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Renal handling of zinc in insulin-dependent diabetes mellitus patients

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Abstract

Hyperzincuria is a common feature in diabetic patients, which is still not understood. Based on the above consideration, the aim of the present study was to investigate the renal handling of zinc in insulin-dependent diabetes mellitus (IDDM) patients. The glomerular filtration rate, urinary zinc excretion, zinc clearance, zinc clearance/creatinine clearance ratio, zinc tubular reabsorption, glycosuria, plasma glucose, C-peptide, glucagon, and cortisol were investigated in 10 normal individuals (Group C1 and Group C2, respectively) and 10 IDDM patients (Group E1: hyperglycemic and glycosuric and Group E2: normoglycemic and aglycosuric) during placebo or venous zinc tolerance test. The results showed that urinary zinc excretion and renal zinc clearance were increased after zinc injection in normal individuals (Group C2) and IDDM patients (Groups E1 and E2) when compared with normal individuals-placebo (Group C1). However, these renal parameters were statistically more significant in the hyperglycemic and glycosuric diabetics (Group E1). Because patients in Group E1 had the lowest plasma C-peptide levels and showed a strong negative correlation between CZn^{++}/Ccr ratio and this hormone, we suggest that in this setting insulin inhibits urinary zinc excretion.

Introduction

Patients with IDDM and non-insulin-dependent diabetes mellitus (NIDDM) have large amounts of zinc in urine, sometimes exceeding more than 1,000 μg 24 h (Maldonado-Martin *et al.* 1991). This large excretion of zinc in diabetics is not clearly understood (Brandão-Neto *et al.* 1995; Chen *et al.* 1995). In normal conditions, the rate of renal zinc excretion is relatively low. For instance, urinary zinc excretion in healthy adults is about 300 to 600 μg 24 h (Pimenta *et al.* 1992). That is due to the fact that zinc is mostly bound to proteins and thus not available for glomerular filtration (Giroux & Henkin 1972). The proximal tubule of the kidney is capable of secreting zinc, and the distal nephron segment is the site for reabsorption (Abu-Hamdan *et al.* 1981; Victery *et al.* 1981). According to Brandão-Neto *et al.* (1995), ~99.77% of the zinc filtered is reabsorbed by the tubules. On the other hand, insulin is thought to be a physiological inhibitor of zinc excretion, whereas glucagon has a stimulatory effect on zinc excretion (Vander *et al.* 1983).

The purpose of the present study was to study the parameters of glomerular filtration and tubular reabsorption in healthy and IDDM patients following venous zinc injection.

Material and methods

Subjects

The study was conducted on 10 normal individuals (5 of each sex, aged 24.10 $\pm$ 1.96) and 10 IDDM (5 of each sex, aged 25.20 $\pm$ 8.10) after obtaining informed consent in writing, and approval by the Medical Ethics Committee. Normal individuals (medical students or staff members) had normal weight with no

history of endocrine disease and not under any medication (Brandão-Neto *et al.* 2000). Diabetic patients received insulin therapy for metabolic control (57.0 ± 20.0 U/day, NPH insulin), except in the morning of the test. Duration of diabetes was 8.00 ± 6.03 years, and they did not have nephropathy or other complications (Brandão-Neto *et al.* 1999). All normal individuals and diabetic patients were studied while on their habitual diet. Diabetic diet consisted of ∼50% carbohydrates, ∼30% fat, and ∼20% protein, calculated as energy content (Brandão-Neto *et al.* 1999). The test was initiated at 7:00 a.m. after an overnight fast. The subjects rested in bed throughout the test. An antecubital vein of each forearm was punctured, and a device for infusion was installed and maintained with saline.

Experimental design (Figure 1)

Group C1: Ten normal individuals were submitted to renal zinc clearance during placebo test. Group C2: The same 10 normal individuals were submitted to renal zinc clearance during venous zinc tolerance test. Group E1: Five IDDM patients (hyperglycemics and glycosurics) were submitted to renal zinc clearance during venous zinc tolerance test. Group E2: Five IDDM patients (normoglycemics and aglycosurics) were submitted to renal zinc clearance during venous zinc tolerance test.

Venous zinc tolerance test

A dose of 25 mg Zn^{++} (2 ml as heptahydrated zinc sulfate) was injected after time 0 min, and over a period of 1 min. Blood samples were collected from contralateral arm at 0, 30, 60, 90, and 120 min after zinc injection (Castro *et al.* 1999).

Glomerular filtration

The procedures were performed between 8:00 and 10:00 a.m. after emptying the bladder and ingestion of 4 ml ultra pure water/kg body wt at 8:00 a.m. The urine sample was collected at 10:00 a.m. for zinc and creatinine measurement. Serum zinc and creatinine were collected at times 0, 30, 60, 90, and 120 min. At the end of the test, weight and height of subjects were measured for the determination of body surface area, because water ingestion. Glomerular filtration rate, urinary zinc excretion, renal creatinine clearance, renal zinc clearance, zinc clearance/creatinine clearance ratio, and zinc tubular reabsorption were calculated according to Brandão-Neto *et al.* (1995), using mathematical parameters. These procedures were performed during glomerular filtration following the ingestion of only ultra pure water plus 2 ml saline, I.V. (Group C1) and ultra pure water plus 2 ml zinc sulfate, I.V. (Group C2, Group E1, and Group E2).

Biological materials and analysis

Blood samples were obtained by puncturing a forearm vein without a tourniquet. Samples showing hemolysis were discarded, because erythrocytes are rich in zinc. All the material used for zinc collection, separation, and storage was plastic and metal free. Serum and urine samples were frozen and stored at $-20°C$ until the time of measurement. Serum and urinary zinc were measured by atomic absorption spectrophotometer (Shimadzu, AA680G, Japan). The sensitivity was 0.01 μg ml^{-1} and the intra assay coefficient of variation was 3.9%. All the other procedures, such as calibration and measurements were done in accordance with the manufacturer (Brandão-Neto *et al.* 2000). Serum and urinary glucose and creatinine, and other biochemical parameters (hemogram, lactate dehydrogenase, alkaline phosphatase, bilirubin, transaminases, total protein, albumin, uric acid, blood urea nitrogen, inorganic phosphate, sodium, chloride, potassium, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides) were measured by autoanalyzer (Cobas-Mira Plus, Switzerland) (Brandão-Neto *et al.* 1999).

Statistical analysis

Statistical analysis was performed using the one-way analysis of variance to compare the results of the fourth groups studied with calculation of F and of its p-value. We calculated the contrasts between pairs of means by the Newman-Keuls multiple comparison test. We also used correlation and unpaired t-test. A P-value of <0.05 was accepted as significant.

Results

Clinical variables

All biochemical parameters, before and after the test, were in the normal range in normal individuals and diabetic patients.

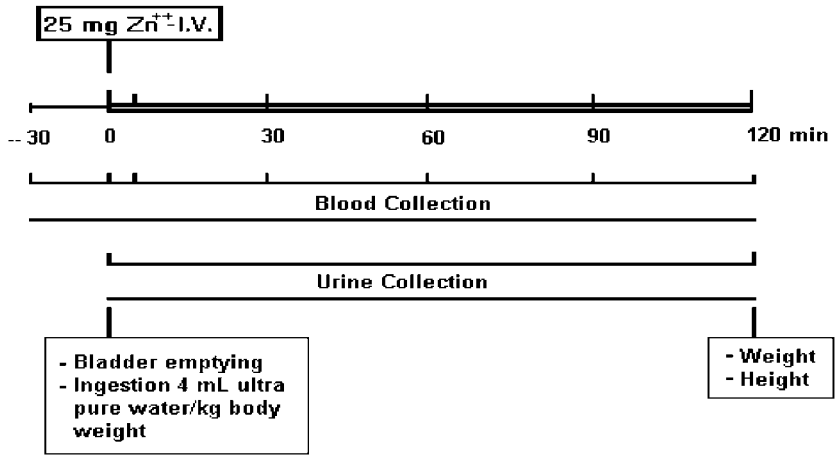

Fig. 1. Experimental design of venous zinc tolerance test, including glomerular filtration test, in 10 normal individuals and 10 IDDM patients.

Table 1. Plasma glucose, urinary glucose, serum zinc, urinary zinc excretion, zinc clearance, zinc clearance/creatinine clearance ratio, and zinc tubular reabsorption in normal individuals and IDDM patients, during venous zinc tolerance test (25 mg Zn^{++})[a]

	GROUP C1	GROUP C2	GROUP E1	GROUP E2
[b]PGlu (mg %)	84.95± 13.52	83.90± 8.14	320.40± 96.60	94.80± 36.94
[c]UGlu (mg %)	–	–	3, 910± 2,540	–
[d]SZn^{++} (μg ml^{-1})	0.96± 0.10	1.04± 0.22	0.96± 0.15	1.08± 0.23
[e]UZn^{++}.V (μg min 1.73 m^2)	0.34± 0.13	1.91± 0.61	2.51± 0.40	1.69± 0.93
[f]CZn^{++} (ml min 1.73 m^2)	0.23± 0.11	0.50± 0.17	0.69± 0.12	0.54± 0.31
[g]CZn^{++}/CCr Ratio	$2.00 \times 10^{-3}\pm$ 1.07×10^{-3}	$6.20 \times 10^{-3}\pm$ 2.14×10^{-3}	$12.32 \times 10^{-3}\pm$ 3.48×10^{-3}	$7.08 \times 10^{-3}\pm$ 3.45×10^{-3}
[h]$TRZn^{++}$ (%)	99.77± 0.13	99.85± 0.88	99.72± 0.11	99.81± 0.12

[a]Results are expressed as means ± SD; group C1 and group C2 contained 10 patients each, and group E1 and group E2 contained 05 patients each.
[b]Groups C1 = C2, $P > 0.05$; C1 < E1, $P < 0.001$; C1 = E2, $P > 0.05$; C2 < E1, $P < 0.001$; C2 = E2, $P > 0.05$; E1 > E2, $P < 0.001$.
[c]Groups E1 > E2, $P < 0.001$.
[d]Groups C1 = C2 = E1 = E2, $P > 0.05$.
[e]Groups C1 < C2, $P < 0.001$; C1 < E1, $P < 0.001$; C1 < E2, $P < 0.001$; C2 = E1, $P > 0.05$; C2 = E2, $P > 0.05$; E1 = E2, $P > 0.05$.
[f]Groups C1 < C2, $P < 0.001$; C1 < E1, $P < 0.001$; C1 < E2, $P < 0.01$; C2 = E1, $P > 0.05$; C2 = E2, $P > 0.05$; E1 = E2, $P > 0.05$.
[g]Groups C1 < C2, $P < 0.001$; C1 < E1, $P < 0.001$; C1 < E2, $P < 0.01$; C2 < E1, $P < 0.001$; C2 < E2, $P > 0.05$; E1 > E2, $P < 0.01$.
[h]Groups C1 = C2 = E1 = E2, $P > 0.05$.
b,d,e,f,g,h: indicate one-way ANOVA analysis.
c: indicates unpaired t-test.

Basal serum zinc (SZn^{++})

SZn^{++} levels were in the normal limits (0.7–1.2 μg ml^{-1}) and not significantly different between normal individuals and diabetic patients (Table 1).

Basal plasma glucose (PGlu) and urinary glucose (UGlu)

Five diabetic patients (Group E1) presented glycosuria ($3,910 \pm 2,540$ mg %) and plasma glucose higher than 244 mg % (320.40 ± 96.60 mg %), whereas the other 5 patients (Group E2) had aglycosuria and plasma glucose lower than 145 mg % (94.80 ± 36.94 mg %). The values (means $\pm$ SD) of plasma glucose between these two groups were significantly different ($P < 0.0001$). There was no correlation between PGlu or UGlu and UZn^{++}.V, and PGlu or UGlu and CZn^{++}/Ccr ratio in all four groups studied ($P > 0.05$) (Table 1).

Glomerular filtration rate (GFR = ml min 1.73 m^2)

GFR did not change during the experiment in all four groups studied, $P > 0.05$.

Urinary zinc excretion ($UZn^{++}.V = \mu g$ min 1.73 m^2)

UZn^{++}.V was lower in Group C1 and higher in Groups C2, E1, and E2. These results indicate that all subjects increased their urinary zinc excretion during the venous zinc tolerance test, particularly in Group E1. Statistical results are showed in Table 1.

Renal zinc clearance (CZn^{++} = ml min 1.73 m^2)

The values of CZn^{++} were higher in Groups C2, E1, and E2 in comparison to Group C1. Statistical results are showed in Table 1.

CZn^{++}/Ccr ratio

CZn^{++}/Ccr ratio values presented the same profile of CZn^{++}. The value of Group C1 was lower in comparison to Group C2, E1 and E2 (Figure 2). Statistical results are showed in Table 1. Strong negative correlation was obtained between CZn^{++}/Ccr ratio and C-peptide only in the Group E1 ($r = -0.9992$, $P < 0.0001$).

Table 2. Values of C-peptide, Glucagon, and Cortisol during venous zinc tolerance test in 10 normal individuals (Group C2), 5 IDDM patients with hyperglycemia and glycosuria (Group E1), and 5 IDDM patients with normoglycemia and aglycosuria (Group E2)[a]

	[b]C-PEPTIDE	[c]GLUCAGON	[d]CORTISOL
Group C2	1.43 ± 0.10	51.66 ± 8.34	12.18 ± 3.93
Group E1	0.03 ± 0.05	65.80 ± 23.01	5.46 ± 1.56
Group E2	0.71 ± 0.87	67.87 ± 18.84	3.98 ± 2.05

[a]Results are expressed as means $\pm$ SD.
[b]C2 > E1, $P < 0.001$; C2 > E2, $P < 0.01$; E1 < E2, $P < 0.05$.
[c]C2 = E1, $P > 0.05$; C2 = E2, $P > 0.05$; E1 = E2, $P > 0.05$.
[d]C2 > E1, $P < 0.01$; C2 > E2, $P < 0.001$; E1 = E2, $P > 0.05$.
[b,c, and d]: indicate one-way ANOVA analysis.

Zinc tubular reabsorption ($TRZn^{++}$ = %)

The values of $TRZn^{++}$ were similar in all four groups studied, with no significant biological and statistical variations, $P > 0.05$ (Table 1).

Plasma C-Peptide (ng ml^{-1}), Glucagon (pg ml^{-1}), and Cortisol (μg dl)

The mean levels of these hormones are showed in Table 2 relating to the Groups C2, E1, and E2 studied. The highest levels of C-peptide and cortisol were observed in Group C2, whereas their lowest levels of C-peptide were observed in Group E1 and cortisol were observed in Group E2. Glucagon was not significantly different in all of the three groups. Statistical results are showed in Table 2.

Discussion

Conflicting data have been reported in the literature about the status of zinc in patients with diabetes mellitus. However, all of these patients lose a large amount of zinc in urine, and, in spite of this phenomenon, there is no good method to establish whether zinc deficient they are (Brandão-Neto *et al.* 2000). The controversy arises because the current methods to characterize marginal zinc nutrition, such as the measurement of zinc in serum, plasma, total blood, red blood cells, granulocytes, platelets, lymphocytes, enzyme activities, hair, nail, sweat, and urine, do not provide consistent results (Brandão-Neto *et al.* 2000). In our experiment, basal serum zinc (SZn^{++}) was similar in all group studied, which probably argues against zinc deficiency (Table 1). These basal serum zinc results are compatible with Maldonado-Martin

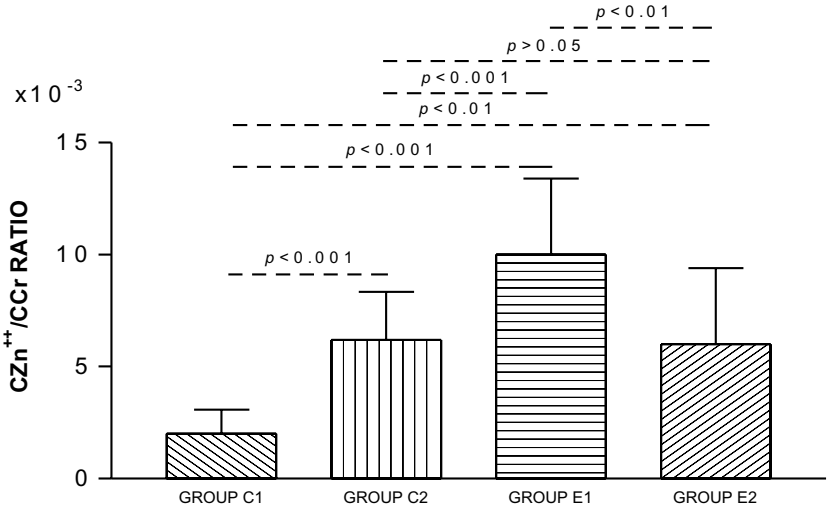

Fig. 2. Values of zinc clearance/creatinine clearance ratio in Group C1, Group C2, Group E1 and Group E2. Values are expressed as mean ± SD. P values were obtained from one-way ANOVA analysis and $P < 0.05$ was accepted as significant (see Table 1).

et al. (1991), and Honnorat *et al.* (1992). A significant increase in renal zinc excretion and renal zinc clearance was observed following venous zinc tolerance test in normal individuals and diabetic patients (Groups C2, E1, and E2; Table 1). These parameters were significantly higher when compared to the normal individuals-placebo group (Group C1), particularly the Group E1 that had high levels of plasma and urinary glucose, and lowest plasma C-peptide levels (Tables 1 and 2 and Figure 2). In this regard, Nakamura *et al.* (1991) reported high loss of zinc in the urine of IDDM patients when compared to normal individuals following intravenous zinc administration (1 μmol kg body weight). On the other hand, as GFR and $TRZn^{++}$ were normal in all four groups studied ($P > 0.05$) the hyperzincuria cannot attributed to alteration in these renal parameters (Table 1).

The association between hyperglycemia and/or glycosuria with hyperzincuria is not well understood. The Group E2 (diabetic patients without high levels of glycemia and glycosuria) showed similar renal zinc excretion and renal zinc clearance when compared with Group C2 (normal individuals following zinc injection). Nevertheless, in spite of the fact that Group E1 showed hyperglycemia, glycosuria, and high loss of urinary zinc, we did not detect any correlation between these parameters ($P > 0.05$). These results are in agreement with those reported by Hägglöf *et al.* (1983) and Kinlaw *et al.* (1983) that demonstrated no correlation between quantitative glycosuria and urinary zinc loss. On the other hand, these results contrast with those of McNair *et al.* (1981), which found that hyperzincuria is correlated with the level of glycosuria in IDDM patients. In this regard, Brandão-Neto *et al.* (1995) reported, in normal subjects, increased renal zinc excretion and renal zinc clearance following intravenous administration of 0.5 ml 50% glucose kg^{-1} body wt, without any zinc contamination.

It is known that glucagon has a stimulatory effect on zinc secretion and insulin and somatostatin has an opposite effect (Vander *et al.* 1983). The mechanisms whereby these hormones affect urinary zinc excretion remain not understood. In our experiment, we observed a strong negative correlation between CZn^{++}/Ccr ratio and plasma C-peptide in the Group E1, but not in the Group E2 ($r = -0.9992$, $P < 0.0001$, and $r = -0.5076$, $P > 0.05$, respectively). Because the mean of plasma C-peptide levels of Group E1 was significantly lower than in Group E2 ($P < 0.05$; Table 2), these results may reflect that insulin, here inferred by the equimolar levels of C-peptide, exerts a tonic inhibitory effect on zinc excretion of greater magnitude than any tonic stimulating effect by glucagon (Vander *et al.* 1983). In addition, the daily injected zinc dose in the form of insulin (0.7–1.2 μg) was negligible and did not influence the plasma and urine levels (Melchior *et al.* 1989). On the other hand, mean plasma glucagon and cortisol levels were similar in the Groups E1 and E2 ($P > 0.05$; Table 2), and no correlation was observed among CZn^{++}/Ccr ratio and these hormones ($P > 0.05$). This is the first time that such phenomenon is report in human beings.

In conclusion, urinary zinc excretion and renal zinc clearance were increased following venous zinc tolerance test in normal individuals and IDDM patients. However, these renal parameters were statistically more significant in the Group E1 (hyperglycemic, glycosuric, and with lowest insulinemic levels) than in others groups (normoglycemic, aglycosuric, and normoinsulinemic levels). Probably the mechanism to explain these exacerbated renal alterations could be the decreased plasma levels of insulin.

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