Original Article

Impaired Insulin Sensitivity and Insulin Secretion in Haemodialysis Patients with and without Secondary Hyperparathyroidism

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Abstract

The aim of our study was to investigate insulin sensitivity and beta cell function in hemodialysis (HD) patients without diabetes. We hypothesized that parathyroid gland function was a determinant of insulin sensitivity and/or beta cell function. The study was a randomized, cross-sectional one and patients were divided into two groups (total 27 patients), Gp.1 being those with relative hypoparathyroidism (iPTH<200 pg/ml) – 9 (33.3%), Gp.2 those with hyperparathyroidism (iPTH≥200 pg/ml) – 18 (66.6%) with Gp.3 (consisting of 43 healthy subjects acting as controls). Insulin resistance and insulin secretion were calculated from fasting serum insulin and glucose concentrations by the Homeostatic Model Assessment score (HOMA IR and HOMA BETA). The value of HOMA IR (3.28±1.3 for Gp.1, 4.80±2.4 for Gp.2, 1.70±0.8 for Gp.3) as well as the glucose level (5.0±1.0mmol/l in Gp.1, 5.2±0.8mmol/l in Gp.2, 4.6±0.4mmol/l in Gp.3) was significantly higher in HD patients than in control subjects. Excessive insulin secretion was present in HD patients (as assessed by HOMA BETA) significantly higher only in Gp.1 (p=0.02). HOMA IR was higher in Gp.2 than in Gp.1 (p=0.05), and both groups had higher levels of HOMA IR than the control group (Gp.1/Gp.1 p=0.03; Gp.2/Gp.3 p=0.00). A positive correlation between HOMA IR and serum iPTH was seen in Gp.2 only (r=0.54, p=0.03). HOMA BETA inversely correlated with Ca x iP product in Gp.1 (r=0.54, p=0.04). The only significant negative correlation between HOMA BETA and age (r= -0.59, p=0.01) was registered in Gp.2. Serum iPTH correlated positively with serum Ca^{2+} (r= 0.49, p=0.03) in Gp.2.

In conclusion, our study demonstrated the presence of a higher level of serum insulin and insulin resistance in HD patients. Serum iPTH directly correlated with the insulin resistance index in hyperparathyroid patients suggesting a possible interaction between PTH and the insulin signaling pathway. Excessive insulin secretion was registered in HD patients, significantly higher only in hypoparathyroid patients. However, beta cells function in both groups of patients was preserved implying relatively good sensitivity of the calcium receptor (CaR) in beta cells.

Keywords

insulin sensitivity, beta cell function, chronic renal failure, secondary hyperparathyroidism, calcium receptor.

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Introduction

A number of studies in uremic patients reported that insulin resistance, reduced beta cell secretion and glucose intolerance leading to dyslipidemia are associated with hyperparathyroidism.[1-3] It was observed that hyperprathyroidism-induced increase in cytosolic calcium could be responsible for beta-cell dysfunction. These alterations were reversed with

verapamil and recurred after discontinuation of the drug.[1] Changes in intracellular calcium concentration have a central role in various cellular processes, from growth and development to hormone secretion. Increases in intracellular ionized calcium (Ca^{2+}) are likely to be sustained by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) or non-selective cation channels (NSCC), depending on the cell type.[4,5] Raised free cytoplasmic Ca^{2+} levels trigger insulin secretion. Squires et al reported that increasing extracellular Ca^{2+} from 1.3 to 5mM increases insulin secretion from human pancreatic islets in *in vitro*.[6] But, later, in perfusion experiments, that transient increase in insulin secretion was followed by a Ca^{2+} concentration-dependent and prolonged inhibition of secretion[7]. That inhibition was fully reversible with a reduced level of extracellular Ca^{2+}. This study demonstrated another, calcium sensing receptor (CaR)-mediated inhibitory mechanism, which may be an important auto-regulatory mechanism in the control of insulin secretion. A novel, small G-protein, Kir/GEM, interacting with the beta3 isoform of the VDCC, inhibits alpha ionic activity and prevents cell-surface expression of alpha subunits VDCC, and could attenuate glucose-stimulated Ca^{2+} increases and insulin secretion in insulin-secreting cells.[8]

Elevated levels of parathyroid hormone (PTH) occur early in renal failure. The onset of secondary hyperparathyroidism is the result of altered mineral and hormone metabolism, especially a diminished synthesis of active vitamin D (calcitriol) metabolite from the failing kidney and resistance of target tissue toward them. A rightward shift of the calcium set-point and an increase of the minimum secretion rate of PTH have been found in secondary hyperparathyroidism, indicating abnormal calcium sensing by parathyroid cells. It is considered that various candidate genes, vitamin D receptor (VDR) gene, CaR gene and PTH gene polymorphisms might contribute to progression of secondary hyperparathyroidism[9-11]. It is well known that extracellular Ca^{2+} influx through L-type VDCC raises free cytoplasmic Ca^{2+} level in beta cells and triggers insulin secretion. The complications associated with chronic secondary hyperparathyroidism are numerous and include "classic effects" of PTH excess on kidney, bone, cardiovascular and erythropoetic system, as well as effects on other "non-classic" targets eg pancreas, adrenal cortex, testis and pituitary gland. Independently of its ability to prevent secondary hyperparathyroidism, or later to decrease elevated PTH level and normalise serum calcium and phosphate, calcitriol is probably a modulator of insulin secretion and insulin sensitivity.[12,13] The treatment of secondary hyperparathyroidism with calcimimetics, new agents which potentiate the effects of extracellular Ca^{2+} on the CaR, besides reducing PTH synthesis and secretion, could make possible the identification of secondary effects of Ca^{2+} on tissues not involved in Ca^{2+} homeostasis, such as pancreatic islets.[14]

Subjects and Methods

Patient population

The total number of subjects involved in the study was 70. Twenty seven of them were stable, end-stage renal patients (17 males and 10 females), on a chronic HD program, 4 hours three times weekly, with HD duration more than six months. All of them were treated in the Hemodialysis Unit of Zemun Clinical Hospital. Exclusion criteria included a history of diabetes mellitus, malignancy and cardiac and vascular failure. The causes of end-stage renal disease were glomerular disease, chronic pyelonephritis, polycystic renal disease, urolithiasis and hypoplastic kidney in 11, 7, 4, 4 and one patient, respectively. None of them had undergone total or subtotal parathyroidectomy or received pulsed intravenous high-dose active vitamin D treatment. The control group consisted of 43 healthy individuals (23 males and 20 females) without renal failure, diabetes or any serious cardiorespiratory disease.

Study design

The study was a randomized cross-sectional one. Each patient was treated with dialyzers containing membranes of cuprofan and polysulfone, and bicarbonate dialysates with a calcium concentration of 1.75mmol/l. Patients received calcium carbonate as phosphate binders. Oral active vitamin D (1,25-dihydroxyvitamin D3) was administered to patients at dosages 0.25 to 0.5 mg/d, if hyperphosphatemia was less than 1.8 mmol/L. With higher serum inorganic phosphate (iP) concentration, phosphate binder therapy was only intensified. Serum intact parathyroid hormone level (iPTH) measurement revealed relative hypoparathyroidism (iPTH <200 pg/ml) in 9 (33.3%) (Gp.1), hyperparathyroidism (iPTH≥200 pg/ml) in 18 (66.6%)(Gp.2) of 27 subjects, with mean ages of 53.7±8.3 and 49.5±7.0 years respectively. The level of iPTH in controls could not be estimated due to lack of sufficient funding. The HD duration did not differ between the two patient groups (67±15 months in Gp.1 and 79±38 months in Gp.2). The mean age of the control (Gp.3) was 50.3±9.6 years.

Methods

Blood samples were obtained during midweek, after 12 hours fasting and immediately prior to dialysis, for measurement of the following variables: serum glucose, insulin, C-peptide, iPTH, total serum proteins, albumin, BUN, creatinine, serum ionized Ca (Ca^{2+}) and inorganic phosphate (iP), which were measured by standard laboratory methods.

Intact PTH was assessed using an immunoradiometric method (CIS-Bio) and insulin and C-peptide were measured using a radioimmunoassay method (INEP Zemun, Belgrade). The normal reference ranges were: for iPTH 11-62pg/ml, for insulin 6-20mU/L, and for C-peptide 0.3-0.8 mmol/L. Estimates of pancreatic beta-cell function and relative insulin resistance were calculated from fasting insulin and glucose concentrations using the Homeostatic Model Assessment score [HOMA BETA (%) = fasting insulin (mU/L) x 20/fasting serum glucose (mmol/L)-3.5 and HOMA IR (mU/L) = fasting insulin (mU/L) x serum glucose (mmol/L)/22.5].[15] Anthropometric measurements were done by one observer. The percentage of body fat mass was estimated from skinfold measurement and was compared to standard anthropometric tables.[18]

Statistical analysis

Data are presented as mean±SD. Differences between groups were evaluated by unpaired two-tailed Student's *t* tests and one-way ANOVA with Bonferroni or Tukey multiple comparison post-test. Linear regression models were used to explore the relationship between HOMA BETA or HOMA IR and other variables. All statistical analyses were done using statistical package SPSS for Windows 8.0.

Results

The clinical characteristics of the participating subjects are reported in Table 1. There were no differences for age, sex, and HD duration between the groups. When we compared clinical profiles, besides significantly higher serum iPTH in Gp.2 than in Gp.1, insulin resistance (HOMA IR) was significantly, but only slightly, higher (p=0.054) in Gp.2 than in Gp.1. In contrast the values for HOMA IR were significantly higher in both groups of HD patients as compared to controls. Insulin secretion (HOMA BETA) was higher in HD patients, but significantly so only in Gp.1. The high SD may have been influenced by the small number of patients in that group. The mean values of glucose (5.0±1.0mmol/l in Gp.1, 5.2±0.8mmol/l in Gp.2) in the HD patients were significantly higher than the value of the control group (4.6±0.4mmol/l in Gp.3). There were no differences for fasting serum Ca^{2+}, iP and Ca x iP product, BUN, creatinine and the indexes of body fat, nutritional status and protein intake (skin folds, BMI, serum protein, albumin and nPCR) between the groups. There was a significant negative correlation between HOMA IR and HD duration, and HOMA IR and the creatinine level. However there was no correlation of IR with other parameters in Gp.1. HOMA IR correlated directly with serum iPTH only in Gp.2 (r=0.544, p=0.03)

Table 1: *Clinical and biochemical characteristics of relative hypoparathyroid (Gp.1) and hyperparathyroid (Gp. 2) HD patients and controls (Gp. 3)*

	Gp. 1	Gp.2	$P_{1/2}$	Gp. 3	$P_{1/3, 2/3}$
N	9	18		43	
Mean Age (year)	53.6±8.3	49.5±7.0	ns	48.2±6.5	ns, ns
Dialysis Duration (month)	67.1±14.7	78.9±38.2	ns		
Albumin (mmol/l)	38.0±5.0	38.3±3.6	ns	41.1±2.1	0.01,0.00
Creatinine (mmol/l)	635.5±179.8	625.5±187.6	ns	94.5±6.4	0.00,0.00
Glucose (mmol/l)	5.0±0.9	5.2±0.7	ns	4.5±0.4	0.04,0.02
Insulin (mU/l)	15.2±5.5	20.1±7.8	ns	9.8±4.4	0.05,0.00
C-peptide (nmol/l)	2.18±1.0	2.25±0.9	ns	0.91±0.3	0.00,0.00
HOMA IR	3.28±1.3	4.80±2.4	0.05	1.70±0.8	0.03,0.00
HOMA BETA	391.6±667.7	266.9±121.4	ns	167.5±139.7	0.02,ns
Ca^{2+} (mmol/l)	1.10±0.9	1.17±0.1	ns		
iP (mmol/l)	1.80±0.3	1.89±0.4	ns		
Ca^{2+} x iP	1.98±0.3	2.19±0.5	ns		
iPTH (pg/ml)	102.8±31.2	876.0±447.5	0.00		
BMI (kg/m2)	23.3±2.1	22.6±3.1	ns	23.5±2.3	ns,ns
WHR	0.87±0.1	0.90±0.1	ns	0.89±0.1	ns,ns
Body fat mass (%)	24.88±7.0	25.9±7.4	ns	27.6±5.2	ns,ns
NPCR (g/Kg/d)	1.19±0.2	1.27±0.3	ns		

Table 2: *Multiple correlation (Pearson's correlation coefficient r) for HOMA IR of relative hypoparathyroid (Gp. 1) and hyperparathyroid (Gp. 2) HD patients*

	Gp.1 **HOMA IR** r	p	Gp. 2 **HOMA IR** r	p
HD duration	**-0.67**	0.04	-0.07	0.78
Creatinine	**-0.65**	0.05	0.12	0.65
iPTH	0.46	0.21	**0.54**	0.03

Table 3: *Multiple correlation (Pearson's correlation coefficient r) for HOMA BETA of relative hypoparathyroid (Gp. 1) and hyperparathyroid (Gp. 2) HD patients*

	Gp.1 **HOMA BETA** r	p	Gp. 2 **HOMA BETA** r	p
Age	0.27	0.48	**-0.59**	0.01
Ca^{2+}	-0.09	0.81	-0.34	0.19
Ca^{2+}x iP	**-0.69**	0.04	-0.27	0.91

(Table 2). Some extent of correlation between HOMA IR and iPTH existed in Gp.1 but this was not significant. Within Gp.3, HOMA IR correlated directly with BMI (r=0.45, p=0.002) and WHR (r=0.63, p=0.00). HOMA BETA inversely correlated with Ca x iP product in Gp.1 (r= -0.689, p=0.04) (Table 3). There was a significant negative correlation between HOMA BETA and age (r=0.594, p=0.01) but there was no significant correlation with serum Ca^{2+} in Gp.2 (Table 3). Serum iPTH correlated positively with serum Ca^{2+} (r= 0.489, p= 0.03) in Gp.2.

Discussion

A number of studies in subjects with end-stage renal failure (ESRF) have confirmed hyperinsulinemia with reduced insulin sensitivity and hyperglycaemia.[2,3] The accepted gold standard of insulin sensitivity, the euglycemic hyperinsulinemic clamp, confirmed reduced insulin sensitivity in end-stage renal failure[2]. In this study, the Homeostatic Model Assessment Method for the calculation of insulin resistance and beta cell function, which is generally accepted for epidemiological studies, was used. Since pure insulin level in ESRF represents the result of both secretion and elimination, reduced elimination by dialyzers, reduced hepatic insulin clearance as in other subjects with insulin resistance, and oversecretion of insulin, with decreased sensitivity to insulin in peripheral tissues, may contribute to hyperinsulinemia. The measurement of C-peptide, with values which correlated excellently with serum insulin and/or the homostatic model of beta cell function, HOMA BETA, has been reported earlier.

We have found reduced insulin sensitivity in both groups of patients but the difference between them was only of borderline significance. In the patients with relative hypoparathyroidism, the index of insulin resistance correlated inversely only with HD duration and directly with creatinine level. In the group with secondary hyperparathyroidism, the degree of insulin resistance was even more marked and a relationship between insulin resistance index and serum iPTH level is reported here. The present findings of reduced insulin sensitivity in the patients with secondary hyperparathyroidism further confirm similar reports by other investigators[1,2,17]. Elevated PTH correlated with high serum ionized calcium concentration suggesting that any resultant insulin resistance concentrations have been already established.[1-3,17] Obesity, differences in body mass index, waist/hip ratio, or in protein and albumin levels were excluded in both groups of HD patients. There was no correlation between body composition and insulin levels, in our patients, as we found in controls, in agreement with the findings reported in another study.[18] Therapeutic and environmental factors, such as dietary calcium, vitamin D, calcitriol use and physical activity were similar in both groups of the ESRF patients. Some studies have shown diminished expression of vitamin D receptors, particularly in hyperplastic parathyroid glands, as a consequence of disturbed genomic effects of active vitamin D induced by the action of a low molecular weight substance in uremia[9]. The study of Kauzcky-Willer reported an effect of biologically active vitamin D on the insulin sensitivity of peripheral tissues which was independent of PTH secretion.[12] The lack of significant correlation between insulin sensitivity and serum PTH in the group with relative hypoparathyroidism may be a consequence of the small number of participants, better mineral and hormone homeostasis, or some other mechanism that might independently affect insulin sensitivity in relatively hypoparathyroid ESRF patients.[10] That group of patients had lower levels of serum iPTH and Ca^{2+}. Better expression of vitamin D receptors in these patients might contribute to reduced insulin sensitivity in peripheral tissue. One could speculate that expression of vitamin D receptor and Ca-sensing receptor may regulate PTH level and insulin sensitivity and /or insulin secretion at the same time, independently. The presence of oxidative stress, as a consequence of the higher plasma glucose present in both HD groups and higher non-esterified free fatty acids (NEFA), could be a potential modulator of the mechanisms involved in insulin resistance in both groups of HD patients. Correlation of vitamin D depletion in secondary hyperparathyroidism has been followed by reductions in NEFA concentrations and insulin resistance.[13]

Beta cell function differed from control subjects, in both

groups of HD patients, with over-secretion of insulin. The HOMA BETA index was significantly higher only in relatively hypoparathyroid patients. The etiology of beta cell dysfunction in ESRF is not clear but it is believed that overactivation of the CaR inhibits basal and nutrient-stimulated insulin secretion.[6,7] In this study, in the relatively hypoparathyroid group, the HOMA BETA index correlated negatively only with the Ca^{2+} x iP product, but in the hyperparathyroid group, HOMA BETA index showed no significant correlation with serum Ca^{2+}. The higher level of serum glucose documented in both groups of patients, could be also implicated as a potential modulator of insulin secretion. Early correction of vitamin D depletion has been shown to restore both insulin secretion and normoglycemia in dialysis patients developing glucose intolerance with failing l-hydroxylation of 25-hydroxy-vitamin D.[19] It could therefore be hypothesised that a low level of active vitamin D and/or high extracellular Ca^{2+}, or a high Ca^{2+} x iP product may result in beta cell dysfunction in those patients. Extracellular Ca^{2+} influx through VDCC raises free cytoplasmic Ca^{2+} levels and triggers insulin secretion. There was a negative correlation between beta cell secretion and the Ca^{2+} x iP product documented in relative hypoparathyroid HD patients. The preserved beta cells function in both groups of our patients, suggests relatively good sensitivity of CaR in beta cells to extracellular calcium. In hyperparathyroid patients one may expect abnormal calcium sensing.

In conclusion, the present study has demonstrated the presence of a higher level of serum insulin and insulin resistance in HD patients, as assessed by the calculation of HOMA IR score. Patient with severe hyperparathyroidism have a higher level of insulin resistance and there is an association between insulin resistance and iPTH levels. Serum iPTH correlated directly with serum Ca^{2+}only in hyperprathyroid patients. Beta cells function was overexpressed in both groups but reached statistical significance only in relatively hypoparathyroid patients. It depended on Ca^{2+} x iP product in relatively hypoparathyroid patients only. Further studies are required using larger numbers of patients to determine whether parathyroidectomy might restore insulin sensitivity and partly beta cell function in hyperparathyroid patients and for definite conclusions to be made in the hypoparathyroid group of HD patients.

References

1. Gadallah MF, el-Shahawy M, Andrews G et al. Factors modulating cytosolic calcium. Role in lipid metabolism and cardiovascular morbidity and mortality in peritoneal dialysis patients. Adv Perit Dial 2001;17:29-36.
2. Mak RH. 1,25 dihydroxycholecalciferol corrects glucose intolerance in hemodialysis patients. Kidney Int 1992;41:1049-54.
3. Mak RH. 1,25-Dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. Kidney Int 1998;53:1353-7.
4. Silva AM, Rosario LM and Santos RM. Background Ca^{2+} influx meditated by dihydropyridine- and voltage-insensitive channels in pancreatic ß-cells. J Biol Chem 1994;269:1795-803.
5. Sharp GWG. Mechanism of inhibition of insulin release. Am J Physiol 1996;271:C1781-99.
6. Squires PE. Non-Ca^{2+} -homeostatic functions of the intracellular Ca^{2+} - sensing receptor (CaR) in endocrine tissues. J of Endocrin 2000;165:173-7.
7. Harris TE, Squires PE, Persaud SJ, Buchan AMJ, Jones PM. The calcium-sensing receptor in human islets: calcium-induced inhibition of insulin secretion. Nephrol Dial Transplant Abstract Suppl 1 2002;17 : Abstract 0493.
8. Beguin P, Nagashima K, Gonoi T, et al. Regulation of Ca^{2+} channel expression at the cell surface by the small G-protein kir/Gem. Nature 2001;411:701-6.
9. Marco MP, Martinez I, Amoedo ML, et al. Vitamin D receptor genotype influences parathyroid hormone and calcitriol levels in predialysis patients. Kidney Int 1999;56:1349-53.
10. Yokoyama K, Shigematsu T, Tsukada T, et al. Calcium-sensing receptor gene polymorphism affects the parathyroid response to moderate hypercalcemic suppression in patients with end-stage renal disease. Clin Nephrol 2002;57:131-5.
11. Tomohito G, Ichiyu S, Mitsumine F, et al. Parathyroid hormone gene polymorphism and secondary hyperparathyroidism in hemodialysis patients. Am J Kidney Dis 2002;39:1255-60.
12. Kautzky-Willer A, Pacini G, Barnas U, et al. Intravenous calcitriol normalizes insulin sensitivity in uremic patients. Kidney Int 1995;47:200-6.
13. Lin SH, Lin YF, Lu KC, et al. Effects of intravenous calcitriol on lipid profiles and glucose tolerance in uremic patients with secondary hyperparathyroidism. Clin Sci 1994;87:533-8.
14. Quaries LD, Sherrard DJ, Adler S, et al. The calcimimetic AMG 073 as a potential treatment for secondary hyperparathyroidism of end-stage renal disease. J Am Soc Nephrol 2003; 14:575-83.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and ß-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28: 412-19.
16. Rashid Qureshi A, Alvestrand A, Danielsson A, et al. Factors predicting malnutrition in hemodialysis patients. A cross-sectional study. Kidney International 1998;53:773-82.
17. Chiu KC, Chuang LM, Lee NP et al. Insulin sensitivity is inversely correlated with plasma intact parathyroid hormone level. Metabolism 2000; 49:15001-5.
18. Garaulet M, Perex-Llamas F, Fuente T, Zamora S, Tebar FJ. Anthropometric computed tomography and fat cell data in an obese population: relationship with insulin, leptin, tumor necrosis factor-alpha, sex hormone-binding globulin and sex hormones. Eur J Endocrinol 2000;143:657-66.
19. Lu KC, Shick SD, Lin SH et al. Hyperparathyroidism, glucose tolerance and platelet intracellular free calcium in chronic renal failure. Q J Med 1994;87:359-65.