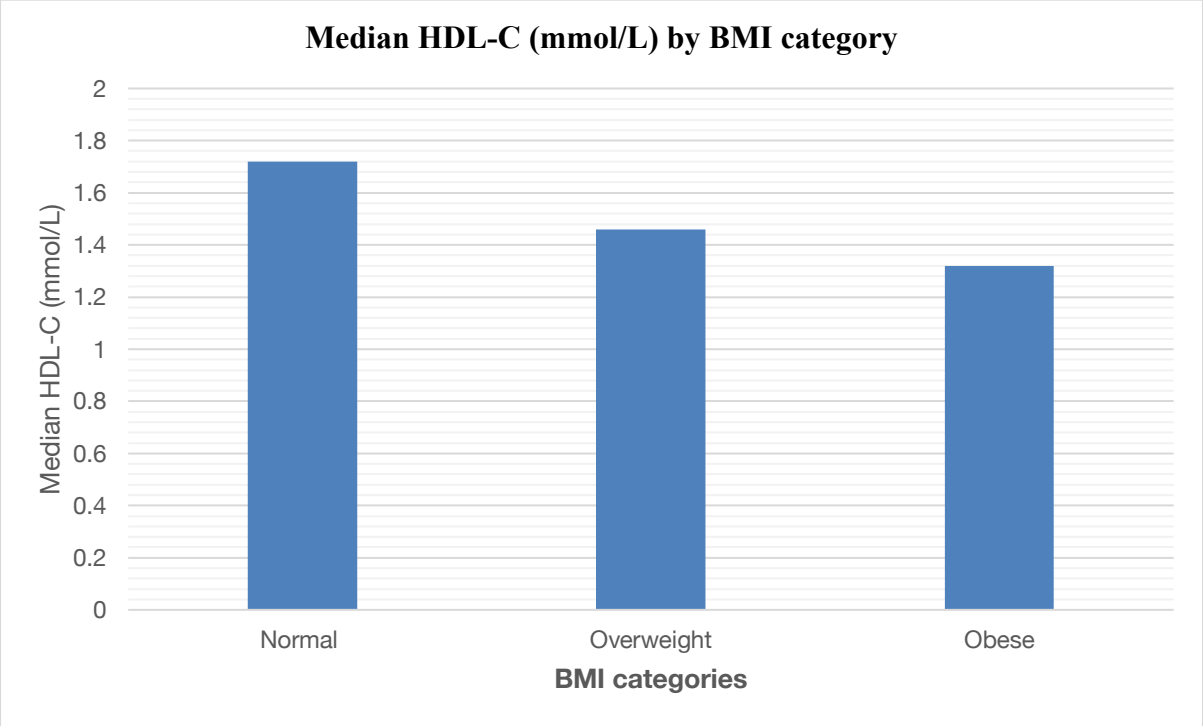



Figure 3.82 Median HDL-C, by BMI categories

A unit increase in BMI (per $Kg/m^2$) was negatively associated with having an elevation in HDL-C level (OR: 0.78 CI 95%: 0.75 – 0.81, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 0.98, CI 95%: 0.98 – 0.99, *p*=<0.01).

Per unit increase in BMI (per $Kg/m^2$) in an obese individual (>30$Kg/m^2$), there was a negative association with having an elevation in HDL-C levels (OR: 0.78 CI 95%: 0.75 – 0.81, *p*=<0.01). The association remained significant on adjustment by age and gender (OR: 0.80, CI 95%: 0.77 – 0.83, p=<0.01). However, the overweight BMI status was not linked with HDL-C levels (*p*=0.14).

#### 3.2.12.4 Triglycerides by body mass index

Median triglyceride levels rose with BMI status from normal (<25$Kg/m^2$) to overweight (25.00 – 29.99 $Kg/m^2$) to obese (>=30 $Kg/m^2$) (*p*=<0.01), as seen in Figure 3.83. On pairwise comparison, a significant difference was present between each BMI status and median triglyceride (*p*=<0.01 respectively). In fact, a positive correlation was present between triglyceride level and BMI (R=0.38, *p*=<0.01).

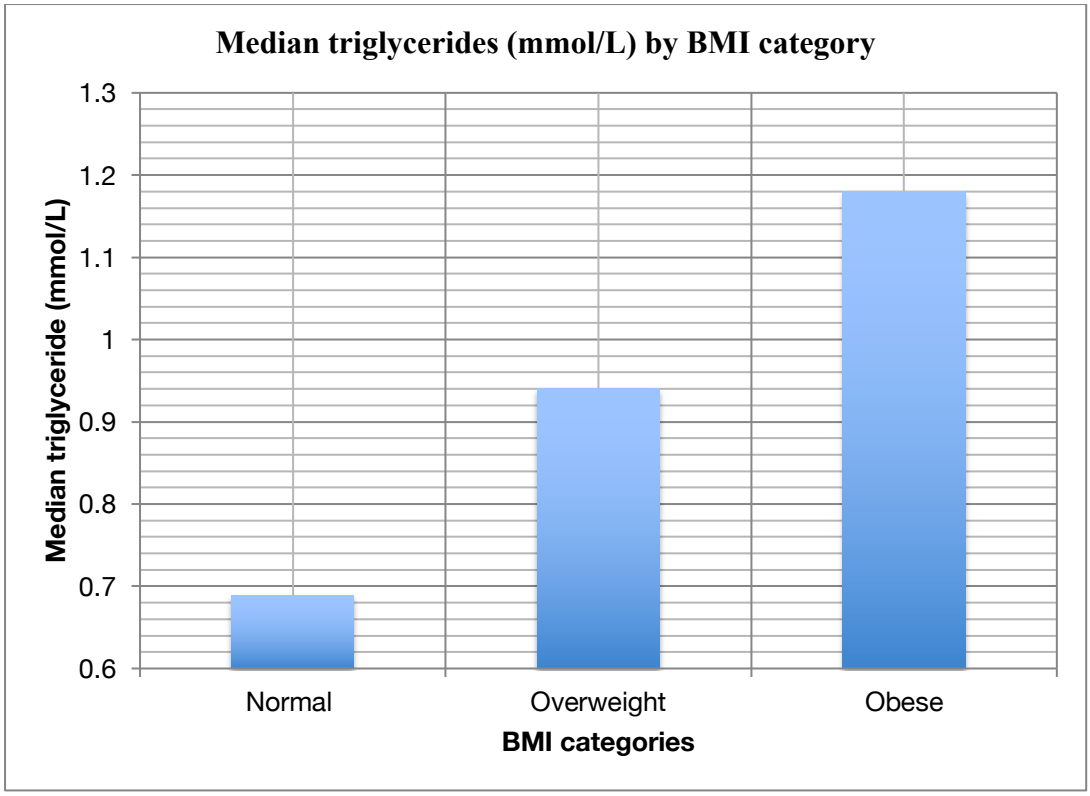



Figure 3.83 Median triglycerides, by BMI categories

A unit increase in BMI (per Kg/m$^2$) was positively associated with having an elevation in triglyceride level (OR: 1.04 CI 95%: 1.03 – 1.04, *p*=<0.01). This relationship remained significant on adjusting for age and gender (OR: 1.03 CI 95%: 1.03 – 1.04, *p*=<0.01).

A unit increase in BMI (per Kg/m$^2$) among obese individuals (>30Kg/m$^2$) was linked with a unit increase in triglycerides level (OR: 1.50 CI 95%: 1.39 – 1.61, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.41, CI 95%: 1.31 – 1.51, *p*=<0.01). However, within the overweight BMI status there was no link between unit increase in BMI and triglyceride levels (*p*=0.83).

### 3.2.12.5 Total cholesterol by body mass index

The median total cholesterol level was significantly higher for the overweight BMI status (25.00 – 29.99 Kg/m$^2$) as compared to that for the normal BMI status (24.99Kg/m$^2$) ($p$=<0.01). There was a subsequently a small dip in the median cholesterol levels for the obese status category when compared to the overweight status ($p$=1.00) as seen in Figure 3.84. The normal BMI status also had a significantly lower median total cholesterol levels than the obese status ($p$=<0.01).

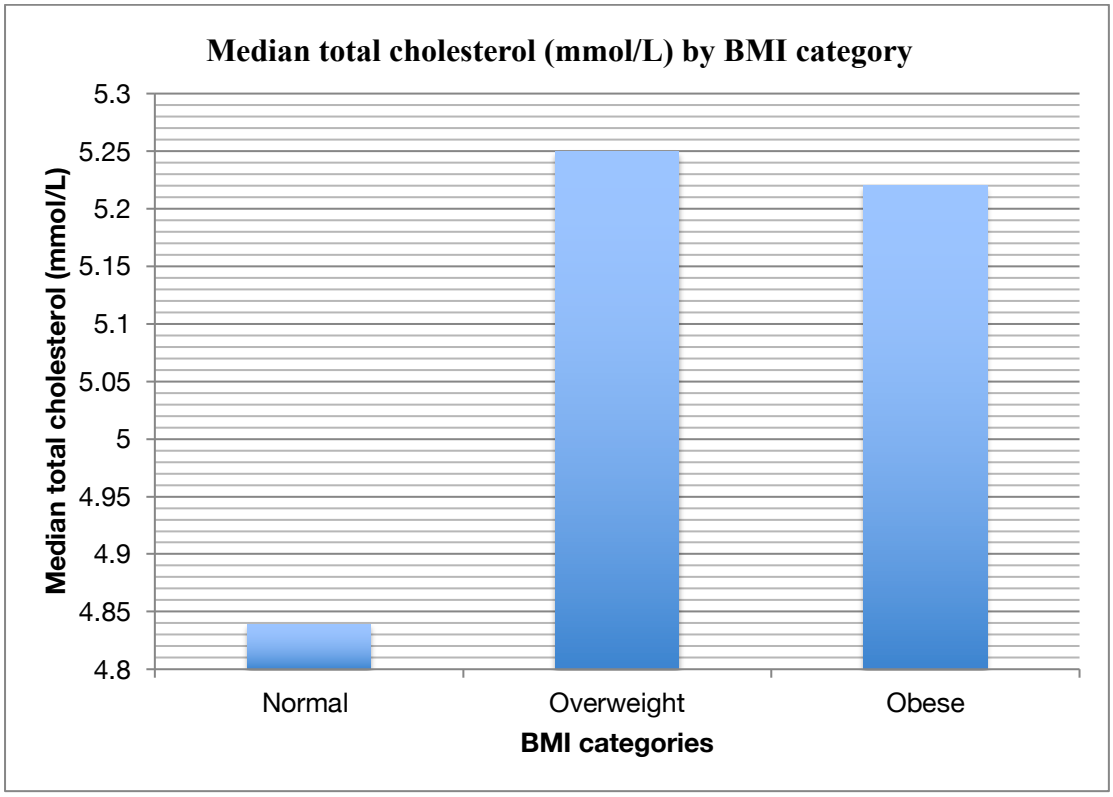



Figure 3.84 Median total cholesterol, by BMI status

A positive correlation was found between total cholesterol and BMI (R=0.15, *p*=<0.01).
For every unit of BMI (per Kg/m$^2$) increase, a positive association was present with total cholesterol (OR: 1.02 CI 95%: 1.01 – 1.03, *p*=<0.01). The association remained significant on adjusting for age and gender (OR: 1.02 CI 95%: 1.01 – 1.03, *p*=<0.01).

A unit increase in obese BMI (per Kg/m$^2$ above 30Kg/m$^2$) was positively associated with having an increase in total cholesterol level (OR: 1.38 CI 95%: 1.14 – 1.67, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.18, CI 95%: 1.07 – 1.31, *p*=<0.01). Similarly, an increase in overweight BMI (per Kg/m$^2$ above 25Kg/m$^2$) was positively associated with a having a rise in total cholesterol level (OR: 1.32 CI 95%: 1.09 – 1.60, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.11, CI 95%: 1.00 – 1.22, *p*=0.05).

### 3.2.13 Relationship between biochemical parameters and glucose regulation

This section considers the relationships between the different biochemical parameters (LDL-C, HDL-C, Triglycerides and Total cholesterol) with glycaemic status among the crude unadjusted diabetes (*n*=219) and IFG (*n*= 460) populations.

#### 3.2.13.1 Biochemical parameters by type 2 diabetes mellitus

When undergoing linkage analysis, the T2DM sub-population was compared to both the non-diabetes sub-population as well as to the 100% metabolically healthy sub-population. By comparing to the metabolically healthy sub-group, the presence of any underlying metabolic or insulin resistance, in the reference group that might affect the linkage analyses was excluded.

#### 3.2.13.1.1 Type 2 diabetes mellitus by LDL-C

The T2DM sub-population exhibited a significantly lower median LDL-C level (median 2.92mmol/L IQR: 1.25) than did the non-T2DM sub-population (median 3.12mmol/L IQR: 1.26, *p*=<0.01).

For every unit increase in LDL-C levels (per 1mmol/L), the odds of being an individual with diabetes rather than without decreased by 24% (OR: 0.76 CI 95% 0.65 – 0.89, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 0.66, CI 95%: 0.56 – 0.79, *p*=<0.01).

However, for every unit increase in LDL-C levels, the odds of being an individual with diabetes rather than metabolically healthy increased by four-fold (OR: 4.15 CI 95%: 2.79 – 6.17, *p* = <0.01). The association was augmented after adjustment for age and gender (OR: 4.94 CI 95%: 2.34 – 10.46, *p*=<0.01).

#### 3.2.13.1.2 Type 2 diabetes mellitus by HDL-C

The T2DM sub-population exhibited a significantly lower median HDL-C level (median 1.23mmol/L IQR: 0.57) than did the non-T2DM sub-population (median 1.50mmol/L IQR: 0.56, *p*=<0.01).

For every unit increase in HDL-C levels (per 1mmol/L), the odds of being an individual with diabetes rather than not decreased by 76% (OR: 0.24 CI 95% 0.17 – 0.36, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 0.31, CI 95%: 0.20 – 0.47, *p*=<0.01). This relationship remained evident when the diabetes sub-group was compared

to the metabolically healthy subgroup (OR: 0.07 CI 95%; 0.03 – 0.13, *p*=<0.01). On adjustment for age and gender, the negative association between HDL-C levels and having T2DM was augmented (OR: 0.10 CI 95%: 0.03 – 0.29, *p*=<0.01).

#### 3.2.13.1.3 Type 2 diabetes mellitus by triglycerides

The T2DM sub-population exhibited a significantly higher median triglyceride levels (median 1.40mmol/L IQR: 0.99) than did the non-T2DM sub-population (median 0.90 mmol/L IQR: 0.68, *p*=<0.01).

For every unit increase in triglyceride levels (per 1mmol/L), the odds of being an individual with diabetes rather than not increased by 72% (OR: 1.72 CI 95% 1.49 – 1.99, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.49, CI 95%: 1.27 – 1.75, *p*=<0.01). This relationship between an increase in triglyceride levels and having T2DM remained strongly associated when comparing to the metabolically healthy sub-group (OR: 75.73, CI 95%: 27.49 – 208.66, *p*=<0.01). The relationship was further augmented on adjustment for age and gender (OR: 87.15, CI 95%: 15.04 – 505.02, *p*=<0.01).

#### 3.2.13.1.4 Type 2 diabetes mellitus by total cholesterol

The T2DM sub-population exhibited a significantly lower median total cholesterol level (median 4.92mmol/L IQR: 1.48) than did the non-T2DM sub-population (median 5.12mmol/L IQR: 1.41, *p*=<0.01).

For every unit increase in total cholesterol levels (per 1mmol/L), the odds of being an individual with diabetes rather than not decreased by 21% (OR: 0.79 CI 95% 0.69 – 0.91,

*p*=<0.01). The association remained significant on adjustment for age and gender (OR: 0.72, CI 95%: 0.62 – 0.84, *p*=<0.01). However, on comparing to the metabolically healthy sub-group, a unit increase in total cholesterol levels (per 1 mmol/L) increased the odds of being an individual with diabetes by 145% (two-fold) (OR: 2.45 CI 95%: 1.84 – 3.33, *p* = <0.01). The association remained significant when adjusted for age and gender (OR: 2.68 CI 95%: 1.53 – 4.70, *p*=<0.01).

#### 3.2.13.1.5 Type 2 diabetes mellitus associations with the biochemical profile

Explanatory analyses were further conducted to discover which biochemical variables (FPG, LDL-C, HDL-C, Triglyceride, Total cholesterol) were linked to global diabetes mellitus (i.e. a combination of previously and newly diagnosed diabetes) once socio-demographic confounding factors (age, gender, education level, residing district, employment status and statin use) were adjusted for, on comparing to the non-diabetes population. This analysis was repeated to explore the linkage analysis with T2DM but on comparing to the metabolically healthy sub-group.

When comparing to the non-diabetes population, an increase in unit FPG level (per mmol/L) was associated with an eleven-fold increased associated risk of having T2DM, while a unit increase in LDL-C (per mmol/L) had the opposite effect, as seen in Table 3.66, in which only significant independent factors were presented.

| DM as dependent variable | | | |
|---|---|---|---|
| **Independent risk factors** | **Odds ratio** | **CI 95%** | ***p*-value** |
| FPG | 11.47 | 8.00 - 16.45 | **<0.01** |
| LDL-C | 0.40 | 0.21 - 0.76 | **<0.01** |
| Age | 1.05 | 1.01 - 1.09 | **<0.01** |
| Statin use* | 3.32 | 1.82 - 6.06 | **<0.01** |
| Southern Harbour district** | 3.26 | 1.06 - 10.01 | **0.04** |
| Western district** | 4.15 | 1.33 - 12.95 | **0.01** |

*No statin uses as reference category
** Gozo district as reference category

Table 3.66 Independent biochemical parameters' associations with having diabetes mellitus diagnosis when compared to the non-diabetes sub-population

The independent variables associated with global T2DM when compared to the metabolically healthy sub-group were found to be triglyceride level, age and gender, as seen in Table 3.67, in which only significant variables were illustrated.

| DM as dependent variable | | | |
|---|---|---|---|
| **Independent risk factors** | **Odds ratio** | **CI 95%** | ***p*-value** |
| Triglycerides | 27.44 | 2.39 - 315.04 | **0.01** |
| Age | 1.15 | 1.06 - 1.24 | **<0.01** |
| Female gender* | 0.06 | 0.01 - 0.46 | **0.01** |

* Male gender as reference category

Table 3.67 Independent biochemical parameters' associations with having diabetes mellitus diagnosis when compared to metabolically healthy sub-group

The exploratory analysis was conducted between previously diagnosed diabetes population and biochemical parameters while comparing to the non-diabetes population and to the metabolically healthy population. A unit increase in FPG level (per mmol/L) was positively associated with being a previously known diabetes, while a unit increase in LDL-C (per

mmol/L) exhibited a negative association, as seen in Table 3.68, when comparing to non-diabetes population.

| Previously DM as dependent variable | | | |
|---|---|---|---|
| **Independent risk factors** | **Odds ratio** | **CI 95%** | ***p*-value** |
| FPG | 2.03 | 1.78 - 2.31 | **<0.01** |
| LDL-C | 0.40 | 0.16 - 1.00 | **0.05** |
| Age | 1.09 | 1.05 - 1.32 | **<0.01** |
| Statin use* | 3.27 | 1.92 - 5.59 | **<0.01** |
| Western district** | 4.58 | 1.37 - 15.31 | **0.01** |

*No statin uses as reference category
** Gozo district as reference category

Table 3.68 Independent biochemical parameters' links with having previously diagnosed diabetes mellitus when compared to the non-diabetes population.

The independent variables associated with previous T2DM when compared to the metabolically healthy sub-group can be seen in Table 3.69.

| Previously DM as dependent variable | | | |
|---|---|---|---|
| **Independent risk factors** | **Odds ratio** | **CI 95%** | ***p*-value** |
| FPG | 1.24 | 1.09 - 1.41 | **<0.01** |
| Age | 1.09 | 1.04 - 1.14 | **<0.01** |
| Statin use* | 2.88 | 1.45 - 5.74 | **<0.01** |
| Female gender** | 0.32 | 0.12 - 0.83 | **0.02** |
| Employed*** | 0.26 | 0.08 - 0.81 | **0.02** |

*No statin uses as reference
** Male gender as reference
*** Domestic tasks as reference

Table 3.69 Independent biochemical parameters' associations with having previously diagnosed diabetes mellitus when compared to the healthy sub-group

Only FPG exhibited any link with having newly diagnosed Diabetes when compared to non-diabetes population, as seen in Table 3.70.

| Newly DM as dependent variable | | | |
|---|---|---|---|
| **Independent risk factors** | **Odds ratio** | **CI 95%** | ***p*-value** |
| FPG | 1.42 | 1.30 - 1.55 | <0.01 |
| Age | 1.06 | 1.03 - 1.10 | <0.01 |

Table 3.70 Biochemical parameters' links with having newly diagnosed diabetes mellitus

Similarly, a strong positive association was evident between an elevated FPG and having newly diagnosed diabetes mellitus when comparing to metabolically healthy sub-group (OR: 64.56, CI 95%: 3.64 – 438.66, *p*=0.01).

### 3.2.13.2 Biochemical parameters by impaired fasting glucose

#### 3.2.13.2.1 Impaired fasting glucose by LDL-C

The IFG sub-population exhibited a significantly higher median LDL-C level (median 3.32mmol/L IQR: 1.23) than the non-IFG (median 3.01mmol/L IQR: 1.27) sub-population (*p*=<0.01). For every unit increase in LDL-C (per 1mmol/L), there was a unit positive increase in the risk of having IFG (OR: 1.43 CI 95% 1.27 – 1.60, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.34, CI 95%: 1.19 – 1.51, *p*=<0.01).

#### 3.2.13.2.2 Impaired fasting glucose by HDL-C

The IFG sub-population had a significantly lower median HDL-C level (1.39mmol/L IQR: 0.50) than did the non-IFG (median 1.50mmol/L IQR: 0.59) sub-population (*p*=<0.01).

For every unit decrease in HDL-C level (per 1mmol/L), there was an associated increase with having IFG (OR: 0.57 CI 95% 0.45 – 0.74, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 0.75, CI 95%: 0.57 – 0.99, *p*=0.04).

#### 3.2.13.2.3 Impaired fasting glucose by triglycerides

The IFG sub-population had a significantly higher median triglyceride level (median 1.08mmol/L IQR: 0.77) than did the non-IFG (median 0.88mmol/L IQR: 0.71) sub-population (*p*=<0.01). An increase in triglyceride levels (per 1mmol/L) was linked with an increased risk of having IFG (OR: 1.28 CI 95% 1.13 – 1.45, *p*=<0.01). However, significance was lost on adjusting for age and gender.

#### 3.2.13.2.4 Impaired fasting glucose by total cholesterol

The IFG sub-population had a significantly higher median total cholesterol level (median 5.35mmol/L IQR: 1.45) than did the non-IFG (median 5.03mmol/L IQR: 1.41) sub-population (*p*=<0.01). An increase in total cholesterol level (per 1mmol/L) was positively associated with having IFG (OR: 1.28 CI 95% 1.16 – 1.41, *p*=<0.01). The association remained significant on adjustment for age and gender (OR: 1.23, CI 95%: 1.11 – 1.36, *p*=<0.01).

#### 3.2.13.2.5 Impaired fasting glucose associations with the biochemical profile

Explanatory analyses were further conducted to discover which biochemical variables (FPG, LDL-C, HDL-C, Triglyceride, Total Cholesterol) were linked to IFG, once statin use and socio-demographic confounding factors (age, gender, education, locality and employment status) were adjusted for.

On adjustment for these confounding factors, none of the biochemical parameters exhibited any associated with having IFG. However, an increase in age (OR: 1.04 CI 95%: 1.03 – 1.05, $p$=<0.01) exhibited a positive association with having IFG, while the female gender exhibited a negative association with having IFG (OR: 0.62 CI 95%: 0.46-0.82 $p$=<0.01).

### 3.2.13.3 Relationship between glucose regulatory sub-groups by biochemical parameters

The median values of measured biochemical variables (FPG, LDL-C, HDL-C, triglycerides and total cholesterol) were compared by gender, for each different glucose regulatory subgroup (i.e. normoglycaemia, impaired fasting glucose, newly diabetes or previously known diabetes), as seen in Table 3.71.

The female population exhibited lower median FPG levels throughout the different glucose regulatory subgroups as compared to the male population. This was statistically significant in all except the *known diabetes mellitus* subgroup (Table 3.71). This gender trend (i.e. lower levels for females than males) was found also for the LDL-C and triglyceride (TG) variables, although significance between genders varied as seen in Table 3.71. Females exhibited statistically higher HDL-C levels than did males, irrespective of their glucose status (Table 3.71). The male population had lower levels of total cholesterol (TC) levels than the female population. This was evident throughout the different glucose regulatory subgroups except for the newly diagnosed diabetes subgroup, however no statistical difference was evident from the female populations in all subgroups (Table 3.71).

Only the normoglycaemic and the IFG subgroups showed significant median differences across all the biochemical variables (except for total cholesterol) between females and males (Table 3.71).

| | | **NGR (*n*=1,182)** | **IFG (*n*=460)** | **NDM (*n*=85)** | **KDM (*n*=134)** | **Mann-Whitney U test** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Median ±IQR** | | | | *p*-value1 | *p*-value2 | *p*-value3 | *p*-value4 |
| Median FPG (mmol/L) | F | 4.98 ±0.37 | 5.95 ±0.3 | 7.54 ±1.07 | 8.39 ±3.11 | **<0.01** | **<0.01** | **<0.01** | 0.16 |
| | M | 5.13 ±0.31 | 6.01 ±0.35 | 9.32 ±3.04 | 9.1 ±3.95 | | | | |
| Median LDL-C (mmol/L) | F | 2.93 ±0.91 | 3.25 ±1.28 | 3.29 ±0.96 | 2.61 ±0.92 | **<0.01** | **<0.01** | 0.17 | 0.67 |
| | M | 3.09 ±0.86 | 3.41 ±0.83 | 3.52 ±1.01 | 2.66 ±0.83 | | | | |
| Median TG (mmol/L) | F | 0.89 ±0.62 | 0.95 ±0.73 | 1.34 ±0.88 | 1.38 ±0.80 | **<0.01** | **<0.01** | **<0.01** | 0.38 |
| | M | 1.11 ±0.78 | 1.12 ±0.73 | 1.86 ±0.99 | 1.48 ±0.85 | | | | |
| Median HDL-C (mmol/L) | F | 1.73 ±0.45 | 1.63 ±0.58 | 1.55 ±0.45 | 1.57 ±0.54 | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| | M | 1.39 ±0.34 | 1.28 ±0.40 | 1.17 ±0.31 | 1.23 ±0.45 | | | | |
| Median TC (mmol/L) | F | 5.04 ±1.09 | 5.38 ±1.48 | 5.44 ±1.19 | 4.71 ±1.08 | 0.10 | 0.08 | 0.70 | 0.26 |
| | M | 4.97 ±0.95 | 5.30 ±0.98 | 5.52 ±1.14 | 4.55 ±0.98 | | | | |

TG - Triglycerides
TC- Total Cholesterol
F - Female
M - Male

*p*-value1:NGT male vs. female
*p*-value2:IFG male vs. female
*p*-value3:NDM male vs. female
*p*-value4:KDM male vs. female

Table 3.71 Glucose regulatory status and median biochemical profile by gender

Comparisons between the biochemical variables and the different glucose regulatory subgroups were performed for each population (female and male separately), as seen in Table 3.72.

Significant differences were exhibited throughout the biochemical variables and their corresponding glucose regulatory subgroups. The only exception was between the newly diagnosed and known diabetes mellitus subgroups, where no significant difference was evident between LDL-C, triglycerides (TG) and total cholesterol (TC) for the females and TG and TC for the males.

| | Female – Kruskal-Wallis test | | | | | |
|---|---|---|---|---|---|---|
| | *p*-value 1 | *p*-value 2 | *p*-value 3 | *p*-value 4 | *p*-value 5 | *p*-value 6 |
| Median FPG (mmol/L) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median LDL-C (mmol/L) | 0.39 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median TG (mmol/L) | 0.82 | **<0.01** | **<0.01** | **0.04** | **<0.01** | **<0.01** |
| Median HDL-C (mmol/L) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median TC (mmol/L) | 0.37 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |

| | Male – Kruskal-Wallis test | | | | | |
|---|---|---|---|---|---|---|
| | *p*-value1 | *p*-value2 | *p*-value3 | *p*-value4 | *p*-value 5 | *p*-value 6 |
| Median FPG (mmol/L) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median LDL-C (mmol/L) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median TG (mmol/L) | 0.82 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median HDL-C (mmol/L) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| Median TC (mmol/L) | 0.76 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |

*p*-value 1: NDM vs KDM
*p*-value 2: NDM vs IFG
*p*-value 3: NDM vs NGR
*p*-value 4: KDM vs IFG
*p*-value 5: KDM vs NGR
*p*-value 6: IFG vs NGR

Table 3.72 Statistical comparisons between the different glucose regulatory subgroups, by median biochemical profiling and gender

On comparing the biochemical variables between the IFG and the diabetes mellitus (previously and newly diagnosed combined) populations, a significant difference was present between each population and biochemical variable, by gender as seen in Table 3.73.

| | | **IFG (*n*=460)** | **T2DM (*n*=219)** | **Mann-Whitney U test** |
|---|---|---|---|---|
| | | Median ±IQR | Median ±IQR | *p*-value |
| FPG (mmol/L) | F | 5.86 ±0.39 | 7.33 ±1.52 | **<0.01** |
| | M | 5.89 ±0.46 | 7.99 ±3.74 | **<0.01** |
| LDL-C (mmol/L) | F | 3.22 ±1.28 | 2.74 ±1.24 | **<0.01** |
| | M | 3.43 ±1.15 | 2.96 ±1.25 | **<0.01** |
| HDL-C (mmol/L) | F | 1.61 ±0.58 | 1.50 ±0.44 | **<0.01** |
| | M | 1.29 ±0.41 | 1.12 ±0.48 | **<0.01** |
| Triglycerides (mmol/L) | F | 0.96 ±0.74 | 1.21 ±0.77 | **<0.01** |
| | M | 1.11 ±0.75 | 1.47 ±1.10 | **<0.01** |
| Total Cholesterol (mmol/L) | F | 5.34 ±1.49 | 4.97 ±1.50 | **<0.01** |
| | M | 5.29 ±1.40 | 4.90 ±1.32 | **<0.01** |

Table 3.73 Comparisons between IFG and Total DM populations, by biochemical parameters and gender

The T2DM population exhibited higher median FPG and median triglycerides levels when compared to IFG population. The LDL-C and total cholesterol median levels were higher in the IFG population. The IFG and T2DM populations' HDL-C levels were within the normal/high range, although the IFG population exhibited higher HDL-C median values than did the T2DM population (Table 3.73).

## 3.3 Association studies

This section explored links between different variables and type 2 diabetes mellitus (T2DM), impaired fasting glucose and metabolic syndrome through multiple binary logistic regression models in order to establish independent risk factors within the crude (unweighted) population.

### 3.3.1 Established independent risk factors for type 2 diabetes mellitus among adults in Malta

The variables that exhibited a univariant statistically significant relationship with T2DM (when compared to non-diabetes population), were analysed using multiple binary logistic regression with backward elimination as seen in Table 3.74.

As expected, an elevation in FPG (per mmol/L) exhibited a positive association with having T2DM. Similarly increasing age (per year), the presence of the metabolic syndrome and a history of hypertension also exhibited a positive association with having T2DM. On the contrary, LDL-C and diastolic blood pressure exhibited an opposite decreased association with having T2DM.

| Variables | Crude model - GLM | | | Adjusted - GLM | | |
|---|---|---|---|---|---|---|
| | Sig. | OR | 95CI% | Sig. | OR | 95CI% |
| FPG | **<0.01** | 8.09 | 6.72 - 9.73 | **<0.01** | 5.06 | 4.21 - 6.10 |
| LDL-C | **<0.01** | 0.82 | 0.73 - 0.91 | **<0.01** | 0.50 | 0.42 - 0.61 |
| HDL-C | **<0.01** | 0.26 | 0.20 - 0.35 | | | |
| Triglycerides | **<0.01** | 1.72 | 1.56 - 1.91 | | | |
| Total cholesterol | **<0.01** | 0.85 | 0.77 - 0.94 | | | |
| Systolic BP | **<0.01** | 1.04 | 1.04 - 1.05 | | | |
| Diastolic BP | **<0.01** | 1.02 | 1.01 - 1.02 | **0.02** | 0.98 | 0.961 - 0.99 |
| BMI | **<0.01** | 1.09 | 1.08 - 1.11 | | | |
| Body Weight | **<0.01** | 1.03 | 1.02 - 1.03 | | | |
| Waist Circumference | **<0.01** | 1.06 | 1.05 - 1.06 | **0.03** | 1.01 | 1.001 - 1.03 |
| Gender (F) | **<0.01** | 0.5 | 0.41 - 0.62 | | | |
| Age | **<0.01** | 1.09 | 1.08 - 1.11 | **<0.01** | 1.05 | 1.034 - 1.07 |
| Metabolic Syndrome | **<0.01** | 9.63 | 7.68 - 12.09 | **<0.01** | 2.07 | 1.398 - 3.06 |
| Hypertension history | **<0.01** | 7.33 | 5.91 - 9.08 | **<0.01** | 2.51 | 1.758 - 3.59 |
| MI history | **<0.01** | 4.81 | 3.05 - 7.58 | | | |
| CHD history | **<0.01** | 4.51 | 3.03 - 6.71 | | | |
| Hyperlipideamia history | **<0.01** | 5.19 | 4.22 - 6.39 | | | |
| Depression history | **0.01** | 1.52 | 1.14 - 2.03 | | | |
| FH of Diabetes | **0.05** | 0.10 | 0.40 - 0.62 | | | |

*MI – myocardial infarction; CHD – coronary heart disease; FH – family history*

Table 3.74 Independent associations for having T2DM, when compared to non-diabetes population, in the Maltese Population

The variables that exhibited a statistically significant relationship with T2DM, (when compared to the metabolically healthy sub-group) were analysed using multiple binary logistic regression and backward elimination as seen in Table 3.75. Of note, only three independent associations with having T2DM were evident.

| Variables | Crude model - GLM | | | Adjusted - GLM | | |
|---|---|---|---|---|---|---|
| | Sig. | OR | 95CI% | Sig. | OR | 95CI% |
| FPG | **<0.01** | 188.72 | 32.44 - 197.86 | **<0.01** | 190.22 | 18.43 - 963.08 |
| LDL-C | **<0.01** | 4.22 | 2.83 - 6.29 | | | |
| HDL-C | **<0.01** | 0.07 | 0.03 - 0.13 | | | |
| Triglycerides | **<0.01** | 75.73 | 27.49 - 208.66 | **<0.01** | 29.14 | 3.93 - 216.23 |
| Total cholesterol | **<0.01** | 2.45 | 1.84 - 3.33 | | | |
| Systolic BP | **<0.01** | 1.26 | 1.20 - 1.34 | | | |
| Diastolic BP | **<0.01** | 1.19 | 1.14 - 1.24 | | | |
| BMI | **<0.01** | 2.44 | 1.95 - 3.05 | | | |
| Body Weight | **<0.01** | 1.27 | 1.20 - 1.34 | | | |
| Waist Circumference | **<0.01** | 1.24 | 1.18 - 1.30 | | | |
| Gender (F) | **<0.01** | 0.07 | 0.04 - 0.13 | **<0.01** | 0.06 | 0.01 - 0.34 |
| Age | **<0.01** | 1.21 | 1.16- 1.25 | | | |
| Hypertension history | **<0.01** | 112.62 | 27.10 - 467.99 | | | |
| MI history | **0.03** | 9.16 | 1.20 - 70.22 | | | |
| CHD history | **0.02** | 6.21 | 1.43 - 27.04 | | | |
| Hyperlipideamia history | **<0.01** | 29.39 | 11.55 - 74.75 | | | |

*MI – myocardial infarction; CHD – coronary heart disease*

Table 3.75 Independent associations for having T2DM, when compared to the healthy sub-group, in the adult population in Malta

### 3.3.2 Established independent risk factors for impaired fasting plasma glucose among adults in Malta

The variables that exhibited a statistically significant relationship with IFG were analysed using multiple binary logistic regression with backward elimination as seen in Table 3.76.

| Variables | Crude model - GLM | | | Adjusted - GLM | | |
|---|---|---|---|---|---|---|
| | Sig. | OR | 95CI% | Sig. | OR | 95CI% |
| FPG | **<0.01** | 1.12 | 1.08 - 1.16 | | | |
| LDL-C | **<0.01** | 1.54 | 1.42 - 1.67 | | | |
| HDL-C | **<0.01** | 0.60 | 0.51 - 0.72 | | | |
| Triglycerides | **<0.01** | 1.31 | 1.20 - 1.42 | **<0.01** | 0.81 | 0.72 - 0.91 |
| Total cholesterol | **<0.01** | 1.37 | 1.28 - 1.47 | | | |
| Systolic BP | **<0.01** | 1.02 | 1.02 - 1.03 | | | |
| Diastolic BP | **<0.01** | 1.03 | 1.03 - 1.04 | **<0.01** | 1.01 | 1.01 - 1.02 |
| BMI | **<0.01** | 1.05 | 1.04 - 1.07 | | | |
| Body Weight | **<0.01** | 1.02 | 1.01 - 1.02 | | | |
| Waist Circumference | **<0.01** | 1.03 | 1.03 - 1.04 | **<0.01** | 1.01 | 1.01 - 1.02 |
| Gender (F) | **<0.01** | 0.58 | 0.50 - 0.67 | **<0.01** | 0.66 | 0.56 - 0.77 |
| Age | **<0.01** | 1.04 | 1.03 - 1.04 | **<0.01** | 1.03 | 1.02 - 1.04 |
| Years of smoking | **<0.01** | 1.01 | 1.01 - 1.02 | | | |
| Hypertension history | **<0.01** | 1.57 | 1.25 - 1.98 | | | |

Table 3.76 Independent associations for having IFG in the Maltese Population

An increase in waist circumference (per cm) exhibited a positive link with having IFG. Similarly, strong associations were evident with an increase in age (per year) and an increase in diastolic blood pressure (mmHg). Conversely, triglyceride levels and female gender exhibited the opposite negative associations.

### 3.3.3 Maltese established independent risk factors for metabolic syndrome

The variables that exhibited a statistically significant relationship with MetS were analysed using multiple binary logistic regression with backward elimination as seen in Table 3.77.

| Variables | Crude model - GLM | | | Adjusted - GLM | | |
|---|---|---|---|---|---|---|
| | *p*-value | OR | 95CI% | *p*-value | OR | 95CI% |
| FPG | **<0.01** | 2.47 | 2.25 - 2.72 | **<0.01** | 1.68 | 1.45 - 1.73 |
| LDL | **<0.01** | 1.58 | 1.46 - 1.70 | **<0.01** | 0.30 | 0.12 - 0.76 |
| HDL-C | **<0.01** | 0.06 | 0.05 - 0.07 | **<0.01** | 0.14 | 0.10 - 0.20 |
| Triglycerides | **<0.01** | 7.55 | 6.46 - 8.82 | **<0.01** | 2.36 | 1.58 - 3.52 |
| Total Cholesterol | **<0.01** | 1.40 | 1.31 - 1.50 | | | |
| Non-HDL | **<0.01** | 1.99 | 1.85 - 2.14 | **0.03** | 3.04 | 1.15 – 8.00 |
| TG/HDL-C | **<0.01** | 1.10 | 1.03 - 1.19 | | | |
| Systolic BP | **<0.01** | 1.08 | 1.08 - 1.09 | **<0.01** | 1.06 | 1.05 - 1.08 |
| Diastolic BP | **<0.01** | 1.08 | 1.07 - 1.09 | | | |
| BMI | **<0.01** | 1.18 | 1.16 - 1.20 | | | |
| Body Weight | **<0.01** | 1.05 | 1.05 - 1.06 | | | |
| Waist Circumference | **<0.01** | 1.08 | 1.08 - 1.09 | **<0.01** | 1.04 | 1.03 - 1.05 |
| Gender (F) | **<0.01** | 0.58 | 0.51 - 0.67 | **<0.01** | 2.72 | 2.14 - 3.48 |
| Age | **<0.01** | 1.06 | 1.05 - 1.06 | | | |
| Years of Smoking | **<0.01** | 1.02 | 1.02 - 1.03 | | | |
| Beer | **0.04** | 1.16 | 1.01 - 1.33 | | | |
| White wine | **0.01** | 0.82 | 0.71 - 0.94 | | | |
| Rose Wine | **<0.01** | 0.70 | 0.61 - 0.81 | | | |
| Hypertension H/O | **<0.01** | 5.47 | 4.68 - 6.39 | **<0.01** | 1.91 | 1.36 - 2.68 |
| MI H/O | **<0.01** | 3.38 | 2.20 - 5.19 | | | |
| CHD H/O | **<0.01** | 2.62 | 1.82 - 3.79 | | | |
| Hyperlipideamia H/O | **<0.01** | 2.20 | 1.88 - 2.58 | | | |
| H/O statin treatment | **<0.01** | 2.71 | 2.08 – 3.53 | | | |

*H/O – history of; MI – Myocardial infarction; CHD – Coronary heart disease*

Table 3.77. Independent associations for having the metabolic syndrome among the adult population in Malta

The independent associated factors for having MetS in the Maltese population were: fasting plasma glucose (FPG); low-density lipoprotein (LDL-C); high-density lipoprotein (HDL-C); triglycerides (TG), non-HDL; systolic blood pressure, diastolic blood pressure; waist circumference, female gender; age; history of hypertension; history of myocardial infarction (MI H/O) and history of hyperlipidemia. These associated factors correspond to the different components that define the metabolic syndrome (Alberti, Zimmet and Shaw, 2006).

## 3.4 Diabetes risk score

This section explored the formulation of a non-invasive predictive diabetes risk model for easy utilization in the Maltese primary health care setting as well as establishing the Malta-specific epidemiological risk factors among adults. Various non-invasive measurements that are easily accessible during a consultation were considered, as show in Table 3.78.

| **Variables** | **Crude model - GLM** | | | **Adjusted - GLM** | | |
|---|---|---|---|---|---|---|
| | *p*-value | OR | 95CI% | *p*-value | OR | 95CI% |
| Gender (F) | **<0.01** | 0.47 | 0.32 -0.70 | **<0.01** | 0.52 | 0.37 - 0.73 |
| Age | **<0.01** | 1.06 | 1.04 - 1.08 | **<0.01** | 1.06 | 1.04 - 1.08 |
| Systolic blood pressure | **<0.01** | 1.02 | 1.01 - 1.03 | **<0.01** | 1.02 | 1.01 - 1.03 |
| Diastolic blood pressure | **<0.01** | 0.97 | 0.96 - 0.99 | **<0.01** | 0.97 | 0.95 - 0.99 |
| Waist circumference | **0.03** | 1.04 | 1.00 - 1.35 | **<0.01** | 1.03 | 1.02 - 1.04 |
| Body mass index (BMI) | 0.15 | 1.01 | 1.00 - 1.03 | | | |
| H/O Smoking * (in years) | 0.24 | 1.01 | 1.00 - 1.02 | | | |
| H/O Coronary Heart Disease * | 0.79 | 0.90 | 0.40 – 2.00 | | | |
| H/O Hypertension * | **<0.01** | 2.27 | 1.58 - 3.24 | **<0.01** | 2.27 | 1.59 - 3.25 |
| H/O Dyslipidaemia * | 0.74 | 0.91 | 0.53 - 1.57 | | | |
| H/O Depression * | 0.85 | 1.05 | 0.645- 1.70 | | | |
| No *Statin* intake | **<0.01** | 0.27 | 0.15 - 0.49 | **<0.01** | 0.30 | 0.21 - 0.44 |

Dependent variable – T2DM
**H/O - history of*
OR – Odd's ratio

Table 3.78 Crude and adjusted variables associated with the onset of diabetes mellitus

The independent associations with type 2 diabetes mellitus were identified after conducting adjusted generalized linear models (GLM) with binary regressions analysis. The independent associated factors were: gender; age; systolic blood pressure, diastolic blood pressure; waist circumference; history of hypertension and history of statin intake.

Considering the continuous variables (systolic blood pressure, diastolic blood pressure, age and waist circumference), receiver-operating characteristic (ROC) curves were constructed, as shown in Figure 3.85, while Table 3.79 illustrates the measured area under the curve values for each variable.

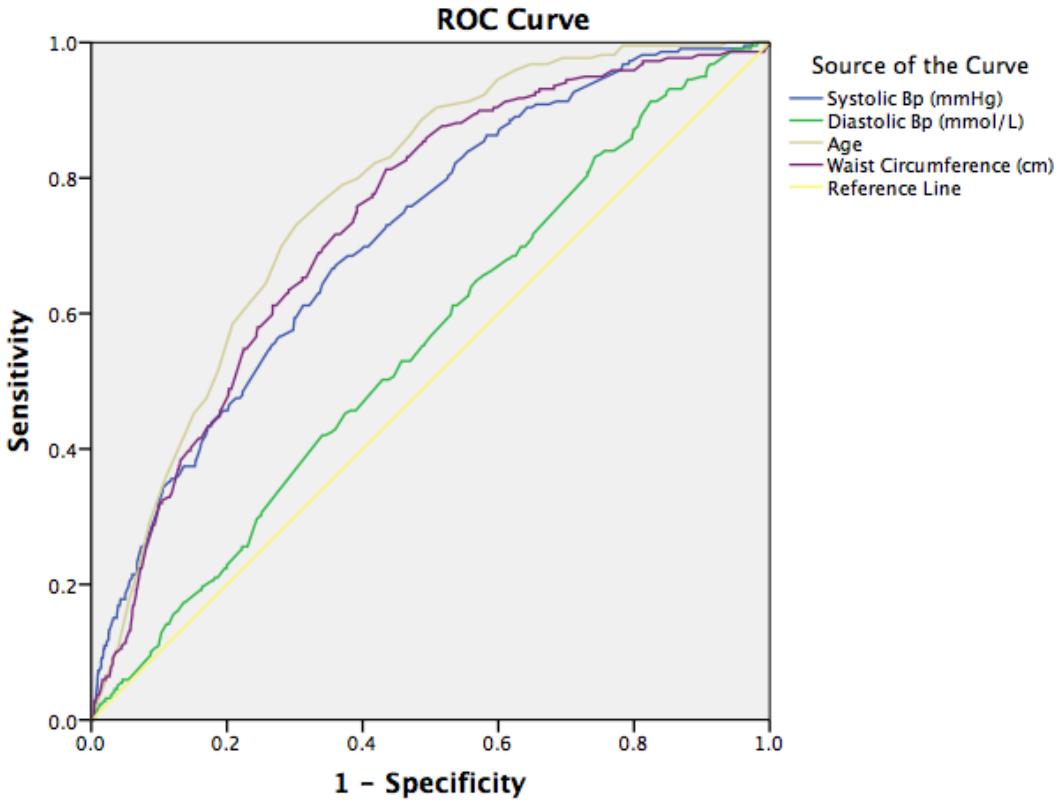



Figure 3.85 Demonstrates the ROC curves for the significant continuous variables for having diabetes mellitus

| Variable | AUC | *p*-value | CI 95% |
|---|---|---|---|
| Age | 0.77 | **<0.01** | 0.74 - 0.80 |
| Waist Circumference | 0.74 | **<0.01** | 0.70 - 0.77 |
| Systolic blood pressure | 0.71 | **<0.01** | 0.68 - 0.75 |
| Diastolic blood pressure | 0.55 | **0.01** | 0.52 - 0.59 |

Table 3.79 Demonstrates the area under the curve (AUC) for each significant continuous variable for having diabetes mellitus

The optimal sensitivities and specificities (best top left point of each ROC curve) for every variable were identified and the SAHHTEK specific cut-off points were established, as seen in Table 3.80.

| Variable | Sensitivity (%) | Specificity (%) | Cut-off point |
|---|---|---|---|
| Age | 76.30 | 66.30 | 55 years |
| Waist Circumference | 71.70 | 63.90 | 95cm |
| Systolic blood pressure | 64.40 | 65.90 | 125mmHg |
| Diastolic blood pressure | 56.60 | 50.00 | 75mmHg |

Table 3.80 Demonstrates the sensitivity, specificity and associated cut-off point for each significant continuous variable for having diabetes mellitus

According to the literature, waist circumference is gender specific, with the European male cut off point at 94cm and the European female cut off point at 80cm (Okosun *et al.*, 1998; World Health Organization, 2008b). Gender stratified ROC curves were created to identify the sensitivity and specificity along with the optimal cut-off points for the waist circumference, by gender for the Maltese diabetes population, as seen in Figure 3.86 and Figure 3.87.

The Malta male waist circumference cut-off point was 100cm with a sensitivity of 64.50% and a specificity of 63.50% (AUC: 0.67, CI 95: 0.63–0.72, $p$=<0.01). The Malta female waist circumference cut-off point was 90cm with a sensitivity of 77.80% and specificity of 65.10% (AUC: 0.76, CI 95%: 0.71 – 0.81, $p$=<0.01).

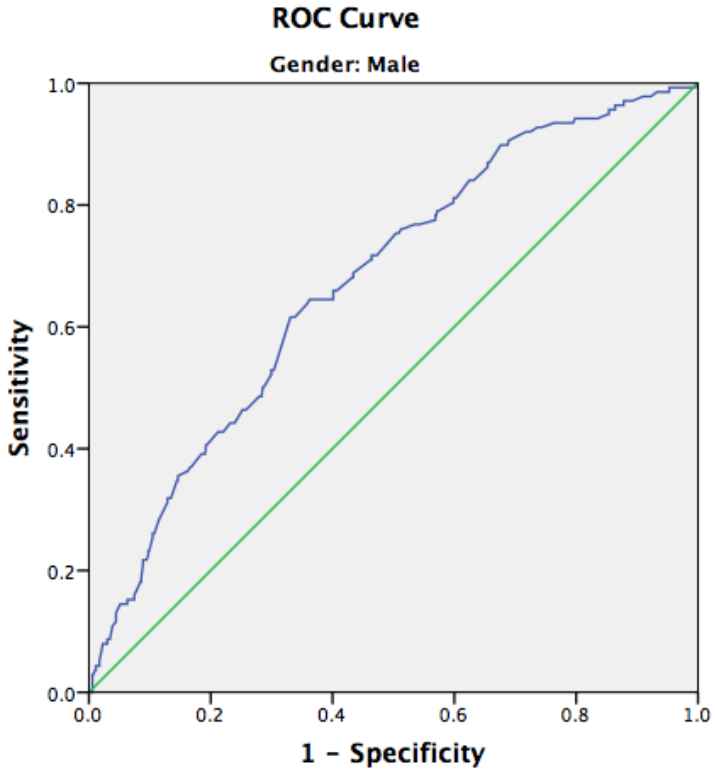



Figure 3.86 Demonstrates the waist circumference ROC curve specific for the male population at risk of T2DM

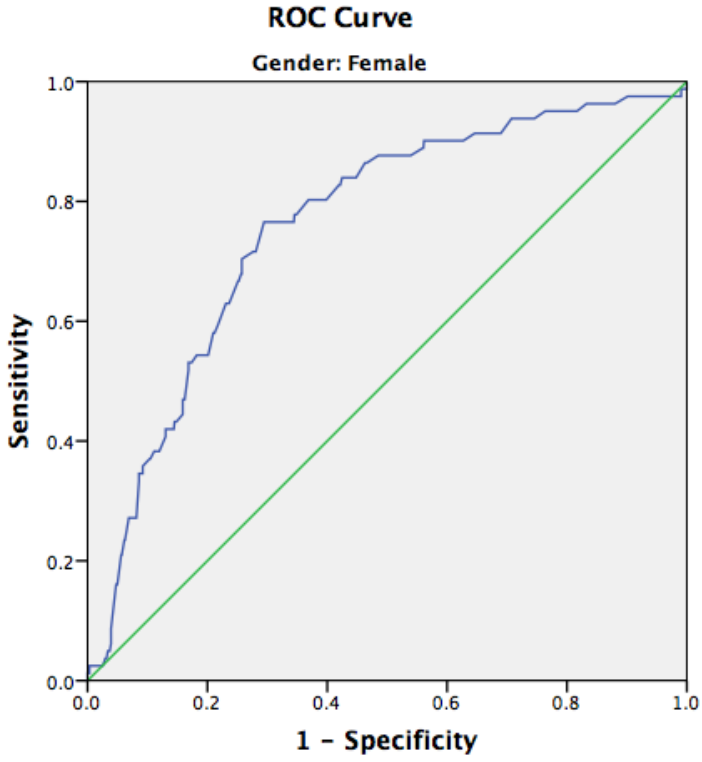



Figure 3.87 Demonstrates the waist circumference ROC curve specific for the female population at risk of T2DM

Conversion of the continuous variables with their identified optimal cut-off points into categorical variables was performed using IBM SPSS software. This was followed by ROC curve analyses for all (significant) categorical variables as seen in Figure 3.88, while Table 3.81 illustrates the AUC for each test variable.

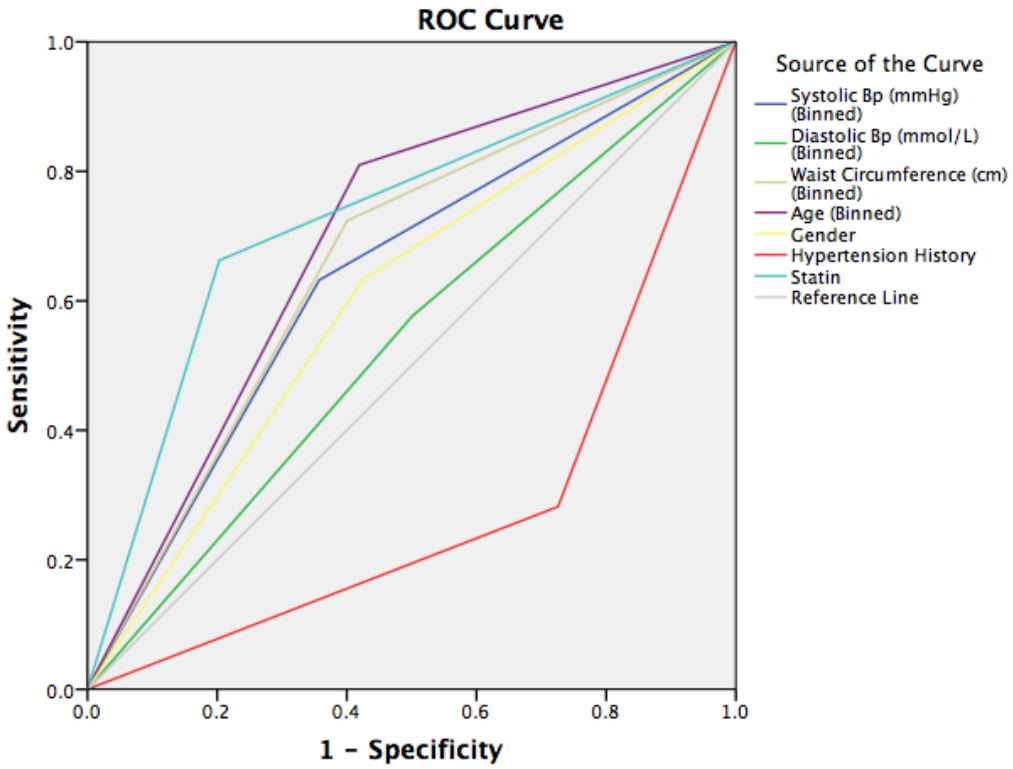



Figure 3.88 Demonstrates the ROC curves for the significant categorical variables for having diabetes mellitus

| Variable | AUC | *p*-value | CI 95% |
|---|---|---|---|
| Age category (>=55 vs. < 54 years) | 0.71 | **<0.01** | 0.68 - 0.75 |
| Statin use (Yes vs. No) | 0.70 | **<0.01** | 0.65 - 0.74 |
| Waist circumference category* | 0.66 | **<0.01** | 0.62 - 0.70 |
| Systolic blood pressure category (>=125 vs. <124 mmHg) | 0.66 | **<0.01** | 0.63 - 0.69 |
| Gender (F vs. M) | 0.60 | **<0.01** | 0.56 - 0.64 |
| Diastolic blood pressure category (>=75 vs. <74 mmHg) | 0.53 | 0.10 | 0.49 - 0.58 |
| Hypertension history (Yes vs. No) | 0.29 | **<0.01** | 0.25 - 0.33 |

* Waist circumference: Males >=100cm vs. <99.99cm; Female >=90cm vs. <=89.99cm

Table 3.81 Demonstrates the AUC for each categorical variable

A history of hypertension (as seen in ROC curve in Figure 3.88) and the mean diastolic blood pressure (as seen in Table 3.81) were both not found to be significant predictors of having type 2 diabetes mellitus. The remaining significant variables were individually, as well as in combination, re-analysed using ROC curves analyses to identify the best predictive diabetes model as seen in Table 3.82 and Figure 3.89. The order of combinations was dependent on the AUC of each variable. The following are the different combinations considered:

- Age + statin use
- Age + statin use + waist circumference*
- Age + statin use + waist circumference* + systolic blood pressure
- Age + statin use + waist circumference* + systolic blood pressure + gender

* Waist circumference: Males >=100cm vs. <99.99cm; Female >=90cm vs. <=89.99cm

| Variable | AUC | *p*-value | CI 95% |
|---|---|---|---|
| Age + Statin use + Waist Circumference* + Systolic blood pressure | 0.82 | <0.01 | 0.80 - 0.85 |
| Age + Statin use + Waist Circumference* | 0.82 | <0.01 | 0.79 - 0.85 |
| Age + Statin use+ Waist Circumference* + Systolic blood pressure + Gender | 0.78 | <0.01 | 0.75 - 0.82 |
| Age + Statin use | 0.78 | <0.01 | 0.75 - 0.82 |
| Age category (>=55 and <54 years) | 0.71 | <0.01 | 0.62 - 0.70 |
| Statin use | 0.70 | <0.01 | 0.65 - 0.74 |
| Waist Circumference category* | 0.66 | <0.01 | 0.62 - 0.70 |
| Gender | 0.60 | <0.01 | 0.56 - 0.64 |

* Waist circumference: Males >=100cm vs. <99.99cm; Female >=90cm vs. <=89.99cm

Table 3.82 Demonstrates the different predictive variables AUC and the combination of these variables for having diabetes mellitus

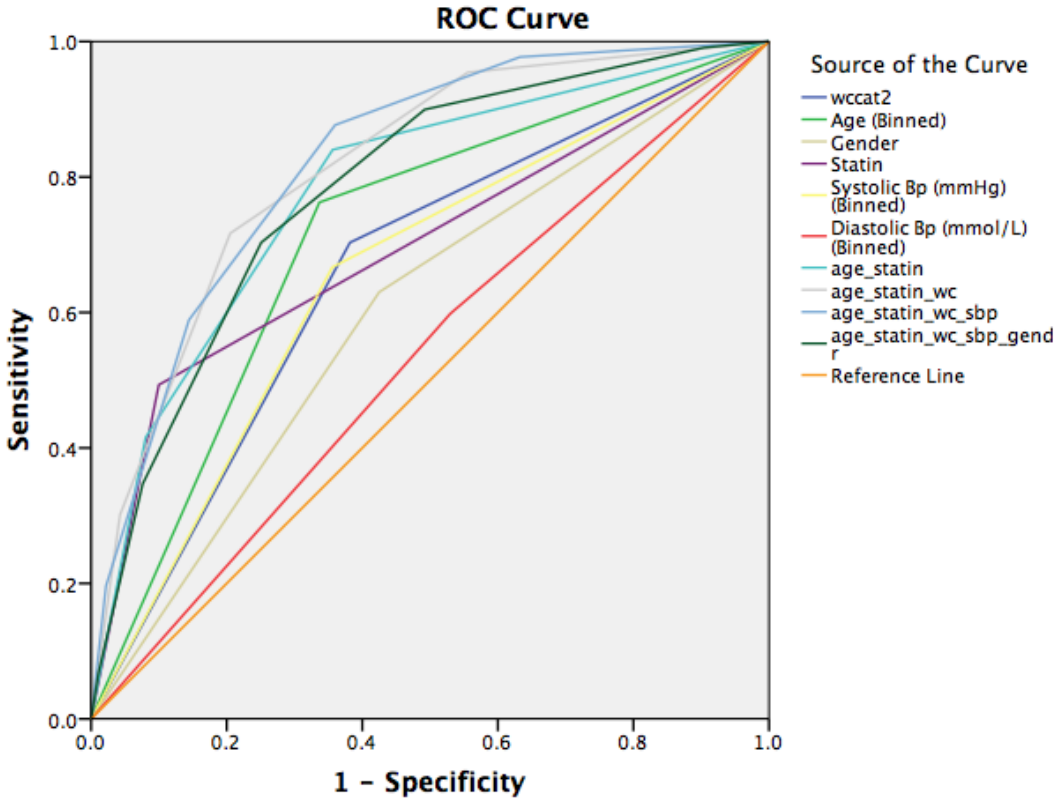



Figure 3.89 Demonstrates the ROC curves of the different individual predictive variables and the combination of variables for having diabetes mellitus

A combination of age, statin use, high waist circumference measurement (>=100cm for males and >=90cm for females) and high systolic blood pressure (>=125mmHg) was identified as the best predictive diabetes combined risk score (highest AUC) for the Malta study population. However, the combination of an increase in age (>=55 years), positive statin usage and a high waist circumference measurement (>=100cm for males and >=90cm for females) had an overlapping AUC value within the confidence intervals for the wider combination (as seen in Table 3.82). For this reason, the latter three-factor combination were selected to be the simplest as well as the most reliable predictive diabetes risk algorithm for Malta. The high diabetes risk score (high age + positive statin use + high

waist circumference) had a sensitivity of 71.70% and a specificity of 79.50% for having type 2 diabetes.

The three- factor combination was re-analysed but now using the combined cohort of persons without diabetes and those with newly diagnosed diabetes i.e. excluding those with known diabetes. Figure 3.90 illustrates the ROC curve established while Table 3.83 presents the AUC of the different predictive variables and the combination of these variables for having diabetes mellitus.

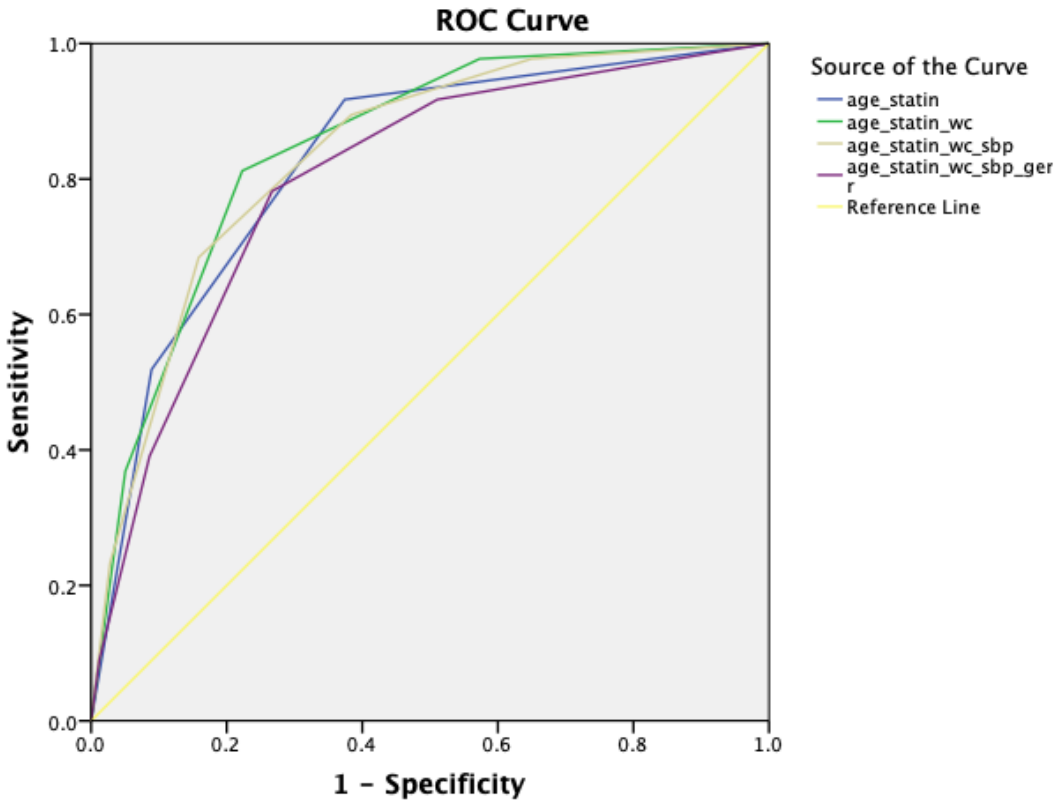



Figure 3.90 Demonstrates the ROC curves of the predictive variables for having diabetes mellitus within the combined cohort of persons without diabetes and those with newly diagnosed diabetes

| Variable | AUC | *p*-value | CI 95% |
|---|---|---|---|
| Age + statin | 0.83 | **<0.01** | 0.79 - 0.86 |
| Age + statin + waist circumference* | 0.85 | **<0.01** | 0.82 - 0.88 |
| Age + statin + waist circumference* + Systolic Bp | 0.84 | **<0.01** | 0.80 - 0.87 |
| Age + statin + waist circumference* + Systolic Bp + Gender | 0.80 | **<0.01** | 0.76 - 0.84 |

Bp – Blood pressure
* Waist circumference: Males >=100cm vs. <99.99cm; Female >=90cm vs. <=89.99cm

Figure 3.83Demonstrates the different predictive variables AUC and the combination of these variables for having diabetes mellitus

Out of all the different variable combinations, the best predictive combination was found to be 'Age + On statin + High waist circumference', with a sensitivity of 81.20% and specificity of 77.70%.

Table 3.84 illustrates the predictive risk score percentage for diabetes mellitus in the presence of each positive variable (age, statin use, waist circumference).

**Diabetes Risk Score for Malta**

| Number of positive variable/s* | Risk of diabetes mellitus |
|---|---|
| 0 | 1.40% |
| 1 | 8.30% |
| 2 | 25.40% |
| 3 | 48.50% |

*Positive variable/s were high age (1), positive statin use (2) and high waist circumference (3)
Table 3.84 Demonstrate the cumulative percentage predictive risk for diabetes in Malta.

### 3.4.1 Diabetes risk factors in the SAHHTEK population

The identified predictive variables for type 2 diabetes mellitus (high age, statin use, high specific waist circumference, systolic blood pressure and diastolic blood pressure) along with their optimal cut-off points, obtained from the ROC curves, were applied to the SAHHTEK adjusted (weighted) population.

It was observed that 32.13% (CI 95%: 30.69 – 33.60) of the SAHHTEK population (50.79% male, CI 95%: 48.04 – 53.53) were above the age of 55 years and automatically exhibited one positive diabetes risk variable predisposing them to 8.30% risk of diabetes. Among these, 23.82% (CI 95%: 21.55 – 26.24) were already diagnosed with diabetes mellitus (64.57% male CI 95%: 59.02 – 69.75).

Considering the Malta-specific waist circumference cut-off points, 38.79% (CI 95%: 36.68 – 40.95) of the male population exhibited a high waist circumference (>=100cm), while 36.33% (CI 95%: 34.22 – 38.49) of the female population also had an elevated waist circumference (>=90cm). Among these, 22.94% (CI 95%: 19.16 – 24.99) of the males and 14.97% (CI 95%: 12.53 – 17.80) of the females had a previous diagnosis of diabetes mellitus.

Comparing the literature-based waist circumference criteria cut-off points (Okosun *et al.*, 1998; World Health Organization, 2008b) to the cut-off points determined for the SAHHTEK population, a wide divergence between the two was observed, as seen in Table 3.85.

| Male - Waist Circumference | | | | |
|---|---|---|---|---|
| | Literature Cut-off | | SAHHTEK Cut-off | |
| | <= 93cm | >=94cm | <= 99cm | >=100cm |
| Male (*n*) | 857 | 1141 | 1223 | 775 |

| Female - Waist Circumference | | | | |
|---|---|---|---|---|
| | Literature Cut-off | | SAHHTEK Cut-off | |
| | <= 79cm | >=80cm | <= 89cm | >=90cm |
| Female (*n*) | 776 | 1173 | 1241 | 708 |

Table 3.85 Comparisons between literature-based and SAHHTEK specific cut-off points for waist circumference, by gender

As expected, when using the SAHHTEK cut-off points, both the male and female populations exhibited a smaller population proportion labelled as having a high waist circumference when compared to those in the literature (Okosun *et al.*, 1998; World Health Organization, 2008b).

A high systolic blood pressure measurement, although not considered as part of the final Maltese diabetes high risk score, still exhibited a high predictive ability. A systolic blood pressure of 125mmHg or above was found to be a risk factor for diabetes mellitus in the Maltese population. 36.61% (CI 95%: 35.12 – 38.13) of the general population (male: 59.31%, CI 95%: 56.75 – 61.81) had a systolic blood pressure above 125mmHg. Among these, 18.48% (CI 95%: 16.56 – 20.56) were previously diagnosed with diabetes mellitus.

On comparing the systolic blood pressure SAHHTEK cut-off points to the literature (American Diabetes Association, 2018a), a higher proportion of the population was found to be at high risk when using the SAHHTEK criteria, as seen in Table 3.86.

| | Systolic Blood Pressure | | | |
|---|---|---|---|---|
| | Literature Cut-off* | | SAHHTEK Cut-off | |
| | <=139mmHg | >=140mmHg | <=124mmHg | >=125mmHg |
| Population (*n*) | 2916 | 1031 | 2502 | 1445 |

** ADA 2018*

Table 3.86 Comparison of literature based systolic blood pressure cut-off points to the established SAHHTEK cut-off criteria

The non-diabetes SAHHTEK population (i.e. previously known + newly diagnosed diabetes population removed) was analysed in accordance with the established diabetes high risk score. This was performed to establish the proportion of the population (not already diabetes) at risk for diabetes mellitus. Table 3.87 exhibits the different diabetes predictor cut-off points, along with the predicted size of the '*at-risk population*', by gender for the non-diabetes population.

| Risk Factor | Female (*n*=1,813) | | Male (*n*=1,727) | | Total (*n*=3,540) | |
|---|---|---|---|---|---|---|
| | Population (%) | At risk for DM (in Persons) | Population (%) | At risk for DM (in Persons) | Population (%) | At risk for DM (in Persons) |
| Age >=55 years | 517 (28.52) | 43 | 449 (26.00) | 37 | 966 (27.29) | 80 |
| On Statins | 147 (8.11) | 12 | 139 (8.05) | 12 | 286 (8.08) | 24 |
| WC* | 602 (33.20) | 50 | 605 (35.03) | 50 | 1207 (34.10) | 100 |
| Age + On Statins | 127 (7) | 32 | 101 (5.85) | 26 | 228 (6.44) | 58 |
| Age + WC* | 293 (16.16) | 74 | 211 (12.22) | 54 | 504 (14.24) | 128 |
| On Statins + WC* | 101 (5.57) | 257 | 61 (3.52) | 15 | 162 (4.58) | 272 |
| Age + On Statins + WC* | 89 (4.91) | 43 | 50 (2.90) | 24 | 139 (3.93) | 67 |

Population refers to the adjusted (weighted) SAHHTEK study population
*WC = waist circumference; Females >=90cm, Males >=100cm

Table 3.87 Population distribution of the non-diabetes SAHHTEK population according to the diabetes predictors and the predictive risk, by gender

The commonest predictive risk factor for diabetes mellitus within the non-diabetes SAHHTEK population (for both males and females, $p$=0.68) was the presence of an elevated waist circumference. This was followed by age above 55 years. The female population exhibiting a significantly higher proportion within the >55 years age group than males ($p$=<0.01).

On utilising the three-risk factor predictive score (Age + statin use + WC), the non-diabetes females exhibited a statistically higher proportion at risk of diabetes mellitus than did the male population ($p$=<0.01).

### 3.4.2 Comparison of SAHHTEK diabetes risk score to International diabetes risk score

The SAHHTEK diabetes predicative risk score established in this study was compared to the FINDRISC diabetes risk score considering they followed a very similar study design (Lindström and Tuomilehto, 2003). This was done by incorporating the different FINDRISC variables to the current study's Maltese population (excluding those with previosuly known diabetes) and ultimately measuring the FINDRISC predictive risk score for the Maltese cohort. The predictive ability was assessed by comparing the ROC curve (Figure 3.91) and the AUC (Table 3.88) of the FINDRISC equation to the SAHHTEK ROC curve (Figure 3.90) and AUC (Table 3.83). The FINDRISC predictive equation within the Maltese population had a sensitivity of 35.50% and specificity of 36.89% for predicting type 2 diabetes.

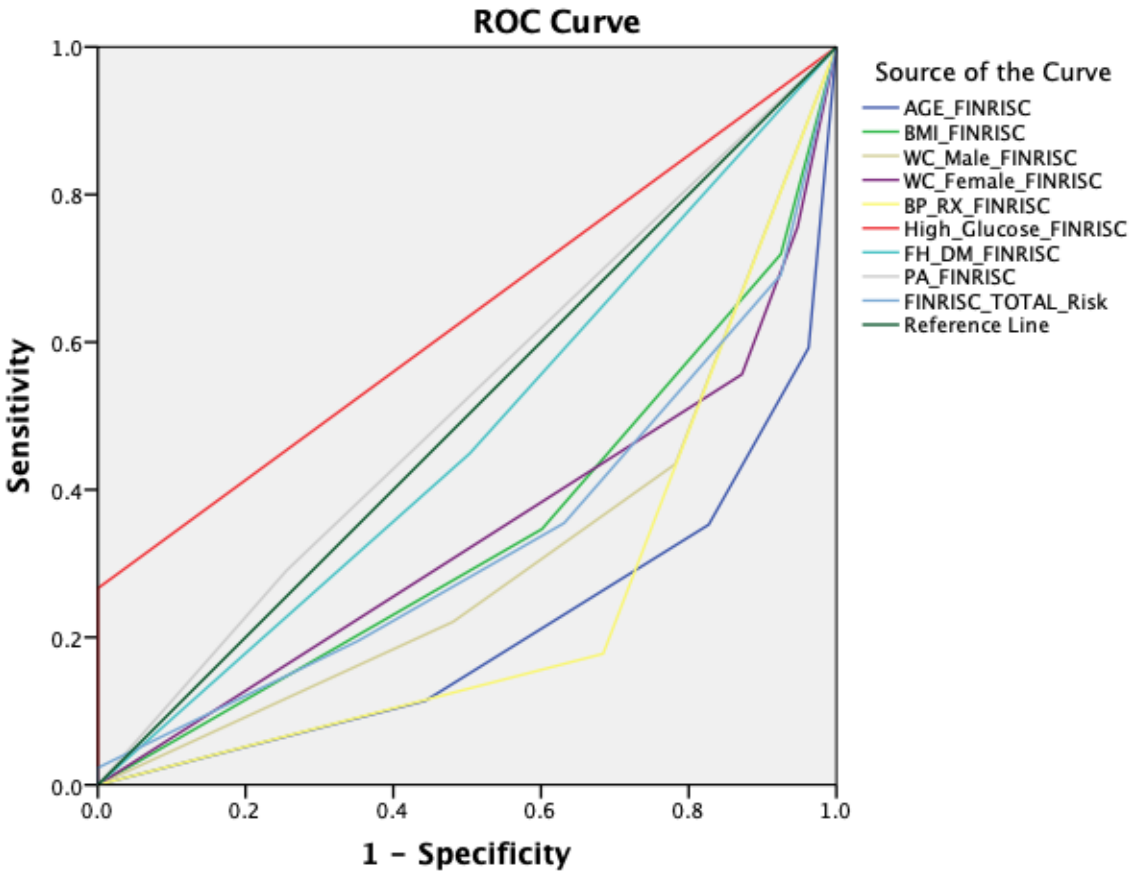



Figure 3.91 Demonstrates the ROC curves of the predictive variables for having diabetes mellitus within the combined cohort of persons without diabetes and those with newly diagnosed diabetes using the FINDRISC variables and equation

| Variable | AUC | *p*-value | CI 95% |
|---|---|---|---|
| Age | 0.21 | **<0.01** | 0.17 - 0.24 |
| BMI | 0.34 | **<0.01** | 0.30 - 0.39 |
| Waist Circumference (Males) | 0.31 | **<0.01** | 0.26 - 0.35 |
| Waist Circumference (Females) | 0.34 | **<0.01** | 0.30 - 0.38 |
| Antihypertensive treatment | 0.25 | **<0.01** | 0.20 - 0.29 |
| History of dysglycaemia | 0.63 | **<0.01** | 0.59 - 0.67 |
| Family history of T2DM | 0.47 | 0.30 | 0.42 - 0.52 |
| Physical activity for at least 30 minutes | 0.52 | 0.50 | 0.47 - 0.57 |
| FINDRISC equation | 0.33 | **<0.01** | 0.29 - 0.37 |

Table 3.88 Demonstrates the different predictive variables AUC and the FINDRISC equation for having diabetes mellitus within the Maltese SAHHTEK study population

The SAHHTEK Maltese specific predictive risk equation (age + statin use + high waist circumference) was observed to have a better predictive ability for T2DM since it was established to have a better AUC value, sensitivity and specificity as compared to the FINDRISC equation within the Maltese population.

## 3.5 Economic burden

This section describes the estimated economic burden of having type 2 diabetes mellitus (T2DM) and obesity in Malta for the year 2016 (year of data collection completion). It also describes the projection for the total population prevalence of T2DM and obesity in Malta for the year 2050. Finally, the estimated projected economic burden of T2DM and obesity for the year 2050 was calculated.

### 3.5.1 Diabetes mellitus and obesity population burden

The established SAHHTEK prevalence rates (including their corresponding confidence intervals) for both diabetes (previously and newly diagnosed) and for obesity were applied to the Maltese population (National Statistics Office 2015). This resulted in an estimate for the 2016 total adult population burden of diabetes mellitus and obesity, by 10-year age groups and by gender. Table 3.89 shows the estimated total adult Maltese diabetes population by gender and age.

| Age | Total Maltese Population by age* | Maltese Population Diabetes Prevalence | | |
|---|---|---|---|---|
| | | **Total (%)** | **Previously Known [%]** | **Unknown diabetes [%]** |
| 25 - 34 | 62,180 | 404 (0.65%) | | 404 (0.65%) |
| 35 - 44 | 56,575 | 2,489 (4.40%) | 933 (1.65%) | 1,556 (2.75%) |
| 45 - 54 | 55,113 | 5,054 (9.17%) | 2,712 (4.92%) | 2,337 (4.24%) |
| 55 - 64 | 59,268 | 11,611 (19.59%) | 6,638 (11.20%) | 4,976 (8.40%) |
| Total | 233,136 | 19,558 (8.39%) | 10,283 (4.41%) | 9,273 (3.98%) |
| Age | **Male Maltese Population by age*** | **Maltese Male Population Diabetes Prevalence** | | |
| | | **Total (%)** | **Previously Known [%]** | **Unknown diabetes [%]** |
| 25 - 34 | 32,359 | | | |
| 35 - 44 | 29,143 | 1,781 (6.11%) | 667 (2.29%) | 1,113 (3.82%) |
| 45 - 54 | 27,728 | 4,204 (15.16%) | 2,426 (8.75%) | 1,777 (6.41%) |
| 55 - 64 | 29,585 | 6,935 (23.44%) | 3,763 (12.72%) | 3,172 (10.72%) |
| Total | 118,815 | 12,920 (10.87%) | 6,856 (5.77%) | 6,062 (5.10%) |
| Age | **Female Maltese Population by age*** | **Maltese Female Population Diabetes Prevalence** | | |
| | | **Total (%)** | **Previously Known [%]** | **Unknown diabetes [%]** |
| 25 - 34 | 29,821 | 397 (1.33%) | | 397 (1.33%) |
| 35 - 44 | 27,432 | 658 (2.40%) | 247 (0.90%) | 411 (1.50%) |
| 45 - 54 | 27,385 | 1,060 (3.87%) | 424 (1.55%) | 630 (2.30%) |
| 55 - 64 | 29,683 | 4,625 (15.58%) | 2,853 (9.61%) | 1,772 (5.97%) |
| Total | 114,321 | 6,740 (5.89%) | 3,524 (3.08%) | 3,210 (2.81%) |

** 2013 Malta Demographic Report*

Table 3.89 Estimation of the population burden of diabetes mellitus by age and gender

An estimated 19,558 (CI 95%: 15,967 – 24,134) adults suffered from diabetes mellitus with a male predominance. Of these 9,273 (CI 95%: 6,716 – 12,911) adults were not aware of their disease, with a male majority (6,062 males CI 95%: 4,216 – 8,661).

Obesity was found to affect 82,066 (CI 95%: 74,397 – 90,076) adult Maltese in the 25 to 64-year age category (35% of the total adult population in this age group). Out of which there were 46,220 (CI 95%: 40,621 – 52,075) males found to be obese and 35,786 (CI 95%: 30,791 – 41,234) females found to be obese. Table 3.90 illustrates the obesity burden in the Maltese islands by age and gender

| Age | Total Maltese Population by age* | Obese BMI (%) |
|---|---|---|
| 25 - 34 | 62,180 | 15,968 (25.68%) |
| 35 - 44 | 56,575 | 18,523 (32.74%) |
| 45 - 54 | 55,113 | 22,315 (40.49%) |
| 55 - 64 | 59,268 | 25,260 (42.62%) |
| Total | 233,136 | 82,066 (35.20%) |

| Age | Male Maltese Population by age* | Obese BMI (%) |
|---|---|---|
| 25 - 34 | 32,359 | 9,569 (29.57%) |
| 35 - 44 | 29,143 | 12,310 (31.45%) |
| 45 - 54 | 27,728 | 12,611 (45.48%) |
| 55 - 64 | 29,585 | 11,730 (39.65%) |
| Total | 155,996 | 46,220 (29.63%) |

| Age | Female Maltese Population by age* | Obese BMI (%) |
|---|---|---|
| 25 - 34 | 29,821 | 6,423 (21.54%) |
| 35 - 44 | 27,432 | 5,914 (21.56%) |
| 45 - 54 | 27,385 | 9,881 (36.08%) |
| 55 - 64 | 29,683 | 13,568 (45.71%) |
| Total | 114,321 | 35,786 (31.30%) |

* 2013 Malta Demographic Report

Table 3.90 Estimation of the population burden of obesity, by age and gender

### 3.5.2 Cost burden of diabetes mellitus

The cost burden incorporating both direct and indirect costs was estimated for new or *undiagnosed diabetes*. The direct medical costs included hospital inpatient costs, physician care, emergency care, outpatients care and pharmaceutical prescriptions. The indirect costs included absence from work, reduced work performance and productivity (Zhang *et al.*, 2009). Considering that the average cost per person (as described in section 2.8.6) was calculated to be approximately €1,052 per person per year (6.67% of the annual mean salary income per person in Malta), the annual burden for the entire population (25 to 64 years) was estimated at €9,755,196 (CI 95%: €7,065,232 – €13,582,372). This contributed to 1.22% (CI 95%: 0.88% - 1.70%) of the total health expenditure (€800,000,000) for Malta for 2016 (Zhang *et al.*, 2009; Mundi., 2016).

The cost burden of known diabetes (as described in section 2.8.6) had been estimated at approximate €1,887 per person annually (11.96% of the annual mean salary income per person in Malta). This amounted to an annual health burden of €19,404,021 (€14,929,944 - €25,336,749) for the entire population (25 to 64 years) (International Diabetes Federation, 2016). This amounts to 2.43% (1.87% - 3.17%) of the Maltese total health expenditure (€800,000,000) (Mundi., 2016).

The Maltese global cost burden for diabetes mellitus (combination of newly diagnosed and previously diagnosed) was estimated at €29,159,217 (CI 95%: €21,994,676 - €38,919,121) for the year 2016. This cost burden contributed to 3.65% (CI 95%: 2.79% - 4.87%) of the total health expenditure (state and private expenditure) for Malta (Mundi., 2016).

### 3.5.3 Cost burden of obesity

The cost burden for obesity (including inpatient stay, day patient stay, general practitioner and specialist consultations but excluding medication and surgical procedures) was estimated to be €23,732,781 (CI 95%: €21,514,972 - €26,049,204) for the year 2016 after incorporating 2% compound interest per annum (Calleja, N; Gauci, 2009). This cost burden contributed to 2.97% (CI 95%: 2.69% - 3.26%) of the total health expenditure for Malta (Mundi., 2016).

### 3.5.4 Total cost burden of 'Diabesity'

The Maltese estimated cost burden for both diabetes mellitus and obesity (Diabesity) for the year 2016 was €52,891,998 (CI 95%: €43,509,648 - €64,968,325) which was equivalent to 6.61% (CI 95%: 5.44% - 8.12%) of Malta's total health expenditure (Mundi., 2016).

### 3.5.5 Projected diabetes and obesity population for the year 2050

The prevalence rates for both diabetes and obesity for those between the ages of 25 to 64 years in Malta were projected for the year 2050. These rates were applied to the EUROSTAT projected 2050 total Maltese population (25 to 64 years) (Eurostat European Commission, 2016).

An estimated 25,071 adults (CI 95%: 21,929 – 27,943) would be suffering from diabetes mellitus in the year 2050, while an estimated 94,034 adults (CI 95%: 87,684 –

100,388) would suffer from obesity in the year 2050. Table 3.91 illustrates the projected prevalence rates for diabetes and obesity for the year 2050, by age groups.

| Age | 2050 Projected Total Maltese Population by age* | Projected Diabetes & Obesity Prevalence for 2050 | |
|---|---|---|---|
| | | Total Diabetes (CI) | Total Obese (CI) |
| 25 - 34 | 56,709 | 601 (255 - 913) | 21,810 (19,791 - 23,829) |
| 35 - 44 | 52,965 | 3,813 (2,961 - 4,666) | 21,175 (19,650 - 22,685) |
| 45 - 54 | 53,819 | 5,457 (4,698 - 6,211) | 22,970 (21,361 - 24,579) |
| 55 - 64 | 62,342 | 15,199 (14,014 - 16,153) | 28,079 (26,882 - 29,295) |
| Total | 225,835 | 25,071 (21,929 - 27,943) | 94,034 (87,684 - 100,388) |

*Demographic Data 2013
CI - 95% Confidence interval

Table 3.91 Demonstrates the projected prevalence rates of diabetes and obese population for the year 2050 in Malta

### 3.5.6 Projected diabetes and obesity cost burden for the year 2050

The projected prevalence rates for both diabetes mellitus and obesity (adults between 25 to 64 years) were applied to the cost burden for both diseases. A 2% compound interest per annum to the established cost burden for the year 2016 was performed. The estimated cost burden for diabetes for 2050 is expected to be €33,751,487 (CI 95%: €25,458,606 - €45,048,473), while for obesity, the cost burden for the year 2050 is expected to be €46,532,294 (CI 95%: 42,183,889 - €51,074,049).

The total adult population (25 to 64 years) projected for 2050 appears to be smaller than that for the examined 2016 adult population. Meanwhile, the diabetes and obese

population is expected to increase by 28% and 15% respectively by 2050. The economic burden is expected to increase proportionately to this increased disease burden.

An exponentiation of 1.2 in the diabetes cost burden and an exponentiation of 2 in obesity cost burden from the 2016 cost is expected to occur by the year 2050. In this way, the estimated total cost burden for the year 2050 for diabetes mellitus type 2 and obesity would amount to one-eighth of the 2016 total health expenditure (€800,000,000).

## 3.6 Genetic studies

This section will explore the effects of the selected ten single nucleotide polymorphisms (SNPs) from candidate genes on a dysglycaemic cohort, a metabolically abnormal cohort and a 100% metabolically healthy cohort. Genotyping was performed on all of the different cohorts (*a more detailed account found below*) followed by the exploration of genotype-phenotype associations of the selected SNPs on the measured biochemical and anthropometric parameters and type 2 diabetes mellitus diagnosis.

### 3.6.1 Characteristics of the cohort selected for the genetic analyses

A total of three hundred and forty-one (*n*=341) Maltese adults aged between 33 and 62 years were selected from the entire crude unadjusted dataset depending on their

glycaemic status and their metabolic status, as described in Chapter 2. The cohort selected for genetic analysis, which will be referred to as the 'genetic sub-cohort', was composed of diabetes and pre-diabetes cases (*n*=198), metabolically abnormal cases (*n*=79) and metabolically healthy cases (*n*=64), as seen in Figure 3.92. Genotyping was performed on all of these cases (*n*=341).

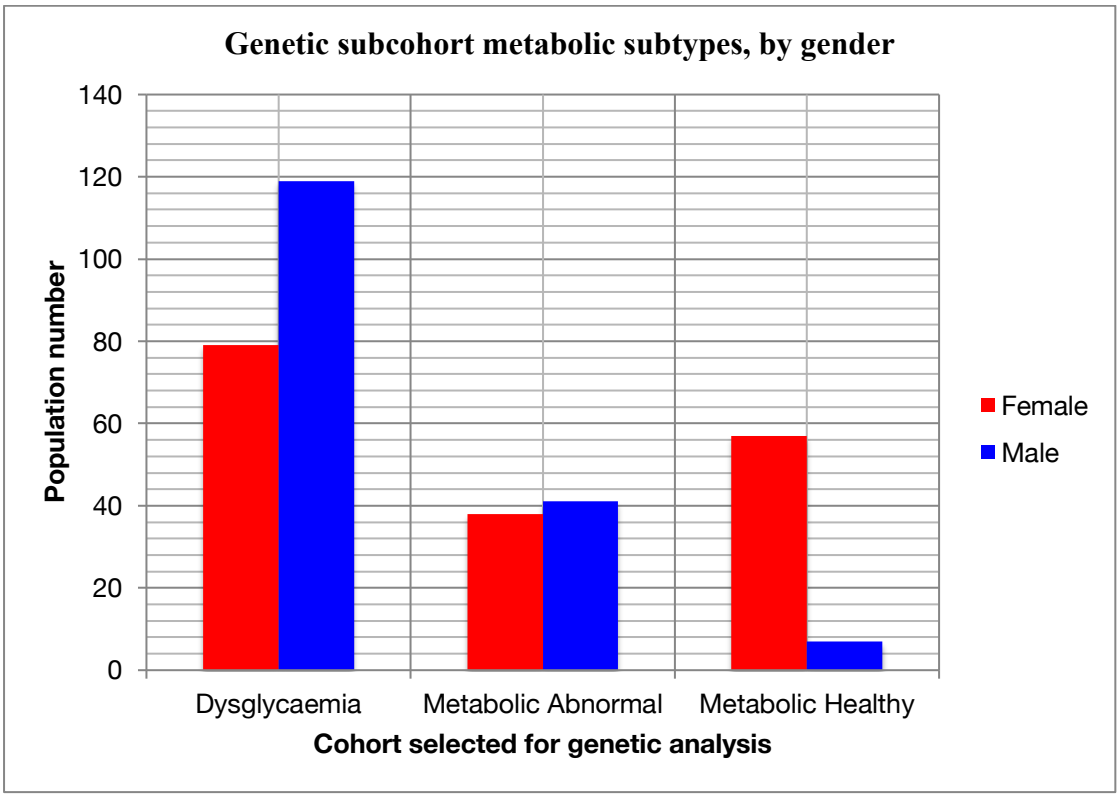



Figure 3.92 Distribution of the total cohort selected for genetic analysis by gender

### 3.6.1.1 The dysglycaemic cohort

The dysglycaemia cohort (*n*=198) was composed of a combination of participants with a diagnosis of type 2 diabetes and pre-diabetes between the ages of 33 to 62 years. The type 2 diabetes mellitus (T2DM) category was composed of previously

known diabetes (*n*=61) and newly diagnosed diabetes (*n*=53). The latter were diagnosed during the health examination survey. The pre-diabetes category was composed of 2 subcategories: impaired fasting plasma glucose – IFG (*n*=67) and impaired glucose tolerance – IGT (*n*=17), with both categories diagnosed during the health examination survey.

The majority of the previously known diabetes population reported a diagnosis of type 2 diabetes since less than 5 years, although 21.05% (CI 95%: 14.52 – 29.48) reported a diagnosis of diabetes for more than 30 years, as seen in Figure 3.93.

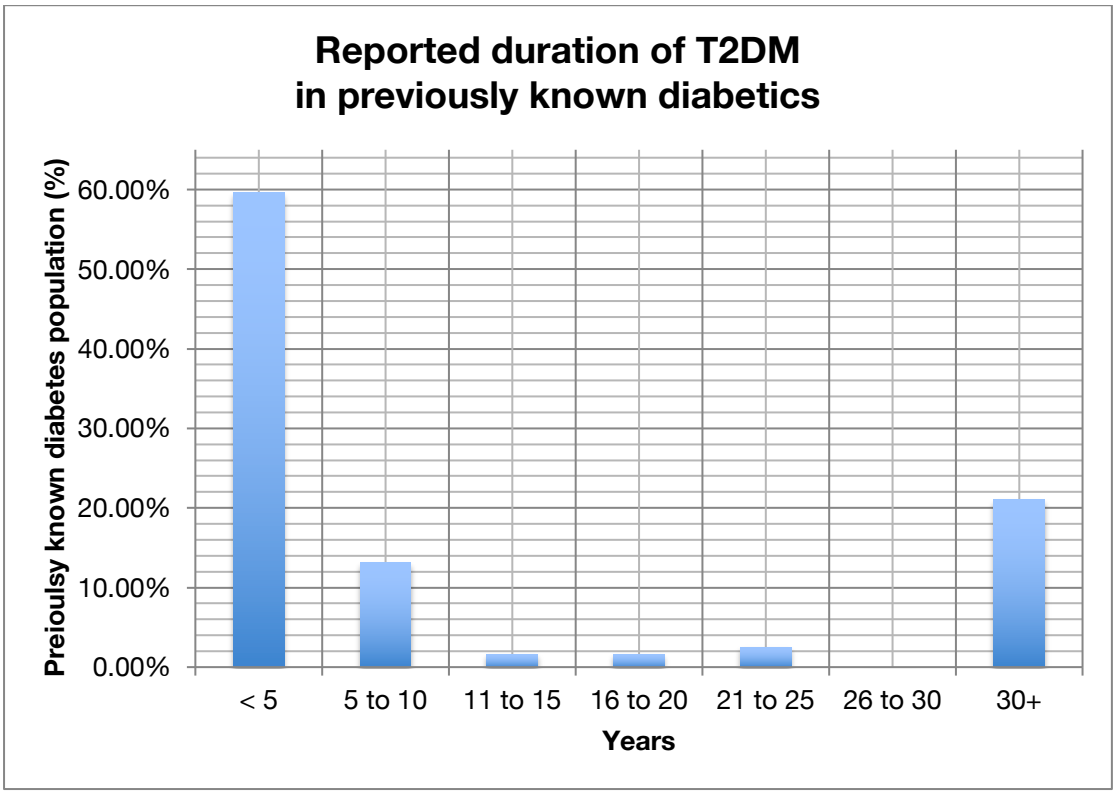



Figure 3.93 Reported duration of T2DM by the previously known diabetes sub-population

Of note, 45.45% (CI 38.67 – 52.41) of the dysglycaemic cohort (*n*=90) reported a family history of T2DM, among whom 56.67% (CI 95%: 46.36 – 66.43) were themselves diagnosed with T2DM. Additionally, 6.06% of the dysglycaemic cohort reported a family history of gestational diabetes.

#### 3.6.1.2 The metabolically abnormal cohort

This cohort was normoglycaemic with at least one other metabolically abnormal parameter (lipid profile, BMI, waist circumference, blood pressure) or having a history of any medical comorbidities or on medical treatment.

It was noteworthy that 37.97% (CI 95%: 28.05 – 49.01) of the 'metabolically abnormal' cohort reported a family history of T2DM, while 15.19% (CI 95%: 8.75% - 24.86) reported a family history of gestational diabetes.

#### 3.6.1.3 The metabolically healthy cohort

All normoglycaemic participants that did not exhibit any metabolic abnormality (FPG, Lipid profile, BMI, waist circumference, blood pressure) and did not report to be on any daily medication or have a medical history were considered as the 'metabolically healthy cohort'.

It was noteworthy that 46.27% (95% 34.86 – 58.09) of the metabolic healthy cohort reported a family history of T2DM, while 9.38% (CI 95%: 4.03 – 19.32) reported a family history of gestational diabetes.

### 3.6.2 Clinical and biochemical comparisons

Two case-control studies were planned to compare the (1) 'Dysglycaemic' cohort (Case) with the 'Metabolically healthy' cohort (Control) and to compare (2) the 'Metabolically abnormal' cohort (Case) with the 'Metabolically healthy' cohort (Control).

#### 3.6.2.1 Clinical and biochemical comparisons between the 'Dysglycaemic' (case) and 'Metabolically healthy' (control) cohorts

The clinical and biochemical parameters were found to be significantly higher within the 'dysglycaemic' cohort when compared to the 'metabolically healthy' cohort, as expected and seen in Table 3.92 and Table 3.93. It was noteworthy that 33.84 % (CI 95%: 27.60– 40.69) of the dysglycaemic cohort (Cases) reported to be on anti-hypertensive medications, which may have had an influence on the median blood pressure measurement of this subgroup unlike the control group, whom none reported to be on any medications.

| | **Case (*n*=198)** | **Control (*n*=64)** | |
|---|---|---|---|
| | Median (IQR) | Median (IQR) | *p*-value* |
| BMI (Kg/m$^2$) | 31.19 (8.20) | 22.68 (3.04) | **<0.01** |
| Systolic BP (mmHg) | 129 (18.00) | 109 (17.00) | **<0.01** |
| Diastolic BP (mmHg) | 78 (15.00) | 68 (10.00) | **<0.01** |
| Waist Circumference (cm) | 102 (16.80) | 72 (8.30) | **<0.01** |
| Waist-index | 1.13 (0.26) | 1.00 (0.31) | 0.83 |

*Mann-Whitney U test; IQR: Interquartile range

Table 3.92 Comparisons of clinical parameters between dysglycaemic cohort and the metabolically healthy cohort

| | **Case (*n*=198)** | **Control (*n*=64)** | |
|---|---|---|---|
| | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 6.71 (1.98) | 5.02 (0.52) | **<0.01** |
| LDL-C (mmol/L) | 3.21 (1.13) | 2.10 (0.48) | **<0.01** |
| HDL-C (mmol/l) | 1.26 (0.47) | 1.80 (0.48) | **<0.01** |
| Triglycerides (mmol/L) | 1.30 (0.77) | 0.56 (0.26) | **<0.01** |
| Total Cholesterol (mmol/L) | 5.16 (1.29) | 4.19 (0.53) | **<0.01** |

*Mann-Whitney U test; IQR: Interquartile range

Table 3.93 Comparisons of biochemical parameters between dysglycaemic cohort and metabolically healthy cohort

It was noteworthy that 23.74% (CI 95%: 18.33 – 30.15) of the dysglycaemic cohort (Cases) reported to be on statin medication, which may have influenced the lipid profile reported in this subgroup, unlike the control group, whom none reported to be on any medications.

On gender stratification, statistically significant differences in both the clinical and biochemical parameters were observed between the Cases and Controls, as seen in Table 3.94 and Table 3.95. Furthermore, statistical comparisons were performed between the females and males within the case and control cohorts respectively, as seen in Table 3.94 and Table 3.95.

The dysglycaemic females had significantly lower median diastolic blood pressure than the dysglycaemic males, which may be due to the fact that a higher female population (24.05% CI: 15.90 – 34.61) reported to be on anti-hypertensive medication as opposed to the males (22.69% CI 95%: 16.04 – 31.05). This may also hold true for the lower median lipid profile exhibited by the dysglycaemic females as opposed to the dysglycaemic males. While 17.72% (CI 95%: 10.73 – 27.70) of the dysglycaemic females were on statins, only 14.29% (CI 95%: 9.02 – 21.79) of dysglycaemic males were on statins.

| | **Case (*n*=198)** | | **Control (*n*=64)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female [*n*=79] Median (IQR) | Male [*n*=119] Median (IQR) | Female [*n*=57] Median (IQR) | Male [*n*=7] Median (IQR) | ***p*-value1** | ***p*-value2** | ***p*-value3** | ***p*-value4** |
| BMI (Kg/m$^2$) | 32.30 (9.54) | 30.50 (6.49) | 22.55 (2.96) | 23.70 (3.42) | **<0.01** | **<0.01** | 0.08 | 0.30 |
| Systolic BP (mmHg) | 127 (15.00) | 130 (19.00) | 110 (17.00) | 103 (17.00) | **<0.01** | **<0.01** | 0.48 | 0.51 |
| Diastolic BP (mmHg) | 75 (11.00) | 80 (15.00) | 68 (11.00) | 70 (7.00) | **<0.01** | **<0.01** | **0.02** | 0.83 |
| Waist Circumference (cm) | 97.50 (20.00) | 104.00 (16.50) | 72.00 (7.50) | 78.00 (11.00) | **<0.01** | **<0.01** | **<0.01** | **0.02** |
| Waist-index | 0.89 (0.27) | 1.09 (0.18) | 0.85 (0.25) | 1.06 (0.27) | 0.83 | 0.80 | 0.10 | 0.09 |

*p*-value1: Mann-Whitney U test Case Females vs. Control Females

*p*-value2: Mann-Whitney U test Case Males vs. Control Males

*p*-value3: Mann-Whitney U test Case Females vs. Case Males

*p*-value4: Mann-Whitney U test Control Females vs. Control Males

IQR: Interquartile range

Table 3.94 Clinical parameters comparisons between the dysglycaemic cohort and the metabolically healthy cohort by gender

| | **Case (*n*=198)** | | **Control (*n*=64)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female [*n*=79] Median (IQR) | Male [*n*=119] Median (IQR) | Female [*n*=57] Median (IQR) | Male [*n*=7] Median (IQR) | ***p*-value1** | ***p*-value2** | ***p*-value3** | ***p*-value4** |
| Fasting plasma glucose (mmol/L) | 6.81 (1.86) | 6.71 (2.42) | 5.04 (0.46) | 4.98 (0.62) | **<0.01** | **<0.01** | 0.32 | 0.61 |
| LDL-C (mmol/L) | 3.02 (1.32) | 3.29 (0.99) | 2.09 (0.47) | 2.18 (0.48) | **<0.01** | **<0.01** | **<0.01** | 0.46 |
| HDL-C (mmol/l) | 1.43 (0.54) | 1.17 (0.42) | 1.79 (0.51) | 1.80 (0.60) | **<0.01** | **<0.01** | **<0.01** | 0.50 |
| Triglycerides (mmol/L) | 1.18 (0.70) | 1.40 (0.87) | 0.56 (0.27) | 0.60 (0.26) | **<0.01** | **<0.01** | **0.02** | 0.71 |
| Total Cholesterol (mmol/L) | 5.13 (1.48) | 5.16 (1.35) | 4.19 (0.45) | 3.92 (0.85) | **<0.01** | **<0.01** | 0.42 | 0.72 |

*p*-value1: Mann-Whitney U test Case Females vs. Control Females

*p*-value2: Mann-Whitney U test Case Males vs. Control Males

*p*-value3: Mann-Whitney U test Case Females vs. Case Males

*p*-value4: Mann-Whitney U test Control Females vs. Control Males

IQR: Interquartile range

Table 3.95 Biochemical parameters comparisons between the dysglycaemic cohort and the metabolic healthy cohort by gender

### 3.6.2.2 Clinical and biochemical comparisons between the 'Metabolically abnormal' (case) and 'Metabolically healthy' (control) cohorts

The clinical and biochemical parameters were found to be significantly higher within the 'Case' sub-group when compared to the 'Control' subgroup, as seen in Table 3.96 and Table 3.97. The 'Case' cohort median FPG was within the normal range and exhibited no statistically significant difference (as expected) but other metabolic parameters including LDL-C, Total cholesterol, BMI and waist circumference were found to be significantly higher than the normal ranges respectively (as stated in section 2.3.7).

| | **Case (*n*=79)** | **Control (*n*=64)** | |
|---|---|---|---|
| | Median (IQR) | Median (IQR) | *p*-value* |
| BMI (Kg/m2) | 28.50 (7.90) | 22.68 (3.04) | **<0.01** |
| Systolic BP (mmHg) | 120 (18.00) | 109 (17.00) | **<0.01** |
| Diastolic BP (mmHg) | 75 (11.00) | 68 (10.00) | **<0.01** |
| Waist Circumference (cm) | 92.60 (21.60) | 72 (8.30) | **<0.01** |
| Waist-index | 1.02 (0.29) | 1.00 (0.32) | 0.89 |

*Mann-Whitney U test; IQR: Interquartile range

Table 3.96 Clinical parameters comparisons between the metabolically abnormal cohort and the metabolic healthy cohort

| | **Case (*n*=79)** | **Control (*n*=64)** | |
|---|---|---|---|
| | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.15 (0.40) | 5.02 (0.52) | 0.24 |
| LDL-C (mmol/L) | 3.34 (1.01) | 2.10 (0.48) | **<0.01** |
| HDL-C (mmol/l) | 1.42 (0.60) | 1.80 (0.48) | **<0.01** |
| Triglycerides (mmol/L) | 0.93 (0.57) | 0.56 (0.26) | **<0.01** |
| Total Cholesterol (mmol/L) | 5.39 (1.13) | 4.19 (0.53) | **<0.01** |

*Mann-Whitney U test; IQR: Interquartile range

Table 3.97 Biochemical parameters comparisons between the metabolically abnormal cohort and the metabolic healthy cohort

Statistical comparisons were performed between the females and males within the case and control cohorts respectively, as seen in Table 3.98 and Table 3.99. In both the Case (metabolically abnormal) and Control (metabolically healthy) cohorts, the females exhibited statistical significantly lower median waist circumference when compared to the male counterparts. This could be explained by the fact that the majority of the females in both cohorts were young (<50 years) and most likely pre-menopausal. This may have contributed to lower visceral fat accumulation and lower waist circumference (Donato *et al.*, 2006).

The metabolically abnormal females exhibited higher median HDL-C and lower median triglycerides levels than their male counterparts, which could be the result of medication. In fact, 26.32% (CI 95%: 14.81 – 42.17) of the metabolically abnormal female cohort reported to be on statin treatment, while 28.95% (CI 95%: 16.88 – 44.88) reported to be on anti-hypertensive medication. On the other hand, 12.20% (CI 95%: 4.86 – 26.01) of the metabolically abnormal male cohort reported to be on statin treatment, while 19.51% (CI 95%: 9.97 – 34.27) reported to be on anti-hypertensive medication.

| | **Case (*n*=79)** | | **Control (*n*=64)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female [*n*=38] Median (IQR) | Male [*n*=41] Median (IQR) | Female [*n*=57] Median (IQR) | Male [*n*=7] Median (IQR) | ***p*-value1** | ***p*-value2** | ***p*-value3** | ***p*-value4** |
| BMI (Kg/m2) | 27.70 (6.08) | 29.40 (7.20) | 22.55 (2.96) | 23.70 (3.42) | **<0.01** | **<0.01** | 0.14 | 0.30 |
| Systolic BP (mmHg) | 120 (14.00) | 120 (18.00) | 110 (17.00) | 103 (17.00) | **<0.01** | **<0.01** | 0.19 | 0.51 |
| Diastolic BP (mmHg) | 75 (10.00) | 74 (11.00) | 68 (11.00) | 70 (7.00) | **<0.01** | **0.02** | 0.81 | 0.83 |
| Waist Circumference (cm) | 88.00 (13.70) | 97.00 (20.60) | 72.00 (7.50) | 78.00 (11.00) | **<0.01** | **<0.01** | **<0.01** | **0.02** |
| Waist-index | 0.91 (0.23) | 1.09 (0.20) | 0.85 (0.25) | 1.06 (0.27) | 0.89 | 0.86 | 0.15 | 0.10 |

*p*-value1: Mann-Whitney U test Case Females vs. Control Females

*p*-value2: Mann-Whitney U test Case Males vs. Control Males

*p*-value3: Mann-Whitney U test Case Females vs. Case Males

*p*-value4: Mann-Whitney U test Control Females vs. Control Males

IQR: Interquartile range

Table 3.98 Clinical parameters comparisons between the metabolic abnormal cohort and the metabolic healthy cohort by gender

| | **Case (*n*=79)** | | **Control (*n*=64)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female [*n*=38] Median (IQR) | Male [*n*=41] Median (IQR) | Female [*n*=57] Median (IQR) | Male [*n*=7] Median (IQR) | ***p*-value1** | ***p*-value2** | ***p*-value3** | ***p*-value4** |
| Fasting plasma glucose (mmol/L) | 5.09 (0.48) | 5.18 (0.39) | 5.04 (0.46) | 4.98 (0.62) | 0.09 | 0.07 | 0.22 | 0.61 |
| LDL-C (mmol/L) | 3.18 (1.09) | 3.43 (0.87) | 2.09 (0.47) | 2.18 (0.48) | **<0.01** | **<0.01** | 0.34 | 0.46 |
| HDL-C (mmol/l) | 1.57 (0.69) | 1.29 (0.37) | 1.79 (0.51) | 1.80 (0.60) | **0.02** | **0.02** | **<0.01** | 0.50 |
| Triglycerides (mmol/L) | 0.87 (0.50) | 1.03 (0.57) | 0.56 (0.27) | 0.60 (0.26) | **<0.01** | **<0.01** | **0.05** | 0.71 |
| Total Cholesterol (mmol/L) | 5.20 (1.17) | 5.40 (0.87) | 4.19 (0.45) | 3.92 (0.85) | **<0.01** | **<0.01** | 0.82 | 0.72 |

*p*-value1: Mann-Whitney U test Case Females vs. Control Females

*p*-value2: Mann-Whitney U test Case Males vs. Control Males

*p*-value3: Mann-Whitney U test Case Females vs. Case Males

*p*-value4: Mann-Whitney U test Control Females vs. Control Males

IQR: Interquartile range

Table 3.99 Biochemical parameters comparisons between the metabolic abnormal cohort and the metabolic healthy cohort by gender

### 3.6.3 Frequencies of allele variants in dysglycaemic cohort (Case) and metabolically healthy cohort (Control)

This section consists of salient genotyping findings including: genotype counts, minor allele frequencies for each variant (in the dysglycaemic and metabolically health study cohorts); tests for deviation from Hardy-Weinberg equilibrium and the tests for association.

Table 3.100 presents the genotype counts, minor allele frequencies and tests for deviation from Hardy-Weinberg equilibrium in the dysglycaemic cohort (Case) compared to the metabolically healthy cohort (control). All SNPs were found to be in Hardy-Weinberg equilibrium except for the *FABP2* and *PPARGY* SNPs in the 'Case' cohort and the FTO and *PPARGY* SNPs in the 'Control' Cohort.

Table 3.101 presents the odds ratio with 95% confidence intervals and *p*-values from the case-control association of the genetic sub-cohort (dysglycaemia against metabolic healthy) using different models. No significant association was identified between each of the alleles under study when comparing the case and control cohorts.

| | | Dysglycaemic Cohort (Case) | | | | | Healthy Cohort (Control) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene / SNP** | **Allele** | **WT** | **HT** | **MT** | **HWE** | **MAF** | **WT** | **HT** | **MT** | **HWE** | **MAF** |
| *ADRB2* rs1042713 | G/**A** | 72 | 96 | 29 | $\chi2 = 0.11$, $p=0.74$ | 0.39 | 30 | 24 | 10 | $\chi2 = 0.82$, $p=0.18$ | 0.34 |
| *NOTCH2* rs10923931 | G/**T** | 150 | 45 | 2 | $\chi2 = 0.47$, $p=0.49$ | 0.12 | 51 | 12 | 1 | $\chi2 = 0.09$, $p=0.76$ | 0.11 |
| *CDKAL1* rs7754840 | G/**C** | 91 | 85 | 20 | $\chi2 = <0.01$, $p=0.98$ | 0.32 | 26 | 34 | 4 | $\chi2 = 2.69$, $p=0.10$ | 0.33 |
| *FABP2* rs1799883 | C/**T** | 84 | 97 | 13 | $\chi2 = 4.64$, ***p*= 0.03** | 0.32 | 28 | 28 | 7 | $\chi2 = 2.25$, $p=1.00$ | 0.33 |
| *FTO* rs9939609 | T/**A** | 72 | 94 | 31 | $\chi2 = <0.01$, $p=0.97$ | 0.40 | 22 | 33 | 2 | $\chi2 = 5.85$, ***p*=0.02** | 0.32 |
| *HHEX* rs1111875 | C/**T** | 30 | 92 | 75 | $\chi2 = 0.04$, $p=0.84$ | 0.61 | 8 | 38 | 18 | $\chi2 = 3.02$, $p=0.08$ | 0.58 |
| *KCNE4* rs1440072 | **C**/T | 124 | 61 | 10 | $\chi2 = 0.48$, $p=0.489$ | 0.21 | 38 | 23 | 2 | $\chi2 = 0.45$, $p=0.50$ | 0.21 |
| *PPARGY* rs1801282 | C/**G** | 139 | 20 | 39 | $\chi2 =105.17$ ***p*=<0.01** | 0.25 | 41 | 8 | 15 | $\chi2 = 31.41$, ***p*=<0.01** | 0.30 |
| *SLC30A8* rs13266634 | C/**T** | 121 | 67 | 6 | $\chi2 = 0.818$, $p=0.37$ | 0.20 | 34 | 23 | 2 | $\chi2 = 0.65$, $p=0.42$ | 0.23 |
| *TCF7L2* rs7903146 | C/**T** | 71 | 98 | 29 | $\chi2 = 0.26$, p=0.61 | 0.39 | 23 | 29 | 11 | $\chi2 = 0.13$, $p=0.72$ | 0.40 |

Observed Genotypes
WT=wild type
HT = Heterozygote
MT = Mutant
HWE = Hardy Weinberg Equation; $p \leq 0.05$ not in HWE
MAF = Minor Allele Frequency
Alleles in **bold** represent the minor allele
Risk allele for all SNPs was the same as the minor allele except for SLC30A8 and HHEX

Table 3.100 Allele frequencies in the dysglycaemic cohort (case) compared to the metabolic healthy cohort (control)

| | | | Armitage's trend test |
|---|---|---|---|
| **Gene / SNP** | **Recessive model [WT<>MT]** | **Dominant model [WT<>HT + MT]** | **Common OR** |
| *ADRB2* rs1042713 | 1.21 [0.52-2.77] *p*=0.66 | 1.53 [0.87-2.71] p=0.14 | 1.16 *p*=0.35 |
| *NOTCH2* rs10923931 | 0.68 [0.06-7.66] *p*=0.75 | 1.23 [0.62-2.45] *p*=0.56 | 1.11 *p*=0.65 |
| *CDKAL1* rs7754840 | 1.43 [0.45-4.55] *p*=0.54 | 0.79 [0.45-1.40] *p*=0.42 | 1.03 *p*=0.84 |
| *FABP2* rs1799883 | 0.62 [0.23-1.71] *p*=0.35 | 1.05 [0.59 - 1.86] *p*=0.87 | 0.87 *p*=0.72 |
| *FTO* rs9939609 | 1.05 [0.44-2.54] *p*=0.91 | 0.91 [0.53-1.64] *p*=0.75 | 1.01 *p*=0.96 |
| *HHEX* rs1111875 | 1.11 [0.44-2.83] *p*=0.83 | 0.80 [0.34-1.84] p=0.59 | 1.12 p=0.46 |
| *KCNE4* rs1440072 | 1.53 [0.32-7.30] *p*=0.59 | 0.87 [0.49-1.56] *p*=0.64 | 1.03 *p*=0.88 |
| *PPARGY* rs1801282 | 0.77 [0.39-1.53] p=0.45 | 0.76 [0.42-1.37] *p*=0.36 | 0.87 *p*=0.40 |
| *SLC30A8* rs13266634 | 0.84 [0.16-4.37] *p*=0.84 | 0.82 [0.45-1.48] *p*=0.51 | 0.86 *p*=0.54 |
| *TCF7L2* rs7903146 | 0.85 [0.37-1.98] *p*=0.71 | 1.03 [0.57-1.85] *p*=0.93 | 0.94 *p*=0.83 |

Table 3.101 Odds ratios, 95% confidence intervals and p-values for the dysglycaemic (case) – metabolic healthy (control) associations

### 3.6.4 Frequencies of allele variants in metabolically abnormal cohort (Case) and metabolically healthy cohort (Control)

This section consists of salient genotyping findings including: genotype counts, minor allele frequencies for each variant (in the metabolically abnormal and metabolically health study cohorts); tests for deviation from Hardy-Weinberg equilibrium and the tests for association.

Table 3.102 presents the genotype counts, minor allele frequency and tests for deviation from Hardy-Weinberg equilibrium in the metabolically abnormal cohort (Case) compared to the healthy cohort (control). All SNPs were found to be in Hardy-Weinberg equilibrium except for the *PPARGY* in the 'Case' cohort and the *FTO* and *PPARGY* SNPs in the 'Control' Cohort.

Table 3.103 presents the odds ratio with 95% confidence intervals and *p*-values from the case-control association of the genetic cohort (metabolically abnormal against metabolically healthy) using different models. No significant association was identified between each of the alleles under study when comparing the case and control cohorts.

| | | Metabolic Abnormal Cohort (Case) | | | | | Healthy Cohort (Control) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene / SNP** | **Allele** | **WT** | **HT** | **MT** | **HWE** | **MAF** | **WT** | **HT** | **MT** | **HWE** | **MAF** |
| *ADRB2* rs1042713 | G/**A** | 25 | 39 | 14 | $\chi2 = 0.03$, p=0.86 | 0.43 | 30 | 24 | 10 | $\chi2 = 0.82$, *p*=0.18 | 0.34 |
| *NOTCH2* rs10923931 | G/**T** | 60 | 18 | 0 | $\chi2 = 1.33$, p=0.25 | 0.12 | 51 | 12 | 1 | $\chi2 = 0.09$, *p*=0.76 | 0.11 |
| *CDKAL1* rs7754840 | G/**C** | 35 | 34 | 8 | $\chi2 = <0.01$, p=0.95 | 0.32 | 26 | 34 | 4 | $\chi2 = 2.69$, *p*=0.10 | 0.33 |
| *FABP2* rs1799883 | C/**T** | 32 | 35 | 11 | $\chi2 = 0.08$, p=0.77 | 0.37 | 28 | 28 | 7 | $\chi2 = 2.25$, *p*=1.00 | 0.33 |
| *FTO* rs9939609 | T/**A** | 23 | 40 | 10 | $\chi2 = 1.27$, p=0.26 | 0.41 | 22 | 33 | 2 | $\chi2 = 5.85$, ***p*=0.02** | 0.32 |
| *HHEX* rs1111875 | C/**T** | 13 | 44 | 22 | $\chi2 = 1.31$, p=0.25 | 0.56 | 8 | 38 | 18 | $\chi2 = 3.02$, *p*=0.08 | 0.58 |
| *KCNE4* rs1440072 | **C**/T | 53 | 24 | 0 | $\chi2 = 2.62$, p=0.11 | 0.16 | 38 | 23 | 2 | $\chi2 = 0.45$, *p*=0.50 | 0.21 |
| *PPARGY* rs1801282 | C/**G** | 51 | 9 | 19 | $\chi2 = 41.80$, **p=<0.01** | 0.30 | 41 | 8 | 15 | $\chi2 = 31.41$, ***p*=<0.01** | 0.30 |
| *SLC30A8* rs13266634 | C/**T** | 41 | 31 | 2 | $\chi2 = 1.90$, p=0.17 | 0.24 | 34 | 23 | 2 | $\chi2 = 0.65$, *p*=0.42 | 0.23 |
| *TCF7L2* rs7903146 | C/**T** | 34 | 32 | 13 | $\chi2 = 1.30$, p=0.25 | 0.37 | 23 | 29 | 11 | $\chi2 = 0.13$, *p*=0.72 | 0.40 |

Observed Genotypes
WT=wild type
HT = Heterozygote
MT = Mutant
HWE = Hardy Weinberg Equation; $p \leq 0.05$ not in HWE
MAF = Minor Allele
Frequency
Alleles in **bold** represent the
minor allele
Risk allele for all SNPs was the same as the minor allele except for SLC30A8 and HHEX

Table 3.102 Allele frequencies in the metabolically abnormal cohort (case) compared to the metabolically healthy cohort (control)

| | | | Armitage's trend test |
|---|---|---|---|
| **Gene / SNP** | **Recessive model [WT<>MT]** | **Dominant model [WT<>HT + MT]** | **Common OR** |
| *ADRB2* rs1042713 | 1.68 [0.64-4.43] *p*=0.29 | 1.87 [0.94-3.71] p=0.07 | 1.33 *p*=0.15 |
| *NOTCH2* rs10923931 | 0.28 [0.01-7.12] *p*=0.28 | 1.18 [0.53-2.63] *p*=0.69 | 1.15 *p*=0.87 |
| *CDKAL1* rs7754840 | 1.49 [0.40-5.47] *p*=0.55 | 0.82 [0.42 - 1.61] *p*=0.56 | 1.07 p=0.95 |
| *FABP2* rs1799883 | 1.38 [0.47-4.03] *p*=0.56 | 1.15 [0.59 - 2.25] *p*=0.68 | 1.16 *p*=0.58 |
| *FTO* rs9939609 | 1.06 [0.36 - 3.11] *p*=0.91 | 1.14 [0.56 - 2.33] *p*=0.72 | 1.05 *p*=0.82 |
| *HHEX* rs1111875 | 0.75 [0.26-2.21] *p*=0.60 | 0.73 [0.28 - 1.86] *p*=0.51 | 0.89 *p*=0.69 |
| *KCNE4* rs1440072 | 0.14 [0.01 - 3.08] p=0.10 | 0.69 [0.34 - 1.38] *p*=0.29 | 0.67 *p*=0.18 |
| *PPARGY* rs1801282 | 1.02 [0.46 - 2.25] *p*=0.96 | 0.97 [0.49 - 1.95] *p*=0.95 | 1.01 *p*=0.99 |
| *SLC30A8* rs13266634 | 0.83 [0.11-6.20] *p*=0.86 | 1.10 [0.55 - 2.18] *p*=0.80 | 1.02 *p*=0.87 |
| *TCF7L2* rs7903146 | 0.80 [0.31 - 2.09] *p*=0.65 | 0.76 [0.39 - 1.50] *p*=0.43 | 0.88 *p*=0.53 |

Table 3.103 Odds ratios, 95% confidence intervals and p-values for the metabolically abnormal (case) – metabolically healthy (control) associations

### 3.6.5 Genotype-phenotype associations

Generalised linear models were used to investigate the associations between each of the ten genetic variants and the corresponding clinical and biochemical parameters in the genetic sub-cohort. This statistical modelling technique enables the characterization of the effect size of each allele on the phenotypic characteristics of the study cohort. Each SNP was analysed in relation to the clinical and biochemical parameters separately using a co-dominant, recessive and dominant genetic model. For the co-dominant model, the wild type allele [WT] of each genotype was considered as the reference category. The recessive model followed the sequence: (AA [WT] +Aa [HT]) vs. aa [MT]; where 'a' is the risk allele; [MT] is the mutant genotype, [WT] is the wild type genotype and [HT] is the heterozygous genotype. The dominant model followed the sequence: AA [WT] vs. (Aa [HT] + aa [MT]).

#### 3.6.5.1 Adrenoceptor beta 2, *ADRB2* [rs1042713]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *ADRB2* locus, as seen in Table 3.104. However, the median BMI was found to be within the overweight range across the different genotypes. Furthermore, the median lipid profiles (especially LDL-C and total cholesterol) were on the high end of the normal range. Only a small proportion of persons within each genotype was on statins (G/G – 15.75% CI 95%: 10.35 – 23.15; A/G – 22.01% CI 95%: 16.24 – 29.10; A/A – 24.53% CI 95% 14.81 – 37.69).

| | *ADRB2* rs1042713 | | | |
|---|---|---|---|---|
| | **GG [WT] (*n*=127)** | **AG [HT] (*n*=159)** | **AA [MT] (*n*=53)** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.80 (01.74) | 5.88 (1.81) | 5.90 (1.88) | 0.82 |
| LDL-C (mmol/L) | 3.08 (1.33) | 3.07 (1.38) | 2.82 (1.28) | 0.70 |
| HDL-C (mmol/l) | 1.42 (0.57) | 1.39 (0.54) | 1.33 (0.56) | 0.55 |
| Triglycerides (mmol/L) | 0.97 (0.77) | 1.02 (0.76) | 1.21 (1.16) | 0.21 |
| Total Cholesterol (mmol/L) | 5.01 (1.32) | 5.05 (1.51) | 5.09 (1.35) | 0.89 |
| BMI ($Kg/m^2$) | 28.60 (10.81) | 29.45 (8.37) | 29.30 (7.73) | 0.58 |
| Systolic Blood pressure (mmHg) | 123.00 (20.00) | 123.00 (18.33) | 120.00 (13.67) | 0.86 |
| Diastolic Blood pressure (mmHg) | 74.00 (13.00) | 75.00 (10.30) | 75.00 (14.00) | 0.59 |
| Waist Circumference (cm) | 94.00 (32.00) | 97.00 (26.000) | 95.00 (18.50) | 0.37 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
A – risk allele

Table 3.104 Biochemical and anthropometric parameters in relation to *ADRB2* genotypes

#### 3.6.5.1.1 *ADRB2* effect on biochemical and clinical parameters following a co-dominant model

The effect size of *ADRB2* on both biochemical and clinical parameters was assessed using a co-dominant genetic model. No statistically significant link with biochemical parameters or with clinical parameters was evident when compared to GG variant as seen in Table 3.105.

#### 3.6.5.1.2 *ADRB2* effect on biochemical and clinical parameters following a recessive model

The effect size of *ADRB2* on both biochemical and clinical parameters was assessed using a recessive genetic model. No statistically significant link with

biochemical parameters or on clinical parameters was evident when following a recessive model (G/G [WT] + G/A [HT]) vs. A/A [MT]), as seen in Table 3.106.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | *p*-value |
| FPG (mmol/L) | AA | -0.65 | 0.74 | -2.11 | 0.81 | 0.38 |
| | GA | -0.34 | 0.55 | -1.43 | 0.74 | 0.54 |
| | GG | Reference | | | | |
| LDL-C (mmol/L) | AA | -0.40 | 0.21 | -0.81 | 0.01 | 0.06 |
| | GA | -0.13 | 0.16 | -0.43 | 0.18 | 0.42 |
| | GG | Reference | | | | |
| HDL-C (mmol/L) | AA | -0.07 | 0.08 | -0.23 | 0.09 | 0.39 |
| | GA | -0.08 | 0.06 | -0.20 | 0.04 | 0.21 |
| | GG | Reference | | | | |
| Triglycerides (mmol/L) | AA | 0.35 | 0.21 | -0.06 | 0.75 | 0.09 |
| | GA | 0.10 | 0.15 | -0.20 | 0.40 | 0.53 |
| | GG | Reference | | | | |
| Total Cholesterol (mmol/L) | AA | -0.35 | 0.23 | -0.81 | 0.11 | 0.131 |
| | GA | -0.16 | 0.17 | -0.50 | 0.18 | 0.37 |
| | GG | Reference | | | | |
| BMI ($Kg/m^2$) | AA | 1.25 | 1.21 | -1.13 | 3.63 | 0.30 |
| | GA | 1.16 | 0.91 | -0.62 | 2.93 | 0.20 |
| | GG | Reference | | | | |
| Systolic Blood Pressure (mmHg) | AA | -2.41 | 3.25 | -8.78 | 3.97 | 0.46 |
| | GA | -0.11 | 2.43 | -4.86 | 4.65 | 0.96 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | AA | 1.21 | 2.18 | -3.06 | 5.48 | 0.58 |
| | GA | -0.02 | 1.63 | -3.21 | 3.17 | 0.99 |
| | GG | Reference | | | | |
| Waist Circumference (cm) | AA | 2.66 | 3.40 | -4.00 | 9.32 | 0.43 |
| | GA | 3.07 | 2.53 | -1.90 | 8.03 | 0.23 |
| | GG | Reference | | | | |

A – risk allele

AA – Mutant genotype

AG – Heterozygous genotype

GG – Wild type genotype

Table 3.105 *ADRB2* [rs1042713] effect on biochemical and clinical parameters in a co-dominant model

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | GG + GA | -0.25 | 0.40 | -0.54 | 1.04 | 0.54 |
| | AA | Reference | | | | |
| LDL-C (mmol/L) | GG + GA | 0.12 | 0.14 | -0.40 | 0.16 | 0.40 |
| | AA | Reference | | | | |
| HDL-C (mmol/L) | GG + GA | 0.01 | 0.07 | -0.15 | 0.13 | 0.88 |
| | AA | Reference | | | | |
| Triglycerides (mmol/L) | GG + GA | -0.21 | 0.12 | -0.03 | 0.45 | 0.08 |
| | AA | Reference | | | | |
| Total Cholesterol (mmol/L) | GG + GA | 0.03 | 0.15 | -0.33 | 0.26 | 0.83 |
| | AA | Reference | | | | |
| BMI ($Kg/m^2$) | GG + GA | -0.04 | 0.94 | -1.81 | 1.88 | 0.97 |
| | AA | Reference | | | | |
| Systolic Blood Pressure (mmHg) | GG + GA | 0.08 | 2.34 | -4.67 | 4.50 | 0.97 |
| | AA | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | GG + GA | -1.73 | 1.52 | -1.25 | 4.71 | 0.25 |
| | AA | Reference | | | | |
| Body Weight (Kg( | GG + GA | -0.33 | 2.92 | -5.39 | 6.05 | 0.09 |
| | AA | Reference | | | | |
| Waist Circumference (cm) | GG + GA | -1.45 | 2.76 | -3.95 | 6.86 | 0.60 |
| | AA | Reference | | | | |
| WHR | GG + GA | -0.01 | 0.01 | -0.01 | 0.03 | 0.35 |
| | AA | Reference | | | | |

A – risk allele

AA – Mutant genotype

AG – Heterozygous genotype

GG – Wild type genotype

Table 3.106 *ADRB2* [rs1042713] effect on biochemical and clinical parameters in a recessive model

#### 3.6.5.1.3 *ADRB2* effect on biochemical and clinical parameters following a dominant model

The effect size of *ADRB2* [rs1042713] on both biochemical and clinical parameters was assessed using a dominant genetic model. No statistically significant effect on biochemical parameters or on clinical parameters was evident when following a dominant model (G/A [HT] + A/A [MT]) vs. GG [WT], as seen in Table 3.107.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | p-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | GA + AA | -0.01 | 0.30 | -0.60 | 0.59 | 0.98 |
| | GG | Reference | | | | |
| LDL-C | GA + AA | 0.01 | 0.11 | -0.20 | 0.21 | 0.82 |
| | GG | Reference | | | | |
| HDL-C | GA + AA | -0.06 | 0.05 | -0.16 | 0.05 | 0.29 |
| | GG | Reference | | | | |
| Triglycerides | GA + AA | 0.14 | 0.09 | -0.04 | 0.32 | 0.13 |
| | GG | Reference | | | | |
| Total Cholesterol | GA + AA | 0.05 | 0.11 | -0.17 | 0.27 | 0.64 |
| | GG | Reference | | | | |
| BMI | GA + AA | 0.37 | 0.71 | -1.01 | 1.76 | 0.60 |
| | GG | Reference | | | | |
| Systolic Blood Pressure | GA + AA | 0.54 | 1.76 | -2.90 | 3.98 | 0.76 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure | GA + AA | 0.32 | 1.14 | -1.92 | 2.56 | 0.78 |
| | GG | Reference | | | | |
| Waist Circumference | GA + AA | 3.893 | 2.0587 | -0.142 | 7.928 | 0.06 |
| | GG | Reference | | | | |

A – risk allele

AA – Mutant genotype

AG – Heterozygous genotype

GG – Wild type genotype

Table 3.107 *ADRB2* effect on biochemical and clinical parameters in a dominant model

### 3.6.5.2 *NOTCH2* [rs10923931]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *NOTCH2* locus, as seen in Table 3.108. However, one can observe a trend of worsening metabolic characteristics between the wild type genotype [G/G] to mutant genotype [T/T] for the majority of the biochemical and clinical parameters. Of note, the median FPG exhibits a transition from an impaired fasting glucose status [G/G and G/T] to a full-blown diabetes [T/T] status. In fact, the T/T genotype (genotype of interest) appears to have all the characteristics contributing to the metabolic syndrome. Interestingly only three individuals exhibited such a genotype, among whom two suffered from diabetes mellitus and one was defined as being metabolically healthy. Table 3.109 illustrates the biochemical and clinical parameters of the T/T genotype.

| | ***NOTCH2* rs10923931** | | | |
|---|---|---|---|---|
| | **GG [WT] (*n*=261)** | **GT [HT] (*n*=75)** | **TT [MT] (*n*=3)** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.85 (1.84) | 5.90 (1.66) | 8.02 (12.36) | 0.39 |
| LDL-C (mmol/L) | 3.02 (1.36) | 3.07 (0.99) | 3.67 (3.63) | 0.36 |
| HDL-C (mmol/l) | 1.43 (0.56) | 1.38 (0.49) | 1.35 (0.57) | 0.77 |
| Triglycerides (mmol/L) | 1.02 (0.85) | 0.96 (0.71) | 1.52 (1.64) | 0.71 |
| Total Cholesterol (mmol/L) | 5.01 (1.43) | 5.11 (1.45) | 5.37 (4.14) | 0.57 |
| BMI ($Kg/m^2$) | 29.30 (9.44) | 29.00 (9.00) | 29.60 (24.09) | 0.84 |
| Systolic Blood pressure (mmHg) | 123.00 (18.70) | 120.00 (20.33) | 131.00 (76.70) | 0.25 |
| Diastolic Blood pressure (mmHg) | 75.00 (12.33) | 73.30 (10.70) | 81.00 (34.00) | 0.50 |
| Waist Circumference (cm) | 95.00 (28.10) | 97.00 (24.30) | 94.80 (51.00) | 0.98 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
T – risk allele

Table 3.108 Biochemical and clinical parameters in relation to *NOTCH2* genotypes

| | ***NOTCH* [rs10923931] - T/T genotype** | |
|---|---|---|
| | **Diabetes (*n*=2)** | **Metabolic healthy (*n*=1)** |
| | Median (IQR) | Median |
| Fasting plasma glucose (mmol/L) | 12.82 (9.60) | 5.26 |
| LDL-C (mmol/L) | 4.84 (2.33) | 2.37 |
| HDL-C (mmol/l) | 1.18 (0.34) | 1.58 |
| Triglycerides (mmol/L) | 1.91 (0.77) | 0.65 |
| Total Cholesterol (mmol/L) | 6.88 (3.02) | 4.25 |
| BMI (Kg/m2) | 37.64 (16.09) | 21.60 |
| Systolic BP (mmHg) | 151.00 (39.00) | 93.00 |
| Diastolic BP (mmHg) | 91.00 (19.00) | 66.00 |
| Waist Circumference (cm) | 104.90 (20.20) | 64.00 |

Table 3.109 Biochemical and clinical parameters of the T/T *NOTCH2* genotype

The metabolically healthy individual carrying the T/T allele still exhibited normal biochemical and clinical parameters as opposed to the diabetes individuals carrying the T/T allele.

#### 3.6.5.2.1 *NOTCH2* effect on biochemical and clinical parameters following a co-dominant model

The effect of *NOTCH2* on both biochemical and clinical parameters was assessed using a co-dominant genetic model. *NOTCH2* showed a statistically significant association with FPG as seen in Table 3.110. Individuals carrying NOTCH2 T/T variant had significantly higher FPG levels (β= 3.72, *p*=0.02) when compared to those with the *NOTCH2* G/G variant.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | p-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | TT | 3.72 | 1.56 | 1.96 | 872.29 | **0.02** |
| | GT | 0.13 | 0.35 | 0.57 | 2.27 | 0.71 |
| | GG | Reference | | | | |
| LDL-C | TT | 0.99 | 0.54 | 0.93 | 7.78 | 0.07 |
| | GT | 0.11 | 0.12 | 0.88 | 1.42 | 0.37 |
| | GG | Reference | | | | |
| HDL-C | TT | -0.16 | 0.28 | 0.49 | 1.47 | 0.56 |
| | GT | -0.01 | 0.06 | 0.88 | 1.12 | 0.89 |
| | GG | Reference | | | | |
| Triglycerides | TT | 0.26 | 0.47 | 0.51 | 3.26 | 0.58 |
| | GT | -0.01 | 0.11 | 0.80 | 1.22 | 0.93 |
| | GG | Reference | | | | |
| Total Cholesterol | TT | 0.98 | 0.58 | 0.86 | 8.22 | 0.09 |
| | GT | 0.10 | 0.13 | 0.85 | 1.42 | 0.46 |
| | GG | Reference | | | | |
| BMI | TT | 2.4 | 3.6 | <0.01 | 14411.3 | 0.50 |
| | GT | -0.5 | 0.8 | 0.1 | 2.9 | 0.50 |
| | GG | Reference | | | | |
| Systolic Blood Pressure | TT | 7.79 | 9.04 | <0.01 | 1198.00 | 0.39 |
| | GT | -3.31 | 2.04 | <0.01 | 1.99 | 0.11 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure | TT | 6.52 | 5.94 | 0.01 | 7745.00 | 0.27 |
| | GT | -1.63 | 1.34 | 0.01 | 2.70 | 0.22 |
| | GG | Reference | | | | |
| Waist Circumference | TT | -2.68 | 10.69 | <0.01 | 8646.00 | 0.09 |
| | GT | -0.37 | 2.41 | 0.01 | 78.18 | 0.46 |
| | GG | | | | | |

T – risk allele

TT – Mutant genotype

TG – Heterozygous genotype

GG – Wild type genotype

Table 3.110 *NOTCH2* [rs10923931] association with biochemical and clinical parameters in a co-dominant model

#### 3.6.5.2.2 *NOTCH2* effect on biochemical and clinical parameters following a recessive model

The effect of *NOTCH2* on both biochemical and clinical parameters was assessed using a recessive genetic model. *NOTCH 2* showed a statistically significant association with FPG, as seen in Table 3.111. *NOTCH2* carriers of the G allele (G/G + G/T) had statistically lower FPG levels (β= -3.69, *p*=0.02) when compared to those with the T/T variant.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | p-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | GG + GT | -3.69 | 1.55 | -6.74 | -0.64 | **0.02** |
| | TT | Reference | | | | |
| LDL-C | GG + GT | -0.96 | 0.54 | -2.03 | 0.10 | 0.08 |
| | TT | Reference | | | | |
| HDL-C | GG + GT | 0.16 | 0.28 | -0.39 | 0.70 | 0.57 |
| | TT | Reference | | | | |
| Triglycerides | GG + GT | -0.26 | 0.47 | -1.18 | 0.66 | 0.58 |
| | TT | Reference | | | | |
| Total Cholesterol | GG + GT | -0.95 | 0.58 | -2.08 | 0.18 | 0.10 |
| | TT | Reference | | | | |
| BMI | GG + GT | -2.55 | 3.64 | -9.69 | 4.58 | 0.48 |
| | TT | Reference | | | | |
| Systolic Blood Pressure | GG + GT | -8.52 | 9.07 | -26.30 | 9.25 | 0.35 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure | GG + GT | -6.89 | 5.95 | -18.54 | 4.77 | 0.25 |
| | TT | Reference | | | | |
| Waist Circumference | GG + GT | 2.60 | 10.68 | -18.33 | 23.53 | 0.81 |
| | TT | Reference | | | | |

T – risk allele
TT – Mutant genotype
TG – Heterozygous genotype
GG – Wild type genotype

Table 3.111 *NOTCH 2* [rs10923931] association with biochemical and clinical parameters in a recessive model

#### 3.6.5.2.3 *NOTCH2* effect on biochemical and clinical parameters following a dominant model

The effect of *NOTCH2* on both biochemical and clinical parameters was assessed using a dominant genetic model. No significant relationship was found with biochemical parameters or with clinical parameters was evident when following a dominant as seen in Table 3.112.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | GT + TT | 0.27 | 0.35 | -0.95 | 0.41 | 0.44 |
| | GG | Reference | | | | |
| LDL-C | GT + TT | 0.14 | 0.12 | -0.38 | 0.09 | 0.24 |
| | GG | Reference | | | | |
| HDL-C | GT + TT | -0.02 | 0.06 | -0.11 | 0.14 | 0.82 |
| | GG | Reference | | | | |
| Triglycerides | GT + TT | -0.01 | 0.11 | -0.21 | 0.21 | 0.99 |
| | GG | Reference | | | | |
| Total Cholesterol | GT + TT | 0.13 | 0.13 | -0.38 | 0.12 | 0.31 |
| | GG | Reference | | | | |
| BMI | GT + TT | -0.42 | 0.81 | -1.17 | 2.01 | 0.60 |
| | GG | Reference | | | | |
| Systolic Blood Pressure | GT + TT | -2.89 | 2.01 | -1.06 | 6.83 | 0.15 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure | GT + TT | -1.32 | 1.32 | -1.27 | 3.92 | 0.32 |
| | GG | Reference | | | | |
| Waist Circumference | GT + TT | -0.46 | 2.38 | -4.2 | 5.12 | 0.85 |
| | GG | Reference | | | | |

T – risk allele

TT – Mutant genotype

TG – Heterozygous genotype

GG – Wild type genotype

Table 3.112 *NOTCH 2* [rs10923931] effect on biochemical and clinical parameters in a dominant model

### 3.6.5.3 CDK5 regulatory subunit associated protein 1 like 1, *CDKAL1*, [rs7754840]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the CDKAL1 locus, as seen in Table 3.113.

| | ***CDKAL1* rs7754840** | | | |
|---|---|---|---|---|
| | **GG [WT]** | **GC [HT]** | **CC [MT]** | |
| | ***n*=152** | ***n*=153** | ***n*=32** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.90 (1.68) | 5.84 (1.82) | 5.93 (1.76) | 0.92 |
| LDL-C (mmol/L) | 3.19 (1.34) | 2.92 (1.27) | 3.16 (1.39) | 0.19 |
| HDL-C (mmol/l) | 1.39 (0.54) | 1.44 (0.58) | 1.29 (0.64) | 0.13 |
| Triglycerides (mmol/L) | 1.10 (0.76) | 0.93 (0.72) | 1.01 (1.28) | 0.12 |
| Total Cholesterol (mmol/L) | 5.11 (1.43) | 4.90 (1.32) | 5.01 (1.89) | 0.42 |
| BMI ($Kg/m^2$) | 29.01 (9.26) | 29.30 (8.93) | 28.74 (6.80) | 0.97 |
| Systolic Blood pressure (mmHG) | 124.00 (16.00) | 122.00 (21.00) | 120.85 (12.65) | 0.50 |
| Diastolic Blood pressure (mmHg) | 75.65 (13.68) | 72.67 (11.00) | 75.65 (10.85) | 0.14 |
| Waist Circumference (cm) | 95.00 (26.75) | 94.40 (94.40) | 97.25 (24.20) | 0.51 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
C – risk allele

Table 3.113 Biochemical and anthropometric parameters in relation to *CDKAL1* genotypes

#### 3.6.5.3.1 *CDKAL1* effect on biochemical and clinical parameters following a co-dominant model

The effect *CDKAL1* on both biochemical and clinical parameters was assessed using a co-dominant genetic model. No statistically significant relationship with biochemical parameters or with clinical parameters was evident when compared to G/G genotype as seen in Table 3.114.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC | -0.52 | 0.86 | -2.20 | 1.16 | 0.55 |
| | GC | -0.39 | 0.54 | -1.44 | 0.67 | 0.47 |
| | GG | Reference | | | | |
| LDL-C (mmol/L) | CC | 0.01 | 0.24 | -0.47 | 0.48 | 0.98 |
| | GC | -0.22 | 0.15 | -0.52 | 0.08 | 0.14 |
| | GG | Reference | | | | |
| HDL-C (mmol/L) | CC | -0.06 | 0.09 | -0.25 | 0.12 | 0.51 |
| | GC | 0.06 | 0.06 | -0.05 | 0.18 | 0.29 |
| | GG | Reference | | | | |
| Triglycerides (mmol/L) | CC | 0.42 | 0.24 | -0.05 | 0.88 | 0.08 |
| | GC | -0.04 | 0.15 | -0.33 | 0.25 | 0.80 |
| | GG | Reference | | | | |
| Total Cholesterol (mmol/L) | CC | 0.16 | 0.27 | -0.37 | 0.69 | 0.56 |
| | GC | -0.19 | 0.17 | -0.52 | 0.14 | 0.26 |
| | GG | Reference | | | | |
| BMI ($Kg/m^2$) | CC | 0.51 | 1.40 | -2.24 | 3.25 | 0.73 |
| | GC | -0.26 | 0.88 | -1.98 | 1.46 | 0.77 |
| | GG | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC | -0.97 | 3.70 | -8.22 | 6.28 | 0.79 |
| | GC | -0.49 | 2.33 | -5.04 | 4.07 | 0.83 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC | 0.74 | 2.50 | -4.17 | 5.64 | 0.77 |
| | GC | -1.49 | 1.57 | -4.57 | 1.58 | 0.34 |
| | GG | Reference | | | | |
| Waist Circumference (cm) | CC | 4.64 | 3.90 | -3.01 | 12.28 | 0.23 |
| | GC | -0.55 | 2.45 | -5.35 | 4.26 | 0.82 |
| | GG | Reference | | | | |

C – risk allele

CC – Mutant genotype

GC – Heterozygous genotype

GG – Wild type genotype

Table 3.114 *CDKAL1* [rs7754840] effect on biochemical and clinical parameters in a co-dominant model

#### 3.6.5.3.2 *CDKAL1* effect on biochemical and clinical parameters following a recessive model

The effect of *CDKAL1* on both biochemical and clinical parameters was assessed using a recessive genetic model. No statistically significant relationship with biochemical parameters or with clinical parameters was evident when G/G + G/C genotypes were compared to C/C genotype as seen in Table 3.115.

#### 3.6.5.3.3 *CDKAL1* effect on biochemical and clinical parameters following a dominant model

The effect of *CDKAL1* on both biochemical and clinical parameters was assessed using a dominant genetic model. No significant relationship with biochemical parameters or with clinical parameters was evident when C/C + C/G genotypes were compared to G/G genotype as seen in Table 3.116.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | GG + GC | 0.19 | 0.50 | -1.18 | 0.79 | 0.70 |
| | CC | Reference | | | | |
| LDL-C (mmol/L) | GG + GC | -0.12 | 0.18 | -0.23 | 0.46 | 0.51 |
| | CC | Reference | | | | |
| HDL-C (mmol/L) | GG + GC | 0.14 | 0.09 | -0.31 | 0.04 | 0.12 |
| | CC | Reference | | | | |
| Triglycerides (mmol/L) | GG + GC | -0.25 | 0.15 | -0.04 | 0.55 | 0.09 |
| | CC | Reference | | | | |
| Total Cholesterol (mmol/L) | GG + GC | -0.13 | 0.19 | -0.24 | 0.50 | 0.48 |
| | CC | Reference | | | | |
| BMI ($Kg/m^2$) | GG + GC | 0.38 | 1.17 | -2.67 | 1.91 | 0.75 |
| | CC | Reference | | | | |
| Systolic Blood Pressure (mmHg) | GG + GC | 1.38 | 2.8 | -7.02 | 4.26 | 0.63 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | GG + GC | -1.55 | 1.89 | -2.15 | 5.24 | 0.42 |
| | CC | Reference | | | | |
| Waist Circumference (cm) | GG + GC | -2.19 | 3.42 | -4.51 | 8.89 | 0.52 |
| | CC | Reference | | | | |

C – risk allele

CC – Mutant genotype

GC – Heterozygous genotype

GG – Wild type genotype

Table 3.115 *CDKAL1* [rs7754840] effect on biochemical and clinical parameters in a recessive model

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CG<br>GG | -0.31<br>Reference | 0.30 | -0.89 | 0.28 | 0.30 |
| LDL-C (mmol/L) | CC + CG<br>GG | -0.17<br>Reference | 0.10 | -0.38 | 0.03 | 0.10 |
| HDL-C (mmol/L) | CC + CG<br>GG | 0.07<br>Reference | 0.05 | -0.03 | 0.18 | 0.17 |
| Triglycerides (mmol/L) | CC + CG<br>GG | 0.01<br>Reference | 0.09 | -0.17 | 0.18 | 0.94 |
| Total Cholesterol (mmol/L) | CC + CG<br>GG | -0.13<br>Reference | 0.11 | -0.35 | 0.09 | 0.24 |
| BMI ($Kg/m^2$) | CC + CG<br>GG | -0.23<br>Reference | 0.69 | -1.58 | 1.13 | 0.74 |
| Systolic Blood Pressure (mmHg) | CC + CG<br>GG | -2.20<br>Reference | 1.69 | -5.51 | 1.12 | 0.20 |
| Diastolic Blood Pressure (mmHg) | CC + CG<br>GG | -1.66<br>Reference | 1.11 | -3.83 | 0.51 | 0.13 |
| Waist Circumference (cm) | CC + CG<br>GG | -0.33<br>Reference | 2.02 | -4.28 | 3.62 | 0.87 |

C – risk allele

CC – Mutant genotype

GC – Heterozygous genotype

GG – Wild type genotype

Table 3.116 *CDKAL1* [rs7754840] relationship with biochemical and clinical parameters in a dominant model

### 3.6.5.4 Fatty acid binding protein 2, *FABP2,* [rs1799883]

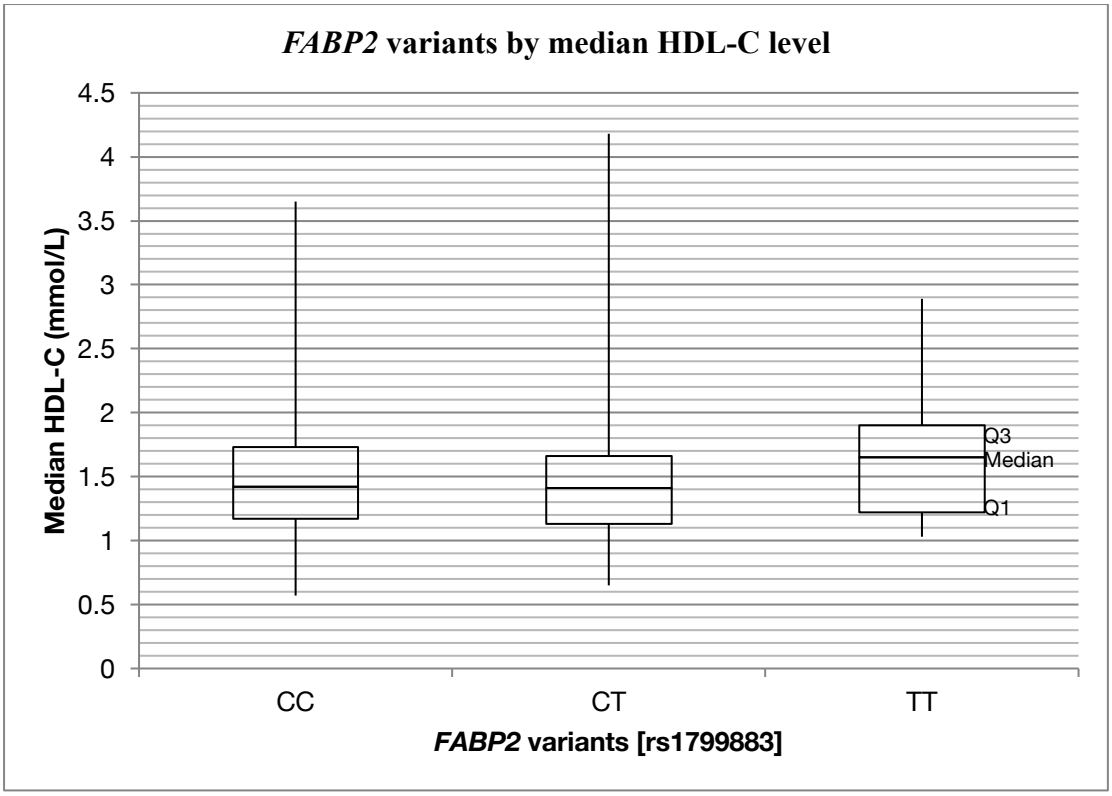
Statistically significant median differences were found between HDL-C levels ($p$=0.05) as well as between triglycerides levels ($p$=0.02) for the different genotypes at the *FABP2* locus, as seen in Table 3.117. The T/T variant exhibited higher median HDL-C levels than both C/C and C/T genotypes ($p$=0.01, $p$=0.04 respectively), as seen in Figure 3.94. However, the T/T variant exhibited lower median triglyceride levels when compared to both C/C and C/T genotypes ($p$=0.01, $p$=0.02 respectively), as seen in Figure 3.95.

| | ***FABP2* rs1799883** | | | |
|---|---|---|---|---|
| | **CC [WT]** | **CT [HT]** | **TT [MT]** | |
| | ($n$=144) | ($n$=160) | ($n$=31) | |
| | Median (IQR) | Median (IQR) | Median (IQR) | $p$-value* |
| Fasting plasma glucose (mmol/L) | 5.88 (2.12) | 5.89 (1.68) | 5.30 (1.21) | 0.17 |
| LDL-C (mmol/L) | 3.09 (1.24) | 3.06 (1.36) | 2.84 (1.47) | 0.59 |
| HDL-C (mmol/l) | 1.38 (0.51) | 1.42 (0.54) | 1.65 (0.68) | **0.05** |
| Triglycerides (mmol/L) | 1.05 (0.91) | 1.02 (0.67) | 0.83 (0.42) | **0.02** |
| Total Cholesterol (mmol/L) | 5.04 (1.35) | 5.08 (1.51) | 4.69 (1.43) | 0.73 |
| BMI (Kg/m$^2$) | 28.90 (8.40) | 29.60 (9.13) | 25.43 (10.17) | 0.06 |
| Systolic Blood pressure (mmHg) | 122.50 (20.32) | 122.85 (18.70) | 120.00 (12.67) | 0.24 |
| Diastolic Blood pressure (mmHg) | 75.17 (11.83) | 74.85 (12.32) | 73.30 (10.00) | 0.73 |
| Waist Circumference (cm) | 94.30 (25.70) | 97.00 (28.50) | 87.30 (32.00) | 0.11 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
T – risk allele

Table 3.117 Biochemical and anthropometric parameters in relation to *FABP2* genotypes




CC – Wild type genotype

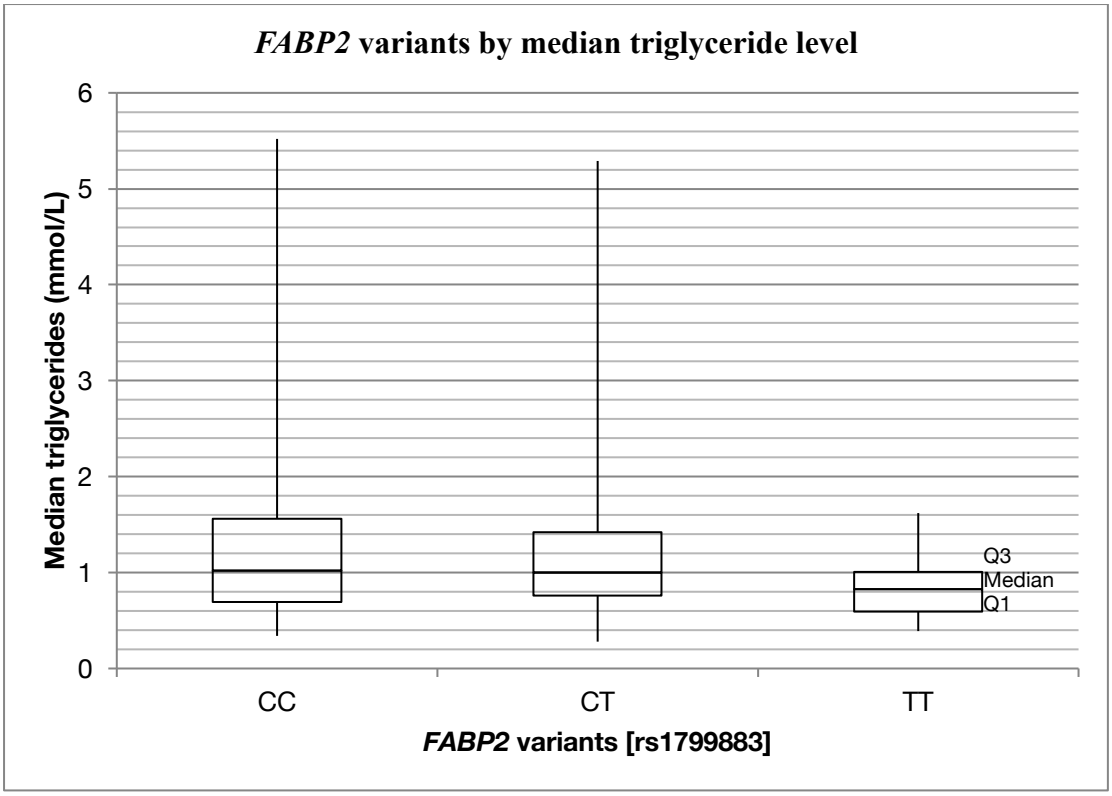
CT – Heterozygous genotype

TT – Mutant genotype

Q1 – 25$^{th}$ percentile (lower quartile)

Q3 – 75$^{th}$ percentile (upper quartile)

Figure 3.94 Median HDL-C, by the different *FABP2* variants




CC – Wild type genotype

CT – Heterozygous genotype

TT – Mutant genotype

Q1 – 25$^{th}$ percentile (lower quartile)

Q3 – 75$^{th}$ percentile (upper quartile)

Figure 3.95 Median triglycerides, by the different *FABP2* variants

Considering that both triglyceride and HDL-C levels were significantly different across the *FABP2* genotype, the values were utilized to calculate the TG/HDL-C ratio. The TG/HDL-C ratio is an indicative insulin resistance risk ratio. Table 3.118 shows the insulin resistance risk across the *FABP2* allele variants. The *FAPB2* variants were explored with regards to the reported intake of statin treatment, as seen in Table 3.119.

| | *FABP2* rs1799883 | | |
|---|---|---|---|
| **TG/HDL-C ratio** | **CC (*n*=144)** | **CT (*n*=160)** | **TT (*n*=31)** |
| Optimal (≤1.99) | 121 (84.00%) | 141 (88.13%) | 30 (96.77%) |
| At Risk (2.00 - 3.99) | 21 (14.58%) | 15 (9.38%) | 1 (3.23%) |
| Very high risk (≥4) | 2 (1.39%) | 4 (2.50%) | 0 |

CC – Wild type genotype

CT – Heterozygous genotype

TT – Mutant genotype

T – risk allele

Table 3.118 Distribution of TG/HDL-C ratio across *FABP2* allele variants

| | *FABP2* rs1799883 | | |
|---|---|---|---|
| | **CC (*n*=144)** | **CT (*n*=160)** | **TT (*n*=31)** |
| On statins | 33 (22.92%) | 27 (16.88%) | 7 (22.58%) |

CC – Wild type

CT – Heterozygous

TT – Mutant

Table 3.119 Distribution of statin treatment across the *FABP2* allele variants

Furthermore, the *FABP2* alleles variants were assessed in relation to their glycaemic and metabolic classification as seen in Table 3.120. The majority of the C/C and C/T variants were persons with diabetes while the majority of T/T variants were metabolically abnormal. No significant difference between the *FABP2* variants and the glycaemic - metabolic classification was identified ($p$=0.40).

| | *FABP2* rs1799883 | | |
|---|---|---|---|
| | **CC [*n*=144] (%)** | **CT [*n*=160]** | **TT [*n*=31]** |
| Diabetes | 53 (37%) | 55 (34%) | 5 (16%) |
| Pre-diabetes | 32 (22%) | 42 (26%) | 8 (26%) |
| Metabolic Abnormal | 32 (22%) | 35 (22%) | 11 (35%) |
| Healthy | 27 (19%) | 28 (18%) | 7 (23%) |

CC – Wild type genotype

CT – Heterozygous genotype

TT – Mutant genotype

T – risk allele

Table 3.120 Distribution of *FABP2* allele variants according to glycaemic and metabolic classification

#### 3.6.5.4.1 *FABP2* effect on biochemical and clinical parameters following a co-dominant model

The effect of *FABP2* on both biochemical and clinical parameters was assessed using a co-dominant genetic model. Individuals carrying the T/T [MT] genotype had a statistically lower triglyceride level (β= -0.42, *p*=0.01) and higher HDL-C level (β= 0.21, *p*=0.02) when compared to the C/C [WT] genotype, as seen in Table 3.121.

#### 3.6.5.4.2 *FABP2* effect on biochemical and clinical parameters following a recessive model

The effect of *FABP2* on both biochemical and clinical parameters was assessed using a recessive genetic model. The carriers of the C allele (C/C + C/T) exhibited a statistically significant effect on HDL-C and triglyceride levels, when compared to the T/T genotype. Individuals carrying the C allele (C/C + C/T) had a statistically lower

HDL-C levels (β= -0.19, *p*=0.03) and higher triglyceride levels (β= 0.39, *p*=0.01) when compared to T/T genotype, as seen in Table 3.122.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | TT | -0.67 | 0.53 | 0.18 | 1.46 | 0.21 |
| | CT | 0.23 | 0.31 | 0.68 | 2.30 | 0.47 |
| | CC | Reference | | | | |
| LDL-C | TT | -0.18 | 0.19 | 0.58 | 1.20 | 0.33 |
| | CT | 0.02 | 0.11 | 0.83 | 1.26 | 0.86 |
| | CC | Reference | | | | |
| HDL-C | TT | 0.21 | 0.09 | 1.03 | 1.48 | **0.02** |
| | CT | 0.04 | 0.05 | 0.93 | 1.15 | 0.50 |
| | CC | Reference | | | | |
| Triglycerides | TT | -0.42 | 0.16 | 0.48 | 0.90 | **0.01** |
| | CT | -0.06 | 0.09 | 0.79 | 1.13 | 0.54 |
| | CC | Reference | | | | |
| Total Cholesterol | TT | -0.14 | 0.20 | 0.59 | 1.28 | 0.47 |
| | CT | 0.04 | 0.12 | 0.83 | 1.30 | 0.74 |
| | CC | Reference | | | | |
| BMI | TT | -1.93 | 1.24 | 0.01 | 1.67 | 0.12 |
| | CT | 0.61 | 0.72 | 0.45 | 7.58 | 0.40 |
| | CC | Reference | | | | |
| Systolic Blood Pressure | TT | -4.08 | 3.10 | 0.00 | 7.45 | 0.19 |
| | CT | -0.36 | 1.80 | 0.02 | 23.75 | 0.84 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure | TT | -1.82 | 2.02 | 0.00 | 8.45 | 0.37 |
| | CT | -0.39 | 1.17 | 0.07 | 6.73 | 0.74 |
| | CC | Reference | | | | |
| Waist Circumference | TT | -4.77 | 3.64 | 0.00 | 10.64 | 0.19 |
| | CT | 2.07 | 2.11 | 0.13 | 500.43 | 0.33 |
| | CC | Reference | | | | |

T – risk allele
TT – Mutant genotype
CT – Heterozygous genotype
CC – Wild type genotype

Table 3.121 *FABP2* [rs1799883] effect on biochemical and clinical parameters in a co-dominant model

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | CC + CT | 0.78 | 0.51 | -0.21 | 1.78 | 0.12 |
| | TT | Reference | | | | |
| LDL-C | CC + CT | 0.19 | 0.18 | -0.16 | 0.54 | 0.28 |
| | TT | Reference | | | | |
| HDL-C | CC + CT | -0.19 | 0.09 | -0.36 | -0.02 | **0.03** |
| | TT | Reference | | | | |
| Triglycerides | CC + CT | 0.39 | 0.15 | 0.09 | 0.69 | **0.01** |
| | TT | Reference | | | | |
| Total Cholesterol | CC + CT | 0.16 | 0.19 | -0.21 | 0.53 | 0.39 |
| | TT | Reference | | | | |
| BMI | CC + CT | 2.25 | 1.18 | -0.08 | 4.57 | 0.06 |
| | TT | Reference | | | | |
| Systolic Blood Pressure | CC + CT | 3.89 | 2.96 | -1.91 | 9.68 | 0.19 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure | CC + CT | 1.62 | 1.92 | -2.15 | 5.39 | 0.40 |
| | TT | Reference | | | | |
| Body Weight | CC + CT | 6.68 | 3.67 | -0.51 | 13.88 | 0.07 |
| | TT | Reference | | | | |
| Waist Circumference | CC + CT | 5.87 | 3.47 | -0.94 | 12.67 | 0.09 |
| | TT | Reference | | | | |
| WHR | CC + CT | 0.02 | 0.01 | -0.01 | 0.05 | 0.13 |
| | TT | Reference | | | | |

T – risk allele

TT – Mutant genotype

CT – Heterozygous genotype

CC – Wild type genotype

Table 3.122 *FABP2* [rs1799883] association with biochemical and clinical parameters in a recessive model

#### 3.6.5.4.3 *FABP2* effect on biochemical and clinical parameters following a dominant model

The effect of *FABP2* on both biochemical and clinical parameters was assessed using a dominant genetic model. Individuals carrying a dominant genotype (C/T + T/T) had a statistically lower HDL-C levels (β= -0.18, *p*=0.04) and higher triglyceride levels (β= 0.39, *p*=0.01) when compared to C/C genotype, as seen in Table 3.123.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | CT + TT | 0.79 | 0.51 | 0.82 | 5.94 | 0.12 |
| | CC | Reference | | | | |
| LDL-C | CT + TT | 0.19 | 0.18 | 0.85 | 1.71 | 0.30 |
| | CC | Reference | | | | |
| HDL-C | CT + TT | -0.18 | 0.09 | 0.70 | 0.99 | **0.04** |
| | CC | Reference | | | | |
| Triglycerides | CT + TT | 0.39 | 0.15 | 1.10 | 1.98 | **0.01** |
| | CC | Reference | | | | |
| Total Cholesterol | CT + TT | 0.16 | 0.19 | 0.81 | 1.71 | 0.39 |
| | CC | Reference | | | | |
| BMI | CT + TT | 2.267 | 1.18 | 0.958 | 97.16 | **0.05** |
| | CC | Reference | | | | |
| Systolic Blood Pressure | CT + TT | 3.88 | 2.93 | 0.15 | 15240.67 | 0.19 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure | CT + TT | 1.66 | 1.93 | 0.12 | 231.52 | 0.39 |
| | CC | Reference | | | | |
| Waist Circumference | CT + TT | 5.94 | 3.45 | 0.44 | 329331.28 | 0.09 |
| | CC | Reference | | | | |

T – risk allele

TT – Mutant genotype

CT – Heterozygous genotype

CC – Wild type genotype

Table 3.123 *FABP2* [rs1799883] association with biochemical and clinical parameters in a dominant model

### 3.6.5.5 Fat mass and obesity association, *FTO* [rs9939609]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *FTO* locus, seen in Table 3.124.

| | ***FTO* rs9939609** | | | |
|---|---|---|---|---|
| | **TT [WT] (*n*=117)** | **AT [HT] (*n*=167)** | **AA [MT] (*n*=50)** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.87 (1.58) | 5.85 (1.85) | 6.07 (2.36) | 0.83 |
| LDL-C (mmol/L) | 3.09 (1.19) | 2.95 (1.37) | 2.86 (1.52) | 0.49 |
| HDL-C (mmol/l) | 1.46 (0.47) | 1.31 (0.58) | 1.49 (0.66) | 0.06 |
| Triglycerides (mmol/L) | 1.01 (0.80) | 1.02 (0.81) | 0.91 (0.83) | 0.88 |
| Total Cholesterol (mmol/L) | 5.08 (1.17) | 4.97 (1.51) | 5.08 (1.69) | 0.42 |
| BMI ($Kg/m^2$) | 29.00 (8.93) | 29.01 (8.95) | 29.30 (10.70) | 0.74 |
| Systolic Blood pressure (mmHg) | 123.00 (19.00) | 121.70 (19.00) | 120.85 (18.00) | 0.78 |
| Diastolic Blood pressure (mmHg) | 74.00 (12.00) | 75.30 (12.00) | 72.83 (10.00) | 0.19 |
| Waist Circumference (cm) | 97.00 (27.00) | 95.00 (29.10) | 94.20 (28.00) | 0.85 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
A – risk allele

Table 3.124 Biochemical and anthropometric parameters in relation to *FTO* genotypes

#### 3.6.5.5.1 *FTO* effect on biochemical and clinical parameters following a co-dominant model

The effect of *FTO* on both biochemical and clinical parameters was assessed using a co-dominant genetic model, as seen in Table 3.125, where no statistically significant differences were present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | AA | 0.27 | 0.45 | -0.62 | 1.15 | 0.55 |
| | AT | -0.14 | 0.32 | -0.77 | 0.49 | 0.66 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | AA | 0.02 | 0.16 | -0.30 | 0.34 | 0.90 |
| | AT | -0.14 | 0.12 | -0.37 | 0.08 | 0.22 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | AA | 0.04 | 0.08 | -0.12 | 0.20 | 0.64 |
| | AT | -0.03 | 0.06 | -0.14 | 0.09 | 0.63 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | AA | 0.03 | 0.14 | -0.24 | 0.30 | 0.82 |
| | AT | 0.01 | 0.10 | -0.19 | 0.20 | 0.92 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | AA | 0.10 | 0.17 | -0.24 | 0.43 | 0.57 |
| | AT | -0.17 | 0.12 | -0.41 | 0.07 | 0.16 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | AA | -0.38 | 1.06 | -2.45 | 1.69 | 0.72 |
| | AT | -0.57 | 0.75 | -2.05 | 0.90 | 0.45 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | AA | -3.19 | 2.68 | -8.44 | 2.06 | 0.23 |
| | AT | -2.12 | 1.91 | -5.86 | 1.62 | 0.27 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | AA | -3.24 | 1.75 | -6.66 | 0.19 | 0.06 |
| | AT | -0.33 | 1.25 | -2.77 | 2.11 | 0.79 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | AA | 1.07 | 3.08 | -4.97 | 7.12 | 0.73 |
| | AT | 1.77 | 2.20 | -2.54 | 6.08 | 0.42 |
| | TT | Reference | | | | |

A – risk allele

AA – Mutant genotype

AT – Heterozygous genotype

TT – Wild type genotype

Table 3.125 *FTO* [rs9939609] association with biochemical and clinical parameters in a co-dominant model

#### 3.6.5.5.2 *FTO* effect on biochemical and clinical parameters following a recessive model

The effect of *FTO* on both biochemical and clinical parameters was assessed using a recessive genetic model, as seen Table 3.126, where no statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | AT + TT<br>AA | -0.37<br>Reference | 0.42 | -0.30 | 1.56 | 0.38 |
| LDL-C (mmol/L) | AT + TT<br>AA | <0.01<br>Reference | 0.15 | -0.75 | 1.33 | 0.99 |
| HDL-C (mmol/L) | AT + TT<br>AA | -0.09<br>Reference | 0.07 | -0.79 | 1.05 | 0.20 |
| Triglycerides (mmol/L) | AT + TT<br>AA | 0.01<br>Reference | 0.13 | -0.23 | 0.26 | 0.93 |
| Total Cholesterol (mmol/L) | AT + TT<br>AA | -0.12<br>Reference | 0.15 | -0.18 | 0.77 | 1.26 |
| BMI ($Kg/m^2$) | AT + TT<br>AA | -0.69<br>Reference | 0.97 | -1.21 | 0.08 | 3.34 |
| Systolic Blood Pressure (mmHg) | AT + TT<br>AA | 1.16<br>Reference | 2.41 | -5.87 | 2.03 | 354.94 |
| Diastolic Blood Pressure (mmHg) | AT + TT<br>AA | 2.25<br>Reference | 1.56 | -5.30 | 2.45 | 199.56 |
| Waist Circumference (cm) | AT + TT<br>AA | -1.47<br>Reference | 2.82 | -4.06 | 0.00 | 57.79 |

A – risk allele

AA – Mutant genotype

AT – Heterozygous genotype

TT – Wild type genotype

Table 3.126 *FTO* [rs9939609] relationship with biochemical and clinical parameters in a recessive model

### 3.6.5.5.3 *FTO* effect on biochemical and clinical parameters following a dominant model

The effect of *FTO* on both biochemical and clinical parameters was assessed using a dominant genetic model, as seen Table 3.127, where no statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | AA + AT<br>TT | 0.07<br>Reference | 0.31 | -0.54 | 0.68 | 0.82 |
| LDL-C (mmol/L) | AA + AT<br>TT | -0.10<br>Reference | 0.11 | -0.31 | 0.12 | 0.35 |
| HDL-C (mmol/L) | AA + AT<br>TT | -0.04<br>Reference | 0.06 | -0.14 | 0.07 | 0.52 |
| Triglycerides (mmol/L) | AA + AT<br>TT | 0.02<br>Reference | 0.09 | -0.17 | 0.20 | 0.86 |
| Total Cholesterol (mmol/L) | AA + AT<br>TT | -0.13<br>Reference | 0.12 | -0.35 | 0.10 | 0.27 |
| BMI ($Kg/m^2$) | AA + AT<br>TT | -0.31<br>Reference | 0.72 | -1.73 | 1.11 | 0.67 |
| Systolic Blood Pressure (mmHg) | AA + AT<br>TT | -1.33<br>Reference | 1.80 | -4.85 | 2.20 | 0.46 |
| Diastolic Blood Pressure (mmHg) | AA + AT<br>TT | -0.53 | 1.17 | -2.82 | 1.75 | 0.65 |
| Waist Circumference (cm) | AA + AT<br>TT | 1.91<br>Reference | 2.11 | -2.22 | 6.04 | 0.37 |

A – risk allele

AA – Mutant genotype

AT – Heterozygous genotype

TT – Wild type genotype

Table 3.127 *FTO* [rs9939609] association with biochemical and clinical parameters in a dominant model

### 3.6.5.6 Hematopoietically expressed homeobox, *HHEX* [rs1111875]

A significant difference was found between different *HHEX* genotypes and median FPG levels ($p$=0.03). However, no significant median differences were present between other biochemical and clinical parameters, as seen in Table 3.128.

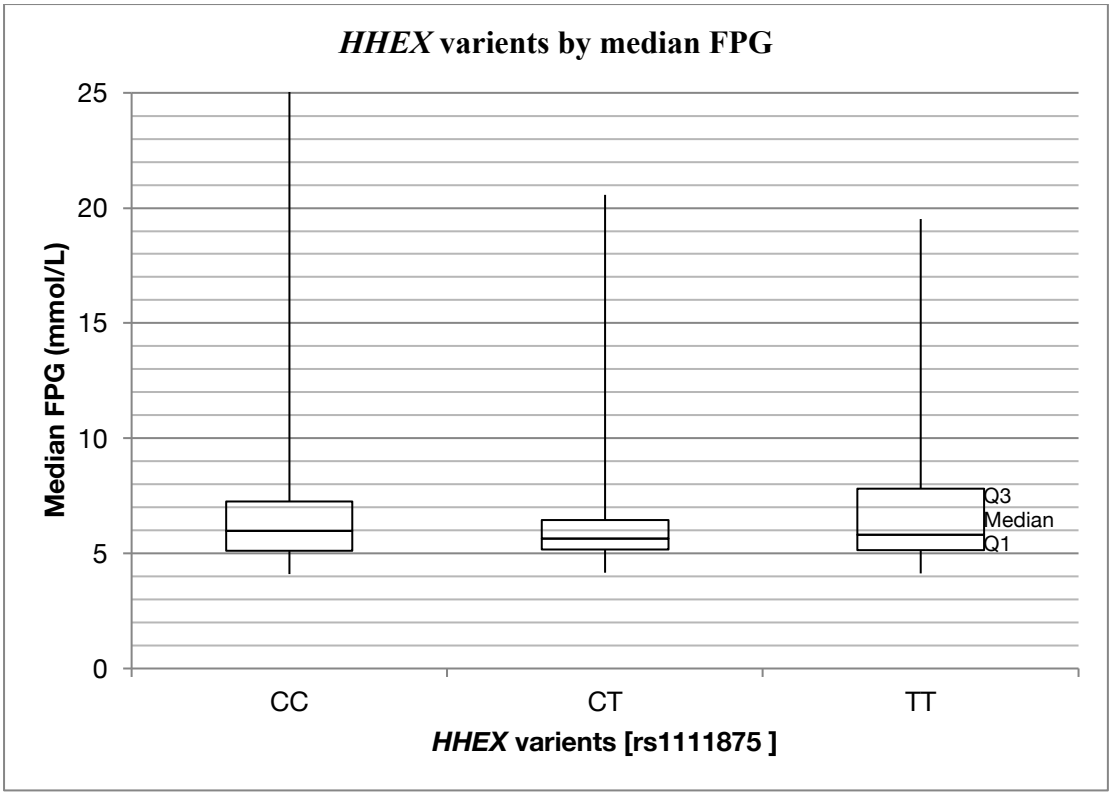
| | ***HHEX* rs1111875** | | | |
|---|---|---|---|---|
| | **TT [WT] (*n*=51)** | **CT [HT] (*n*=174)** | **CC [MT] (*n*=115)** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.95 (2.81) | 5.70 (1.36) | 6.11 (2.27) | **0.03** |
| LDL-C (mmol/L) | 3.09 (1.72) | 2.93 (1.29) | 3.10 (1.24) | 0.62 |
| HDL-C (mmol/l) | 1.26 (0.62) | 1.43 (0.53) | 1.40 (0.57) | 0.50 |
| Triglycerides (mmol/L) | 1.00 (0.79) | 0.97 (0.80) | 1.02 (0.76) | 0.56 |
| Total Cholesterol (mmol/L) | 4.90 (1.76) | 4.97 (1.43) | 5.11 (1.25) | 0.50 |
| BMI ($Kg/m^2$) | 29.60 (8.10) | 28.60 (9.33) | 29.37 (9.24) | 0.27 |
| Systolic Blood pressure (mmHg) | 124.00 (18.30) | 120.70 (18.67) | 123.00 (20.00) | 0.41 |
| Diastolic Blood pressure (mmHg) | 75.00 (12.63) | 73.32 (12.00) | 76.00 (12.00) | 0.13 |
| Waist Circumference (cm) | 99.80 (24.00) | 93.50 (28.00) | 97.00 (25.50) | 0.22 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
C – risk allele

Table 3.128 Biochemical and anthropometric parameters in relation to *HHEX* genotypes

The C/C allele (mutant genotype and genotype of interest) exhibited a higher median FPG level than C/T allele (heterozygous genotype) ($p$=0.03) but no significant difference from T/T allele (wild type). The median FPG across the *HHEX* genotypes can be seen in Figure 3.96.




CC – Mutant genotype

CT – Heterozygous genotype

TT – Wild type genotype

Q1 – 25$^{th}$ percentile (lower quartile)

Q3 – 75$^{th}$ percentile (upper quartile)

Figure 3.96 Median fasting plasma glucose by *HHEX* variants

The *HHEX* variants were categorized into different classifications according to the glycaemic and metabolic status, as seen in Table 3.129. The majority of subjects with the mutant variant (C/C) were persons with diabetes. No significant difference between the *HHEX* variants and the glycaemic - metabolic classification was identified ($p$=0.16).

| | ***HHEX* - rs1111875** | | |
|---|---|---|---|
| | **TT [n=51] (%)** | **CT [n=174] (%)** | **CC [n=115] (%)** |
| Diabetes | 21 (41%) | 46 (26%) | 46 (40%) |
| Pre-diabetes | 9 (18%) | 46 (26%) | 29 (25%) |
| Metabolic Abnormal | 13 (25%) | 44 (25%) | 22 (19%) |
| Healthy | 8 (16%) | 38 (22%) | 18 (16%) |

C – Risk allele
CC- Mutant genotype
CT – Heterozygous genotype
TT – Wild type genotype

Table 3.129. Distribution of the *HHEX* variants, by glycaemic and metabolic classification

The *HHEX* variants were explored with regards to the TG/HDL-C ratio, which is an indicative ratio for insulin resistance risk, as seen in Table 3.130. The majority of the *HHEX* variants exhibited an optimal TG/HDL-C ratio, indicating a normal insulin resistance in this subgroup. However, the T/T variant had a higher proportion of subjects falling within the "at risk" for insulin resistance subgroup than the other variants. Conversely, the C/T variant had the highest proportion of subject within the "very high risk" for insulin resistance subgroup.

| | ***HHEX* rs1111875** | | |
|---|---|---|---|
| | **TT (*n*=51)** | **CT (*n*=174)** | **CC (*n*=115)** |
| Optimal (<= 1.99) | 40 (78.43%) | 156 (89.67%) | 99 (86.09%) |
| At Risk (2.00 - 3.99) | 9 (17.65%) | 14 (8.05%) | 15 (13.04%) |
| Very high risk (4+) | 1 (1.96%) | 4 (2.30%) | 1 (0.87%) |

C – Risk allele
CC- Mutant genotype
CT – Heterozygous genotype
TT – Wild type genotype

Table 3.130. Distribution of the *HHEX* variants, by the TG/HDL-C risk ratio

#### 3.6.5.6.1 *HHEX* effect on biochemical and clinical parameters following a co-dominant model

The effect of *HHEX* on both biochemical and clinical parameters was assessed using a co-dominant genetic model, as seen in Table 3.131. However, no statistically significant differences were evident.

#### 3.6.5.6.2 *HHEX* relationship with biochemical and clinical parameters following a recessive model

The effect of *HHEX* on both biochemical and clinical parameters was assessed using a recessive genetic model, as seen in Table 3.132. An individual carrying a T allele (T/T + C/T) had a statistically lower diastolic blood pressure (β= -2.29, $p$=0.05) when compared to C/C genotype.

#### 3.6.5.6.3 *HHEX* effect on biochemical and clinical parameters following a dominant model

The effect of *HHEX* on both biochemical and clinical parameters was assessed using a dominant genetic model, as seen in Table 3.133. No statistically significant differences between the diverse genotypes was present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC | -0.02 | 0.44 | -0.89 | 0.85 | 0.96 |
| | CT | -0.61 | 0.42 | -1.43 | 0.21 | 0.15 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC | -0.03 | 0.16 | -0.34 | 0.29 | 0.87 |
| | CT | -0.11 | 0.15 | -0.40 | 0.19 | 0.47 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC | 0.001 | 0.081 | -0.16 | 0.16 | 1 |
| | CT | 0.02 | 0.08 | -0.13 | 0.18 | 0.75 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC | -0.12 | 0.14 | -0.39 | 0.15 | 0.37 |
| | CT | -0.11 | 0.13 | -0.37 | 0.14 | 0.39 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC | -0.02 | 0.17 | -0.35 | 0.31 | 0.90 |
| | CT | -0.11 | 0.16 | -0.42 | 0.21 | 0.50 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC | 0.28 | 1.04 | -1.77 | 2.33 | 0.79 |
| | CT | -0.003 | 0.99 | -1.93 | 1.93 | 0.99 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC | 2.12 | 2.63 | -3.03 | 7.27 | 0.42 |
| | CT | -1.48 | 2.48 | -6.34 | 3.38 | 0.55 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC | 1.49 | 1.74 | -1.92 | 4.90 | 0.39 |
| | CT | -0.95 | 1.64 | -4.16 | 2.27 | 0.56 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC | -1.16 | 3.05 | -7.15 | 4.82 | 0.70 |
| | CT | -1.42 | 2.88 | -7.07 | 4.23 | 0.62 |
| | TT | Reference | | | | |

C – Risk allele

CC- Mutant genotype

CT – Heterozygous genotype

TT – Wild type genotype

Table 3.131 *HHEX* [rs1111875] relationship with biochemical and clinical parameters in a co-dominant model

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CT + TT<br>CC | -0.56<br>Reference | 0.31 | -0.31 | 1.05 | 0.07 |
| LDL-C (mmol/L) | CT + TT<br>CC | -0.08<br>Reference | 0.11 | -0.75 | 1.14 | 0.47 |
| HDL-C (mmol/L) | CT + TT<br>CC | 0.01<br>Reference | 0.05 | -0.99 | 1.13 | 0.83 |
| Triglycerides (mmol/L) | CT + TT<br>CC | 0.01<br>Reference | 0.09 | -0.84 | 1.21 | 0.92 |
| Total Cholesterol (mmol/L) | CT + TT<br>CC | -0.10<br>Reference | 0.12 | -0.72 | 1.13 | 0.37 |
| BMI ($Kg/m^2$) | CT + TT<br>CC | -0.54<br>Reference | 0.72 | -0.14 | 2.40 | 0.46 |
| Systolic Blood Pressure (mmHg) | CT + TT<br>CC | -2.75<br>Reference | 1.78 | -0.02 | 2.60 | 0.12 |
| Diastolic Blood Pressure (mmHg) | CT + TT<br>CC | -2.29<br>Reference | 1.17 | -0.01 | 1.00 | **0.05** |
| Waist Circumference (cm) | CT + TT<br>CC | 0.32<br>Reference | 2.11 | -3.81 | 4.46 | 0.88 |

C – Risk allele

CC- Mutant genotype

CT – Heterozygous genotype

TT – Wild type genotype

Table 3.132 *HHEX* [rs1111875] relationship with biochemical and clinical parameters in a recessive model

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CT<br>TT | -0.56<br>Reference | 0.41 | -1.36 | 0.25 | 0.17 |
| LDL-C (mmol/L) | CC + CT<br>TT | -0.07<br>Reference | 0.14 | -0.35 | 0.22 | 0.65 |
| HDL-C (mmol/L) | CC + CT<br>TT | 0.07<br>Reference | 0.07 | -0.07 | 0.21 | 0.33 |
| Triglycerides (mmol/L) | CC + CT<br>TT | -0.15<br>Reference | 0.12 | -0.39 | 0.10 | 0.23 |
| Total Cholesterol (mmol/L) | CC + CT<br>TT | -0.02<br>Reference | 0.15 | -0.32 | 0.28 | 0.90 |
| BMI ($Kg/m^2$) | CC + CT<br>TT | -0.86<br>Reference | 0.95 | -2.73 | 1.02 | 0.37 |
| Systolic Blood Pressure (mmHg) | CC + CT<br>TT | -1.10<br>Reference | 2.37 | -5.74 | 3.55 | 0.64 |
| Diastolic Blood Pressure (mmHg) | CC + CT<br>TT | -0.21<br>Reference | 1.56 | -3.26 | 2.84 | 0.89 |
| Waist Circumference (cm) | CC + CT<br>TT | -3.94<br>Reference | 2.79 | -9.41 | 1.52 | 0.16 |

C – Risk allele

CC- Mutant genotype

CT – Heterozygous genotype

TT – Wild type genotype

Table 3.133 *HHEX* [rs1111875] relationship with biochemical and clinical parameters in a dominant model

### 3.6.5.7 Potassium voltage-gated channel subfamily E regulatory subunit 4, *KCNE4*, [rs1440072]

No statistically significant biochemical and clinical differences were observed between the different variants at the *KCNE4* locus, as seen in Table 3.134.

| | ***KCNE4* rs1440072** | | | |
|---|---|---|---|---|
| | **TT [WT]** | **CT [HT]** | **CC [MT]** | |
| | (*n*=215) | (*n*=108) | (*n*=12) | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.87 (1.68) | 5.80 (1.85) | 6.76 (1.49) | 0.11 |
| LDL-C (mmol/L) | 3.05 (1.29) | 3.02 (1.37) | 3.11 (1.39) | 0.94 |
| HDL-C (mmol/l) | 1.43 (0.54) | 1.35 (0.63) | 1.24 (0.49) | 0.20 |
| Triglycerides (mmol/L) | 0.97 (0.78) | 1.08 (1.00) | 1.16 (0.63) | 0.15 |
| Total Cholesterol (mmol/L) | 5.02 (1.41) | 5.06 (1.49) | 4.78 (1.09) | 0.77 |
| BMI (Kg/m$^2$) | 29.37 (9.57) | 28.80 (7.67) | 27.35 (9.74) | 0.58 |
| Systolic Blood pressure (mmHg) | 123.00 (21.00) | 120.67 (17.68) | 125.32 (10.67) | 0.68 |
| Diastolic Blood pressure (mmHg) | 75.00 (12.66) | 74.85 (11.00) | 75.00 (9.30) | 0.91 |
| Waist Circumference (cm) | 95.00 (26.90) | 94.35 (26.50) | 98.40 (22.00) | 0.65 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
C – risk allele

Table 3.134 Biochemical and anthropometric parameters, by *KCNE4* genotypes

#### 3.6.5.7.1 *KCNE4* effect on biochemical and clinical parameters following a co-dominant model

The effect of *KCNE4* on both biochemical and clinical parameters was assessed using a co-dominant genetic model. *KCNE4* genotypes exhibited a statistically significant association with triglycerides, as seen in Table 3.135. Individuals carrying the C/T genotype had a significantly higher triglyceride levels (β=0.20, *p*=0.04) compared to those with the T/T genotype.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC | 0.27 | 0.82 | -1.35 | 1.88 | 0.75 |
| | CT | 0.05 | 0.31 | -0.57 | 0.66 | 0.88 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC | 0.12 | 0.29 | -0.46 | 0.70 | 0.69 |
| | CT | -0.13 | 0.11 | -0.35 | 0.09 | 0.25 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC | -0.07 | 0.15 | -0.37 | 0.23 | 0.65 |
| | CT | -0.04 | 0.06 | -0.15 | 0.07 | 0.46 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC | -0.05 | 0.25 | -0.55 | 0.44 | 0.84 |
| | CT | 0.20 | 0.10 | 0.01 | 0.38 | **0.04** |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC | 0.06 | 0.32 | -0.56 | 0.68 | 0.84 |
| | CT | -0.06 | 0.12 | -0.30 | 0.17 | 0.61 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC | -2.63 | 1.92 | -6.39 | 1.13 | 0.17 |
| | CT | -0.80 | 0.73 | -2.22 | 0.63 | 0.27 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC | 0.90 | 4.83 | -8.57 | 10.37 | 0.85 |
| | CT | -1.43 | 1.83 | -5.02 | 2.16 | 0.44 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC | -1.77 | 3.19 | -8.03 | 4.49 | 0.58 |
| | CT | 0.44 | 1.21 | -1.94 | 2.81 | 0.72 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC | -2.30 | 5.31 | -12.70 | 8.11 | 0.67 |
| | CT | -3.07 | 2.01 | -7.02 | 0.87 | 0.13 |
| | TT | Reference | | | | |

C – Risk allele

CC – Mutant genotype

CT – Heterozygous genotype

TT – Wild type genotype

Table 3.135 *KCNE4* [rs1440072] relationship with biochemical and clinical parameters in a co-dominant model

#### 3.6.5.7.2 *KCNE4* effect on biochemical and clinical parameters following a recessive model

The effect of *KCNE4* on both biochemical and clinical parameters was assessed using a recessive genetic model, as seen in Table 3.136. No statistically significant differences between genotypes was present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | TT + CT | -0.26 | 0.80 | 1.30 | 1.81 | 0.75 |
| | CC | Reference | | | | |
| LDL-C (mmol/L) | TT + CT | -0.01 | 0.28 | 0.53 | 0.56 | 0.96 |
| | CC | Reference | | | | |
| HDL-C (mmol/L) | TT + CT | 0.17 | 0.14 | 0.45 | 0.11 | 0.23 |
| | CC | Reference | | | | |
| Triglycerides (mmol/L) | TT + CT | 0.12 | 0.24 | 0.59 | 0.35 | 0.63 |
| | CC | Reference | | | | |
| Total Cholesterol (mmol/L) | TT + CT | 0.18 | 0.30 | 0.76 | 0.41 | 0.55 |
| | CC | Reference | | | | |
| BMI ($Kg/m^2$) | TT + CT | 1.80 | 1.84 | 5.41 | 1.81 | 0.33 |
| | CC | Reference | | | | |
| Systolic Blood Pressure (mmHg) | TT + CT | -1.01 | 4.55 | -7.90 | 9.92 | 0.82 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure (mmH) | TT + CT | 1.03 | 2.98 | 6.87 | 4.81 | 0.73 |
| | CC | Reference | | | | |
| Waist Circumference (cm) | TT + CT | 1.16 | 5.11 | 11.18 | 8.85 | 0.82 |
| | CC | Reference | | | | |

C – Risk allele
CC – Mutant genotype
CT – Heterozygous genotype
TT – Wild type genotype

Table 3.136 *KCNE4* [rs1440072] relationship with biochemical and clinical parameters in a recessive model

#### 3.6.5.7.3 *KCNE4* effect on biochemical and clinical parameters following a dominant model

The effect of *KCNE4* on both biochemical and clinical parameters was assessed using a dominant genetic model, with no statistically significant differences between the diverse genotypes present, as seen in Table 3.137.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CT | -0.56 | 0.41 | -1.36 | 0.25 | 0.17 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC + CT | -0.07 | 0.14 | -0.35 | 0.22 | 0.65 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC + CT | 0.07 | 0.07 | -0.07 | 0.21 | 0.33 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC + CT | -0.15 | 0.12 | -0.39 | 0.10 | 0.23 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC + CT | -0.02 | 0.15 | -0.32 | 0.28 | 0.90 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC + CT | -0.86 | 0.95 | -2.73 | 1.02 | 0.37 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC + CT | -1.10 | 2.37 | -5.74 | 3.55 | 0.64 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC + CT | -0.21 | 1.56 | -3.26 | 2.84 | 0.89 |
| | TT | Reference | | | | |
| Body Weight (Kg) | CC + CT | -4.47 | 2.94 | -10.24 | 1.30 | 0.13 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC + CT | -3.94 | 2.77 | -9.41 | 1.52 | 0.16 |
| | TT | Reference | | | | |
| WHR | CC + CT | -0.02 | 0.01 | -0.04 | 0.01 | 0.18 |
| | TT | Reference | | | | |

C – Risk allele
CC – Mutant genotype
CT – Heterozygous genotype
TT – Wild type genotype

Table 3.137 *KCNE4* [rs1440072] relationship with biochemical and clinical parameters in a dominant model

### 3.6.5.8 Peroxisome proliferator activated receptor gamma. *PPARG*, [rs1801282]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *PPARG* locus, as seen in Table 3.138.

| | ***PPARGY* rs1801282** | | | |
|---|---|---|---|---|
| | **CC [WT]** | **CG [HT]** | **GG [MT]** | |
| | (*n*=231) | (*n*=37) | (*n*=73) | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.90 (1.81) | 5.76 (1.08) | 5.81 (1.82) | 0.42 |
| LDL-C (mmol/L) | 3.05 (1.33) | 3.08 (1.19) | 3.05 (1.42) | 0.76 |
| HDL-C (mmol/l) | 1.39 (0.53) | 1.48 (0.58) | 1.46 (0.54) | 0.36 |
| Triglycerides (mmol/L) | 1.01 (0.78) | 0.96 (0.49) | 0.98 (0.92) | 0.37 |
| Total Cholesterol (mmol/L) | 5.02 (1.48) | 4.99 (1.35) | 5.09 (1.37) | 0.77 |
| BMI (Kg/m$^2$) | 29.45 (9.33) | 29.10 (6.00) | 28.80 (9.57) | 0.91 |
| Systolic Blood pressure (mmHg) | 123.00 (18.70) | 120.00 (23.00) | 120.00 (18.00) | 0.24 |
| Diastolic Blood pressure (mmHg) | 75.00 (11.67) | 75.00 (12.00) | 73.33 (13.33) | 0.76 |
| Waist Circumference (cm) | 97.00 (27.00) | 96.00 (25.00) | 93.00 (28.00) | 0.54 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
G – risk allele

Table 3.138 Biochemical and clinical parameters, by *PPARG* genotypes

#### 3.6.5.8.1 *PPARG* effect on biochemical and clinical parameters following a co-dominant model

The effect of *PPARGY* on both biochemical and clinical parameters was assessed using a co-dominant genetic model, as seen in Table 3.139. No statistically significant differences were present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | p-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | GG | 0.39 | 0.36 | 0.23 | 3.00 | 0.27 |
| | CG | -0.72 | 0.47 | 0.19 | 1.23 | 0.13 |
| | CC | Reference | | | | |
| LDL-C | GG | -0.08 | 0.13 | -0.72 | 1.18 | 0.52 |
| | CG | -0.10 | 0.17 | -0.65 | 1.26 | 0.56 |
| | CC | Reference | | | | |
| HDL-C | GG | -0.01 | 0.06 | -0.88 | 1.13 | 0.92 |
| | CG | 0.15 | 0.08 | -0.98 | 1.37 | 0.08 |
| | CC | Reference | | | | |
| Triglycerides | GG | 0.10 | 0.11 | -0.90 | 1.37 | 0.35 |
| | CG | -0.17 | 0.14 | -0.64 | 1.12 | 0.24 |
| | CC | Reference | | | | |
| Total Cholesterol | GG | -0.06 | 0.14 | -0.72 | 1.23 | 0.66 |
| | CG | -0.14 | 0.18 | -0.61 | 1.23 | 0.42 |
| | CC | Reference | | | | |
| BMI | GG | 0.07 | 0.84 | -0.20 | 5.59 | 0.94 |
| | CG | -0.54 | 1.11 | -0.67 | 5.16 | 0.63 |
| | CC | Reference | | | | |
| Systolic Blood Pressure | GG | 1.65 | 2.09 | 0.00 | 11.56 | 0.43 |
| | CG | 2.81 | 2.76 | 0.00 | 13.48 | 0.31 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure | GG | 0.19 | 1.38 | 0.06 | 12.27 | 0.89 |
| | CG | 0.30 | 1.82 | 0.02 | 25.89 | 0.87 |
| | CC | Reference | | | | |
| Waist Circumference | GG | 2.49 | 2.47 | 0.00 | 10.41 | 0.31 |
| | CG | 0.87 | 3.25 | 0.00 | 246.96 | 0.79 |
| | CC | Reference | | | | |

G – Risk allele

GG – Mutant genotype

CG – Heterozygous genotype

CC – Wild type genotype

Table 3.139 *PPARG* [rs1801282] relationship with biochemical and clinical parameters in a co-dominant model

#### 3.6.5.8.2 *PPARG* effect on biochemical and clinical parameters following a recessive model

The effect of *PPARGY* on both biochemical and clinical parameters was assessed using a recessive genetic model, as seen in Table 3.140. No statistically significant differences were present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CG | -0.45 | 0.35 | -1.13 | 0.24 | 0.20 |
| | GG | Reference | | | | |
| LDL-C (mmol/L) | CC + CG | 0.06 | 0.12 | -0.18 | 0.30 | 0.62 |
| | GG | Reference | | | | |
| HDL-C (mmol/L) | CC + CG | 0.02 | 0.06 | -0.10 | 0.15 | 0.70 |
| | GG | Reference | | | | |
| Triglycerides (mmol/L) | CC + CG | -0.12 | 0.10 | -0.32 | 0.08 | 0.25 |
| | GG | Reference | | | | |
| Total Cholesterol (mmol/L) | CC + CG | 0.03 | 0.13 | -0.23 | 0.28 | 0.84 |
| | GG | Reference | | | | |
| BMI ($Kg/m^2$) | CC + CG | -0.01 | 0.81 | -1.60 | 1.58 | 0.99 |
| | GG | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC + CG | 1.62 | 2.02 | -2.34 | 5.57 | 0.42 |
| | GG | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC + CG | 0.20 | 1.32 | -2.38 | 2.79 | 0.88 |
| | GG | Reference | | | | |
| Waist Circumference (cm) | CC + CG | 2.28 | 2.35 | -2.34 | 6.89 | 0.33 |
| | GG | Reference | | | | |

G – Risk allele

GG – Mutant genotype

CG – Heterozygous genotype

CC – Wild type genotype

Table 3.140 *PPARGY* [rs1801282] relationship with biochemical and clinical parameters in a recessive model

#### 3.6.5.8.3 *PPARG* effect on biochemical and clinical parameters following a dominant model

The effect of *PPARGY* on both biochemical and clinical parameters was assessed using a dominant genetic model, as seen in Table 3.141. No statistically significant differences were present.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | GG + CG | -0.03 | 0.31 | -0.58 | 0.64 | 0.92 |
| | CC | Reference | | | | |
| LDL-C (mmol/L) | GG + CG | -0.12 | 0.11 | -0.10 | 0.33 | 0.29 |
| | CC | Reference | | | | |
| HDL-C (mmol/L) | GG + CG | 0.06 | 0.05 | -0.17 | 0.04 | 0.24 |
| | CC | Reference | | | | |
| Triglycerides (mmol/L) | GG + CG | 0.03 | 0.09 | -0.21 | 0.15 | 0.76 |
| | CC | Reference | | | | |
| Total Cholesterol (mmol/L) | GG + CG | -0.08 | 0.12 | -0.14 | 0.31 | 0.47 |
| | CC | Reference | | | | |
| BMI ($Kg/m^2$) | GG + CG | -0.04 | 0.72 | -1.37 | 1.45 | 0.95 |
| | CC | Reference | | | | |
| Systolic Blood Pressure (mmHg) | GG + CG | 1.73 | 1.79 | 1.78 | 5.24 | 0.33 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | GG + CG | 0.07 | 1.17 | -2.37 | 2.23 | 0.95 |
| | CC | Reference | | | | |
| Waist Circumference (cm) | GG + CG | -1.87 | 2.09 | -2.24 | 5.97 | 0.37 |
| | CC | Reference | | | | |

G – Risk allele

GG – Mutant genotype

CG – Heterozygous genotype

CC – Wild type genotype

Table 3.141 *PPARGY* [rs1801282] effect on biochemical and clinical parameters in a dominant model

### 3.6.5.9 Solute carrier family 30 member 8, *SLC30A8* [rs13266634]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *SLC30A8* locus, as seen in Table 3.142.

| | ***SLC30A8* rs1326634** | | | |
|---|---|---|---|---|
| | **TT [WT] (*n*=10)** | **CT [HT] (*n*=121)** | **CC [MT] (*n*=196)** | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.73 (0.74) | 5.89 (1.95) | 5.90 (1.79) | 0.72 |
| LDL-C (mmol/L) | 3.07 (0.68) | 3.01 (1.41) | 3.15 (1.33) | 0.28 |
| HDL-C (mmol/l) | 1.51 (0.36) | 1.42 (0.59) | 1.39 (0.51) | 0.55 |
| Triglycerides (mmol/L) | 0.96 (0.83) | 1.04 (0.81) | 0.98 (0.8) | 0.99 |
| Total Cholesterol (mmol/L) | 4.90 (0.59) | 5.01 (1.46) | 5.13 (1.51) | 0.35 |
| BMI ($Kg/m^2$) | 32.88 (11.16) | 29.40 (9.30) | 28.85 (8.82) | 0.36 |
| Systolic Blood pressure (mmHg) | 130.83 (31.30) | 120.70 (22.00) | 123.00 (17.03) | 0.99 |
| Diastolic Blood pressure (mmHg) | 76.35 (9.33) | 73.33 (11.00) | 75.00 (12.45) | 0.92 |
| Waist Circumference (cm) | 104.00 (27.50) | 95.00 (27.50) | 95.00 (27.00) | 0.87 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
C – risk allele

Table 3.142 Biochemical and clinical parameters by *SLC30A8* genotypes

#### 3.6.5.9.1 *SLC30A8* effect on biochemical and clinical parameters following a co-dominant model

The effect of *SLC30A8* on both biochemical and clinical parameters was assessed using a co-dominant genetic model, as seen in Table 3.143. No statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC | 0.72 | 0.86 | -0.97 | 2.42 | 0.40 |
| | CT | 0.71 | 0.88 | -1.01 | 2.43 | 0.42 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC | 0.14 | 0.31 | -0.46 | 0.75 | 0.64 |
| | CT | 0.04 | 0.31 | -0.57 | 0.66 | 0.89 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC | -0.10 | 0.16 | -0.41 | 0.20 | 0.50 |
| | CT | -0.08 | 0.16 | -0.39 | 0.23 | 0.63 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC | 0.22 | 0.27 | -0.31 | 0.74 | 0.41 |
| | CT | 0.25 | 0.27 | -0.29 | 0.78 | 0.36 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC | 0.13 | 0.33 | -0.52 | 0.78 | 0.69 |
| | CT | 0.02 | 0.34 | -0.64 | 0.68 | 0.96 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC | -3.52 | 2.01 | -7.46 | 0.41 | 0.08 |
| | CT | -2.66 | 2.04 | -6.66 | 1.33 | 0.19 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC | 3.14 | 5.13 | -6.92 | 13.20 | 0.54 |
| | CT | 5.46 | 5.21 | -4.75 | 15.67 | 0.30 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC | 2.73 | 3.38 | -3.89 | 9.36 | 0.42 |
| | CT | 4.10 | 3.43 | -2.63 | 10.82 | 0.23 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC | -4.73 | 5.82 | -16.15 | 6.68 | 0.42 |
| | CT | -3.12 | 5.91 | -14.71 | 8.47 | 0.60 |
| | TT | Reference | | | | |

C- Risk allele

CC – Mutant

CT – Heterozygous

TT – Wild type

Table 3.143 *SLC30A8* [rs1326634] relationship with biochemical and clinical parameters in a co-dominant model

### 3.6.5.9.2 *SLC30A8* effect on biochemical and clinical parameters following a recessive model

The effect of *SLC30A8* on both biochemical and clinical parameters was assessed using a recessive genetic model, with no significant difference as seen in Table 3.144.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | TT +CT | -0.03 | 0.30 | -0.57 | 1. 62 | 0.93 |
| | CC | Reference | | | | |
| LDL-C (mmol/L) | TT +CT | -0.12 | 0.11 | -0.10 | 0.13 | 0.28 |
| | CC | Reference | | | | |
| HDL-C (mmol/L) | TT +CT | 0.02 | 0.05 | -0.12 | 1.08 | 0.74 |
| | CC | Reference | | | | |
| Triglycerides (mmol/L) | TT +CT | 0.01 | 0.09 | -0.20 | 1.17 | 0.89 |
| | CC | Reference | | | | |
| Total Cholesterol (mmol/L) | TT +CT | -0.12 | 0.11 | -0.11 | 0.34 | 0.31 |
| | CC | Reference | | | | |
| BMI ($Kg/m^2$) | TT +CT | -0.64 | 0.71 | -2.03 | 0.75 | 0.37 |
| | CC | Reference | | | | |
| Systolic Blood Pressure (mmHg) | TT +CT | 0.77 | 1.78 | -4.21 | 2.68 | 0.66 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | TT +CT | 0.33 | 1.15 | -2.59 | 1.93 | 0.77 |
| | CC | Reference | | | | |
| Waist Circumference (cm) | TT +CT | 0.46 | 2.045 | -4.47 | 3.55 | 0.82 |
| | CC | Reference | | | | |

C- Risk allele
CC – Mutant
CT – Heterozygous
TT – Wild type

Table 3.144 *SLC30A8* [rs1326634] relationship with biochemical and clinical parameters in a recessive model

### 3.6.5.9.3 *SLC30A8* effect on biochemical and clinical parameters following a dominant model

The effect of *SLC30A8* on both biochemical and clinical parameters was assessed using a dominant genetic model, with no significant differences found as seen in Table 3.145.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CT | 0.75 | 0.86 | -0.94 | 2.44 | 0.39 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC + CT | 0.18 | 0.31 | -0.42 | 0.78 | 0.56 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC + CT | -0.10 | 0.15 | -0.38 | 0.19 | 0.52 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC + CT | 0.21 | 0.26 | -0.31 | 0.73 | 0.43 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC + CT | 0.16 | 0.32 | -0.47 | 0.79 | 0.62 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC + CT | -2.81 | 2.01 | -6.75 | 1.13 | 0.16 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC + CT | 3.71 | 5.00 | -6.08 | 13.51 | 0.46 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC + CT | 1.47 | 3.29 | -4.97 | 7.91 | 0.65 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC + CT | -2.08 | 5.82 | -13.48 | 9.33 | 0.72 |
| | TT | Reference | | | | |

C- Risk allele

CC – Mutant

CT – Heterozygous

TT – Wild type

Table 3.145 *SLC30A8* [rs1326634] relationship with biochemical and clinical parameters in a dominant model

### 3.6.5.10 Transcription factor 7 like 2, *TCF7L2,* 10q25.3, [rs7903146]

No statistically significant biochemical and clinical differences were observed between the different genotypes at the *TCF7L2* locus, as seen in Table 3.146.

| | ***TCF7L2* rs7903146** | | | |
|---|---|---|---|---|
| | **CC [WT]** | **CT [HT]** | **TT [MT]** | |
| | (*n*=128) | (*n*=159) | (*n*=53) | |
| | Median (IQR) | Median (IQR) | Median (IQR) | *p*-value* |
| Fasting plasma glucose (mmol/L) | 5.84 (1.66) | 5.87 (1.73) | 5.92 (2.48) | 0.97 |
| LDL-C (mmol/L) | 3.04 (1.28) | 3.02 (1.40) | 3.22 (1.33) | 0.60 |
| HDL-C (mmol/l) | 1.41 (0.69) | 1.43 (0.48) | 1.35 (0.54) | 0.72 |
| Triglycerides (mmol/L) | 0.96 (0.82) | 1.04 (0.80) | 0.98 (0.7) | 0.32 |
| Total Cholesterol (mmol/L) | 5.09 (1.29) | 4.96 (1.55) | 5.20 (1.46) | 0.54 |
| BMI (Kg/m$^2$) | 29.13 (10.45) | 29.40 (8.77) | 28.56 (6.23) | 0.48 |
| Systolic Blood pressure (mmHg) | 122.35 (20.00) | 123.00 (18.33) | 121.70 (14.30) | 0.92 |
| Diastolic Blood pressure (mmHg) | 75.00 (15.52) | 74.67 (11.67) | 75.00 (10.00) | 0.77 |
| Waist Circumference (cm) | 95.50 (29.00) | 95.00 (29.10) | 96.00 (22.22) | 0.92 |

*Kruskal-Wallis test; IQR: Interquartile range

WT – Wild Type genotype
HT – Heterozygous genotype
MT – Mutant genotype
T – risk allele

Table 3.146 Biochemical and clinical parameters by, *TCF7L2* genotypes

#### 3.6.5.10.1 *TCF7L2* effect on biochemical and clinical parameters following a co-dominant model

The effect of *TCF7L2* on both biochemical and clinical parameters was assessed using a co-dominant genetic model, as seen in Table 3.147. No statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG | TT | 0.35 | 0.44 | -0.60 | 3.38 | 0.42 |
| | CT | -0.01 | 0.32 | -0.53 | 1.86 | 0.98 |
| | CC | Reference | | | | |
| LDL-C | TT | 0.14 | 0.15 | -0.85 | 1.56 | 0.37 |
| | CT | 0.05 | 0.11 | -0.84 | 1.31 | 0.66 |
| | CC | Reference | | | | |
| HDL-C | TT | -0.03 | 0.08 | -0.83 | 1.13 | 0.68 |
| | CT | -0.06 | 0.06 | -0.84 | 1.05 | 0.26 |
| | CC | Reference | | | | |
| Triglycerides | TT | 0.06 | 0.13 | -0.82 | 1.38 | 0.65 |
| | CT | 0.14 | 0.10 | -0.96 | 1.39 | 0.14 |
| | CC | Reference | | | | |
| Total Cholesterol | TT | 0.12 | 0.16 | -0.81 | 1.55 | 0.48 |
| | CT | 0.01 | 0.12 | -0.80 | 1.27 | 0.97 |
| | CC | Reference | | | | |
| BMI | TT | 1.15 | 1.02 | 0.04 | 2.36 | 0.26 |
| | CT | -0.12 | 0.75 | -0.21 | 3.84 | 0.88 |
| | CC | Reference | | | | |
| Systolic Blood Pressure | TT | -0.28 | 2.55 | -0.01 | 110.99 | 0.912 |
| | CT | 0.92 | 1.8516 | 0.01 | 14.97 | 0.618 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure | TT | 1.51 | 1.67 | 0.01 | 5.85 | 0.37 |
| | CT | -0.69 | 1.22 | -0.05 | 5.46 | 0.57 |
| | CC | Reference | | | | |
| Waist Circumference | TT | 0.32 | 2.95 | 0.00 | 445.46 | 0.92 |
| | CT | 1.35 | 2.15 | 0.06 | 257.70 | 0.53 |
| | CC | Reference | | | | |

T – Risk allele
TT – Mutant
CT – Heterozygous
CC - Homozygous

Table 3.147 *TCF7L2* [rs7903146] relationship with biochemical and clinical parameters in a co-dominant model

### 3.6.5.10.2 *TCF7L2* effect on biochemical and clinical parameters following a recessive model

The effect of *TCF7L2* on both biochemical and clinical parameters was assessed using a recessive genetic model, as seen in Table 3.148. No statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | CC + CT | -0.36 | 0.40 | -1.15 | 0.43 | 0.37 |
| | TT | Reference | | | | |
| LDL-C (mmol/L) | CC + CT | -0.11 | 0.14 | -0.39 | 0.17 | 0.43 |
| | TT | Reference | | | | |
| HDL-C (mmol/L) | CC + CT | -0.01 | 0.07 | -0.14 | 0.14 | 0.97 |
| | TT | Reference | | | | |
| Triglycerides (mmol/L) | CC + CT | -0.02 | 0.12 | -0.22 | 0.26 | 0.87 |
| | TT | Reference | | | | |
| Total Cholesterol (mmol/L) | CC + CT | -0.11 | 0.15 | -0.41 | 0.18 | 0.45 |
| | TT | Reference | | | | |
| BMI ($Kg/m^2$) | CC + CT | 1.09 | 0.94 | -0.75 | 2.93 | 0.25 |
| | TT | Reference | | | | |
| Systolic Blood Pressure (mmHg) | CC + CT | -0.23 | 2.33 | -4.80 | 4.34 | 0.92 |
| | TT | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | CC + CT | 1.13 | 1.53 | -1.87 | 4.13 | 0.46 |
| | TT | Reference | | | | |
| Waist Circumference (cm) | CC + CT | 0.43 | 2.70 | -4.87 | 5.73 | 0.87 |
| | TT | Reference | | | | |

T – Risk allele
TT – Mutant
CT – Heterozygous
CC – Homozygous

Table 3.148 *TCF7L2* [rs7903146] relationship with biochemical and clinical parameters in a recessive model

### 3.6.5.10.3 *TCF7L2* effect on biochemical and clinical parameters following a dominant model

The effect of *TCF7L2* on both biochemical and clinical parameters was assessed using a dominant genetic model, as seen in Table 3.149. No statistically significant differences were evident.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| FPG (mmol/L) | TT + CT | 0.08 | 0.30 | -0.51 | 1.67 | 0.79 |
| | CC | Reference | | | | |
| LDL-C (mmol/L) | TT + CT | -0.07 | 0.11 | -0.76 | 1.15 | 0.49 |
| | CC | Reference | | | | |
| HDL-C (mmol/L) | TT + CT | 0.06 | 0.05 | 0.95 | 1.17 | 0.30 |
| | CC | Reference | | | | |
| Triglycerides (mmol/L) | TT + CT | -0.12 | 0.09 | -0.74 | 1.06 | 0.18 |
| | CC | Reference | | | | |
| Total Cholesterol (mmol/L) | TT + CT | -0.03 | 0.11 | -0.78 | 1.21 | 0.77 |
| | CC | Reference | | | | |
| BMI ($Kg/m^2$) | TT + CT | 0.37 | 0.70 | -0.37 | 5.77 | 0.60 |
| | CC | Reference | | | | |
| Systolic Blood Pressure (mmHg) | TT + CT | 0.76 | 1.75 | 0.07 | 65.64 | 0.66 |
| | CC | Reference | | | | |
| Diastolic Blood Pressure (mmHg) | TT + CT | 0.89 | 1.15 | 0.26 | 23.07 | 0.44 |
| | CC | Reference | | | | |
| Waist Circumference (cm) | TT + CT | 1.09 | 2.02 | 0.01 | 17.72 | 0.59 |
| | CC | Reference | | | | |

T – Risk allele
TT – Mutant
CT – Heterozygous
CC – Homozygous

Table 3.149 *TCF7L2* [rs7903146] relationship with biochemical and clinical parameters in a dominant model

### 3.6.6 Associations between the ten selected SNPs and type 2 diabetes mellitus

Generalized linear models, following a step-wise backwards regression model, were used to investigate the association between the ten SNPs and a diagnosis of type 2 diabetes mellitus.

In a co-dominant model, a significant association between *FTO* [rs9939609] and a diagnosis of diabetes mellitus was exhibited. Individuals carrying the A/A and A/T variant exhibited negative associations with having a diagnosis of type 2 diabetes mellitus, as seen in Table 3.150.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| ***FTO*** | AA | -0.73 | 0.35 | -1.41 | -0.05 | **0.04** |
| [rs9939609] | AT | -0.66 | 0.33 | -1.30 | -0.02 | **0.05** |
| | TT | Reference | | | | |

A – Risk allele

AA – Mutant genotype

AT – Heterozygous genotype

TT – Wild type genotype

Table 3.150 Significant association with a diagnosis of diabetes mellitus following a co-dominant model

On adjusting for potential confounding factors including age, gender and body mass index, the A/A variant (mutant genotype) remained significantly negatively associated with having a diagnosis of diabetes, as seen in Table 3.151.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| ***FTO*** | AA | -0.84 | -1.638 | -1.64 | -0.05 | **0.04** |
| [rs9939609] | AT | -0.71 | -1.462 | -1.46 | 0.05 | 0.07 |
| | TT | Reference | | | | |
| **Gender** | Female | 0.33 | 0.27 | -0.20 | 0.86 | 0.22 |
| | Male | Reference | | | | |
| **BMI** | | 0.13 | 0.02 | 0.08 | 0.17 | **<0.01** |
| **Age** | | 0.09 | 0.02 | 0.06 | 0.12 | **<0.01** |

A – Risk allele

AA – Mutant genotype

AT – Heterozygous genotype

TT – Wild type genotype

Table 3.151 Associations with a diagnosis of diabetes mellitus following a co-dominant model after adjustments for confounding factors

In a recessive model, a significant association between *FTO* [rs9939609] and a diagnosis of diabetes mellitus was found. Similarly (in a recessive model), a relationship was found between *FABP2* [rs1799883] and a diagnosis of diabetes mellitus. Individuals carrying the T-allele variant of the *FTO* genotype were negatively associated with having a diagnosis of diabetes mellitus, as seen in Table 3.152. Individuals carrying the C-allele variant of the *FABP2* genotype were positively associated with having a diagnosis of diabetes mellitus, as seen in Table 3.152.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| ***FTO*** | TT + AT | -0.64 | 0.33 | -1.28 | 0.00 | **0.05** |
| [rs9939609] | AA | Reference | | | | |
| ***FABP2*** | CC + CT | 1.27 | 0.56 | 0.18 | 2.36 | **0.02** |
| [rs1799883] | TT | Reference | | | | |

***FTO:*** A- Risk allele; AA – Mutant genotype; AT – Heterozygous genotype; TT – Wild type genotype

***FABP2:*** T- Risk allele; TT – Mutant genotype, CT – Heterozygous genotype, CC – Wild type genotype

Table 3.152 Significant associations with a diagnosis of diabetes mellitus following a recessive model

On adjusting for potential confounding factors including age, gender, body mass index and lipid profile, the *FABP2* variants lost their significance ($p$=0.20) whereas the *FTO* variants [TT+AA vs. AA] remained significant associated negatively with having a diagnosis of diabetes mellitus (B: -1.01, SE: 0.42, CI 95%: -1.83 to -0.18 $p$= 0.02).

In a dominant model, a statistically significant association between *KCNE4* and a diagnosis of diabetes mellitus was exhibited. Individuals carrying the *KCNE4* T-allele variant were significantly associated negatively with having a diagnosis of diabetes mellitus, as seen in Table 3.153.

| Parameter | Variants | B | Std. Error | 95% Confidence intervals | | *p*-value |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| *KCNE4* | TT + CT | -1.41 | 0.62 | -2.64 | -0.19 | **0.02** |
| [rs1440072] | CC | Reference | | | | |

T – Risk allele
TT – Mutant
CT – Heterozygous
CC – Wild type

Table 3.153 Significant association with a diagnosis of diabetes mellitus following a dominant model

On adjusting for potential confounding factors including age, gender, body mass index and waist circumference, the *KCNE4* variant [TT + CT vs. CC] remained significantly negatively associated with having a diagnosis of diabetes mellitus (B: -1.85, SE: 0.70, CI 95%: -3.22 to -0.47, $p$=<0.01).

# Chapter four – Discussion

## 4.1 Critical appraisal of the Maltese epidemiological studies

Over the last fifty years, four epidemiological studies on non-communicable diseases were conducted in Malta. The sample population for the first epidemiological study conducted by Prof. Zammit Maempel was not representative of the Maltese population as households in only two areas (one rural and one urban) participated. Conversely, all the other conducted studies, including the current study (SAHHTEK), had a randomized stratified sample population by age and gender. Only the Maltese 2010 European Health Examination Survey (EHES) pilot study was not conducted on a nationally representative sample of the Maltese population. All the surveys were cross-sectional. Table 4.1 compares the study designs across the different Maltese epidemiological studies. The participating population age groups varied across all studies, which makes it difficult to compare the studies together. However, a common adult cohort between the ages of 25 and 64 years was present throughout all the studies, which makes this age group fitting for comparison.

All Maltese epidemiological studies followed a different study protocol, although similarities were present. A socio-demographic questionnaire was distributed across all studies. Measurements for blood pressure, weight and height were also examined throughout all studies. The current study was the first that performed genetic analysis on a sub-population of the study population. Meanwhile the WHO (1981) study performed specialised examinations unlike the rest of the studies, as seen in Table 4.1.

| Study | Study design | Population | Data collected | Response |
|---|---|---|---|---|
| Zammit Maemple - 1964 | • All households in one urban area and one rural area<br>• House visits | All ages | **Phase I:** Urine for glycosuria (dipstick)<br>**Phase II:** Questionnaire, Physical exam, 50g OGTT<br>**Phase III:** Statistical analyses | 5757 subjects |
| Katona *et al.* - 1981 (WHO study) | • Randomised representative sample from the electoral list<br>• Stratified according to age, gender, occupation & education<br>• Cross-sectional study | >=15 years | **Phase I:** Fasting capillary sample from ear lobe, 75g OGTT, questionnaire, measurements for: blood pressure, weight, height & skinfold thickness<br><br>**Phase II:** Repeat OGTT in those with abnormal results + Bloods for insulin, C-peptide, HLA-type, blood groups, renal profile, uric acid, lipid profile, HbA1C. Ophthalmological exam, Neurological exam, ECG, Oscillographic recoding, Anthropometry, Cardiological interview & Nutritional interview<br><br>**Phase III:** Statistical analyses | 2945 subjects |
| Cacciattolo - 1984 (MONICA study) | • Randomised representative sample from the electoral list<br>• Stratified according to age & gender<br>• Cross-sectional study | 25 to 64 years | **Phase I:** Questionnaire, Measurements for: blood pressure, weight, height & pulse rate<br>Bloods for total cholesterol, HDL-C, gamma-glutamyl transferase & serum thiocyanate<br>**Phase II:** Statistical analyses | 2042 subjects |

OGTT – Oral glucose tolerance test;

| Study | Study design | Population | Data collected | Response |
|---|---|---|---|---|
| Pilot study EHES - 2010 | • Randomised sample from population register of NSO<br>• Stratified according to age, gender and region of residence | ≥18 years | **Phase I:** Questionnaire, Measurements for: blood pressure, weight, height, waist circumference<br><br>visual acuity, bloods for plasma glucose, HbA1C & total cholesterol. Spirometery test<br>**Phase II:** Statistical analyses | 221 subjects |
| Cuschieri *et al.* 2016 (SAHHTEK study) | • Randomised representative sample from ID cards & passport register<br>• Stratified according to age, gender and town of residence<br>• Cross-sectional study | 18 to 70 years | **Phase I:** Questionnaire, Measurements for: blood pressure, weight, height, waist circumference, hip circumference, bloods for fasting plasma glucose, lipid profile, whole blood sample for genetics analysis<br><br>**Phase II**: 75g OGTT on indeterminate fasting plasma glucose subjects (FPG between 5.60 – 6.99mmol/L)<br><br>**Phase III:** Genetic analyses: DNA extraction and Genotyping of 10 SNPs on a sub-population<br><br>**Phase IV:** Statistical analyses | 1861 subjects |

NSO – National statistical office; EHES – European health examination survey

Table 4.1 Comparisons between the Maltese epidemiological studies over the years

### 4.1.1 Response rate

In SAHHTEK, a 47.15% response rate was obtained which was considered as relatively good taking into consideration that an invasive health examination was being proposed. This is also in keeping with the 2010 Malta Health Examination Survey response rate, as well as with other health examination surveys conducted throughout Europe (Groves, 2004; Directorate for Health Information and Research., 2012; Mindell *et al.*, 2015; Jølle *et al.*, 2018). A decline in response rates for both health interview and health examination surveys have been reported across time, with response rates varying from 80% in the 1980s to 50-60% and even lower as the years progressed (Tolonen *et al.*, 2008). In fact, in the Czech edition of the European Health Examination Survey (EHES) in 2014/5, a nationally representative sample of 3,850 individuals were approached, with a response rate of 31.69% (Čapková *et al.*, 2017). The better-established SHeS in Scotland managed a response rate of 64% from all across Scotland (Scottish Government, 2016).

A similar response rate was obtained among the individuals that were offered the OGTT, even though these had already participated in the health examination survey earlier. Those not participating provided a number of reasons for declining the invitation, the most common being that "they were afraid to know more" and that "they were too busy to attend a 2-hour test". In such cases, the principle researcher advised them to visit their family doctor to verify their glycaemic status by follow-up. The OGTT response rate obtained in the current study was at par with that of a Norwegian study also performing OGTTs (Jølle *et al.*, 2018).

The steep decline in participation rates in epidemiological studies worldwide has been attributed to various reasons, including the increase in the number of surveys over recent years, as well as to the invasive procedures involved (Galea and Tracy, 2007). In fact, this decline can be observed across local epidemiological studies as seen in Table 4.1, where, as the years progressed, response rate declined.

The response rate for the health examination survey in this study rose with increasing participant age, which is in keeping with the established age and gender bias in response rates illustrated in other European surveys (Eagan *et al.*, 2002; Dunn *et al.*, 2004; Mindell *et al.*, 2015).

## 4.2 Type 2 diabetes mellitus

### 4.2.1 Screening for type 2 diabetes mellitus

The type 2 diabetes mellitus diagnostic criteria for this study followed the American Diabetes Association (ADA) and the World Health Organization (WHO) standard recommendations to use a fasting plasma glucose (FPG) ≥7mmol/l for the estimation of diabetes prevalence, with no repeat testing if a diagnosis of diabetes was established (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; World Health Organization, 2006; American Diabetes Association, 2018b). This case definition was reported to provide a good estimate of diabetes prevalence since on repeat testing of those with a FPG ≥7mmol/l, an approximate 75% of those previously tested had a confirmed clinical diabetes diagnosis (Mooy *et al.*, 1996; Christensen *et al.*, 2004; World Health Organization, 2006).

### 4.2.2 Prevalence of type 2 diabetes mellitus in Europe

The 2017 estimated European diabetes adult population prevalence was 8.80% (CI: 7.00 – 12.00%) for 20 to 79 year olds, with a projected increase to 10.20% (CI: 8.20 – 13.70%) by 2045 (International Diabetes Federation, 2017). According to the 2017 IDF estimates, Malta had the highest diabetes prevalence in Europe with 13.81% (CI: 7.93 – 16.89) of the total Maltese population suffering from the disease, followed by Cyprus with a prevalence of 10.43% (CI: 7.05 – 17.47) (International Diabetes Federation, 2017). Interestingly, the IDF reported a lower diabetes prevalence for Italy (8.45% CI: 7.61 – 10.09), which is the closest neighbouring country to Malta. Meanwhile, for the Northern country, the United Kingdom (UK), the T2DM prevalence rate was reported to be much lower (5.95% CI: 5.27 – 7.59).

The IDF prevalence rate is an overestimation of the true prevalence of diabetes in Malta, which might also be true in other countries. The IDF estimates are based on a number of sources with projections conducted to establish the prevalence rates (Guariguata *et al.*, 2011). Similar IDF overestimation of the actual diabetes prevalence rate was reported by a study performed in Switzerland to evaluate whether diabetes prevalence could be estimated using routine data. This concluded that although they (the authors) might have underestimated diabetes prevalence, it still gave a more realistic prevalence rate when compared to the IDF estimation (Bopp, Zellweger and Faeh, 2011). In contrast, a study conducted in Saudi Arabia comparing a national estimate study with IDF national estimates, concluded that the IDF estimates for 2011 and 2030 were underestimations for that country (Al-Quwaidhi *et al.*, 2014). These discrepancies have been attributed to the methods utilized by the IDF to calculate their predictions. These include logistic regression methods based on predictors of

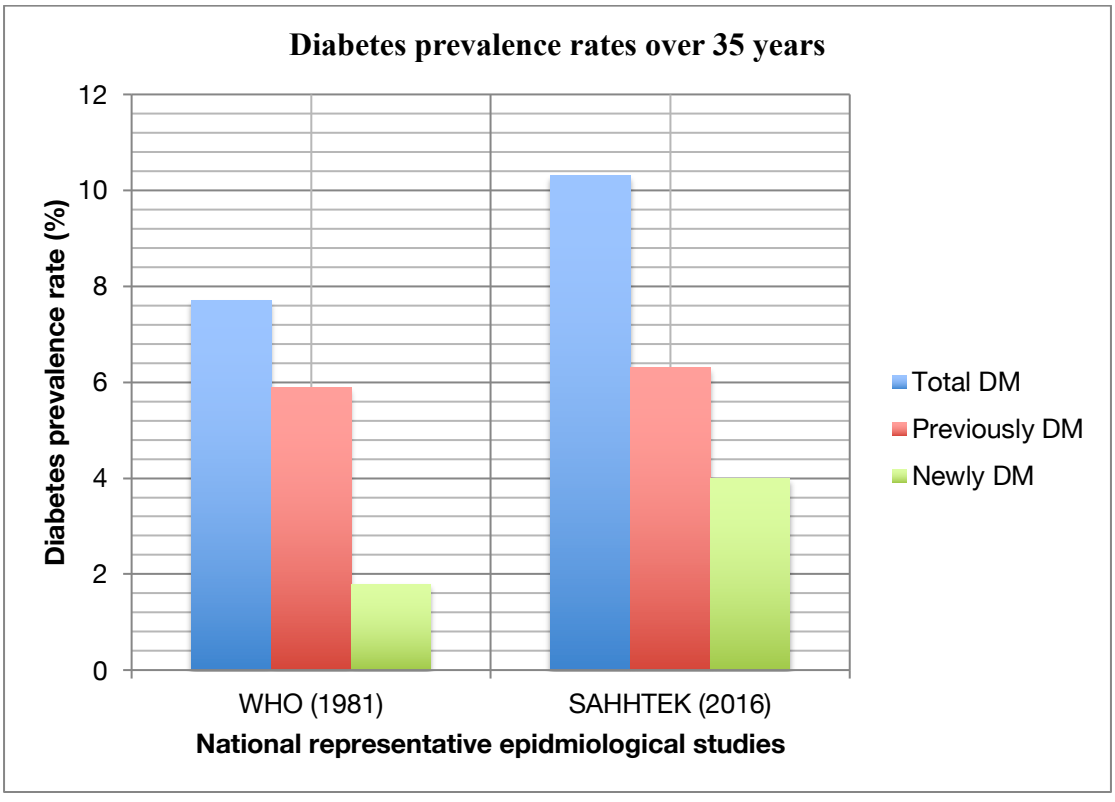
demographic changes of urbanization and aging rather than accounting for changes in the diabetes mellitus risk factors (Al-Quwaidhi *et al.*, 2014).

In 2015, the neighbouring country of Italy recorded a self-reported type 2 diabetes prevalence rate of 5.5% across all age groups (Istituto superiore di sanità (ISTAT), 2015). Interestingly the Italian Southern regions and the Islands exhibited higher T2DM prevalence rates when compared to the Northern Italian regions. In fact, Sicily, the closest Italian region to Malta, was reported to have a T2DM prevalence of 6% (Istituto superiore di sanità (ISTAT), 2015). On the other hand, the prevalence of T2DM in the UK (a Northern country) for both diagnosed and undiagnosed diabetes was estimated to be 8.6% of the population (16 years and over) in 2015 (Public Health England, 2016). These country specific prevalence rate estimates follow a different study design and age groups between themselves however the growing T2DM epidemic can be clearly illustrated across Europe.

Interestingly Malta, a Southern European country and in close proximity to Sicily (from where Malta imports the majority of its goods) appears to have higher T2DM prevalence rates than Sicily. In fact, it appears that the Malta T2DM prevalence was more similar to the UK's T2DM estimate. This may suggest that the Malta T2DM epidemic is not totally based on geographical orientation but could be related to other behavioural and lifestyle influences acquired during the British colonisation. However, these prevalence rates can only provide an indication and one cannot directly compare Malta to the other countries due to the difference in study design and age groups.

### 4.2.3 Prevalence of type 2 diabetes mellitus in Malta

An increase in the adult prevalence of type 2 diabetes mellitus in Malta was registered over the last 35 years, culminating in the rate being at a par to that of other Mediterranean Islands. In fact, Cyprus has estimated its diabetes mellitus prevalence at 10.30% and Sardinia at a 13.37% (Loizou *et al.*, 2006; Muntoni *et al.*, 2009). Similarly, in Italy an increase in T2DM prevalence was registered from 3.8% to 5.3% between 2000 and 2016 (Gargiulo, L; Burgio, A; Grippo, 2017). Additionally, a drastic increase in newly diagnosed diabetes prevalence rate can be appreciated in Malta when comparing the last national representative study conducted in 1981 to the present study, as seen in Figure 4.1.




DM – Diabetes mellitus

Figure 4.1 Comparisons between the 1981 WHO study and 2014 – 2016 SAHHTEK study of the diabetes sub-populations

Over 35 years, there has also been a shift in the gender predominance of type 2 diabetes mellitus. The current study demonstrates a male diabetes predominance contrasting with findings of the 1981 and 2010 surveys, which showed a female diabetes predominance. Similarly, a female predominance was also reported by a Norwegian health examination survey conducted between 1984 and 1986 with a gender shift on repeating the survey between 1995 and 1997 (Gale and Gillespie, 2001). This gender shift is in keeping with the rest of the world, where diabetes seemingly now affects more males than females (International Diabetes Federation, 2017). The male diabetes predominance has been reported to be due to an increasing obesity rates, from a young age, when compared to the female population. This increase in obesity susceptibility could have been the result of a change in social factors (Gale and Gillespie, 2001). Nowadays, the majority of the jobs are sedentary in contrast to the early part of the past century where jobs were more labour intensive, and travelling was done by foot or bicycle (Gale and Gillespie, 2001). Furthermore, males have greater hepatic and visceral fat stores and are physiologically less insulin sensitive than females (Geer and Shen, 2009). Therefore, one can hypotheses that males require less weight gain than females to develop T2DM, which would explain the male diabetes predominance. In fact, it was reported that biological differences between males and females are the fundamental components for the development of T2DM (Karastergiou *et al.*, 2012; Kautzky-Willer, Harreiter and Pacini, 2016). However, environmental, socioeconomical and cultural factors also play a role in T2DM susceptibility and gender differences (Karastergiou *et al.*, 2012; Kautzky-Willer, Harreiter and Pacini, 2016; Krag *et al.*, 2016).

Ageing is a risk factor in the development of diabetes and it has been established as being a contributor towards the ever-higher diabetes prevalence in Europe (International Diabetes Federation, 2017). The association between the increase in age and the increased diabetes prevalence has been found to be statistically relevant in the Maltese population (Nakagami *et al.*, 2003). This has also been exhibited in the 1981 study (Katona, G, Aganovic, I, Vuskan V, 1983). Such an association was confirmed in this study.

In the SAHHTEK study, the female with diabetes exhibited an earlier age of onset than males unlike the findings of the 1981 study. In 1981, males exhibited an earlier age of onset when compared to the current study (Katona, G, Aganovic, I, Vuskan V, 1983). One particular female age group within the current study's T2DM female population (30 – 49 years) did not report a history of gestational diabetes nor of a family history of T2DM. Therefore a genetic predisposition for their T2DM may be debatable (Yoon *et al.*, 2002). However, the median BMI of this subgroup was within the overweight-obese category. This might be the underlying cause of their insulin resistance with an eventual beta cell failure and the development of T2DM. Consequently, the early onset of T2DM among the Maltese female population could possibly be more due to environmental and behavioural factors (Kautzky-Willer, Harreiter and Pacini, 2016).

This study's female with diabetes also exhibited a higher gender prevalence at the older age spectrum (60 – 69 years). The females at this age range had the highest obesity prevalence rate, which may have contributed to this higher diabetes prevalence rate. A high adipose concentration resulting from weight gain (obesity) leads to a cascade of metabolic events including, but not limited to, promoting inflammation and increasing

circulatory free fatty acids resulting in a low grade chronic inflammation and the development of peripheral and hepatic insulin resistance (González-Muniesa *et al.*, 2017; Rocca *et al.*, 2018). This is known to eventually lead to beta cell failure and hyperglycaemia. An increase in adipose tissue is also a feature of menopause. Menopause is not directly associated with the development of T2DM, but the associated features of menopause are potential risk factors of T2DM. Apart from an increase in body adiposity, other related factors like androgenicity, sleep disturbances and midlife depression have all been found to increase the glucose levels and predispose to T2DM (Kim, 2012; Slopien *et al.*, 2018).

### 4.2.4 Co-morbidity and Type 2 diabetes mellitus in Malta

As expected, the previously known diabetes sub-population reported a number of cardiovascular co-morbidities, predominantly in the male population while a reported history of hypothyroidism predominated within the female population. It is a well-known fact that diabetes mellitus is a risk factor for the development of cardiovascular disease including stroke, coronary heart disease and peripheral arterial disease (Cooper, S; Caldwell, 1999; Dokken, 2008; Aronson and Edelman, 2014; Al-Nozha, Ismail and Al Nozha, 2016). This is supported by this study's findings where the diabetes population had a substantially higher prevalence of global (examined + self-reported) hypertension. However, since this is a retrospective study, it is difficult to assess the temporal relationship between diabetes and hypertension here.

The thyroid hormones are insulin antagonists and like insulin, are involved in cellular metabolism. The excess or deficiency of either hormones (thyroid or insulin) can lead

to a derangement of the other hormone (Sugrue, McEvoy and Drury, 1982). Therefore, presence of insulin resistance has been associated with thyroid dysfunction and in return, can be a major cause of impaired glucose metabolism (Wang, 2013). As exhibited in this SAHHTEK study, thyroid dysfunction was more prevalent in diabetes females than males (Perros *et al.*, 1995). A link appears to be present between thyroid disease and diabetes in the SAHHTEK study, especially in the female population, although further research is required in order to assess for temporal relationships. However, this link has already been reported previously (Perros *et al.*, 1995).

A substantial proportion of the study's diabetes population reported a first-degree family history of dysglycaemia, which is consistent with the well-established literature findings including local data (Schranz, 1989; Groop *et al.*, 1996). The risk of developing T2DM in first-degree relatives was found to be around 3.5 times that of the general average in the middle-aged population (Gloyn and McCarthy, 2001; Weires *et al.*, 2007). However, a family history of diabetes mellitus was not found to have an association with T2DM in this study. This may suggest that within the Maltese population, environmental and behavioural factors may be the leading risk factors contributing to the development of T2DM rather than genetic factors. In fact, in the genetic analyses performed as part of this study, the majority of the examined SNPs did not show any associated effect for the development of T2DM. However, considering this was a retrospective study and family history was self-reported, further research is required.

Furthermore, a number of female with diabetes reported to have suffered from gestational diabetes in this study. The relationship between gestational diabetes and the

increased risk of developing T2DM later on in life has been well explored in the literature and confirmed with this study's findings (Bellamy *et al.*, 2009; Bentley-Lewis, 2009).

### 4.2.5 The burden of type 2 diabetes mellitus in the Maltese population

Small islands, such as Malta, have cultural and geographical stressors that influence the developmental risk of certain metabolic diseases including type 2 diabetes mellitus and obesity (Formosa, Savona-Ventura and Mandy, 2012). The geographical position of Malta in the middle of the Mediterranean Sea brought about a genetic influx from all around the Mediterranean (Formosa, Savona-Ventura and Mandy, 2012). In fact, molecular SNPlotypes representing the metabolic syndrome, inflammatory response and maturity onset diabetes of the young (MODY) have shown similarities between the Maltese and the Libyan populations (Al-Ashtar, 2008). This suggests a similar genetic make-up between these two populations. Local studies have identified significant positive relationships between maternal and paternal family histories of diabetes with the presence of diabetes in adult females and males (Schranz, 1989; Savona-Ventura, Schranz and Chircop, 2003). In the present study, a proportion (less than half) of diabetes individuals had a first-degree family history of type 2 diabetes mellitus, which is a common finding in the literature (Katulanda *et al.*, 2015; Vornanen *et al.*, 2016). This reflects the genetic susceptibility of individuals along with shared, disease-enhancing environments and behaviours that could eventually influence the development of T2DM (Yoon *et al.*, 2002). The SAHHTEK survey, in contrast with several other studies, including European ones, showed that the presence of a positive family history was not significantly associated with having diabetes (InterAct

Consortium *et al.*, 2013; Katulanda *et al.*, 2015; Vornanen *et al.*, 2016). It has been reported that although populations with chronic diseases tend to have family history characteristics, it is not typical of the majority of the people with chronic diseases to have a family history of the disease (Yoon *et al.*, 2002).

The factors with a positive association with T2DM in this study were found to be an increase in fasting plasma glucose (FPG), an increase in age, a diagnosis of the metabolic syndrome (MetS) and a history of hypertension. These associations were established when the T2DM sub-population was compared to the non-T2DM population. However, when comparing the T2DM sub-population to the metabolically healthy sub-group (100% metabolically normal), several differences were observed. In fact, only an increase in FPG and an increase in triglyceride levels exhibited a positive association with the presence of T2DM, while the female gender had a negative association with the presence of T2DM. *All the different factors and their positive and negative relations to T2DM will be discussed individually in depth in the following sections of the discussion*.

Two sets of multiple regression analyses were performed in order to explore for potential metabolic confounding factors within the non-diabetes population that might affect the outcome. The non-diabetes sub-population still exhibit considerable underlying metabolic abnormalities, such as dyslipidaemia, hypertension, obesity and insulin resistance. Such metabolic abnormalities may predispose to eventual dysglycaemia (Nyenwe and Dagogo-Jack, 2011; Grundy, 2012). Non-alcoholic liver disease (NAFLD) is a metabolic abnormality associated with insulin resistance, dyslipidaemia, obesity, hypertension as well as with an increase in circulatory glucose

and lipid substrates (Marchesini *et al.*, 2001; Hazlehurst *et al.*, 2016). The lipotoxicity present in NAFLD might affect the pancreatic beta cells and contribute to eventual T2DM (Firneisz, 2014). In fact, it has been reported that individuals with NAFLD are at high risk of developing T2DM (Balkau *et al.*, 2010; Sung *et al.*, 2012; Kotronen *et al.*, 2013; Jäger *et al.*, 2015). Therefore, when analysing for associations with T2DM while comparing to the healthy sub-group (100% metabolically normal), any underlying insulin resistance or NAFLD factors were eliminated.

In summary, FPG is one of the diagnostic/screening tests to identify T2DM (World Health Organization, 2006; American Diabetes Association, 2018b), so it is expected to exhibit a positive association with T2DM, even when underlying metabolic abnormalities are excluded. Ageing is a well-established risk factor for the development of T2DM (Kirkman *et al.*, 2012; International Diabetes Federation, 2017). This follows the fact, that as age advances, there is an increased burden on the pancreas, resulting in a progressive decline in its function. Consequently, glucose metabolism impairment will result (Basu *et al.*, 2003; Bryhni, Arnesen and Jenssen, 2010). Such pancreatic decline and glucose impairment may be due to underlying metabolic abnormalities such as lipotoxicity, which correspond to the established lack of association between ageing and T2DM when comparing to the metabolically healthy sub-group in this study.

The presence of T2DM or of an elevated FPG level constitutes one of the diagnostic components for a diagnosis of the metabolic syndrome (Alberti, Zimmet and Shaw, 2006). So it comes to as no surprise that a diagnosis of the metabolic syndrome had an association with the diagnosis of T2DM (when comparing to the non-diabetes population) in this study (Hayashi *et al.*, 1999; Conen *et al.*, 2007; Kim *et al.*, 2015).

However, a diagnosis of the metabolic syndrome can be established by the presence of other abnormal components (rather than dysglycaemia), including dyslipidaemia and hypertension along with obesity. These components have been reported to be strongly associated with NAFLD and regarded as the liver manifestation of metabolic syndrome (Marchesini *et al.*, 2001). Therefore, as expected, when analysing for associations with T2DM while comparing to the metabolically healthy sub-group, no associations between a history of metabolic syndrome and T2DM were established.

Conversely, LDL-C was found to have a negative association for having T2DM even after adjusting for statin medication when comparing to the non-diabetes population. This association could be the result of metabolic confounders, lifestyle interventions and environmental factors that were not quantified in this study. Another plausible cause could be the presence of an underlying type B LDL-C phenotype that may not be picked up on routine biochemical testing (Feingold *et al.*, 1992). (*This will be discussed in further detail when discussing the relationship between biochemical parameters and T2DM*.) However, this association between LDL-C and T2DM was abolished when T2DM population was compared to the metabolically healthy sub-group. This brings forward the possibility that the LDL-C negative association with T2DM was confounded by the presence of metabolic abnormalities found within the non-diabetes population.

A raised diastolic blood pressure was negatively associated with the presence of a diagnosis of T2DM, when compared to the non-diabetes population. Such an association could not be found in the literature and therefore could be a sporadic result or due to the underlying metabolic confounding factors. In fact, this association was

lost when comparing the T2DM outcome to the metabolically healthy sub-group. However, a possible explanation for such an association could be due to the fact that T2DM individuals are usually middle-aged or elderly and it has been reported that diastolic blood pressure decreases with age. This results from the fact that ageing affects arterial stiffness, contributing to early pressure wave recoil. This results in a decrease in diastolic pressure and an increase in systolic pressure (Osher and Stern, 2008). Hence, physiologically an increase in diastolic blood pressure is more likely to occur in younger individuals and these are less likely to have developed T2DM yet.

### 4.2.6 Risk factors for Malta's type 2 diabetes mellitus

Different entities and countries have proposed various diabetes predictive models, but a Malta-specific diabetes predictive model has never yet been established. A two-stage predictive model has been found to be effective, where the initial stage consists of a non-invasive and easy to perform model (Tabák *et al.*, 2012). The second stage (include laboratory testing) is performed only if the first stage resulted in a positive predictive outcome (Tabák *et al.*, 2012).

Research tools utilising anthropometric parameters have been previously used to assess for the presence of non-communicable diseases in populations. These are inexpensive and easy to use while being useful for monitoring at community levels (Seidell *et al.*, 2001). In fact, these have evolved to be reliable predictive indicators for various non-communicable diseases risk factors. However, different populations have different predictive anthropometric variables and threshold cut-off values (Himabindu *et al.*, 2013).

Defining individualized cut-off points for health examination measurement indices (such as BMI, waist circumference, blood pressure) is essential for effective disease screening (Berber *et al.*, 2001). Over the years, different entities including the WHO, have provided different anthropometric cut-off points according to the population under study (Molarius *et al.*, 1999; World Health Organization, 2006).

The established diabetes risk score for Malta was the combination of age (≥55 years), high waist circumference (male ≥100cm, female ≥90cm) and a history of statin therapy. These three factors contribute to the Maltese diabetes's characteristics.

Age is a well-established risk factor for diabetes mellitus and thus establishing age as a predictor of diabetes mellitus is justified (Mooradian, AD; McLaughlin, S; Boyer, CC; Winter, 1999; American Diabetes Association, 2018b).

Different international organisations have established the use of the waist circumference as a good diagnostic criterion for metabolic complications (Alberti, Zimmet and Shaw, 2006; Kagawa, Byrne and Hills, 2008). In this study, when non-invasive parameters were assessed, the waist-circumference measurement was found to have a significant independent predictive ability for diabetes in Malta, unlike the body mass index (BMI) and coincides with other studies (Hardy *et al.*, 2017; Luo *et al.*, 2018). This confirms that within the Maltese population, the presence of central abdominal fat is more important than the total amount of body fat as a predictor of type 2 diabetes mellitus. The waist-circumference measure is a good surrogate marker for visceral fat since it reflects the presence of fat around the visceral organs and abdomen (Molarius and Seidell, 1998). Visceral fat has endocrine functions and is known to be

an independent risk factor for T2DM (Bray *et al.*, 2008). Furthermore, central obesity has been associated with alterations in glucose insulin homeostasis, with decrease glucose tolerance, with a decrease in metabolic insulin clearance and with a decrease in insulin-stimulated glucose disposal (Vazquez *et al.*, 2007).

Comparably, Deloumeaux *et al.* (2004) in their study exhibited the same higher discriminatory ability of the waist circumference over the BMI for predicting T2DM (Deloumeaux, Ninin and Foucan, 2004). Other studies, including a German study, exhibited the same findings with the waist circumference as being a superior predictive tool for diabetes than BMI (Folsom *et al.*, 2000; Hartwig *et al.*, 2016). The waist circumference has however, been found to be liable to differences in its cut-off points depending on ethnicity and gender (Lear *et al.*, 2010). In fact, different international organizations put forward different cut-off points, the commonest being 94cm for the European males and 80cm for the European females (International Diabetes Federation, 2006; Lau *et al.*, 2007). The optimal sensitivity and specificity for the waist circumference as a predictor of T2DM found for the SAHHTEK Maltese population, by gender, resulted in a higher waist circumference cut-off points than those reported in European survey cut-off points. This possibly follows the fact that the majority of the Maltese population, irrespective of any other existing comorbidity, is either overweight or obese as the norm, as was found in this study (Cuschieri *et al.*, 2016). A higher than normal waist circumference is a common finding among overweight and obese individuals. Another probable explanation is that international organizations could have shifted the cut off in order to maximize sensitivity at the cost of specificity.

A medical history of statin use implies that the individual has been diagnosed with previous dyslipidaemia or has experienced a cardio-metabolic event, or is at higher risk of cardiovascular disease (Shah and Goldfine, 2012). All of these different possibilities have been established as being risk factors for diabetes mellitus (Alberti, Zimmet and Shaw, 2006; Pathak and Pathak, 2012; Ali *et al.*, 2015; American Diabetes Association, 2018b). In spite of the fact that this study (SAHHTEK) could not assess for temporal relationships between cardiovascular disease and T2DM or the intake of statins and T2DM, a link could be observed between intake of statins and T2DM. More research to identify whether cardiovascular disease, high lipid profile or intake of statins are the possible causation of T2DM is required.

The Maltese specific predictive risk score was found to have adequate sensitivity and specificity when based on the entire diabetes cohort as well as when excluding the previously known diabetes cohort. The latter exhibited a much better sensitivity and specificity for establishing diabetes risk within high risk population and could be considered as a valid public health intervention tool. On comparing the sensitivity and specificity of the predictive ability of the Maltese specific score to a well-established international score (FINDRISC diabetes risk score) within the Maltese study population, it was observed that the Maltese specific risk score had a higher sensitivity and specificity. The desirable predicative ability of risk scores usually dependent on the specific population characteristics that the score was formulated on. However, some scores, such as the FINDRISC have been found to have adequate predictive ability if used in other populations (Bernabe-Ortiz *et al.*, 2018). Alas, this it is not always the case (Glumer *et al.*, 2006) as illustrated in this study. In fact, this study population was found to have phenotypic and genotypic differences from neighbouring countries as

well as between the Maltese islands districts. These differences may explain the reason as to why the FINDRISC exhibited very low sensitivity and specificity for type 2 diabetes risk prediction within the Maltese cohort. This justifies the generation of a Malta-specific equation. In fact, different countries have taken up a similar task in order to establish their own country specific predictive tool to enable the best predictive ability for their specific population characteristics (Glümer *et al.*, 2004; Kengne *et al.*, 2014; Paprott *et al.*, 2016).

However, one needs to keep in mind that although this risk score will assist physicians to identify individuals at higher risk of diabetes, some individuals may be wrongly labelled as being at higher risk given that sensitivity is less than 100%. It is therefore suggested that those individuals found to be at risk of diabetes mellitus (positive predictive outcome) using this three-predictor risk score, are further investigated by having laboratory testing performed, as was previously suggested (Tabák *et al.*, 2012).

### 4.2.7 Type 2 diabetes mellitus and depression

Depression is a very common non-communicable disease, with the WHO claiming that an estimated 350 million people worldwide suffer from this disease in 2016 (World Health Organization, 2016). The depression (self-reported) prevalence rate in this study was found to be lower than that of six other European countries (14%) that were studied in a pan-European study (Munizza *et al.*, 2013). However, the Maltese depression rate appeared to be higher than that of the neighbouring country of Italy (4.2%) (Munizza *et al.*, 2013). The Maltese population followed the well-established

trend where females were more likely to suffer from depression when compared to the male population (Kornstein *et al.*, 2000; Roy and Lloyd, 2012).

Diabetes individuals are commonly associated with a diagnosis of depression (Anderson *et al.*, 2001; Fisher *et al.*, 2007; Mommersteeg *et al.*, 2013). However, this was not the case in the current study (SAHHEK). Similar results were previously reported in European, American and other Ethnic studies (Aujla *et al.*, 2009; Roy and Lloyd, 2012).

The diabetes individuals in the SAHHTEK study portraying a history of depression were mostly in the older age bracket (50+ years in males and 60+ in females). It could be hypothesised that diabetes mellitus might have led to socio-psychological problems over time (Johnson and Wolinsky, 1993). Furthermore, this study's findings show that diabetes depressed individuals exhibited a low educational level and were mostly retired, coinciding with a Spanish study (Salinero-Fort *et al.*, 2018). This could partly be explained by the fact that the majority of this subgroup was above the age of 60 years. It was evident that there was a cohort effect with younger generations being better educated than their older cohorts. Another probable reason accounting for the depression distribution within SAHHTEK study follows the fact that the longer the duration of T2DM diagnosis the higher the odds of developing depression (Almeida *et al.*, 2016).

As expected, the SAHHTEK diabetes population who reported to suffer from depression had a poorer glycaemic control than the diabetes population without a history of depression (Lustman *et al.*, 2000). This poor glycaemic control could be

related to the effect of depression on the individual's diabetes self-care including maintaining a healthy diet, physical activity and adherence to medication (Lin *et al.*, 2004; Gonzalez *et al.*, 2008).

## 4.3 Impaired fasting glucose

### 4.3.1 Screening for impaired fasting glucose

The SAHHTEK protocol followed the ADA cut-off point in view of the fact that there is a lack of IFG data within the Maltese population. This is similar to the European scenario where data is mostly based on predictions and assumptions (International Diabetes Federation, 2017). The only prior Maltese data available is from the 1981 WHO survey, in which the impaired glucose tolerance (IGT) prevalence rate (15+ years) was 5.60% with a female predominance (Katona *et al.* 1983). Approximately 20% of OGTT diagnosed diabetes have a FPG less than 6.10mmol/L, especially in females and the elderly (Shaw, Zimmet and Alberti, 2006). Following this reported fact and that Malta lacked specific IFG base-line, the IFG cut-off point for SAHHTEK was taken as an ≥5.60mmol/L. The SAHHTEK study protocol did not include an OGTT on every participant due to limited human resources and financial constraints.

### 4.3.2 Impaired fasting glucose in Malta

The Malta IFG prevalence was more than twice that of diabetes mellitus, indicating that a large proportion of the Maltese non-diabetes population was at high

risk of developing type 2 diabetes mellitus. Similarly, other European countries reported high IFG prevalence rates when following the same IFG criteria as SAHHTEK. In fact, a Danish study reported a 37.6% prevalence among 30 to 60 years olds while a German study reported a 26.4% prevalence among the adults of the North-eastern region and a 17.2%. prevalence among the adults of the Southern region (Borch-Johnsen *et al.*, 2004; Tamayo *et al.*, 2014). A European meta-analysis reported that the average IFG prevalence rate was of 8.4% for adults from 18 year and above (Eades, France and Evans, 2016). Diabetes is the tip of the proverbial iceberg when it comes to impaired glucose metabolism.

The Maltese IFG population characteristics followed the typical phenotypes of a pre-diabetes population with the majority being overweight or obese and suffering from dyslipidaemia (Valensi *et al.*, 2005).

The typical age of onset for pre-diabetes is 45 years of age for both genders. It is also the age at which screening is recommended to be initiated for pre-diabetes and diabetes (American Diabetes Association, 2018b). In the Maltese population, the average age of onset of IFG was much younger than that found in some of the literature (Yin *et al.*, 2015; Vaidya *et al.*, 2016; American Diabetes Association, 2018b). However, the SAHHTEK IFG age of onset coincided with published pan-European meta-analysis data (Eades, France and Evans, 2016).

Approximately half of the IFG population in this study reported a family history of T2DM. In fact, across four German studies, the IFG population was found to be significantly associated with a family history of T2DM (Wagner *et al.*, 2013). Such

findings may point to an element of genetic inheritability for this dysglycaemic state. However, in this study, no association with family history of T2DM was established even though a substantial proportion of the IFG population reported a family history. Furthermore, only a small proportion of the IFG population reported co-morbidities. Glycaemic related co-morbidities, such as cardiovascular disease, might still be in their infancy stage of development due to the possible mild dysglycaemic state and atherosclerosis may still have not developed (Grundy, 2012). However, the relationship between fasting plasma glucose and cardiovascular disease is continuous or J-shaped which implies that as the glucose level increases over a substantial period of time, it is more likely for cardiovascular co-morbidities to emerge (DECODE Study Group, 2003; Lawes *et al.*, 2004).

Those individuals with an established pre-diabetes diagnosis have been reported to stand at higher than average risk (approximate 30%) of developing full-blown diabetes mellitus, cardiovascular disease, cardiovascular mortality as well as increased all-cause mortality (Valensi *et al.*, 2005; Wen *et al.*, 2005). In fact, it was reported that IFG individuals (FPG 5.6 – 6.99mmol/L) had an annual relative risk of developing T2DM of 4.7% (CI 95%: 39 – 46) when compared to normoglycaemic individuals (Tirosh *et al.*, 2005). Hence, given that in Malta the age of onset of IFG appeared to be substantially lower than elsewhere as shown in the literature, this raises the discussion as to whether an early screening program should be considered for high-risk individuals.

### 4.3.3 Impaired fasting glucose risk in Malta

The established associated risk predictors for IFG were different from those of T2DM for this study population. These findings coincide with those from an American study that reported discrepancies present between risk factors contributing to IFG and to T2DM (Okwechime, Roberson and Odoi, 2015).

This study confirms the findings from another study performed in Spain, which strongly links abdominal obesity with pre-diabetes (Díaz-Redondo *et al.*, 2015). An increase in central adiposity, which is measured by waist circumference, leads to an increase in circulatory free fatty acids and this induces insulin resistance. This contributes to an increase in plasma glucose and can present as IFG. However, a long duration of circulating high free fatty acids would impair the beta cell function due to lipotoxicity and promote higher glucose concentration with an eventual development of T2DM (Grundy, 2012).

High concentrations of free fatty acids initiate triglyceride production. The triglyceride levels are considered as markers of glucose control and insulin resistance (Lee *et al.*, 2016; Kang *et al.*, 2017). In IFG individuals, the insulin resistance level may still be potentially low as is the triglyceride levels to reach pathological levels. In fact, the median triglyceride levels for the IFG population of this study were within the normal range. Hence, this may be a probable explanation for the negative link between triglyceride levels and IFG. Another probable explanation could be the small sample size of the IFG population. Additionally, there may have been other unknown potential environmental and genetic factors affecting the understudy IFG sub-population that were not considered here as potential confounders.

Ageing is a well-known risk factor for progressive pancreatic impairment, especially with the associated increased insulin sensitivity and loss of beta cell function (Bryhni, Arnesen and Jenssen, 2010; Okwechime, Roberson and Odoi, 2015). This results in defective insulin secretion followed by an elevation in plasma glucose levels (Basu *et al.*, 2003). This metabolic physiology of ageing coincides with the established associated effect of age on IFG in this study. In fact, this Malta based population study showed an association between the increase in age and the risk of developing pre-diabetes. This is similar to a previously reported population-based study, although in that study the association was especially true for women irrelevant of their body mass status (Hilawe *et al.*, 2016), which was not the case in the SAHHTEK study.

It has been reported that IFG is commoner in males than in females across all age groups, which was also found in this study (The DECODE Study Group., 1998, 2003; Unwin *et al.*, 2002). These gender differences may be due to differences arising from body size, genetics and fasting glucose levels, where females have been reported to have an overall better insulin sensitivity than the males (Williams *et al.*, 2003; Glechner *et al.*, 2015; Graham *et al.*, 2015). The Maltese population had a lower prevalence of females with pre-diabetes and diabetes, as well as a lower female overweight-obese prevalence rate (Cuschieri *et al.*, 2016). All of these factors may have had a contributing effect on this finding.

This study (SAHHTEK) established an opposite link between the diastolic blood pressure and IFG when compared to the link between diastolic blood pressure and T2DM. Such a finding coincides with a Mediterranean study reporting an independent association between diastolic dysfunction and IFG (Milwidsky *et al.*, 2015),

# 4.4 Overweight-Obese population

## 4.4.1 Prevalence of overweight-obese population

The overweight-obese epidemic is affecting all European countries, with the southern countries exhibiting a higher overweight problem than some of their northern counterparts (Brandt and Erixon, 2013). It comes as no surprise, therefore, that Malta - a southern European country, was declared to have one of the highest obesity rates in Europe in a pan-European study (World Health Organization, 2014). In effect, the SAHHTEK study revealed a great majority of the adult population to be suffering from either overweight or obesity, which is in keeping with other international data (World Health Organization, 2018b). Similarly, a Southern Italian study reported that the overweight (34.5%) and obesity (16.1%) prevalence rates are on the rise within the adult population (18+ years) (Osella *et al.*, 2014). However, the obesity epidemic is a global European problem with the highest self-reported overweight prevalence rates found to be in Czech Republic (45.2%) and the highest obesity rates in Slovenia (20.8%) when the adult population (18+ years) of twenty Northern and Eastern European countries were compared together (Marques *et al.*, 2018). However, there are a number of factors that may be contributing to higher overweight-obesity rates in certain countries, such as in Malta, when compared to other European countries. The food consumption and the physical activity patterns of a population along with cultural values, environmental structures, social inequalities, social gradients, economic factors and stress, are all modulating factors that contribute to the overweight-obesity prevalence of a country (Blundell *et al.*, 2017). In fact, one of the cultural norms of Malta is to indulge in a large meal portion size as well as the tendency to engage in feasts and celebrations with abundance of food and drink typically high in saturated

fats and sugars (Malta Standards Authority, 2010; Formosa, Savona-Ventura and Mandy, 2012). The Maltese also exhibit a tendency to dine out when socialising with friends and family (Piscopo, 2004). Malta has also undergone a cultural shift from the traditional Mediterranean lifestyle to an Anglo-Saxon lifestyle (Tessier and Gerber, 2005). Unfortunately, Malta being a small island, depends highly on food imports, which also has a negative impact on the dietary habits and choices (Atkins and Gastoni, 1997). Furthermore, Malta is a high-densely populated island with lack of open spaces, unlike other European countries, along with a hilly geographical terrain (Price waterhouse Coopers, 2010; National Statistics Office., 2017) which potentially act as hindrance factors from undergoing physical activity and so enhancing the obesogenic environment in Malta. Additionally, the high vehicle density and traffic congestion hinders individuals in engaging in walking and cycling attitudes, especially due to the limited infrastructure available (Price waterhouse Coopers, 2010; Directorate-General for Communication, 2013).

On comparing this study's (SAHHTEK) results with the last health examination study (2010 pilot) conducted in Malta, there appears to have been a reduction in the prevalence of overweight individuals (not significant $p$=0.55) but a significant increase in the prevalence of obesity ($p$=0.05) (Directorate for Health Information and Research., 2012; Cuschieri *et al.*, 2016). One needs to be cautious when interpreting the 2010 EHES results since this was a pilot study based on a relatively small sample size. A similar comparison with the 1981 WHO survey was performed (Katona, G, Aganovic, I, Vuskan V, 1983). This comparison showed interesting differences. There was primarily an increase in the obese population ($p$=<0.01) (especially in males, $p$=<0.01) and a relative decrease in the overweight ($p$=0.02) and normal weight

($p$=0.14) categories in the SAHHTEK study when compared to 1981 WHO study. The SAHHTEK female normal weight category increased ($p$=<0.01) while the male normal weight category decreased ($p$=<0.01). Overall in recent decades, the obesity rate among adults in Malta has increased, especially in males (Cuschieri *et al.*, 2016). Furthermore, the female population were more within normal weight category may be the result of being more health conscious and somehow are managing to maintain a healthier lifestyle than their male counterparts. The increase in male obesity is in keeping with other European countries (Iceland and Norway) including the neighbouring country of Italy (OECD, 2012; Osella *et al.*, 2014; Marques *et al.*, 2018), although the opposite is true for Latvia, Turkey and Hungry (OECD, 2012).

The Malta overweight-obese epidemic is also present in children. In the most recent Health Behaviour in School-Aged Children (HBSC) survey (2013-2014), Malta ranked as the country with the highest prevalence of obese children aged between the ages of 11 and 15 (World Health Organization., 2016). Italy also reported a childhood obesity crisis in 2016, where obesity accounted for 9.3% of Italy's children, while 22.5% were overweight (Silano, Agostoni and Fattore, 2018). This childhood obesity European problem was set as a priority action by Malta's health authorities in the 2017 EU Presidency hosted by Malta (Independent News, 2016).

Regretfully, such an obesogenic status is expected to decrease the non-communicable disease-free years projected for adults populations, independent of the different socioeconomic level, physical activity and smoking habits (Nyberg *et al.*, 2018).

# 4.5 Metabolic syndrome

## 4.5.1 Metabolic syndrome prevalence

The metabolic syndrome is an established public health epidemic with an estimated quarter of all European adults suffering from this condition (Grundy, 2008; International Diabetes Federation, 2015). Southern European countries tend to exhibit higher overweight and hypertension rates than their Northern counterparts, predisposing these populations to a greater risk for MetS development (Brandt and Erixon, 2013; Scuteri *et al.*, 2015). The current study (based in Malta, which is a Southern European country) confirms the high prevalence of overweight and obesity (Cuschieri *et al.*, 2016). The study's adult MetS prevalence rate was found to be higher than in several other Mediterranean countries, notably in Italy (females 18%; males 15%) and Greece (23.6%), even if these are based on older studies (Athyros *et al.*, 2005; Miccoli *et al.*, 2005).

The male population in this study had a statistically higher prevalence of MetS when compared to the female population, in contrast to the dominating global female MetS prevalence trends (Beigh and Jain, 2012). However, it was reported that overall, European studies report little gender disparity (Rochlani, Pothineni and Mehta, 2015). The reason for Malta's male to female discrepancy could be related to the fact that, unlike in most of Southern Europe where the females predominantly exhibit abdominal obesity, in Malta, males are more obese than their female counterparts (Scuteri *et al.*, 2015; Cuschieri *et al.*, 2016). However, the female gender was found to be an independent risk factor for MetS on adjusting for various confounding factors. This finding is in keeping with the general literature trend (Beigh and Jain, 2012).

Our study showed an increase in MetS prevalence with age, similar to the pattern shown in other Mediterranean countries (Anagnostis, 2012). In fact, age was found to be an independent risk factor for having MetS in Malta.

Pre-menopausal females (<60 years) tended to have a lower MetS prevalence rate when compared to males of similar age in Malta. This was in keeping with the findings of other studies published (Song *et al.*, 2015, 2016). However, with increasing age, the prevalence of females with MetS was found to increase and can range from 20 to 60% especially in elderly females (Liu *et al.*, 2013). This is consistent with what is known of the pre- to post-menopausal hormonal transition. The process of the menopause presents with altered hormonal milieu whereby the oestrogen levels decline while the testosterone levels persists (Lasley *et al.*, 2002; Hopper *et al.*, 2014). In fact, it was found that higher testosterone levels had an effect on the components of MetS, namely central adiposity, triglycerides and HDL-C levels (Haffner and Valdez, 1995; Pugeat *et al.*, 1995). Menopausal females tend to increase in abdominal adiposity, develop insulin resistance and dyslipidaemia leading to an increased risk to develop MetS (Song *et al.*, 2016). The associate effects found in this study are in keeping with this trend.

### 4.5.2 Metabolic syndrome components

The factors contributing to the development of the metabolic syndrome depend on the environment, gender, age, region and ethnicity of the population (Kaur, 2014). However, insulin resistance has been declared to be the key mediator for this cardio-metabolic disease with a direct relationship to obesity (Lotta *et al.*, 2017).

In this study, all the different metabolic components showed independent predictive ability towards the presence of MetS. The triglyceride level was found to have the highest predictive ability for MetS from all the MetS components. Hypertriglyceridemia is an established risk factor for MetS and is responsible for the enzyme 'cholesterol ester transfer protein' stimulation. This enzyme facilitates the exchange of cholesteryl esters to triglycerides. It also facilitates the movement of triglyceride-rich lipoproteins to HDL-C and LDL-C (Tao *et al.*, 2016). This results in an elevated triglyceride and reduced HDL-C levels. Both components contribute to the development of MetS as well as act as risk factors for cardiovascular diseases (Singh, Gupta and Khajuria, 2015; Amin *et al.*, 2016).

Low-density lipoprotein (LDL-C), especially the small dense LDL-C component, although not a direct contributor to MetS, still has metabolic and atherogenic effects (Gazi *et al.*, 2006). In fact, in our study, the MetS population exhibited a high prevalence of elevated LDL-C levels, although it was established to have an association effect against having MetS for the Maltese MetS population. A reason for this effect could be due to the presence of high dense LDL-C particles rather than small dense LDL-C particles in our study population. It has been reported that high dense LDL-C is not associated with MetS (Holvoet *et al.*, 2008). Another possible explanation for this LDL-C effect could be due to the increased likelihood of being prescribed statins, which alter the LDL-C physiology. In fact, statins are prescribed for a number of reasons; as a primary prevention against cardiovascular disease; for the management of cardiovascular disease and in those with a diabetes mellitus diagnosis (Bonetti *et al.*, 2003; Bibbins-Domingo *et al.*, 2016). One needs to keep in mind that this association

could have been confounded by unmeasured confounding factors such as environmental and genetic elements.

Non-HDL has also been found to be an independent risk factor for MetS in the Maltese population. The non-HDL category includes all the apolipoprotein B-containing lipoproteins, which consist of cholesterol, LDL-C as well as triglyceride-rich chylomicrons, chylomicron remnants, and very low-density lipoprotein (VLDL) (Liu and Reaven, 2013). Considering that dyslipidaemia forms the basis of MetS and that the non-HDL variable is an indicator of all apolipoproteins, it stands to reason that it was found to be a predictor for MetS. In fact, the non-HDL value has been reported to serve as a useful identification tool for MetS in children and adolescents (Li *et al.*, 2011).

The triglyceride to high-density lipoprotein ratio (TG/HDL-C) has been established as a marker for both insulin resistance and for small dense LDL-C, with strong associations with MetS components (Amin *et al.*, 2016). However, the TG/HDL-C ratio has not been intensely investigated as a MetS predictor and different population cut-off points have been proposed. In this study (SAHHTEK), the cut-off points adopted for the TG/HDL-C ratio were those established for insulin resistance in view of the fact that insulin resistance is part of MetS development. This ratio (TG/HDL-C) appeared to have a significant association effect for the Maltese population before adjusting for different cofounding factors, after which, TG/HDL-C was not found to be an independent risk factor for MetS. The outcome could have been brought about by the presence of a higher proportion of high-dense LDL-C, which is not a recognized marker of TG/HDL-C (Janiszewska, Kubica and Odrowąż-sypniewska, 2015).

### 4.5.3 Metabolic syndrome and medical history

A medical history of hypertension was found to be an independent risk factor for MetS in the Maltese population. In fact, a history of hypertension is a well-established risk factor and contributor for the syndrome (Alberti, Zimmet and Shaw, 2006).

### 4.5.4 Metabolic syndrome and type 2 diabetes mellitus

The metabolic syndrome predisposes to the development of type 2 diabetes mellitus, which in turn is a diagnostic criterion for MetS (Alberti, Zimmet and Shaw, 2006). This study showed that more than a quarter of the MetS population do in fact suffer from diabetes mellitus. This corresponds well with the fact that insulin resistance is the basis of the pathophysiology of both conditions (Lotta *et al.*, 2017). Both MetS and diabetes also exhibit similar dyslipidaemia characteristics, which consist of low levels of HDL-C and elevated levels of triglycerides, (Singh, Gupta and Khajuria, 2015) as seen in this study (SAHHTEK).

### 4.5.5 Metabolic syndrome and obesity

Obesity is a major component of the metabolic syndrome. This study's findings demonstrate that a high percentage of obese individuals also suffer from the MetS. The positive correlation between BMI and MetS clearly indicates that as the BMI increases so too does the presence of MetS, although here (in SAHHTEK study) the actual BMI was not found to be an independent risk factor for MetS.

On the other hand, waist circumference was found to be an independent risk factor of MetS within the Maltese population. By direct inference, individuals with an increased waist circumference stand at higher risk of developing cardio-metabolic risks than those who are simply overweight but had a normal waist circumference.

Different studies have proposed different conclusions with regards to the best index for diagnosing MetS. Suggestions put forward include ethnicity and racial variations (Ko *et al.*, 2012; Bener *et al.*, 2013). Waist circumference is a measure of central fat distribution while BMI combines both fat mass and lean mass in its measurements. This led to the argument that waist circumference was a better predictor for MetS (Czernichow *et al.*, 2011). The SAHHTEK study confirms the finding that waist circumference remains a more telling indicator of metabolic risk (as well as for diabetes) rather than BMI in the Maltese population.

### 4.6 Effects of socio-demographic status

Socioeconomic status (SES) is a complex term combining a number of variables, including employment status, education level, income, wealth as well as place of residence. SES has been reported to impact on a number of diseases (Winkleby *et al.*, 1992). In fact, SES is a well-established cardiovascular risk factor and means for predicting behaviour (Minor, Wofford and Wyatt, 2008; Lam, 2011). Poor socioeconomic status has been associated with poorer health outcome, although it is important to consider underlying heterogeneity (Kennedy *et al.*, 1998; Adler and Ostrove, 1999; Smith, 2003). The education level was established as the best marker of

SES since it offers the most stable measure at an individual level and is immune to reverse causation such as income and wealth status (Minor, Wofford and Wyatt, 2008).

### 4.6.1 Socio-demographic status by anthropometric and biochemical factors

#### 4.6.1.1 Socio-demographic status by body mass index and waist circumference

##### 4.6.1.1.1 Education levels, body mass index and waist circumference

A low socioeconomic status has been associated with an increased risk of developing an overweight and obese status (McLaren, 2007). An inverse relationship was established in the SAHHTEK study between the education level and BMI. The lower the education levels within this study population, the higher the BMI status measured. This relationship was also present for waist circumference, especially in females, and is consistent with some other studies (Molarius *et al.*, 2000; Hermann *et al.*, 2011). The majority of the low education levels sub-populations had an overweight-obese BMI status. In fact, an associated risk was established, in this study, between those reporting education level only till primary school as well as those stopping school before finishing secondary school and an increased BMI. No significant links with the "no formal education" sub-population was established, which could be due to the small sample size within this sub-category.

Persons with low education and hence low socioeconomic status population in a developed country such as Malta are less likely to want or be able to choose a healthy lifestyle since such a lifestyle cannot be afforded. A healthy lifestyle implies the consumption of natural foods over processed foods; as well as engaging in physical

activity that might require the investment in sports equipment and/or joining a health club (Kim, Symons and Popkin, 2004).

#### 4.6.1.1.2 Districts, body mass index and waist circumference

Residence in the different districts within the Maltese Islands did not appear to impinge on the BMI status nor on waist circumference, even though socioeconomic differences may be present across the different localities. Studies conducted in Europe and in America investigating the socioeconomic differences across districts found a relationship between the different districts and BMI status (Cummins *et al.*, 2007; Do and Finch, 2008). A probable reason for this study's findings could be related to the small area (316Km$^2$) that makes up the Maltese Islands. However, on establishing the prevalence rates for overweight-obesity, differences were found between the different districts. The Gozo district exhibited the highest overweight-obese prevalence rate when compared to the other Maltese districts. This finding coincides with another local study examining the overweight-obese situation among young school children, where it was reported that boys residing in Gozo were significantly more overweight and obese than the Maltese districts (Sant'angelo *et al.*, 2011). Behavioural, social, environmental and genetic factors might be contributing to such findings (García-Mendizábal *et al.*, 2009), which are difficult to quantify. Therefore, the lack of potential associations between the different districts and BMI / waist circumference in this study could have been confounded by any of the above-mentioned factors (i.e. behavioural, social, environmental and genetics).

#### 4.6.1.1.3 Employment status, body mass index and waist circumference

Current employment was negatively associated with having obesity in this study, irrespective of age and gender. This relationship is supported by other studies (Morris, 2007; Lindeboom, Lundborg and van der Klaauw, 2010). Conversely, those employed in this study showed an associated link with having an increase in waist circumference, irrespective of age and gender. These contradictory findings could be due to the presence of underlying cofounding factors that were not accounted for. However, this study established that BMI and the waist circumference measurements consider different aspects of the human biology. Waist circumference is the more ideal measurement for visceral and abdominal adiposity (Kok, Seidell and Meinders, 2004) and therefore a probable marker for health problems. On the other hand, the BMI does not take into consideration the body fat distribution apart from not differentiating between body weight due to muscle bulk and fat (Burkhauser and Cawley, 2008). Therefore, the associated effects established with employment could be related to differences between the BMI and waist circumference.

An employed individual may be considered as leading a more "active" daily lifestyle due to the daily commute to work. The tasks performed during the day may contribute to a large proportion of their daily activities, which might be in contrast to those who are unemployed. Even those with a sedentary employment are more likely to be more physically active than those unemployed (Van Domelen *et al.*, 2011). The employed are more likely to be able to afford affluent lifestyles. The consumption of fruit, vegetables and pertaining to a healthy lifestyle is more likely to be sustainable by the employed (Dave and Kelly, 2012). These factors may explain the negative link between obese status and employment. However, the employed population is more likely to have

access to and indulge in refined sugary foods as well as to consume larger meals that will lead to an increase in adipose deposition and hence increased waist circumference. On the contrary, the unemployed are more likely to consume cheap processed high fat, salt and sugar food that is readily available in the communities. Such food consumption would also contribute to an increase in waist circumference.

It was observed that both the retired population and those performing domestic tasks (housewives) exhibited the highest overweight-obese prevalence rates when compared to the other employment categories. The retired population was mostly composed of adults between the ages of 60 to 70 years. Physiologically, as the individual gets older, the fat mass increases while the fat-free mass (primarily skeletal muscle) decreases (Baumgartner *et al.*, 1995; Gallagher *et al.*, 1996). The increase in total fat mass has been attributed to a decrease in energy expenditure, an increase in energy intake or a combination of both. Physical activity tends to decrease as the age increases for various biological reasons such as the presence of osteoarthritis, cardio-vascular or musculoskeletal co-morbidities. This decrease in physical activity accounts for about one-half of the decrease in total energy expenditure that occurs physiologically with ageing (Tzankoff and Norris, 1977). An increase in energy intake and deliberate decrease in physical activity due to sedentary life, in combination to the physiological increase in fat mass and physiological decrease in physical activity, tend to promote an overweight-obese status. Consequently, the overweight-obese status exacerbates the age-related decline in physical function including the activities of daily living, contributing to a further increase in body mass and waist circumference (Hubert, Bloch and Fries, 1993). This may be a probable explanation to the high prevalence rate of overweight-obese within the study's retired population. In fact, a positive association

between retired females and an increase in waist circumference has been established. This association was only significant for the female population, in contrast to the male population, possibly due to the biological changes (menopause) that occur in females during the retirement period. Post-menopausal females have been associated with an increase in intra-abdominal fat mass, which supports this study's findings (Toth *et al.*, 2000; Donato *et al.*, 2006).

Housewives/men were also found to have high overweight-obese prevalence rates. These are often portrayed as always being on the go to maintain a sustainable household (Yuhaniz and Jusan, 2016). However, the amount and type of household activities along any other physical activity need to be considered. If the individual effectively does little aerobic work, calories are not sufficiently burnt leading to weight gain and eventually obesity (Lombard, Catherine; Teede, 2009). Different household activities are related to different calorie usage. Manually sweeping the floor can burn 40 – 45 calories while utensils cleaning can burn 22 to 26 calories (Saboo *et al.*, 2014). Nutritional intake and habits need to be considered as well. Individuals who spend a lot of time alone at home might end up frequently snacking on readily available processed foods. Another scenario may be that meals are skipped and then after a long period of time a big meal is consumed. Both scenarios can contribute towards an increase in body mass (Chung *et al.*, 2011).

#### 4.6.1.2 Socio-demographic status by blood pressure

##### 4.6.1.2.1 Education level and blood pressure

An inverse relationship was established between blood pressure and education level in the SAHHTEK study, with those having stopped their education at primary education exhibiting the highest blood pressure measurements. In fact, this was further enhanced by the presence of a significant associated effect of low education levels on having hypertension. Age and gender did however have confounding effects on some education levels. The relationships between the level of education and blood pressure levels has already been reported in other studies (Ordunez *et al.*, 2005; Wang *et al.*, 2006). A low education level is associated with decreased awareness on the importance of following a good, healthy and wellness lifestyle. Persons with low levels of education tend to also have a decreased ability for blood pressure maintenance (Wang *et al.*, 2006). However, paradoxically, in the current study those reporting no formal education were found to have a significantly lower blood pressure when compared to those with primary school education. This seemingly strange finding might be accounted for by the fact that a very small population sample reported 'no formal' education. This might have been caused by type 2 error, although further investigation of this sub-group is required.

##### 4.6.1.2.2 Districts and blood pressure

Residing in particular districts within the Maltese Islands (Gozo, Western and South Eastern districts) was linked with an elevation in blood pressure. This coincides with findings elsewhere (Kiefe *et al.*, 1997; Levine *et al.*, 2011; Wang *et al.*, 2018).

However, on adjusting for potential confounding factors, only the Western district remained significantly associated with an elevation in blood pressure. Even though potential confounding factors were adjusted for, other unaccounted environmental and biological confounding factors might still distort this link.

The Western district is less densely populated than other Maltese districts (National Statistics Office., 2017). In a recent study, it was reported that a rural dwelling was associated with higher blood pressure compared to an urbanized dwelling (Wang *et al.*, 2018). This suggests that the association observed in this study might be related to the level of population density and environmental exposures within the district. Furthermore, the Western district population reported the highest "no formal education" when compared to the rest of the districts. Lower education contributes to lower knowledge on healthy lifestyle as well as on preventive measures of hypertension (Wang *et al.*, 2018). Having a low education level might contribute to lower employment rate or low income that consequently leads to the inability to afford a healthy lifestyle. In fact, the Western district population was ranked the second highest population for unemployment status (self-reported), when compared to the other Malta districts. All these factors might be contributing to the observed associated effects between the Western district and blood pressure.

#### 4.6.1.2.3 Employment status and blood pressure

Those retired had the highest systolic blood pressure measurements. It is a well-known fact that as the age progresses, arterial stiffening occurs due to atherosclerotic deposits leading to the development of secondary hypertension (Jani and Rajkumar

2006). The arterial stiffening is accelerated by the presence of other co-morbidities such as smoking and the presence of type 2 diabetes mellitus (Leone 2011, Epstein and Sowers 1992). However, effectively no independent link between being retired population and hypertension was found in this study, as this relationship was completely confounded by age and gender

#### 4.6.1.3 Socio-demographic status by biochemical profile

##### 4.6.1.3.1 Districts and biochemical profile

Significant differences in the median FPG, LDL-C and total cholesterol levels were evident between Gozo and the other Maltese districts. The population of Gozo also had higher biochemical profile levels. It was noteworthy that place of residence (especially Gozo) was linked with variable biochemical profiles (FPG, LDL-C and total cholesterol) in our study. Residence in Gozo (as compared to the Southern Harbour) was independently linked with having an increase in FPG, LDL-C and total cholesterol. Additionally, both the Northern Harbour and Northern district also exhibited an independent increased risk of having a higher total cholesterol. This suggests that a genetic element (possibly due to higher inter-breeding) could be predisposing the Gozo population to a higher risk of dyglycaemia and dyslipidemia unless there are other different environmental factors that are unaccounted for. Notwithstanding the fact that both the Maltese and Gozo populations remain part of one nation, differences in the environment, in cultural attitudes towards health and behavioural influences may be contributing to these biochemical variations. In fact, it is known that social and environmental influences contribute between 45% and 60% of health status variations (Donkin *et al.*, 2017).

#### 4.6.1.3.2 Education level and biochemical profile

Higher education levels have long been associated with better health and quality of life (Backlund *et al.* 1999, Baker *et al.* 2011). In fact, low education levels were associated with an increased risk of having elevated FPG and LDL-C levels, along with an associated risk of low HDL-C levels within the current study. HDL-C levels increased with higher education levels, suggesting that the higher the education level, the more the individuals were equipped with the right health information and were able to maintain a healthier lifestyle with a better biochemical outcome although this could only be borne out from a prospective study (Bachmann *et al.*, 2003).

#### 4.6.1.3.3 Employment status and biochemical profile

Students exhibited better biochemical profiles when compared to all other employment categories in our study. This is in contrast with findings elsewhere, where young adults have had more health-related problems such as obesity and dyslipidaemia (Liang *et al.* 2015).

Being retired was linked to having higher FPG levels, however this relationship was lost after adjusting for age and gender. The retired population is normally elderly (>60 years), which coincides with the physiological changes of pancreatic impairment and insulin resistance, leading in turn to an increase in plasma glucose levels (Lyssenko *et al.* 2005). However, a retired status was associated with an associated effect against the elevation in total cholesterol levels, even after adjusting for age and gender. This finding could be the result of confounding factors such as due to medication taken for

preventive measures or else for management due to other co-morbidities (Pedro-Botet *et al.* 2015).

#### 4.6.1.4 Social determinants of health and health inequalities

Differences in the social determinants of health and health inequalities could be contributing to the observed differences in the health status, the biochemical and the anthropometric parameters between the Malta and the Gozo districts. Notwithstanding the fact that this study was not aimed to explore social determinants of health or health inequalities in Malta, it is a requisite to consider these factors as potential contributors as well as confounding influences for the findings established in this study.

Social determinants of health, defined as the conditions in which people are born, grow, live, work and play, influence health (World Health Organization, 2008a). Evidence shows that the lower the socioeconomic status of a population the worse is the health status (Marmot and Commission on Social Determinants of Health, 2007). The main indicator for the socioeconomic status of participants was the highest education level. The Gozo district population had the highest population proportion (when compared to the Malta districts) reporting '*education till primary school*', which is a low education level. However, the Gozo district population reported the lowest population proportion with '*education level till secondary school*', which was the commonest educational level reported by the study population. Lower socioeconomic status in Gozo district may be contributing to the observed poor metabolic profile.

Another possible explanation to the health inequalities within the Gozo district follows the geographical limitations, where Gozitan students are limited in their options where to further their education post-secondary school level. This leads to children and adolescents enduring possible higher academic pressure in order to guarantee entrance to institutes located in Gozo. This would prevent very young students from having to commute to Malta on a daily basis to continue with their education. This is however inevitable when considering University studies. It was reported that this additional academic pressure might enforce an increasing sedentary lifestyle on children and adolescents leading to overweight and obesity (Sant'angelo *et al.*, 2011). In fact, it was established that Gozitan boys were more obese than Maltese boys in a recent study (Sant'angelo *et al.*, 2011). Considering the established link between childhood obesity and adult obesity (Eriksson *et al.*, 2003; Lobstein *et al.*, 2004), this may project to the higher morbidity observed within the Gozo population.

It is understood that although the majority of Gozitans reported to be employed (similar to people in Malta), a proportion needs to commute daily or weekly between Gozo and Malta for work. This internal migration brings with it a number of hardships to the involved Gozitans, including but not limited to; early rising, fatigue, costs of travelling, wasted time in travelling, separation from family and accommodation costs (Mizzi, 1988). Such a lifestyle "stressor" results in a disturbed circadian rhythm. This in turn results in health inequalities and possible predisposition to health problems. In fact, altered circadian rhythms have been associated with an increase in blood pressure, in heart rates as well as an increased predisposition to multiple cardiovascular disease, diabetes and obesity (Muller, Tofler and Stone, 1989; Farhud and Aryan, 2018).

Social inequalities may also be responsible for the uncontrolled fasting plasma glucose within the diabetes population. In fact, a proportion of the study's diabetes population reported to have no formal education, and these were found to have the highest FPG levels. Education levels have been linked to the population social gradient. The higher the education, the better the net income and the more empowered the population is to: (1) seek medical help, (2) have higher awareness of prevention, (3) recognise early signs and symptoms of diseases (4) as well as maintain a healthy lifestyle (Marmot, 2016).

#### 4.6.1.4.1 Gender inequalities

Gender inequality was observed in this study. The female population (>50 years) reporting their highest education level up till secondary school was mostly unemployed or followed a domestic role only. On the contrary, the male population reporting education level till secondary school was mostly employed. The situation differed for the younger female population, especially for the 30 to 40-year cohort, as these reported to be mostly employed. Linked to this, younger females reported higher levels of education than the older females. These observations could be linked to gender inequality that was mostly evident in the older generation. In past decades, males dominated the educational and employment scene with females tasked mostly with domestic tasks and low education opportunities. This attitude is changing hand in hand with a cultural shift over the more recent decades, leading to a steady reduction in the gender and age equality gap, as seen in most European countries (Gehringer and Klasen, 2017).

# 4.7 Effects of lifestyle status

## 4.7.1 Effect of smoking habits

### 4.7.1.1 Epidemiology of smoking

Daily and occasional smoking prevalence rates were similar to those reported in the Maltese 2008 and 2014 Health Interview Surveys (HIS) as well as to the smoking prevalence of Italy and Germany (Directorate for Health Information and Research., 2008; Eurostat, 2014, 2015; Eurostat European Commission, 2017). The SAHHTEK study is not fully comparable to the HIS studies due to a variation in the respective age bands (SAHHTEK 18 to 70 years while HIS population 15 years onwards). However, over the years the smoking rate has remained relatively stable. This was further confirmed by the fact that the SAHHTEK study reported similar findings to those of the latest HIS (2014-2015) (Eurostat European Commission, 2017). Of note, the SAHHTEK smoking prevalence rate (24.22%) was higher than the estimated WHO (20.40%) prevalence rate for Malta (World Health Organization, 2017). The WHO estimates are usually based on projections and estimations from published studies, which is probably the reason for the slight discrepancy in prevalence rates as well as the age ranges varied between both studies.

The study's results position Malta as being one of the lowest tobacco consuming Mediterranean country in comparison to other Mediterranean countries such as Greece (32%) and Cyprus (29%) (Gallus *et al.*, 2013; Eurostat, 2014). However, this is not the case when comparing to our neighbouring country, Italy and to the UK. In 2016, a representative cross-sectional study (15+ years) reported the Italian smoking

prevalence to be 21.4%, while in 2018 the UK's statistic office reported the current smokers' prevalence to be 14.9% (Lugo *et al.*, 2017; National Statistics, 2018). Considering that Malta is highly influenced by the British system as well as by our neighbouring country, it appears that Malta still carries a higher tobacco prevalence than both countries. Similar to Malta, both countries have tobacco legislations enforcing smoke free areas in public places, in cars in presence of minors, printing of pictorial shocking images on tobacco packets and hefty fines for breaking the law (Department of Health and Social Care, 2016; Lugo *et al.*, 2017). This may suggest that tobacco smoking in Malta may have underlying cultural and behavioural influences that need to be identified and targeted.

The SAHHTEK study revealed a higher male proportion that smoked as compared to females. This is in keeping with the 2008 and 2014 HIS findings as well as a local study by Sant Portanier *et al*. (Sant Portanier, Sant Fournier and Montefort, 2004; Directorate for Health Information and Research., 2008; Eurostat, 2014).

#### 4.7.1.2 Education level and smoking

A link between education levels and smoking habits has been reported elsewhere, with smoking habits mostly prevalent at low education levels (Giovino *et al.*, 1995; Escobedo and Peddicord, 1996). In fact, this was observed in this study, where one fourth of the adults reporting '*no formal education*' also reported to be smokers. However, the highest smoking prevalence was within the population reporting '*education up till secondary school*'. This could possibly be due to the fact that the majority of the study population fell within this educational level. Of note, the lowest

smoking prevalence cohorts were two extremes: those reporting educations only till '*primary school*' and those with a '*post-graduate education*'. Those reporting a '*post-graduate education*' followed the expected low smoking trend. The low education-low smokers' cohort could be due to the small sample size or a remnant of those being less able to afford cigarettes, since Malta is one of the lowest per capita salaried population. Further research is needed to explore the causes for this relationship between low education level and smoking habit.

Recently, there has been some debates as to whether a causal link exists between smoking and education levels. It was reported that factors contributing to smoking habits start from a very early age, most probably from childhood. The childhood environment may be the dominant influence to the adoption of smoking habits and this makes it difficult to conceptualize or study the causal pathway in a simplified way (Maralani, 2014). In fact, it is common practice that initiation of smoking starts at an early age, usually before the age of 20 years (Chassin *et al.*, 1996; Lantz, 2003). This means that smoking starts before key educational transitions such as the completion of secondary school or tertiary school entry and/or completion. The difference between those individuals who start smoking and those who do not might depend on the acquisition of analytical or self-efficacy skills or higher social integration from an early stage in their lives. Higher education may act as a proxy for gaining such resources (Maralani, 2014). Acquiring such skills and resources may also enable the individual to stop smoking, although this may be more difficult than anticipated. Furthermore, individuals with higher education levels are more likely to land into more demanding and stressful jobs, making it more difficult to stop smoking. Meanwhile, others may

pick up their smoking habit as a stress reliever after they engage in a stressful job (Ayyagari and Sindelar, 2010).

#### 4.7.1.3 Body mass and smoking

A general perception is that active smokers have lower BMIs than their non-smoker counterparts. Body mass has been reported to increase drastically on quitting (Munafò, Tilling and Ben-Shlomo, 2009). In smokers, weight reduction has been attributed to a less efficient calorie absorption and storage process with an increased metabolic rate as well as heightened thermogenesis (Zhang *et al.*, 2001; Chiolero *et al.*, 2008). The SAHHTEK study findings support this theory, as the study's smoking population had a lower BMI status than did non-smokers. This finding is consistent with other population studies, including cross-sectional and cohort studies, although there have been some contradictory reports published (Jitnarin *et al.*, 2008; Dvorak *et al.*, 2009; Macera *et al.*, 2011; Dare, Mackay and Pell, 2015). This study showed a negative relationship between waist circumference and smoking, which does not coincide with the literature, where it was reported that central adiposity accumulates in smokers and may lead to an increase in waist circumference (Morris *et al.*, 2015). The negative relationship found in our study may have been confounded by unknown and uncontrolled influences.

#### 4.7.1.4 Blood pressure and smoking

The current study established a negative correlation between diastolic blood pressure and smoking habits. A number of cross-sectional studies also reported a lower blood pressure in smokers (Gordon and Kannel, 1982; Green, Jucha and Luz, 1986;

Leone, 2011). Smoking has been reported to be responsible for an initial transient nicotine-mediated vasoconstriction leading to an increase in the blood pressure. This is followed by a decrease in blood pressure, which is mediated by the chronic nicotine depressant effects. The decrease in blood pressure could also be attributed to the loss in body weight, which is also linked with smoking (Leone, 2011). However, although a correlation was present between smoking and diastolic blood pressure, no independent link between smoking habit and elevated blood pressure was established in this study. Smoking is an established risk factor for coronary heart disease. However no consensus has been reached as to the casual mechanisms of the smoking effects on blood pressure (Frati, Iniestra and Ariza, 1996; Leone, 2011).

### 4.7.2 Effect of alcohol habits

#### 4.7.2.1 Epidemiology of alcohol

Alcohol consumption goes back to prehistoric times, although nowadays, recreational higher alcohol consumption has spread widely across the globe. This is resulting in more social and health problems (Poli *et al.*, 2013). The SAHHTEK study identified that the majority of the adult population drink alcohol, although these consume alcohol in low to moderate amounts and on an occasional basis. The SAHHTEK study is not directly comparable to the HIS of 2008, although similar trends were exhibited in both surveys (Directorate for Health Information and Research., 2008). Southern European countries including Malta, Italy, Cyprus and Greece tend to have relatively lower alcohol consumption levels at approximately 7 – 8 litres of pure alcohol per adult per year when compared to the Northern European countries (World Health Organization, 2015). In fact, Malta's average alcohol consumption per capita

was found to be only a third of that found in most other countries like the Czech Republic and Hungary (European Commission, 2015). A country's average per capita alcohol consumption is directly related to the prevalence of alcohol dependence and alcohol-related harm (World Health Organization, 2018a). In contrast to Southern European countries, the mean consumption of alcohol in the whole of Europe has been declared to be the highest in the world (World Health Organization, 2018a). Unfortunately, recent high alcohol consumption rates found in 15-year-old children in Malta ranked them as the highest ('*at least once a week*') alcohol consumers across Europe (32% boys, 26% girls) (World Health Organization., 2016).

In this study it was observed that the highest alcohol consumption prevalence rate was within the male population between 30 to 39 years of age. This is the same gender and age band observed to have the highest smoking habit prevalence rate. A strong association has long been established between alcohol and nicotine use (Zacny, 1990). Both of these habits have similar reasons for initiation including: peer pressure, sensation seeking and impulsivity (Little, 2000). Nicotine reduces the sedative properties of alcohol. This may lead to nicotine and alcohol habits kick starting together (Perkins, 1997). Despite the well-known dangers of smoking and drinking, a peak in young men has been reported (Ahlström and Österberg, 2005). This habit has been entitled "The young male cigarette and alcohol syndrome" and has been related to the male short-term mating strategy (Vincke, 2016). Such plausible reasons could explain the high prevalence rates of alcohol and smoking habits within the 30 to 39 years age group in this study.

#### 4.7.2.2 Body mass index and alcohol

All those among the alcohol drinkers were classified as having a moderate to low frequency drinking habit. On comparing the SAHHTEK alcohol drinkers to the non-alcohol drinkers, the alcohol drinking population were less obese then the non-drinkers. In fact, within the SAHHTEK study, the BMI was found to be negatively associated with alcohol frequency. This is not the first cross-sectional study to report a lack of a positive association between BMI and alcohol intake (Sayon-Orea, Martinez-Gonzalez and Bes-Rastrollo, 2011). It was also reported that alcohol consumers did not appear to gain weight when compared to non-alcohol consumers even though alcohol is a high calorie dense substance (Jéquier, 1999). Other studies, from Denmark and the US have also reported negative correlations between BMI and drinking frequency (SAHHTEK study's findings coincide with such literature), suggesting that low frequent alcohol consumption may exhibit protective effects on BMI (Tolstrup *et al.*, 2005; French *et al.*, 2009). Considering that the majority of the SAHHTEK population consumed frequent albeit low to moderate quantities of alcohol, it comes as no surprise that alcohol consumption was found to be associated with a negative effect on the having obesity, even on adjusting for several confounding factors. A possible reason for this effect follows the fact that alcohol users may skip or diminish calorific meals, leading in the extreme to alcohol intake as their sole energy source (French *et al.*, 2009). Another suggestion is that since the majority of the population followed a low-to-moderate alcohol intake, they were also following a more healthy lifestyle (French *et al.*, 2009).

In this study, waist circumference was not linked with alcohol consumption. The lack of a prospective study limits the understanding of the true relationship between alcohol

consumption and BMI. In fact, French *et al.* (2009) reported that over a period of time, the frequency or the amount of alcohol intake might vary, leading to corresponding changes in BMI (French *et al.*, 2009).

#### 4.7.2.2.1 Body mass index, metabolic conditions and alcohol

Moderate alcohol use has been reported to have positive effects on metabolic syndrome components other than obesity. An increase in HDL-C level has been associated with moderate alcohol consumption by inducing the hepatic synthesis of HDL-C as well as by increasing the activity of lipoprotein lipase enzyme (Nishiwaki *et al.*, 1994). In return, an increase in HDL-C leads to cardio-protective actions (Rye and Barter, 2014). Moderate alcohol has also been associated with beneficial effects on blood pressure especially among red wine consumers (Di Castelnuovo *et al.*, 2002; Kloner and Rezkalla, 2007). This suggests that there is a positive overall effect of moderate alcohol consumption on the metabolic syndrome.

Another component of the metabolic syndrome is insulin resistance and the presence of type 2 diabetes mellitus. Alcohol in moderate use was also found to be negatively linked to the development of diabetes mellitus both in the SAHHTEK study as elsewhere (Poli *et al.*, 2013). This has been attributed to the fact that alcohol is associated with the enhancement of insulin sensitivity (Facchini, Chen and Reaven, 1994).

Of note, the diabetes population that reported to be moderate alcohol consumers in this study had a lower median BMI but a higher median FPG when compared to diabetes

teetotallers. As expected, alcohol consumption is associated with a lower BMI among those that do not consume alcohol (*as discussed previously*). The higher median FPG could be explained by the fructose content of the alcoholic beverage. Fructose undergoes first pass metabolism within the liver (insulin independent), which initiates fatty acid synthesis through Acetyl-CoA resulting in lipid deposition within the liver and eventual hepatic insulin resistance through the activation of inflammatory pathways (Bremer, Mietus-Snyder and Lustig, 2012). In a diabetes individual, there is already the presence of insulin resistance so that the addition of fructose (originating from the alcohol beverage) further enhances the insulin resistance and enhances hyperglycaemia. This could lead to higher median FPG levels, as observed in this study.

### 4.7.3 Effects of physical activity

It is a well-known fact that physical activity is health beneficial (National Heart, Lung, 2016). Its positive effects include a decreased incidence of type 2 diabetes and obesity, improved metabolic control in diabetes individuals, a positive effect on depressive moods as well as a reduction in all-cause mortality (Kesaniemi *et al.*, 2001). The majority of the Maltese population exhibited low to moderate levels of physical activity. An interesting fact was that physical activity (low, moderate and high) was not found to be associated with the presence or absence of diabetes mellitus, impaired fasting plasma glucose or overweight-obesity. This is in contrast to what has been reported elsewhere (Hemmingsson and Ekelund, 2007; Ghaderpanahi *et al.*, 2011; Brugnara *et al.*, 2016; van der Berg *et al.*, 2016). It is a well-established that assessing physical activity is extremely complex and that no single tool is capable of capturing all the subcomponents (Warren *et al.*, 2010). In fact, the physical activity assessment

method used in this study (SAHHTEK), followed the method used in the survey by Pantelic *et al*. (Pantelić *et al.*, 2012). Such an assessment had not been followed by previous Maltese surveys (Directorate for Health Information and Research., 2008). The SAHHTEK physical activity data was self-reported through interviewer-administered questions. This may have been biased and played a role in the results obtained. Such data gathering has limitations, including participants having difficulties in recalling and ascertaining their frequency, duration and intensity of physical activity as well as difficulties in capturing all domains of physical activity (Sallis and Saelens, 2000). Also, self-reported physical activity data collected through interviews is known to be subject to a substantial social desirability bias (Helmerhorst *et al.*, 2012). For this reason, other methods have been suggested for a more accurate assessment of physical activity, including the use of accelerometers, heart rate monitoring and pedometers (Warren *et al.*, 2010). These measures went beyond the scope and resource capacity of the SAHHTEK survey.

## 4.8 Effect of anthropometric parameters

### 4.8.1 Body mass index vs. waist circumference

Disagreements were found on comparing the BMI categories to waist circumference cut-off points in both the men and women populations within this study (SAHHTEK). A proportion of the population with normal waist circumference fell into either overweight or obese BMI categories. Similar findings have been reported in another study (Gierach *et al.* 2014). Further analysis to identify the possible reasons contributing to these disagreements were performed by considering glucose regulation

(diabetes mellitus and impaired fasting plasma glucose status). However, the disagreements between BMI-waist circumference could not be addressed by considering just dysglycaemia. Another probable explanation for these disagreements is due to the fact that the body mass index measures the body weight without considering the body fat distribution, whilst the waist circumference considers exclusively visceral and abdominal obesity (Kok, Seidell and Meinders, 2004; Burkhauser and Cawley, 2008). In fact, it was reported that individuals with a normal weight BMI but with differences in waist circumference were associated with an increased metabolic risk, even if the waist circumference was considered to be within the normal range (Eckel *et al.*, 2015).

### 4.8.2 Body mass index, waist circumference and blood pressure

An increase in the clinical measurements of body mass index and waist circumference showed a link with an increase in blood pressure in this study. This was consistent with another study in the U.S (Roka, Michimi and Macy, 2015). The current study showed that that higher BMI and higher waist circumference were associated with an elevated blood pressure as with previously published studies (Ghosh and Bandyopadhyay, 2007; Chen *et al.*, 2015). There are a number of proposed mechanisms that link adiposity with an increase in blood pressure. General adiposity (measured by BMI) can result from dysfunctional adipose tissue resulting in an increase in leptin levels, an increase in sympathetic nervous system activity, as well as an increase in the renin-angiotensin-aldosterone system activity, all of which increase blood pressure (Landsberg *et al.*, 2013).

Conversely, central adiposity (measured by waist circumference) can lead to an increase in blood pressure through several potential mechanisms. Physical compression of the kidney, systemic inflammation and oxidative stress leading to artery stiffness are possible mechanisms. Central obesity leads to the development of hyperinsulinaemia as a consequence of insulin resistance which in return affects the blood pressure (Kotsis *et al.*, 2010; Purkayastha, Zhang and Cai, 2011; Dorresteijn, Visseren and Spiering, 2012; Kalil and Haynes, 2012).

### 4.8.3 Body mass index, impaired fasting glucose and type 2 diabetes mellitus

The study's IFG and diabetes mellitus populations were found to exhibit a higher degree of obesity when compared to normoglycaemic population. These findings are consistent with other national surveys conducted in Europe as well as in Asia (Qian *et al.*, 2010; Zatońska *et al.*, 2011; Rahmanian *et al.*, 2015; Tsirona *et al.*, 2016). In this study, being obese was associated with having IFG, coinciding with the conclusions published in yet another study (Choudhary and Antal, 2013). In fact, a link has been established between obesity and the development of IFG with eventual development of type 2 diabetes mellitus (Choudhary and Antal, 2013; Hagman *et al.*, 2014). Indeed, the current study found that an increase in BMI was an independent associated risk factor for having newly diagnosed diabetes. However, once diabetes mellitus had been established (represented as the previously diagnosed diabetes sub-population), BMI was no longer an independent risk factor in this study. This follows the fact that the mechanism behind the links between obesity and dysglycaemia involve the establishment of insulin resistance, pro-inflammatory cytokines, derangement of fatty acid metabolism as well as development of mitochondrial and endoplasmic

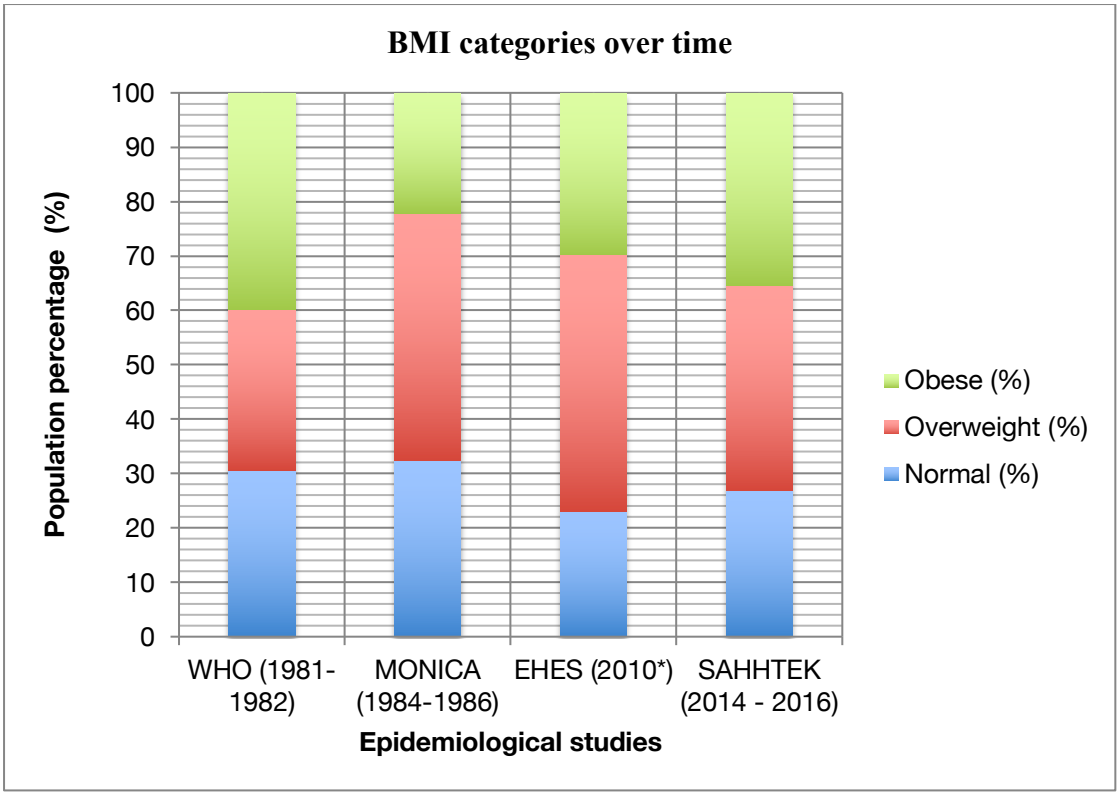
reticulum dysfunctional stress (Eckel *et al.*, 2011). One can therefore argue that once type 2 diabetes mellitus has been established, insulin resistance is at its maximum and the associated risk of BMI to the development of T2DM would be null. An increase in weight in T2DM individuals leads to increasing difficulties in managing the hyperglycaemia, apart from enhancing the overall complication rates, morbidity and mortality (Wilding, 2014).

The deposition of body fat, including visceral adiposity contributes to an enhanced waist-to-hip ratio and is associated with the development of the metabolic syndrome as well as type 2 diabetes mellitus (Björntorp, 1991). Indeed, an increase in waist circumference was established to be an independent risk factor for having previously diagnosed diabetes mellitus (but not newly diagnosed diabetes) when comparing to the non-diabetes subpopulation. However, this could have been confounded by underlying metabolic abnormalities, since this relationship was lost on comparing to the metabolically healthy subpopulation. Hence, these finding merits further investigation including investigation with a large diabetes population size to ensure that Type II error did not occur.

### 4.8.4 Comparisons of body mass index measurements across the diverse epidemiological studies in Malta

Over recent decades three population based epidemiological studies have been conducted (WHO 1981, MONICA 1984, EHES 2010) all of which measured BMI by means of height and weight examinations (Katona, G, Aganovic, I, Vuskan V, 1983; Cacciottolo, 1989; Directorate for Health Information and Research., 2012). These

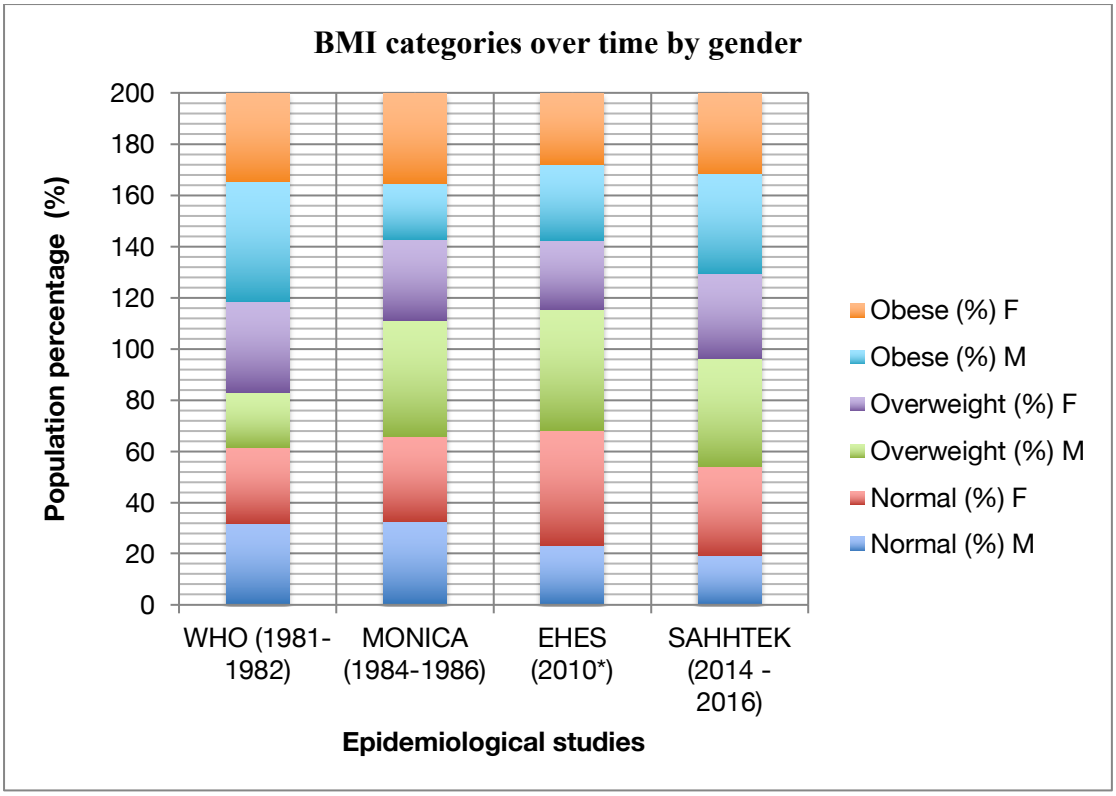
studies, along with the SAHHTEK study followed the same BMI definition and were available in age strata between 25 and 64 years (but were not age standardized) for ease of comparison. All the studies (WHO, MONICA and SAHHTEK) were nationally representative surveys except for the EHES, which was a smaller pilot study. However, this pilot study still gives an indication on the BMI situation in Malta over the period of time. As can be observed in Figure 4.2, over the period of 35 years there has been a general increase in the obesity rate and a corresponding decline in the overweight and normal weight categories. A similar shift in body weight was pervasive over the years across the globe. In fact, in a U.S. study it was reported that the overall obesity rates increased significantly from the 1980's to the present day (Flegal *et al.*, 2016). The same phenomenon was observed in Europe where more than half of the adult population (52%) within the European Union was found to be either overweight or obese during the past decade (OECD, 2012).




*EHES – pilot study

Figure 4.2 Comparisons of BMI between epidemiological studies in Malta

Interestingly, different countries exhibited divergent gender predominance. Between the year 2013 to 2014 the U.S. registered a significant linear increase obesity rates among women as compared to men (Flegal *et al.*, 2016). This is in keeping with the European surveys conducted in Latvia, Turkey and Hungry (OECD, 2012). Conversely, the situation in Malta differs across all other studies in that males had higher obesity prevalence than did females, as seen in Figure 4.3. The same trend was observed in the European countries of Iceland and Norway (OECD, 2012). However, in all studies females always exhibited a higher prevalence of normal BMI status than did the males.




*EHES – pilot study

Figure 4.3 Comparisons between Maltese epidemiological studies by BMI categories and gender

### 4.8.5 Elevated blood pressure

Hypertension is one of the leading causes of cardiovascular disease and mortality worldwide (Tao *et al.*, 2015). The European prevalence of hypertension was reported to be 23.20% (Eurostat, 2016). The SAHHTEK survey prevalence of hypertension was extrapolated to the whole of the Maltese population (18 to 70 years), where an approximate 61,000 Maltese adults suffered from hypertension. This was compared to a 5 European country study which had a slightly older age band (20 to 79 years). It was reported that Germany (another high meat/meat products consumption population) had a higher hypertension prevalence than did the United Kingdom, Italy,

France and Spain (Eichmann, Potthoff and Schmidt, 2012). The SAHHTEK survey showed that the rates for Malta were higher for both genders as compared to all countries in this European study. This despite the fact that Malta's estimate should be an underestimate given the age range difference.

On comparing this study's self-reported hypertension prevalence to that reported in the 2008 Malta HIS self-reported study, there appeared to be no difference in the overall total hypertension prevalence rates (HIS 2008 - 23.70%; SAHHTEK - 23.92%) (Directorate for Health Information and Research., 2008). Despite the similarities between the self-reported prevalence rates between the HIS 2008 and the SAHHTEK survey, on incorporating the newly identified hypertensive population during the health examination to the self-reported hypertension group, an overall higher hypertension prevalence was obtained in SAHHTEK study as could be expected, emphasising the existence of a larger number of undiagnosed hypertensives.

A proportion of the SAHHTEK hypertensive population did not report being on any medication despite being aware of having the disease. Furthermore, some of those who were on medication were found to have a poorly controlled blood pressure during the health examination. It is a well-known fact that most hypertensive individuals are not effectively controlled in accordance to the recommended blood pressure targets (Kearney *et al.*, 2004).

Prehypertension (systolic blood pressure 120 – 139mmHg or diastolic blood pressure 80 – 89mmHg) was the more prevalent form of hypertension present in Malta, which is consistent with the literature (Greenlund, Croft and Mensah, 2004; Wang and Wang,

2004). It has been reported that over a third of prehypertensive adults will progress to full-blown hypertension over a 4-year period (Vasan *et al.*, 2001). Interestingly, in this Malta study, pre-hypertension was already evident from an early age (20 years) in both genders and exhibited the expected increase in prevalence as age increased. It has been reported that young adults with pre-hypertension are increasingly common occurrence in research studies and that this factor has been associated with an increased susceptibility to atherosclerosis later on in life (Pletcher *et al.*, 2008).

The current study showed a strong link between the presence of hypertension and a high BMI which is consistent with findings elsewhere (Poirier *et al.*, 2006; Ong *et al.*, 2007). The literature suggests that an increase in body weight eventually leads to an increase in blood pressure (Ong *et al.*, 2007). The ideal management for obesity-hypertension is known to be a substantial weight reduction. In fact, it was reported that a weight loss of 5 to 10% improved the blood pressure and reduced the amount of medication required (Poirier *et al.*, 2006).

### 4.8.6 Blood pressure and type 2 diabetes mellitus

Hypertension is a common co-morbidity of type 2 diabetes (Matheus *et al.*, 2013). Inflammation, oxidative stress, insulin resistance and obesity are the common pathways between hypertension and type 2 diabetes mellitus (Cheung and Li, 2012). Insulin resistance induce hyperinsulinaemia, leading to an increased sodium reabsorption from the kidney's renal tubule. This increase in circulatory volume along with peripheral vascular resistance results in an increase in blood pressure and eventual hypertension (Martinez and Sancho-Rof, 1993; Shimamoto *et al.*, 2014). Indeed, our

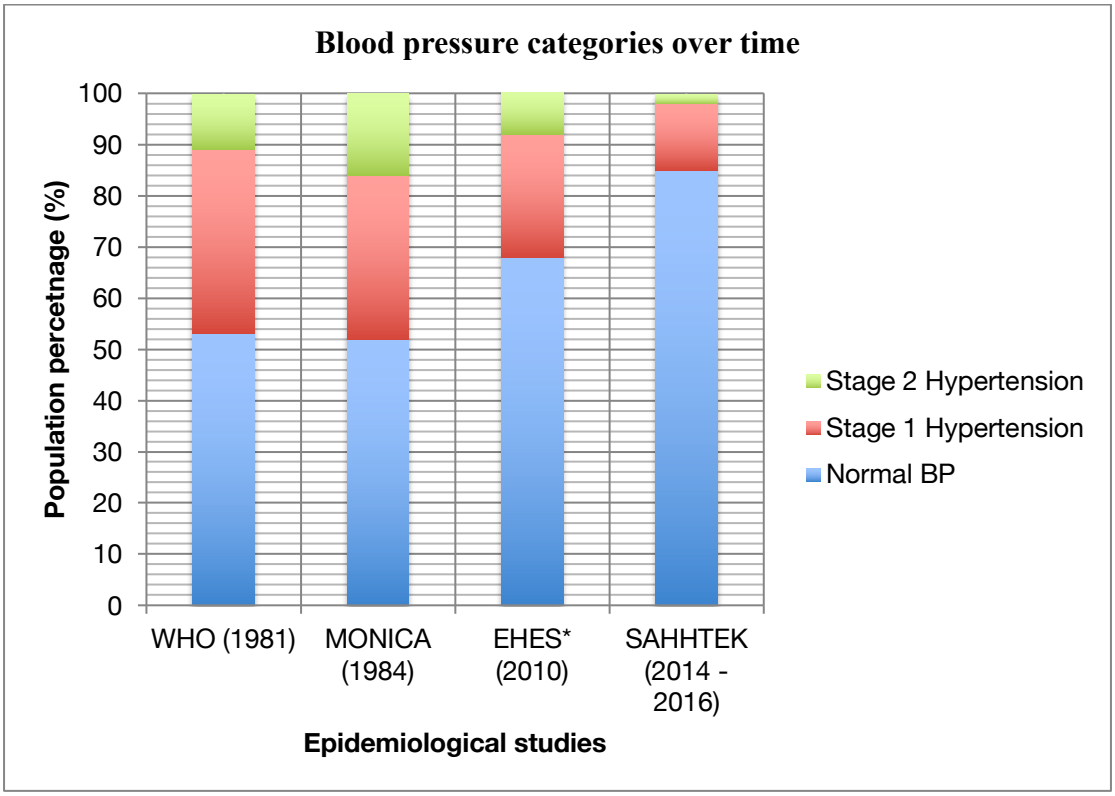
study exhibited an associated link between an increase in systolic blood pressure and diabetes mellitus, which coincided with finding from other European studies (Lindholm *et al.*, 2002; Gupta *et al.*, 2008; Aikens *et al.*, 2017). A recent study evaluated the impact of blood pressure on diabetes mellitus by associating 28 genetic variants linked with systolic blood pressure. It was concluded that there may be a genetic element contributing to this association (Aikens *et al.*, 2017).

### 4.8.7 Comparisons of elevated blood pressure across the Maltese epidemiological studies

Blood pressure measurements were taken in all the three previously conducted Maltese epidemiological studies (WHO 1981, MONICA 1984, EHES 2010). The population cohorts between the ages of 25 and 64 years were analysed across all the epidemiological studies including the current study (SAHHTEK). Blood pressure was categorized into three groups as follows: Normal blood pressure (systolic <140mmHg and diastolic <90mmHg); Stage 1 hypertension (systolic ≥140mmHg but ≤159mmHg *Or* diastolic ≥90mmHg but ≤99mmHg) and Stage 2 hypertension (systolic ≥160mmHg *Or* diastolic ≥100mmHg). These were in accordance to the latest guidelines (Whelton *et al.*, 2017).

On comparing the blood pressure categories across the four epidemiological studies, it was observed that the normal blood pressure prevalence increased over time while the hypertension prevalence decreased, as seen in Figure 4.4. This finding coincides with global trends across high-income countries (NCD Risk Factor Collaboration (NCD-RisC), 2017). Similar findings were also recorded in Italy and in the UK, where it was

reported that this decrease in blood pressure was due to the reduction in salt intake within the British population (He, Pombo-Rodrigues and MacGregor, 2014; Tocci and Presta, 2017).

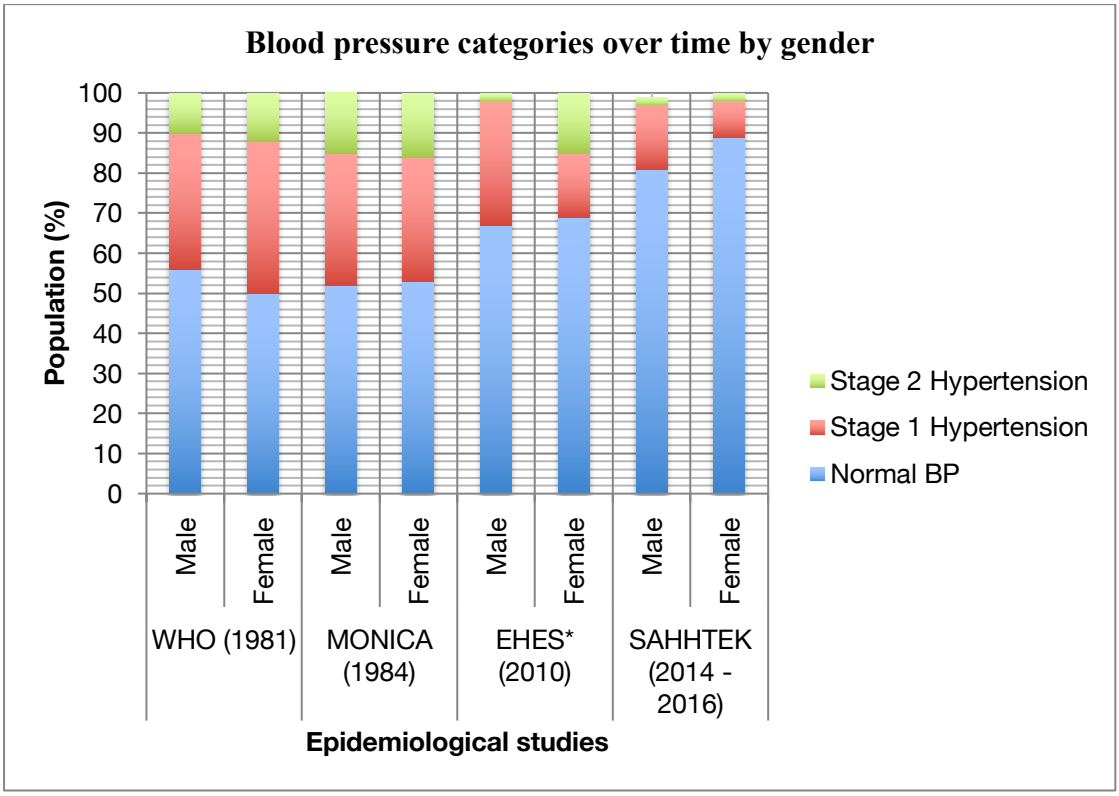



*EHES – pilot study

Figure 4.4 Comparisons between blood pressure measurements across studies in Malta over the past 35 years

On gender stratification, it was observed that in 1981 the female population alone had predominantly stage 1 hypertension. This trend changed with the male population becoming predominantly stage 1 hypertensive in the subsequent studies, as seen in Figure 4.5. The finding of a male predominance for hypertension is consistent with those of previous studies elsewhere (Reckelhoff, 2001; NCD Risk Factor Collaboration (NCD-RisC), 2017). A substantial proportion of the female population until 2010 fell within stage 2 hypertension category but this was down to a mostly normal blood

pressure by the 2014 – 2016 SAHHTEK study. It is important to note that data on hypertension medication was not available for the previous epidemiological studies and therefore the effect of such medication on the hypertension prevalence rates could not be evaluated.




*EHES – pilot study

Figure 4.5 Comparisons between Maltese epidemiological studies by blood pressure categories and gender

# 4.9 Effect of biochemical parameters

## 4.9.1 Biochemical parameters by age and gender

An increase in mean fasting plasma glucose (FPG) with age was observed in this study, which coincides with numerous global studies (DeFronzo, 1981; Shimokata *et al.*, 1991; Ferrannini *et al.*, 1996; Ko, Wai and Tang, 2006; Kalyani and Egan, 2013).

In fact, a positive association was established between age and elevated FPG level. Ageing is a well-known risk factor for progressive pancreatic impairment, especially due to loss of beta cell function (Bryhni, Arnesen and Jenssen, 2010). This results in defective insulin secretion followed by an elevation in plasma glucose levels (Basu *et al.*, 2003).

The lipid profile components (LDL-C, Triglycerides, Total cholesterol) in this study population were also higher as the age increased. This coincided with findings in already published literature (Sikandar Hayat Khan, 2012). It also explains the observation established in this study, whereby students had lower median LDL-C levels than the rest of the employment status subgroups. Students are typically of a young age and therefore are expected to have normal lipid profiles, unless underlying co-morbidities are present. In fact, during this study's association analysis, the link between student status and LDL-C was lost on adjusting for age as a confounder.

The HDL-C levels within this study were higher in the younger age groups and decreased as the age groups advanced. This could be explained by the fact that the younger population is usually more physically active than the older population, therefore favouring such an HDL-C distribution (Healy *et al.*, 2011). HDL-C was reported to undergo a functional impairment with age due to alteration in its' composition. This predisposes the older population to possible cardiovascular disease onset or the progression of such disease (Holzer *et al.*, 2013).

In general, the female population exhibited greater dyslipidaemia at an older age than males. This is in keeping with the pre-menopause effect, where the oestrogens exhibit

a protective role in females. In the current study there was a link between female gender and decreased FPG, LDL-C and triglycerides levels (even after adjustment for potential confounders). These associations are similar to previously published results in the literature (Anagnostis *et al.*, 2015; Manafa and Ihim, 2015). Furthermore, the female population (unlike the male population) in the current study exhibited an increase in HDL-C levels with increasing age until the fifth decade followed by a decrease in the level of HDL-C above 60 years of age. This is consistent with the findings that a decrease in oestradiol levels in post-menopausal females is associated with a decrease in HDL-C levels (Sultan *et al.*, 2003). It could further be explained by a decrease in physical activity with increasing age, as reported in a Mediterranean study (Skoumas *et al.*, 2003). The Mediterranean study concluded that females, but not males, exhibited an independent rise in HDL-C in relation to physical activity (Skoumas *et al.*, 2003).

The SAHHTEK male population exhibited a generally higher median lipid levels (except for total cholesterol and HDL-C) when compared to the female population. This was evident across all the different glucose regulatory categories, which is in keeping with another study (Khan, Sobki and Khan, 2007). An explanation for the current study's male lipid abnormalities could be related to the recent finding (unveiled in this study) that the vast majority of the Maltese male population was either overweight or obese (Cuschieri *et al.*, 2016). This male trend was consistent with the literature, where males tend to have a less favourable lipid profile with advancing age, resulting in an increased risk for cardiovascular disease (Kolovou *et al.*, 2009).

### 4.9.2 Biochemical parameters by body mass index

Glucose, a simple carbohydrate, consumed in excess volumes leads to the synthesis of fatty acids, which in turn contribute towards an increase in body fat (Nelson, DL; Cox, 2005). This increased body fat deposition promotes an increase in the body mass index (BMI). The study population showed a positive relationship between fasting plasma glucose (FPG) and BMI, where an increase in FPG levels was linked to a similar increase in BMI. An increase in plasma glucose has been associated with an increase in lipogenesis which further contributes to an increase in weight (Nelson, DL; Cox, 2005). Hence, this corroborates the SAHHTEK findings, whereby as the various lipid profile component (LDL-C, triglycerides and total cholesterol) levels increased so too did the BMI level. On the other hand, HDL-C levels exhibited a decreased level with an increase in BMI level, which is consistent with a dyslipidaemia pathophysiology (Wang and Peng, 2011). In fact it was reported that obesity has an effect on both the intrinsic and extrinsic HDL-C particles causing rapid clearance of HDL-C hence resulting in a decrease in HDL-C levels (Wang and Peng, 2011).

Furthermore, the mentioned biochemical associations with BMI, form part of the metabolic syndrome definition. An increase in adiposity is the main contributing component for the metabolic syndrome along with the presence of at least two of the biochemical components (elevated FPG, elevated triglycerides or decreased HDL-C) or an elevated blood pressure (Alberti, Zimmet and Shaw, 2006). Although an elevation in LDL-C level does not fall within the metabolic syndrome definition, an elevated LDL-C is related to an increased insulin resistance (Ginsberg, 1991). As previously

stated, insulin resistance forms the basis of the metabolic syndrome pathophysiology (Reaven, 1988).

### 4.9.3 Biochemical parameters and type 2 diabetes mellitus

As expected, higher median FPG levels within the SAHTTEK diabetes population were present when compared to the non-diabetes population (International Diabetes Federation, 2017). A link exists between an elevation in FPG and having type 2 diabetes mellitus. These findings are consistent with the pathophysiology of type 2 diabetes mellitus which underlies the inability of the pancreatic beta cells to compensate for the development of insulin resistance. As a consequence, insulin sensitivity and secretion are altered (Lyssenko *et al.*, 2005). Insulin deficiency leads to a decrease in glucose utilization and increased hepatic glucose production, in turn leading to hyperglycaemia (Valensi *et al.*, 2005).

The majority of the study's previously known diabetes population reported to be aware of their diabetes condition and was on treatment. However, more than half had an elevated FPG (above 7mmol/L) during the health examination survey. There are a number of probable reasons contributing to such an elevation. The first to be considered is whether these individuals were actually fasting prior to attending for their health examination. Anyone having breakfast prior to attending the health examination may have had an elevated post-prandial glucose level. Another factor could be that their prescribed treatment had not yet controlled their disease adequately. Yet another factor could be non-compliance to medication. In fact, a small percentage of the reported previously known diabetes in the current study (SAHHTEK) reported not to be on any

treatment. Non-adherence to medication is a common public health challenge with as many as 50% of the patients discontinuing their medications within a year of initial prescription (World Health Organization, 2003; Vanelli *et al.*, 2009; Granger and Bosworth, 2011). In fact, at a systemic level only 80% of the persons with diabetes are considered as adequately taking their medication (Raebel *et al.*, 2013). This non-compliance can be attributed to various factors including but not limited to, patient-centred factors, therapy-related or healthcare system related factors (Khan *et al.*, 2012). The patient centred factors can be due to demographic (education level, age, gender) and psychological (patient beliefs, knowledge) related factors. Therapy related factors include the duration of therapy as well as side effects from other medications the patient may be on. Healthcare system related aspects could be present and results from lack of frequent follow-ups. Such practices could result in long-term complications, co-morbidities and premature mortality, which also incur additional costs to the health care system (Zullig *et al.*, 2015). Furthermore, T2DM is a progressive disease and most patients will eventually require an increase in medication dose or a combination of medications apart from maintaining a healthy lifestyle. It is suggested that achieving an agreement between the diabetes patient and the healthcare provider on a targeted approach for a specific barrier would subside the problem of non-compliance (Katon *et al.*, 2010).

Individuals suffering from T2DM can exhibit a spectrum of dyslipidaemic disorders (Hachem and Mooradian, 2006). However, the most common phenotype attributed to insulin resistance and insulin deficiency is the presence of high triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C) and high small dense lipoprotein low-density lipoprotein cholesterol (sdLDL-C) levels (Ginsberg, 1991). In fact, it has been

reported that diabetes individuals are more prone to develop dyslipidaemia when compared to normoglycaemic individuals (Pathak and Pathak, 2012). In this study, the diabetes female population exhibited a higher median lipid profile as compared to their male counterparts, which coincides with the findings published in another study (Ali *et al.*, 2015).

Conversely, the situation in Malta differed from the literature in that the diabetes population had a lower median lipid profile than the non-diabetes population. A possible reason for this finding may be the existence of a care protocol by which all diabetes individuals are started on statin therapy and lifestyle interventions on diagnosis of diabetes, in accordance with international organisation recommendations (American Diabetes Association, 2018a). It has been stated that statin treatment can have a major pharmacological effect on the reduction of the LDL-C levels, although statins also have some effects on the reduction of triglyceride levels and the elevation of the HDL-C levels (Bonetti *et al.*, 2003). This may explain the higher prevalence of normal LDL-C levels as well as low/normal triglyceride levels within the SAHHTEK diabetes population. The low/normal triglyceride levels suggest that the diabetes population had a good glycaemic control since plasma triglyceride levels are directly correlated with glycaemic control (Lopes-Virella *et al.*, 1981; Pfeifer *et al.*, 1983). However as insulin deficiency sets in, triglyceride clearance decreases with an increase in triglyceride-rich lipoprotein synthesis (Reaven and Greenfield, 1981; Gibbons, 1989). In fact, this study established a positive association between elevated triglyceride levels and having T2DM when compared to the metabolically healthy sub-group.

However, at times a normolipidaemic profile may be present in T2DM individuals with an underlying LDL-B phenotype (Feingold *et al.*, 1992). The LDL-B phenotype results when a difference in glycosylation of LDL-C occurs. The LDL-C is taken up in an unregulated manner by macrophage scavenger receptors ultimately contributing to the formation of foam cells (Austin and Krauss, 1995).

Considering that the SAHHTEK diabetes population exhibited a mild hypertriglyceridemia and a majority with a normal LDL-C concentration, it further supports the possibility that the SAHHTEK diabetes population exhibited a 'B profile pattern' of LDL (smaller, denser particles) rather than an 'A profile pattern'. This makes these LDL-C particles more susceptible to oxidative modification and catabolism through the macrophage scavenger receptors and therefore, still holds an atherogenic effect (Austin and Krauss, 1995; Austin *et al.*, 1995; Miller *et al.*, 2011). In fact, the 'B profile pattern' LDL-C (small dense LDL-C) has been associated with an increased risk of cardiovascular disease, which is a common complication of diabetes mellitus (Austin *et al.*, 2000; Borch-Johnsen *et al.*, 2004; Arai *et al.*, 2013; American Diabetes Association, 2018b).

On statistical analysis it was found that LDL-C had a negative association with previously diagnosed diabetes mellitus even after adjustment for statin therapy (when compared to non-diabetes population). This could be the result of lifestyle interventions that were not quantified in this study. In fact it was reported that LDL B phenotype frequency decreases with exercise induced weight loss due to a decrease in sdLDL-C concentration (Williams *et al.*, 1989). However, this association was lost when

previously T2DM was compared to metabolically healthy sub-group. This may suggest that the negative association was influenced by metabolic confounders.

No association between the LDL-C and newly diagnosed diabetes was present which is a similar finding to the IFG sub-population. This may suggest that in the early stages of diabetes dysglycaemia, LDL-C may have lower pathological effects, possibly due to lesser amounts of small dense LDL-C within the circulation.

### 4.9.4 Biochemical parameters and impaired fasting glucose

The IFG population in our study had significantly elevated LDL-C, triglyceride and total cholesterol levels when compared to the non-IFG population. It is known that pre-diabetes populations are at a higher risk for elevated LDL-C levels as well as exhibiting a dyslipidaemia profile similar to that for the diabetes mellitus population (Miyazaki *et al.*, 2012; Kansal and Kamble, 2016). A common feature of the pre-diabetes state is the presence of high triglyceride (TG) levels, which is a known risk factor for the development of cardiovascular disease and type 2 diabetes mellitus (Ginsberg, Zhang and Hernandez-Ono, 2005; Alberti, Zimmet and Shaw, 2006). In fact, a link was established between raised triglyceride levels and IFG within the current study.

## 4.10 Economic burden

Considering that a substantial proportion of the younger population (<55 years) was found to suffer from hitherto unknown diabetes as well as an increased body weight (obese > overweight), and given the expected morbidity and life quality loss, action at population level to address this ought to be a priority. It is established that the higher the obesity and diabetes prevalence rates, the higher the expected hospitalization rates and other related costs to individuals and populations (Van Den Bos, 1995; Y. C. Wang *et al.*, 2011).

The obesity epidemic in its own right is of utmost importance in order to prevent further obesity-related illnesses. It is well established that the risk of co-morbidity increases with the increase in body mass index (BMI) (Van Den Bos, 1995). Obesity contribute towards a decrease in the quality of life and mortality of many, but also, to an estimated 8 to 10% of the total health expenditure in many European countries (Brandt and Erixon, 2013). The current Maltese obesity impact on the total health expenditure relates well to other European countries (Brandt and Erixon, 2013).

Malta appears to have the same diabetes health care expenditure burden as do other countries (International Diabetes Federation, 2015). Our results are in keeping with the IDF's report that type 2 diabetes mellitus accounts for 5 to 20% of the majority of the countries' total health care expenditure (International Diabetes Federation, 2015).

As part of this study extrapolations from the calculated 2016 obesity and diabetes burden for the year 2050, lead to an expected major health care concern for Malta. This

is especially with the expected high diabetes prevalence rise and related economical costs.

It is essential that health care services in Malta consider effective preventive strategies and if necessary, assess the cost effectiveness of population screening to target these conditions from an early age, before the disease has given rise to complications (Sammut, A; Calleja, N; Cachia, 2016). Although this could impose an even larger health budget for the country, such early action could help decrease the health burden contributed to by diabetes, obesity and their associated complications and ultimately save money for an economy that is already under stress.

## 4.11 Genetics

### 4.11.1 Summary of findings

The genetic sub-cohort was selected from the crude unadjusted population after sub-categorizing the population according to the dysglycaemic profile, metabolic characteristics and age. Individuals having a diagnosis of diabetes mellitus, those with pre-diabetes (IFG + IGT), those having at least one abnormal metabolic parameter (metabolically abnormal) and metabolically healthy between the ages of 33 and 62 years were considered. The ‘metabolically abnormal’ sub-category included an exceptionally larger number of participants than the rest of the sub-categories. Therefore, a sample from this subgroup was obtained by random selection using the SPSS software to obtain a comparative sample to the other subgroups.

The cohort selected for genetic analysis (the genetic sub-cohort) was composed of dysglycameic (diabetes and pre-diabetes) cases, metabolically abnormal cases and metabolically healthy cases. On comparing the dysglycaemic cohort to the metabolically healthy cohort, significantly different biochemical and clinical parameters were present. This coincides with similar findings reported describing changes in biochemical and clinical characteristics, such as increase in weight, lead to metabolic changes with possible development of dysglycaemia (Hartwig *et al.*, 2015). Insulin resistance is the contributing factor resulting in these biochemical and clinical characteristics of the dysglycaemic status. Insulin resistance leads to high circulatory levels of triglycerides and small dense LDL-C along with a decrease in HDL-C levels (Eisenberg, 1984; Gray *et al.*, 1997; Adiels *et al.*, 2006, 2007, 2008). Furthermore, insulin resistance is associated with an elevation in body mass index, waist circumference and blood pressure (Hwang *et al.*, 1987; Reaven, 1988; Tiikkainen *et al.*, 2003).

Similarly, the metabolically abnormal subgroup was significantly different from the metabolically healthy subgroup, with the exception of the fasting plasma glucose levels. This was expected since both the ‘metabolically abnormal’ and the ‘metabolically healthy’ subgroups were defined as having a fasting plasma glucose less than 5.60mmol/L. The metabolically abnormal subgroup, although exhibiting normoglycaemia, may still have underlying subclinical insulin resistance. This subgroup is not only at higher risk of developing diabetes mellitus but may also start featuring insulin resistance characteristics (Nyenwe and Dagogo-Jack, 2011).

Therapeutic medication such as statins and anti-hypertensive medication may influence the biochemical and clinical characteristics of the receiving individual (Xian-Yu *et al.*, 2015). Statin medication has pleiotropic effects with established anti-inflammatory properties (Wierzbicki, Poston and Ferro, 2003). Such properties may have an effect on specific gene expression/s (Gbelcová *et al.*, 2017). Furthermore, genetic factors may have an effect on therapeutic effects of medication (Belle and Singh, 2008).

#### 4.11.1.1 Summary of the genotype-phenotype findings

The *NOTCH2* (rs10923931) T/T genotype variant was associated with higher fasting plasma glucose than those carrying the G-allele.

The *FABP2* (rs1799883) T/T genotype exhibited a significantly higher median HDL-C but lower median triglyceride level than the C/T and C/C variants. Following a co-dominant model, the *FABP2* T/T variant exhibited a higher HDL-C and lower triglyceride levels when compared to C/T and C/C variants. Following a recessive model, the *FABP2* recessive [C/C + C/T] variant exhibited lower HDL-C and higher triglyceride levels compared to T/T variant. While following a dominant model, the *FABP2* dominant [T/T + C/T] variant exhibited lower HDL-C and higher triglyceride levels compared to C/C variant.

The *HHEX* (rs1111875) C/C genotype exhibited higher median fasting plasma glucose level compared to C/T and T/T variants.

#### 4.11.1.2 Summary of the associations with diabetes mellitus

Under a co-dominant model, the *FTO* A/A variant showed an independent association with a lower risk of having a diagnosis of T2DM after adjustment for confounders.

The recessive model exhibited the *FTO* T-allele variant (T/T + T/A) having an independent association with a lower risk of having a diagnosis of T2DM after adjustment for confounders.

The dominant model exhibited the *KCNE4* T-allele variant (T/T + T/C) having an independent association with a lower risk of having a diagnosis of T2DM after adjustment for confounders.

### 4.11.2 Type 2 diabetes mellitus susceptibility loci

The T2DM susceptibility loci mainly affect insulin sensitivity or beta-cell function. These loci are typically represented by a common lead single nucleotide polymorphism (SNP) with a minor allele frequency (MAF) of more than 5%. The majority of the lead SNPs at T2DM susceptibility loci, with some exceptions of loci such as *SLC30A8*, affect non-coding sequences. Multiple association signals at a particular locus may be present and are derived from variants that act independently of each other or through haplotypes. These association signals usually extend over hundreds of kilobases through linkage disequilibrium (LD) between common variants composed of multiple genes that have an effect on T2DM susceptibility.

A case-control study is usually performed in order to establish associations of T2DM susceptibility with genetic variants. Case control study design provides good comparison information between multiple outcomes for a given exposure. Such design enables the researchers to establish whether phenotype-genotype differences are present between those with diabetes and those without. This study design is inexpensive and requires few subjects for comparative analysis to be performed (Song and Chung, 2010). One of the approaches used is that of the "logistic regression model", which typically assumes an additive effect of each allele on the disease (multiplicative effect on odds ratio). A major advantage to this approach is that it takes into account potential non-genetic confounding risk factors as covariates such as age, sex, central obesity measured through BMI and waist circumference that affect T2DM susceptibility (Morris, 2014). In fact, such an approach was applied in this study and these confounders, amongst others, were tested in accordance to the SNP physiology. However, this approach has an inherent disadvantage due to the fact that the significance tests (and *p*-value obtained) do not take into account the power of the association test (Burton *et al.*, 2007). Consequently, one is not able to sufficiently quantify statistically how confident the variants could be causally linked to the disease. Such a problem can be overcome by undergoing GWAS analysis (Morris, 2014). However, a GWAS analysis was beyond the scope of this study. Other disadvantages to case-control studies is that they are susceptible to recall and information bias, as well as it is difficult to validate information.

Discovery of the T2DM susceptibility loci have led to initial fine mapping of the genetic diabetes architecture and identifying the pathophysiology basis of the disease. However, the majority of these loci causal variants and transcripts are still yet to be

determined (Morris, 2014). A casual variant influences the molecular or cellular process leading to an effect on the phenotype. Moreover, GWAS identified variants do not normally predict the disruption of protein-coding regions. In fact, the majority of the GWAS tag SNPS are within intronic regions (intergenic) and are likely to influence gene regulation which makes biological interpretation difficult (Edwards *et al.*, 2013).

Family studies, even though was outside the scope of this current study and study design, can also be used to investigate the genetic factors of diabetes. Such genetic epidemiological studies are based on families and are used to investigate the familial trait aggregation and localised the genes that are responsible for diabetes within the human genome. Furthermore, family studies can identify the physiological role of candidate genes for diabetes mellitus as well as their impact on the predisposition of gene expression (Mansour-Chemaly *et al.*, 2002).

### 4.11.3 Single nucleotide polymorphisms (SNPs) and genotyping

SNPs are markers found at particular loci that contribute to the identification of the genetic determinants of complex traits. The genetics of common complex diseases are elucidated by means of SNPs by using genome-wide association studies, candidate gene case-control association studies as well as genome-wide linkage analyses (Shen, Abdullah and Wang, 2009).

The genetic analysis for this study followed a replication of established loci after reviewing the published literature especially from genome-wide association studies (GWAS). Also reviewed the previous work published locally from the Laboratory of

Molecular Genetics at the University of Malta studies (Grant *et al.*, 2006; Zeggini *et al.*, 2007; Pace, 2013; Craus, 2016). Due to financial and time constrains only ten SNPs were chosen. However, the selection was based on known or the presumed role of the risk alleles in diabetes (insulin secretion, beta cell function, insulin sensitivity, effect on glucagon) and the obesity pathophysiology, which constitute further to the aim of the study to understand the burden of diabetes, dysglycaemia and their co-determinants. A candidate gene study was the appropriate study design for the study's genetic analyses.

### 4.11.4 Candidate gene studies vs. Genome-Wide Association Studies (GWAS)

Candidate gene studies are based on known or presumed biological function of selective putative genes that are related to the disease under study (Gloyn and McCarthy, 2001). Hence, candidate gene association studies consider the statistical efficiency of the biological understanding of the phenotype and the association analysis of the complex disease under study (Tabor, Risch and Myers, 2002). Candidate gene analysis utilizes custom arrays of SNPs, which enable the researcher to select the target SNPs to be investigated, as was performed in this study. These analyses are also performed when budget constrains are present as opposed to GWAS (Peters *et al.*, 2010). However, limitations to such studies are present, such as the fact that the biology of the disease needs to be known. Population stratification (which also occurs in GWAS studies) may lead to false positive results due to non-random mating or population subdivision. This leads to variations in marker allele frequencies among population segments as a result of founder effects or genetic drift (Slatkin, 1991). In such cases, a positive result occurs due to a difference in ancestry rather than the association between

genes and the disease (Freedman *et al.*, 2004). Moreover, candidate gene studies may be non-replicable between studies due to variations in study design and different relative risks in different populations.

Genome-wide association studies (GWAS) have enabled the identification of a number of variants that are contributing to common diseases that were previously unrecognized in candidate gene studies (Hirschhorn and Daly, 2005). The GWAS have been characterized as "hypothesis-free" as this method offers the opportunity to overcome all hurdles of incomplete understanding of the pathophysiology of the disease while scanning large chunks of the genome (Hunter, Altshuler and Rader, 2008; Pearson and Manolio, 2008). GWAS are mainly used for the discovery of common variants conferring low to moderate risks of the disease (Reich and Lander, 2001). The most common type of variants identified by GWAS are the gains and losses of DNA (copy number variants) that are known to have phenotypic effects on gene expression as well as function (Estivill and Armengol, 2007). However, a number of variants are not picked up by GWAS, namely structural variants, noncoding RNAs and epigenetic changes (Kitsios and Zintzaras, 2009). Furthermore, GWAS tests associations between each individual variant and a specific phenotype while assuming independent effects. This is despite the fact that complex diseases are caused by the interplay of multiple genetic and environmental functions (Cordell, 2002). It has been reported that many SNPs have small individual size effects and it is only in combination that their genetic effect is enhanced (Cordell, 2009). Therefore, when analysing individual SNPs, the GWAS misses such signals since these are usually embedded in a genome-wide sea of noise. Rare variants are usually also missed by GWAS, following the fact that existing GWAS platforms are not designed to capture such variants. These rare variants may

contribute to the heritability of many traits and diseases, and missing such variants may contribute to "missing heritability" (Maher, 2008). Missing heritability has been attributed to the gene-environment, gene-gene interactions and epigenetics. The epigenetic factors include DNA methylations and histone modifications that might be contributing to the environmental exposures leading to T2DM risk (Stančáková and Laakso, 2016).

Furthermore, as already mentioned, the majority of the GWAS tagged SNPs are within intronic regions (intergenic) and are likely to influence gene regulation and makes biological interpretation difficult (Edwards *et al.*, 2013). Therefore, SNPs identified through GWAS such as FTO, CDKAL1 and NOTCH2, which were studied in this study, may exhibit phenotypic characteristic that may be difficult to interpret, which may be the case in this study.

### 4.11.5 Interpretation of genotype assay

The study followed a candidate gene study through the utilization of the commercially readily available SNPs. The results obtained from the TaqMan® assay were analysed by following a case-control study design. The 'case' cohort consisted of the dysglycaemic subgroup (diabetes + pre-diabetes) and the metabolically abnormal cohort (normoglycaemia but at least one abnormal biochemical or anthropometric parameter) separately and compared to the 'control' cohort, which consisted of metabolically healthy subgroup. Two case cohorts were considered in order to evaluate the allele frequencies and associations present in the dysglycaemic Maltese population as well as those at metabolic risk. The 'metabolically abnormal' cohort represented an intermediate cardio-metabolic state with the possible presence of insulin resistance

(Nyenwe and Dagogo-Jack, 2011; Grundy, 2012). The control cohort was made up of a small population that exhibited a metabolic healthy profile. This metabolically healthy cohort consisted of a small sample size with a predominantly female population. The metabolically healthy cohort was considered to have a lack of underlying metabolic factors that may influence the genetic analyses. However, no significant associations were found when comparing both case cohorts to the metabolically healthy control. This may be due to the small sample size as well as to possible unknown confounding factors such as epigenetic and environmental factors. This could also be due to possible different allele frequencies between the cases and controls due to population differences unrelated to the metabolic status. In fact, population stratification is a known potential problem in association studies giving rise to false positive or false negative findings (Marchini *et al.*, 2004; Palmer, Burton and Smith, 2011).

The Hardy-Weinberg equilibrium states that alleles and genotype frequencies in a population will remain constant from one generation to another (Crow, 1999). The majority of the allele variants in this study's subgroups (dysglycaemia, metabolically abnormal, metabolically healthy) followed the Hardy-Weinberg equilibrium. However, the *FABP2, PPARGY* and *FTO* exhibited disequilibrium. Such disequilibrium could be due to the large influx of migrations experienced by the Maltese Islands over the past couple of decades. Other possible reasons could arise from genetic drift, which is a common occurrence in small populations or from population stratification. The presence of mutations or natural selection can also contribute to disequilibrium from Hardy-Weinberg.

For each of the three cohorts (dysglycaemia, metabolically abnormal and metabolically healthy), the minor allele frequencies (MAF) of each of the ten SNPs were established. As expected, minor variations between the MAFs were present across the three cohorts (Knowler *et al.*, 1988). Considering the location of the Maltese Islands, which are situated between the European continent and the African continent, the allele frequencies for each SNP were compared to both continents. The 1000 Genomes Phase 3 was the source for the reference MAF (http://www.ensembl.org/index.html.) Table 4.2 compares the established MAF across the cohort selected for genetic analysis (dysglycaemic, metabolically abnormal and metabolically healthy) with the reported MAF of Tuscany Italy and Africa.

The majority of the SNP's allele frequencies were similar to those reported for Tuscany, Italy. However, the MAF for *FABP2* (rs1799883), *HHEX* (rs1111875) and *PPARGY* (rs1801282) were found to be different from those found in Tuscany, Italy and African MAFs. Of note, both *FABP2* and *PPARGY* were also found not to be in Hardy-Weinberg equilibrium. Such differences may be due to spurious associations resulting from genetic ancestry (Knowler *et al.*, 1988). Another explanation could be due to a founder effect, which is a common occurrence in islands. The Founder effect occurs when there is loss of genetic variation as a new population is formed from a small number of individuals originating from a larger population. During the centuries, the Maltese Islands were conquered by multiple nations that may have had an effect on the population contributing to the formation of such a Founder effect. More recently, the Maltese Islands have experienced a large influx of immigrations from all across the globe, especially from the Eastern part of the Mediterranean Sea, the Eastern part of Europe and from the Northern Africa. In fact, a small proportion of this study's genetic

cohort reported to be ‘non-native born’, which further points to the potential effect these individuals might have had on the results established. Furthermore, considering the population diversity that is constituting the Maltese population could also explain the divergence from Hardy-Weinberg equation for some of the SNPs within the different cohorts understudy including the ‘Metabolically Healthy’ cohort. Another potential reason for these finding is interbreeding, which might be occurring in some geographical areas of Malta as well as between the migrating population settling in Malta.

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| | | Dysglycaemic cohort | Metabolic Abnormal | Healthy | Tuscany Italy | Africa |
|---|---|---|---|---|---|---|
| **Gene / SNP** | **Allele** | **MAF** | **MAF** | **MAF** | **MAF** | **MAF** |
| *ADRB2* rs1042713 | G/**A** | 0.39 | 0.43 | 0.34 | 0.37 | 0.52 |
| *NOTCH2* rs10923931 | G/**T** | 0.12 | 0.12 | 0.11 | 0.09 | 0.38 |
| *CDKAL1* rs7754840 | G/**C** | 0.32 | 0.32 | 0.33 | 0.27 | 0.63 |
| *FABP2* rs1799883* | C/**T** | 0.32 | 0.37 | 0.33 | 0.25 | 0.23 |
| *FTO* rs9939609 | T/**A** | 0.40 | 0.41 | 0.32 | 0.46 | 0.49 |
| *HHEX* rs1111875* | C/**T** | 0.61 | 0.56 | 0.58 | 0.41 | 0.18 |
| *KCNE4* rs1440072 | **C**/T | 0.21 | 0.16 | 0.21 | 0.14 | 0.04 |
| *PPARGY* rs1801282* | C/**G** | 0.25 | 0.30 | 0.30 | 0.08 | 0.01 |
| *SLC30A8* rs13266634 | C/**T** | 0.20 | 0.24 | 0.23 | 0.25 | 0.07 |
| *TCF7L2* rs7903146 | C/**T** | 0.39 | 0.37 | 0.40 | 0.37 | 0.26 |

MAF = Minor Allele Frequency
Alleles in **bold** represent the minor allele
* MAF divergent from Tuscany Italy and African

Table 4.2 Comparisons of the minor allele frequencies of the ten SNPs between the different study's cohorts with Italy and Africa

Genetic drift occurs when the true allele frequency of a certain population is not accurately presented in the new population. Genetic drift may have also occurred by the genetic bottleneck following World War II (WWII). This genetic bottleneck can occur when the number of individuals in a population is reduced drastically. During the war, the Maltese Islands suffered from famine and selection pressure that led to a reduction in the population (both in mortality and birth rate). A severe post war economic depression also contributed to a substantial emigration and a further population reduction. The presence of selection pressure is another possible explanation to the difference in allele frequencies. Selection pressure occurs when external agents such as resource availability, environmental conditions and biological factors affect the individual's ability to survive in a given environment. Furthermore, population stratification may have possibly had an effect on the results. This follows the fact that less than half the study's genetic sub-cohort reported a history of T2DM. Population stratification occurs from the admixture of two populations, which can lead to spurious associations between a genetic marker and the disease phenotype.

In summary, the established variations of the allele frequency from neighbouring countries could be a consequence of the Maltese population being a product of a unique genetic and social history along with established ancestral patterns of geographic migration, mating practices and reproductive expansions (such as the baby boom after WWII). Furthermore, bottlenecks and stochastic variation may have yielded the established allele frequencies

### 4.11.6 Heterogeneity of type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a heterogeneous disease with a genetic predisposition and environmental influences. Rapid changes in environmental factors such as lifestyle factors are the most likely contributing factors resulting in the global T2DM epidemic (Tuomi *et al.*, 2014). T2DM heterogeneity can be classified into different heterogeneity categories, namely clinical heterogeneity, locus heterogeneity and allelic heterogeneity.

Clinical heterogeneity incorporates clinical related factors that increase susceptibility to development of diabetes. Obesity is a strong modifiable factor contributing to T2DM development. Obesity leads to ectopic fat deposition in insulin-sensitive organs such as skeletal muscle and liver that affect the insulin-signalling pathway (Petersen *et al.*, 2007). This enhances insulin resistance due to a defective non-oxidative glucose pathway metabolism; increased intramyocellular lipid content in the liver and viscera (Weiss *et al.*, 2003; Liska *et al.*, 2007; Taksali *et al.*, 2008). Therefore, an obese individual is more likely to develop T2DM. Interestingly, an obese adolescent has a faster beta cell failure with a deterioration rate of approximately 15% per year when compared to an obese adult beta cell deterioration rate of 7% yearly (Saad *et al.*, 1988; Edelstein *et al.*, 1997; Gungor and Arslanian, 2004). In this study, both of the ‘case’ cohorts (i.e. the dysglycaemic and metabolically abnormal) exhibited significantly higher median body mass index and waist circumference when compared to the ‘control’ cohort (i.e. normal metabolic characteristics and presumed lack of insulin resistance), which correlates to the pathophysiology of obesity as a susceptibility factor for T2DM.

The age of onset of diabetes plays a role in the clinical heterogeneity of diabetes. Traditionally, the age of onset of uncontrolled hyperglycaemia was used as the distinguishing factor to

identify the type of diabetes mellitus an individual was suffering from. However, nowadays this is of minimal clinical value especially between 20 and 50 years, where different types of diabetes can present, as discussed below.

Very young children are those most likely to suffer from type 1 diabetes mellitus due to autoimmune destruction of the pancreatic beta cell and insulin deficiency, in which case these require lifelong insulin. In fact, administering of insulin improves their hyperglycaemic state (Alberti, 2010; Libman IM, LaPorte RE, Libman AM, 2011).

The latent autoimmune diabetes of adults (LADA) presents with hyperglycaemia in middle age individuals. Until some time ago, the development of hyperglycaemia in middle age implied that the individual was suffering from T2DM. However, administering oral hypoglycaemic agents does not improve this condition (LADA) over a period of time. LADA has an autoimmune pathophysiology with the presence of GAD autoantibodies and ultimately results in an insulin-dependent state (Tuomi *et al.*, 1993).

Hyperglycaemia presenting at an early age (before 25 years) with a strong family history of hyperglycaemia, is liable to be classified as Type 1 diabetes due to its early onset. In this study, a small proportion of the diabetes population reported an early onset of diabetes (>30 years from time of conduction of the study) with a first-line family history of hyperglycaemia, which may point to the presence of monogenic diabetes. In a recent Italian study, it was reported that 23% of adults with a multigenerational T2DM had monogenic disease (Pezzilli *et al.*, 2018). The study by Pezzilli *et al*. (2018) considered a population in close geographic proximity of the Maltese Islands and this further support the hypothesis that some of this study's diabetes individuals may have had monogenic diabetes. Unfortunately, it was beyond the scope of this

study to test for such a condition. Monogenic diabetes (MODY) occurs due to beta cell dysfunction and results from one genetic mutation unlike T2DM, which is a multigenetic disease and therefore shows 'locus heterogeneity' (Tattersall, 1974, 1998; McCarthy and Hattersley, 2008).

Locus heterogeneity implies that mutations in different genes may explain one variant phenotype. T2DM also shows 'allelic heterogeneity', which is a feature of genetic architecture where different alleles in the same gene contribute to similar phenotype variant. An example of allelic heterogeneity was reported at the TCF7L2 locus (Klimentidis, Zhou and Wineinger, 2014).

### 4.11.7 Genotype-phenotype associations

The prevalence of T2DM in Malta has been reported to be exceptionally high when compared to other European countries and this study further supported this fact (Katona, G, Aganovic, I, Vuskan V, 1983; Cuschieri and Mamo, 2014; International Diabetes Federation, 2017). Considering the high T2DM prevalence, the Maltese population is an ideal population to conduct T2DM genotype-phenotype association studies. Of the ten SNPs under study, only NOTCH2 (rs10923931), FABP2 (rs1799883) and HHEX (rs1111875) exhibited significant genotype-phenotype characteristics. Considering that the Maltese population might have undergone genetic drift across recent decades and founder effects are known to affect islands along with the fact that a small proportion of the genetic cohort (4.09%) were non-native born these factors could all have contributed to the limited associations presented in this study. Such genetic diversity in the genetic population might have led to confounding effects on the case-control association studies due to population stratification (Wang *et al.*, 2010).

#### 4.11.7.1 *NOTCH2* (rs10923931)

The expression of the *NOTCH2* gene activates the HES/HEY family, which is positively correlated with hepatic glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase (PCK1) as well as with the regulation of hepatic glucose output. *NOTCH* signalling is present in all adults' hepatocytes. However, in the presence of insulin resistance there is enhanced signalling activation. In fact, *NOTCH* activation has been reported to be present in hepatic insulin-resistance leading to hyperglycaemia and eventual development of diabetes. Furthermore, *NOTCH* signalling is correlated with hepatic lipid content, necro-inflammation markers and an abnormal metabolic state. (Valenti *et al.*, 2013). This correlates with the study's findings where the mutant T/T genotype was found to be associated with higher fasting plasma glucose levels than those carrying the 'G-allele'. The phenotype of the T/T genotype was expressed mostly in those individuals with a diagnosis of diabetes and being obese. These individuals exhibited a 'metabolic syndrome' profile with an elevated lipid profile, which all correlated with possible over-activation of *NOTCH* signalling. Conversely, those with a normal metabolic state (defined as those metabolically healthy) and carrying the T/T allele still exhibited a normal metabolic phenotype. These subjects may be prone to develop hyperglycaemia and diabetes at a later stage. However, a longitudinal cohort study is required to establish such an association.

#### 4.11.7.2 *FABP2* (rs1799883)

The *FABP2* gene encodes for the intestinal fatty acid binding protein (IFABP) and is a family member of the intracellular lipid binding proteins (Baier *et al.*, 1995; Hertzel and Bernlohr, 2000; Furuhashi and Hotamisligil, 2008; Qiu *et al.*, 2014). The *FABP2* transports hydrophobic fatty acids from the plasma membrane through aqueous cytosol to the

endoplasmic reticulum, where the fatty acids are esterified to form triglycerides and transported into the circulation as chylomicrons (Weiss *et al.*, 2002). An allele transition from G to A (rs1799883) contributing to a substitution of amino acids from Alanine (Ala) to Threonine (Thr) results in an increased binding affinity of long chain fatty acids as well as enhanced triglyceride secretion (Baier, Bogardus and Sacchettini, 1996; Qiu *et al.*, 2014). The *FABP2* affect lipid metabolism and does not have a direct effect on insulin resistance. This corresponds to the fact that in this study's T/T genotype (mutant), it was more common in the 'metabolically abnormal' cohort as opposed to the 'Dysglycaemic' cohort. Dysglycaemic individuals tend to have dyslipidaemic abnormalities following hepatic insulin resistance or adipocyte saturation (Mooradian, 2009; González-Muniesa *et al.*, 2017; Rocca *et al.*, 2018). *FABP2* has an effect on the intestinal fatty acid binding protein and does not affect the liver, which further supports this study's findings. This puts forward the suggestion that the dyslipidaemic abnormalities within the 'Metabolically Abnormal' cohort are arising from abnormalities at the intestinal level.

In this study, the mutant genotype (T/T) had significantly higher HDL-C and lower triglycerides than the C/T and C/C alleles. This phenotype characteristic could be the result of statin therapy or be due to other unaccounted confounding factors. In fact, statin therapy (Rosuvastatin) was reported to decrease IFABP levels, although the subjects under study were suffering from HIV rather than from T2DM (Funderburg *et al.*, 2016). Statins were also reported to influence liver-FABP expression which contributed to lower triglyceride levels (Landrier *et al.*, 2004). Thus, the contradictory effects of the T/T genotype on the *FABP2* pathophysiology could be due to potential pharmacogenomics effect of statin therapy on FABP transporters. The same phenomenon was exhibited when evaluating the biochemical parameters effect on the *FABP2* variants in a co-dominant model. However, on evaluating the

biochemical parameters effect on the FABP2 variants in a recessive model (C/C + C/T vs. T/T) and in a dominant model (T/T + C/T vs. C/C), both exhibited a lower HDL-C and higher triglyceride levels. These findings correspond to the *FABP2* pathophysiology. Of special interest, the T-allele variant in both models appeared to "overcome" the potential statin effect or had other confounding factors that might have led to the co-dominant model results. These effects could also be due to the small sample size of the study as well as possible founder effects.

#### 4.11.7.3 *HHEX* (rs1111875)

The *HHEX* gene is involved in the encoding of transcription factor of the Wnt signalling pathway, which is the fundamental pathway for cell growth and development of the pancreas (Morgutti *et al.*, 2001; Foley and Mercola, 2005; Smith, 2006). It was reported that HHEX-IDE (rs1111875) variant was associated with insulin secretion response following glucose load (Grarup *et al.*, 2007). Thus, a *HHEX* mutation may affect the β- cell function leading to the inability to compensate for hyperglycaemia. In fact, this was the case in this study, where the mutant C/C genotype exhibited a higher median FPG when compared to the heterozygous and homozygous variants. Furthermore, the C/C genotype was mostly present in the diabetes subgroup, which further correlates with the pathophysiology of *HHEX* on the beta-cell function. Serum insulin was not measured in this study. Due to this, insulin resistance could not be calculated. However, the insulin resistance risk was calculated by utilizing the triglyceride to HDL-C (TG/HDL-C) risk ratio. Individuals carrying the C/C genotype exhibited a high TG/HDL-C ratio corresponding to high-risk of insulin resistance. This furthers correlates with the HHEX pathophysiology.

### 4.11.8 Associations with type 2 diabetes mellitus

The *FTO* variant was independently associated negatively with having a diagnosis of T2DM after adjusting for BMI, age and gender following both a co-dominant model (A/A variant significant) and a recessive model (T/T + T/A vs. A/A). Considering that *FTO* is related to obesity, BMI was considered as a confounding factor and adjusted for in the model. The established association is in contrast to the findings of the reported literature, where the *FTO* was associated with both obesity and T2DM (Yajnik *et al.*, 2009). *FTO* polymorphism predisposes to an increased weight again (high BMI) and subsequently increases the risk of developing diabetes (Frayling *et al.*, 2007). A possible explanation for this study's result could be due to the small sample size of the study, the effect of environmental factors or other confounding factors that were not adjusted for during modelling. A possible confounding factor could be the level of physical activity performed by the individuals. Although physical activity was measured in this study, results appeared to be inaccurate and were not used for these associations. However, it has been reported that those carrying the *FTO* mutant or were heterozygous, had lower BMI if they were physically active (Andreasen *et al.*, 2008). Possible founder effects may be at play and contributing to such a result. The *FTO* has an intronic effect and it was reported that such genes may exhibit biological phenotypes that may be difficult to interpret (Edwards *et al.*, 2013). This may be another possible explanation to this study's results.

In a dominant model the *KCNE4* T-allele (T/T + T/C vs. C/C) was associated with lower risk of having a diagnosis of T2DM. Both BMI and waist circumference have been related to *KCNE4* and therefore were considered as confounding factors (Croteau-Chonka *et al.*, 2011). The association remained significant after adjusting for age, gender, BMI and waist circumference. *KCNE4* has been reported to have an inhibitory effect on the potassium-voltage

channel *KCNQ1*, where in return the *KCNQ1* has been associated with T2DM in both European and Asian populations (Grunnet *et al.*, 2002; Unoki *et al.*, 2008; Yasuda *et al.*, 2008). Thus, the inhibitory effect of *KCNE4* on *KCNQ1* reduces the risk of T2DM, which coincides with this study's resulting association.

### 4.11.9 Implications of genetic findings

The genetic findings in this study exhibit some unique and unexpected findings when compared to other similar studies performed locally and internationally. One needs to keep in mind that the genetic cohort under study was derived from a national representative sample obtained during a cross-sectional study, which albeit having its own limitations, follows a different study design than previous work done at a local level (Pace, 2013; Craus, 2016). The current genetic cohort was composed of a small sample size due to financial constraints and limited human resources, which might have contributed to the established findings. Nevertheless, the genetic cohort was composed of varied ancestral backgrounds (native born 95.91%, EU-born 2.34% and non-EU born 1.75%). Hence, different ancestral genetic profiles might have been the driving factor for these unique findings. It was also established during this study, that significant phenotypical profile differences were present between different geographical districts. This points to a potential geographical effect on the Maltese population that might be affecting the genetic make-up. On needs to also consider the fact that social, cultural and environmental factors were not considered or adjusted for during this study. These might have significant effects on the genotypical profile. Such findings have an important public health implication and should be considered in further analyses in the future. It is recommended that the associations established should be re-validated using a larger national representative sample population keeping in mind the ancestral origin of the population along

with different social, cultural and environmental factors that might potentially have an effect on the genetic profile. Furthermore, considering the advances in the genetic field, the effect of potential epigenetics could also be considered. Such evidence-based data would enable the formulation of public health interventions that are genetic and phenotypic specific.

The genetic findings in this study, even if based on a small sample size carrying a number of limitations, already presents a dysglycaemic and a dyslipidaemic genetic susceptibility profile. This goes in accordance to the epidemiological findings of this study, where a metabolically unhealthy profile predominated with a substantial proportion of the study population exhibiting a dysglycaemic and dyslipidaemic phenotypical profile. The current study furthermore points to the possibility of the genetic profile of the dysglycaemic individuals being different to that of the metabolically unhealthy but normoglycaemic profile. In fact, the *FABP2* mutant allele exhibited a higher prevalence within the metabolically abnormal cohort rather than the dysglycaemic cohort which might suggest that a dyslipidaemic profile within the metabolic abnormal cohort was originating from a different genetic predisposition from the diabetes dyslipidaemic profile. Hence, a larger genetic population study would not only re-assess the associations that were analysed in this study but might enable the formulation of a combined phenotypic and genetic risk scores that could be utilized as a means of a population-based preventive screening strategy for the high-risk population

## 4.12 Study strengths and limitations

The SAHHTEK study was a cross-sectional (retrospective) study and therefore unable to consider temporal relationships with dysglycaemia in Malta. In spite of this, a number of links were established between the different measured and self-reported factors with dysglycaemic and other conditions identified in this study.

The strength of this study is that it was a nationally representative sample with an adequate response rate considering the invasive measurements performed. In fact, as discussed previously the Czech edition of the European Health Examination Survey (EHES) obtained a response of 31.69% (Čapková *et al.*, 2017). While the better-established SHeS in Scotland managed a response rate of 64% from all across Scotland (Scottish Government, 2016). However, potential selection bias might still have occurred. Responders may have been different to non-responders and it remains difficult to remove this bias altogether. The decision to conduct weighting of the data by age, gender and towns was an effort to partly overcome selection bias given a worse no response in a specific age-gender stratum. Even though the population data was weighted, some subgroups still remained with small numbers. This may have affected the power of specific subgroups statistical testing, resulting in possible type II errors. Potential known confounding factors were adjusted for during multiple regression analysis, although the presence of other unknown confounding factors may still have been present such as environmental, behavioural and genetic factors.

Health examination hubs were set up across all the towns of Malta. However, only one hub was set up for the island of Gozo, which was considered as feasible since the island is relatively small compared to Malta. By setting up hubs within towns, it enabled easy access for participation. Those accepting the invitation did not require to travel far from their home. In

fact, for most of the participants the examination hub (*berga* or health centre) was within walking distance. During the pilot study it was observed that the older population (45+ years) preferred the early appointments (7am till 8.30am) while the younger population preferred the later appointments (8.30am till 9.30am). Such appointment scheduling was followed for the health examination survey. However, appointments were amended to the participant's request. This appointment flexibility and setting examination hubs within the participant's residential area played to the strength of the study and contributed to a relatively good response rate.

Another strength of this study is the limited sources of information bias. The data collection was performed by the same small number of trained fieldworkers ($n$=10). Revalidation and training seasons with all fieldworkers were conducted every couple of months to ensure validity and standardization throughout the data collection. Furthermore, prior to the start of each data collection day, the project coordinator reminded the fieldworkers of the study's protocols. Most of the study's data was measurable and therefore provided more accurate data than self-reported data, which is most likely to be overestimated or underestimated. None of the examined measurements were rounded up but the actual reading was recorded and hence this eliminated digital preference. Validated tools of measurement were used following the EHES guidelines. However, the demographic data, lifestyle habits, medical histories and family histories were all inevitably compiled via self-reporting by participants. Such data carried the risk of human bias or recall bias. Smoking habits data was not validated through biochemical assays. It has, however been reported that self-reported smoking exhibits good agreement with nicotine concentrations in population studies (Yeager and Krosnick, 2010). Data on alcohol intake was subject to the memory and reporting bias. The alcohol data gathered was categorized which may have influenced to some degree the interpretation of results. The information gathered on depression was based on self-reporting, which might have been

subject to data inaccuracy or false statements given it is a sensitive subject with social stigma. Information on physical activity was self-reported and although both the interviewers and the participants had a readily prepared explanation sheet for each physical activity category (mild, moderate and vigorous), the data gathered showed no relevant correlations or associations with any metabolic condition. The probable reason for this is that sedentary individuals over-reported their daily activities while those leading an active lifestyle did not consider certain activities as being a form of physical activity. This made the physical activity data inaccurate and could not be used, as it was intentioned. Similarly, the self-reported food frequency section was not used in data analyses due to questionability of its reliability, especially in the obese participants. Seasonal variations may have impacted on the reported physical activity patters as well as food consumption frequency given that the data gathering lasted in excess of 12 months.

Blood pressure measurement is very sensitive to the state of mind or behaviour of the participant during the measurement, the environment setup and to the aneroid sphygmomanometer device used. The aneroid sphygmomanometers used for this study were validated to start with and calibrated regularly. However, some effect on accuracy may still have taken place despite the fact that the fieldworkers were trained, and their readings were double-checked with other experienced blood pressure measurers prior to initiation of the fieldwork. Thus, observer auscultation errors may have occurred causing systematic error such as through reaction times to auditory and visual cues. Different cuff sizes were available, and fieldworkers were trained to measure the cuff size prior to taking the blood pressure. Measurement of blood pressure was taken after 20 minutes of sitting down; this minimising blood pressure fluctuations. All participants were asked to sit up straight with both feet touching the floor and their arms resting at the heart level, as these techniques were known to

enable more accurate readings. For ease of this study, participants scoring three consecutive high blood pressure measurements during the health examination survey, were considered to be hypertensives. Patients were advised to seek medical follow up by their family physician since their high blood pressure could have been due to white coat hypertension. Hence, the global hypertension prevalence rate in this study could have been overinflated.

Participants were asked to remove heavy clothing and accessories only without compromising privacy during the anthropometric measurements for weight, height, hip and waist circumference. Because of this, the measured weight may have been slightly overestimated, particularly in the winter months. However, although light clothing was still present, palpation for landmarks was performed when measuring for the hip and waist circumference to ensure that the measuring tape was placed in the correct position.

The SAHHTEK protocol involved measurement of the fasting plasma glucose (FPG) and lipid profile for the whole sample population (using the left arm since the right arm was used for the blood pressure). The oral glucose tolerance test was only offered to those with impaired fasting plasma glucose (IFG) obtained during the health examination stage. Participants obtaining normal fasting plasma glucose but with underlying impaired glucose tolerance or type 2 diabetes could have been missed. Furthermore, participants obtaining an impaired fasting glucose during the health examination and not accepting their invitation for the oral glucose tolerance test (OGTT), could have led to underlying unaccounted impaired glucose tolerance or type 2 diabetes. All diabetes tests including fasting plasma glucose have both a pre-analytic and analytic variability. There is a possibility that on repeating the test/s, a value below the diagnostic cut-off point may be present. Since this was an epidemiological study, a repeat FPG was not performed in those newly diagnosed as diabetes (FPG >=7mmol/L). However, these

individuals were advised to seek medical help from their personal family doctor. Furthermore, individuals obtaining an impaired fasting plasma glucose in the health examination survey might have been normoglycaemic on repeating the FPG. In fact, this was the case in some of individuals with IFG accepting the invitation for an OGTT. Considering the low percentage of participants obtaining a normal fasting plasma glucose result during the OGTT stage, descriptive and analytic analysis were based on the initially diagnosed IFG population.

A potential pre-analytic variability may have occurred, even though great care was taken to ensure that the blood samples were transported to the biochemistry laboratory within 2 hours from bloodletting. All bloodletting was performed while the participant was in the sitting position, this limiting any inter-subject variability results that are known to occur due to posture variance, especially for total cholesterol measurement. Attention was also given not to prolong the use of a tourniquet to prevent the vein from being occluded beyond 120 seconds so as not to affect the total cholesterol levels.

The data collection took place during weekends and public holidays between November 2014 till November 2015, excluding the Christmas-New Year weekends, Easter Weekend and the hottest summer months between June and August. The summer months were excluded due to the expected decline in response rate (Potoski *et al.*, 2015). During summer the weather is pretty hot, and a sizeable proportion of the Maltese population are known to undergo internal and external migration to summer homes or holiday destinations. However, seasonal variations may still have had an effect on the response rate as well as on the biological measures, such as blood pressure, FPG, blood lipid levels, BMI and waist circumference. The study was a health examination survey and hence clinical diagnosis could not be established. However, being a health examination survey high-risk population for particular conditions could be identified.

Differences in the socioeconomic and metabolic parameters between the Gozo district and the other Maltese districts could be subject to Type II errors due to small numbers involved.

The literature provides various definitions for the metabolic syndrome and the established prevalence figures could be subject to the definition used by the study.

The study does not cover the whole population but only a subset of the adult population. General demographic data was based on the published reports from 2013.

The health cost for undiagnosed diabetes was based on a U.S. study, as no data was available for the Maltese population, although the GPD per capita for both countries was taken in consideration. The cost of illness data was obtained from secondary sources and this may have affected the validity of the cost of illness in the Maltese health care setting. The previously known diabetes cost was obtained from the 2015 IDF estimated costs for Malta but no detailed description was available for the breakdown of the health care costs. All costs from the secondary sources lacked confidence intervals. Therefore, sensitivity analysis could not be performed on the unit costs. Comparisons of the whole study population with other studies (local and internationally) were difficult due to the fact that the age groups and the total population samples were different from other studies and not comparable. Projections for the year 2050 were based on current conditions with an assumption that all demographic and risk factors would continue at their current rates. The changing migratory patterns across Europe can indeed result in a different impact on a very changed situation by 2050 both in terms of demography, as well as on the economic situation of that time. The economic burden excluded certain society burdens, including intangibles from pain and a decreased quality of life, as well as the impact on caregivers.

The diabetes risk score was based on the general population with T2DM as the outcome. Although the prediction model predicts the outcome of interest it was not internally validated or external validated. The model was not evaluated for its impact on healthcare; individual behaviour or care; patient's health outcome or cost-effectiveness. Furthermore, there may have been risk factors or medication therapies (confounders) that were not considered when creating the model. This may therefore have biased the risk estimation. Therefore, further research and validation is recommended before utilizing this risk score.

The genetic population consisted of a small population number, which may have had an effect on the results. The case and control populations were age matched as much as possible although there was still a discrepancy in the gender population. There were more females that fell within the healthy population, which again could have affected the genotyping results. Considering that both case populations (dysglycameic and metabolically unhealthy) may have been on medication including metformin (in previously diagnosed diabetes), statins and aspirin, such medication may have interacted on gene expression. Such drugs have an effect on multiple tissue types through signalling pathways that eventually alter the systemic metabolic and inflammatory profile of the individual. Genotyping was limited to just 10 SNPs and exhibited limited associated data between genetics and diabetes.

# Chapter five – Conclusion

## 5.1 Conclusions

A cross-sectional study was conducted among a nationally representative sample of adults (aged 18-70 years) in Malta to evaluate the burden of type 2 diabetes mellitus (T2DM) and dysglycaemia while analysing for their co-determinants among the Malta adult population. The Malta prevalence burden of diabetes (T2DM) has substantially increased since the last nationwide epidemiological study conducted in 1981, especially considering the amount of hitherto yet undiagnosed diabetes. This T2DM increase was comparable to findings in several other European countries, although the Malta T2DM burden was found to be amongst the highest in Europe. Furthermore, the burden of impaired fasting glucose (IFG) within the adult population of Malta was fully explored for the first time as part of this study. It was concluded that dysglycaemia (T2DM and IFG) was affecting approximately one in four adults in this population (aged 18 to 70 years), at the time of study. In fact, dysglycaemia was found from as early as the third decade of life for IFG and from the fourth decade of life for T2DM. The prevalence for both dysglycaemic conditions was naturally higher with advancing age.

This study also shed light for the first time on the full extent to which the current adult population was afflicted by the modern epidemics of overweight and obesity. Most of the Maltese adult population in the study, was either overweight or obese. Furthermore, approximately one in four adults were also found to suffer from the metabolic syndrome. The prevalence for both the overweight and obese conditions naturally increased with advancing age. The overweight-obese epidemic was predominately found within the male population. Furthermore, on comparing to the previously conducted local epidemiological studies, those

classified as obese were more prevalent, while those classified as overweight were less prevalent. This held true across the age range studied among the adult population.

A substantial proportion of the Malta adult population suffered from global hypertension (a combination of self-reported and newly diagnosed on examination). However, the hypertension situation (at the time of the study) appeared to be less prevalent as compared to the previously reported local epidemiological studies. Hypertension was predominant within the diabetes population, affecting more than three-quarters of this sub-population, with a male predominance. Meanwhile almost half of the IFG population exhibited a hypertensive profile, again with a male predominance.

The lipid profile within the Malta adult population was explored for the first time as part of this study. Most adults studied exhibited a characteristic normolipidaemic profile, although as expected the situation differed (and was increased) in the presence of dysglycaemia.

The burden of depression in Malta was also explored for the first time on a population level. This was further considered with regards to the depression burden among the diabetes population. At a population level, one in ten adults reported to be depressed, with a higher prevalence reported by the type 2 diabetes sub-population.

Approximately half of the Malta adult population under study reported a moderate alcohol consumption habit, although the majority of the diabetes population did not report such a habit. Similarly, the diabetes population was mostly self-reported as non-smokers in contrast to a quarter of the general population reporting to have the tobacco smoking habit.

The socio-economic status (in particular, the educational level) of the population was closely linked to the profile in the different biochemical and anthropometric parameters studied. In fact, the diagnosis of diabetes, IFG and overweight-obese status were all mostly prevalent within the lowest socioeconomic categories. The study exhibited an inverse relationship between education level and metabolic abnormalities (including BMI, waist circumference, FPG and lipid profile).

The population residing in the district of Gozo exhibited a significantly higher metabolically abnormal profile than did the five other districts making up the Island of Malta. On exploring relationships in this study, it was revealed that the population residing in the district (island) of Gozo had a higher risk for having a higher fasting plasma glucose, a higher LDL-C level and a higher total cholesterol level, even after adjustments for confounders were made.

While exploring the available relationships in this study, it was revealed that a higher abdominal adiposity and a higher median weight were linked to various metabolic abnormalities (diabetes mellitus, impaired fasting plasma glucose and hypertension) within the Maltese population. The presence of having the obese status, along with dyslipidaemia predominated as characteristics in the dysglycaemic populations (T2DM and IFG). In fact, the overwhelming majority of the newly diagnosed diabetes were either overweight or obese. The youngest adults (in their fourth decade) diagnosed with diabetes by this study were *all obese*. Furthermore, the obese status was also positively linked to the presence of hypertension.

The independent factors associated with having T2DM (as compared to the non-diabetes population) were an increase in fasting plasma glucose (FPG) level, a higher age, a

higher waist circumference, having a diagnosis of the metabolic syndrome and having a history of hypertension. On the contrary, higher levels of LDL-C and higher diastolic blood pressure levels were associated with lower levels of T2DM.

Independent factors associated with having T2DM (as compared to metabolically healthy individuals) were in the presence of higher fasting plasma glucose (FPG) levels, higher triglyceride levels and being male.

The independent factors associated with having IFG were a higher age, a higher waist circumference and a higher diastolic blood pressure level. It was found that higher levels of triglycerides and being a female were less likely to be associated with the presence of IFG.

The typical characteristics of the adult Type 2 Diabetes were found to be (1) age above 55 years, (2) the presence of an elevated waist circumference (male > 100cms and females > 90cms) and (3) the reporting of being on lipid lowering statin therapy. An adult presenting with all three characteristics at primary health care in Malta could be considered as at 'higher risk' of having diabetes mellitus.

The estimated economic burden of diabetes mellitus and obesity is considerable and contributes to a substantial proportion of the total health expenditure in Malta. It's projected increase by one-eight by the year 2050 (should all demographic and risk factors continue at their current rates) suggests that much of the health budget is amenable to reduction by effective preventive action.

Overall this study has demonstrated a change in the metabolic profile across time, with a gender shift from a female to a male predominance in diabetes at population level. The Maltese adult population, when compared to other European countries, had a high prevalence

of diabetes mellitus, of overweight and obese individuals. An increase in visceral adiposity appeared to be centre stage for the Maltese dysregulated metabolic profile. Health inequalities were evident between the close districts of the Maltese Islands. Regretfully, even though validated tools of measure were utilized for the determination of population physical activity and food frequency habits, the data gathered was found to be inconclusive, possibly due to reporting bias and hence was used sparingly. Furthermore, the study characterized the Malta diabetes adults and estimated the economic burden of both T2DM and obesity.

## 5.2 Recommendations

Both diabetes mellitus and obesity have been established as being major health burdens within the Malta population. Data such as this could be beneficial for politicians and public health specialists in order to plan health strategies and services. However, this study was retrospective and therefore temporal relationships could not be assessed. For this reason, the author puts forward the recommendation for further research, specifically including longitudinal studies, possibly on higher risk individuals as identified in this study.

Prospective studies could potentially elucidate the important links between diabetes and obesity and establish the temporal relationships between them. When considering the Maltese diabetes characteristics, further research could identify which (if at all) among the presence of cardiovascular disease risk factors identified - a high lipid profile or the actual intake of statins were causatively linked to the development of T2DM. It is more likely that statin use follows the diagnosis of diabetes (given the prevalent clinical policies) rather than the opposite scenario. It is also suggested that other factors such as behaviour and the environment could be

explored further as potential causative factors contributing to the early onset of diabetes within the Maltese population.

A gender effect was clearly observed, with the male population exhibiting an increased tendency for metabolic abnormalities, namely T2DM, obesity and hypertension. Such male gender dominance merits targeted further public health research and eventually, action that is gender sensitive, including interventions that reach out to the male population specifically. Age was also observed to be a contributing factor to metabolic abnormalities. This implies the need for policy interventions that are earlier and preventive in nature and which to try to reduce the evident burden of increasing weight gain with increasing age.

Further research studies are needed to establish whether the TG/HDL-C ratio could be used as a definitive marker for MetS in this country as well as to establish the TG/HDL-C Malta-specific cut-off points.

Application of the screening principles set out in 1968 by Wilson and Jungner to type 2 diabetes mellitus in Malta suggests that T2DM could be a candidate for screening in the adult Maltese population. Certainly, it fits the criteria better than do some of the conditions that Malta has initiated national screening for at EU instigation (Azzopardi-Muscat *et al.*, 2017). The WHO has suggested that when the prevalence of undiagnosed T2DM was high at a population level and significant associated cardiovascular disease risk was evident, then screening could be beneficial. This study among adults in Malta confirms the high prevalence and burden of T2DM. Population-based screening using a multi-stage screening process could well be feasible for Malta. It is therefore recommended that policy makers in Malta might reconsider the feasibility of screening for diabetes in Malta. It is also recommended that the diabetes risk

score established in this study be externally validated and pilot performance of its application be measured for discrimination, calibration and (re) classification before it could be considered for implementation in the community among adults in Malta.

Further research is also needed to investigate the characteristics among the population of Gozo, which this study has established as at relatively higher risk for diabetes and indeed for cardiovascular disease than the population of Malta. Significant differences in biochemical and anthropometric parameters were established between the adult populations of the two islands in this study. A larger and specifically Gozo population cohort would enable a better understanding of whether such differences and links are worthy of further action. However, island-specific rates (incidence and mortality) should be assessed and compared for cardiovascular disease and diabetes in a relatively easier ecological study with geographical comparisons.

Further research is also required to quantify and qualify the nutrition and physical activity of the Maltese population in a better way than this study has been able to do. This is required given the evident risks posed by the other cardiovascular risk profiles among adults in Malta. Other physical activity tools of measurement can be investigated such as the use of accelerometers, pedometers and heart-rate monitors, to assess for validity and thus possible application in any future study, in order to have a more accurate understanding of the adult Maltese physical activity profile. Similarly, other nutrition tools of measurement such as food diaries ought to be piloted locally for use to better assessment of the nutritional intake.

The genetic cohort studied in this study was, on practical grounds, composed of a small population cohort. However, the established significant links could be interpreted as having

important genetic roles within the Maltese population. Therefore, a larger genetic study could be undertaken for revalidation of the established significant links between different SNPs and anthropometric, biochemical and diabetes phenotypes. The genes that were found to have significant associations should be further investigated. It is known that each gene is composed of multiple base-pair variations on the same chromosome. The variations in the alleles are referenced using Reference SNP cluster ID. In this study, only one specific allele variant for each gene was analysed. Therefore, it is recommended, that other allele variants are analysed for the same genes (*NOTCH2, FABP2* and *HHEX*). This would enable the evaluation of the Maltese population for potential significant associations with different allele variants of the same genes.

Furthermore, one may consider undergoing genetic analysis using Next Generation Sequencing to enable mapping of entire Maltese genomes.

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# Appendix A – Permissions

- Research ethics committee
- Information and data protection commissioner
- Mater Dei hospital administration
- Mater Dei hospital pathology department
- Laboratory of Molecular Genetics

# Research Ethics Committee

**L-UNIVERSITÀ TA' MALTA**

Msida – Malta
Skola Medika
Sptar Mater Dei

**UNIVERSITY OF MALTA**

Msida – Malta
Medical School
Mater Dei Hospital

Ref No: **19/2014**

Friday, 30th May 2014

Dr Sarah Cuschieri
2, Il-Holma
Triq il-Marg
Attard ATD2380

Dear Dr Cuschieri,

Please refer to your application submitted to the Research Ethics Committee in connection with your research entitled:

**Prevalence and determinants of the type 2 diabetes and impaired glucose tolerance in Malta**

The University Research Ethics Committee granted ethical approval for the above mentioned protocol.

Yours sincerely,

Dr. Mario Vassallo
Chairman
Research Ethics Committee

***Email:*** umms@um.edu.mt • ***Web:*** http://www.um.edu.mt/ms

# Information and Data Protection Commissioner

IDPC
INFORMATION AND DATA PROTECTION COMMISSIONER

27th March 2014

Dr Sarah Cuschieri MD PG Dip. Diabetes (Cardiff)
Department of Public Health
Faculty of Medicine and Surgery
University of Malta
Msida.

Dear Dr Cuschieri,

I make reference to your letter dated 22nd March 2014 concerning the cross-sectional survey on diabetes in Malta which will be undertaken by the University of Malta, through the Department of Public Health.

Having recognised the benefits, the Maltese population, the Department and the Ministry of Health, will derived from this national project in the process of addressing the disease of diabetes;

Having considered that the study has already been approved by the Health Ethics Committee, the committee recognised by the Commissioner, in terms of article 16(2)(b) of the Data Protection Act, to approve non-academic studies involving health data;

Having confirmed that participation in this study shall be on a voluntary basis and the processing of participants' personal and sensitive personal data shall be legitimised on the legal criterion of informed consent;

Having considered that access to the collected participants' information, specifically the identification of blood samples, shall only be limited to two medical professionals, namely, Dr Mamo and Dr Cuschieri;

Having considered that the availability of such information to named co-researchers shall be carried out in an anonymous manner by coding the identification data pertaining to the participants' blood samples;

Having ascertained that any further research, carried out by other researchers, shall only be permissible with the consent of the respective data subjects involved in the study;

It is hereby being concluded that there shall be no data protection impediments for the retention of the blood samples for a period of ten (10) years at the University of Malta's BioBank.

Yours sincerely,

Ian Deguara
Head - Technical

Airways House, Second Floor, High Street, Sliema SLM 1549, MALTA. **Tel:** (+356) 2328 7100. **Fax:** (+356) 2328 7198. **E-mail:** idpc.info@gov.mt **Web:** www.idpc.gov.m

# Mater Dei Hospital Administration


Mater Dei Hospital Administration
Chief Executive Office

Ing. Joe Caruana,

Chief Executive Officer

Administration

Mater Dei Hospital

24th March 2014

To Whom It May Concern,

Mater Dei Hospital endorses the University of Malta Ph.D project "Prevalence and Determinants of the type 2 diabetes and impaired glucose tolerance in Malta" to be conducted by Dr. Sarah Cuschieri (ID: 23788M) and grants access to blood results from iSoft Programme.

Thank you

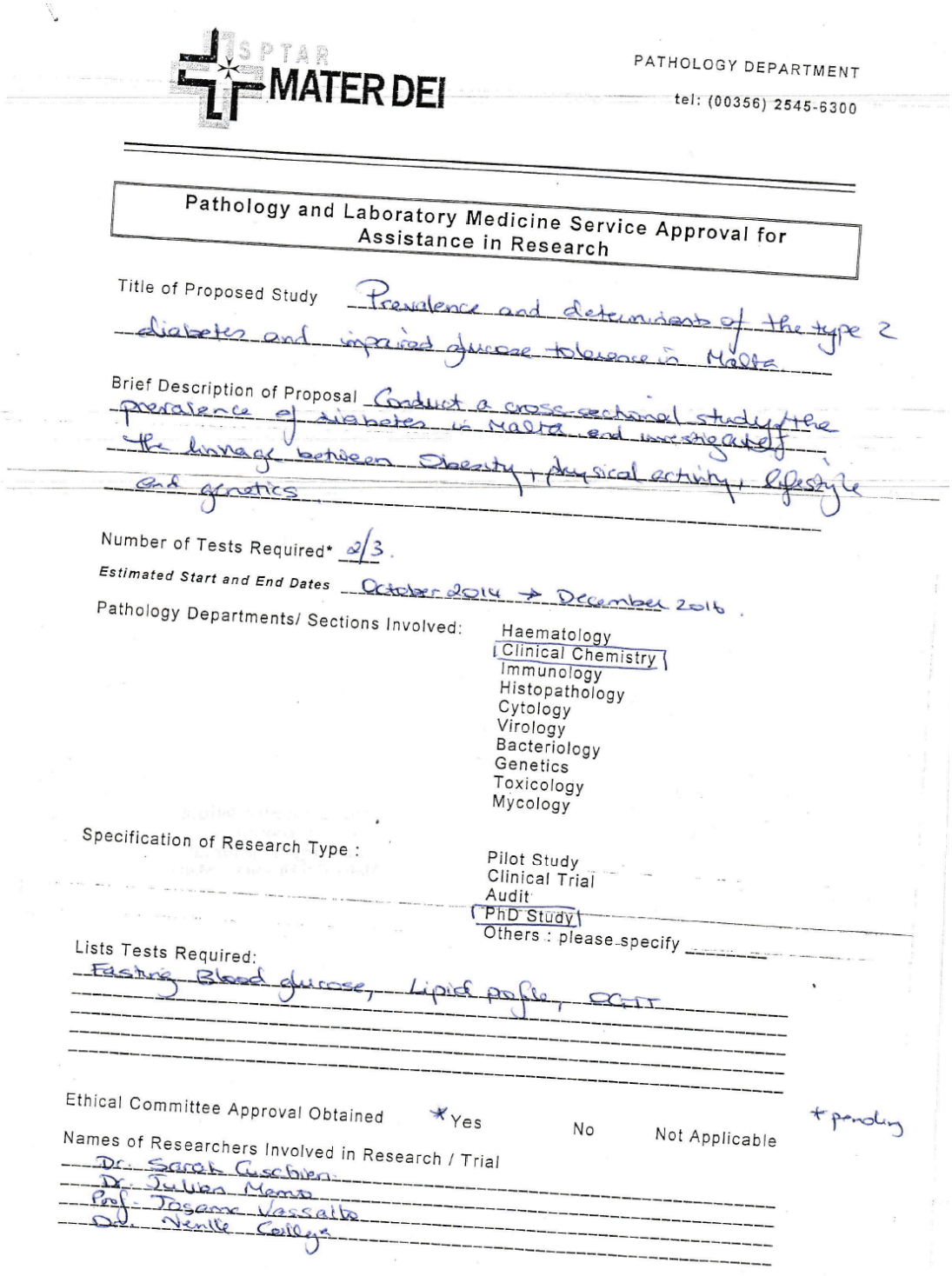
Yours truly,

Ing. Joe Caruana, CEO 23/3/14

MATER DEI HOSPITAL, TAL-QROQQ, MSIDA, MSD 2090, MALTA
tel (+356) 2545 0000 facsimile (+356) 21240 176 DDI: (+356) 2545 + Extension No.

Ministry for Social Policy

# Mater Dei Hospital Pathology Department



SPTAR MATER DEI

PATHOLOGY DEPARTMENT
tel: (00356) 2545-6300

**Pathology and Laboratory Medicine Service Approval for Assistance in Research**

Title of Proposed Study: Prevalence and determinants of the type 2 diabetes and impaired glucose tolerance in Malta

Brief Description of Proposal: Conduct a cross-sectional study of the prevalence of diabetes in Malta and investigate the linkage between Obesity, physical activity, lifestyle and genetics

Number of Tests Required* 2/3.

*Estimated Start and End Dates* October 2014 → December 2016.

Pathology Departments/ Sections Involved:
- Haematology
- [Clinical Chemistry]
- Immunology
- Histopathology
- Cytology
- Virology
- Bacteriology
- Genetics
- Toxicology
- Mycology

Specification of Research Type :
- Pilot Study
- Clinical Trial
- Audit
- [PhD Study]
- Others : please specify

Lists Tests Required:
Fasting Blood glucose, Lipid profile, OGTT

Ethical Committee Approval Obtained *Yes No Not Applicable *pending

Names of Researchers Involved in Research / Trial
Dr. Sarah Cuschieri
Dr. Julian Mamo
Prof. Josanne Vassallo
Dr. Neville Calleja

PATHOLOGY DEPARTMENT

Signatue of Lead Researcher / Applicant **

Contact Details

e-mail Sarah.Cuschieri@um.edu.mt pager number _____

phone number 79415298 Department of Anatomy

Who should be contacted in the case of Panic Values / Results if Applicable

Principal Investigation Dr. Cuschieri Tick where appropriate

On Call Physician ___

Other ___ Contact details : 79415298

Date of Application 18/2/14

Declaration of Acceptance of Specimens

For laboratory Use Only

Dr. Gerald Bahagiar
M.D., M.Sc., F.R.C.P., M.R.C.P.C.H., F.R.C. Path.,
Consultant Chemical Pathologist

Signature of Head of Department

Dr. Christopher Barbara
Chairman
Pathology Department
Mater Dei Hospital - Malta

Signature of Chairman of Pathology

Estimated Cost of Research

For managerial Use only

Estimated Materials and Equipment Cost

Estimated Human Resources Cost

Estimated Total Cost

** The applicant / researcher undersigned above acknowledges the necessity of the role of the Laboratory Service as an integral component of the above mentioned Trial / Research / Study / Audit and binds himself / herself to liase with the relevant laboratory personnel to ensure smooth provision of service which includes appropriate notification of samples to be sent and appropriate identification of such specimens

# Laboratory of Molecular Genetics

**Laboratory of Molecular Genetics**
**Department of Physiology and Biochemistry**
**Biomedical Sciences Building**
**University of Malta**
**Msida MSD 2080 Malta**

Professor Alex. E. Felice M.D., Ph.D.

Phone: (+356) 23402774 Fax: (+356) 21343535 Email: alex.felice@um.edu.mt Web: www.biotech.um.edu.mt

17 February, 2014.

Chairman,
Research Ethics Committee,
University of Malta,
CAMNPUS.

Dr. Sarah Cuschieri, Departent of Anatomy is authorized to use the facilities of this laboratory to pursue her doctoral studies.

Best regards

# Appendix B –
# Tools of measurements and forms

- Consent form – English version
- Consent form – Maltese version
- Questionnaire
- Invitation letter
- Blood result letter
- Oral glucose tolerance test result letter

# Consent form – English version

**CONSENT FORM**

I am a Maltese citizen and am over eighteen (18) year of age.

I have been asked to participate in a research study entitled:

**Prevalence and determinants of the type 2 diabetes and impaired glucose tolerance in Malta**

The purpose and details of the study have been explained to me by Dr. Sarah Cuschieri
And any difficulties, which I raised, have been adequately clarified.

I give my consent to the Principal Investigator and her delegate/s to undergo a physical examination (blood pressure, height and weight) and to take the necessary samples (12 mls), to work fasting blood glucose, lipid profile, serum analysis and isolate DNA from white blood cells for analysis for the presence of mutations in the genetic makeup which predispose to diabetes, obesity, inflammation and/or insulin resistance. The DNA sample may be stored at University of Malta for further analysis later on.

I also give my consent that if my fasting blood glucose is found to be between 5.6 to 6.9mmol/L I would be informed and called back for further tests. I therefore also give my consent to undertake an oral glucose tolerance test (OGTT) as well have 6mls of blood taken for analysis of serum insulin, high sensitive CRP and autoimmune markers for type 1 diabetes, which will determine my glucose tolerance. I am aware of the inconveniences, which this may cause.

I understand that the results of this study may be used for medical or scientific purposes and that the results achieved from this study in which I am participating may be reported or published: however, I shall not be personally identified in any way, either individually or collectively, without my express written permission.

Should any further sampling/analyses of blood be required, I request to be informed and I reserve the right to refuse to continue to participate or to withdraw from the study. Should any further analyses unrelated to the purpose indicated above be considered, I wish to be contacted and informed of the purpose of those studies. I am aware of the inconveniences, which this may cause.

I am under no obligation to participate in this study and I am doing so voluntarily.

I may withdraw from the study at any time, without giving any reason. This will not influence in any way the care and attention and treatment normally given to me *(applicable only in case of patients receiving treatment)*.

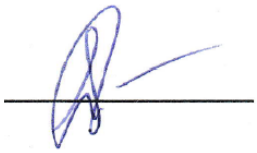
I understand that any complications and/or adverse effects, which may arise during or as a consequence of the study, will be recorded and my family doctor would be informed on all the results so as I would receive the appropriate medical attention and management.

In case of queries during the study I may contact Dr. Sarah Cuschieri Tel No. 79847970

Signature of participant _____

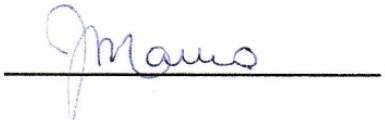
Name of participant _____
(in block letters)

ID. No: _____

Signature of Principle Investigator 

Name of Principle Investigator

Dr. Sarah Cuschieri

Signature of Principle Supervisor 

Name of Principle Supervisor

Dr. Julian Mamo

_____
Date

# Consent form – Maltese version

**Formola tal-kunsens**

Jien ċittadin/a Malti/ja u ghandi l-fuq min tmintax (18) il-sena.

Jien ġejt mitlub/a sabiex nipparteċipa fi-studju ta' riċerka bit-titolu:

**Prevalenza u determinanti tad-dijabete tip 2 u dwar it-tolleranza ghaz-zokkor f'Malta**

L-iskop u d-dettalji ta'dan l'istudju ġew spjegati lili minn Dr Sarah Cuschieri u jekk kellhi xi diffikultajiet kienu iċċarati.

Jien nagħti l-kunsens tiegħi lill-investigatur prinċipali u d-delegati tagħha li jsir eżami fiżiku fuqi (tittiehed il-pressjoni u jitkejlu it-tul u piż) u li jieħdu l-kampjuni meħtieġa (12 mls ta' demm), li minnhom jinhadem testijiet bhal dawk taz-zokkor fid-demm, il-profil tal-kolesterol u analazi tas-*serum* waqt li jien sajjem/a u jiżolaw id-DNA tieghi miċ-ċelluli bojod tad-demm għall-analiżi u għat-tfittxija tal-preżenza ta' mutazzjonijiet fil-għamla ġenetika li jippredisponu ghad-dijabete, ghall-obeżità, infjammazzjoni u / jew ghal reżistenza għall-insulina. Il-kampjun tad-DNA tista' tinħażen fl-Università ta' Malta għal analiżi aktar tard.

Jien ukoll nghati l-kunsens tieghi li jekk il-livell taz-zokkor fid-demm tieghi jigi bejn 5.6 u 6.9mmol/L jien nigi informata biex jitiehdu aktar testijiet. Ghalhekk jien naghti l-kunsens tieghi li jinhadem it-test tal- *Oral glucose tolerance* (OGTT), kif ukoll jiehdu kampjun ta-demm (6mls) ghal-analizi ghal-level tal-insulina fid-demm, *high sensitive CRP* u *autoimmune markers* ta' diabete tip 1, li jidderminaw it-tolerenza tieghi taz-zokkor. Jiena konxju ta' l-inkonvenjenzi, li dan jista' jikkawża.

Nifhem li r-riżultati ta' dan l-istudju jistgħu jintużaw għal għanijiet mediċi jew xjentifiċi, u li r-riżultati miksuba minn dan l-istudju li fih jiena se nipparteċipa jistgħu jiġu rrappurtati jew pubblikati: madankollu, jien m'għandiex niġi identifikat/a personalment fi kwalunkwe mod, individwalment jew kollettivament, mingħajr permess bil-miktub minni.

Jekk ikun mehtieg li jittiehdu aktar kampjuni tad-demm ghal-analiżi, jien nitlob li nigi infurmat/a. Jien ghandi id-dritt li nirrifjuta li nkompli nipparteċipa jew li nista nirtira mill-istudju. Jekk kwalunkwe analiżi mhux relatati mal-għan indikat hawn fuq jigu konsidrati mir-ricerkatur, nixtieq li niġi kkuntattjat/a u infurmat/a dwar l-iskop ta' dawn l-istudji. Jiena konxju ta' l-inkonvenjenzi, li dan jista' jikkawża.

Nifhem li jien ma ghandi l-ebda obbligu biex niehu sehem f'dan l-istudju u jekk nippartecipa, jien se nagħmel dan fuq bazi volontarju.

Jien nista' nirtira mill-istudju fi kwalunkwe ħin, mingħajr ma nagħti ebda raġuni ghal dan. Dan mhux se jinfluwenza bl-ebda mod il-kura, attenzjoni u trattament normalment mogħtija lili (*applikabbli biss fil-każ ta' pazjenti li jirċievu kura*).
Nifhem li xi kumplikazzjonijiet u / jew effetti ħżiena, li jistgħu jinqalgħu matul jew bħala konsegwenza ta' dan l-istudju, ser jiġu rreġistrati u t-tabib tal-familja tieghi se jkun informat/a dwar ir-rizulti kollha f'dan l-istudju biex jien jkolli l-attenzjoni u ghajnuna medika mehtiega.

Fil-każ ta' mistoqsijiet matul l-istudju jien nista' nikkuntattja lil Dr Sarah Cuschieri
Tel No 79847970

Firma ta' parteċipant _____

Isem tal-Parteċipant _____
(b'ittri kbar)

ID. Nru: _____

Firma tal- Investigatur _____

Isem tal- Investigatur Dr. Sarah Cuschieri

Firma tas-Superviżur Prinċipali _____

Isem tas- Superviżur Prinċipali Dr. Julian Mamo

_____
Data

# Questionnaire – English version

Name & Surname:_____ Code number _____ 1

**Prevalence and determinants of the type 2 diabetes and impaired glucose tolerance in Malta Questionnaire**

Date of interview |___|___||___|___||___|___|___|___| (dd/mm/yyyy)

## A. General Information

**1 a. Name of Family doctor & Contact number** _____

**1b. Gender**

| | |
|---|---|
| Male | |
| Female | |
| Not Applicable | |

**1c. Locality:** _____

**1d. Age:** (last birthday)

|___|___| Years

**2. What is your country of birth?**

| | |
|---|---|
| Native-born | |
| Born in another EU Member State | |
| Born in a non-EU country | |

**3. What is the highest education leaving certificate, diploma or education degree you have obtained? Please include any vocational training.**

| | | |
|---|---|---|
| ISCED 0 | No formal education | |
| ISCED 1 | Primary education | |
| ISCED 2 | Lower secondary education (did not finish secondary school) | |
| ISCED 3 | Upper secondary education (finish 5th form) | |
| ISCED 4 | Post-secondary but non-tertiary education (6th form, MCAST, ITS) | |
| ISCED 5 | First stage of tertiary education (Undergraduate University) | |
| ISCED 6 | Second stage of tertiary education (Postgraduate Diploma, Masters, PhD) | |

**4a. How would you define your current labour status?**

| | | |
|---|---|---|
| Working for pay or profit (including unpaid work for a family business or holding, an apprenticeship or paid traineeship, currently not at work due to maternity, parental, sick leave or holidays) | | Go to 4b. |
| Unemployed | | |
| Pupil, student, further training, unpaid work experience | | |
| In retirement or early retirement or has given up business | | |
| Permanently disabled | | |
| Giving a community service as ordered by court | | |
| Fulfilling domestic tasks | | |
| Other; Please specify | | |

**4b. Are (Were) you an employee, self-employed or working without payment as a family worker?**

| | | | |
|---|---|---|---|
| Employee | Yes | No | |
| Self- employed | Yes | No | Go to 4d |
| Family worker | Yes | No | Go to 4d |

**4c. What type of work contract do (did) you have?**

| | | |
|---|---|---|
| Permanent job / work contract of unlimited duration | Yes | No |
| Temporary job / work contract of limited duration | Yes | No |

**4d. In your (main) job do (did) you work full-time or part-time?**

| | | |
|---|---|---|
| Full time | Yes | No |
| Part time | Yes | No |

**4e. What is (was) your occupation in this job?**

Job title: _____

Describe what you mainly do (did) in your job:

---

## B. Smoking Habits

**5a. Do you smoke at all nowadays? (Tick most appropriate)**

| | | |
|---|---|---|
| Yes, daily | | |
| Yes Occasionally | | **Go to 5d** |
| Not at all | | **Go to 5d** |

**5b. What tobacco product do you smoke each day? Fill in ONE box for each line.**

| | | |
|---|---|---|
| Manufactured cigarettes | Yes | No |
| Hand-rolled cigarettes | Yes | No |
| Cigars | Yes | No |
| Pipefuls of tobacco | Yes | No |
| Other | Yes | No |

**5c. On average, how many cigarettes, cigars or pipefuls do you smoke each day? If you fill in one answer at least: →GO TO 5e**

|___|___| Manufactured cigarettes
|___|___| Hand-rolled cigarettes
|___|___| Cigars
|___|___| Pipefuls of tabacco
|___|___| Other

**5d. Have you ever smoked (cigarettes, cigars, pipes) daily, or almost daily, for at least one year?**

| | | |
|---|---|---|
| | Yes | |
| | No | Go to 5f |

**5e. For how many years have you smoked daily? Count all separate periods of smoking daily. If you don' t remember the exact number of years, please give an estimate.**

|___|___| Years

**5f. How often are you exposed to tobacco smoke indoors at home?**

| | |
|---|---|
| Never or almost never | |
| Less than 1 hour per day | |
| 1-5 hours a day | |
| More than 5 hours a day | |

**5g. How often are you exposed to tobacco smoke indoors in public places and transport (bars, restaurants, shopping malls, bingo halls, bowling alleys, bus)?**

| | |
|---|---|
| Never or almost never | |
| Less than 1 hour per day | |
| 1-5 hours a day | |
| More than 5 hours a day | |

**5h. How often are you exposed to tobacco smoke indoors at your workplace?**

| | |
|---|---|
| Never or almost never | |
| Less than 1 hour per day | |
| 1-5 hours a day | |
| More than 5 hours a day | |
| Not relevant (don't work or don't work indoors) | |

## C. Alcohol Intake

**6a. Indicate with a circle which type of Alcohol you consume:**

| | | | | |
|---|---|---|---|---|
| Beer | Red wine | White wine | Rose wine | Liquor- Spirits |

**6b. When did you last have a beer or a glass of wine or liquor?**

| | | |
|---|---|---|
| Today or yesterday | Yes | No |
| During the last week | Yes | No |
| During the last month | Yes | No |
| More than 1 month ago | Yes | No |
| No alcohol last 12 months | Yes | No |

**6c. How often do you drink beer, wine or liquor?**

| | | |
|---|---|---|
| Daily or almost daily | Yes | No |
| 3 to 6 times per week | Yes | No |
| 1 to 2 times per week | Yes | No |
| 1 to 3 times per month | Yes | No |
| Less often | Yes | No |
| No alcohol last 12 months | Yes | No |

**6d. How many days a week do you drink alcohol? (Circle the appropriate answer)**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

**6e. How much did you drink on one typical occasion?**

| | | |
|---|---|---|
| 1 beer (341mls) / 1 wine glass (142mls) / 1 liquor (43mls) | Yes | No |
| 2 - 5 beers / glasses of wine / liquor | Yes | No |
| 6 - 10 beers / glasses of wine / liquor | Yes | No |
| More than 10 beer / glasses of wine / liquor | Yes | No |
| No alcohol last 12 months | Yes | No |

**6f. During the past 12 months, how often have you had 5 or more drinks on the same occasion (same evening, same party etc)?**

| | | |
|---|---|---|
| More than once a week | Yes | No |
| Once a week | Yes | No |
| 2 to 3 times a month | Yes | No |
| Once a month | Yes | No |
| Less than once a month | Yes | No |
| Never | Yes | No |
| No alcohol last 12 months | Yes | No |

## D. Physical Activity

**7a. During the past 7 days, on how many days did you do vigorous physical activities?**

|___| Days per week **Or** Don't Know |___| Refusal |___|

**7b. During the past 7 days, how much time did you spend in total doing vigorous physical activities?**

|___|___| hours |___|___| minutes **Or** Don't Know |___| Refusal |___|

**7c. During the past 7 days, on how many days did you do moderate physical activities?**

|___| Days per week **Or** Don't Know |___| Refusal |___|

**7d. During the past 7 days, how much time did you spend in total doing moderate physical activities?**

|___|___| hours |___|___| minutes **Or** Don't Know |___| Refusal |___|

**7e. During the past 7 days, on how many days did you walk for at least 10 minutes at a time?**

|___| Days per week **Or** Don't Know |___| Refusal |___|

**7f. During the past 7 days, how much time did you spend walking in total?**

|___|___| hours |___|___| minutes **Or** Don't Know |___| Refusal |___|

## E. Dietary Intake

**8a. How often have you been eating potatoes/pasta/rice in the past week?**

| | None | 1 – 2 times/week | 3 – 4 times/week | 5 – 7 times/week |
|---|---|---|---|---|
| Potatoes | | | | |
| Pasta | | | | |
| Rice | | | | |
| White Bread | | | | |
| Brown Bread | | | | |
| Other: | | | | |

**8b. How often have you been eating vegetables in the past week?**

| | None | 1 – 2 times/week | 3 – 4 times/week | 5 – 7 times/week |
|---|---|---|---|---|
| Salad / raw vegetables | | | | |
| Boiled vegetables | | | | |
| Vegetables in hot dishes | | | | |
| Legumes | | | | |
| Other: | | | | |

**8c. How much fruit do you usually eat during a day/week? 1 portion = 1 piece**

| | |
|---|---|
| | None |
| | 1 – 2 per week |
| | 3 – 4 per week |
| | 5 – 6 per week |
| | 1 -2 per day |
| | 3 – 4 per day |
| | 5 – 6 per day |
| | More than 6 per day |

**8d. How often have you been eating the following foods with bread the past week?**

| | None | 1 – 2 times/week | 3 – 4 times/week | 5 –7 times/week |
|---|---|---|---|---|
| Cottage Cheese | | | | |
| Cheese | | | | |
| Meat | | | | |
| Fish | | | | |
| Egg | | | | |
| Mayonnaise salad | | | | |
| Vegetables | | | | |
| Olive Oil | | | | |
| Marmalade / Honey/ Jam | | | | |

| | Not at all (0) | Several days (1) | More than half the days (2) | Nearly every day (3) |
|---|---|---|---|---|
| Poor appetite or overeating | | | | |
| Feeling bad about yourself – or that you are a failure or have let yourself or your family down | | | | |
| Trouble concentrating on things, such as reading the newspaper or watching TV? | | | | |
| Moving or speaking so slowly that other people could have noticed? Or being so fidgety or restless that you gave been moving around a lot more than usual | | | | |
| Thoughts that you would be better off dead or of hurting yourself in some way | | | | |

**10b. If you checked off any problems (above), how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people? Tick most appropriate.**

| Not difficult at all | Somewhat difficult | Very difficult | Extremely difficult |
|---|---|---|---|
| | | | |

**11a. During the past two weeks, have you used any medicines that were prescribed for you by a doctor?**

☐ Yes

☐ No

**11b. Were the medicines for … ? (Answer if Q. 11a is "Yes")**

| | | |
|---|---|---|
| High blood pressure | Yes | No |
| Lowering blood cholesterol level (Statins) | Yes | No |
| Diabetes (pills) | Yes | No |
| Diabetes (Insulin) | Yes | No |
| Polycystic ovarian syndrome | Yes | No |
| Slow thyroid | Yes | No |
| Fast thyroid | Yes | No |
| Depression | Yes | No |
| Heart problems | Yes | No |

# Invitation letter

UNIVERSITY OF MALTA
L-Università ta' Malta

SAHHTEK
The University of Malta Health and Wellbeing Study

THE ALFRED MIZZI FOUNDATION
CELEBRATING 100 YEARS
ALF MIZZI & SONS LTD.
1915-2015

Dear _____,

I am writing to invite you to participate in a national health survey that is being organised across Malta. Your name has been selected randomly by computer to participate in this study that is being organised by the University of Malta in collaboration with Alf Mizzi Foundation as the main sponsor, the Ministry for Energy and Health and with the support of a number of private sponsors.

The aim of this survey is to study in detail health in Malta as well as determines the frequency of diabetes (type 2), high blood pressure (hypertension) and obesity. It will also assist us to identify how many Maltese have a predisposition for these diseases, which is of great importance to health.

Should you accept to take part in this study, you will be offered a Free health check-up, including blood pressure, body weight and height measurements and will undergo a short interview. You will also have your blood taken to assess sugar and cholesterol levels. This is expected to last around 35-45 minutes.

Your results will be kept in confidentiality and will be forwarded to you. Participating in this study would provide you with a better understanding of your health and your risk to develop a number of conditions.

The Ethics Research Committee of the University of Malta has approved this study. You will be offered some food and drink in view of the fact that you had to come in the fasting state as well as some tokens of our appreciation at the end of the appointment.

Your appointment has been set for the _____

_____

Your participation would be of great help for the study of our population health and for the improvement of our health services. You are required to come for your appointment having fasted for 9 hours. You can drink water and take any medications that you may be already taking earlier in the morning. For more information visit: www.sahhtek.com

**It would be appreciate it if you ALWAYS phone either to Confirm or to Refuse or to Re-schedule your appointment on**: Mobile number **99776011** or via e-mail **diabetesstudymalta@gmail.com**

Thank you very much for agreeing to learn more about your health and to help us to plan better for further improvements to our health care services!

Sincerely yours,

Dr. Sarah Cuschieri MD, MSc Diabetes (Cardiff), PG Dip. Diabetes (Cardiff)

MINISTRY FOR ENERGY AND HEALTH

RIDT

Atlas Insurance

Appendix

UNIVERSITY OF MALTA
L-Università ta' Malta

SAHHTEK
The University of Malta Health and Wellbeing Study

THE ALFRED MIZZI FOUNDATION
CELEBRATING 100 YEARS
ALF MIZZI & SONS LTD.
1915-2015

Ghaziez/a _____,

Qed niktiblek biex nistidnek tippartecipa f'servej nazzjonali fuq is-saħħa li qed jiġi organizzat madwar Malta u Ghawdex. Ismek ġie magħżul b'sistema tal-kompjuter biex tipparteċipa f'dan l-istudju li qed jiġi organizzat mill-Università ta' Malta b'kollaborazzjoni mal' Alf Mizzi Foundation, mal-Ministeru għall-Enerġija u s-Saħħa u bl-appoġġ ta' numru ta' sponsors privati.

L-għan ta' dan l-istudju hu li nistudjaw fid-detal is-sahha f'Malta u niddetermina l-frekwenza tad-dijabete (tip 2), pressjoni għolja u l-obeżita f'Malta, kif ukoll biex tgħin lilna biex nidentifikaw kemm Maltin għandhom predispożizzjoni għal dawn il-mard, li huma ta' importanza kbira għas-saħħa.

Jekk inti taċċetta li tieħu sehem f'dan l-istudju, inti ser tiġi offrut *health check-up* b'xejn, inkluż li tittiehed il-pressjoni tieghek, jitkejjel il-piż u l-għoli tieghek, u tipparteċipa f'intervista qasira. Jittiehed ukoll id- demm tiegħek ghal testijiet ghal- livel taz-zokkor u tal-kolesterol. Dan il- *health check-up* huwa mistenni li jdum madwar 35-45 minuta.

Riżultati tiegħek ser jinżammu f'kunfidenzjalità u ser jintbagħtu lilek. Meta tipparteċipa f'dan l-istudju se tigi provdut/a fehma aħjar ta' saħħtek u tar-riskju tiegħek li tiżviluppa numru ta' kundizzjonijiet.

Il-Kumitat tar-Riċerka u tal- Etika tal-Università ta' Malta approvat dan l-istudju. Inti ser tiġi offrut/a xi ikel u xorb minhabba li inti tigi ghal-appuntament fi stat ta' sawm. Se jinghataw ukoll xi rigali xirqa bhala apprezzament tagħna fl-aħħar tal-appuntament.

L-appuntament tiegħek ġie stabbilit ghal _____
_____

Il- parteċipazzjoni tiegħek tkun ta' għajnuna kbira għall-istudju tas-saħħa tal-popolazzjoni u għat-titjib tas-servizzi tas-saħħa tagħna. Inti mitlub li tigi għall-appuntament tiegħek sajjem/a għal 9 sigħat. Tista' tixrob l-ilma u tieħdu xi mediċini li inti tista' tkun diġà qed tieħdu, aktar kmieni fil-għodu. Ghal aktar informazzjoni: www.sahhtek.com

**Napprezzaw hafna li <u>DEJJEM</u> Cempel kemm biex Tikkonferma jew Tirrifjuta jew biex Tibiddel l-appuntament tieghek fuq**: Mobile number: **99776011** jew e-mail: **diabetesstudymalta@gmail.com**

Grazzi ħafna talli qbilt biex titgħallem aktar dwar is-saħħa tiegħek u biex tgħinna nippjanaw aħjar għal aktar titjib fis-servizzi u fil- kura tas-saħħa tagħna!

Dejjem tiegħek,

Dr. Sarah Cuschieri MD, MSc Diabetes (Cardiff), PG Dip. Diabetes (Cardiff)

MINISTRY FOR ENERGY AND HEALTH

RIDT

Atlas Insurance

# Blood results letter

Date

Dear

Thank you for participating in the study "SAHHTEK". The blood tests performed included fasting plasma glucose and lipid profile that are being attached below. The values within the brackets are the normal values. Any values above the normal ranges should be discussed with your family doctor.

| | |
|---|---|
| Fasting plasma glucose | (3.88 – 6.38 mmol/L) |
| Cholesterol | (2.0 – 5.0 mmol/L) |
| Triglycerides | (0.1 – 2.26 mmol/L) |
| HDL Cholesterol | |
| Non-HDL Cholesterol | (0.00 – 3.36 mmol/L) |
| LDL Cholesterol | |

If you got fasting plasma glucose between 5.6 – 6.9mmol/L, your plasma glucose is considered as borderline and indeterminate. This means in the coming days you would be contacted by our team in order to have the gold standard glucose test in order to have a clear indication of your glucose status.

Thank you
With sincere regards,
Dr. Sarah Cuschieri
Survey coordinator

# Oral glucose tolerance test result letter

UNIVERSITY OF MALTA
L-Università ta' Malta


THE ALFRED MIZZI FOUNDATION
CELEBRATING 100 YEARS
ALF MIZZI & SONS LTD.
1915-2015

Dear

Thank you for participating in the study "SAHHTEK". The diabetes blood test performed to determine your sugar status is being attached below.

Fasting plasma glucose
First hour plasma glucose
Second hour plasma glucose

The table below shows the sugar status according to the value obtained in the second hour.

| OGTT value in second hour | Sugar status |
|---|---|
| < 7.8 mmol/L | Normal |
| >= 7.8 – 11.0 mmol/L | Pre-diabetes |
| > = 11.1 mmol/L | Diabetes |

Thank you for participating in this study

With sincere regards,

Dr. Sarah Cuschieri
Survey coordinator

MINISTRY FOR
ENERGY AND HEALTH


# Appendix C – Data collection and genetic analysis protocols

- Blood pressure measurement
- Weight
- Height
- Waist-Hip circumference
- Bloodletting
- Blood collection and testing
- Oral glucose tolerance test
- DNA quantification using UV spectrophotometry
- Genotyping by Sanger Sequencing
- Health examination survey sequence

## Blood pressure measurements

Using a validated (following British Hypertension Society Protocol) aneroid sphygmomanometer Welch Allyn® aneroid sphygmomanometer model 7670-04 to take blood pressure (Saxena et al. 2012).

Limitations:

Some persons have a tendency to have high blood pressure levels when the blood pressure is measured in the physician's office than at home or when measured by a physician rather than by a nurse. This is called "while coat hypertension". Environmental factors affecting the health examination room could not be regulated due to the nature of the data collection protocol and utilization of small governmental clinics found in each town. Therefore temperature of the room, possible environmental disturbance such as traffic or people entering the room could have had an effect on the blood pressure measurements.

Precautions taken:

Caution was exhibited throughout the data collection to take the blood pressure measurement immediately after the interview had terminated, to ensure that the participant had been resting sitting down for at least 20 minutes. All participants had their blood pressure measurements taken in sitting down position while the participants back was supported and both feet resting firmly on the floor. Participants were advised not to talk during the measurements and the cuff was placed on bare arm.

Exclusion criteria:

Blood pressure was to be measured from all participants except if a person had:

- Amputation of both arms,
- Cast on both arms,
- Open wounds/sores on both arms,
- Rash on both arms,
- Malformation of both arms preventing to place the cuff, or
- Lymph node malfunction affecting both arms and preventing to place the cuff properly.

Instructions to participants and fieldworkers:

1. Before examination, participants were instructed to abstain from eating. Drinking (except water), smoking and heavy exercise for one hour before measurement.
2. The subject removed outer garments and all other tight clothing. Sleeves rolled up so that the upper arm was bare. Caution was given to make sure that the remaining garments did not cause any constrictive effect, and the blood pressure cuff was not placed over any garment.
3. The participant was in a sitting position so that the arm and back was supported. The participant feet were resting firmly on the floor, not dangling. If the participant could not sit and the measurement was taken in supine posture, this was recorded. (This was never the case in this study)
4. The measurements were taken on the right arm whenever possible.

5. The arm was rested on the desk so that the antecubital fossa was at the level of the heart and palm was facing up. The cuff was placed 2-3cm above the antecubital fossa.
6. The greatest circumference of the upper arm was measured to ensure the right cuff-size was used.
7. During blood pressure measurement, participants were instructed not to move or talk. (To prevent blood pressure from increasing due to these activities).

Note: Quality control of both the stethoscope and aneroid sphygmomanometer was done with every new examination site set up.

Measurements:

- The cuff was inflated to a peak inflation level according to when the pulse of the participant was no longer felt. The pressure was then deflated at a rate of 2mmHg per second.

- The systolic pressure and the diastolic pressure were recorded accurately according to the pointer of the aneroid meter e.g. 120/90 if the Korotkoff Phase 1 is head over the 120mmHg mark. If it is heard e.g. at 123mmHg than 123mmHg as systolic blood pressure would be recorded. The same accurate recoding was performed for the diastolic reading. The diastolic pressure was recorded when there was disappearance of the repetitive sound (Phase 5).

Reference:

Saxena, Y., Saxena, V., Gupta, R. (2012) Clinical validation of aneroid sphygmomanometer. Indian J Physiol Pharmacol. 56 (3): 255 – 361.

Tolonen, H., Koponen, P. (2013) EHES Manual. Part B. Fieldwork procedures. National Institute for Health and Welfare.

Tolonen, H.A., Koponen, P., Naska, A., Mannisto, S., Broda, G., Palosaari, T., Kuulasmaa, K. (2015) Challenges in standardization of blood pressure measurement at the population level. BMC Medical Research Methodology 15:33 DOI: 10.1186/s12874-015-0020-3.

## Weight Protocol

A calibrated certified ADAM® MDW-250L physical digital scale with height rod was used to measure the body weight in kilograms and the height in centimetres.

Exclusion criteria

- Immobile or in a wheelchair,
- Severe difficulties in standing steady,
- Heavier than the upper limit of the scale,
- Refused

The reason for exclusion was recorded.

Instructions to participants and fieldworkers:

1. The participant was asked to undress to his/her underwear. If the participant refused or felt uncomfortable undressing, he/she was asked to take off the shoes, heavy garments such as jacket, pullover, belts, heavy jewelry and to empty his/her pockets. The clothing worn during the measurement was to be recorded.
2. The participant was asked to stand in the center of the platform, arms hanging loosely at his/her sides.
3. Checked that the weight was distributed evenly on both feet.
4. The participant was asked to stand still facing ahead (not looking down).

Note: The equipment undergone regular calibration, every time a new location was set up.

Reference:

Maki-Opas J, Koponen P, Tolonen H. EHES Manual. Part B. Fieldwork procedures. National Institute for Health and Welfare, 2013.

## Height Protocol

Setting up the standardized length rod:

A standardized length rod part of a calibrated certified ADAM® MDW-250L physical digital scale was used. The scale rod was placed vertical to a hard wall surface.

Exclusion criteria

Height was measured from all participants, except if a person was:

- Immobile or in a wheelchair;
- Had difficulties in standing straight;
- Had a hairstyle or head gear (e.g. turban) which prevented the proper measurement;
- Was taller than the maximum height of the stadiometer;
- Refused

Any reasons for the exclusion were recorded.

Instructions for participants and fieldworkers:

1. The participant was asked to remove his/her shoes, heavy outer garments, and hair ornaments and headdress
2. The participant stood with his/her back to the height rule or to the wall.

3. The back of the head, shoulder blades, buttocks and heels were touching or in line with the stadiometer or the wall.
4. The participant was asked to stand in a natural straight standing position, weight evenly on both feet and arms hanging loosely by his/her side.
5. The participant was instructed to look straight ahead.
6. The participant was instructed to keep his/her eyes focused ahead, to breathe in deeply and to stretch to his/her full height.
7. The standing position was checked from the front in order to verify that the participant was standing straight and in the middle of the stadiometer.
8. The headpiece of the stadiometer was lowered so that the hair was pressed flat.
9. When the participant was taller than the measurer, steps were used so as to read the height rule properly.

Reference:

Maki-Opas J, Koponen P, Tolonen. EHES Manual. Part B. Fieldwork procedures. National Institute for Health and Welfare, 2013.

## Waist-Hip Circumference

Exclusion criteria

1. Immobile or in a wheelchair,
2. Had difficulties standing straight
3. Had a hernia, a colostomy or ileostomy (wears a stoma bag/ostomy bag), recent abdominal surgery or other problems/devices which prevented the proper measurements,
4. Refused

Instructions to participants and fieldworkers:

1. The participant was asked to reveal the waist, by loosening the belt, lowering the pants/skirt and lifting the shirt while making sure that nothing was straining the waist. The measurement was done on bare skin or on top of the least possible clothes.
2. The participant stood in front of the measurer.
3. The participant was asked to stand with their weight evenly balanced on both legs with a small gap between the legs.
4. The participant's hands were hanging loosely beside the body.
5. The waist was palpated to find the right measurement place: midway between the lower rib margin and the iliac crest. The position was checked from both the right and left sides of the body.
6. The measuring tape was positioned at the participant's waist. The tape was not be too tight or too loose. One could place one finger between the tape and the subject's

body.

7. The participant was instructed to breathe normally; the reading was taken at the end of light exhalation.
8. The waist circumference was recorded to the nearest millimeter.

For Hip measurement:

The same instructions as the waist circumference measurement was give but the measuring tape was placed at the tip of the iliac crest instead.

Reference:

Maki-Opas J, Koponen P, Tolonen. EHES Manual. Part B. Fieldwork procedures. National Institute for Health and Welfare, 2013.

## Bloodletting Protocol

The core measurements in stage 1 of the data collection were fasting plasma glucose (FPG), lipid profile (total cholesterol, triglycerides, LDL, HDL) and a whole blood sample for DNA extraction.

Equipment

- Blood sampling vacuum tubes
- Needle for vacuum tubes
- Needle holder / adapter into which the needle was pushed.
- Needle disposal container
- Disinfection solution
- Swabs, gauze pads
- Skin tape
- Disposable gloves

Blood collection tubes

- Serum tubes containing Z serum Sep Clot Activator (4ml) – Yellow bottle
- FX Sodium Fluoride / Potassium Oxalate plasma tube (2ml) – Grey bottle
- EDTA plasma tube (3ml) - Purple bottle

Notes:

Preferably, the blood was not collected from the same arm that was used for blood pressure measurement, (i.e. blood usually be drawn from the left arm).

Participants were instructed to fast at least 9 hours before blood was withdrawn.

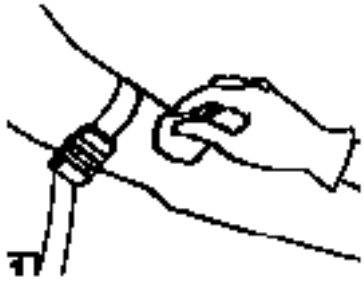
For safety reasons disposable gloves were worn during blood sampling and when processing serum, plasma and blood.

One serum bottle and one plasma bottle were transferred to the Pathology laboratory of Mater Dei Hospital within 2 hours of bloodletting. The EDTA bottle containing whole blood was transferred to a -20°C freezer at University of Malta.

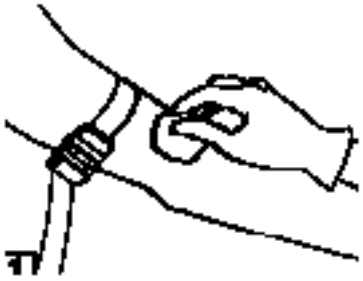
Procedure:

1. Identification of the participant against the participants lists
2. The participant code was written on the blood bottles or the printed labels were put when available
3. Gently the sample needle was twisted to break the seal. The cover was released from the end of the needle. The holder and sample needle were connected while the needle protector was left intact.

4. The tourniquet was placed around the participant's upper arm.



5. The arm was placed in a downward position and disinfected the puncture site.



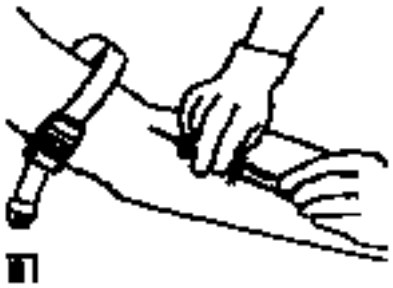
6. The protector case was removed from the needle.

7. Venipuncture was performed using the accepted technique (below).

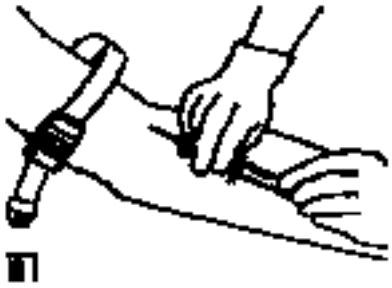
8. The blood draw took place in the following order; Serum tubes (yellow bottle), EDTA tube (purple bottle) and Sodium Fluoride tube (grey bottle).

9. The tube was pressed at the bottom of the holder; the needle punctured the stopper and start to fill. The tube was supported during filling.

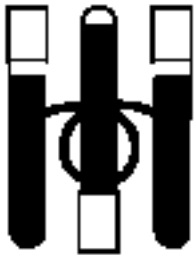
10. The tourniquet was removed when the bloodstream stopped.



11. The tube was taken out of the holder and checked that the tube was filled to an accurate volume.



12. Immediately after the serum tube was adequately mixed 5 times by inverting the tube completely top-down. The tubes were stored in a vertical position on ice.



5 times

Reference:

Alfthan G, Tolonen H, Lund L, Sundvall J. EHES Manual. Part B. Fieldwork procedures. National Institute for Health and Welfare, 2013.

# Blood Collection and Testing

The clinical laboratory testing was kept to the minimum possible 'total error' in order to reflect the true biological condition being evaluated (glucose and lipids). During the fieldwork, the same phlebotomists performed bloodletting. They followed the same protocol, where the blood tubes were rotated for five times before placing the tubes on ice.

All blood tubes were transported on ice in a cooling bag within 2 hours of bloodletting. The blood tubes were deposited immediately at the laboratory so as the blood testing processing would be initiated immediately. The same automated COBAS INTEGRA® analysers and kits were used throughout the study.

The COBAS INTEGRA® analysers are maintained with frequent internal and external quality controls in order to verify the performance of the tests in accordance to the pre-established specifications. The laboratory performs the internal quality control by periodically assaying the quality control materials, where if the result is within acceptable limits, then the patient samples can be reported with good probability that they are suitable for clinical use. On the other hand, if the results are not within the acceptable limits, corrective action is necessary before patient results can be reported. The laboratory also performs calibration of the machines and laboratory methods by using manufacturer's calibrator materials and verification procedures.

## Glucose measurement

A sodium fluoride preservative containing tube (grey bottle) was used for the specimen collection. This is done to inhibit glycolysis that usually takes place at a rate of 0.4mmol/L per hour at room temperature (Weissman and Klein 1958). When the preservative is used, the specimen remains clinically acceptable up to 90 minutes before separation of cells and serum need to take place. Although the glucose metabolism continues to occur at a slower rate than if no preservative was present (Mikesh and Bruns 2008). The FPG measure was obtained by following a hexokinase and glucose oxidase enzyme reactions and the result was reported in millimoles per liter (mmol/L).

## Lipid profile measurement

A serum clot activator tube was used for the collection of the lipid profile test. Using an aliquot serum samples, various lipid measures were obtained as follows: high-density lipoprotein (HDL) by following the clearance method; triglyceride by following an enzymatic colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenozone reaction; total cholesterol by a cholesterol oxidase enzymatic reaction while the low-density lipoprotein (LDL) value was measured by following the Friedewald formula calculation. All blood measures were reported in millimoles per liter (mmol/L).

# Oral glucose tolerance test

A WHO consultation report stated that a FPG value of 7mmol/l had an equal diagnostic significance to a 2hr-glucose load. ADA also recommends the use of FPG as a primary diagnostic tool and that those subjects obtaining an FPG value >=7mmol/l are to be considered as having diabetes. On the other hand, ADA suggests that subjects with FPGs values less than 5.60mmol/L have normal glucose tolerance (American Diabetes Association 2016). Appendix Table 1. illustrates the different glucose subgroups according to the fasting plasma glucose result.

| **Fasting plasma glucose value** | **Glucose tolerance** |
|---|---|
| < 5.60 mmol/L | Normal glucose |
| 5.60 – 6.99 mmol/L | Impaired glucose – Needs further investigations |
| > = 7 mmol / L | Diabetes |

Appendix Table 1. Fasting plasma glucose classification

**OGTT procedure**

a) The participant fasted for at least 6 hours prior to the test. The participant was previously advised not to change the dietary pattern prior to the test.
b) On arrival to the allocated place, the participant had the first glucose bloodletting sample. This grey bottle was labeled as ‘Fasting plasma glucose – 0hr’.

c) A 75g glucose load was mixed with 300ml of water and stirred well. On obtaining a clear liquid, it was given to the participant and advised to drink it as soon as possible.

d) On consuming the whole glucose load, the time was noted and written down.

e) Bloodletting for glucose was repeated at 1 hour and 2 hours after consumption of the glucose load. The grey bottles were labeled "1st hour" and "2nd hour" accordingly.

f) Once the test was ready, the samples were passed immediately to the Biochemistry laboratory.

g) The results of the OGTT were evaluated according to the WHO and ADA criteria as shown in Appendix Table 2.. The results were documented in a designated spreadsheet.

h) Each participant got the OGTT results through a letter with explanation of the results obtained (Appendix B). Participants were advised to talk to their medical doctor.

| **OGTT value** | **Glucose tolerance** |
|---|---|
| < 7.8 mmol/L | Normal |
| >= 7.8 – < 11.1 mmol/L | Impaired glucose tolerance (IGT) |
| > = 11.1 mmol/L | Diabetes |

Appendix Table 2. OGTT result in accordance to the glucose tolerance

References

Alberti, K.G. and Zimmet, P.Z. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and definition of diabetes mellitus provisional report of WHO consultation. Diabetes Medicine. 15:539 – 53.

American Diabetes Association (2016) Classification and Diagnosis of Diabetes. Diabetes Care 39(Suppl. 1): S13-S22

Mikesh LM and Bruns DE. Stabilization of glucose in blood specimen: mechanism of delay in fluoride inhibition of glycolysis. Clinical Chemistry 54(5): 930 – 932.

Weissman M and Klein B. (1958) Evaluation of glucose determinations in untreated serum samples. Clin. Chem 4(5): 420 – 422.

WHO (2006) WHO International Diabetes Foundation. Definition and diagnosis of diabetes mellitus and intermediate hyper glycaemia: report of a WHO/IDF consultation. Genève: World Health Organization

# DNA quantification using UV spectrophotometry

Microvolume nucleic acid quantification was performed using the following protocol:

1. The upper and lower optical surfaces of the microvolume spectrophotometer sample retention system was cleaned by pipetting 2 to 3 µL of deionized water onto the lower optical surface.
2. The lever arm was closed, ensuring that the upper pedestal comes in contact with the deionized water. The lever arm was lifted and both optical surfaces were wiped with a clean, dry, lint-free lab wipe.
3. Using the Nanodrop software, the Nucleic Acid application was selected. Using a small-volume, calibrated pipettor a blank measurement was performed by dispensing 1µL of buffer onto the lower optical surface. The lever arm was lowered and "Blank" selected in the Nucleic Acid application.
4. Once the blank measurement was complete, both optical surfaces were wiped with a clean, dry, lint-free lab wipe.
5. 1 µL of nucleic acid sample were pipetted onto the lower optical pedestal and the lever arm was closed. Because the measurement is volume independent, the sample only needs to bridge the gap between the two optical surfaces for a measurement to be made.
6. "Measure" in the application software was selected. The software will automatically calculate the nucleic acid concentration and purity ratios (A260:280 and A260:230 Ratios). Following sample measurement, the spectral image was reviewed to assess sample quality.

To accurately assess sample quality, 260/280 or 260/230 ratios should be analysed in combination with overall spectral quality. Pure nucleic acids typically yield a 260/280 ratio of ~1.8 and a 260/280 ratio of ~2.0 for DNA and RNA, respectively. This ratio is dependent on the pH and ionic strength of the buffer used to make the blank and sample measurements. Significantly different purity ratios may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. The 260/230 purity ratio is a second measure of DNA purity with values for a "pure" nucleic acid commonly in the range of 1.8-2.2.

# Genotyping by Sanger Sequencing

PCR Primer design

The genotype of rs1801282 polymorphism in *PPRAy* was confirmed by Sanger sequencing of PCR amplicons. Briefly, PCR primers were designed to amplify a short sequence surrounding the polymorphism of interest.

A PCR primer is a short oligonucleotide sequence synthesized artificially to enable the selective and repeated amplification of the target region of interest during a polymerase chain reaction. Primers for conventional PCR were designed using Primer3 Software – an open source software available online at http://frodo.wi.mit.edu/primer3/input.htm (Rozen and Skaletsky, 2000). Several factors are taken into consideration when designing PCR primers. The primers were chosen to be between 18-25bp long, to have a random distribution of bases and avoid nucleotide sequence repeats. Primer pairs were chosen to have similar melting temperatures ($T_m$) to ensure simultaneous annealing of primers. The approximate annealing temperature is calculated from $T_m = 2 \Sigma (A+T) + 4 \Sigma (G+C)$ (Suggs *et al.*, 1981). The distance between the primers was chosen to be between 200 and 500 bp and care was taken to ensure that restriction enzyme digestion of the PCR product resulted in fragments of unequal size, to make separation by gel electrophoresis easier. All primers used in a PCR should have similar melting temperatures and GC content (guanine-cytosine content). Typically, primers with melting temperatures in the range of 60-62°C are chosen. GC content should be near 50%. Primers should have little intramolecular and intermolecular secondary structure, which can interfere with primer annealing to the template. Primers with intramolecular complementarity can form secondary structure within the same primer molecule.

Intermolecular complementarity allows a primer molecule to anneal to another primer molecule rather than the template.

Primers were purchased from Integrated DNA Technologies (IDT), Belgium. The lyophilized primers were dissolved in the appropriate volume of sterile water to form a 100μM stock solution. This was then sub-aliquoted and diluted to form working solutions at a concentration of 50μM (unless specified otherwise) and stored at -20$^0$C.

The sequence of the designed primers is shown below. These primers amplify a 565bp region in *PPARy* that flanks the rs1801282 variant selected for this study.

Forward: 5' AAA AAT GCA AGT GGA TAT TGA ACA 3'

Reverse: 3' CAC AAC CTG GAA GAC AAA CTA CAT 5'

Preparation of the PCR reaction

The polymerase chain reaction (PCR) is a process mediated by DNA-dependent DNA polymerase that exponentially amplifies strands of template DNA within a relatively short period of time. The PCR reaction mixture consisted of an appropriate buffer having the right concentration of magnesium chloride, the four exclusive nucleotides (dATP dCTP, dATP and dGTP), forward and reverse primers, genomic DNA, and finally *Taq* polymerase that catalyses the actual reaction.

The final volume of the reaction mixture used was 20 μl. A PCR mix containing 1.5mM MgCl2, 0.4M Tris-HCl, 0.1M NH42SO4, 0.1%w/v Tween-20, 200uM of each dNTP

and ready to load dyes was used the polymerase chain reaction (5x FIREPol® Master Mix, Solis BioDyne, Estonia).

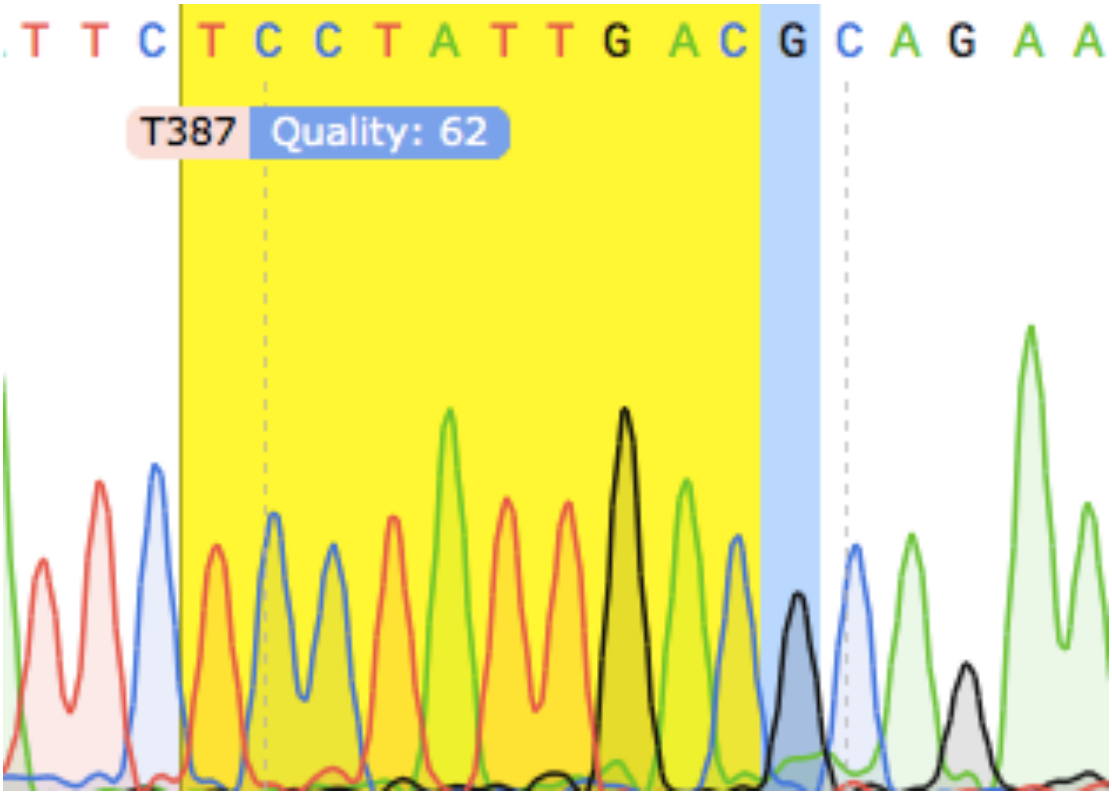
To each single PCR tube, the following was pipetted: 4µl of 5x PCR mix, 0.1 µl of each forward and reverse primer (50 µM) required for the specific PCR, and finally the addition of 1 µl (80 ng) of genomic DNA sample. Water was added to the reaction to a total of 20uL.

PCR reactions were prepared in sterile 96-well plates. The plates were placed into the thermal cycler equipment (Biometra T3000 thermal cycler) and thermal cycling was initiated. The PCR reaction was optimized, and the following thermal cycling protocol was carried out for 30 cycles; denaturation at a temperature of $95^0$C for 50 seconds, annealing temperature of $57^0$C for 30 seconds and elongation at a temperature of $72^0$C for 45 seconds. At the end of the last cycle, a final extension cycle of 10 minutes at a temperature of $72^0$C was performed. Once PCR was complete, all samples were removed from the thermal cycler and stored at $–20^0$C prior to analyses.

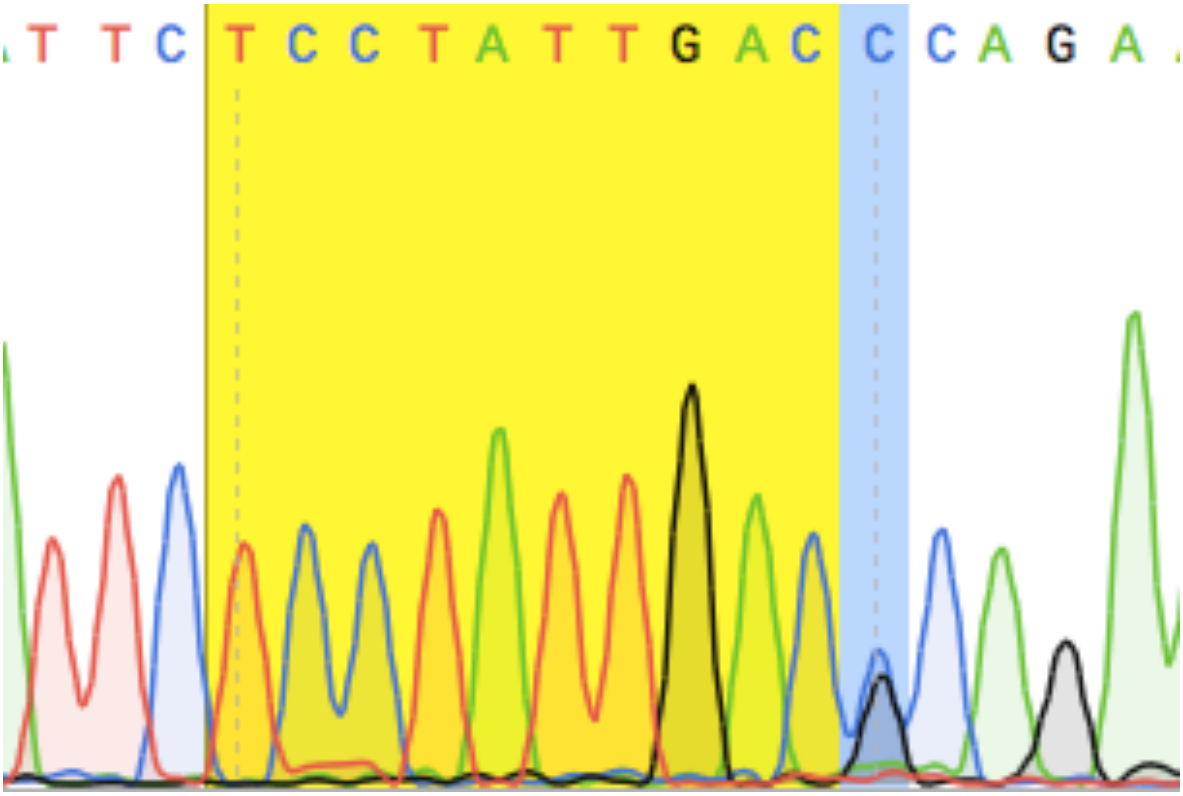
Post PCR analysis

Following thermal cycling, the purity of selected PCR products was confirmed by agarose gel electrophoresis, using a 2% agarose gels stained with ethidium bromide. Electrophoresis was carried out at a voltage of 15 volts/cm and a current of 300 mA till desirable migration was observed. The gels were then viewed on a UV scanner and photographed for records. PCR amplicons that were pure, lacking in primer dimers and of the required size were submitted for Sanger sequencing at GATC, Germany. Sequencing traces were individually analysed for the presence or absence of the variant

of interest using SnapGene Viewer (available online at: http://www.snapgene.com/products/snapgene_viewer/) as seen in Appendix Figure 1 and Appendix Figure 2.




Appendix Figure 1. SnapGene Viewer showing an absence of variant of interest (blue shading)




Appendix Figure 2. SnapGene Viewer showing the presence of variant of interest (blue shading)

# Data Collection sequence

The following step-wise sequence was followed during the health examinations (Appendix Figure 3).

Step 1: Registration

a. Participant was greeted and identification matched to the participation list allocated for the day and ticked as "Attended".
b. Unique code number for the participant was written on the questionnaire sheet
c. Participant was directed towards the consent and interview station

Step 2: Informed consent station

a. Participant sat down in front of the interviewer
b. An overview of the study, its aims and what was required of each participant was explained in detail.
c. A consent form was given to each participant. Enough time was allocated for them to read it and ask any further clarifications. The health checkup wasn't undertaken if the participant refused to consent to it.

Step 3: Interview station

a. The trained interviewer went through the questionnaire and ticked the appropriate answers.

b. Once ready, the participant was directed to the blood pressure station (right next to the interview station).

Step 4: Blood pressure station

a. Participant was asked to sit down and roll up the sleeves (they were asked to remove their shirts if the cuff was tight)

b. The blood pressure measurement was taken three times in a row.

c. Once ready, participant was directed to the measurements station.

Step 5: Measurements station

a. Participant was asked to remove any jacket, coat, hat, scarf, bulky belt and shoes he/she was wearing. The clothing was kept at a minimum without compromising privacy or decency. Pockets were emptied
b. Participant stepped onto the scales and weight was noted in kilogram.
c. While on the scales, the height was measured using the in-built height rod in meters.
d. Next, the hip and waist circumference were measured using a measuring tape.
e. Once finished from the measurement station, the participant was directed towards the bloodletting station.

Step 6: Bloodletting station

a. The participant was asked to sit down comfortably as the process of blood taking was explained.
b. The sleeve was rolled and 3 blood sample bottles (yellow serum bottle; purple EDTA bottle; grey fluoride bottle) were taken. The time of bloodletting was noted and written down for research purposes only.
c. Once ready, a plaster was applied to the puncture wound and care taken that the participant was well and fit to leave.
d. The blood bottles taken were placed under ice in cooler bag after participant's code was written on the blood bottles.

Step 7: Farewell station

a. The participant was thanked for his/her attendance and appreciation tokens were given.
b. The measurements taken during the health check-up were explained and advice given accordingly. A copy of all the measurements was given to the participant.
c. Participant was advised that should any abnormal blood result be obtained, the project coordinator would personally contact him/her, provided that the relevant contact details were given.
d. Participant was also advised that the blood results obtained would be sent by post to their respective address and to show these results to their own family doctor. (Appendix B)
e. He / She was informed that if their fasting plasma glucose was between 5.60 and 6.99mmol/L, the plasma glucose was indeterminate and the gold standard

glucose test (oral glucose tolerance test) would be offered at a later date, should he/she wish to have it performed.

Step 8: Transfer of blood samples

a. As soon as the health check-up session ended and the venue was cleared up, the project coordinator personally took the bloods for fasting plasma glucose and lipid profile to the pathology laboratory at Mater Dei Hospital.
b. After which, the project coordinator took the remaining blood tube (EDTA tube) to be used for later extraction of DNA, to the University of Malta to be stored in a -20 degrees census freezer.

Step 9: Recording of blood test results

a. The day after each data collection session, the project coordinator checked the blood results (fasting plasma glucose & lipid profile) for each participant on the NHS software (iSoft).
b. Each value was recorded on a designated spreadsheet according to the participant identifier.
c. Anyone with fasting plasma glucose (FPG) more than 7mmol/L was contacted and advised to seek medical help.
d. Each participant results were printed on a result template document and sent to the participant through post. (Appendix B)


Appendix Figure 3. The stepwise sequence followed during the health examination

# Appendix D – Results

- Crude population by age (single years) and gender
- Response rate by towns and gender
- Adjusted population by age (single years) and gender
- Dietary intake

# Crude population

Appendix Table 3. Distribution of the crude population by age (single years) and gender

| | Participants | |
|---|---|---|
| Age | Females (*n*=1,025) | Males (*n*=836) |
| 18 | 4 | 4 |
| 19 | 3 | 2 |
| 20 | 10 | 7 |
| 21 | 9 | 7 |
| 22 | 10 | 3 |
| 23 | 13 | 6 |
| 24 | 16 | 7 |
| 25 | 11 | 8 |
| 26 | 11 | 3 |
| 27 | 14 | 10 |
| 28 | 8 | 13 |
| 29 | 13 | 7 |
| 30 | 19 | 14 |
| 31 | 18 | 12 |
| 32 | 19 | 14 |
| 33 | 19 | 20 |
| 34 | 13 | 10 |
| 35 | 10 | 13 |
| 36 | 15 | 16 |
| 37 | 17 | 11 |
| 38 | 20 | 17 |
| 39 | 20 | 10 |
| 40 | 20 | 19 |
| 41 | 19 | 22 |
| 42 | 22 | 18 |
| 43 | 23 | 22 |
| 44 | 18 | 20 |
| 45 | 27 | 10 |
| 46 | 24 | 17 |
| 47 | 22 | 18 |
| 48 | 26 | 15 |
| 49 | 20 | 13 |
| 50 | 33 | 21 |
| 51 | 21 | 19 |
| 52 | 27 | 20 |
| 53 | 24 | 14 |

| | Participants | |
|---|---|---|
| Age | Females (*n*=1,025) | Males (*n*=836) |
| 54 | 33 | 28 |
| 55 | 28 | 36 |
| 56 | 19 | 24 |
| 57 | 30 | 20 |
| 58 | 26 | 25 |
| 59 | 26 | 16 |
| 60 | 21 | 29 |
| 61 | 23 | 14 |
| 62 | 21 | 15 |
| 63 | 21 | 13 |
| 64 | 26 | 30 |
| 65 | 30 | 22 |
| 66 | 21 | 17 |
| 67 | 15 | 21 |
| 68 | 24 | 17 |
| 69 | 14 | 20 |
| 70 | 29 | 27 |
| Total | 1025 | 836 |

# Response rate by towns and gender

Appendix Table 4. The response rate by town and gender.

| Town | Total Population attendance | % Male attendance | % Female attendance |
|---|---|---|---|
| Attard | 48% | 43.14 | 54.17 |
| Balzan | 48% | 38.46 | 55.56 |
| B'Kara | 42% | 30.61 | 52.04 |
| Lija | 65% | 58.33 | 72.73 |
| Marsa | 49% | 38.89 | 57.89 |
| Mgarr | 35% | 36.84 | 33.33 |
| Mosta | 52% | 48.86 | 54.55 |
| Mtarfa | 54% | 41.67 | 66.67 |
| Naxxar | 45% | 45.00 | 45.76 |
| San Pawl il- Bahar | 38% | 33.82 | 43.08 |
| Valletta | 25% | 30.00 | 20.00 |
| Hamrun | 40% | 36.36 | 44.74 |
| Santa Venera | 51% | 45.45 | 59.38 |
| Floriana | 48% | 40.00 | 54.55 |
| Rabat - Malta | 58% | 62.00 | 53.85 |
| Dingli | 45% | 42.86 | 47.37 |
| Safi | 42% | 44.44 | 40.00 |
| Kirkop | 63% | 55.56 | 70.00 |
| Qrendi | 27% | 30.77 | 23.08 |
| Mqabba | 63% | 52.94 | 73.33 |
| Zurrieq | 38% | 32.00 | 44.90 |
| Gharghur | 24% | 20.00 | 27.27 |
| Iklin | 81% | 88.89 | 72.22 |
| San Gwann | 52% | 48.21 | 56.52 |
| Pembroke | 61% | 53.33 | 68.75 |
| Swieqi | 40% | 34.15 | 47.22 |
| Sliema | 42% | 32.08 | 53.06 |
| St. Julian's | 57% | 38.24 | 74.29 |
| Msida | 32% | 15.63 | 48.39 |
| Pieta' | 21% | 15.79 | 26.67 |
| Ta' Xbiex | 33% | 36.36 | 28.57 |
| Gzira | 48% | 43.75 | 51.72 |
| Marsaskala | 53% | 45.28 | 60.78 |
| Marsaxlokk | 53% | 38.89 | 66.67 |
| Birzebbuga | 61% | 69.05 | 52.38 |
| Gudja | 60% | 50.00 | 68.75 |
| Ghaxaq | 40% | 30.00 | 50.00 |

| Town | Total Population attendance | % Male attendance | % Female attendance |
|---|---|---|---|
| Mellieha | 51% | 43.90 | 58.97 |
| Siggiewi | 57% | 65.79 | 47.37 |
| Zebbug | 59% | 50.91 | 67.31 |
| Qormi | 50% | 42.17 | 58.23 |
| Zabbar | 51% | 39.47 | 59.15 |
| Fgura | 61% | 46.43 | 75.44 |
| Zejtun | 53% | 37.50 | 70.00 |
| Tarxien | 62% | 66.67 | 56.41 |
| Poala | 61% | 47.06 | 75.00 |
| Birgu | 46% | 58.33 | 33.33 |
| Bormla | 47% | 50.00 | 44.00 |
| Isla | 46% | 46.15 | 50.00 |
| Xaghjra | 42% | 33.33 | 50.00 |
| Kalkara | 56% | 50.00 | 61.54 |
| Luqa | 40% | 30.77 | 50.00 |
| Santa Lucija | 64% | 53.33 | 71.43 |
| Fontana | 43% | 66.67 | 25.00 |
| Gharb | 44% | 22.22 | 71.43 |
| Kercem | 53% | 40.00 | 66.67 |
| Ghajnsielem | 23% | 28.57 | 18.75 |
| Nadur | 40% | 39.13 | 40.00 |
| Qala | 44% | 55.56 | 33.33 |
| Rabat - Ghawdex | 74% | 53.13 | 96.55 |
| Sannat | 35% | 50.00 | 20.00 |
| Xaghra | 51% | 39.13 | 62.50 |
| Xewkija | 30% | 31.25 | 28.57 |
| Zebbug | 19% | 18.18 | 20.00 |
| Munxar | 38% | 33.33 | 42.86 |

# Adjusted population

Appendix Table 5. Distribution of adjusted population by age (single years) and gender

| Age | Female (*n*=1,949) | Male (*n*=1,998) |
|---|---|---|
| 18 | 16 | 33 |
| 19 | 10 | 6 |
| 20 | 33 | 27 |
| 21 | 34 | 47 |
| 22 | 36 | 26 |
| 23 | 44 | 30 |
| 24 | 55 | 50 |
| 25 | 36 | 40 |
| 26 | 34 | 13 |
| 27 | 43 | 53 |
| 28 | 19 | 56 |
| 29 | 42 | 28 |
| 30 | 51 | 43 |
| 31 | 35 | 36 |
| 32 | 44 | 39 |
| 33 | 40 | 61 |
| 34 | 31 | 30 |
| 35 | 22 | 38 |
| 36 | 30 | 38 |
| 37 | 35 | 34 |
| 38 | 45 | 43 |
| 39 | 42 | 30 |
| 40 | 32 | 40 |
| 41 | 32 | 45 |
| 42 | 34 | 36 |
| 43 | 35 | 46 |
| 44 | 28 | 43 |
| 45 | 40 | 20 |
| 46 | 36 | 36 |
| 47 | 34 | 40 |
| 48 | 38 | 34 |
| 49 | 30 | 26 |
| 50 | 50 | 39 |
| 51 | 32 | 35 |
| 52 | 40 | 35 |
| 53 | 38 | 25 |

| Age | Female (*n*=1949) | Male (*n*=1998) |
|---|---|---|
| 54 | 50 | 53 |
| 55 | 41 | 65 |
| 56 | 29 | 43 |
| 57 | 43 | 36 |
| 58 | 39 | 46 |
| 59 | 42 | 30 |
| 60 | 33 | 50 |
| 61 | 37 | 23 |
| 62 | 36 | 26 |
| 63 | 37 | 27 |
| 64 | 49 | 56 |
| 65 | 53 | 42 |
| 66 | 36 | 31 |
| 67 | 26 | 42 |
| 68 | 47 | 36 |
| 69 | 23 | 39 |
| 70 | 54 | 52 |
| Total | 1949 | 1998 |

# Dietary intake

The weekly dietary intake of the SAHHTEK population was evaluated through the food frequency questionnaire covering carbohydrate, protein, fat and dairy products consumed in a typical week.

## Consumption of carbohydrate-based food

The carbohydrate-based food data was gathered through the food frequency questionnaire, which included the assessment of weekly intake of potatoes, pasta, rice, white bread and brown bread. The weekly consumption of these carbohydrate products exhibited similar trends throughout the general population, overweight, obese, IFG and diabetes mellitus populations.

The majority of the populations reported eating potatoes, pasta and rice '*once to twice per week'* but white bread '*5 to 7 times per week*'. Brown bread was rarely consumed in a typical week. Males among all the different weight and glucose regulation populations (general, overweight obese, IFG and diabetes) exhibited higher weekly carbohydrate consumption than did females. The male to female frequencies for carbohydrate-based food for each different population (general population, diabetes, IFG, overweight and obese) can be seen below in Appendix Tables 6 to 10.

Appendix Table 6. Carbohydrate based food frequency within the general population, by gender

| | | Female [N=1949] (%) | Male [N=1998] (%) | Chi Test *p*-value |
|---|---|---|---|---|
| Potatoes per week | None | 252 (13) | 120 (6) | **<0.01** |
| | 1 - 2 times/week | 1060 (54) | 1182 (59) | |
| | 3-4 times/week | 470 (24) | 566 (28) | |
| | 5 -7 times/week | 166 (9) | 130 (7) | |
| Pasta per week | None | 297 (15) | 159 (8) | **<0.01** |
| | 1-2 times/week | 1383 (71) | 1364 (68) | |
| | 3-4 times/week | 236 (12) | 436 (22) | |
| | 5-7 times/week | 34 (2) | 40 (2) | |
| Rice per week | None | 848 (44) | 860 (42) | 0.35 |
| | 1-2 per week | 1007 (51) | 1030 (52) | |
| | 3-4 per week | 58 (3) | 79 (4) | |
| | 5 - 7 per week | 35 (2) | 30 (2) | |
| White bread per week | None | 524 (27) | 431 (22) | **<0.01** |
| | 1-2 per week | 464 (23) | 337 (17) | |
| | 3-4 per week | 303 (16) | 268 (13) | |
| | 5-7 per week | 658 (34) | 961 (48) | |
| Brown bread per week | None | 1322 (68) | 1496 (74) | **<0.01** |
| | 1-2 per week | 316 (16) | 214 (11) | |
| | 3-4 per week | 131 (7) | 116 (6) | |
| | 5-7 per week | 179 (9) | 172 (9) | |

Appendix Table 7. Carbohydrate based food frequency within diabetes population, by gender

| | | Female [N=136] (%) | Male [N=271] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Potatoes per week | None | 13 (10) | 20 (7) | 0.72 |
| | 1 - 2 times/week | 74 (54) | 146 (54) | |
| | 3-4 times/week | 40 (29) | 80 (30) | |
| | 5 -7 times/week | 9 (7) | 25 (9) | |
| Pasta per week | None | 19 (14) | 35 (13) | 0.26 |
| | 1-2 times/week | 100 (74) | 182 (67) | |
| | 3-4 times/week | 14 (10) | 48 (18) | |
| | 5-7 times/week | 3 (2) | 6 (2) | |
| Rice per week | None | 61 (45) | 129 (48) | **0.02** |
| | 1-2 per week | 75 (55) | 126 (46) | |
| | 3-4 per week | 0 | 14 (5) | |
| | 5 - 7 per week | 0 | 3 (1) | |
| White bread per week | None | 35 (26) | 46 (17) | 0.14 |
| | 1-2 per week | 15 (11) | 40 (15) | |
| | 3-4 per week | 19 (14) | 33 (12) | |
| | 5-7 per week | 67 (49) | 152 (56) | |
| Brown bread per week | None | 83 (61) | 174 (64) | 0.07 |
| | 1-2 per week | 18 (13) | 33 (12) | |
| | 3-4 per week | 6 (4) | 27 (10) | |
| | 5-7 per week | 29 (21) | 37 (14) | |

Appendix Table 8. Carbohydrate based food frequency within IFG population, by gender

| | | Female [N=361] (%) | Male [N=564] (%) | Chi test p-value |
|---|---|---|---|---|
| Potatoes per week | None | 49 (14) | 36 (6) | **<0.01** |
| | 1 - 2 times/week | 171 (47) | 363 (64) | |
| | 3-4 times/week | 109 (30) | 137 (24) | |
| | 5 -7 times/week | 32 (9) | 28 (5) | |
| Pasta per week | None | 47 (13) | 34 (6) | **<0.01** |
| | 1-2 times/week | 270 (75) | 394 (70) | |
| | 3-4 times/week | 39 (11) | 131 (23) | |
| | 5-7 times/week | 5 (1) | 5 (1) | |
| Rice per week | None | 149 (41) | 248 (44) | **<0.01** |
| | 1-2 per week | 188 (52) | 304 (54) | |
| | 3-4 per week | 9 (2) | 10 (2) | |
| | 5 - 7 per week | 15 (4) | 2 (1) | |
| White bread per week | None | 90 (25) | 103 (18) | **0.05** |
| | 1-2 per week | 75 (21) | 108 (19) | |
| | 3-4 per week | 51 (14) | 83 (15) | |
| | 5-7 per week | 145 (40) | 268 (48) | |
| Brown bread per week | None | 236 (65) | 448 (79) | **<0.01** |
| | 1-2 per week | 72 (20) | 56 (10) | |
| | 3-4 per week | 30 (8) | 22 (4) | |
| | 5-7 per week | 23 (6) | 38 (7) | |

Appendix Table 9. Carbohydrate based food frequency within overweight population, by gender

| | | Female [N=620] )(%) | Male [N=788] (%) | Chi test p-value |
|---|---|---|---|---|
| Potatoes per week | None | 69 (1!) | 31 (4) | **<0.01** |
| | 1 - 2 times/week | 324 (52) | 498 (63) | |
| | 3-4 times/week | 170 (27) | 214 (27) | |
| | 5 -7 times/week | 57 (9) | 45 (6) | |
| Pasta per week | None | 90 (15) | 72 (9) | **<0.01** |
| | 1-2 times/week | 447 (72) | 553 (70) | |
| | 3-4 times/week | 77 (12) | 152 (19) | |
| | 5-7 times/week | 6 (1) | 11 (1) | |
| Rice per week | None | 271 (44) | 356 (45) | 0.08 |
| | 1-2 per week | 328 (53) | 393 (50) | |
| | 3-4 per week | 12 (2) | 32 (4) | |
| | 5 - 7 per week | 9 (1) | 7 (1) | |
| White bread per week | None | 188 (30) | 150 (19) | **<0.01** |
| | 1-2 per week | 131 (21) | 160 (20) | |
| | 3-4 per week | 100 (16) | 119 (15) | |
| | 5-7 per week | 201 (32) | 359 (46) | |
| Brown bread per week | None | 407 (66) | 576 (73) | **0.01** |
| | 1-2 per week | 111 (18) | 102 (13) | |
| | 3-4 per week | 40 (6) | 50 (6) | |
| | 5-7 per week | 62 (10) | 60 (8) | |

Appendix Table 10. Carbohydrate based food frequency within obese population, by gender

| | | Female [N=609] (%) | Male [N=736] (%) | Chi test p-value |
|---|---|---|---|---|
| Potatoes per week | None | 93 (15) | 57 (8) | **<0.01** |
| | 1 - 2 times/week | 331 (54) | 448 (61) | |
| | 3-4 times/week | 137 (22) | 176 (24) | |
| | 5 -7 times/week | 48 (8) | 55 (7) | |
| Pasta per week | None | 98 (16) | 57 (8) | **<0.01** |
| | 1-2 times/week | 442 (73) | 503 (68) | |
| | 3-4 times/week | 56 (9) | 165 (22) | |
| | 5-7 times/week | 13 (2) | 11 (1) | |
| Rice per week | None | 273 (45) | 305 (41) | 0.09 |
| | 1-2 per week | 302 (50) | 383 (52) | |
| | 3-4 per week | 15 (2) | 33 (4) | |
| | 5 - 7 per week | 19 (3) | 15 (2) | |
| White bread per week | None | 160 (26) | 163 (22) | **<0.01** |
| | 1-2 per week | 126 (21) | 134 (18) | |
| | 3-4 per week | 91 (15) | 85 (12) | |
| | 5-7 per week | 232 (38) | 354 (48) | |
| Brown bread per week | None | 434 (71) | 540 (73) | 0.77 |
| | 1-2 per week | 74 (12) | 77 (10) | |
| | 3-4 per week | 38 (6) | 43 (6) | |
| | 5-7 per week | 63 (10) | 76 (10) | |

## Consumption of main meals

The consumption of main meals data was gathered through the food frequency questionnaire, which included the assessment of weekly intake of beef/veal, pork, chicken, fish, ready-made meals, cheesecakes (‘*pastizzi’*) and vegetable dishes. The weekly consumption of these products exhibited similar trends throughout the general population, overweight, obese, IFG and diabetes mellitus populations. (Appendix Tables 11 to 15).

## Dairy based food

The weekly food frequency analysis for dairy products included light cheese, cheese, cheeselets (ġbejniet), ricotta, skimmed milk, full milk, light yogurt, yogurt, and mozzarella.

The majority of the dysglyceamia and weight category populations (including whole, normal body weight, overweight, obese, IFG, diabetes) reported a low consumption of dairy products, with some exceptions (Appendix Tables 16 to 20). The majority consumed cheese at a frequency of ‘*1 to 2 times per week*’ except for normal weight males and diabetes females, who reported ’*less than weekly’* consumption. On the other hand, the majority of diabetes females, reported to consume cheeselets ‘*1 to 2 times per week*’. This contrasts with the rest of the population, among whom cheeselets were consumed less than once weekly. All female populations, unlike males, reported a majority favouring skimmed milk.

Appendix Table 11. Consumption of main meals food frequency within the general population, by gender

| | | Female [N=1949] (%) | Male [N=1998] (%) | p-value |
|---|---|---|---|---|
| Beef / Veal per week | None | 715 (37) | 576 (29) | **<0.01** |
| | 1-2 per week | 1149 (4) | 1266 (63) | |
| | 3-4 per week | 77 (4) | 150 (8) | |
| | 5-7 per week | 8 (1) | 6 (0) | |
| Pork per week | None | 988 (51) | 770 (39) | **<0.01** |
| | 1-2 per week | 924 (47) | 1179 (59) | |
| | 3-4 per week | 34 (2) | 44 (2) | |
| | 5-7 per week | 3 (0) | 5 (0) | |
| Chicken per week | None | 179 (9) | 217 (11) | **<0.01** |
| | 1-2 per week | 1144 (59) | 1238 (62) | |
| | 3-4 per week | 549 (28) | 455 (23) | |
| | 5-7 per week | 77 (4) | 88 (4) | |
| Fish per week | None | 788 (40) | 864 (43) | **<0.01** |
| | 1-2 per week | 1015 (52) | 1035 (52) | |
| | 3-4 per week | 121 (6) | 93 (5) | |
| | 5-7 per week | 25 (1) | 6 (0) | |
| Ready-made meals per week | None | 1353 (69) | 1255 (63) | **<0.01** |
| | 1-2 per week | 530 (27) | 654 (33) | |
| | 3-4 per week | 41 (2) | 46 (2) | |
| | 5-7 per week | 25 (1) | 43 (2) | |
| Cheesecakes/pastizzi per week | None | 1586 (81) | 1362 (68) | **<0.01** |
| | 1-2 per week | 339 (17) | 569 (28) | |
| | 3-4 per week | 11 (1) | 47 (2) | |
| | 5-7 per week | 13 (1) | 20 (1) | |
| Vegetable dishes per week | None | 689 (35) | 964 (48) | **<0.01** |
| | 1-2 per week | 704 (36) | 781 (39) | |
| | 3-4 per week | 317 (16) | 146 (7) | |
| | 5-7 per week | 239 (12) | 107 (5) | |

Appendix Table 12. Consumption of main meals food frequency within the diabetes population, by gender

| | | Female [N=136] (%) | Male [N=271] (%) | *p*-value |
|---|---|---|---|---|
| Beef / Veal per week | None | 66 (49) | 96 (35) | **0.02** |
| | 1-2 per week | 68 (50) | 157 (58) | |
| | 3-4 per week | 2 (1) | 14 (5) | |
| | 5-7 per week | 0 | 4 (1) | |
| Pork per week | None | 60 (44) | 111 (41) | 0.73 |
| | 1-2 per week | 74 (54) | 152 (56) | |
| | 3-4 per week | 2 (1) | 7 (3) | |
| | 5-7 per week | 0 | 1 (0) | |
| Chicken per week | None | 14 (10) | 34 (13) | 0.47 |
| | 1-2 per week | 82 (60) | 173 (64) | |
| | 3-4 per week | 36 (26) | 54 (20) | |
| | 5-7 per week | 4 (3) | 10 (4) | |
| Fish per week | None | 48 (35) | 122 (45) | **0.02** |
| | 1-2 per week | 84 (62) | 130 (48) | |
| | 3-4 per week | 4 (3) | 19 (7) | |
| | 5-7 per week | 0 | 0 | |
| Ready-made meals per week | None | 94 (69) | 193 (71) | 0.34 |
| | 1-2 per week | 40 (29) | 67 (25) | |
| | 3-4 per week | 2 (1) | 7 (3) | |
| | 5-7 per week | 0 | 4 (1) | |
| Cheesecakes/ pastizzi per week | None | 96 (71) | 142 (52) | **0.01** |
| | 1-2 per week | 38 (28) | 121 (45) | |
| | 3-4 per week | 2 (1) | 7 (3) | |
| | 5-7 per week | 0 | 1 (0) | |
| Vegetable dishes per week | None | 47 (35) | 116 (43) | 0.24 |
| | 1-2 per week | 63 (46) | 113 (42) | |
| | 3-4 per week | 17 (13) | 21 (8) | |
| | 5-7 per week | 9 (7) | 21 (8) | |

Appendix Table 13. Consumption of main meals food frequency within the IFG population, by gender

| | | Female [N=361] (%) | Male [N=564] (%) | *p*-value |
|---|---|---|---|---|
| Beef / Veal per week | None | 124 (34) | 142 (25) | **<0.01** |
| | 1-2 per week | 214 (59) | 390 (69) | |
| | 3-4 per week | 20 (6) | 32 (6) | |
| | 5-7 per week | 3 (1) | 0 | |
| Pork per week | None | 150 (42) | 240 (43) | 0.36 |
| | 1-2 per week | 207 (57) | 321 (57) | |
| | 3-4 per week | 2 (1) | 3 (1) | |
| | 5-7 per week | 2 (1) | 0 | |
| Chicken per week | None | 30 (8) | 62 (11) | 0.38 |
| | 1-2 per week | 228 (63) | 364 (65) | |
| | 3-4 per week | 86 (24) | 117 (21) | |
| | 5-7 per week | 17 (5) | 21 (4) | |
| Fish per week | None | 149 (41) | 250 (44) | 0.27 |
| | 1-2 per week | 190 (53) | 286 (51) | |
| | 3-4 per week | 20 (6) | 28 (5) | |
| | 5-7 per week | 2 (1) | 0 | |
| Ready-made meals per week | None | 274 (76) | 400 (71) | 0.22 |
| | 1-2 per week | 75 (21) | 149 (26) | |
| | 3-4 per week | 9 (2) | 10 (2) | |
| | 5-7 per week | 3 (1) | 5 (1) | |
| Cheesecakes/pastizzi per week | None | 285 (79) | 429 (76) | 0.34 |
| | 1-2 per week | 72 (20) | 123 (22) | |
| | 3-4 per week | 4 (1) | 8 (1) | |
| | 5-7 per week | 0 | 4 (1) | |
| Vegetable dishes per week | None | 123 (34) | 286 (51) | **<0.01** |
| | 1-2 per week | 134 (37) | 207 (37) | |
| | 3-4 per week | 69 (19) | 51 (9) | |
| | 5-7 per week | 35 (10) | 20 (4) | |

Appendix Table 14. Consumption of main meals food frequency within the overweight population, by gender

| | | Female [N=620] (%) | Male [N=788] (%) | *p*-value |
|---|---|---|---|---|
| Beef / Veal per week | None | 218 (35) | 210 (27) | **<0.01** |
| | 1-2 per week | 369 (60) | 523 (66) | |
| | 3-4 per week | 30 (5) | 53 (7) | |
| | 5-7 per week | 3 (1) | 2 (1) | |
| Pork per week | None | 327 (53) | 312 (40) | **<0.01** |
| | 1-2 per week | 282 (45) | 462 (59) | |
| | 3-4 per week | 10 (2) | 14 (2) | |
| | 5-7 per week | 1 (0) | 0 | |
| Chicken per week | None | 47 (8) | 100 (13) | **<0.01** |
| | 1-2 per week | 378 (61) | 484 (61) | |
| | 3-4 per week | 179 (29) | 170 (22) | |
| | 5-7 per week | 16 (3) | 34 (4) | |
| Fish per week | None | 200 (32) | 338 (43) | **<0.01** |
| | 1-2 per week | 361 (58) | 396 (50) | |
| | 3-4 per week | 49 (8) | 54 (7) | |
| | 5-7 per week | 10 (2) | 0 | |
| Ready-made meals per week | None | 444 (72) | 512 (65) | 0.06 |
| | 1-2 per week | 162 (26) | 251 (32) | |
| | 3-4 per week | 6 (1) | 13 (2) | |
| | 5-7 per week | 8 (1) | 12 (2) | |
| Cheesecakes/ pastizzi per week | None | 532 (86) | 582 (74) | **<0.01** |
| | 1-2 per week | 82 (13) | 196 (25) | |
| | 3-4 per week | 4 (1) | 9 (1) | |
| | 5-7 per week | 2 (1) | 1 (0) | |
| Vegetable dishes per week | None | 203 (33) | 413 (52) | **<0.01** |
| | 1-2 per week | 215 (35) | 280 (36) | |
| | 3-4 per week | 110 (18) | 59 (7) | |
| | 5-7 per week | 92 (15) | 36 (5) | |

Appendix Table 15. Consumption of main meals food frequency within the obese population, by gender

| | | Female [N=609] (%) | Male [N=736] (%) | *p*-value |
|---|---|---|---|---|
| Beef / Veal per week | None | 232 (38) | 198 (27) | **<0.01** |
| | 1-2 per week | 354 (58) | 479 (65) | |
| | 3-4 per week | 20 (3) | 55 (7) | |
| | 5-7 per week | 3 (1) | 4 (1) | |
| Pork per week | None | 286 (47) | 276 (38) | **0.01** |
| | 1-2 per week | 307 (50) | 440 (60) | |
| | 3-4 per week | 14 (2) | 19 (3) | |
| | 5-7 per week | 2 (1) | 1 (0) | |
| Chicken per week | None | 64 (11) | 82 (11) | **<0.01** |
| | 1-2 per week | 309 (51) | 438 (60) | |
| | 3-4 per week | 201 (33) | 182 (25) | |
| | 5-7 per week | 35 (6) | 34 (5) | |
| Fish per week | None | 270 (44) | 342 (46) | 0.45 |
| | 1-2 per week | 302 (50) | 363 (49) | |
| | 3-4 per week | 30 (5) | 25 (3) | |
| | 5-7 per week | 7 (1) | 6 (1) | |
| Ready-made meals per week | None | 428 (70) | 466 (63) | **<0.01** |
| | 1-2 per week | 145 (24) | 239 (32) | |
| | 3-4 per week | 25 (4) | 23 (3) | |
| | 5-7 per week | 11 (2) | 8 (1) | |
| Cheesecakes/pastizzi per week | None | 457 (75) | 447 (61) | **<0.01** |
| | 1-2 per week | 139 (23) | 270 (37) | |
| | 3-4 per week | 2 (1) | 11 (1) | |
| | 5-7 per week | 11 (2) | 8 (1) | |
| Vegetable dishes per week | None | 221 (36) | 317 (43) | **<0.01** |
| | 1-2 per week | 229 (38) | 303 (41) | |
| | 3-4 per week | 90 (15) | 71 (10) | |
| | 5-7 per week | 69 (11) | 45 (6) | |

Appendix Table 16. Dairy food frequencies within the general population, by gender

| | | Female [N=1949] (%) | Male [N=1998] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Cheese | None | 1406 (72) | 1599 (80) | **<0.01** |
| | 1 - 2 times per week | 348 (18) | 214 (11) | |
| | 3 - 4 times per week | 92 (5) | 96 (5) | |
| | 5 - 7 times per week | 103 (5) | 89 (4) | |
| Cheese | None | 627 (32) | 493 (25) | **<0.01** |
| | 1 - 2 times per week | 737 (38) | 671 (34) | |
| | 3 - 4 times per week | 299 (15) | 375 (19) | |
| | 5 - 7 times per week | 286 (15) | 459 (23) | |
| Cheeselets (Gbejniet) | None | 1005 (52) | 1034 (52) | 0.32 |
| | 1 - 2 times per week | 770 (40) | 765 (38) | |
| | 3 - 4 times per week | 134 (7) | 165 (8) | |
| | 5 - 7 times per week | 40 (2) | 34 (2) | |
| Ricotta | None | 1061 (54) | 1301 (65) | **<0.01** |
| | 1- 2 times per week | 755 (39) | 629 (31) | |
| | 3 - 4 times per week | 100 (5) | 49 (2) | |
| | 5 - 7 times per week | 33 (2) | 19 (1) | |
| Skimmed milk | None | 770 (40) | 1058 (53) | **<0.01** |
| | 1 -2 times per week | 128 (7) | 95 (5) | |
| | 3 - 4 times per week | 98 (5) | 67 (3) | |
| | 5 - 7 times per week | 953 (49) | 778 (39) | |
| Full milk | None | 1380 (71) | 1124 (56) | **<0.01** |
| | 1 - 2 times per week | 94 (5) | 124 (6) | |
| | 3 -4 times per week | 54 (3) | 76 (4) | |
| | 5 - 7 times per week | 421 (22) | 674 (34) | |

| | | Female [N=1949] (%) | Male [N=1998] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Yogurt | None | 1133 (58) | 1558 (78) | **<0.01** |
| | 1 -2 times per week | 319 (16) | 169 (8) | |
| | 3 - 4 times per week | 207 (11) | 127 (6) | |
| | 5- 7 times per week | 290 (15) | 144 (7) | |
| Yogurt | None | 1663 (85) | 1682 (84) | 0.24 |
| | 1 -2 times per week | 169 (9) | 174 (9) | |
| | 3 - 4 times per week | 71 (4) | 73 (4) | |
| | 5 - 7 times per week | 46 (2) | 69 (3) | |
| Mozzarella | None | 1364 (70) | 1373 (69) | **<0.01** |
| | 1 -2 times per week | 552 (28) | 567 (28) | |
| | 3 - 4 times per week | 26 (1) | 56 (3) | |
| | 5 - 7 times per week | 7 (0) | 2 (0) | |

Appendix Table 17. Dairy food frequencies within the diabetes population, by gender

| | | Female [N=136] (%) | Male [N=271] (%) | Chi test p-value |
|---|---|---|---|---|
| Light Cheese | None | 86 (63) | 192 (71) | **<0.01** |
| | 1 - 2 times per week | 24 (18) | 45 (17) | |
| | 3 - 4 times per week | 2 (1) | 15 (6) | |
| | 5 - 7 times per week | 24 (18) | 19 (7) | |
| Cheese | None | 48 (35) | 70 (26) | 0.16 |
| | 1 - 2 times per week | 35 (26) | 86 (32) | |
| | 3 - 4 times per week | 18 (13) | 48 (18) | |
| | 5 - 7 times per week | 35 (26) | 67 (25) | |
| Cheeselets (Gbejniet) | None | 60 (44) | 158 (58) | **<0.01** |
| | 1 - 2 times per week | 68 (50) | 82 (30) | |
| | 3 - 4 times per week | 4 (3) | 26 (10) | |
| | 5 - 7 times per week | 4 (3) | 5 (2) | |
| Ricotta | None | 58 (43) | 181 (67) | **<0.01** |
| | 1- 2 times per week | 73 (54) | 80 (30) | |
| | 3 - 4 times per week | 3 (2) | 6 (2) | |
| | 5 - 7 times per week | 2 (1) | 4 (1) | |
| Skimmed milk | None | 41 (30) | 140 (52) | **<0.01** |
| | 1 -2 times per week | 3 (2) | 8 (3) | |
| | 3 - 4 times per week | 0 | 8 (3) | |
| | 5 - 7 times per week | 92 (68) | 115 (42) | |
| Full milk | None | 119 (88) | 171 (63) | **<0.01** |
| | 1 - 2 times per week | 3 (2) | 12 (4) | |
| | 3 -4 times per week | 1 (1) | 8 (3) | |
| | 5 - 7 times per week | 13 (10) | 80 (30) | |

| | | Female [N=136] (%) | Male [N=271] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Yogurt | None | 77 (57) | 221 (82) | **<0.01** |
| | 1 -2 times per week | 25 (18) | 35 (13) | |
| | 3 - 4 times per week | 10 (7) | 9 (3) | |
| | 5- 7 times per week | 24 (18) | 6 (2) | |
| Yogurt | None | 123 (90) | 240 (89) | 0.39 |
| | 1 -2 times per week | 11 (8) | 28 (10) | |
| | 3 - 4 times per week | 2 (1) | 1 (1) | |
| | 5 - 7 times per week | 0 | 2 (1) | |
| Mozzarella | None | 101 (74) | 198 (73) | 0.35 |
| | 1 -2 times per week | 31 (23) | 70 (26) | |
| | 3 - 4 times per week | 4 (3) | 3 (1) | |
| | 5 - 7 times per week | 0 | 0 | |

Appendix Table 18. Dairy food frequencies within the IFG population, by gender

| | | Female [N=361] (%) | Male [N=564] (%) | Chi test p-value |
|---|---|---|---|---|
| Light Cheese | None | 284 (79) | 461 (82) | 0.36 |
| | 1 - 2 times per week | 42 (12) | 49 (9) | |
| | 3 - 4 times per week | 22 (6) | 28 (5) | |
| | 5 - 7 times per week | 13 (4) | 26 (5) | |
| Cheese | None | 109 (30) | 130 (23) | **<0.01** |
| | 1 - 2 times per week | 147 (41) | 196 (35) | |
| | 3 - 4 times per week | 40 (11) | 115 (20) | |
| | 5 - 7 times per week | 65 (18) | 123 (22) | |
| Cheeselets (Gbejniet) | None | 181 (50) | 276 (49) | 0.43 |
| | 1 - 2 times per week | 161 (45) | 243 (43) | |
| | 3 - 4 times per week | 17 (5) | 38 (7) | |
| | 5 - 7 times per week | 2 (1) | 7 (1) | |
| Ricotta | None | 203 (56) | 359 (64) | **0.01** |
| | 1- 2 times per week | 134 (37) | 189 (34) | |
| | 3 - 4 times per week | 21 (6) | 11 (2) | |
| | 5 - 7 times per week | 3 (1) | 5 (1) | |
| Skimmed milk | None | 127 (35) | 300 (53) | **<0.01** |
| | 1 -2 times per week | 26 (7) | 22 (4) | |
| | 3 - 4 times per week | 18 (5) | 26 (5) | |
| | 5 - 7 times per week | 190 (53) | 216 (38) | |
| Full milk | None | 273 (76) | 331 (59) | **<0.01** |
| | 1 - 2 times per week | 14 (4) | 28 (5) | |
| | 3 -4 times per week | 5 (1) | 21 (4) | |
| | 5 - 7 times per week | 69 (19) | 184 (33) | |

| | | Female [N=361] (%) | Male [N=564] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Yogurt | None | 219 (61) | 432 (77) | **<0.01** |
| | 1 -2 times per week | 59 (16) | 45 (8) | |
| | 3 - 4 times per week | 42 (12) | 43 (8) | |
| | 5- 7 times per week | 41 (11) | 44 (8) | |
| Yogurt | None | 308 (85) | 516 (91) | **0.03** |
| | 1 -2 times per week | 28 (8) | 27 (5) | |
| | 3 - 4 times per week | 12 (3) | 9 (2) | |
| | 5 - 7 times per week | 13 (4) | 12 (2) | |
| Mozorella | None | 266 (74) | 387 (69) | 0.21 |
| | 1 -2 times per week | 92 (25) | 172 (30) | |
| | 3 - 4 times per week | 3 (1) | 3 (1) | |
| | 5 - 7 times per week | 0 | 2 (1) | |

Appendix Table 19 Dairy food frequencies within the overweight population, by gender

| | | Female [N=620] (%) | Male [N=788] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Cheese | None | 435 (70) | 618 (78) | **<0.01** |
| | 1 - 2 times per week | 127 (20) | 97 (12) | |
| | 3 - 4 times per week | 27 (4) | 37 (5) | |
| | 5 - 7 times per week | 31 (5) | 36 (5) | |
| Cheese | None | 195 (31) | 190 (24) | **<0.01** |
| | 1 - 2 times per week | 239 (39) | 301 (38) | |
| | 3 - 4 times per week | 94 (15) | 134 (17) | |
| | 5 - 7 times per week | 92 (15) | 163 (21) | |
| Cheeselets (Gbejniet) | None | 327 (53) | 397 (50) | 0.84 |
| | 1 - 2 times per week | 243 (39) | 328 (42) | |
| | 3 - 4 times per week | 42 (7) | 53 (7) | |
| | 5 - 7 times per week | 8 (1) | 10 (1) | |
| Ricotta | None | 322 (52) | 512 (65) | **<0.01** |
| | 1- 2 times per week | 264 (43) | 254 (32) | |
| | 3 - 4 times per week | 27 (4) | 13 (2) | |
| | 5 - 7 times per week | 7 (1) | 9 (1) | |
| Skimmed milk | None | 219 (35) | 382 (48) | **<0.01** |
| | 1 -2 times per week | 48 (8) | 47 (6) | |
| | 3 - 4 times per week | 34 (5) | 24 (3) | |
| | 5 - 7 times per week | 319 (51) | 335 (43) | |
| Full milk | None | 454 (73) | 465 (59) | **<0.01** |
| | 1 - 2 times per week | 29 (5) | 48 (6) | |
| | 3 -4 times per week | 20 (3) | 29 (4) | |
| | 5 - 7 times per week | 117 (19) | 246 (31) | |

| | | Female [N=620] (%) | Male [N=788] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Yogurt | None | 348 (56) | 605 (77) | **<0.01** |
| | 1 -2 times per week | 99 (16) | 72 (9) | |
| | 3 - 4 times per week | 64 (10) | 60 (8) | |
| | 5- 7 times per week | 109 (18) | 51 (6) | |
| Yogurt | None | 539 (87) | 685 (87) | 0.08 |
| | 1 -2 times per week | 46 (7) | 77 (10) | |
| | 3 - 4 times per week | 21 (3) | 17 (2) | |
| | 5 - 7 times per week | 14 (2) | 9 (1) | |
| Mozzarella | None | 459 (74) | 527 (67) | **0.01** |
| | 1 -2 times per week | 156 (25) | 242 (31) | |
| | 3 - 4 times per week | 4 (1) | 17 (2) | |
| | 5 - 7 times per week | 1 (0) | 2 (1) | |

Appendix Table 20. Dairy food frequencies within the obese population, by gender

| | | Female [N=609] (%) | Male [N=736] (%) | Chi test p-value |
|---|---|---|---|---|
| Light Cheese | None | 422 (69) | 577 (78) | <0.01 |
| | 1 - 2 times per week | 103 (17) | 81 (1!) | |
| | 3 - 4 times per week | 39 (6) | 36 (5) | |
| | 5 - 7 times per week | 45 (7) | 42 (6) | |
| Cheese | None | 218 (36) | 168 (23) | <0.01 |
| | 1 - 2 times per week | 228 (37) | 242 (33) | |
| | 3 - 4 times per week | 82 (13) | 144 (20) | |
| | 5 - 7 times per week | 81 (13) | 182 (25) | |
| Cheeselets (Gbejniet) | None | 312 (51) | 378 (51) | 0.45 |
| | 1 - 2 times per week | 242 (40) | 281 (38) | |
| | 3 - 4 times per week | 38 (6) | 61 (8) | |
| | 5 - 7 times per week | 17 (3) | 16 (2) | |
| Ricotta | None | 314 (52) | 479 (65) | **<0.01** |
| | 1- 2 times per week | 248 (41) | 218 (30) | |
| | 3 - 4 times per week | 31 (5) | 28 (4) | |
| | 5 - 7 times per week | 16 (3) | 11 (1) | |
| Skimmed milk | None | 205 (34) | 370 (50) | **<0.01** |
| | 1 -2 times per week | 29 (5) | 15 (2) | |
| | 3 - 4 times per week | 32 (5) | 36 (5) | |
| | 5 - 7 times per week | 343 (56) | 315 (43) | |
| Full milk | None | 445 (73) | 457 (62) | **<0.01** |
| | 1 - 2 times per week | 25 (4) | 38 (5) | |
| | 3 -4 times per week | 11 (2) | 31 (4) | |
| | 5 - 7 times per week | 128 (21) | 210 (29) | |

| | | Female [N=609] (%) | Male [N=736] (%) | Chi test *p*-value |
|---|---|---|---|---|
| Light Yogurt | None | 312 (51) | 560 (76) | **<0.01** |
| | 1 -2 times per week | 114 (19) | 58 (8) | |
| | 3 - 4 times per week | 73 (12) | 60 (8) | |
| | 5- 7 times per week | 110 (18) | 58 (8) | |
| Yogurt | None | 546 (90) | 653 (89) | **0.02** |
| | 1 -2 times per week | 27 (4) | 52 (7) | |
| | 3 - 4 times per week | 26 (4) | 15 (2) | |
| | 5 - 7 times per week | 10 (2) | 16 (2) | |
| Mozorella | None | 429 (70) | 511 (69) | 0.82 |
| | 1 -2 times per week | 173 (28) | 214 (29) | |
| | 3 - 4 times per week | 7 (1) | 11 (1) | |
| | 5 - 7 times per week | 0 | 0 | |

# Appendix E – Publications

global health, epidemiology and genomics

ORIGINAL RESEARCH ARTICLE

# Diabetes, pre-diabetes and their risk factors in Malta: a study profile of national cross-sectional prevalence study

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**Background** Type 2 diabetes mellitus constitutes a global epidemic and a major burden on health care systems across the world. Prevention of this disease is essential, and the development of effective prevention strategies requires validated information on the disease burden and the risk factors. Embarking on a nationally representative cross-sectional study is challenging and costly. Few countries undertake this process regularly, if at all.

**Method** This paper sets out the evidence-based protocol of a recent cross-sectional study that was conducted in Malta. Data collection took place from November 2014 to January 2016.

**Results** This study presents up-to-date national data on diabetes and its risk factors (such as obesity, smoking, physical activity and alcohol intake) that will soon be publicly available.

**Conclusion** This protocol was compiled so that the study can be replicated in other countries. The protocol contains step-by-step descriptions of the study design, including details on the population sampling, the permissions required and the validated measurement tools used.

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**Key words:** Cross-sectional studies, diabetes mellitus, evidence-based medicine, health care survey, Malta.

## Introduction

Type 2 diabetes mellitus (T2DM) is a global epidemic, and the International Diabetes Federation (IDF) declared it a 'global emergency' in 2015. The estimated global prevalence of T2DM in 2015 was 8.8% and this is predicted to rise to 10.4% by 2040 [1]. Across Europe, ageing, inadequate physical activity levels, high calorie diets and rising levels of obesity continue to contribute to the disease, which has a substantial impact on individuals' quality of life [2]. Another established risk factor for diabetes is pre-diabetes, where an individual's blood glucose level is above the normal level but not high enough for a diagnosis of diabetes [3]. Those with pre-diabetes are at an increased risk of developing diabetes, with a 5-year conversion rate from 10 to 23% depending on the diagnostic criteria used [4]. Therefore, a greater understanding of pre-diabetes is essential for the prevention of diabetes [3]. In addition, identifying individuals with pre-diabetes at an early stage can have a positive impact on health outcomes [5].

In order for countries to be in a position to manage the increasing burden of diabetes and to be able to develop evidence-based prevention strategies, regular evaluations of the prevalence of diabetes, its risk factors and its impact need to be carried out at the national level [6]. The IDF has strongly recommended that individual countries carry out research (including prevalence studies) in order to obtain an accurate and up-to-date picture of the local diabetes situation [1].

Malta is an archipelago of islands in the Mediterranean Sea, at the crossroads between Europe and Africa, and it

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has been known to have a high prevalence of T2DM since the 18th century [7]. It is a small archipelago of islands (<316 $km^2$) with an accessible population (<500 000 people), which makes it a relatively easy location in which to carry out nationally representative studies on diabetes and other common non-communicable diseases. The increase in the prevalence of diabetes in the country has been attributed to the shift in its population from a Mediterranean lifestyle to a more Westernized lifestyle [8].

Prevalence studies are not conducted regularly in Malta. Effectively, this study protocol is the *only* national cross-sectional study on diabetes and its risk factors to be conducted in the past 35 years [9]. Considering the extensive changes in lifestyles, culture and demographics of the Maltese population over the recent decades and considering the improvements in genetic technology, conducting national surveys of non-communicable diseases such as diabetes that utilize the advanced genetic technology is imperative in order to gather up-to-data data [8]. These national studies should also identify the most important current risk factors for the disease in question.

The study based on this protocol was supported by Malta's Minister for Health who highlighted that it would provide key evidence for the development of prevention strategies and it would provide information to inform future research [10]. As a result of the vital nature of the data collected in the study, a campaign (with stringent ethical criteria) to raise funds from academic and private sources was launched, thereby avoiding the need to rely on the extremely limited state funding.

The study was conducted by the University of Malta. It was entitled 'SAHHTEK' (Your Health) – The University of Malta Health and Wellbeing Study' and it took place from November 2014 to January 2016 with encouragement and in-kind support from the government of Malta. The aim of the study was to obtain up-to-date information on the prevalence of T2DM in Malta, to investigate the pre-diabetic population and to shed light on the important links between pre-diabetes and diabetes and its determinants [11]. This paper presents the study protocol and the lessons learnt from the study in order to aid other countries that wish to undertake a similar survey.

## Definition of cases

The participants that had been previously diagnosed with diabetes and were on medication were categorized as having diabetes irrespective of their fasting blood glucose (FBG) levels. Participants who had not previously been diagnosed but who had an FBG level of ≥7 mmol/L were categorized as having newly diagnosed diabetes. This was in line with the standard recommendations to use an FBG level of ≥7 mmol/L for the estimation of the prevalence of diabetes (with no repeat testing if a diagnosis of diabetes is established) [12–14]. Participants with an FBG level of 5.6–6.9 mmol/L who had not previously been diagnosed with diabetes were categorized as having impaired fasting glucose (IFG) [11]. The FBG cut-off point of 5.6 mmol/L has been proven to be the optimal value for the prediction of future diabetes [15]. Participants with a FBG level of <5.59 mmol/L were assumed to have normal carbohydrate metabolism [12].

A 2-h oral glucose tolerance test (OGTT) was undertaken by those who had IFG in order to assess whether the participants had impaired glucose tolerance (IGT) or diabetes (despite not having FBG levels of ≥7 mmol/L). This is in line with the fact that approximately 31% of individuals with diabetes are not picked up by the FBG criteria [13, 16]. In line with the OGTT cut-off criteria of the World Health Organization (WHO), those with a 2-h blood glucose level of 7.8–11.0 mmol/L were categorized as having IGT, whereas those with a level of ≥11.1 mmol/L were categorized as having diabetes [13].

## Sampling methodology

The sample was randomly selected from the official population register in order to be representative of the Maltese resident population (including foreign-born residents) who had lived in Malta at least 6 months. The sample was selected using a stratification strategy which depended on age (between 18 and 70), gender and location. Based on the American Diabetes Association (ADA) estimate of the prevalence of pre-diabetes (25%), the prevalence of pre-diabetes in Malta was estimated to be 25% [17]. Since a primary aim of the study was to identify the subpopulation of individuals with pre-diabetes, it was assumed that the size of the sample would have to be approximately four times the number of individuals with pre-diabetes required. Should the prevalence of individuals with pre-diabetes in Malta be higher than 25%, it would be to the study's advantage. Based on local health examination studies, a response rate of around 50% was expected [18]. Therefore, the size of the sample had to be eight times the required number of individuals with pre-diabetes. In addition, as one of the aims of the study was to explore the associations between a large numbers of factors, for the purpose of sample size estimation, it was assumed that the worst-case scenario was a 50% response rate.

Using the PiFace® software with a maximum confidence interval of ±5%, it was estimated that a sample of 384 participants with pre-diabetes should be recruited [17, 19]. Therefore, a minimum sample of 3072 had to be selected from the population. In order to allow for a lower response rate compared to that in Malta's 2010 European Health Examination Survey, and for deaths and emigration during the fieldwork period, a sample of 4000 was chosen [18]. This amounted to approximately 1% of the adult population of Malta. In fact, the demographics of the study population were similar to the representative data obtained from the latest Maltese demographic report issued by Malta's


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National Statistics Office, as seen in Table 1 that demonstrates the sample population in relation to the total population of Malta by age groups [20].

The selection of a sample drawn from the population register allowed nationally representative data to be collected that means that conclusions can be drawn for the whole population, and it also means that in-depth analyses of subgroups (such as the diabetic and pre-diabetic subgroups) can be carried out. As single-stage random sampling was used, there was no clustering effect. Pregnant female participants were excluded from the survey

### Permissions

Both the Research Ethics Committee of the Faculty of Medicine and Surgery at the University of Malta and Malta's Information and Data Protection Commissioner gave their permission for the study to be run. The Ministry for Energy & Health, through the Parliamentary Secretary and the Department of Primary Care, provided support in the form of the use of local government health clinics and the use of the Biochemistry Laboratory of the public Mater Dei Hospital.

### Recruitment of fieldworkers

Interviewers and health assessors (who were trained phlebotomists who collected the blood samples as well as carrying out the health examinations) were recruited using adverts in local newspapers. The ability to communicate in both Maltese and English was considered to be essential. Training sessions on the measurement tools were organized quarterly to ensure that the assessments were conducted uniformly to avoid inter- and intra-observer variability. The interviewers underwent frequent revalidation of their interviewing techniques to avoid biases and to ensure the quality of the data.

### Measurement tools

Invitation letters and consent forms were composed in Maltese and English. Back translation from the English version to Maltese version then back to English version was performed. In line with the WHO STEPS framework, a validated questionnaire-based assessment, simple physical measurements (blood pressure, height, weight and waist and hip circumferences) and biochemical measurements (FBG, lipid

**Table 1.** *Size of the study sample compared to the size of the national population in 2013*

| Age group | Total population[a] | Total sample (approximately 1% of population) |
|---|---|---|
| 18–24 | 40 564 | 504 |
| 25–34 | 62 180 | 747 |
| 35–44 | 56 575 | 725 |
| 45–54 | 55 113 | 749 |
| 55–64 | 59 268 | 804 |
| 65–70 | 33 480 | 471 |

| Age group | Male population[a] | Male sample (approximately 1% of population) |
|---|---|---|
| 18–24 | 21 045 | 260 |
| 25–34 | 32 359 | 391 |
| 35–44 | 29 143 | 377 |
| 45–54 | 27 728 | 366 |
| 55–64 | 29 585 | 408 |
| 65–70 | 16 136 | 239 |

| Age group | Female population[a] | Female sample (approximately 1% of population) |
|---|---|---|
| 18–24 | 19 519 | 244 |
| 25–34 | 29 821 | 356 |
| 35–44 | 27 432 | 348 |
| 45–54 | 27 385 | 383 |
| 55–64 | 29 683 | 396 |
| 65–70 | 17 344 | 232 |

[a] Demographic data from Malta's National Statistics Office.


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profile and glucose tolerance, as measured using an OGTT for the selected participants) were carried out [21]. The questionnaire used was a composite of a number of validated tools, details of which are available in the online Supplementary File. The validated measurement equipment was calibrated in accordance with WHO regulations, details of which can also be found in the online Supplementary File [22].

### Age of participants

The study aimed to assess the adult population in Malta. For this reason and also to avoid the requirement for parental consent, the lower age limit was set at 18. Even though both pre-diabetes and T2DM are being diagnosed at increasingly early ages in many countries, the prevalence in those under 18 would still be relatively low compared with that in the older population [23]. The upper age limit was set at 70 years as beyond this age, the presence of long-standing diabetes or incident disease is more common [24]. Approximately 20% of those older than 65 have diabetes and studying older age groups may skew the true prevalence of diabetes [25]. Research on diabetes in those aged over 70 has shown that glucose diagnoses and glucose control showed no significant cardiovascular disease reduction and some studies even showed excessive deaths in those are undergoing intensive glucose control [24].

### Health examinations

The randomly selected participants were each asked to attend an appointment at various locations in Malta, having fasted for at least 9 h and having abstained from cigarette smoking and physical activity for at least an hour. On registering, each participant was issued with a unique code. An interviewer explained the consent form and allowed time for the participant to read it and ask questions. If the subject agreed to participate, the questionnaire was filled in by the interviewer in accordance with the participant's responses.

While still sitting down, three consecutive blood pressure measurements were taken, and the third blood pressure measurement was recorded (to reduce the impact of white coat hypertension). This was followed by measurements of weight and height using validated weighing scales with a built-in height rod. The participants removed their shoes and excess clothing and emptied their pockets. The waist and hip circumferences were measured using a measuring tape. The waist circumference was measured midway between the lower rib margin and the iliac crest and the hip circumference was measured at the tip of the iliac crest.

Sets of three venous blood samples were collected from each participant for measuring FBG levels, for assessing lipid profiles, and for carrying out a genotyping analysis. The blood samples were collected by skilled phlebotomists, using butterfly needles and a Vacutainer® system. Each blood sample was labelled with the participant's code and put on ice in a cooler bag. Great care was taken with the samples to avoid haemolysis or glycolysis. This involved using preservative tubes and delivering them to the laboratory within 2 h of collection. This was feasible as Malta is a very small country and the laboratory is in a central location, so travelling distances were short.

Each participant was given a copy of all the results of the measurements taken during the examination and they were advised that they would receive a copy of their blood test results by mail within a week and that their results should be discussed with their general practitioner. They were informed that, should their FBG level be 5.6–6.9 mmol/L, this was considered to be a borderline result and so an OGTT would be offered at a later date.

### Blood tests

The assessments of FBG levels and lipid profiles were performed at the Biochemistry Laboratory of the Mater Dei Hospital. Automated and regularly quality controlled COBAS INTEGRA® Analysers machines were used to carry out the tests.

The fasting blood samples to be used to measure FBG levels were collected in fluoride-containing tubes in order to minimize the levels of glycolysis. The FBG levels were measured using hexokinase and glucose oxidase enzyme reactions.

A serum clot activator tube was used to collect blood samples for the lipid profile assessment. This involved assessing each participant's total serum cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides. These assessments were carried out in light of the link between lipid parameters and diabetes, including the association between the diagnostic criteria for metabolic syndrome (which involves multiple lipid parameters) and the development of T2DM [26]. At the time of formulating this protocol, there was no clear consensus in the literature as to the appropriate fasting period prior to lipid profile testing [27, 28]. Therefore, a 9-h fasting period was chosen in line with the preliminary recommendations in the literature [29].

Using aliquots from each serum sample, the lipid variables were measured as follows: HDL was measured using the clearance method, triglycerides were measured using an enzymatic colorimetric analysis with glycerol phosphate oxidase and 4-aminophenazone total cholesterol was measured using a cholesterol oxidase enzymatic reaction and LDL was measured using the Friedewald formula.

A third venous blood sample was taken in an EDTA tube and stored at −20 °C for later use in a genotyping analysis. This would help us identify any links between different genetic mutations and diabetes mellitus on a population level.

Those participants who were invited to undergo the OGTT were advised to follow their regular diet before the test but to fast the night and the morning before the


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test. An initial FBG sample was taken from each participant in a fluoride-containing tube. The participants were then given 75 g of glucose and additional blood glucose samples were collected after 1 and 2 h.

### Data input and analysis

A single fieldworker inputted the data in order to avoid bias. The data inputting was performed using a secure online software program that matched the format of the survey questionnaire and also allowed each participant's measurements to be added. The software was programmed to carry out data validation (which included setting upper and lower limits for the various variables such as age) to ensure the quality of the data. The data were then transferred to a spreadsheet program for analysis. The analysis was carried out using IBM SPSS version 21 for Mac.

The participants' questionnaires were cross-referenced with the online versions to check the accuracy of the online data. This process involved cross-referencing several randomly selected questionnaires with the online version at the end of each month.

## Results

A number of lessons were learnt while conducting the survey, as shown in Table 2. A pilot test was conducted to identify each fieldworker's strengths and ensure that the fieldworkers focused on the tasks that they excelled in during the actual survey. Another lesson learnt was that the waist and hip circumferences should be measured after the height and weight measurements. This was because once the participants had removed all their excess clothes and other items, it was easier to continue with the circumference measurements and it helped to prevent inter-observer variability. Care was taken to take each participant's waist circumference measurement as the participant breathed out.

**Table 2.** *Summary of the lessons learnt from the survey*

| |
|---|
| Early appointments were appreciated by the participants aged 50 and over |
| Holding health examinations in each town centre led to good response rates |
| Flexible appointment dates and times led to good the response rates |
| Measuring the waist and hip circumference just after measuring weight and height was found most appropriate and acceptable by participants |
| Carrying out an interview, followed by health examination measurements and then the collection of blood was acceptable to the participants |
| Explanations of the whole process prior to and during the survey made the experience more enjoyable for the participants |
| Explanations of the participants' measurements at the end of their health examinations were appreciated by the participants |

A health examination hub (where the interviews, collection of blood samples and measurements took place) was set up every weekend at different local government health clinics situated throughout the Maltese Islands. Holding the health examinations in each town health clinic was an effective approach as the participants found it easier to attend when they did not need to travel large distances. The randomly selected participants from each town received a postal invitation letter 2 weeks prior to their appointment, together with an explanation of the aim of the study, the benefits of attending, and an appointment date and time. Participants were given the choice to change their appointment date and time to a more suitable alternative (including a weekday appointment) and to attend a clinic in a different town. This flexibility encouraged those who wanted to attend to participate. The early appointments (7.00 am–7.30 am) were found to be favoured by those over 50, while the later appointments (8.30 am–9.00 am) were favoured by the younger population. The last appointment was set at 9.30 am for two reasons: participants needed to be fasted so late appointments would not be well tolerated and the blood samples needed to be taken to the laboratory within 2 h after they were collected.

The interviews were challenging despite the fact that the interviewers were well trained because there was a risk of information bias as some of the answers depended on the participants' knowledge and memory skills.

At the end of the health examinations, participants often expressed their gratitude for the experience, for being kept informed of their measurement results throughout the processes (where possible) and for being given a copy of their measurement results.

## Discussion

Regular cross-sectional prevalence studies of non-communicable diseases can provide an evidence base to aid public health professionals (including policy makers) in developing strategies to prevent and manage the diseases. Prevalence studies can be technically challenging, laborious and expensive, so they are rarely conducted. Our protocol used a multidisciplinary approach and was based on up-to-date findings from the literature in order to collect cross-sectional epidemiological data at minimal costs and with a minimal number of fieldworkers. The evaluation of the study showed that the protocol set out an effective method for obtaining high-quality data. Although the protocol focused on how to conduct a T2DM prevalence study and to collect data on the determinants of this disease, the protocol can be modified to accommodate the collection of data on other non-communicable diseases.

### Study limitations

Although great care was taken to reduce bias, the study involved data collected from people who may have provided


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inaccurate data and may not have fasted as advised. Also, participants who had normal FBG levels but abnormal post-prandial levels (indicative of pre-diabetes or diabetes) could have been missed by the study.

## Conclusion

This protocol sets out the methods used in a prevalence study in Malta, which provided essential data that will be used to develop an up-to-date national policy on diabetes (which will include the development of prevention strategies). The study utilized validated measurement tools and was generally found to be acceptable by the participants. The study provided data on the national prevalence of diabetes, IFG, IGT, obesity, regular physical activity, alcohol consumption and tobacco consumption, together with information on the important associations between the potential risk factors and diabetes, and, for the first time, information on the genetics associated with the development of diabetes.

## Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/gheg.2016.18.

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## Declaration of Interest

None.

## Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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# Getting to grips with the obesity epidemic in Europe

**Sarah Cuschieri[1] and Julian Mamo[2]**

**Abstract**

Obesity is a global epidemic. It is responsible for increased patient morbidity and mortality. Significant related pathologies including diabetes mellitus compound the overall risks. Obesity is a significant financial burden. This includes direct and indirect medical costs, amounting to millions of euros each year. Multiple European studies have outlined a steady incline in obesity prevalence rates. Tackling obesity is no easy task. Policy makers aiming to reduce obesity rates should adopt an evidence-based approach. This entails adopting both micro- and macro-interventions tweaked to each country's individual requirements. The ideal way forward would be to tackle obesity from the individual, population-wide and food industry angles. The key towards a successful intervention is for each country to carry out well-planned health examination studies, in an attempt to pin point local risk factors. Having a correct individualized picture, each country can move forward and draw policies and interventional procedures. The aim should be to primarily improve the quality of life. Second, the country's capital expenditure is also reduced.

**Keywords**

Obesity, burden of illness, economic burden of disease, epidemics, disease management, policy

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## The Obesity situation

Non-communicable diseases (NCDs) have been on the rise for several decades and by 2010 had been found to account for 86% of deaths and 77% of the disease burden in Europe.[1] Obesity is a major cause for the development of NCDs. According to the World Health Organization (WHO), the obesity epidemic has more than doubled between 1980 and 2014.[2] In 2014, over 1.9 billion adults (18+ years) were overweight, with over 600 million being obese. Worryingly, a similar picture was unveiled in children. Around 41 million children under the age of 5 years were overweight or obese.[2] In Europe, obesity accounted for more than 1 million deaths and 12 million life-years of ill health in 2010.[3]

Some NCDs such as type 2 diabetes mellitus (T2DM) and cardiovascular disease have been strongly linked with obesity. In fact, the prevalence rates of diseases such as T2DM are on the increase throughout all age groups.[1] Obesity is also one of the key factors in the development of metabolic syndrome. This affects 20%–30% of the European population.[1]

Obesity alone has been labelled as the contributor of 80% of all T2DM, 35% of ischaemic heart disease and 55% of hypertension among European adults.[4] It has also been directly linked to the development of other pathologies. Among which, we find malignancies, gallbladder stones and impaired reproductive performance.[5,6] Higher health care expenditures are anticipated due to the increased rates of obesity and its association with other chronic diseases.[7]

Obesity is the result of multifactorial elements. Genetic, environmental and behavioural interactions each play a contributing aspect.[8] Before estimating the burden and the rate of increase, an accurate knowledge of prevalence (the amount of people affected at a specific point in time) and incidence (rate of new onset of obesity over time)

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is essential. Prevalence studies are way less expensive to conduct compared to incidence studies. This accounts for the wider availability of prevalence data.

The majority of European obesity prevalence studies utilize the body mass index (BMI) as their defining tool. Most are based on self-reported surveys.[7] The use of BMI for prevalence data can be misleading. This is the case in individuals with high muscle to body fat ratio and in certain ethnic groups.[9] Another possible fault is the self-reporting feature of these studies. This undermines the accuracy of the reported results. People tend to underreport their body measurements.[1] These can be up to 50% less than objective measurements.[6] Therefore, regular health examination surveys should be conducted by European countries for accurate overweight–obesity data.[7] For ease of comparison, homogeneous methods between different countries health examination surveys should be present. The ultimate accurate overweight–obesity data could be obtained by conducting updated pan-European studies comparing all European countries utilizing representative population samples and homogeneous methods.[10]

This study presents an overview of the overweight–obesity epidemic in Europe in order to provide professionals including policy makers, evidence-based literature on the epidemiological and economical burden of obesity in a single document.

### *Method*

A literature search using specialized libraries including PubMed and 'Google Scholar' was performed. The following keywords 'Obesity in Europe', 'Obesity Epidemic in Europe', 'Europe Obesity Cost', 'Prevention of obesity burden' and 'Physical activity to prevent obesity in Europe' were considered. These search criteria resulted in many 'hits'. Those articles with relevant titles to the research phrases and keywords published between 2000 and 2015 were considered. Each abstract falling within these categories was reviewed and relevant articles to the study's aim were obtained and fully analysed. Only studies that claimed to be representative of the population under study were considered. Small sample size studies with sample population less than 2000 individuals were excluded in view of low statistical power. Also any studies published outside the above year bracket were excluded.

Systematic reviews with data pertaining to European countries were given the highest consideration. Malta was the centre of focus, since it has the highest childhood (11–15 years) and adult male obesity rates in Europe and second highest in adult females.[11,12] Policies and strategies set up in Malta to combat this highly prevalent condition were considered in this study.

## How much of a burden?

Obesity is responsible for direct medical costs (e.g. physician's fees, clinical tests) and non-medical costs (e.g. transporting patients to treatment centres). Unsurprisingly, the direct per capita cost of a normal weight person is less than the overweight/obese counterpart. Costs for overweight and obese individuals were, respectively, 9.9% and 42.7% higher when compared to normal weight adults.[13] The whole of Europe spends between 1.9% and 4.7% of the total annual health care costs and 2.8% of the annual hospital costs in dealing with overweight or obese patients.[14]

In Malta, it was estimated that ill health due to obesity alone accounted for an annual cost of €20 million in 2009.[15] This accounted for around 5.7% of the total Maltese health expenditure.[15] Should the obesity rate remain stable (an underestimate), the annual health costs attributable to obesity in 2020 would amount to €27 million.[16]

Indirect costs attributed to the overweight/obesity epidemic relate to the person's absence from work or disease-related productivity loss. Intangible costs, such as the person's quality of life, including one's social life, are also present.[17,18]

A study carried out in Germany estimated the costs due to obesity and related comorbidities in 2002. The authors incorporated the costs of four different comorbidities arising due to obesity. These were T2DM, hypertension, stroke and myocardial infarction. The direct medical costs were €1343–2699 million. The costs incorporating the comorbidities amounted to €2701–5682 million.[19]

In 2012, it was estimated that the obesity cost (direct and indirect) in Europe was around €81 billion per year. This is in keeping with the WHO estimates on obesity expenditure of 2%–8% of the total national expenditure in the 53 European countries.[20]

With the increasing prevalence of obesity, the total health burden expenditure has been increasing proportionately and inevitably. There is no expectation that the obesity figures will plateau anytime in the near future. This warrants immediate and effective strategies to counteract the constantly increasing financial burden and decreasing quality of life.

## Action against obesity

Governments and international organizations over the years have developed policies and programmes aiming to deflate obesity rates. Unfortunately, such action has not been effective. The factors causing uncontrolled obesity rates remain unremedied.[21]

Evidence-based micro- and macro-interventions have been considered. Micro-interventions are aimed at individual, localized and community levels. They can rely on at least partial scientific evidence for their effectiveness. Macro-interventions are spread against larger entities including the food industry. The food industry influence on obesity needs to be targeted in an intelligent, cost-effective manner. Measures should include price policies, industry-based action as well as taxation and marketing regulatory mechanisms.

These are aimed at reducing the high fat, high sugar (and high salt) content of food.[6,22]

## Micro-interventions

Micro-interventions can be further subdivided into various approaches targeting the family, school, workplace and community levels.

The family approach is aimed at young children and their families. The aim is to encourage the maintenance of a desirable weight and prevent obesity from an early age. The effectiveness is inversely proportional to the age of the child. This approach is intensive and individually targeted, as well as expensive to maintain. It has, however, shown to be effective.[6,23] Weight control during pregnancy is essential. Women with a pre-pregnancy overweight/obese status are at risk to develop diabetes mellitus, hypertension and preeclampsia during pregnancy.[24,25] This also increases the chance of instrumental delivery and caesarean sections. The child is at an increased risk of developing various health issues. These range from congenital anomalies to hypoglycaemia.[26]

Targeting school-age children in an attempt to promote a healthier lifestyle is a difficult feat unless the whole school environment is incorporated in the strategy. This requires multiple, simultaneous interventions. Examples are as follows: promoting a healthy breakfast prior to lessons, installing a fruit-tuck shop, banning fatty and unhealthy foods within and near school premises and many more. In conjunction with physical activity and promotion activities, this was found effective only to a variable degree.[6]

An example of a successful multi-component school intervention was 'CATCH' in the United States. This consisted of an individual-level behavioural classroom curriculum and cafeteria environmental changes. These resulted in positive effects on the students' dietary intake.[27] In Malta, a campaign 'Lunchbox' was launched in April 2015. The aim was to encourage school children to a healthier lunchbox consisting of cereals, vegetables, fruits, low-fat milk products and plenty of water.[28]

A holistic approach to the problem incorporates the psychological aspect. Efforts to enhance self-esteem and avoid unhealthy weight loss measures are essential. These should enhance and complement obesity prevention measures in schools. Care should be exercised in order to prevent the development of stigma associated with eating disorders and obesity.[29,30]

A similar approach can be adopted in the workplace. Employers are encouraged to promote healthy eating habits and increase the physical activity at the workplace.[31] An example of a worksite intervention was the 'Working Well Trail'. This was the largest theory-based nutrition intervention performed in 108 worksites over a 2-year period. It included individual-level and environmental interventions in a randomized portion of the worksite workers. Overall, there was a positive outcome. There was a modest increase in fruit, vegetable and fibre intake among workers assigned to the interventional group.[32] Other programmes have been implemented such as the Five-A-Day research programme, which resulted in an increase in fruit and vegetable intake among the workers.[33]

Vending machines are a common encounter in both schools and workplaces. These are well-known sources of unhealthy food and beverages.[34] Interventions should target a shift towards healthier products and better display of nutritional information pre-purchase.[35]

## Macro-interventions

Macro-interventions are an umbrella term, incorporating all actions to tackle obesity through a population-based approach. These consist of policies, strategies and population-based obesity programmes aiming for a longer lasting change in the population. The main targets are twofold: first, to bring a change in the eating habits towards healthier food; second, to promote an increase in physical activity. Different strategies could be implemented such as the increase in the price of unhealthy food and/or decreasing the price of healthy food. These strategies worked out effectively in both China and the United States, respectively.[36,37]

In the US study 'Changing Individuals' Purchase of Snacks – CHIPS', the reduction in price of healthy food significantly affected the sales. The authors concluded that price is a major factor influencing the food choice. A small price reduction resulted in a major shift towards healthier food choices. A potential risk when drastically chopping food prices is a counter effect of increased food volume consumed. This results in an overall increased total energy intake, which is the exact opposite of the ideal scenario. A thorough financial evaluation is essential to address all the potential effects of a price reduction on the population.[37] Another option is to increase taxation on unhealthy food. This has been the theme behind a recent large debate between European countries. Among others, United Kingdom has already introduced taxation on sugar drinks.[38] These are long-term plans which take time to be adopted by the population. The effects are beneficial in the immediate and future time frames.[6]

A national strategy 'A Healthy Weight for Life 2012–2020' was set up in Malta, with the aim to assess obesity and its determinants locally and implementing action plans. Various interventions were proposed. One is to analyse the social impact of subsidies on healthy and targeted taxation on specific unhealthy foods and drinks. Another possibility is to increase the availability of healthy food outlets such as smoothie bars and at the same time restrict outlets selling fast foods. A further plan is to set up a 'Healthy Food Scheme' where food is colour coded according to the nutritional status of the item. There are plans to tackle the workplace as well. A proposed 'Healthy Workplace Scheme' was conceptualized to help and incentivize employers to promote healthy eating and support weight management programmes.[16]

Over the years, other European countries have also set up different initiatives to try to halt obesity and promote healthy eating and drinking. A Danish programme was set up to try to promote fruit and vegetables in the workplace, schools and restaurants.[39] While in the United Kingdom, a national campaign was set up by the Education and Resources for Improving Childhood continence (ERIC) to improve the access of fresh drinking water in all primary and secondary schools.[40]

## The food industry and obesity

The obesity epidemic requires an interdisciplinary approach involving not only the establishment of individualized and population-targeted strategies but also the involvement and the cooperation of other stakeholders including the food industry.[41] A critical factor in the prevention of obesity is to target the food and beverage industry.[42]

On average, consumers perform more than 200 food-related decisions per day but only recall less than 10% of these.[43] The most important motivators, determining which food is consumed, depend on the food's taste, quality, convenience and price.[41] It is therefore essential that the food industry applies these motivators to healthier food choices. According to the WHO, a healthy diet is one that is low in fats, sugar and salt. The total energy intake is balanced against the energy expenditure.[44] The role of the food industry is to favour the production of low energy dense foods as well as better nutritional quality foods. The readily available products should be evaluated and their energy content be reduced with responsible marketing and labelling of the nutrients.[42] Daily-recommended nutritional values and meal portions should be established as part of a normal daily food routine.[41] The challenge is to produce foods with lower energy contents while retaining the essential nutrients.[42]

It is imperative that governments work hand in hand with the food industry and science-based communities for the development of healthy food.[41] Food retails and caterers also have a role in obesity prevention.[42] It is of utmost importance that all the different food industry players work in collaboration for a healthy food delivery with the aim to halt the burden of obesity.

Social media is a major influence to the obesity epidemic. There should be better control and guidelines as to the manner in which unhealthy food products are presented to the general public. This might mean applying restrictions to TV adverts during peak children hours. Government policies should aim to promote healthy food advertisements in an effort to reduce fast foods and sugar-rich items.[45,46]

Children are more sedentary nowadays during their 'free time' with the introduction of advanced and interactive technology. The obvious consequence is a higher obesity risk. Strategies targeting physical activity at schools should be implemented. School attendance is compulsory up to teenage years. Enrolling physical activity in the mainstream curriculum safeguards a minimum level of exercise for all children in this age group.[47]

The Global Action Plan for Prevention and Control of Non-communicable diseases 2013–2020 has been set up. The aim is to increase the surveillance and monitoring of the NCDs that are most pressing including obesity. They plan to offer help and support to governments in developing preventative policies.[1] Also, in 2013, the WHO established a global monitoring framework to follow the preventative actions against the major NCDs.[1]

Obesity remains a major health concern for countries across Europe. Despite efforts through multiple initiatives and actions, the epidemic remains on the rise. Country-based strategies offer a valid framework but early tangible action in individual communities is generally lacking. The ideal situation would see initiatives based on and targeted towards each country's risk profile. The relationships between obesity and closely related pathologies such as diabetes and cardiovascular disease should be part of the planned strategies.

## Study strengths and limitations

The study targeted the obesity epidemic from different aspects of public health. It provided insight of how this epidemic could be prevented with the aid of evidence-based policies and strategies. Only papers and policies that were found using a set of keywords were considered. Any other literature falling outside the research criteria was not considered.

## Conclusion

The first step in the path against obesity is to obtain accurate baseline prevalence figures for all countries across Europe. These will portrait a better picture of the local risks and determinants across the different age groups. All this is only possible through well-planned health examination studies. These will help each individual country develop national guidelines based on their particular requirements. Once these are completed, a multi-level approach to promote prevention and strategies to reduce obesity will be the way forward. The establishment of clear primary and secondary care obesity preventative programmes would help reduce the large economic expenditure and improve the quality of life of obese people.

### Contribution

Both authors gave equal contribution to formulation of this article including conception and design of article as well as drafting and revising of the article. All authors approve the final article that is being submitted.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

## Ethical approval

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## New contribution

This article brings together the current obesity situation and preventive recommendations in one place, making it feasible for policy officials to understand the epidemiology and the health burden including medical costs attributed to obesity. As well as be equipped with evidence-based recommendations to halt the obesity epidemic from an individualized to a national perspective.

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Original Article

# Malta: Mediterranean Diabetes hub – a journey through the years

Sarah Cuschieri, Julian Mamo

**Abstract**

Introduction: Diabetes mellitus in Malta has been an established major health problem for years. It has been linked with cultural, geographical, historical, genetic as well and a change from a Mediterranean diet to a Westernized diet. This diabetes burden has lead to establishment of diabetes clinics both in the central general hospital, as well as in the community. Over the past 50 years, there have been two major epidemiological diabetes studies conducted to evaluate diabetes in the Maltese population.

Diabetes in Malta: To date, there is no established national diabetes plan or diabetes register in Malta, although there has been the formation of a governmental diabetes focus group. The time is right for an updated prevalence study to look at the current Maltese generation and their changing determinants including diabetes risk factors and genetics, followed by the development of preventative strategies and policies.

Conclusion: Over the years, diabetes burden has increased and become a public health and national financial concern. It is of utmost importance to address this national disease. An updated prevalence study would provide the evidence-based backbone for the development of diabetes preventive strategies and policies. The combination of which will enable the Maltese health services to be improved and better equipped to come to grips with this epidemic.

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Diabetes Mellitus; Policy; Malta; Diet, Mediterranean

## Introduction

Diabetes mellitus type 2 (T2DM) is a growing epidemic globally. By 2013 it was affecting 382 million people worldwide with an estimated 135 million currently unaware of their disease status.[1] Those suffering from T2DM are known to have a significant reduction in life quality as well as life expectancy, directly caused by this disease.[2] Moreover, in 2013 diabetes was estimated to be responsible for 5.1 million deaths worldwide.[1]

As in other European countries, diabetes mellitus is a major health problem in the Mediterranean island of Malta. T2DM is not a recent occurrence in Malta and it is known that by the eighteenth century, diabetes was already documented in medical literature. [3] Diabetes has been documented to have a negative impact on the lives of many Maltese since 1886, where diabetes was noted to be responsible for 2.1 per 10,000 deaths of the population. Diabetes related mortality rate continued to increase to 4.5 per 10,000 of population in 1900 and 8.7 per 10,000 in 1942. By 1955, Malta had the leading recorded diabetes mortality rate in the world with 26.1 deaths per 100,000 population followed by Belgium with 23.9, the USA with 15.5 and Italy with 11.1. [4]

The scope of the present paper is to review diabetes mellitus type 2 in Malta over the years along with the various local factors contributing to the high prevalence of diabetes in Malta and the current diabetic epidemiological situation.

## Predisposing factors for Diabetes Mellitus in Malta

Malta is a small island in the center of the Mediterranean Sea, positioned at the crossroads between Europe and North Africa. Over the years, Malta sustained different cultural changes as one dominating empire took over from another, leaving an ethnic mixture, substantial socio-diversity and varied genetic imprints on the Maltese population. [5] In 2008, as part of his PhD studies Al-Ashtar A. reports that both the Maltese and the Libyan populations had similar genetic

diabetic and metabolic profiles. [6] In a recent PhD study, Pace N. found as many as ten candidate genes significantly associated with type 2 diabetes mellitus and the metabolic syndrome among adult Maltese newly diagnosed diabetic persons. [7] In Malta, a strong family history of diabetes among Diabetics was already established with a statistical significant relationship between Diabetes and both maternal and paternal diabetes history.[8-9]

Having a restricted irrigated agricultural land has meant that Malta had to import most of its food supplies from overseas. The dependence on imports and their inconsistent supply had led to a tendency for chronic food deprivation in years gone by, especially during the period of world war II (1937 – 1949) affecting all of the population including pregnant women and their unborn children. [10] This most probably led to the development of the *Thrifty Diet Phenotype*. This phenotype is a protective mechanism developed to adapt for periods of starvation and food deprivation.[11]

It was suggested that a strong link between Malta and Diabetes contributed to a change from a Mediterranean diet to a more British type of diet in the late 19$^{th}$ to early 20$^{th}$ century, which may have led to this increase in T2DM prevalence. The theory suggests that an increased intake of fat and refined carbohydrates led to an overload of the *Thrifty Diet Physiology* which is in turn responsible for the increase in peripheral insulin resistance as well as to an increase in the prevalence of obesity.[3] This forms the *Baker's hypothesis* whereby those children originally adapted to surviving in a starved situation, now faced rich foods predisposing towards childhood and adulthood obesity and adult T2DM.[12] This link was found to be present in the Maltese population when the 2001-02 Health Behaviour in School children Study (HSBC) showed that 33.3% of the Maltese population was either overweight or obese.[13]

In more recent times, pregnant women in Malta have been found to be overfeeding their unborn child while in utero. This predisposes the child to foetal obesity or macrosomia. This is the basis of the *Pedersen's hypothesis*, which suggests that such a situation leads to the foetal pancreas and hypothalamus being adapted to this nutritional state with a predisposition to obesity and T2DM later on in their lives.[14]

**Past epidemiological studies in Malta**

A high diabetes occurrence in the Maltese population was first documented in 1927, and in his book, Debono JE. describes the prevalence estimated to be 4.5% of the population and the disease firmly linked with obesity.[15]

The increasing disease burden over the years led to the establishment of a special Diabetes Clinic in 1939 at the only general hospital in the island. By the 1950s, T2DM was considered to be of major public health concern, during which time there was an extension of diabetic clinics in the community.[3, 16] This heightened the keen interest of local academia and by 1964, the first epidemiological diabetes 'pilot study' was conducted by Prof. J. Zammit Maemple. Table 1. Summaries the study design, results and outcomes of this study.[17]

***Table 1**: Summaries the first epidemiological study (1964) design and outcome*

| | | **JV Zammit Maempel - 1964** |
|---|---|---|
| | | |
| Population Sample | | 5757 subjects |
| Population demographics | | All ages |
| Study Design | | All households in Urban area of Floriana & Rurar area of Gharghur, Madliena and Bahar ic-Caghaq |
| Screening methods | | |
| | Phase I | Urine dipstick for glucose, reducing substance, ketone bodies & albumin |
| | Phase II | Glycosurics (from phase I) undergone a 50g OGTT, questionnaire and physical examination, along with an equal number of age-matched non-glycosuria individuals |
| | Phase III | Statistical Study of the findings |
| | | |
| Results | Phase I | Glycosuria - 8.9% (9% males; 8.8% females); Albuminuria - 23% |
| | Phase II | Of the glycosuric - 70.1% had DM; 7.4% lag storage curve; 7.4% had renal glycosuria; remaining had normal OGTT. |
| | | Of the non-glycosurics - 15% had DM, 15.7% lag storage curve; 1.5% had renal glycosuria; 67.8% had normal OGTT |
| Risk factors | | Obesity (60 out of 100 diabetics) |
| Complications | | Peripheral Vascular disease, Coronary disease, Cerebrovascular disease, Hypertension |
| T2DM prevalence | | 19.9% |
| Newly Diagnosed T2DM | | 1 out of every 10 inhabitants |

*T2DM – Type 2 Diabetes Mellitus; DM – Diabetes Mellitus; OGTT – Oral Glucose Tolerance Test*

In 1981, the World Health Organization (WHO) performed the second prevalence study on Diabetes in Malta. During the same year, the Maltese Diabetes Association was set up and a year later (1982) became part of the International Diabetes Federation (IDF). Table 2. Summaries the study design, results and outcomes of this study.[18]

***Table 2**: Summaries the 1981 WHO epidemiological study design and outcomes*

| | | **World Health Organization Study - 1981** |
|---|---|---|
| | | |
| Population Sample | | 2945 subjects |
| Population demographics | | > 15 years |
| Study Design | | Randomized from electoral list, stratified according to age, gender, occupation & education |
| | | |
| Screening methods | | If not previously diagnosed with DM: |
| | Phase I | Fasting blood capillary sample from ear lobe, Fasting urine sample (glucose, proteins, blood & ketones), 75g OGTT, questionnaire, blood pressure, weight, height, and skinfold thickness. |
| | Phase II | Repeat of OGTT in those with abnormal or indeterminate result in phase I. Blood for Insulin, C-peptide, HLA-type, blood groups, Renal profile, Uric acid, lipid profile, |
| | Phase III | Clinical follow up of complications |
| | | |
| Risk factors | | Obesity linked with high calorie intake. Hypertension |
| Complications | | DM patients showed: Higher mortality rate; Blindness more common; Acute MI more common; Left Ventricular failure more common; Neuropathy in lower limbs more |
| T2DM prevalence | | 7.7% (5.9% previously know, 1.8% newly diagnosed) |
| Newly Diagnosed T2DM | | 1.8% |
| IGT | | 5.6% |

*T2DM – Type 2 Diabetes Mellitus; DM – Diabetes Mellitus; OGTT – Oral Glucose Tolerance Test; IGT- Impaired Glucose Tolerance; MI- Myocardial Infarction.*

The *pilot study* conducted by Prof. Zammit Maempel in 1964 could today be critically appraised for its design, whereby the sample population studied was non-randomized and not a representative sample of the Maltese population. There was no stratification for the different social factors and age, making it more difficult to differentiate the different types of diabetes. One can appreciate however, that as a first attempt to establish diabetes prevalence, it gave a clear idea of the relatively high diabetes burden among the Maltese population and of the poor control of the disease among sufferers at the time as well as of the strong local link of the disease with obesity.

The measurement tools used comprising seeking out glycosuria and subsequently confirming with an oral glucose tolerance test (OGTT) was also quite unique.

When considering the study conducted by WHO in 1981, one appreciates the improved epidemiological approach and the use of a randomized stratified sample of the population selected from the electoral list.

The screening method used by the WHO was the 75g OGTT - the gold standard screening tool, unlike the 50g OGTT used in the Zammit Maempel study earlier. The WHO study gave a more reliable and comparable diabetes profile, with appropriate distribution by age and gender.

No further population based studies have since elaborated on the changing Diabetes picture and burden in Malta until 2010, when the local centre of the European Health Examination Survey pilot study examined 400 randomized adult participants (18+ years) and, on the basis this time of a fasting glucose level, obtained a diabetes prevalence of 9.8% for this population. Females (10.7%) had a higher blood glucose average as compared to males (9%). This study, in common with the previous 2 studies, reported that those already diagnosed with diabetes had a generally poor diabetic management; with 38.5% of the known diabetics having an elevated blood glucose level.[19] This study utilised a relatively small population sample size and consequently, results exhibited wide confidence intervals. The results must therefore be considered with caution.

During the same year, the Department of Health Promotion and Disease Prevention issued "A Strategy for the Prevention and Control of Non-communicable Disease in Malta", which recommended local diabetes targets for 2020. [20]

**Diabetes burden in Malta - Nowadays**

To date, there is no national diabetes plan or diabetes register in Malta. Similarly, there is a lack of established preventative or screening protocols for diabetes. The lack of any updated diabetes prevalence data for the Maltese population is a clear hindrance to the formulation of any such plans and protocols.

A recent survey conducted on the attitudes and habits of Malta's general practitioners (GPs) reported that there is a lack of consistency in their diabetic preventative and management practices. The screening methods used by GPs studied were varied, with a large percentage using capillary blood glucose as the screening test for diabetes. A correlation was found between the different generations of GPs and the screening tests used. Thus, older GPs (21years+ since graduation) tended to use the HbA1C test more as a screening method when compared to younger GPs.[21]

**Diabetes action plan**

With the recent establishment of a governmental diabetes focus group and the first national diabetes plan being in the pipeline, the time is right to update the situation on the prevalence of diabetes type 2 and stop basing pharmacological and therapeutic plans on estimates of prevalence that are now no longer viable due to the time and changes in the population in terms of aging and changing risk factor profiles. [22]

A prevalence study at this point in time would be an opportunity to look at current generation of Maltese and their changing determinants and diabetes risk factors. Among these are the growing problems of obesity, the earlier onset of insulin resistance as seen globally.[2] It is also an opportunity to engage hitherto unavailable technology to study the underlying genetic predisposition among the Maltese population.

It is also a critical point in which to study the Maltese pre-diabetic population by linking prevalence to predisposition and risk factors - which may ultimately lead to eventual type 2 diabetes. Acquiring knowledge on the local precursor situation of diabetes (pre-diabetes) among a representative sample of adult Maltese today would be of great public health importance. Such a cross-sectional prevalence study has been proposed and is set to start at the end of 2014.[23] This epidemiological study aims to study a representative 1% of the adult Maltese population. The aim is to come up with valid and reliable updated diabetes type 2 prevalence figures as well as to have the first obesity, hypertension, smoking and alcohol consumption prevalence study with the power to give reliable figures. It is also set to understand the current Maltese dietary lifestyle and identify the risk factors predisposing the Maltese population to pre-diabetes and diabetes. This data can in turn be used to establish a diabetes risk score by age, gender and other factors for persons living in Malta.

With reliable updated diabetes prevalence figures as well as the establishment of the frequency of Diabetes risk factors in the Maltese Islands, a prevention strategy, a Diabetes policy and achievable population targets can then be accurately established to enable an evidence basis for Diabetes control plans.

Of the two broad strategies for the prevention of any disease –high risk and population strategies, none can yet be said to be underway in the Maltese Islands, historical hub of Diabetes in the Mediterranean. Both can, however, be employed side by side.

Employing the high-risk strategy for which screening is essential would enable the identification of high-risk individuals so that early action can be taken for them and ensure effective therapy. On the positive side, this tends to be an acceptable way forward for patients and health professionals while efficiently reducing disease and risk. The negative side is that this is not really a radically effective prevention strategy and is more geared at secondary prevention. The 1968 Wilson and Jungner criteria, universally accepted for any screening programme, are all satisfied in the case of Diabetes and its impact on the Maltese population: Diabetes is an important disease; there is an acceptable and an effective screening tool which can be performed regularly - one which is acceptable to patients and health professionals alike; there is a recognizable early detectable stage and the natural history of the disease is well known; there exist facilities to bring about effective investigation and control of the disease and the costs should be balanced relative to other health costs.[25]

The other option - the population strategy, involves the broad action needed to prevent the primary causes of the disease (lack of exercise, poor diet, obesity) for all individuals in the Maltese population, irrespective of their current risk status. This would involve a multi-sectoral approach including targeted taxation, aimed this time at high fat, high calorie foods and coupled with initiatives in the environment. It would also involve influencing the access to a healthier lifestyle - restrictions on importation and on the food manufacturing industry, an educational drive and action in other related areas for a concerted action. [24]

Given the sustained negative impact of Diabetes on health and the prospect of an ever greater impact of the disease on the lives of adults in Malta with each passing year, there is no reason why both strategies should not be initiated and combined for the prevention and control of Diabetes in the coming years

**Conclusion**

Diabetes Mellitus type 2 has been a sustained health burden among the Maltese population probably for a long time, but certainly, over the past century with more accurate measures gauging the problem in the 1960s and 1980s. Over the last 33 years estimates were used to gauge the diabetes burden in Malta. Today, we do not really know how many diabetics and pre-diabetics reside in the Maltese islands and whether their needs are being largely met. A national plan and an accurate age-gender disease profile are essential for the planning of control measures for Diabetes among adults in Malta, especially given the high impact of this disease locally.

A proposed diabetes prevalence study now getting underway aims to provide the evidence basis for updated health policies and to furnish health care workers with validated information about this disease.

Establishing profiles of the different risk groups (for pre-diabetes and diabetes) and their associated anthropometric, biochemical markers and genetic factors is another achievable key goal.

Preventive strategies can combine the benefits of screening high-risk controls with pan-population initiatives; bring healthy food and regular exercise within easier access of every Maltese.[26]

The development of a predictive tool such as an "app" for mobile phones and computers could help individuals measure their risk of developing or having a disease such as diabetes. This could be incorporated into primary care practice by patients, health insurers and health professionals. Identifying those in the population with highest predisposing pre-diabetes risk factors and formulating a pre-diabetic risk score would help pick up susceptible subjects at an early stage. Such risk scores reduce the cost and inconvenience of unnecessary screening.[27]

This information, placed within a national diabetes plan, would enable the Maltese health services to be better equipped to come to grips with this ever growing epidemic while aiding to improve the quality of life of those affected by the disease.

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REVIEW

# Prevalence of obesity in Malta

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## Summary

### *Background*

Obesity is a global epidemic with the Mediterranean island of Malta being no exception. The World Health Organization (WHO) has identified Malta as one of the European countries with the highest obesity prevalence.

### *Method*

A cross-sectional study was conducted (2014–2016) under the auspices of the University of Malta. The prevalence of overweight-obesity in Malta was calculated and then age stratified for comparisons with previous studies.

### *Results*

The study identified 69.75% (95% CI: 68.32–71.18) of the Maltese population to be either overweight or obese. The men overweight/obese prevalence (76.28% 95% CI: 74.41–78.14) was statistically higher than that for women (63.06% 95% CI: 60.92–65.20) ($p=0.0001$). Age stratification revealed that both genders had the highest overweight prevalence rates between 55 and 64 years (Men=23.25% 95% CI: 20.43–26.33; Women=24.68% 95% CI: 21.44–28.22). Men obesity prevalence rates were highest in the 35 to 44 years group (22.52% 95% CI: 19.65–25.68) while for women it was highest in the 55 to 64 years group (28.90%, 95% CI: 25.44–30.63).

### *Conclusion*

Over a 35-year period, an overall decrease in the normal and overweight BMI categories occurred with an increase in the prevalence of obesity. An exception was observed in the women, where the prevalence of normal BMI increased over this time period. Also, it appears that while the total population obesity prevalence increased (for 2016), a percentage of the women have shifted from an obese to an overweight status.

**Keywords:** Epidemics, Malta, obesity, overweight.

## Introduction

Obesity is a well-established global epidemic with an estimated 50% of the European population being overweight (1). Multifactorial elements result in this epidemic with the environmental and behavioural interactions declared to contribute a major role in the development of obesity (2). The increase in obesity and overweight among adults is seen across most European Countries (1). Southern European countries tend to exhibit a higher overweight population than their northern counterparts (1). One such southern country is the island of Malta, located in the middle of the Mediterranean Sea. Malta has been declared to have one of the highest European obesity rates in Europe (3). Malta is an archipelago between Sicily and North Africa, with an area of 316 $km^2$ and a GDP per capita of 22,779.91 USD (4). The Maltese Islands have a total population of 425,384 (median age 40.9 years), out of which 212,424 are men (median age 39.7 years) and 212,960 are women (median age 42.1 years). In fact, Malta is one of the highest densely populated countries in the world with about 1,265 inhabitants per square kilometre (5).

Over the years, Malta has experienced a change in culture, behavioural attitudes and lifestyle. In the 19th century, Malta was concurred by the British Empire

resulting in the introduction of a Westernized diet. Over the years, a shift from a Mediterranean to a Westernized diet was evident.

Technology advances lead to a change in lifestyles, with the population becoming more sedentary. A cultural and social change gradually occurred because of a migration shift. Malta nowadays hosts a number of different sub populations as residents. These include European, African and Asian natives. All of these social, cultural and behavioural changes could have had a determinant impact on the obesity epidemic within the Maltese Islands (6).

Data for these obesity observations stem from population surveys. The last population representative surveys undertaken were in 1981 conducted by the World Health Organization (WHO) and in 1984 as part of the MONICA project, both of which used a measured height and weight to calculate the body mass index (BMI) (7,8). Apart from a EU pilot Health Examination study conducted by the Department of Health Research and Information in 2010 ($n=200$), there have not been any other recent national surveys (9). Representative population monitoring surveys should be conducted on regular basis to assess the weight gain epidemic (10). Conducting such surveys measures the effectiveness of health promotion policies as well as identifies and targets high-risk population groups that would benefit from prevention strategies (10). In order to update the Maltese picture, a study entitled 'SAHHTEK' set up by the University of Malta undertook a nation-wide cross-sectional health examination survey over the past 2 years (2014–2016).

## SAHHTEK—the Malta Health and Wellbeing survey

SAHHTEK was a cross-sectional survey utilizing a randomized age (18–70 years) and gender representative data that was obtained from the national registry. The data was further stratified to represent an approximate 1% of the population from each Maltese town. The randomized population ($n=4,000$) was invited to participate in a free health check-up. A letter of invitation along with an explanatory pamphlet was sent via post. The check-ups were held in each Maltese town health clinic. Among the different measurements taken during the survey, trained personnel measured height and weight using validated machines. These measurements were used to calculate the body mass index (BMI) by dividing the weight (in kilograms—kg) over the height squared (in metres—$m^2$). The Research Ethics Committee of the Faculty of Medicine and Surgery at the University of Malta together with the Information and Data protection commissioner gave their permission for this study.

The SAHHTEK population was divided into three categories according to the established BMI, where $<24.99$ kg/$m^2$ was labelled as normal BMI, 25–29.99 kg/$m^2$ as overweight and $>30$ kg/$m^2$ as obese (11). The BMI prevalence rate was calculated for each weight category, age and gender category (Table 1). Statistical analysis was conducted using IBM SPSS vs. 21 for Mac software. Chi-square statistical test was used to compare the men and women subgroups by age and BMI status. Statistical significance was considered as $p$-value $<0.05$. The sample population was weighted according to gender, age

**Table 1** Age stratification of the adult population according to gender and BMI groups and showing the total prevalence and gender prevalence according the different BMI groups

| Age | Gender | Normal | Overweight | Obese | Total |
|---|---|---|---|---|---|
| | | $<25$ kg/$m^2$ | 25–29.99 kg/$m^2$ | $\geq30$ kg/$m^2$ | |
| 18–24 | Men | 147 | 34 | 38 | 219 |
| | Women | 148 | 41 | 38 | 227 |
| 25–34 | Men | 122 | 159 | 118 | 399 |
| | Women | 189 | 106 | 81 | 376 |
| 35–44 | Men | 59 | 168 | 166 | 393 |
| | Women | 164 | 98 | 72 | 334 |
| 45–54 | Men | 54 | 133 | 156 | 343 |
| | Women | 111 | 137 | 140 | 388 |
| 55–64 | Men | 59 | 183 | 159 | 401 |
| | Women | 56 | 153 | 176 | 385 |
| 65–70 | Men | 33 | 110 | 100 | 243 |
| | Women | 52 | 85 | 102 | 239 |
| Total | | 1194 | 1407 | 1346 | 1194 |
| Prevalence (%) | | 30.25 | 35.65 | 34.10 | |
| Prevalence | Men (%) | 23.72 | 39.39 | 36.89 | |
| | Women (%) | 36.94 | 31.81 | 31.25 | |

and locality in order for the data to be statistically representative of the whole Maltese population and to take into consideration the non-respondents.

## Results

Out of the total number of people invited ($n$=4,000), 49% participated in this study. This positive population response rate to participate was considered adequate and valid ($<p$=0.05). The majority of the population was found to be either overweight (35.65% 95% CI: 34.27–37.15) or obese (34.10% 95% CI: 32.64–35.60), with only 30.25% (95% CI: 28.84–31.70) having a normal body weight. Thus, Malta has 69.75% (95% CI: 68.32–71.18) of the total adult population (18–70 years old) suffering from an abnormally high body weight.

The men had higher overweight (39.39% 95% CI: 37.27–41.55) and obese (36.89% 95% CI: 34.80–39.03) prevalence rates when compared to women. These in turn had an overweight rate of 31.81% (95% CI: 29.78–33.91) and obese prevalence of 31.25% (95% CI: 29.23–33.34) ($p$=0.0001). This gender difference is in keeping with a recent study marking southern European countries as having high overweight/obese rates among men (1).

Men with *normal* body weight (BMI) were within the 18–24 age group (67.12% 95% CI: 60.65–73.01). For the women, this was between the 25 and 34 age group (50.27% 95% CI: 45.24–55.29) ($p$=0.0001). The highest age group exhibiting overweight rates was within the 55–64 age group for both genders (Men 45.64% 95% CI: 40.83–50.53; Women 39.74% 95% CI: 34.98–44.71) ($p$=0.006).

Regarding obesity, the highest prevalence rate for men was in the 35–44 age group (42.24% 95% CI: 37.45–47.18), whereas for women it was in the 55–64 age group (45.71% 95% CI: 40.80–50.71) ($p$=0.0001).

### Time trends of overweight and obesity prevalence in Malta

With the passage of time, three epidemiological studies have been performed (WHO 1981, MONICA 1984, EHES 2010), all of which measured BMI by means of height and weight examinations. These studies along with SAHHTEK study followed the same BMI definition and were age stratified between 25 and 64 years (but were not age standardized) for ease of comparison. Figure 1 shows the BMI distribution over time for the total population in each study.

On direct age standardization using the 1981 study rates and comparing with the current study (Table 2), there was an increase in the *obese* population (1.21). On the other hand, *overweight* and *normal* weight categories decreased (0.96; 0.9, respectively). On gender stratification, the men with obesity ratio showed an increase (1.88) while the women with obesity ratio declined (0.88) over 35 years. The overweight men and women exhibited a decline in expected rates (0.91, 0.97, respectively). The normal weight women showed an increase in expected rate (1.18), while the normal weight men showed a decrease in the expected rate (0.41).

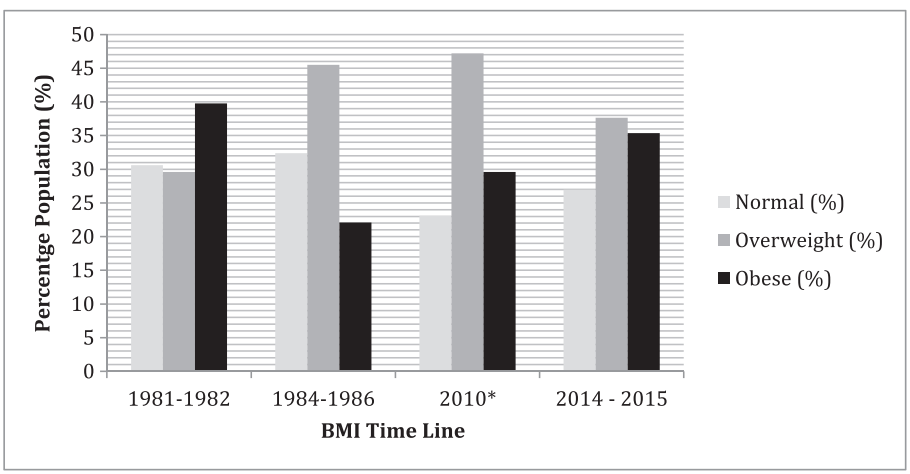


**Figure 1** BMI distribution for the Malta population aged 25–64 years 1981–2015. * Pilot study performed in 2010 ($n$=200).

**Table 2** Direct age standardization between the two national representative epidemiological studies by BMI

| | Total population—Normal BMI | | | Men—Normal BMI | | | Women—Normal BMI | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1981 | 2014–2016 | | 1981 | 2014–2016 | | 1981 | 2014–2016 | |
| | % | Expected | Actual | % | Expected | Actual | % | Expected | Actual |
| Sum total | 29.9 | 905 | 814 | 37.96 | 588 | 520 | 24.13 | 356 | 520 |
| | Total population—Overweight BMI | | | Men—Overweight BMI | | | Women—Overweight BMI | | |
| | 1981 | 2014–2016 | | 1981 | 2014–2016 | | 1981 | 2014–2016 | |
| | % | Expected | Actual | % | Expected | Actual | % | Expected | Actual |
| Sum total | 39.15 | 1181 | 1137 | 45.94 | 705 | 643 | 34.24 | 508 | 494 |
| | Total population—Obese BMI | | | Men—Obese BMI | | | Women—Obese BMI | | |
| | 1981 | 2014–2016 | | 1981 | 2014–2016 | | 1981 | 2014–2016 | |
| | % | Expected | Actual | % | Expected | Actual | % | Expected | Actual |
| Sum total | 29.23 | 882 | 1068 | 20.95 | 318 | 599 | 35.51 | 532 | 469 |

## Discussion

The Maltese population is predominately overweight or obese. Over 35 years, there has been a general increase in the obesity rate and a decline in the overweight and normal weight rates. This increase in body weight has been a gradual but progressive problem over the years across the world. It was observed in a recent U.S. study where the overall obesity rates increased from the 1980s to the present day (12). The same phenomenon is observed in Europe where more than half of the adult population (52%) within the European Union are either overweight or obese (13).

Interestingly, different countries exhibit divergent gender predominance. Between 2013 and 2014 the U.S. has had a significant linear increase in women's obesity rates as compared to men (12). This is in keeping with the European countries of Latvia, Turkey and Hungry (13). But the contrary has been found in Malta, where the men exhibited a higher obesity proportion than the women. The same trend was observed in the European countries of Iceland and Norway (13).

The overweight-obese epidemic is also present in children. In the most recent Health Behaviour in School-Aged Children (HBSC) survey (2013–2014), Malta ranked as the country with the highest prevalence of obese children aged between 11 and 15 (14).

Obesity can contribute to the development of other chronic diseases such as diabetes mellitus type 2 and cardiovascular disease (15). This leads to higher overall health care costs (13). Expenditures caused by obesity are the result of direct and indirect costs. This impacts both the individual person as well as the whole country. The WHO stated that the obesity expenditure contributed to 2–8% of the total national expenditure in the 53 European countries (16). In Malta, the estimated (underestimated) annual obesity health costs by 2020 amounted to €27 million (17).

Although public health policies and strategies for prevention and management of obesity are in place, still more work need to be done (1). Maltese diet relies to a big extent on imported foods. Therefore, taxation and importation regulations may need to be revised to meet the growing needs of Malta's population (1). An inter-sectoral approach to prevent the obesity epidemic needs to be undertaken. This includes the conduction of regular prevalence studies such as SAHHTEK. These enable the targeting of high-risk groups, while making it easier to address inequalities that warrant immediate action in any European country. This obesity epidemic is highlighted for priority action in the 2017 EU Presidency hosted by Malta (aimed to target childhood obesity) (18).

## Conflict of interests

None

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RESEARCH LETTER

# Relationship of past, present, and passive smoking with sociodemographic, anthropometric, biochemical, and dysglycemic profiles

Highlights
- Former smokers exhibited a significantly higher metabolic risk compared with subjects with other smoking habits.
- Current smokers with diabetes exhibited significantly higher fasting blood glucose and triglyceride levels.
- Only former smoking was independently associated with increased dysglycemic risk after adjustment for potential confounders

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**Keywords:** diabetes mellitus, Malta, prediabetes state, risk, smoking.

## To the editor

Cigarette smoking has been associated with the development of many chronic diseases, including type 2 diabetes mellitus (T2DM), hypertension, and stroke.[1,2] This has been attributed to tobacco-induced oxidants, inflammation, and insulin resistance.[2,3]

The Mediterranean island of Malta has an established high prevalence of dysglycemia.[4] Malta is a small island nation. This makes it feasible to construct and conduct a cross-sectional study to establish the smoking determinants of diabetes mellitus at a population level. The aim of this study was to establish the prevalence of current, former, and passive smoking and their relationships with anthropometric and biochemical variables within the general and the dysglycemic population of Malta. Study objectives included the establishment of any association between smoking habits and dysglycemic status, namely impaired fasting blood glucose (IFG) and T2DM.

## Methods

A national representative health examination survey was conducted among adults in Malta (2014–2016). Detailed methodology is provided elsewhere.[5] The different smoking habits (current, former, and passive smoking) were gathered through a validated questionnaire. Biochemical tests incorporating measurement of fasting blood glucose (FBG) and lipid profile were performed.

Participants with FBG between 5.6 and 6.9 mmol/L were classified as IFG, whereas those with FBG ≥7 mmol/L were categorized as newly diagnosed T2DM. Participants with a previous history of diabetes mellitus or on oral hypoglycemic agents, regardless of their plasma glucose concentrations, were classified as cases of known T2DM. The newly diagnosed T2DM and known T2DM populations were combined to represent the total T2DM population.

Participants reporting smoking more than 1 cigarette per day were considered current smokers. Individuals who had quit smoking for at least a year were considered former smokers. Smokers reporting to have stopped smoking for <1 year were still considered current smokers based on the World Health Organization

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report.[6] Individuals who denied active smoking but reported exposure to cigarette smoke regularly at work or at home for more than an hour each day were considered passive smokers. The remaining individuals were categorized as non-smokers.

Prevalence rates of each smoking subpopulation were calculated. The significance of differences between the different smoking subpopulations was evaluated using non-parametric tests. Generalized binary logistic regression models were performed to identify any associations between different smoking statuses (current, former, and passive) and the risk of developing T2DM and IFG. The non-smoker subgroup was used as the reference category for these models.

Ethics and data protection approvals were granted by the University of Malta Research Ethics Committee and the Information and Data Protection National Commissioner, respectively. Written informed consent was obtained from all participants.

## Results

In this study (n = 3947; 1998 males), the prevalence of current tobacco smoking was 24.30% (95% confidence interval [CI] 22.98-25.66), with a male preponderance (males 29.73% [95% CI 27.77-31.77]; females 18.73% [95% CI 17.06-20.52]). The prevalence of former smokers was 19% (95% CI 17.81-20.26), again with a male preponderance (males 24.92% [95% CI 23.08-26.87]; females 12.93% [95% CI 11.51-14.49]). The passive smokers accounted for 27.24% (95% CI 25.87-28.65) of the total population (males 56.65%; 95% CI 53.67-59.58).

The biochemical and anthropometric profile for each smoking subgroup is given in Table 1. Statistically significant differences were found between every biochemical and anthropometric parameter (except for total cholesterol) and the smoking subgroups.

Multivariate analysis revealed that only the former smokers exhibited an independent positive association (odds ratio [OR] 1.67; 95% CI 1.10-2.54; $P = 0.02$) of having dysglycemia (IFG and T2DM) compared with non-smokers and adjusting for confounding factors (ie, sex, education duration, employment status, alcohol habit, physical activity, obesity status, history of hypertension, history of dyslipidemia, and locality of residence).

## Comments

Less than one-quarter of Maltese adults reported to be current smokers in this study. This proved to be a

**Table 1** Pairwise comparisons of biochemical and anthropometric parameters among different smoking subgroups

| | Non-smoker (n = 1781) | Current smoker (n = 959) | Former smoker (n = 750) | Passive smoker (n = 457) | *P*-value (Kruskal-Wallis) | Pairwise analysis (Dunn's test) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ |
| FBG (mmol/L) | 5.35 (4.98-5.73) | 5.26 (4.88-5.75) | 5.56 (5.18-6.05) | 5.22 (0.71) | <0.01 | <0.01 | NS | NS | <0.01 | <0.01 | NS |
| LDL-C (mmol/L) | 3.05 (2.98-3.60) | 3.21 (2.44-3.83) | 3.14 (2.52-3.72) | 2.99 (1.07) | <0.01 | NS | 0.03 | <0.01 | NS | NS | NS |
| HDL-C (mmol/L) | 1.52 (1.27-1.84) | 1.34 (1.1-1.63) | 1.44 (1.23-1.79) | 1.51 (0.65) | <0.01 | <0.01 | <0.01 | <0.01 | NS | NS | NS |
| TG (mmol/L) | 0.87 (0.59-1.26) | 1.00 (0.70-1.48) | 1.02 (0.8-1.41) | 0.88 (0.63) | <0.01 | NS | 0.02 | <0.01 | 0.02 | <0.01 | NS |
| TC (mmol/L) | 5.05 (4.31-5.76) | 5.22 (4.4-5.83) | 5.14 (4.52-5.79) | 5.07 (1.21) | 0.14 | | | | | | |
| SBP (mm Hg) | 121.00 (110-130) | 120.00 (110-127) | 123.00 (112-132) | 120.00 (20) | <0.01 | <0.01 | NS | NS | 0.01 | NS | NS |
| DBP (mm hg) | 76.00 (69-80) | 75.00 (67-80) | 76.00 (69-82) | 74.00 (11.00) | 0.04 | NS | NS | NS | NS | NS | NS |
| BMI (kg/m$^2$) | 28.31 (25.30-31.20) | 27.82 (23.26-31.09) | 28.83 (25.2-32.56) | 28.03 (7.90) | <0.01 | <0.01 | NS | NS | NS | 0.04 | NS |
| WC (cm) | 91.00 (78-100) | 91.00 (78-101) | 95.00 (85-104) | 91.00 (22.80) | <0.01 | <0.01 | NS | NS | 0.01 | <0.01 | NS |
| WHR | 0.90 (0.84-0.96) | 0.92 (0.87-0.97) | 0.94 (0.0.88-0.98) | 0.91 (0.11) | <0.01 | NS | NS | <0.01 | 0.01 | <0.01 | NS |
| Age (y) | 50.00 (36-59) | 47.00 (35-54) | 55.00 (39-63) | 42.00 (24.00) | <0.01 | <0.01 | 0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

Abbreviations: $P_1$, current vs former smoker; $P_2$, current vs passive smoker; $P_3$, current vs non-smoker; $P_4$, former vs passive smoker; $P_5$, former vs non-smoker; $P_6$, passive vs non-smoker. BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, Systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist: hip ratio.
Unless indicated otherwise, data are given as the median (interquartile range).

higher prevalence than the neighboring country of Italy.[7] The former smoker population in this study exhibited increased body mass index, waist circumference, and waist: hip ratios compared with the other smoker subpopulations, which corresponds to the literature.[8] Furthermore, former smokers exhibited higher median FBG and triglyceride levels. In fact, former smoking was the only smoking status associated with concurrent dysglycemia. The causation for this relationship could not be established due to the nature of this study, but it appears to be independent of socioeconomic or lifestyle factors. On smoking cessation, individuals tend to consume high-sugar and fat-rich snacks to satisfy their cravings, which may be the case in this study. This will lead to an increase in adipose tissue (resulting in insulin resistance and eventual dysglycemia) and derangement of the lipid profile, including triglycerides. Other possible causes contributing to an increase in adipose tissue, leading to eventual dysglycemia, could be related to physiological changes, such as a reduction in metabolic rate following smoking cessation.

The benefits of quitting smoking by far outweigh the downside of the association with dysglycemia. Quitting is by no means an easy feat. Weight management programs, along with physiological support, should be incorporated into smoking cessation initiatives in order to maintain a successful smoke-free lifestyle.

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## Disclosure

The authors declare that they have no conflict of interest. The funding bodies had no influence on the survey protocol or data analysis.

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Archives of Public Health

RESEARCH Open Access

# The diabesity health economic crisis—the size of the crisis in a European island state following a cross-sectional study

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## Abstract

**Background:** Diabetes type 2 and obesity are well-established global epidemics and contributors to clinical, social and economic health burdens. The prevalence rates of these diseases are still on the rise among countries resulting in a corresponding public health burden. The Mediterranean island of Malta, known for it's high diabetes and obesity rates, provides a good fundamental basis to portray the economical health burden of these diseases.

**Method:** A recent randomised stratified representative cross-sectional survey conducted in Malta tackling diabetes, obesity and other determinants, was used to work out the population prevalence of these diseases. The cost burden of diabetes and obesity, based on published data, was incorporated to the established population prevalence rates, in order to estimate the Maltese economical burden. Projections to the year 2050 by a bottom-up prevalence based design were performed.

**Results:** One eight of the Maltese adults (25 to 64 years) suffered from diabetes out of which approximately 10,000 adults were unaware of the disease. Alarmingly, more than a third of the Maltese population suffer from obesity. The approximate health care costs (direct and indirect) for the diabetic adult population was of €29,159,217 (€21,994,676 - €38,919,121) annually, amounting to 3.64% (2.75–4.875%) of the total health expenditure in Malta. The obesity cost burden was of €23,732,781 (€21,514,972-€26,049,204) annually contributing for 2.97% (2.69–3.26%) of the total health expenditure. The projected prevalence and costs for 2050 exhibited an estimated cost burden increase of €33,751,487 (€25,458,606–€45,048,473) for the diabetes mellitus population and €46,532,294 (€42,183,889–€51,074,049) for the obese population. These projected cost burdens are expected to increase exponentially the total health care expenditure in Malta by 2050.

**Conclusion:** Having an understanding of the prevalence and the economic cost burden of diabetes and obesity within a country, along with projections of the expected burden will enable policy and public health officials to clearly visualize this growing problem. It also helps in establishing effective preventive strategies and screening programs targeting these epidemics.

**Keywords:** Economic burden of disease, Burden of illness, Diabetes mellitus, Obesity, Malta

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## Background

The global obesity and diabetes mellitus type 2 prevalence rates have increased exponentially over recent years [1, 2]. Globally an estimated 2 billion people are overweight, a third of which being obese [1]. The increasing prevalence of obesity is predicted to lead to other chronic related diseases [3]. The link between obesity and type 2 diabetes is well established through the development of obesity related insulin resistance. It follows that the prevalence of the two diseases is directly proportional [3]. However, only about 20–25% of the overweight and obese population is expected to develop diabetes [4]. Both diseases lead to heavy increases in clinical, social and economic health burdens as well as to increments in morbidity and mortality [5, 6]. They also impact on employment and productivity, both of which have significant direct impacts on a country's productivity and economical growth [7].

Malta is a European island nation in the middle of the Mediterranean Sea with a total population of 425,384 (median age 40.9 years). Its history is littered with diverse dominating powers and shifts in its culture throughout the centuries. Up to 150 years ago, the population followed a Mediterranean lifestyle and diet, although this has now shifted to a more Westernized lifestyle and diet [8]. The World Health Organization (WHO) conducted the last Maltese national prevalence study in Diabetes in 1981 [9]. Subsequently, a health examination pilot study was conducted on the Island in 2010. However, the latter study had a small sample size ($n = 221$) and offered only a glimpse of the actual diabetes and obesity burden [10]. This has led to Maltese policies and economic burden extrapolations being based on outdated sources, despite strong emigrational, cultural and lifestyle changes over recent decades [8]. Between 2014 and 2016, the University of Malta "SAHHTEK" (*Your Health*) cross-sectional study was conducted. This provided valid, updated prevalence rates, through which the diabetes mellitus type 2 and obesity health burden in Malta could be established. The aim of this study was to establish the current (for 2016) prevalence and economic burden of diabetes type 2 and obesity in Malta. The objectives were: 1) to establish the body mass distribution within the diabetic sub-population by age and gender; 2) to estimate the projected total population prevalence of type 2 diabetes and obesity in Malta for 2050; 3) estimate the economic burden of diabetes and obesity for the year 2050 and; 4) provide evidence based data for policy makers to target and implement prevention strategies concerning these non-communicable diseases. This study will serve as a fundamental basis for the burden estimation in neighbouring Mediterranean countries.

## Method

A cross-sectional health examination survey was conducted between November 2014 and January 2016. The sample population was obtained from the national registry after performing randomization and stratification of the data by age (18 to 70 years) and gender [11]. The data was further stratified according to residence locality, to represent an approximate 1% population from each Maltese town. The total randomized and stratified population ($n = 4{,}000$) was invited to participate in a health examination survey set up in every Maltese town through an invitation letter sent out via post. The health examination survey consisted of a validated questionnaire and health examination including weight and height as well as blood letting for fasting blood glucose and lipid profile.

The measured weight and height was used to calculate the body mass index (BMI) by dividing the weight (in kilograms - kg) over the height squared (meters squared - $m^2$). Those participants with a BMI <24.99Kg/$m^2$ were considered as normal BMI; 25–29.99Kg/$m^2$ as overweight and >30Kg/$m^2$ as obese [12]. Participants were considered as suffering from '*previously known diabetes mellitus*' if they gave a medical history of diabetes or were on oral hypoglycemic agents, irrelevant of the measured fasting blood glucose. Meanwhile, all those obtaining measured fasting blood glucose above 7 mmol/L were considered as newly diagnosed diabetics [13].

The diabetes population (total) was defined as the sum of the previously known diabetes mellitus participants to the newly diagnosed participants. Prevalence rates of diabetes (by gender and 10-year age groups) were calculated by dividing the total diabetes population by the total participants who attended the study. The prevalence rate was applied to the latest officially recorded total Maltese population demographic statistics to obtain the overall population with diabetes [12]. Similar calculations were used to estimate the prevalence rates of those with normal, overweight or obese status in the Maltese population [14, 15]. Sensitive analyses based on the confidence intervals of the prevalence rates were preformed. The study only considered type 2 diabetes mellitus individuals. The Research Ethics Committee of the Faculty of Medicine and Surgery at the University of Malta together with the Information and Data protection commissioner gave their consent for this study.

The estimated cost burden for diabetes in Malta was calculated by applying the established prevalence rate of *newly diagnosed diabetics* and that for the *previously known diabetics* (individually) to the cost burden found in the literature for each diabetes subgroup. The cost burden for the newly diagnosed diabetics population was based on estimates performed by Zhang Y et al. (2009)

in the USA after adjusting for the gross domestic poduct (GDP) per capita and by adjusting for deflation between the USA and Malta [16]. The cost burden for the *previously known diabetes population* was obtained from published International Diabetes Federation (IDF) data on Malta [17]. The cost attributed for the obese population was obtained from a study conducted in Malta by the Department of Health Information and Research in 2008, based on the 2008 European Health Information Survey, after 2% compound interest per annum was performed [18].

Projections to the year 2050 were performed by a bottom-up prevalence-based design for diabetes and for obese rates (1981 to 2014–2015 surveys). For purposes of comparative and statistical analysis, only the subgroup of adults between 25 and 64 years of age were utilized from SAHHTEK. This was required to keep a valid comparison with the 1981 study. By hypothesizing that no other factors distorted linear projections for both disease prevalence rates, an estimation of the affected population and their economic burden was established based on the EUROSTAT 2050 population projections for Malta [19]. Statistical analysis including chi squared was calculated by means of IBM SPSS version 21. Demographic and lifestyle data was gathered by means of a combination of validated tools used during the "SAHHTEK" survey [20–22].

## Results

The sample population that attended the study (response rate of 49% $p = <0.05$) was weighted according to age, gender and locality. This enabled the data to be statistically representative of the whole population of Malta as well as to take in consideration the non-responders. After statistically weighting the sample, the final population dataset was of 3,947 (1998 males, 1949 females). Out of which, 10.39% (95% CI: 9.47–11.38) suffered from diabetes, among which 6.31% (95% CI: 5.59–7.11) were previously known diabetics and 4.08% (CI: 3.50–4.74) were newly diagnosed. 69.77% (95% CI: 68.32–71.18) of the total population was found to be either overweight or obese (Table 1) with a heavy male predominance. The diabetic population was predominantly either overweight or obese (92.20% CI 95%: 89.16–94.45) as seen in Table 1.

It was noted that younger (<55 years) diabetics were predominately (54% CI 95%: 44.30–63.12%) new (unknown) diabetics, while for those aged 55 and over, only 34% (CI 95%: 29.21–39.80) were new diabetics with the vast majority (66% CI 95%: 60.20–70.79) being known diabetics. This finding is in keeping with published data evaluating the degree of diabetes awareness between the 1981 and 2010 examination surveys conducted in Malta [23].

Further subdivision of the diabetic population by age and body weight revealed younger diabetics (<55 years) to be predominately obese (50% CI 95%: 40.56–59.44) or overweight (46% CI 95%: 36.88–55.70), with only 4% (CI 95%: 2–8.9) having normal weight. Conversely, a higher proportion of older diabetics (≥55 years) were found to belong to the normal weight category (9% CI 95%: 6.37–12.50) with 57% (CI 95%: 51.50–61.61) of these found

**Table 1** BMI prevalence rates by population (diabetic and non-diabetic) population

| BMI category | | | |
|---|---|---|---|
| Total Population | Normal % (95% CI) | Overweight % (95% CI) | Obese % (95% CI) |
| Total ($n = 3947$) | 30.26% (28.84–31.70) | 35.66% (34.17–37.15) | 34.11% (32.64–35.60) |
| Male ($n = 1998$) | 23.72% (21.91–25.64) | 39.39% (37.27–41.55) | 36.89% (34.80–39.03) |
| Female ($n = 1949$) | 36.94% (34.83–39.11) | 31.81% (29.79–33.91) | 31.25% (29.23–33.34) |
| *p*-value* | 0.0001 | 0.0001 | 0.0001 |
| Diabetic Population | Normal % (95% CI) | Overweight % (95% CI) | Obese % (95% CI) |
| Total ($n = 410$) | 7.8% (5.55–10.84) | 36.83% (32.30–41.60) | 55.37% (50.53–60.11) |
| Male ($n = 274$) | 8.39% (5.61–12.33) | 39.78% (34.16–45.68) | 51.82% (45.92–57.57) |
| Female ($n = 136$) | 6.62% (3.36–12.26) | 30.88% (23.71–39.10) | 62.50% (54.12–70.19) |
| *p*-value* | 0.73 | 0.84 | 0.327 |
| Non-Diabetic Population | Normal % (95% CI) | Overweight % (95% CI) | Obese % (95% CI) |
| Total (3537) | 32.85% (31.31–34.42) | 35.51% (33.76–36.90) | 31.64% (30.12–33.19) |
| Male (1724) | 26.16% (24.14–28.29) | 39.33% (37.05–41.65) | 34.51% (32.31–36.79) |
| Female ($n = 1813$) | 39.22% (36.99–41.48) | 31.88% (29.78–34.06) | 28.9% (26.86–31.03) |
| *p*-value* | 0.0001 | 0.0001 | 0.0001 |

*Chi squared between each BMI category against gender

obese and 34% (CI 95%: 28.59–39.13) found to be overweight ($p = 0.001$).

On incorporating the established prevalence rates and their corresponding confidence intervals from this study to the Maltese population, an estimate of the present total population burden of diabetes mellitus and obesity by 10-year age groups and gender was established [14]. Table 2 shows the estimated total Maltese diabetes population by gender and age.

In the Maltese adult population between 25 and 64 years of age, approximately 20,000 adults suffer from diabetes mellitus type 2. Out of which, approximately 10,000 adults suffer from diabetes and are yet unaware of it, with a male predominance (approx. 6,000). On comparing the Maltese diabetes population to the IDF Atlas (7th Edition) European diabetes population, the Maltese diabetic population contributes to an approximate 0.03% of diabetes within Europe [2]. This prevalence percentage is an estimate comparison since the adult population age groups for the IDF (20 to 79 years) was different when compared to SAHHTEK study (25 to 64 years).

On the other hand, approximately 82,000 of the 233,136 adults (35% of the adult population) in this age category (25–64 years) in Malta are obese, with a persisting male predominance (approx. 46,000) (Table 3). This ranks the Maltese population as the most obese country in the Mediterranean (Italy – 10.5%; Cyprus – 13.9%; Greece – 16.9%) and in Europe [24].

Estimating the cost burden (both direct and indirect) of an undiagnosed diabetic to be approximately €1,052 per person per year (6.67% of the annual mean salary income per person in Malta), the annual burden for the entire population (25 to 64 years) was estimated at €9,755,196 (€7,065,232–€13,582,372), which contributes to 1.22% (0.88–1.70%) of the total health expenditure for Malta [16, 25]. The total health expenditure for Malta (for 2016) is approximately €800,000,000 [25]. The direct medical costs include hospital inpatient costs, physician care, emergency care, outpatients care and prescriptions, while the indirect costs included absence from work, reduced work performance and productivity [16]. Conversely, a known diabetic person's disease cost burden was approximately €1,887 per person annually (11.96% of the annual mean salary income per person in Malta), attributing to an annual health burden of €19,404,021 (€14,929,944–€25,336,749) for the entire population (25 to 64 years) [17]. This is consistent with 2.43% (1.87–

**Table 2** Estimation of the diabetes mellitus population burden by applying the prevalence rates to Maltese population data by age and gender

| Maltese Population Diabetes Prevalence | | | | |
|---|---|---|---|---|
| Age | Total Maltese Population by age[a] | Total (CI) | Previously Known (CI) | Unknown Diabetics (CI) |
| 25–34 | 62,180 | 404 (143–964) | | 404 (143–964) |
| 35–44 | 56,575 | 2,489 (1,765–3,491) | 933 (515–1,641) | 1,556 (996–2,399) |
| 45–54 | 55,113 | 5,054 (4,007–6,332) | 2,712 (1,962–3,726) | 2,337 (1,642–3,296) |
| 55–64 | 59,268 | 11,611 (10,052–13,347) | 6,638 (5,435–8,060) | 4,976 (3,935–6,253) |
| Total | 233,136 | 19,558 (15,967–24,134) | 10,283 (7,912–13,427) | 9,273 (6,716–12,911) |
| Age | Male Maltese Population by age[a] | Maltese Male Population Diabetes Prevalence | | |
| | | Total (CI) | Previously Known (CI) | Unknown Diabetics (CI) |
| 25–34 | 32,359 | | | |
| 35–44 | 29,143 | 1,781 (1,195–2,611) | 667 (332–1,271) | 1,113 (664–1,821) |
| 45–54 | 27,728 | 4,204 (3,252–5,368) | 2,426 (1,708–3,397) | 1,777 (1,173–2,651) |
| 55–64 | 29,585 | 6,935 (5,784–8,236) | 3,763 (2,896–4,840) | 3,172 (2,379–4,189) |
| Total | 118,815 | 12,920 (10,231–16,216) | 6,856 (4,937–9,507) | 6,062 (4,216–8,661) |
| Age | Female Maltese Population by age[a] | Maltese Female Population Diabetes Prevalence | | |
| | | Total (CI) | Previously Known (CI) | Unknown Diabetics (CI) |
| 25–34 | 29,821 | 397 (143–945) | | 397 (143–945) |
| 35–44 | 27,432 | 658 (313–1,300) | 247 (49–749) | 411 (148–977) |
| 45–54 | 27,385 | 1,060 (633–1,733) | 424 (173–934) | 630 (318–1,210) |
| 55–64 | 29,683 | 4,625 (3,648–5,806) | 2,853 (2087–3,856) | 1,772 (1,178–2,624) |
| Total | 114,321 | 6,740 (4,736–9,785) | 3,524 (2,309–5,539) | 3,210 (1,787–5,756) |

CI – 95% Confidence intervals
[a]2013 Malta Demographic Report

**Table 3** Estimation of the obese population burden by applying the prevalence rates to Maltese population data by age and gender

| Maltese Population | | |
|---|---|---|
| Age | Total Maltese Population by age[a] | Obese BMI (CI) |
| 25–34 | 62,180 | 15,968 (14,127–17,951) |
| 35–44 | 56,575 | 18,523 (16,644–20,497) |
| 45–54 | 55,113 | 22,315 (20,386–24,299) |
| 55–64 | 59,268 | 25,260 (23,239–27,328) |
| Total | 233,136 | 82,066 (74,397–90,076) |
| | | |
| Age | Male Maltese Population by age[a] | Obese BMI (CI) |
| 25–34 | 32,359 | 9,569 (8,187–11,076) |
| 35–44 | 29,143 | 12,310 (10,914–13,750) |
| 45–54 | 27,728 | 12,611 (11,172–14,078) |
| 55–64 | 29,585 | 11,730 (10,349–13,171) |
| Total | 155,996 | 46,220 (40,621–52,075) |
| | | |
| Age | Female Maltese Population by age[a] | Obese BMI (CI) |
| 25–34 | 29,821 | 6,423 (5,272–7,747) |
| 35–44 | 27,432 | 5,914 (4,792–7,212) |
| 45–54 | 27,385 | 9,881 (8,615–11,222) |
| 55–64 | 29,683 | 13,568 (12,111–15,052) |
| Total | 114,321 | 35,786 (30,791 - 41,234) |

CI - 95% Confidence intervals
[a]2013 Malta Demographic Report

3.17%) of the Maltese total health expenditure. Therefore the global cost burden of diabetes mellitus for the Maltese health system is approximate a total of €29,159,217 (€21,994,676–€38,919,121) annually. The diabetes cost burden contributes to 3.65% (2.79–4.87%) of the total health expenditure (state and private expenditure) for Malta [25]. On the other hand, the attributed cost burden (included inpatient stay, day patient stay, general practitioner and specialist consultations but not medication and surgical procedures) for obesity in Malta (2% compound interest per annum), was estimated to be €23,732,781 (€21,514,972–€26,049,204) for the year 2016 [18]. The obesity cost burden contributes to 2.97% (2.69–3.26%) of the total health expenditure for Malta [25]. Therefore the total 'diabesity' cost burden for Malta, in 2016, is an approximate total of €52,891,998 (€43,509,648–€64,968,325) which amounts to 6.61% (5.44–8.12%) of the Malta's total health expenditure.

The projected EUROSTAT 2050 Maltese population (25 to 64 years) was incorporated within the projected prevalence rates for diabetes and obesity rates as seen in Table 4. On incorporating these projected prevalence rates within the projected cost burden (2% compound interest per annum from the 2016 costs) for diabetes and obesity, we were able to estimate the likely economic burden caused by these diseases for 2050 (Table 5). The total adult population (25 to 64 years) projected for 2050 appears to decrease from the current 2016 adult population. Meanwhile, the diabetes and obese population will increase by approximately 28 and 15% respectively by 2050. The economic burden is expected to increase in association with this disease burden. An exponentiation of 1.2 in the diabetes cost burden and exponentiation of 2 in obese cost burden from the current (2016) is expected to occur by 2050. This contributes to an estimated cost burden of €33,751,487 (€25,458,606–€45,048,473) for diabetes mellitus and €46,532,294 (€42,183,889–€51,074,049) for the obese. Therefore the estimated total cost burden for 2050 for diabetes mellitus type 2 and obesity would amount to one-eight of the current (2016) total health expenditure (€800,000,000).

## Discussion

Diabetes mellitus type 2 and obesity prevalence rates are on the rise with the majority of diabetic patients being obese [26]. This is observed in the current study where the bulk of the Maltese population was either overweight or obese. Apart from this, a tenth of the total adult population (18 to 70 years) suffered from diabetes mellitus type 2. Another worrying feature is that the majority of the Maltese diabetic population was obese. The projected Maltese estimates for 2050, also exhibit an exponential increase in the obese population, along with a drastic increase in diabetes mellitus. Although diabetes might not develop in all those who are obese, the accumulation of adipose tissue initiates a cascade of

**Table 4** Projected prevalence rates of diabetes and obese population for 2050 in Malta

| Age | 2050 Projected Total Maltese Population by age[a] | Projected Diabetes & Obesity Prevalence for 2050 | |
|---|---|---|---|
| | | Total Diabetes (CI) | Obese (CI) |
| 25–34 | 56,709 | 601 (255–913) | 21,810 (19,791–23,829) |
| 35–44 | 52,965 | 3,813 (2,961–4,666) | 21,175 (19,650–22,685) |
| 45–54 | 53,819 | 5,457 (4,698–6,211) | 22,970 (21,361–24,579) |
| 55–64 | 62,342 | 15,199 (14,014–16,153) | 28,079 (26,882–29,295) |
| Total | 225,835 | 25,071 (21,929–27,943) | 94,034 (87,684–100,388) |

CI - 95% Confidence interval
[a]Demographic Data 2013

**Table 5** Comparison of the total cost burden for diabetes and obese population for 2016 and those projected for 2050

| Maltese Population (25 to 64 years) | | |
|---|---|---|
| | Total Diabetes | Obese |
| 2016 Costs | €29,159,217 | €23,732,781 |
| | (€21,994,676–€38,919,121) | (€21,514,972–€26,049,204) |
| 2050 Projected Costs with 2% Compound interest per annum | €33,751,487 | €46,532,294 |
| | (€25,458,606–€45,048,473) | (€42,183,889–€51,074,049) |

metabolic events. These in turn have the ability to initiate or exacerbate insulin resistance in those more predisposed. All this can subsequently lead to the development of various metabolic diseases including hypertension, dyslipideamia and cardiovascular disease [27].

Tackling the obesity epidemic in its own right is of utmost importance in order to prevent further obesity-related illnesses that are contributing not only to a decrease in the quality of life and mortality of many, but also attributing to an estimated 8 to 10% of the total health expenditure in many European countries [28]. In Malta, the cost burden attributed to overweight-obesity was estimated to be 5.7% of total health expenditure in 2008 [18]. Considering that only the obesity burden was evaluated in the current study, one cannot directly compare to the 2008 (Malta) health expenditure. However, no drastic exponential increase appears to have occurred. On comparing the current obesity impact on the total health expenditure to other European countries, Malta relates well to the obesity related expenditure reported [28].

The International Diabetes Federation estimated the 2009 cost burden for diabetes mellitus to contribute to 11% of the total health expenditure in Malta [29]. Our results showed that diabetes (direct and indirect) cost burden on the total health expenditure has decreased by approximately three-fold from the IDF estimate for Malta. According to the IDF, diabetes mellitus type 2 accounts for 5 to 20% of the majority of the countries' total health care expenditure, which is in keeping to the study's results [2]. In Malta, diabetes appears to have the same burden effect on the total health care expenditure as other countries.

The diabetes and obesity epidemics present as a great burden of care and an economic challenge to the Maltese population from the relatively young age of 35 years onwards [30]. In fact, extrapolations from the current (2016) obese and diabetes burden to 2050, exhibit a major health care concern for Malta, especially with the expected high diabetes prevalence rise and related economical costs. It is questionable whether the overall health budget will also swell proportionately, given the current economic situation. This is more so considering that by 2050, diabetes and obesity health burdens are expected to amount to one-eight of the current (2016) total health care expenditure. The planned Malta EU Presidency for 2017 (January to June) reflects the obesity burden and is planned to tackle Childhood obesity. Considering that a substantial proportion of the young population (<55 years) was found to have unknown diabetics and an increased body weight (obese > overweight), this brings forward the need for immediate action. The higher the obesity and diabetes prevalence rates, the higher the expected hospitalization rates and other related costs [5]. It is well established that the risk of co-morbidity increases with the increase in body mass index (BMI) [31]. Per capita costs reach their maximum among the obese population [32]. This phenomenon has been recorded in various European countries [33–35]. It is essential that health services in Malta and elsewhere in Europe embark on effective preventive strategies and opportunistic screening to target these conditions from an early age, before the disease has been ingrained for too long [23]. Although this could impose an even larger health budget request on the country, such early action could help decrease the health burden contributed to diabetes, obesity and their associated complications. In fact, disease prevention should always be the primary target of any health management strategy.

### Study limitations

The response rate obtained was 49%. This can present as a potential non-response bias but the data was statistically weighted according to age, gender and locality in order to overcome this potential bias. The study does not cover the whole population but only a subset of the adult population. Since the prevalence of diabetes is substantial within the elderly population, costs may be affected by other underlying comorbidities. General demographic data was based on the latest published reports from 2013. The health cost for undiagnosed diabetes was based on a U.S. study, as no data was available for the Maltese population, although the GPD per capita for both countries was taken in consideration. The cost of illness data was obtained from secondary sources and this may affect the validity of the cost of illness in the Maltese health care setting. The previously known diabetes cost was obtained from the 2015 IDF estimated costs for Malta but no detailed description was available to the health care costs constitution. All costs from the

secondary sources lacked confidence intervals; therefore sensitivity analysis could not be performed on the unit costs. Comparisons with other studies were difficult due to the fact that the age groups and the total population samples were different from the current study and not comparable. Projections for the year 2050 were based on current conditions with an assumption that all demographic and risk factors would continue at their current rates. The economical burden excluded society burden including intangibles from pain and decreased quality of life, as well as the impact on caregivers.

## Conclusion

The study explored the present disease burden and economic costs for diabetes and obesity in Malta as well as those projected for 2050. A substantial prevalence and economical burden increase was estimated for 2050. The current Maltese health care expenditure for both diseases was similar to other European countries estimates. The data obtained should encourage policy makers to further explore the situation and bring forward preventative strategies considering the present 'diabesity' burden and the expected exponential increase. Implementing early screening tests for diabetes and obesity might be one of the best strategies to counteract the epidemic and reduce the overall costs.

#### Abbreviations

BMI: Body mass index; GDP: Gross domestic product; IDF: International diabetes federation; WHO: World Health Organization

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#### Availability of data and materials

Data analysed during this study are included in this published article. More detailed datasets analysed during the current study available from the corresponding author on reasonable request.

#### Authors' contributions

SC, JV, NC, NP and JM were responsible for the design of the cross-sectional protocol. These authors were also involved in the writing and reviewing of the article. SC, JA, BA, FA and EZ were the fieldworkers and data collectors of the cross-sectional study. These also contributed to formulation of the article. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no conflict of interest. The funding body had no influence on the protocol of the survey or on the data analysis. No author received any personal funding.

#### Consent for publication

Not applicable.

#### Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics approval was granted from Research Ethics Committee of the Faculty of Medicine and Surgery at the University of Malta. Every participant gave his or her informed consent.

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Original article

# The effect of age, gender, TG/HDL-C ratio and behavioral lifestyles on the metabolic syndrome in the high risk Mediterranean Island population of Malta

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ABSTRACT

*Aims:* Metabolic syndrome (MetS) is a public health epidemic, typically with female predominance. The aim was to analyse the effect of gender and age on MetS and its components; analyse effects of lifestyle, diabetes mellitus and identify predictors for MetS including TG/HDL ratio, on a national level in a Mediterranean island. Findings will provide evidence-based data for neighboring countries to aid in combat of this epidemic.

*Method:* A cross-sectional survey was conducted in Malta (2014–2016) on a randomized adults population sample. Various components of MetS were measured along with lifestyle habits (smoking, alcohol and physical activity) and family history (cardiovascular and diabetes). Both descriptive and statistical analyses were performed.

*Results:* A total of 80,788 Maltese adults estimated to suffer from MetS. Males were predominantly affected with significant difference from females. All MetS components were found to be significant predictors along with alcohol habits but not smoking. Neither physical inactivity nor family history of cardiovascular disease, showed any predictive ability for MetS even after adjustment. Elevated triglyceride levels exhibited highest predictive effect on MetS. TG/HDL ratio showed predictive ability in the Maltese population.

*Conclusions:* Males were at higher risk for MetS in Malta. A number of predictors were established but not sedentary lifestyle. TG/HDL ratio may provide to be a good indicator for development of MetS.



## 1. Introduction

The metabolic syndrome (MetS) is a public health concern with an estimated quarter of the world's adults suffering from this condition with European adults being no exception [1,2]. The prevalence varies considerably and depends on the environment, region, gender, age, race and ethnicity of the studied population [3]. The MetS is composed of a cluster of risk factors including central obesity, insulin resistance, hypertension and dyslipidemia, which predispose towards the development of cardiovascular disease and diabetes mellitus type 2 [4]. Family and twin studies exhibit a heritability element to the development of MetS [5]. The presence of MetS increases the individual risk to develop cardiovascular disease by threefold and diabetes mellitus type 2 by fivefold [4]. Globally, the prevalence of MetS is higher in females, except in Eastern populations. However, European studies report little gender differences in the prevalence of MetS [6–8]. European studies overall reported little gender disparity [8].

Central obesity and insulin resistance are the underlying causes for the development of MetS and have been related to low physical activity levels, aging, polycystic ovarian syndrome and a high dietary intake of fats and carbohydrates [6,9]. Increased added sugar in food and beverages (during processing and preparation) is also an independent risk factor for MetS [10].

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Insulin resistance is a crucial risk factor for MetS. The identification of a predictive marker for insulin resistance could enable professionals to preemptively assess the risk of MetS development. Considering that waist circumference is not a routine measurement by physicians but that a lipid profile is more often performed, a lipid-derived ratio may be suggested [11]. The triglyceride to high-density lipoprotein (TG/HDL-C) ratio is an easy, non-invasive test that has been considered an independent marker for insulin resistance [12,13]. Epidemiological studies have also shown an association of high TG/HDL-C ratio with increased cardiovascular disease risk [13]. A limited number of studies have investigated this ratio as a metabolic syndrome indicator for particular populations [14,15].

The Maltese Islands within the Mediterranean Sea, positioned at the crossroads between the European and African continents, are a high-risk population for the metabolic syndrome [16]. A recent study reported that 69.75% of the Maltese adult population is either overweight or obese [18]. This corroborates with previously reported data that the Mediterranean populations have a heavy overweight-obese burden, linked to cardiovascular disease [18]. Considering that the Maltese archipelago amounts to an area of 316 $km^2$, with a total population of 425,384 inhabitants, it presents itself as an ideal location for epidemiological representative population studies to be conducted. Such studies provide essential findings that could enable other Mediterranean countries to relate to. An exploration of such risk factors for MetS in the adult population of Malta was the main aim of this study.

The objectives were to; (a) determine the prevalence of the metabolic syndrome within the Maltese Islands, (b) examine the sex differences in the prevalence of the metabolic syndrome and its components, (c) determine the age effect on the prevalence of metabolic syndrome and its components by gender, (d) identify the effect, if any, of triglyceride/high-density lipoprotein ratio (TG/HDL-C ratio) on the prediction of the metabolic syndrome within the Maltese population, (e) determine whether behavioral lifestyles in this population had an effect on the metabolic syndrome, (f) determine the effect of diabetes mellitus on the metabolic syndrome and (e) determine the predictors for the development of MetS within the Maltese population. This study will serve as a fundamental basis for understanding metabolic syndrome risk factors for neighbouring Mediterranean countries.

## 2. Subjects, materials and methods

A nation-wide cross-sectional survey under the auspices of the University of Malta with the name "SAHHTEK" (*your health*) was conducted from November 2014 to January 2016 in Malta. A population of 4060 adults was recruited from the national registry after randomization, stratification by age (18–70 years) and gender from every town. The detailed protocol of this survey has been published elsewhere [19]. This survey consisted of a validated questionnaire as well as a health examination, including measurements for waist circumference (cm), body mass index (weight over height squared – kg/$m^2$), blood pressure (mmHg) and serum blood measurements for fasting blood glucose (FBG), triglycerides (TG) and high-density-lipoprotein cholesterol (HDL-C).

The questionnaire gathered information on demographic data, lifestyle habits (including smoking habits, alcohol consumption, physical activity), medical history (including previous diagnosis of diabetes mellitus and related medication) and family history (including family history of cardiovascular disease and diabetes). A physical activity questionnaire was used to work out the metabolic equivalent (MET) and categorise physical activity into low, moderate and high. Details on this protocol are found in the supplement material.

The definition of the metabolic syndrome (MetS) that the SAHHTEK study adhered to was that of the International Diabetes Federation Consensus [20]. Metabolic syndrome was present if participants exhibited an increased waist circumference (male $\geq$94 cm, female $\geq$80 cm) and the presence of any two of the following features: TG $\geq$1.7 mmol/L *or* HDL-C $<$1.03 mmol/L in male, $<$1.29 mmol/L in female *or* High blood pressure (Systolic blood pressure $\geq$130 mmHg or Diastolic blood pressure $\geq$85 mmHg or on hypertensive treatment) *or* FBG $\geq$5.6 mmol/L *or* previously diagnosed diabetes mellitus type 2 *or* on diabetic treatment. The definition of diabetes mellitus population in the SAHHTEK survey included those participants with a previous history of diabetes type 2 or who were on oral hypoglycemic agents for diabetes or obtained a FBG $\geq$7 mmol/L during the health examination. The subset of the SAHHTEK population obtaining an FBG between 5.6–6.9 mmol/L was invited for an OGTT. Those who scored a 2nd hour serum glucose level $\geq$11.1 mmol/L were also considered as suffering from diabetes mellitus type 2 [19].

The Triglyceride to High-density lipoprotein (TG/HDL-C) ratio was also estimated. Due to a lack of established specific cut-off points for the TG/HDL-C ratio as a predictor of MetS, the established insulin resistance cut-off points were considered. A TG/HDL-C ratio $<$2 was considered as optimal, 2–3.99 as at risk of insulin resistance and >4 as at very high risk of insulin resistance [13]. Statistical analyses were performed using IBM SPSS vs. 21, where the mean and standard deviations of all the metabolic components were calculated. An independent *t*-test and one-way ANOVA tests were performed to analyse the significance of mean values. A Chi-squared test was performed to analyse for any

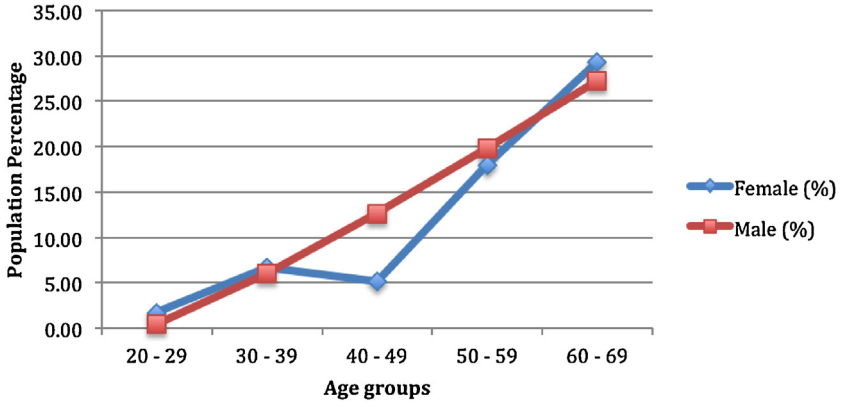


**Fig. 1.** Distribution of MetS prevalence rates by age groups and gender.
Age distribution within the MetS population.

significant comparisons between gender and the various components of MetS. Different predictive models were performed using multiple binary logistic regressions (including hierarchical) to assess for predictors of MetS. A *p*-value of less than 0.05 was considered as significant. Ethical and data protection permissions were granted from the University of Malta Research and Ethical Committee (UREC) and the Information and Data protection commissioner respectively.

## 3. Results

The data obtained from SAHHTEK study was statistically weighted by age, gender and locality to compensate for the non-responders (50%). The final weighted representative population of 3947 (1998 male) was obtained.

The prevalence of MetS within the entire adult population of SAHHTEK study was of 26.30% (CI 95%: 24.95–27.69) ($n = 1038$). On incorporating this prevalence to the demographic population data of the total adult Maltese population (18–70 years), it was estimated that 80,788 (76,641–85,058) adults suffered from MetS in Malta [21]. The male population exhibited a higher MetS prevalence (31.63% CI 95%: 29.63–33.70) than did the female population (20.83% CI 95%: 19.09–22.69) ($p = 0.0001$). In fact, the males exhibited 72% increased odds (OR: 1.72; CI 95%: 1.494–1.981; $p = 0.0001$) to develop MetS while the females had reduction in risk of MetS (OR: 0.581; CI 95%: 0.505–0.669; $p = 0.0001$).

The prevalence of MetS escalated with an increase in age in both genders with one exception in females between the ages of 40 to 49 years (Fig. 1). On age stratification, the females at both ends of the age spectrum (20–39 years and 60–69 years) had a higher prevalence of MetS than the male population as observed in Fig. 1. On binary logistic regression modeling, increase in age (per year) exhibited a predictive effect on development of MetS within both the female population and male population (OR: 1.058; CI 95%: 1.052–1.064 for both males and females).

The MetS population had an elevated waist circumference (mean female – 97.70 cm ± 13.55 SD, mean male – 105.36 cm ± 10.18 SD) with the male mean waist circumference (WC) being significantly higher than that of females ($p = 0.0001$). For every increased centimeter in waist circumference, it showed 1.082 (95% CI 95%: 1.076–1.089) times increase in odds of having MetS ($p = 0.0001$). On the other hand, the population without MetS (non-MetS) had a normal waist circumference (mean female – 79.87 cm ± 14.79 SD, mean male 91.29 cm ± 14.96). A comparison between the general MetS population to the non-MetS population is illustrated in Table 1. A significant difference was found within all

**Table 1**
Distribution of the components (other than waist circumference) making up the metabolic syndrome, within the MetS population and the population without MetS (non-MetS).

| | Metabolic Syndrome Population | | Non-Metabolic Syndrome Population | | |
|---|---|---|---|---|---|
| | Total (*n = 1038*) | | Total (*n = 2909*) | | |
| | Mean ± SD | *n* (%) | Mean ± SD | *n* (%) | p-value |
| HDL Cholesterol (mmol/L) | 1.24 ± 0.39 | | 1.62 ± 0.42 | | 0.0001[f] |
| Normal/High | | 537 (51.73%) | | 2725 (93.67%) | 0.0001[g] |
| Low[a] | | 501 (48.27%) | | 184 (6.33%) | |
| Triglycerides (mmol/L) | 1.75 ± 1.02 | | 0.88 ± 0.48 | | 0.0001[f] |
| Normal | | 557 (53.66%) | | 2792 (95.32%) | 0.0001[g] |
| High[b] | | 481 (46.34%) | | 117 (4.02%) | |
| Systolic Blood pressure (mmHg) | 132 ± 14 | | 116 ± 17 | | 0.0001[f] |
| Normal[c1] | | 273 (26.30%) | | 2326 (79.96%) | 0.0001[g] |
| Normal[c2] | | *120* | | 197 (6.77%) | |
| High[c3] | | *645* | | 386 (13.27%) | |
| MetS – Systolic | | 765 (73.70%) | | | |
| Diastolic Blood pressure (mmHg) | 81 ± 9 | | 73 ± 11 | | 0.0001[f] |
| Normal[d1] | | 393 (37.86%) | | 2413 (82.95%) | 0.0001[g] |
| Normal[d2] | | *295* | | 247 (8.49%) | |
| High[d] | | *350* | | 249 (8.56%) | |
| MetS – Diastolic | | 645 (62.14%) | | | |
| Fasting blood glucose (mmol/L) | 6.71 ± 2.48 | | 5.30 ± 1.04 | | 0.0001[f] |
| Normal[e1] | | 238 (22.93%) | | 439 (15.09%) | 0.0001[g] |
| Normal[e2] | | *8* | | 102 (3.51%) | |
| High[e3] | | *792* | | 2368 (81.40%) | |
| MetS – FBG | | 800 (77.07%) | | | |

[a]Low HDL is defined as HDL <1.03 mmol/L (male), HDL <1.29 mmol/L (female).
[b]High Triglycerides is defined as triglycerides >1.70 mmol/L.
[c1]Normal systolic blood pressure <130 mmHg and not on anti-hypertensive.
[c2]Normal systolic blood pressure <130 mmHg and on anti-hypertensives.
[c3]High Systolic blood pressure is defined as > = 130 mmHg.
MetS – Systolic: combination of C2 and C3 make up a component of MetS.
[d1]Normal diastolic blood pressure <85 mmHg and not on anti-hypertensive.
[d2]Normal diastolic blood pressure <85 mmHg and on anti-hypertensive.
[d3]High Diastolic blood pressure is defined as > = 85 mmHg.
MetS – Diastolic: combination of D2 and D3 make up a component of MetS.
[e1]Fasting blood glucose <5.6 mmol/L and No history of diabetes.
[e2]Fasting blood glucose <5.6 mmol/L and a history of diabetes.
[e3]High Fasting blood glucose is defines as > = 5.6 mmol/L.
MetS – FBG: combination of e2 and e3 make up a component of MetS.
[f]student *t*-test comparing means per category.
[g]Chi squared comparing the different categories.

the MetS components between the MetS and the non-MetS populations. On analyzing the Maltese MetS population by gender, significant gender difference was exhibited within all other MetS components as seen in Table 2.

The MetS components were age stratified by 10-year age groups according to gender as illustrated in Table 3. The female population showed a significantly higher mean FBG value than that of males between 30 and 49 years of age ($p = 0.0001$), while from 50 years onwards, the male population had a higher mean FBG ($p = 0.002$). A significantly higher mean HDL-C value was exhibited throughout all age groups within the female population when compared to the male population ($p = 0.0001$). The male population had a higher TG mean value throughout all the age groups except between 20 and 29 years ($p = 0.05$). Only the 60 to 69 year age group exhibited an elevated mean systolic blood pressure in both genders, while the 20–29 years male age group had an elevated diastolic blood pressure.

Models were performed to analyse for the predictive ability of the various MetS components for the development of MetS individually and by gender (Table 4). A decrease in HDL was found to result in a 94.6% reduction in the risk of having MetS (OR: 0.054, 95% CI 95%: 0.042 to 0.07, $p = 0.0001$). Per unit increase in TG level resulted in seven-fold increase in the risk of developing MetS (OR: 7.547, 95% CI 95%: 6.449–8.832, $p = 0.0001$). An increase in systolic blood pressure was found to increase the risk of MetS by 8.2% (OR: 1.082, CI 95%: 1.075–1.088, $p = 0.0001$). Meanwhile for every 1 mmHg elevation in diastolic blood pressure, it was found to result in 8.1% increase in the risk of MetS (OR: 1.081, CI 95%: 1.072–1.090, $p = 0.0001$). While per unit of elevation in the FBG level, it resulted in a two-fold increase in the risk of developing MetS (OR 2.386, CI 95%: 2.172–2.621, $p = 0.0001$).

Table 5 demonstrates the different reported lifestyle behavioural patterns and family history distributions within the MetS population, by gender. The majority of the MetS Maltese population did not exhibit a family history of cardiovascular disease. On the other hand, a high proportion of the population (with a female majority) had a family history of diabetes mellitus type 2. Both cardiovascular disease and diabetes mellitus family history independently, showed no significant predictor ability towards the development of MetS ($p = 0.101$ and $p = 0.061$ respectively). A family history of diabetes mellitus was, however, found to show MetS predictive ability on adjusting for gender ($p = 0.02$) and after adjusting for both gender and age ($p = 0.03$).

The Maltese MetS population was predominantly non-smoking and being a smoker did not exhibit a predictive effect for MetS (OR: 0.965; CI 95%: 0.804–1.159; $p = 0.705$). This held true after modeling with gender as well as with gender and age ($p = 0.687$, $p = 0.447$ respectively). The male MetS population showed significantly higher weekly alcohol consumption than did females ($p = 0.0001$). Alcohol consumption (days per week) was only found to be a predictor of MetS ($p = 0.137$) on adjusting for both age and gender

**Table 2**
Distribution of metabolic syndrome components (prevalence and mean) other than high waist circumference (100%), by gender.

| | MetS whole population | | | | | |
|---|---|---|---|---|---|---|
| | Total (*n* = *1038*) | | Male (*n* = 632) | | Female (*n* = 406) | |
| | Mean ± SD | *n* (%) | Mean ± SD | *n* (%) | Mean ± SD | *n* (%) | p-value |
| HDL Cholesterol (mmol/L) | 1.24 ± 0.39 | | 1.14 ± 0.32 | | 1.40 ± 0.44 | | 0.0001[f] |
| Normal | | 537 (51.73%) | | 340 (53.80%) | | 197 (48.52%) | 0.0001[g] |
| Low[a] | | 501 (48.27%) | | 292 (46.20%) | | 209 (51.48%) | |
| Triglycerides (mmol/L) | 1.75 ± 1.02 | | 1.85 ± 1.06 | | 1.59 ± 0.93 | | 0.0001[f] |
| Normal | | 557 (53.66%) | | 310 (49.05%) | | 247 (60.84%) | 0.0001[g] |
| High[b] | | 481 (46.34%) | | 322 (50.95%) | | 159 (39.16%) | |
| Systolic Blood pressure (mmHg) | 132 ± 14 | | 132 ± 13 | | 133 ± 15 | | 0.403[f] |
| Normal[c1] | | 273 (26.30%) | | 184 (29.11%) | | 89 (21.92%) | 0.0102[g] |
| Normal[c2] | | *120* | | *76* | | *44* | |
| High[c3] | | *645* | | *372* | | *273* | |
| MetS – Systolic | | 765 (73.70%) | | 448 (70.89% | | 317 (78.08% | |
| Diastolic Blood pressure (mmHg) | 81 ± 9 | | 82 ± 9 | | 79 ± 9 | | 0.0001[f] |
| Normal[d1] | | 393 (37.86%) | | 224 (35.44%) | | 169 (41.63%) | 0.0451[g] |
| Normal[d2] | | *295* | | *164* | | *131* | |
| High[d] | | *350* | | *244* | | *106* | |
| MetS – Diastolic | | 645 (62.14%) | | 408 (64.56%) | | 237 (58.37% | |
| Fasting blood glucose (mmol/L) | 6.71 ± 2.48 | | 6.93 ± 2.62 | | 6.36 ± 2.21 | | 0.0001[f] |
| Normal[e1] | | 238 (22.93%) | | 125 (19.78%) | | 113 (27.83% | 0.0026[g] |
| Normal[e2] | | *8* | | *5* | | *3* | |
| High[e3] | | *792* | | *502* | | *290* | |
| MetS – FBG | | 800 (77.07%) | | 507 (80.22%) | | 293 (72.17%) | |

[a]Low HDL is defined as HDL < 1.03 mmol/L (male), HDL < 1.29 mmol/L (female).
[b]High Triglycerides is defined as triglycerides >1.70 mmol/L.
[c1]Normal systolic blood pressure <130 mmHg and not on anti-hypertensive.
[c2]Normal systolic blood pressure <130 mmHg and on anti-hypertensive.
[c3]High Systolic blood pressure is defined as >= 130 mmHg.
MetS – Systolic: combination of C2 and C3 make up a component of MetS.
[d1]Normal diastolic blood pressure <85 mmHg and not on anti-hypertensive.
[d2]Normal diastolic blood pressure <85 mmHg and on anti-hypertensive.
[d3]High Diastolic blood pressure is defined as >= 85 mmHg.
MetS – Diastolic: combination of D2 and D3 make up a component of MetS.
[e1]Fasting blood glucose <5.6 mmol/L and No history of diabetes.
[e2]Fasting blood glucose <5.6 mmol/L and a history of diabetes.
[e3]High Fasting blood glucose is defines as >= 5.6 mmol/L.
MetS – FBG: combination of e2 and e3 make up a component of MetS.
[f]Student *t*-test comparing means of males vs. females per category.
[g]Chi squared test comparing males vs. females with and without abnormality.

**Table 3**
Distribution of the MetS component mean values, by age and gender.

| | | 20–29 Mean ± SD | 30–39 Mean ± SD | 40–49 Mean ± SD | 50–59 Mean ± SD | 60–69 Mean ± SD | ANOVA p-value |
|---|---|---|---|---|---|---|---|
| FBG (mmol/L) | F | 4.87 ± 0.44 | 7.53 ± 5.15 | 6.08 ± 0.94 | 5.91 ± 2.46 | 6.39 ± 1.59 | 0.0001 |
| | M | 4.94 ± 0.40 | 5.62 ± 0.32 | 5.98 ± 1.76 | 7.29 ± 3.69 | 6.85 ± 2.36 | |
| HDL (mmol/L) | F | 1.01 ± 0.01 | 1.11 ± 0.17 | 1.24 ± 0.37 | 1.27 ± 0.32 | 1.48 ± 0.45 | 0.0001 |
| | M | 0.94 ± 0.01 | 1.04 ± 0.21 | 1.14 ± 0.27 | 1.07 ± 0.25 | 1.14 ± 0.32 | |
| Triglycerides (mmol/L) | F | 1.53 ± 0.41 | 1.91 ± 1.27 | 1.76 ± 1.53 | 1.61 ± 0.86 | 1.43 ± 0.7 | 0.05 |
| | M | 0.78 ± 0.1 | 2.02 ± 1.53 | 1.92 ± 0.97 | 1.95 ± 0.94 | 1.78 ± 1.1 | |
| Systolic Bp (mmHg) | F | 118 ± 3 | 119 ± 12 | 122 ± 15 | 128 ± 16 | 136 ± 10 | 0.0001 |
| | M | 120 ± 1 | 128 ± 5 | 127 ± 10 | 128 ± 14 | 135 ± 11 | |
| Diastolic Bp (mmol/L) | F | 78 ± 8 | 74 ± 10 | 76 ± 9 | 80 ± 11 | 81 ± 9 | 0.0001 |
| | M | 87 ± 2 | 84 ± 4 | 84 ± 7 | 83 ± 9 | 82 ± 9 | |
| WC (cm) | F | 80.2 ± 0.86 | 88.07 ± 9.81 | 96.32 ± 12.78 | 100.5 ± 13.91 | 99.88 ± 12.79 | 0.001 |
| | M | 107.4 ± 0.3 | 106.2 ± 14.05 | 102.85 ± 9.51 | 105.1 ± 10.31 | 106.57 ± 9.73 | |

**Table 4**
Predictive models analyzing the different MetS components by gender as predictors for the development of MetS.

| Model | | b | 95% Confidence intervals Low | Odds | High | sig. |
|---|---|---|---|---|---|---|
| 1 | Waist circumference | 0.081 | 1.077 | 1.084 | 1.091 | 0.0001 |
| | Male | 0.125 | 1.133 | 0.957 | 1.133 | 1.341 |
| 2 | Waist circumference | 0.08 | 1.077 | 1.084 | 1.091 | 0.0001 |
| | Female | −0.125 | 0.746 | 0.883 | 1.044 | 0.146 |
| 3 | FBG | 0.846 | 2.122 | 2.331 | 2.562 | 0.0001 |
| | Male | −0.282 | 0.644 | 0.754 | 0.883 | 0.0001 |
| 4 | FBG | 0.846 | 2.122 | 2.331 | 2.562 | 0.0001 |
| | Female | 0.282 | 1.132 | 1.326 | 1.553 | 0.0001 |
| 5 | HDL | −2.712 | 0.052 | 0.066 | 0.085 | 0.0001 |
| | Male | −0.144 | 0.737 | 0.866 | 1.018 | 0.081 |
| 6 | HDL | −1.984 | 0.166 | 0.138 | 0.163 | 0.0001 |
| | Female | −0.138 | 0.734 | 0.871 | 1.033 | 0.113 |
| 7 | Triglycerides | 2 | 6.303 | 7.389 | 8.662 | 0.0001 |
| | Male | −0.138 | 0.735 | 0.871 | 1.032 | 0.11 |
| 8 | Triglycerides | 2 | 6.303 | 7.389 | 8.662 | 0.0001 |
| | Female | 0.138 | 0.969 | 1.148 | 1.361 | 0.11 |
| 9 | Systolic blood pressure | 0.077 | 1.074 | 1.08 | 1.087 | 0.0001 |
| | Male | −0.334 | 0.609 | 0.716 | 0.842 | 0.0001 |
| 10 | Systolic blood pressure | 0.077 | 1.074 | 1.08 | 1.087 | 0.0001 |
| | Female | 0.334 | 1.187 | 1.396 | 1.641 | 0.0001 |
| 11 | Diastolic blood pressure | 0.075 | 1.069 | 1.078 | 1.088 | 0.0001 |
| | Male | −0.319 | 0.624 | 0.724 | 0.847 | 0.0001 |
| 12 | Diastolic blood pressure | 0.075 | 1.069 | 1.078 | 1.088 | 0.0001 |
| | Female | 0.319 | 1.18 | 1.375 | 1.603 | 0.0001 |

b = b – coefficient.
sig. = Wald statistics significance.

($p = 0.035$). The majority of both the male and female population followed a sedentary lifestyle. Interestingly, low-level physical activity did not show any predictive effect for MetS ($p = 0.829$) even on adjusting for gender ($p = 0.715$), age ($p = 0.321$), BMI ($p = 0.985$), WC ($p = 0.862$) and even following hierarchical logistic regression for the mentioned parameters ($p = 0.431$). As expected, the majority of the MetS population (both females and males) was obese. An increase in BMI was found to result in 17.7% increased in risk of having MetS (OR: 1.177, CI 95%: 1.160–1.195, $p = 0.0001$).

The TG/HDL-C ratio was calculated for the MetS population according to gender as found in Table 6. The mean TG/HDL-C ratio showed significant difference between males and females ($p = 0.001$), although both means fell within the optimal range. The majority of the MetS population (both males and females) had an optimal TG/HDL-C ratio, although only the male population exhibited a proportion being at high-risk of insulin resistance and subsequently, of MetS. In effect, an elevation in TG/HDL-C ratio had 11.6% increased risk of developing MetS (OR: 1.116, CI 95%: 1.038–1.2, $p = 0.003$), although the relationship seen on adjusting for age was lost on adjusting for gender (age $p = 0.004$, gender $p = 0.15$).

Among the adult Maltese metabolic syndrome population, 28.13% (CI 95%: 25.48–30.94) also had a diagnosis of diabetes mellitus, with a male predominance (70.55% CI 95%: 65.07–75.49). A diagnosis of diabetes mellitus had a five-fold increase in risk of developing MetS (OR: 5.06, CI 95%: 4.63-5.48, $p = 0.0001$). On adjusting for both age and gender, the significant predictive ability remained equally evident ($p = 0.0001$).

## 4. Discussions

Southern European countries tend to exhibit higher overweight populations and hypertension than the Northern counterparts, predisposing these populations to greater risk for MetS development [22,23]. Malta is a Southern European country with the adult MetS prevalence found to be higher than several other Mediterranean countries, notably Italy and Greece [24,25]. In Europe, only a small gender difference has been exhibited in MetS prevalence rates distribution [23]. A Portuguese study exhibited a predominantly male MetS tendency although the majority of the literature claims that MetS is commoner in females including in the Mediterranean country of Greece [18,25–28].

In Malta, there was a more significant male to female discrepancy in contrast to what had been reported elsewhere in Europe. This could relate to the fact that, unlike the majority of Southern Europe, where the females predominantly exhibit abdominal obesity, in Malta, males are more obese than their female counterparts [19,23]. The study further confirms that the Maltese males have higher abdominal obesity, since the waist circumference was significantly higher for males than the female population.

Adults in Malta showed an increase in mean MetS prevalence as age increased similar to the pattern shown by other Mediterranean countries [29]. Females tended to have a lower MetS prevalence rate before the age of 60 years when compared to males, in Malta as elsewhere [30]. This is consistent with the menopausal oestrogen deficiency theory; where females tend to increase in abdominal adiposity, develop insulin resistance and dyslipidemia in menopause. All this predisposes individuals towards postmenopausal MetS development [30].

In Malta, young female population between 20 and 39 years of age showed higher prevalence of MetS than did their male counterparts. This female age group also exhibited the highest mean FBG within the entire female population, with a higher TG mean value than that of males. These young Maltese women exhibited a worse mean metabolic profile than older females. This may be related to a possible underlying polycystic ovarian syndrome (PCOS) diagnosis, which is linked to MetS [6]. Further research is required to assess this age group and to explore the consideration of possible initiation of screening programmes for both MetS and PCOS at an early age among females in Malta should be considered.

**Table 5**
Distribution of behavioral lifestyle habits, family history and BMI statuses in the MetS population by gender.

| | MetS whole population | | | Chi squared |
|---|---|---|---|---|
| | Total ($n$ = 1038) | Male ($n$ = 632) | Female ($n$ = 406) | p-value |
| Family History of Cardiovascular Disease | | | | |
| No | 784 (76.49%) | 486 (77%) | 308 (75.90%) | 0.667 |
| Yes | 243 (23.41%) | 145 (23%) | 98 (24.10%) | |
| Family History of Diabetes Mellitus | | | | |
| No | 543 (52.31%) | 356 (56.40%) | 187 (46.20%) | 0.001 |
| Yes | 494 (47.59%) | 275 (43.60%) | 219 (53.80%) | |
| Smoking status | | | | |
| Non-Smoker | 781 (75.24%) | 453 (71.68%) | 328 (80.79%) | 0.001 |
| Current Smoker | 257 (24.76%) | 179 (28.32%) | 78 (19.215) | |
| Alcohol consumption | | | | |
| 1 to 2 times per week | 247 (23.80%) | 178 (28.16%) | 69 (17%) | 0.0001 |
| 1 to 3 times per month | 106 (10.21%) | 81 (12.82%) | 25 (6.16%) | |
| 3 to 6 times per week | 57 (5.49%) | 45 (7.12%) | 12 (2.96%) | |
| Daily or almost daily | 130 (12.52%) | 110 (17.41%) | 20 (4.93%) | |
| Less often | 324 (31.21%) | 155 (24.53%) | 169 (41.63%) | |
| No alcohol last 12 months | 174 (16.76%) | 63 (9.97%) | 111 (27.34%) | |
| Physical activity | | | | |
| High level | 146 (14.07%) | 75 (11.87%) | 71 (17.49%) | 0.038 |
| Moderate level | 349 (33.62%) | 216 (34.18%) | 133 (32.76%) | |
| Low level | 543 (52.31%) | 341 (53.96%) | 202 (49.75%) | |
| BMI status | | | | |
| Normal (<25 kg/m$^2$) | 52 (5.01%) | 18 (2.85%) | 34 (8.37%) | 0.0001 |
| Overweight (25–29.99 kg/m$^2$) | 350 (33.72%) | 227 (35.92%) | 123 (30.30%) | |
| Obese (> = 30kg/m$^2$) | 636 (61.27%) | 387 (61.23%) | 249 (61.33%) | |

**Table 6**
Distribution of the TG/HDL ratio in MetS population by gender.

| | Male ($n$ = 632) | | Female ($n$ = 406) | | |
|---|---|---|---|---|---|
| | Mean ± SD | $n$ (%) | Mean ± SD | $n$ (%) | p-value |
| TG/HDL ratio[c] | 1.06 ± 1.01 | | 0.86 ± 0.71 | | 0.001[a] |
| <2 (optimal) | | 559 (88.45%) | | 371 (91.385) | 0.001[b] |
| 2–3.99 (at risk) | | 50 (7.91%) | | 35 (8.62%) | |
| 4 (high risk) | | 23 (3.64%) | | 0 | |

[a] student $t$-test comparing means of males vs. females.
[b] Chi squared test comparing males vs. females between different ratio categories.
[c] Marker for Insulin Resistance.

The majority of adult Maltese persons diagnosed with the metabolic syndrome were non-smokers, low alcohol consumers with low physical activity. This is consistent with findings in Portugal. However, unlike Portugal, in Malta, alcohol habits had a predictive effect on the development of MetS, in keeping with established links between alcohol and the development of MetS [6,26]. Only low physical activity was unable to show a predictive effect for MetS development in the Maltese, which seems to contradict the established links of sedentary lifestyle and MetS [31]. This may suggest the possibility that the Maltese are predisposed to stronger genetic factors leading to the development of MetS, although more research is warranted to confirm this.

The TG/HDL-C ratio has not been intensely investigated as a MetS predictor and different population cut-off points have been proposed. In this study, the cut-off points considered were those established for insulin resistance in view of the fact that insulin resistance is part of MetS development. TG/HDL-C appeared to have a significant predictive effect for the Maltese population and inevitably a risk for MetS, even on adjusting for age. This ratio is a suggestive measure to be used by the Maltese physicians to identify adults at risk of MetS. Further longitudinal research on a larger population is needed to establish whether this ratio could be used as a definitive marker for MetS as well as to identify Maltese-specific cut-off points. Another established tool for MetS prediction is the visceral adiposity index [32]. This was not investigated in this study following the fact that it is not customary for physicians to measure the waist circumference and calculate the BMI in a normal consultation visit.

All `the metabolic syndrome components along with female gender and alcohol consumption showed a predictive effect on the development of MetS. An elevation in the triglyceride level had the highest odds prediction ability for MetS in the Maltese population followed by a diagnosis of type 2 diabetes. Considering that the prevalence of diabetes mellitus in Malta is one of the highest within Europe, compounded by the presence of very high overweight and obese rates, the Maltese population is at a very high cardiometabolic risk [17,33].

## 5. Conclusions

The Maltese male population exhibited the highest MetS prevalence and predictive risk for MetS when compared to females. The presence of an increased waist circumference and 2 other MetS risk factors exhibited the highest risk of developing MetS. The development of MetS is further increased by the presence of alcohol consumption and if the individual suffered from diabetes mellitus type 2. Even though this study found no direct relationship between a sedentary lifestyle and the development of MetS, this could relate to small numbers in the subgroups. The TG/HDL-C ratio may be a good predictive MetS tool to be used by physicians in clinics. The Maltese population should be analysed further to establish for any links between physical activity and MetS. Also, further research on the TG/HDL-C predictive ability and its utilization as a MetS diagnostic tool is recommended. Health professionals should also keep an eye on young females exhibiting

MetS components since they appeared also to be at a higher risk. This study provides evidence that the Maltese population has a very high cardiometabolic risk and brings forward the very real possibility of a need for screening and other preventive services to further combat this epidemic.

## 6. Study limitations

There are various definitions for the metabolic syndrome in the literature and the prevalence rate may be subject to the definition used. The different metabolic components measurements were recorded by trained skilled fieldworkers but still could have been subject to human error. The study population included 1038 participants suffering from MetS, which may have had an effect on the power of the study's results. In particular, certain subgroups such as those adults regularly exercising might be composed of exceptionally small numbers and here Type 2 errors may come into play. This was a cross-sectional study and not a longitudinal study. The latter would be required in order to explore more fully the association of metabolic syndrome and TG/HDL-C ratio further and to assess its effects over time.

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## Disclosure statement

The authors declare that they have no conflict of interest. The funding body had no influence on the protocol of the survey or on the data analysis. No author received any personal funding.

## Author's contributions

S.C., J.V., N.C., N.P. and J.M. were responsible for the design of the cross-sectional protocol. S.C. was responsible for the data collection and analysis, data interpretation and manuscript writing. J.V., N.C., N.P. and J.M. were responsible for the reviewing of the article including data analysis.

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*Research Article*

# The Effects of Socioeconomic Determinants on Hypertension in a Cardiometabolic At-Risk European Country

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*Background.* A relationship has been established between socioeconomic status and hypertension. The aim of this study was to determine the prevalence of hypertension and to explore the links between hypertension and socioeconomic factors in the adult population of Malta. *Methods.* A national representative cross-sectional health examination study was performed between 2014 and 2016. Sociodemographic and medical history data was gathered by validated questionnaires while blood pressure was measured. Prevalence rates of known hypertension, newly hypertension, and global hypertension were calculated. Associations between sociodemographic characteristics and hypertension were identified through logistic regression models. *Results.* Hypertension contributed to 30.12% (CI 95%: 28.71–31.57) of the study population, with a male preponderance. The majority was known hypertensive (73.59% CI 95%: 71.01–76.02), with only three-quarters on medication. Multivariant analyses showed that increasing age and body mass index, male gender, and living in Gozo, Western district, and Northern Harbour district were associated with having hypertension. *Conclusion.* Hypertension is a problem in Malta especially in the male population and with increasing age and body mass index. Education did not exhibit any associated risk for having hypertension, which is inconsistent with the literature, while habitat localities played a role in hypertension development.

## 1. Introduction

High blood pressure is a serious public health concern, with the World Health Organization (WHO) reporting a hyper-tension global mortality rate of 13% [1]. Hypertension is estimated to contribute to 25% of all European myocardial infarctions [2]. In fact, the WHO European Region document *Health 2020 Policy* gave hypertension high priority in order to tackle the epidemic and to reduce its prevalence [3]. High blood pressure is a preventable disease and has been associated directly with lifestyle habits, including tobacco smoking, a lack of physical activity, and alcohol consumption
[2]. In fact, the relationship between hypertension and socioeconomic status has been well established [4].

Socioeconomic status (SES) is a complex term combining a number of variables, including employment status, educational level, income, and wealth as well as place of residence. SES is a well-established cardiovascular risk factor and means for predicting behaviour [5, 6]. The educational level has been established as the best marker of SES since it offers the most stable measure at an individual level and does not have the problem of reverse causation such as income and wealth status [6].

Malta is a Mediterranean island at crossroads between Europe and Africa, with 40.1% of the mortality rate attributed to cardiovascular disease [7]. Considering the small size of this island and its crucial location, a representative adult health examination cross-sectional study was considered fea-sible to provide much needed information on health effects. The aim of this study was to determine the prevalence of hypertension and to explore the links between hypertension and socioeconomic factors in the adult population of Malta.

## 2. Method

A cross-sectional study was conducted by the University of Malta between November 2014 and January 2016, with the survey name of "SAHHTEK" *(your health)*. The detailed survey methodology was described elsewhere [8]. In summary, a randomized stratif ied sample, by age (18 to 70 years) and gender, representing approximately 1% of the total population from each Maltese town, was invited to participate in the health examination survey. A validated sociodemographic and medical history questionnaire was filled in during the survey [8]. The health examination consisted of three con-secutive blood pressure readings after a 20-minute rest (sit-ting down), besides anthropometric measurements. The aver-age of the three blood pressure readings was recoded and used for this study. Informed consent was obtained from every participant. Ethical and data protection approvals were granted from the University of Malta Research Ethical Com-mittee (UREC) and the Information and Data Protection Commissioner, respectively.

The population reporting a history of hypertension or who was on antihypertensive medication was labeled as *"known hypertensive."* Participants with a systolic blood pressure over 140 mmHg or a diastolic blood pressure above 90 mmHg and who were not *known hypertensives* were labeled as *"newly hypertensive."* Those reporting to suffer from *known hypertension* but did not report to be on treatment and were found to have a normal blood pressure during health examination were labeled as *normotensive*. All popula-tion with no history of hypertension and a normal blood pressure during health examination was also labeled as *normotensive*.

The *known hypertensive* and the *newly hypertensive* subpopulations were considered collectively as the global *hypertension population* for this study. The prevalence rates of *known hypertension*, *newly hypertension*, and the global *hypertension population* were calculated. The study popula-tion was age-stratified by 10-year age groups (20 to 69 years), and considering the representative prevalence rates in these age groups, an estimate of the total Maltese adult population suffering from hypertension was calculated.

The links between self-reported sociodemographic, lifestyle variables (residing district, highest educational level, employment status, smoking habits, alcohol consumption habits, and physical activity levels), body mass index (BMI), and hypertension were explored. Univariate and multiple variant logistic regression models were performed to assess any associations between these sociodemographic character-istics and hypertension.

## 3. Results

The survey response rate was of 49% ( = 3947), out of which 30.12% (CI 95%: 28.71–31.57) contributed to the hypertension population ( = 1189). Hypertensive males accounted for 35.04% (CI 95%: 32.97–37.15) of the total study male pop-ulation, while hypertensive females accounted for 25.09% (CI 95%: 23.21–27.06) of the total female study population.

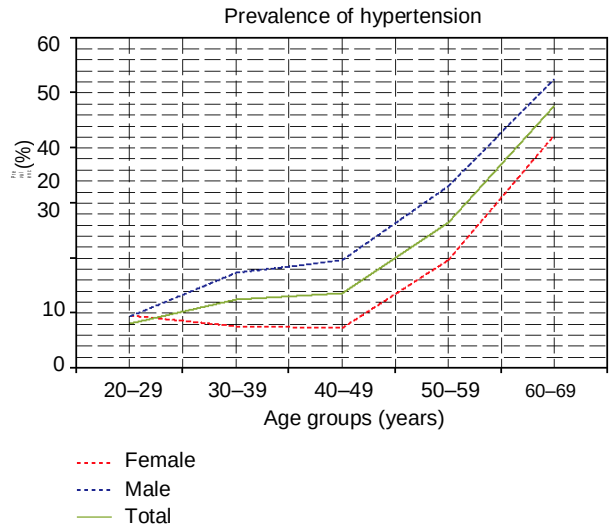


Figure 1: Distribution of hypertension prevalence by age groups and gender.

On age and gender stratification, a male predominance was present throughout all age groups, as seen in Figure 1. With increasing age in the 20- to 39-year bracket, males had higher blood pressure levels, while the female population actually had slightly lowered prevalence with increasing age in this bracket. However, from the age of 40 years, a higher prevalence rate of hypertension was exhibited for both genders with every age increase. On applying the prevalence rates for each age group and gender to the total Maltese population by age and gender [9], an estimated distribution of hypertension within the Maltese population was established, as seen in Table 1.

The majority of the hypertension population consisted of *known hypertensives* (73.59% CI 95%: 71.01–76.02), with the male prevalence of 71.29% (CI 95%: 67.82–74.52) and a female prevalence of 76.89% (CI 95%: 72.95–80.42). Among the *known hypertensives*, 85.60% (CI 95%: 83.11–87.78) were on medication. However, 5.34% (CI 95%: 3.93–7.21) were found to have uncontrolled systolic blood pressure (>140 mmHg) while 1.20% (CI 95%: 0.60–2.30) had uncontrolled diastolic blood pressure (>90 mmHg) during the health examination survey. Out of those reporting to suffer from hypertension, 5.52% (CI 95%: 4.19–7.23) did not report to be on treatment and were found to have a normal blood pressure during the health examination. This subgroup may have exhibited an error in their medical history recall and were therefore considered to be normotensive.

The *newly hypertensive* population contributed to 26.41% (CI 95%: 23.98–28.99) of the hypertension population. The majority (64.01% CI 95%: 58.56–69.13) of the newly diagnosed hypertensives were male. Table 2 demonstrates differ-ent hypertension characteristics by gender. The Maltese males tend to be more likely to have an elevated blood pressure, be unaware of their hypertension, be untreated, or have uncontrolled blood pressure.

Table 1: Distribution of the estimated Maltese hypertension population, by age groups and gender.

(a)

| Total Maltese population by age | | Total population with hypertension | Percentage with hypertension, by age |
|---|---|---|---|
| 20–29 | 55,218 | 5,345 | 9.68% |
| 30–39 | 56,184 | 7,193 | 12.80% |
| 40–49 | 48,406 | 6,466 | 13.36% |
| 50–59 | 57,291 | 15,223 | 26.57% |
| 60–69 | 55,986 | 26,395 | 47.15% |
| *Total* | 273,085 | 60,622 | |

(b)

| Total male Maltese population by age | | Male with hypertension | Percentage with hypertension, by age |
|---|---|---|---|
| 20–29 | 28,228 | 2,646 | 9.38% |
| 30–39 | 28,837 | 5,005 | 17.36% |
| 40–49 | 24,546 | 4,796 | 19.54% |
| 50–59 | 28,822 | 9,529 | 33.06% |
| 60–69 | 27,388 | 14,384 | 52.52% |
| *Total* | 137,821 | 36,360 | |

(c)

| Total female Maltese population by age | | Female with hypertension | Percentage with hypertension, by age |
|---|---|---|---|
| 20–29 | 26,990 | 2,699 | 10.00% |
| 30–39 | 27,347 | 2,188 | 8.00% |
| 40–49 | 23,860 | 1,670 | 7.00% |
| 50–59 | 28,469 | 5,694 | 20.00% |
| 60–69 | 28,598 | 12,011 | 42.00% |
| *Total* | 135,264 | 24,262 | |

Demographic data 2013.

Table 2: Demonstrating hypertension characteristics, by gender.

| Hypertension characteristics | Male (%) [ = 1998] | Female (%) [ = 1949] | Total (%) [ = 3947] |
|---|---|---|---|
| Normotensive | 1298 (64.96) | 1460 (74.91) | 2758 (69.88) |
| Hypertensive *(global hypertension)* | 700 (35.04) | 489 (25.09) | 1189 (30.12) |
| Unaware *(newly hypertensive)* | 201 (10.06) | 113 (5.80) | 314 (7.96) |
| Aware *(known hypertensive)* | 499 (24.97) | 376 (19.29) | 875 (22.17) |
| Untreated | 81 (4.05) | 45 (2.31) | 126 (3.19) |
| Treated | 418 (20.92) | 331 (16.98) | 749 (18.98) |
| Uncontrolled | 32 (1.60) | 15 (0.77) | 47 (1.19) |
| Controlled | 386 (19.32) | 316 (16.21) | 702 (17.79) |
| Total with elevated blood pressure | 668 (33.43) | 474 (24.32) | 1142 (28.93) |

## 4. Hypertension Population and Sociodemographic Characteristics

On comparing the sociodemographic characteristics and lifestyle factors to the hypertensive and normotensive popula-tions, significant differences were observed. These differences are outlined in Table 3.

Multivariant analyses showed that increasing age and increasing BMI, the male gender, physical activity, and living in Gozo, Western district, and Northern Harbour were associated with the development of hypertension (Table 4).

Alcohol consumption, smoking habit, education level, and employment status were not found to have a signif icant associated risk on hypertension after adjustment for all soci-odemographic and lifestyle habits.

## 5. Discussion

Hypertension is one of the leading causes of cardiovascular disease and mortality worldwide [10]. The prevalence of hypertension varies from one global region to another, with the highest prevalence being in the African region (46%) and

Table 3: Distribution of the sociodemographic characteristics in the normotensive and the hypertensive populations by gender.

| | | Hypertensive (total) | Normotensive | value | Hypertensive | | value |
|---|---|---|---|---|---|---|---|
| | | | | | Male ( = 729) | Female ( = 529) | |
| | Southern Harbour | 585 | 215 | | 124 | 91 | |
| | Northern Harbour | 731 | 340 | | 195 | 145 | |
| Locality | Southeastern | 382 | 220 | **0.0001** | 122 | 97 | **0.0001** |
| | Western | 360 | 186 | | 109 | 77 | |
| | Northern | 423 | 163 | | 91 | 72 | |
| | Gozo | 208 | 134 | | 87 | 46 | |
| | Low educational level | 361 | 273 | | 160 | 201 | |
| Education | Medium educational level | 497 | 1034 | **0.0001** | 284 | 213 | **0.0001** |
| | High educational level | 399 | 1382 | | 285 | 114 | |
| Employment status | Employed | 625 | 1898 | **0.0001** | 473 | 153 | **0.0001** |
| | Not in employment | 632 | 791 | | 256 | 376 | |
| Smoking habit | Smoking | 268 | 691 | **0.003** | 192 | 76 | **0.001** |
| | Nonsmoking | 990 | 1999 | | 537 | 453 | |
| Alcohol consumption | Alcohol consumption | 1487 | 625 | **0.001** | 458 | 167 | **0.0001** |
| | No-alcohol consumption | 1203 | 633 | | 271 | 361 | |
| | No activity | 290 | 98 | | 45 | 53 | |
| Physical activity | Walk | 522 | 230 | **0.015** | 138 | 91 | 0.084 |
| | Moderate activity | 1585 | 783 | | 458 | 325 | |
| | Vigorous activity | 292 | 147 | | 88 | 59 | |

Chi-square.

Table 4: Demonstrates the crude and adjusted sociodemographic independent variables for the development of hypertension.

| Sociodemographic factors | Crude analysis | | | Adjusted analysis | | |
|---|---|---|---|---|---|---|
| | OR | CI | value | OR | CI | value |
| Male | 1.521 | 1.332–1.737 | **0.0001** | 1.83 | 1.544–2.168 | **0.0001** |
| Female | Reference | | | Reference | | |
| High educational level | 0.218 | 0.181–0.264 | **0.0001** | 0.935 | 0.731–1.195 | 0.592 |
| Medium educational level | 0.369 | 0.307–0.445 | **0.0001** | 0.923 | 0.741–1.149 | 0.471 |
| Low educational level | Reference | | | Reference | | |
| No-alcohol use | 1.247 | 1.093–1.423 | **0.001** | 1.018 | 0.866–1.197 | 0.827 |
| Alcohol use | Reference | | | Reference | | |
| Nonsmoking | 1.285 | 1.097–1.505 | **0.002** | 0.981 | 0.815–1.181 | 0.839 |
| Smoking | Reference | | | Reference | | |
| Vigorous activity | 1.433 | 1.065–1.929 | **0.018** | 1.492 | 1.057–2.104 | **0.023** |
| Moderate activity | 1.41 | 1.111–1.789 | **0.005** | 1.341 | 1.023–1.758 | **0.034** |
| Walk | 1.264 | 0.965–1.655 | 0.09 | 1.143 | 0.842–1.553 | 0.391 |
| No activity | Reference | | | Reference | | |
| Gozo | 1.727 | 1.329–2.245 | **0.0001** | 1.771 | 1.303–2.408 | **0.0001** |
| Northern | 1.032 | 0.817–1.304 | 0.794 | 1.124 | 0.857–1.474 | 0.399 |
| Western | 1.424 | 1.125–1.802 | **0.003** | 1.473 | 1.121–1.937 | **0.005** |
| Southeastern | 1.541 | 1.229–1.9332 | **0.0001** | 1.674 | 1.287–2.178 | **0.0001** |
| Northern Harbour | 1.241 | 1.02–1.51 | **0.031** | 1.204 | 0.961–1.509 | 0.107 |
| Southern Harbour | Reference | | | Reference | | |
| Not in employment | 2.421 | 2.114–2.772 | **0.0001** | 1.181 | 0.974–1.432 | 0.091 |
| Employed | Reference | | | Reference | | |
| BMI | 1.127 | 1.113–1.141 | **0.0001** | 1.104 | 1.088–1.210 | **0.0001** |
| Age | 1.07 | 1.07–1.08 | **0.0001** | 1.066 | 1.058–1.073 | **0.0001** |

Reference category.

the lowest in America (35%), with the European region prevalence rate reported to be somewhere in between these two continents [11, 12]. The male gender, in our study, exhibited the highest hypertension prevalence, which is consistent with what is normally found within high-income countries [13].

On comparing the hypertension prevalence found in our study to a Pan-European study conducted in 5 Euro-pean countries, Malta exhibited the highest hypertension prevalence [14]. This Pan-European study had reported that Germany had the highest hypertension prevalence among UK, Italy, France, and Spain. Malta showed higher prevalence rates for each gender (Germany male: 25.1%, female: 22.9%; Malta male: 36.49%, female: 27.14%), although one needs to keep in mind that different age bands (Malta study: 18 to 70 years, Pan-European study: 20–79 years) were examined in both studies [14]. Conversely, our study population reported Maltese hypertensives having a higher antihypertensive phar-macological prescription frequency when compared to the average European prescription frequency [15, 16]. Nonad-herence to antihypertensive medication is a global issue and leads to loss of clinical ef f icacy as well as to loss of economic ef f iciency resulting in an increase in hospitalization costs [16].

Hypertension determinants are well established and include age, gender, sociodemographic factors, obesity, smoking, excess alcohol, and sedentary lifestyle [17, 18]. On univariant analysis, education level, which is considered as the most stable determinant of socioeconomic status, was found to exhibit an associated protective effect against having hypertension in our study. This effect was lost on adjusting for age and gender, which could be the result of a change in education opportunities over time and more females are continuing their education. This finding is inconsistent with other association reports where the level of education was found to have an inverse association with blood pressure [19, 20].

The female gender in our study was found to exhibit an associated protective effect on hypertension. This is partially consistent with findings from other studies within other high-income countries [21]. However, this protective effect exhibited by our female population was previously reported in middle-income countries and not high-income countries like Malta [22–24].

Intense physical activity was found to increase the risk of having hypertension in our study, which is not consistent with the literature [25]. A possible reason to this f inding follows the fact that the Maltese population is well known to be at high risk of metabolic diseases. A proportion of the population follows lifestyle interventions to try to halt the development of these noncommunicable diseases, which may have led to this finding.

A link was established between three different districts (Gozo, Western, and Northern Harbour) and having hypertension. Further research is required to assess whether an environmental or a genetic cause may be contributing to this relationship. However, a possible explanation for the link between the Northern Harbour and hypertension could result from the fact that the majority of the Maltese popula-tion lives in this district.

A link has been reported between alcohol consumption and blood pressure, with chronic drinkers having a longer impact on blood pressure outcomes [26]. A linear relationship had been reported between alcohol intake and blood pressure [27]. This was not the case in our study, where non-alcohol drinkers were found to be at risk of having hypertension. However, this associated effect of alcohol consumption on hypertension development was lost once various confounder factors were adjusted for, suggesting that no direct link between alcohol and hypertension was evident in our population.

## 6. Study Limitations and Strengths

The response rate for the health examination was rather low due to the invasive blood measurement required, which may have affected the results. The demographic data and lifestyle habits were self-reported by participants. This predisposes the risk of human bias or inaccurate recollection of information. Blood pressure measurement is very sensitive; accuracy may have been affected by the behaviour of the participant, the environment of the setup, and the aneroid sphygmomanome-ter device used even though sphygmomanometers were calibrated regularly. Auscultation fieldworker observer errors may have occurred including systematic error in auscultation method if the observer did not hear well enough or had slow reactions to auditory and visual cues, even though fieldworkers were trained and their readings double checked with other experienced blood pressure measurers prior to initiation of the fieldwork. Measurement of blood pressure was taken after 20 minutes of sitting down; this avoided any blood pressure fluctuations. All participants were asked to sit up straight with both feet touching the f loor and their arms resting at the heart level, where these techniques were found to enable more accurate readings. The study was a cross-sectional study, which does not adjust for changes in behaviour af ter the diagnoses of hypertension or other diseases. There is thus great difficulty in linking exposures with outcomes from this study in any causative way.

## 7. Conclusion

Approximately a third of the adult population in Malta (mostly males) suffered from hypertension. Aging males, increase in BMI, and living in Gozo, Western district, and Northern Harbour exhibited a higher associated risk to develop hypertension. Education levels were not found to be associated with the hypertension development.

## Disclosure

The funding body had no influence on the protocol of the survey or on the data analysis. No author received any personal funding.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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RESEARCH ARTICLE

# The interaction of dyslipidaemia with glycaemia in an adult population study

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## Abstract

**Purpose** Individuals with dysglycaemic are prone to dyslipidaemia. Understanding the dyslipidaemic status of dysglycaemic individuals is essential for monitoring and early prevention. The aim was to assess the control of lipidaemia by glycaemic status in a representative adult population.

**Methods** A retrospective health examination survey was performed on a sample of adults ($n = 3947$) in Malta in 2014–6. Sociodemographic data, biochemistry blood tests and anthropometric measurements were gathered. Statistical analysis was performed to evaluate the lipidaemic status and its control across the glycaemic spectrum (normoglycemic, impaired fasting glucose individuals, new diabetics and known diabetics).

**Results** The prevalence of *uncontrolled dyslipidaemi*a was 7.75% (CI 95%: 6.69–8.63), among whom 6.97% (CI 95%: 6.21–7.81) were naïve dyslipidaemic. A progressive elevation in both LDL-C and total cholesterol but not triglycerides was present among *uncontrolled dyslipidaemi*a individuals across the glycaemic spectrum. *Global dyslipidaemia* was present in 19.26% (CI 95%: 18.05–20.52) of the total general population and in 46.59% (CI 95%: 40.49–52.69%) of known diabetics. Most individuals irrespective of lipid status were normoglycaemic.

**Conclusions** Dyslipidaemia occurs in the presence of insulin resistance. Dyslipidaemia predominated in the normoglycaemic state irrespective of statins use, indicating the need to manage dyslipidaemia prior to dysglycaemia.

**Keywords** Hyperlipidaemia · Diabetes mellitus, type 2 · Insulin resistance, policy · Epidemiology · Malta

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## Introduction

Understanding the dyslipidaemic status and its metabolic correlations at population level is essential for monitoring of health status, planning and evaluating healthcare. Dyslipidaemia is the presentation of combined elevation of low-density lipoprotein cholesterol (LDL-C) level and an elevated triglyceride level and a decreased high-density lipoprotein cholesterol (HDL-C) level [1]. This is a common occurrence in dysglycaemic individuals, especially among those with an established diabetes mellitus type 2, also known as diabetic dyslipidaemia [2]. Furthermore, dyslipidaemia is a well-established contributor to the development of cardiovascular disease especially in dysglycaemic individuals [2, 3]. Individuals suffering from diabetes mellitus have been reported to have a 2- to 4-fold increased risk for the developing of cardiovascular disease. In fact, diabetes has been identified as a coronary artery disease risk factor [4, 5].

Individuals with diabetes tend to lose the ability to metabolise lipids and lipoproteins, increasing the risk of elevated low-density lipoprotein (LCL-C), elevated triglycerides and a

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decreased level of high-density lipoprotein (HDL-C) [6]. The diabetic LDL-C particle tend to be smaller and denser due to the simultaneous presence of high triglyceride levels. This contributes to the documented "atherogenic lipid pattern", which is present in both pre-diabetic and diabetic individuals (impaired fasting glucose and impaired glucose tolerance) [7, 8]. These smaller, denser LDL-C particles have greater ability to penetrate into the blood vessels, thereby potentiating the risk for thrombus formation [9]. A reduction of 1 mmol/L of the mean LDL-C plasma level at population level is thought to contribute to a 21% risk reduction in cardiovascular mortality [10, 11].

The most commonly used lipid-lowering medications are statins. Statins block the 2-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) conversion to mevalonic acid and therefore limit cholesterol synthesis [12]. In turn, lower hepatic cholesterol levels result, leading to an increase in low-density lipoprotein (LDL)-receptor expression in hepatocytes. This leads to an enhanced LDL-particle clearance from the blood [13]. Statins also act on reducing the triglyceride levels whilst increasing the high-density lipoprotein [12, 14].

The Mediterranean Island of Malta has been reported to have high diabetes mellitus type 2, obesity and hypertension prevalence rates when compared to other European countries [15–18]. This makes the adult population of Malta an ideal cohort in which to analyse the effects of glycaemia on lipidaemic status. Furthermore, considering that the total population of Malta is less than half a million and that the Maltese Islands are small (316Km [2]), the short distances between towns made it feasible for a nationally representative health examination survey to be conducted with all participants undergoing fasting lipid profile testing. Commonly, epidemiological studies are unable to undergo such an extensive examination at a population level [3].

The aim of this study was to assess the glycaemic status of the population in relation to their lipidaemia control with or without the use of statins, while establishing the prevalence of *uncontrolled* and *global* dyslipidaemia among the high-risk Maltese population. The hypothesis was that as the glycaemic spectrum shifts from normoglycaemia to full-blown diabetes mellitus, the lipid profile would become more dyslipidaemic without the use of statins, while it would stay more within the normal range for those on statins. Such data can facilitate a comparative review by neighbouring Mediterranean countries as well as among other high diabetes prevalence countries. The data will aid in the identification of dyslipidaemic changes at population level which may relate to the onset of cardiometabolic complications and to understand the relationships between dyslipidaemia and dysglycaemia.

## Method

A retrospective cross-sectional survey on diabetes mellitus type 2 was conducted between November 2014 and January 2016 under the auspices of the University of Malta. The detailed study protocol was described elsewhere [19]. In summary, a nationally representative health examination survey was performed on a randomised, stratified sample. Stratification was affected by age (18–70 years), gender and town, with individuals selected from across all towns within the Maltese Islands. The sample population under study represented approximately 1% of each town's adult population. Blood tests performed as part of the health survey included fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and total cholesterol (TC). Informed written consent was obtained from every participant. Ethical and data protection approvals were granted from the University of Malta Research Ethical Committee (UREC) and the Information and Data protection national commissioner respectively.

The segment of the study population obtaining a dyslipidaemic status (high LDL-C + high triglycerides + low HDL-C levels) at the time of the health examination survey was labeled as *uncontrolled dyslipidaemia.* The dyslipidaemia status was defined as an elevated LDL-C of ≥3 mmol/l, an elevated triglyceride (TG) level of ≥1.69 mmol/L and a low HDL-C level of ≤1.03 mmol/L for males and ≤ 1.29 mmol/L for females [5, 20, 21]. The label of *global dyslipidaemia* was given to the proportion of the study population that reported to be on statin medication irrespective of current measured lipid profile, in combination with those participants that were found to have dyslipidaemia during the health examination survey but were not on statin medication. Therefore, this population (global dyslipidaemia) was hypothesized to represent the whole Maltese dyslipidaemic population with potential atherogenic changes and susceptibility to dysglycaemia.

The study population was subdivided into four glucose regulatory subgroups, namely normoglycaemia, impaired fasting glucose (IFG), newly diagnosed diabetes mellitus (NDM) and previously known diabetes mellitus (KDM) subgroups. This was performed in order to represent a continuum in the transition from normal to disordered glucose metabolism, which enabled a more accurate biochemical analyses across the glucose transition. The subdivision was based on the fasting blood glucose results obtained during the health examination survey while incorporating any self-reported history of diabetes mellitus. Those participants obtaining a fasting blood glucose (FBG) level between 5.6–6.9 mmol/L and not reporting to be on oral hypoglycaemic agents were labeled as *Impaired Fasting Glucose* (IFG). All IFG individuals reported to have not been aware of their dysglycaemia before the health examination survey. Those participants with a FBG > =7 mmol/L were labeled as *Newly diagnosed diabetes mellitus* (NDM), provided they were not previously diagnosed as diabetics or were on oral hypoglycaemic agents [22]. Identifying newly diagnosed diabetics following a single fasting blood glucose reading is a common practice in population-based health examination surveys [23]. The

participants with a previous history of diabetes mellitus or on oral hypoglycemic agents, irrespective of their measured fasting plasma glucose, were labeled as cases of *known diabetes mellitus* (KDM). Those individuals who did not fall within these glucose dysglycaemic categories were considered as having normoglycaemia.

The Kolmogorov-Smirnov test for normality confirmed that the blood test measurements for the population were not normally distributed. Statistical analyses using non-parametric tests were performed using IBM SPSS version 21. The median and interquartile ranges (IQR) were calculated for the lipid profile variables within each of the four-glycaemic subgroups. The Kruskal-Wallis test was performed to establish any significant differences between each lipid profile variable and the corresponding glycaemic subgroup within the different dyslipidaemic populations (uncontrolled and global). Pairwise comparisons (Dunn's test) between the four-glycaemic subgroups for each dyslipidaemic population were performed.

## Results

A total sample population of 3947 adults was included in the study after weighting for non-responders (response rate of 49%, $p = <0.05$). The prevalence of high LDL-C levels was 51.25% (CI 95%: 49.69–52.81), that of high triglyceride levels was 15.05% (CI 95%: 13.97–16.20) and that of high cholesterol levels was 52.31% (CI 95%: 49.19–52.31) for the entire population. The prevalence of *uncontrolled dyslipidaemi*a (high LDL-C + high TG + Low HDL-C) at the point of the study was of 7.75% (CI 95%: 6.69–8.63). The *uncontrolled dyslipidaemi*a population was composed of naïve dyslipidaemic individuals ($n = 275$) and individuals reported to be on statins ($n = 485$) yet still with uncontrolled dyslipidaemia during the examination ($n = 31$). Thus, the 7.75% of prevalent current uncontrolled dyslipidemia consisted of a proportion with naïve dyslipidaemia - 6.97% (CI 95%: 6.21–7.81) and a proportion of those with known dyslipidaemia, on statins and yet uncontrolled - 0.79% (CI 95%: 0.55–1.12). The naïve dyslipidaemia sub-population was predominantly male (76.73% CI 95%: 71.37–81.35) aged between 30 and 70 years.

Considering that the *uncontrolled dyslipidaemi*a population was predominantly composed of naïve dyslipidaemic individuals, it was hypothesized that this population represented the uncontrolled lipidaemia adult population of Malta who remained mostly without dyslipidaemic awareness and without the effect of statins.

Participants on statins may have been taking these due to either a known dyslipidaemic status or else had been started as a preventive measure following certain conditions such as cardiovascular disease or at onset of diabetes mellitus [24]. Regrettably, data pertaining to the reason the participants were on statins was not available.

The *global dyslipidaemia* population contributed to 19.26% (CI 95%: 18.05–20.52) of the total general population. The *global dyslipidaemic* population was considered as representative of the total adult population of Malta with a dyslipidaemic status with or without statin medication at population level. This *global dyslipidaemia* population consisted of the combination of individuals that were already on statin treatment ($n = 485$, out of which $n = 31$ had uncontrolled dyslipidaemia on examination) in addition to naïve cases ($n = 275$).

*Global dyslipidaemia* was found to be present in 46.59% (CI 95%: 40.49–52.69%) of this study population's known diabetes sub-population.

Both populations (*uncontrolled* and *global* dyslipidaemia) were sub-categorised according to their glycaemic status that was established during the health examination survey, as seen in Table 1. For both the dyslipidaemic populations (*uncontrolled* and *global*), the majority of the individuals exhibited a normoglycaemic status (FBG <5.6 mmol/L with no history of diabetes) with a female predominance, followed by impaired fasting glucose (IFG) with a male predominance. As expected, the newly diagnosed diabetic (NDM) subgroup exhibited a slightly higher proportion with *uncontrolled dyslipidaemia* when compared to previously known diabetics (KDM). This follows the standard practice where statin therapy is initiated with the onset of diabetes mellitus [25]. This finding contrasted with the *global dyslipidaemia sub*-population, where the KDM sub-group contributed a higher proportion when compared to the NDM subgroup.

Table 2 illustrates the reported statin use across the different glycaemic status sub-groups in relation to the two dyslipidaemic (*uncontrolled* and *global*) populations while being contrasted with the general population. A progressive steady increase in statin use was observed within the general population across the glycaemic spectrum from normoglycaemia to previously known diabetes mellitus. However, on evaluating those with *uncontrolled dyslipidaemia* status, the IFG subgroup individuals reported a predominance for statin medication. Interestingly 2.94% (CI 95%: 1.47–5.58) of the *uncontrolled dyslipidaemic* population was cognizant of their diabetes mellitus status and were already on statins, yet still had uncontrolled dyslipidaemia. The highest reported statin use within the *global dyslipidaemic* population was amongst the normoglycaemic sub-group, which coincides with the established fact that the *global dyslipidaemic* population was predominated by the normoglycaemic status.

### Uncontrolled dyslipidaemia population

The median lipid profile components of the *uncontrolled dyslipidaemia* population were evaluated across the

**Table 1** Summarizes the different lipid status sub-population according to their glycaemic status

| Population | | Glycaemic status | | | |
|---|---|---|---|---|---|
| | | Normoglycemic | IFG | NDM | KDM |
| Uncontrolled dyslipidaemia ($n = 306$) | Total | 49.35% | 32.03% | 9.48% | 9.15% |
| | Female ($n = 77$) | 61.04% | 28.57% | 2.60% | 7.79% |
| | Male ($n = 229$) | 45.41% | 33.19% | 11.79% | 9.61% |
| Global dyslipidaemia* ($n = 760$) | Total | 40.39% | 27.37% | 10.39% | 21.84% |
| | Female ($n = 285$) | 46.32% | 26.32% | 7.02% | 20.35% |
| | Male ($n = 475$) | 36.84% | 28.00% | 12.42% | 22.74% |
| Normal lipidaemia** ($n = 1550$) | Total | 75.74% | 1.61% | 7.16% | 15.48% |
| | Female ($n = 824$) | 81.92% | 1.21% | 5.10% | 11.77% |
| | Male ($n = 726$) | 68.73% | 2.07% | 9.50% | 19.70% |
| General ($n = 3947$) | Total | 66.28% | 23.44% | 4.03% | 6.31% |
| | Female ($n = 1949$) | 74.50% | 18.57% | 2.82% | 4.16% |
| | Male ($n = 1998$) | 58.26% | 28.18% | 5.21% | 8.41% |

*Global dyslipidaemia – the combination of uncontrolled dyslipidemia found during health examination, controlled dyslipidaemia found during health examination but on reported statin treatment

**Normal LDL-C + Triglycerides + HDL-C

*IFG*, Impaired fasting glucose; *NDM*, Newly diagnosed diabetes mellitus; *KDM*, Previously known diabetes mellitus

glycaemic transition, from normoglycaemia to full-blown known diabetes mellitus, as seen in Table 3. As expected the new DM sub-group exhibited a significantly higher LDL-C and total cholesterol levels when compared to all other glycaemic status groups (except when total cholesterol was compared to normoglycaemic sub-group). There was no significant difference between the lipid profile components of the normoglycaemic and the impaired fasting glucose sub-categories. On further sub-analysing the *uncontrolled dyslipidaemia* population by gender, the female population showed a tendency for higher lipid profile components when compared to the male proportion within the normoglycaemic (except for triglycerides), NDM and KDM sub-groups, as seen in Table 4.

## Global dyslipidaemia population

The median lipid profile components of the *global dyslipidaemic* population across the glycaemic status are shown in Table 5. The previously known diabetes (KDM) dyslipidaemic sub-group exhibited significantly lower LDL-C, triglycerides and total cholesterol levels when compared to normoglycaemic, IFG and newly diagnosed (NDM) sub-groups. Conversely, similarities were present across all lipid profile components between normoglycaemic, IFG and NDM dyslipidaemic sub-groups. On sub-analysing the *global dyslipidaemic* population by gender, similarities between the females and males were evident. However, across all the glycaemic spectrum females exhibited significantly higher HDL-C levels than did males, as seen in Table 6.

### Uncontrolled dyslipidaemia vs. global dyslipidaemia populations

On comparing the lipid profile components of the *uncontrolled dylipidaemia* population to *the global dyslipidaemia* population, significant differences were found between the lipid profile components across all the glycaemic status sub-groups, as shown in Tables 3, 4, 5, and 6. The median LDL-C, triglycerides and total cholesterol values were significantly higher within the *uncontrolled dylipidaemia* population when compared to the *global dyslipidaemia* population. This held true also on gender stratification ($p = <0.01$ respectively). The HDL-C median levels were significantly lower within the *uncontrolled dylipidaemia* population when compared to the

**Table 2** Summarises the reported statin uses across the different glycaemic status, by the different lipidaemia populations under study

| Populations | Glycaemic status - reported to be on statins | | | | Total on statin |
|---|---|---|---|---|---|
| | Normoglycemic | IFG | NDM | KDM | |
| Uncontrolled dyslipidaemia ($n = 306$) | 1.63% | 4.90% | 0.65% | 2.94% | 10.13% |
| Global dyslipidaemia* ($n = 760$) | 21.18% | 16.45% | 6.71% | 19.34% | 63.68% |
| Normal lipidaemia ($n = 1550$) | 4.97% | 0.77% | 5.42% | 2.58% | 13.74% |
| General ($n = 3947$) | 6.15% | 13.51% | 32.08% | 59.44% | 12.29% |

*Global dyslipidaemia – the combination of uncontrolled dyslipidemia found during health examination, controlled dyslipidaemia found during health examination but on reported statin treatment

**Table 3** Uncontrolled dyslipidaemic population median lipid profile components, by glycaemic status

| | Uncontrolled dyslipidaemia | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normoglycemic ($n = 151$) | IFG ($n = 98$) | NDM ($n = 29$) | KDM ($n = 28$) | *Kruskal-Wallis* | *Dunn's test* | | | | | |
| | Median (IQR) | Median (IQR) | Median (IQR) | Median (IQR) | p value | p value1 | p value2 | p value3 | p value4 | p value5 | p value6 |
| Age (years) | 40 (18) | 57 (19) | 51 (19) | 55 (17) | **<0.01** | **<0.01** | **0.01** | **<0.01** | 1 | 1 | 1 |
| LDL (mmol/L) | 3.83 (1.09) | 3.85 (1.02) | 4.17 (1.52) | 3.43 (0.84) | **<0.01** | 1 | **<0.01** | **0.01** | **<0.01** | 0.08 | **<0.01** |
| HDL (mmol/L) | 1.09 (0.24) | 1.02 (0.23) | 1.02 (0.25) | 0.98 (0.17) | **0.01** | 0.17 | 0.16 | 0.06 | 1 | 1 | 1 |
| Triglycerides (mmol/L) | 2.16 (0.91) | 2.49 (1.29) | 2.20 (1.24) | 2.21 (0.97) | 0.18 | | | | | | |
| Total Cholesterol (mmol/L) | 6.05 (1.02) | 6.10 (0.96) | 6.47 (1.66) | 5.61 (0.89) | **<0.01** | 1 | 0.18 | **<0.01** | **0.04** | **0.01** | **<0.01** |
| BMI (Kg/m2) | 30.80 (7.90) | 30.00 (5.90) | 31.10 (11.38) | 29.30 (7.69) | 0.84 | | | | | | |

*p value1*, Normoglycaemic vs. IFG; *p value2*; Normoglycaemic vs. NDM; *p* value3, Normoglycaemic vs. KDM; *p value4*, IFG vs. NDM; *p value5*, IFG vs. KDM; *p value6*, NDM vs. KDM; *IQR*, Interquartile range; *IFG*, Impaired fasting glucose; *NDM*, Newly diagnosed diabetes mellitus; *KDM*, Known diabetes mellitus

**Table 4** Uncontrolled dyslipidaemic population median lipid profile components, by glycaemic status and gender

| | Normoglycaemic | | *p* value* | IFG | | *p* value* | NDM | | *p*-value* | KDM | | *p*-value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female ($n = 47$) Median (IQR) | Male ($n = 104$) Median (IQR) | | Female ($n = 22$) Median (IQR) | Male ($n = 76$) Median (IQR) | | Female ($n = 2$) Median (IQR) | Male ($n = 27$) Median (IQR) | | Female ($n = 6$) Median (IQR) | Male ($n = 22$) Median (IQR) | |
| Age (years) | 45 (23) | 40 (18) | 0.99 | 63 (18) | 55 (21) | **<0.01** | 65 (10) | 51 (19) | **0.01** | 61 (10) | 52 (17) | 0.11 |
| LDL-C (mmol/L) | 4.05 (1.08) | 3.76 (1.00) | **<0.01** | 3.98 (1.08) | 3.85 (0.95) | 0.66 | 5.65 (0.89) | 4.17 (0.92) | **0.01** | 3.34 (0.21) | 3.75 (0.84) | 0.13 |
| HDL-C (mmol/L) | 1.13 (0.28) | 1.08 (0.26) | **0.01** | 1.03 (0.12) | 0.99 (0.22) | 0.24 | 1.12 (0.25) | 1.02 (0.24) | **0.01** | 1.29 (0.25) | 0.88 (0.14) | **<0.01** |
| Triglycerides (mmol/L) | 1.95 (0.57) | 2.19 (0.86) | **<0.01** | 2.42 (1.11) | 2.70 (1.33) | 0.60 | 4.49 (1.26) | 2.11 (1.24) | **0.02** | 2.60 (0.88) | 1.80 (0.78) | **0.03** |
| Total Cholesterol (mmol/L) | 6.40 (0.89) | 5.88 (1.02) | **0.01** | 6.22 (0.80) | 6.06 (1.03) | 0.54 | 8.69 (1.33) | 6.47 (1.35) | **0.01** | 5.69 (0.61) | 5.61 (0.89) | 0.14 |

*Mann-Whitney U test

**Table 5** Global dyslipidaemic population median lipid profile components, by glycaemic status

| | Global dyslipidaemia | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normoglycemic ($n = 307$) | IFG ($n = 208$) | NDM ($n = 79$) | KDM ($n = 166$) | *Kruskal-Wallis* | *Dunn's test* | | | | | |
| | Median (IQR) | Median (IQR) | Median (IQR) | Median (IQR) | p-value | p-value1 | p-value2 | p-value3 | p-value4 | p-value5 | p-value6 |
| Age (years) | 54 (24) | 59 (12) | 62 (14) | 64 (10) | **<0.01** | **<0.01** | **<0.01** | **<0.01** | 1 | **0.02** | 0.54 |
| LDL (mmol/L) | 3.37 (1.19) | 3.32 (1.21) | 3.60 (1.45) | 2.47 (1.07) | **<0.01** | 1 | 1 | **<0.01** | 1 | **<0.01** | **<0.01** |
| HDL (mmol/L) | 1.23 (0.47) | 1.18 (0.49) | 1.12 (0.48) | 1.23 (0.65) | 0.25 | | | | | | |
| Triglycerides (mmol/L) | 1.84 (1.15) | 1.77 (1.48) | 1.83 (1.50) | 1.35 (0.90) | **<0.01** | 1 | 0.46 | **<0.01** | 0.77 | **<0.01** | **<0.01** |
| Total Cholesterol (mmol/L) | 5.63 (1.43) | 5.43 (1.46) | 5.47 (1.55) | 4.53 (1.34) | **<0.01** | 1 | 1 | **<0.01** | **<0.01** | 1 | **<0.01** |
| BMI (Kg/m2) | 28.83 (6.90) | 29.61 (5.77) | 31.40 (9.98) | 31.05 (6.03) | **<0.01** | 0.1 | **<0.01** | **<0.01** | **0.05** | 1 | 0.7 |

*p-value1*, Normoglycaemic vs. IFG; *p value2*, Normoglycaemic vs. NDM; *p value3*, Normoglycaemic vs. KDM; *p value4*, IFG vs. NDM; *p value5*, IFG vs. KDM; *p value6*, NDM vs. KDM; *IQR*, Interquartile range; *IFG*, Impaired fasting glucose; *NDM*, Newly diagnosed diabetes mellitus; *KDM*, Known diabetes mellitus

**Table 6** Global dyslipidaemic population median lipid profile components, by glycaemic status and gender

| | Normoglycaemic | | *p*-value* | IFG | | *p*-value* | NDM | | *p*-value* | KDM | | *p*-value* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female ($n = 132$) Median (IQR) | Male ($n = 175$) Median (IQR) | | Female ($n = 75$) Median (IQR) | Male ($n = 133$) Median (IQR) | | Female ($n = 20$) Median (IQR) | Male ($n = 59$) Median (IQR) | | Female ($n = 58$) Median (IQR) | Male ($n = 108$) Median (IQR) | |
| Age (years) | 58 (20) | 51 (37) | **<0.01** | 63 (10) | 58 (15) | **<0.01** | 64 (8) | 60 (14) | **0.02** | 64 (8) | 64 (13) | 0.92 |
| LDL-C (mmol/L) | 3.10 (1.32) | 3.50 (1.10) | 0.16 | 3.32 (1.13) | 3.37 (1.23) | 0.44 | 3.43 (1.64) | 3.66 (1.14) | 0.80 | 2.36 (0.91) | 2.62 (1.18) | 0.61 |
| HDL-C (mmol/L) | 1.37 (0.51) | 1.13 (0.30) | **<0.01** | 1.44 (0.60) | 1.12 (0.31) | **<0.01** | 1.47 (0.26) | 1.09 (0.36) | **<0.01** | 1.48 (0.44) | 1.03 (0.44) | **<0.01** |
| Triglycerides (mmol/L) | 1.46 (0.97) | 1.97 (1.27) | **<0.01** | 1.40 (1.23) | 1.81 (1.54) | **<0.01** | 1.44 (1.17) | 1.85 (1.29) | 0.21 | 1.30 (0.75) | 1.46 (0.91) | 0.17 |
| Total Cholesterol (mmol/L) | 5.41 (1.74) | 5.66 (1.36) | 0.98 | 5.41 (1.35) | 5.50 (1.65) | 0.59 | 5.47 (2.04) | 5.48 (1.46) | 0.61 | 4.61 (1.26) | 4.48 (1.55) | 0.26 |

*Mann-Whitney U test

*global dyslipidaemia* population, even after gender stratification ($p = <0.01$ respectively). Of note was the fact that the *global dyslipidaemia* population was significantly older (in years) when compared to the *uncontrolled dylipidaemia* population.

## Discussion

Dyslipidemia is a physiological occurrence in the presence of insulin resistance with or without the presence of hyperglycaemia [26, 27]. Lipoprotein abnormalities typically initiate in the pre-diabetic state [28]. In our study, the highest population proportion contributing to both the *uncontrolled* and *global* dyslipidaemic populations were found to have normoglycaemic status. However, when compared, the normoglycaemic and the IFG (pre-diabetes) lipid profile components were found to be similar with both exhibiting a high triglyceride level. This may suggest underlying insulin resistance that will lead to a shift from normoglycaemia to dysglycaemia in a matter of time even though the current FBG was within the normal range. In fact, it has been reported that dysregulation of lipids indicates underlying pathophysiological abnormalities such as insulin resistance and abdominal obesity which contribute to hyperinsulinaemia. This in turn leads to hyperglycaemic status and enhances hepatic gluconeogenesis and glucose output. Furthermore, a reduction in the suppression of adipose tissue lipolysis occurs resulting in an eventual hypertriglyceridemia and reduced HDL-C levels [29]. In our study, the body mass indexes (BMI) of both the normoglycaemic and IFG sub-groups were found to have comparable obesity states, which further supports the fact that insulin resistance may have been present. It is a well-known fact that both insulin resistance and obesity are features of the metabolic syndrome that is involved in both the lipid and glucose metabolism [21, 30].

The *uncontrolled dyslipidaemia* population lipid components showed a progressive median incline across the glycaemic spectrum (Normoglycaemia-IFG vs. NDM) for both the LDL-C and total cholesterol levels but not for triglycerides. The triglyceride levels across the glycaemic spectrum, although elevated, did not show any significant changes across the glycaemic spectrum, which is not in keeping with the literature [31].

Considering that the pre-diabetic subgroup (IFG) within our study reported to have not been previously aware of their dysglycaemia; it can be hypothesized that the use of statins (within this subgroup) prior to their knowledge of dysglycaemia suggests that diabetic dyslipidaemia had already started to develop and supports the literature in that lipoprotein changes occur in the pre-diabetic state [28]. In fact, this observation was further supported by the progressive statin usage trend across the glycaemic spectrum within our study population. It is documented that as dysglycaemia appears more evident, so too does dyslipidaemia and/or related co-morbidities such as cardiovascular disease [32]. For this reason, initiating statin therapy as a preventive measure against dyslipidaemic complications is a common practice along with lifestyle management for newly diagnosed diabetics [24]. It is important to note that lower lipid profile component targets are set for certain conditions, such as in dysglycaemic individuals [5]. However, a considerable number of individuals treated with statins do not achieve the treatment goal values envisaged by physicians. This could be observed in our study, where a percentage of previously known diabetic individuals were found to have uncontrolled dyslipidaemic despite their statin medication [3]. One possible reason for the presence of uncontrolled dyslipidaemia in this group is non-adherence to medication. This is a common public health challenge with as many as 50% of patients discontinuing their medications within a year of initial prescription [33, 34]. Such practices could result in long-term complications, co-morbidities and premature mortality, which also incur additional costs to the health care system [35]. This non-adherence to medication may explain why not every previously known diabetic individual attending our study reported to be on statin medication. This follows the fact that it is of standard practice in Malta and internationally that all newly diagnosed diabetic individuals are started on lifestyle modifications followed by statin treatment [25, 36]. It appeared that within our KDM population, the males were even less adherent to their medication when compared to their female counterparts as age progressed. Conversely, non-adherence to medication was reported to be multi-factorial and gender-specific [37, 38]. However, the lack of data on the heterogeneity of statin dose among the compliant participants may have had an effect on the study's outcome.

## Conclusions

Understanding the dylipidaemic pathophysiology with its early onset prior to the development of any co-morbidities, including dysglycaemia, is essential. Our study provides the evidence that dyslipidaemia predominates in normoglycaemic states irrespective of statins use, with those on statin medications having lower lipid profile components. Initiating educational outreaches to the population to undergo medical check-ups for the presence of dyslipidaemia, especially in high-risk populations, is essential. Physicians should supplement lifestyle modification with statin therapy depending on the plasma lipid profile results. Managing dyslipidaemia in its early stages, prior to the presence of dysglycaemia should be the norm according to current clinical guidelines. This could prevent co-morbidities including diabetes and cardiovascular diseases from developing, as well as lowering related mortality rates.

## Study limitations

The study has the usual temporal limitations associated with cross-sectional studies i.e. it is unable to predict the exposure, disease onset and outcome time relationships because both have been collected at the same time. Establishing a diagnosis of diabetes mellitus following a single fasting blood glucose has been reported to be satisfactory for an epidemiological study, such as this one. However, such a protocol could have erroneously diagnosed a proportion of pre-diabetic individuals as newly diagnosed diabetics since a second confirmatory test was not conducted. Data on the type and dose of statin therapy, as well as the reason for statin prescription were not available and therefore could not be taken into consideration during data analysis and interpretation. Oral glucose tolerance testing was not conducted as part of the population health examination survey and therefore impaired glucose tolerance (IGT) status could not considered as part of the pre-diabetes status in this study. Furthermore, since this data is self-reported, human error in drug medication reporting as well as regarding drug compliance to medication could have been in place. A small sample population in certain sub-groups could have had an effect on the power of the statistical comparisons and analysis and led to type 1 errors.

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## Compliance with ethical standards

**Conflict of interests** The authors declare that they have no conflict of interest.

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Review

# Type 2 diabetes – An unresolved disease across centuries contributing to a public health emergency☆

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ABSTRACT

*Aims:* Type 2 diabetes mellitus (T2DM) is a global epidemic. However, T2DM is not a new, 21st century disease. Different populations have been struggling with this disease across a number of centuries. The question lies as to why humanity has never succeeded in keeping it in check over the course of its history.
*Materials and methods:* In this review, the history of T2DM and its evolution throughout the ages are revisited. The review then investigates the growing burden of T2DM across the past fourteen years within the European continent, while comparing this epidemic with the obesity burden.
*Results:* Various explanations for the emergence of this public health epidemic were explored ranging from lifestyle factors, high sugary food and drink, disrupted sleep pattern to obesity. Over a fourteen-year period, an evident steady incline in both T2DM and obesity prevalence rates across Europe was evident.
*Conclusion:* It is essential for public health officials and researchers alike to have a good grip of the past and the present diabetes epidemiology and its co-determinants. This will provide the basis for new and improved strategies to target and prevent this epidemic.


## 1. Introduction

Type 2 diabetes mellitus (T2DM) has been declared a public health emergency back in 2015, where 7.3 billion adults were estimated to suffer from this disease [1]. In 2017, T2DM was estimated to affect 7.5 billion adults (8.8% of adults between 20 and 79 years). This rate is expected to rise to 9.9% by the year 2045 which would then affect 9.5 billion adults [2]. However, T2DM is not a disease of this era and its existence has been documented for centuries.

## 2. History of diabetes mellitus

Diabetes mellitus is a disease of antiquity with the initial description attributed to the Egyptian period. An Egyptian papyrus dating back to 1550 BC described the polyuric state. Between 400 and 500 BC, Hindau physicians Charak and Sushrut, first recognized the sweetness of the diabetic urine. They also noted that this condition was prevalent in those who over indulged in sweet and fatty food, who exhibited a sedentary lifestyle and were overweight. In the second century AD, Aretaeus of Cappadocia was the first to coin the Greek word “syphon” for “diabetes”, since he noticed a man's body was not retaining fluid but rather, the body was used as a channel to release fluid [3]. Matthew Dobson from Liverpool in 1776 was the first to describe hyperglycaemia in the serum along with the presence of sweet urine in a diabetic patient [4]. The surgeon John Rollo was the pioneer in applying the adjective “mellitus”, which is a derivative of a Latin word meaning “honey” [5]. In 1815 the French Chemist Michel Chevreul discovered that the sugar in diabetic urine was glucose, leading to a change in the diagnostic methods for diabetes, from tasting the urine to measuring of glucose levels. In fact, physician Ivar Christian Bang identified a method of measuring glucose repeatedly, leading to the development of the glucose tolerance test between 1913 and 1915 [3].

In 1869, Paul Langerhans discovered “islands” of cells within the pancreas parenchyma which, later on in 1893 were named the “islets of Langerhans” by Gustave Laguesse. He suggested that these cells were responsible for pancreatic secretions, which were later on named “insulin” [6–8].

In 1921, the orthopaedic surgeon Frederick Banting and the medical student Charles Best under the supervision of Professor McLeod made the discovery of injectable insulin as a treatment for diabetes [9]. The first human experiment using injectable insulin

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1871-4021/

was performed in January 1922 on a 14-year-old diabetic boy. It was noted that the clinical symptoms and biological abnormalities were reversed to normal [9]. However, it was observed that not everyone with diabetes had a positive outcome with insulin injections. In fact, in 1930s, both Wilhelm Falta and Harold Himsworth proposed that there were individuals who were insulin-sensitive and others who were insulin-insensitive [10,11].

The development of the "insulin clamp" technique in 1970s enabled the measurement of the hypoglycaemic action of insulin. This technique led to countless research studies to investigate insulin resistance and its relationship with T2DM [12]. As the years progressed, diabetes management became more refined. Insulin preparations proliferated with genetic engineering leading to the production of designer insulin such as fast-acting analogues *lispo* and *aspart* and the peakless basal insulin such as *glargine* and *detemir*. The revolutionary invention of the "pen" injection devices by John Ireland in 1981 made glucose management by insulin more patient friendly [13]. The oral hypoglycemic agent sulfonylureas originated in the early 1940s. These drugs proved to be insulin secretagogues [14]. The first biguanide *Phenformin* was introduced in 1959 and *Metformin* was available in the European market in 1960 [15]. More hypoglycaemic drug classes have been developed recently such as the glitazones, glucagon-like peptide 1 (GLP-1) agonists and inhibitors of the enzyme dipeptidylpeptidase-4 (DPP-4).

## 3. Susceptibility for type 2 diabetes

The underlying T2DM pathophysiology includes pancreatic beta cell dysfunction and development of insulin resistance [16]. An interaction between genetics (non-modifiable) and environmental (modifiable) factors increase the risk for obesity, insulin resistance and beta cell dysfunction, which ultimately lead to the development of T2DM [17–22]. Insulin resistance and cardiovascular disease risk factors (commonly associated with adiposity) appear to contribute to a substantial risk for the development of diabetes [23]. Lifestyle habits also increase the risk of T2DM. It has been reported that smoking reduces insulin sensitivity and predisposes the smoker to T2DM. Meanwhile dysfunctional sleep, with long (>8 h) and short (<6 h) stints of sleep have also been associated with increased risk of insulin resistance and T2DM, although gender differences were noted [24,25]. It is well known that dietary habits including fatty and sugary foods have an adverse effect on insulin sensitivity. Food products with added sugar, being a soda can or preserved food tin, also increase the risk of T2DM. This results from the presence of fructose, which is a component of added sugar. Fructose undergoes first pass metabolism within the liver (insulin independent), which initiates fatty acid synthesis, resulting in lipid deposition within the liver and eventual hepatic insulin resistance. In return, gluconeogenesis is promoted resulting in hyperglycaemia and the development of T2DM [26]. However, the onus of the T2DM epidemic rests on the increasing obesity rate. In fact, the T2DM epidemic is intertwined with the increasing obesity crisis and one epidemic cannot be dealt with without addressing the other.

## 4. The obesity epidemic

As discussed previously, the concept of excess adiposity (obesity) leading to T2DM is not something innovative to this era but it has been noted and reported as far back as the early ages. What is intriguing is the fact that only recently has this been given any real attention.

The obesity epidemic is mostly attributed to an increase in body fat deposition as a result of reduced physical activity and unhealthy dietary habits. This obesity crisis is now originating within the intra-uterine period and continuing to the end of life. In fact, the global obesity epidemic has been linked to simultaneous aging of the population, which has been occurring over the past few decades [27]. The obesity epidemic has also been unveiled amongst children worldwide, where an approximate 41 million children under the age of 5 years were estimated to be either overweight or obese in 2016 [28]. With the increasing obesity rates especially associated with high sugar and fatty food consumption, it is becoming a common occurrence to have fertile mothers providing a hyperglycaemic foetal environment to their unborn child. Such an environment predisposes the child to develop obesity, insulin resistance and T2DM later on in life [29,30]. Over the years there have been an increase in both portion size and consumption of food mostly at home and at fast-food restaurants [31]. Both of these factors promote weight gain and obesity [32]. Furthermore, the socioeconomic status of an individual has also a contributing effect on the development of obesity. Low socioeconomic status individuals are more likely to consume low cost energy-dense food rather than healthy diets based on fish, lean meat, fruit and vegetables, which tend to be higher in price [33].

## 5. The burden of type 2 diabetes

The pathophysiology of T2DM was established throughout the centuries along with the identification of the various diagnostic and treatment modalities. One would expect that as the years progressed the emergence of T2DM would have subsided to nothing. This is however, not the case as shown in Fig. 1, which presents a graphical illustration of the progressive prevalence of T2DM in adults (20–79 years) across eight European countries between the year 2003 and the year 2017 [34]. This brings forward the notion that T2DM prevalence is being affected by a multiplex of conditions that also need attention to, with special attention to the obesity epidemic. Table 1 illustrates clearly that as the prevalence of obesity increased across the years in Europe, a corresponding increase in T2DM prevalence occurred [35].

## 6. The way forward

Both the public and private global sectors, individually or in collaboration, have been working to end this epidemic. Multiple strategies and policies have been implemented across nations but unfortunately both the T2DM and obesity epidemics are on the rise.

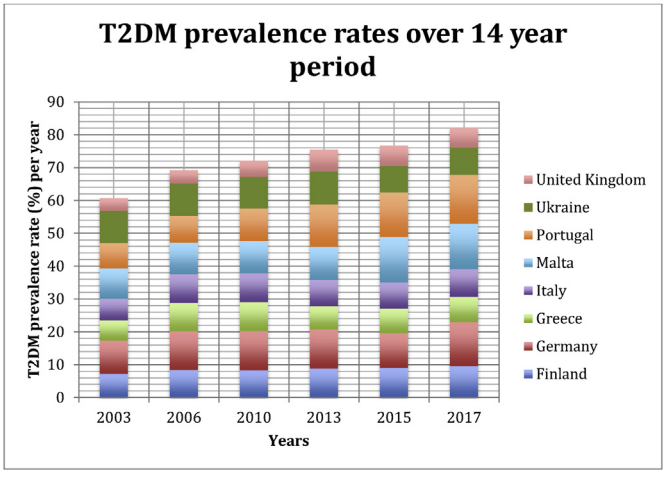


**Fig. 1.** T2DM prevalence rates over a 14- year period in 8 European countries [34].

**Table 1**
Prevalence rates of obesity and T2DM across 14 years in Europe. Adopted from WHO Global Observatory data and International Diabetes Federation Atlases [34,35].

| Country | 2003 | | 2006 | | 2010 | | 2013 | | 2015 | | 2017 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Obesity (%) | T2DM (%) | Obesity (%) | T2DM (%) | Obesity (%) | T2DM (%) | Obesity (%) | T2DM (%) | Obesity (%) | T2DM (%) | T2DM (%) |
| Albania | 14.4 | 3.8 | 16.2 | 4.8 | 18.7 | 4.8 | 20.5 | 2.8 | 21.7 | 12.0 | 11.93 |
| Andorra | 23.9 | 7.7 | 24.8 | 7.8 | 26.0 | 8.8 | 27.0 | 7.6 | 27.7 | 11.9 | 12.7 |
| Armenia | 15.1 | – | 16.0 | – | 17.5 | 8.7 | 19.0 | 2.64 | 20.2 | 7.2 | 7.56 |
| Austria | 16.2 | 9.6 | 17.5 | 11.1 | 19.2 | 11.2 | 20.5 | 9.27 | 21.5 | 9.5 | 9.93 |
| Azerbaijan | 12.8 | 6.9 | 14.0 | 6.9 | 15.9 | 7.1 | 17.8 | 2.28 | 19.2 | 6.3 | 6.92 |
| Belarus | 20.9 | 6.9 | 22.1 | 9.2 | 23.7 | 9.1 | 25.1 | 6.26 | 26.1 | 6.5 | 7.14 |
| Belgium | 19.7 | 4.2 | 20.8 | 7.9 | 22.2 | 8.0 | 23.3 | 6.45 | 24.1 | 6.7 | 6.81 |
| Bosnia and Herzegovina | 14.5 | 9.6 | 15.5 | 9.0 | 17.0 | 9.1 | 18.1 | 12.4 | 19.0 | 12.3 | 12.63 |
| Bulgaria | 21.1 | 10 | 22.4 | 10.1 | 24.3 | 9.0 | 25.8 | 7.63 | 26.9 | 8.4 | 8.16 |
| Croatia | 20.4 | 5.8 | 21.8 | 9.5 | 23.8 | 9.2 | 25.4 | 6.97 | 26.5 | 6.8 | 6.9 |
| Cyprus | 17.6 | 5.1 | 18.7 | 10.3 | 20.1 | 10.4 | 21.3 | 10.24 | 22.2 | 10.4 | 10.43 |
| Czech Republic | 23.2 | 9.5 | 24.3 | 9.7 | 25.8 | 8.7 | 27.1 | 9.23 | 28.0 | 9.9 | 9.63 |
| Denmark | 16.3 | 6.9 | 17.5 | 7.5 | 19.0 | 7.7 | 20.1 | 8.58 | 20.9 | 9.9 | 9.86 |
| Estonia | 19.7 | 9.7 | 20.4 | 9.9 | 21.6 | 9.9 | 22.7 | 7.71 | 23.4 | 6.0 | 6.11 |
| Finland | 19.5 | 7.2 | 20.8 | 8.4 | 22.4 | 8.3 | 23.6 | 8.85 | 24.5 | 9.0 | 9.56 |
| France | 18.1 | 6.2 | 19.3 | 8.4 | 20.8 | 9.4 | 22.0 | 7.5 | 22.8 | 7.4 | 7.26 |
| Georgia | 15.5 | 9.0 | 16.9 | 9.1 | 19.0 | 9.2 | 21.0 | 2.96 | 22.5 | 7.5 | 7.98 |
| Germany | 19.7 | 10.2 | 21.0 | 11.8 | 22.8 | 12.0 | 24.2 | 11.95 | 25.2 | 10.6 | 13.4 |
| Greece | 20.9 | 6.1 | 22.4 | 8.6 | 24.4 | 8.8 | 25.9 | 7.01 | 26.9 | 7.5 | 7.71 |
| Hungary | 21.9 | 9.7 | 23.3 | 9.8 | 25.3 | 8.8 | 26.9 | 7.61 | 28.1 | 9.3 | 9.64 |
| Iceland | 17.7 | 2.0 | 19.0 | 2.0 | 20.5 | 2.1 | 21.8 | 3.96 | 22.7 | 7.6 | 8.05 |
| Ireland | 17.9 | 3.4 | 19.7 | 5.6 | 22.4 | 5.7 | 24.7 | 6.47 | 26.2 | 5.3 | 4.65 |
| Israel | 21.9 | 7.1 | 23.0 | 7.8 | 24.5 | 7.1 | 25.6 | 6.65 | 26.3 | 8.5 | 8.63 |
| Italy | 17.9 | 6.6 | 19.1 | 8.7 | 20.6 | 8.8 | 21.7 | 7.95 | 22.5 | 7.9 | 8.45 |
| Kazakhstan | 14.6 | 5.5 | 15.8 | 5.6 | 17.6 | 5.6 | 19.3 | 4.87 | 20.6 | 6.2 | 6.9 |
| Kyrgyzstan | 9.5 | 4.3 | 10.4 | 4.3 | 11.9 | 4.3 | 13.5 | 5.02 | 14.7 | 5.2 | 6.05 |
| Latvia | 21.5 | 9.9 | 22.3 | 10.0 | 23.5 | 9.9 | 24.5 | 6.17 | 25.3 | 7.3 | 7.63 |
| Lithuania | 23.8 | 9.4 | 24.6 | 9.7 | 25.9 | 9.7 | 27.1 | 4.9 | 27.9 | 5.5 | 5.56 |
| Luxembourg | 18.4 | 3.8 | 19.8 | 6.9 | 21.5 | 7.0 | 22.8 | 5.78 | 23.7 | 5.7 | 5.89 |
| Malta | 25.6 | 9.2 | 27.0 | 9.7 | 28.7 | 9.8 | 29.9 | 10.14 | 30.6 | 13.9 | 13.81 |
| Montenegro | 18.5 | – | 20.1 | – | 22.1 | 8.4 | 23.5 | 12.51 | 24.4 | 12.8 | 12.85 |
| Netherlands | 15.9 | 3.7 | 17.6 | 7.3 | 19.9 | 7.7 | 21.5 | 7.5 | 22.6 | 7.9 | 8.16 |
| Norway | 18.7 | 6.7 | 20.2 | 4.7 | 22.0 | 4.7 | 23.5 | 5.9 | 24.5 | 7.8 | 8.11 |
| Poland | 19.6 | 9.0 | 20.7 | 9.1 | 22.5 | 9.3 | 24.0 | 6.5 | 25.0 | 7.6 | 7.82 |
| Portugal | 16.1 | 7.8 | 17.7 | 8.2 | 19.9 | 9.9 | 21.5 | 12.96 | 22.7 | 13.6 | 14.9 |
| Romania | 18.0 | 9.3 | 19.1 | 9.4 | 21.0 | 8.4 | 22.7 | 5.14 | 23.9 | 10.6 | 12.48 |
| Russian Federation | 21.1 | 9.2 | 21.9 | 9.0 | 23.2 | 9.0 | 24.4 | 10.03 | 25.2 | 11.1 | 8.12 |
| Serbia | 17.5 | – | 18.8 | – | 20.6 | 8.6 | 22.0 | 12.35 | 23.0 | 13.2 | 13.35 |
| Slovakia | 16.9 | 8.7 | 17.9 | 8.8 | 19.5 | 7.7 | 20.9 | 10.16 | 21.9 | 9.9 | 10.69 |
| Slovenia | 17.1 | 9.6 | 18.2 | 9.8 | 19.8 | 9.9 | 21.1 | 10.33 | 22.0 | 10.7 | 10.81 |
| Spain | 20.8 | 9.9 | 22.1 | 7.5 | 24.0 | 8.7 | 25.5 | 10.83 | 26.6 | 10.4 | 11.23 |
| Sweden | 16.9 | 7.3 | 18.1 | 7.2 | 19.6 | 7.3 | 20.8 | 6.36 | 21.6 | 6.3 | 7.2 |
| Switzerland | 16.1 | 9.5 | 17.2 | 11.2 | 18.8 | 11.3 | 19.9 | 7.45 | 20.8 | 7.7 | 7.89 |
| Tajikistan | 7.3 | 3.7 | 8.1 | 3.5 | 9.5 | 3.6 | 10.9 | 4.48 | 12.0 | 4.5 | 5.39 |
| Turkey | 22.4 | 7.0 | 24.5 | 7.1 | 27.4 | 7.4 | 29.8 | 14.58 | 31.4 | 12.5 | 12.54 |
| Turkmenistan | 10.7 | 4.0 | 11.9 | 4.0 | 13.8 | 4.1 | 15.5 | 4.05 | 16.8 | 5.2 | 5.96 |
| Ukraine | 21.1 | 9.7 | 22.0 | 9.8 | 23.3 | 9.6 | 24.6 | 2.99 | 25.6 | 8.0 | 8.23 |
| United Kingdom | 21.5 | 3.9 | 23.2 | 4.0 | 25.7 | 4.9 | 27.5 | 6.57 | 28.9 | 6.2 | 5.95 |
| Uzbekistan | 9.3 | 4.0 | 10.3 | 4.0 | 11.9 | 4.0 | 13.5 | 5.05 | 14.7 | 5.2 | 6.48 |

Having a good understanding of the available knowledge along with keeping up to date with the continuous advancement in research and technology should empower a multidisciplinary approach towards this epidemic. Collaborations between different stakeholders, both at a local and international setting are required. In fact, the control of T2DM needs to consider the biological (genetics) factors, the behavioural factors, the socioeconomic conditions as well as the health care services available. Every sector provides a challenge, but it is the combination of all aspects that will provide the basis towards success.

## Conflicts of interest

None.

## Data availability

http://www.diabetesatlas.org and http://apps.who.int/gho/data/node.main.A896?lang=en.

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The author performed the literature review and constructed the manuscript.

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