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Zinc kinetics in insulin-dependent diabetes mellitus patients

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Abstract

Zinc has an important role in the control of carbohydrate metabolism, and diabetic patients are at risk for zinc deficiency. However, there are conflicting data concerning nutritional zinc status. In order to investigate this topic, 10 normal and 10 insulin-dependent diabetic patients were studied following venous zinc tolerance test. Our results found no evidence of zinc deficiency or of changes on the kinetic parameters of zinc in patients with insulin-dependent diabetes mellitus following a venous zinc tolerance test.

Introduction

Zinc is a micronutrient that is involved in the metabolism of glucose and insulin. Zinc is found in high concentrations in the endocrine pancreas of many species where it modulates the synthesis, secretion and degradation of insulin as well as the formation of insulin hexamers. Zinc may also regulate insulin sensitivity in peripheral tissues (Brandão-Neto *et al.* 1990; Faure *et al.* 1992; Brun *et al.* 1995). On the other hand, zinc has also been implicated in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) and in the development of its complications (Jameson *et al.* 1985; Sandstead & Egger 1997). It is been reported that these patients commonly present hyperzincuria (Honnorat *et al.* 1992; Bhanot *et al.* 1994). Thus, it is important to understand the kinetics of zinc in diabetic patients. The available data on zinc status in patients with diabetes mellitus are controversial. The controversy arises because the current methods to characterize marginal or sub-optimum zinc nutrition, such as the measurement of zinc in serum, plasma, total blood, red blood cells (RBC), granulocytes, platelets, lymphocytes, enzyme activities, hair, nail, sweat and urine, do not provide consistent results (Walsh *et al.* 1994). In this regard, Nakamura *et al.* (1991) have proposed that the measurement of total-body zinc clearance, following injection of 0.066 mg Zn^{++}/kg body weight, may be an efficient parameter to characterize marginal or severe zinc deficiency. Based on these considerations, the aim of the present study was to evaluate the zinc kinetics in normal and IDDM individuals following a venous zinc tolerance test.

Materials and methods

Subjects

Ten normal individuals (5 of each sex, aged 24.10 ± 1.96) and 10 IDDM (5 of each sex, aged 25.20 ± 8.10) were studied after obtaining informed consent in writing, and approval by the Medical Ethics Committee. Normal individuals (medical students or staff members) had body mass index of 19.54 ± 2.76 kg/m^2 (mean $\pm$ SD), had no history of endocrinopathy and were not under any medications. Diabetic patients had body mass index of 19.94 ± 4.15 kg/m^2 (mean $\pm$ SD) and received insulin therapy for metabolic control (57.0 ± 20.0 U/day, NPH insulin), except in the morning of test, and they did not have nephropathy or other complications. Duration of diabetes was 8.00 ± 6.03 years. All control and diabetic patients

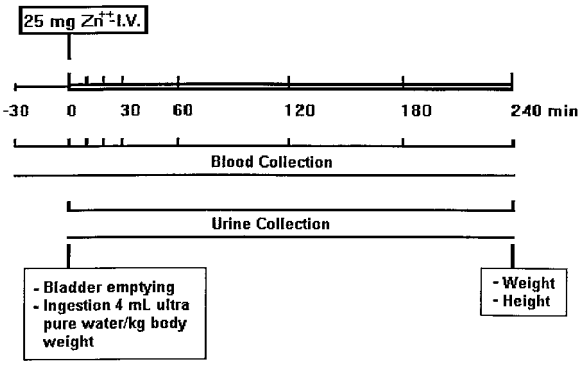

Fig. 1. Experimental design of venous zinc tolerance test and glomerular filtration test.

were studied while on their usual diet. Diabetic diet consisted of 50% carbohydrates, 30% fat, and 20% protein, calculated as energy content (Brandão-Neto *et al.* 1999).

Experimental design (Figure 1)

Group 1 (control): Ten healthy subjects
Group 2 (experimental): Ten IDDM patients

Venous zinc tolerance test

We investigated zinc kinetics in these patients during venous zinc tolerance test (25 mg Zn^{++}, 2 mL as zinc sulfate) at 8:00 a.m., after 12-h fast. The subjects rested in bed throughout the experiment. An antecubital vein of each forearm was punctured and a device for infusion was installed and maintained with physiological saline. Zinc was injected over a period of 1 min. Blood samples were collected from the 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 measurements. Serum zinc was collected at times 0, 30, 60, 90, and 120 min. Serum creatinine was collected at time 0 and 120 min. At the end of the test, weight and height of subjects were measured for the determination of body surface area. These procedures were performed during glomerular filtration following the ingestion of ultra pure water plus 25 mg Zn^{++} (2 mL as zinc sulfate), i.v., in the Group 1 and 2 (Brandão-Netoit *et al.* 1995).

Kinetics approach

The parameters of the kinetics study were calculated as follows: Serum zinc: SZn^{++}; volume of distribution: $Vd = \text{dose i.v.}/\Delta Co$; elimination constant: $Kel = 0.693/t1/2$; and total-body zinc clearance: $CZn^{++} = Kel \times Vd$ (Nakamura *et al.* 1991).

Metal ion analysis

Venipuncture was performed using plastic syringes without a tourniquet. All procedures regarding manipulation of zinc samples were done according with the international standards for prevention of Zn^{++} contamination of the environment (Subramanian 1995). Blood samples: Immediately after collection the blood samples were maintained in metal-free tubes without anticoagulants and kept at 37 °C, during 120 min, until clot formation. Next, 500 μL of the serum was collected with plastic pipets and transferred to plastic tubes containing 2000 μL of ultra pure water (Milli-Q plus, Millipore, USA) to dilute the serum. The samples were then kept at −20 °C, up to 2 months until analysis. Samples with signs of hemolysis were discarded. Urinary samples: Urine was collected in a recipient made out of stainless steel, the volume was measured in a cylinder and then 500 μL was collected and diluted with 2000 μL of ultra pure water.

All serum and urinary samples of each individual from control and experimental phases were analyzed within the same assay in duplicate using spectrophotometer of atomic absorption (AA-680G - Shimadzu - Japan). Normal values for the assay were within 0.7–1.2 μg/mL, the sensitivity was 0.01 μg/mL and the intra assay coefficient of variation was 3.9%. The standard solution of Zn^{++} (0.5 ppm) was obtained by diluting the stock solution of Zn^{++} (500 ppm) that was prepared from 0.5 g of Zn^{++} powder purchased from Merck (Germany), and dissolved in a small volume of hydrochloric acid (HCl), which was later reconstituted to 1 L, with HCl 1% (v/v). The Zn^{++} concentration of the samples were determined using a standard solution of our laboratory as quality control, which has been in use routinely since 1975. All the other procedures, such as calibrations and measurements were done in accordance with the manufacturer.

Table 1. Daily intakes of nutrients by dietary recalls during the study in 10 normal individuals and 10 insulin-dependent diabetes mellitus patients.

	Control		IDDM	
	Male	Female	Male	Female
Energy (kcal)	2345.38± 148.68	1632.23± 43.04	2357.60± 313.53	1670.45± 134.08
Protein (g)	55.72± 7.28	41.77± 2.19	56.32± 15.36	43.72± 6.83
Animal Protein (%)	83		72	
Vegetal Fiber (g)	14.11 ± 1.10		16.90 ± 1.20	
Zinc (mg)	14.80 ± 1.23		12.8 ± 2.05	
Zinc from Animal Protein (%)	97		86	

Table 2. Kinetic characteristics of the patients studied after the administration of the zinc load (25 mg Zn^{++})[a].

	SZn^{++}[b] (μg/dL)	t1/2[c] (h)	Kel[d] (h^{-1})	Vd[e] ($L.kg^{-1}$)	CZ^{++}[f] (mL.min)	AUC[g] ($mL.min^{-1}$)
Control	104.20± 22.08	3.34± 0.62	0.21± 0.05	0.07± 0.01	16.19± 4.16	1643.07± 437.85
IDDM	102.00± 19.28	2.93± 0.41	0.24± 0.00	0.07± 0.01	17.90± 4.65	1449.49± 373.05

[a]Results are expressed as means ± SD, each group contained 10 patients. [b, c, d, e, f, g]Control $=$[b, c, d, e, f, g] IDDM, $p > 0.05$.

Biochemical analysis

Serum and urinary glucose, creatinine, and other biochemical parameters were measured by autoanalyzer (Cobas-Mira Plus, Switzerland) as previously described by Brandão-Neto *et al.* (1999).

Statistical analysis

Statistical analysis was performed using the Student's *t*-test for unpaired data and linear regression test.

Results

Nutritional assessment showed that healthy and diabetic patients were maintained in a diet with adequate caloric contents and were all in a good clinical nutritional state. No patient showed signs of zinc deficiency as assessed by food intake, reported symptoms, and medical examination. Daily intakes of nutrients by dietary recalls showed that both groups had the mean energy intake within the normal limits of the recommended dietary allowances. Protein intake was in the normal range in both groups that presented a high intake of food from animal sources. Zinc intake was more than 13 mg in both groups (86% provided by protein of animal sources). Our patients were fed normal-fiber amounts (more than 14 g/day), which did not affect zinc bioavailability (Table 1).

Basal serum zinc levels were similar in both groups and within the normal limits (70–120 μg/dL), $p > 0.05$ (Figure 2 and Table 2). There were no differences between sexes in these two groups. During the zinc injection, serum zinc levels showed the same profile in the two groups (Figure 2), and serum glucose levels were also not changed in both groups (Brandão-Neto *et al.* 1999). Half-life of serum zinc (t1/2), Kel, CZn^{++}, Vd, and AUC were similar in these groups, $p > 0.05$, respectively (Figure 2 and Table 2).

Discussion

IDDM patients are at risk for zinc deficiency (Sandstead & Egger 1997). However, it is very difficult to establish the zinc status, and there is not a good method available to determine marginal or

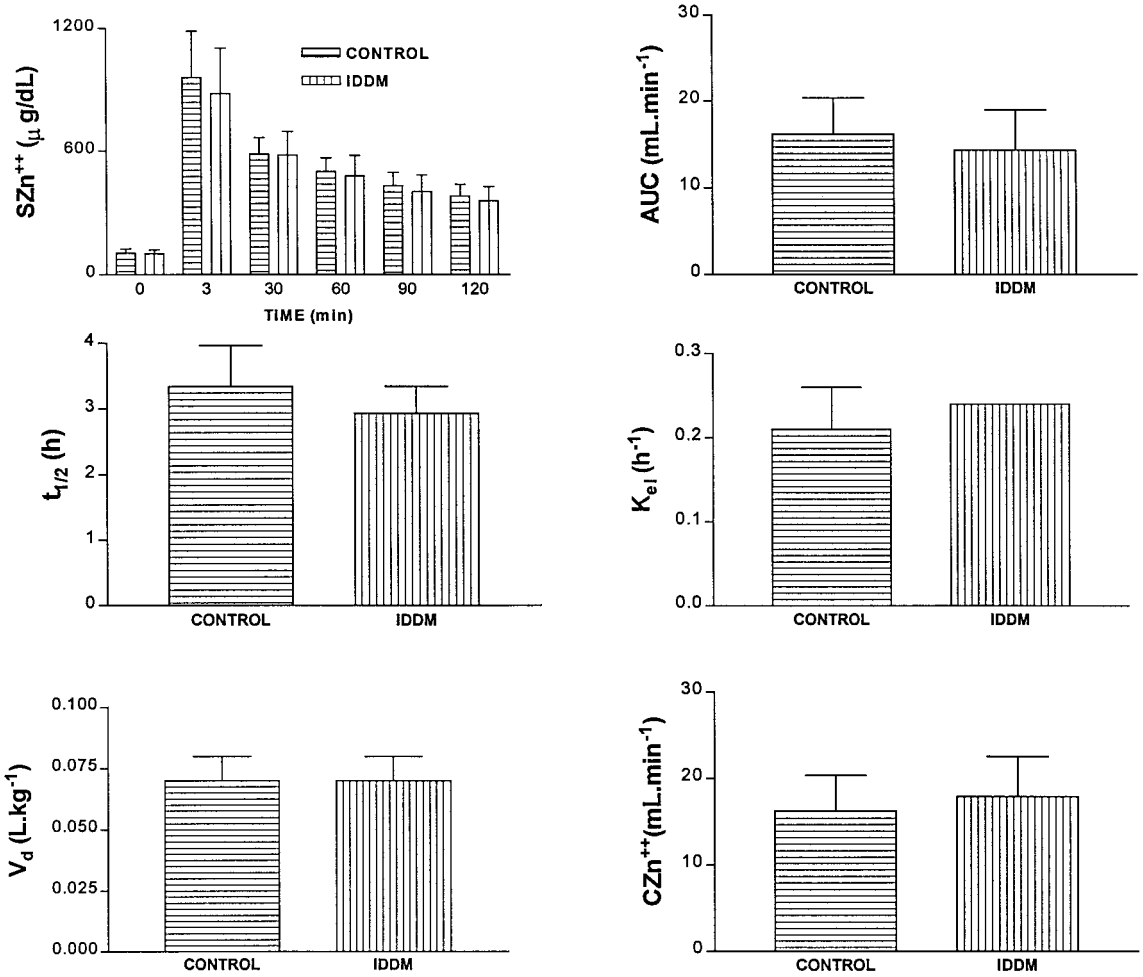

Fig. 2. Values of serum zinc (SZn^{++}), area under the curve (AUC), half-life of serum zinc ($t_{1/2}$), elimination constant of serum zinc (k_{el}), volume of distribution (V_d), and total-body zinc clearance (CZn^{++}) in 10 control subjects and 10 insulin-dependent diabetes mellitus patients following 25 mg Zn^{++} intravenously.

sub-optimum zinc deficiency (Walsh *et al.* 1994). Recently, Nakamura *et al.* (1991) proposed a kinetics approach to study zinc metabolism and reported evidence of zinc deficiency in children with IDDM.

In this study, energy intake, protein intake, zinc intake, mainly from animal sources, and fiber intake were found at the recommended dietary allowance in both groups investigated (FAO/WHO/UNO, 1985) (Table 1). Thus, basal serum zinc concentrations were in the normal limits (70–120 μg/dL) and similar in the healthy and diabetic patients (Figure 2 and Table 2). Furthermore, there was no difference between sexes in these two groups. These results are consistent with those obtained by Maldonado-Martin *et al.* (1991). With respect to glucose, the infusion of a pharmacological dose of zinc (25 mg for both groups) was incapable to increase acutely serum glucose levels as reported by Etzel & Cousins (1983). This lack of effect on serum glucose was previously reported by us elsewhere (Brandão-Neto *et al.* 1990, 1991).

With respect to the zinc kinetics study, we observed no statistical difference among (t1/2) Kel, Vd, CZn^{++}, and AUC in these groups (Figure 2 and Table 2), in agreement with our previous report (Brandão-Neto *et al.* 1996). These results differ from the findings reported by Nakamura *et al.* (1991), who argue that CZn^{++} is a better parameter than serum zinc level. Furthermore, their IDDM patients had the $CZn^{++} \sim 3$ times higher than control subjects, and the longer the duration of IDDM the higher were the CZn^{++} values. In our case, we did not observe any differences in CZn^{++} between control and dia-

betic patients, even in patients with a longer duration of diabetes than the ones reported from Nakamura (9.3 years). In this regard, Maldonado-Martin *et al.* (1991), injecting 8 mg Zn^{++} in healthy and IDDM patients, reported that serum zinc in IDDM patients initially increased and subsequently showed a more significant decline than in controls. Although these authors have not investigated zinc clearance, they reported that urinary zinc excretion 24 h after zinc injection was significantly increased in controls, but not in diabetics. Taken all together, we argue that our diabetic patients are not zinc-deficient.

In conclusion, total-body zinc clearance is not a reliable parameter to detect zinc deficiency in humans and that the interrelationship between zinc and diabetes mellitus needs further studies to clarify the zinc status of these patients.

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