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Lack of acute zinc effects in glucose metabolism in healthy and insulin-dependent diabetes mellitus patients

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Abstract

Acute or chronic zinc administration may cause hyperglycemia in experimental animals. These findings are attributed to permissive actions of glucocorticoids and glucagon upon hepatic gluconeogenesis and glycogenolysis. The effect of Zn^{++} on plasma glucose, C-peptide, glucagon, and cortisol was investigated in healthy and insulin-dependent diabetes mellitus (IDDM) patients. 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 tested at 7:00 AM after 12-h fast. Twenty-five mg of Zn^{++} were administered intravenously during 1 min, and blood samples were collected from the contralateral arm at 0, 3, 30, 60, 90 and 120 min after Zn^{++} injection. The plasma levels of glucose, C-peptide, and glucagon remained constant throughout the experimental period in both groups studied. Plasma cortisol levels decreased significantly, which is consistent with our previous findings. These results suggest that, in contrast to experimental animals, acute Zn^{++} administration, despite decreasing cortisol levels, does not change carbohydrate metabolism in human beings.

Introduction

Zinc is an essential micronutrient that is involved in carbohydrate metabolism. Hypozincemia and hyperzincemia have powerful influences on glucose and insulin metabolism through the pancreatic β-cells or peripheral action of insulin (Faure *et al.* 1992; Kubisch *et al.* 1994; Brun *et al.* 1995; Chen *et al.* 1998). On the other hand, patients with diabetic complications had significantly lower serum zinc levels in comparison to patients without diabetic complications (Jameson *et al.* 1985; Winterberg *et al.* 1989). These are reasons to suspect that abnormal zinc metabolism may play a role in the pathogenesis of diabetes mellitus and some of its complications. Also, it has been reported that acute administration of zinc promotes hyperglycemia in experimental animals (Horak & Sunderman 1975; Etzel & Cousins 1983). On the other hand, oral or intraperitoneal administration of Zn^{++} normalized hyperglycemia in diabetic rats within 2, 3 or 24 h (Lin & McCormick 1986; Shisheva *et al.* 1992). The mechanism by which this micronutrient produces this effect is not clear. On the basis of these considerations, the aim of the present study was to determine, in humans, the possible existence of an acute effect of zinc on glucose metabolism and on the complexity of the mechanisms involved.

Materials and methods

Subjects

The study group was comprised of 10 normal individuals (5 of each sex, aged 24.10 ± 1.96) and 10 IDDM (5 of each sex, aged 25.20 ± 8.10). Normal individuals (medical students or staff members) were free from endocrine disease, all were of normal weight, and none used any type of medication. Food intake was evaluated at the beginning of the study and after 2 months using a weekly history dietary recalls. It was based on frequency and amount of intake for each food and

exact 24-h dietary recalls, obtained from both groups. Nutrient intake was calculated using the Nutritional Evaluation computer program (V. 2.0), developed by the Department of Nutrition, Universidade Federal de São Paulo, Brazil) that takes into account the composition of Brazilian foods. Diabetic patients received insulin therapy for metabolic control, except in the morning of test, and they did not have nephropathy or other complications. Duration of diabetes was 8.00 $\pm$ 6.03 years. Initial screening included electrocardiogram, hemogram, liver and kidney function tests, and they were normal for all patients studied. The protocol, which was approved by the Medical Ethics Committee, was explained to the patients and written consent has obtained from all participants.

Experimental design

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

Venous zinc tolerance test

This test was started at 7:00 AM after 12-h fast, with the subjects maintaining dorsal decubitus throughout the test. Number 19 infusing devices were implanted into the antecubital veins of both forearms and maintained with physiological saline. Basal blood samples were collected at -30 and 0 min for zinc, glucose, C-peptide, glucagon, and cortisol. Intravenous administration of 25 mg Zn^{++} (2 mL zinc sulfate) over a period of 1 min was started at time 0 min (8:00 h). Blood samples were then immediately collected from another vein in the contralateral arm at 3, 30, 60, 90 and 120 min for above element and substances.

Sample collection and analysis

Venipuncture was performed using plastic syringes without a tourniquet. All material used for zinc collection, separation, and storage was propylene plastic and metal free. Serum and plasma samples were frozen and stored at $-20\,^{\circ}$C until the time of measurement. Serum zinc was measured by atomic absorption spectrophotometer (Shimadzu, AA680G, Japan) according to the instructions of manufacturers. Hemolyzed samples were discarded. The intraassay error was 2.4% and sensitivity was 0.02 μg/mL. Serum glucose was measured by the glucose oxidase method using an autoanalyzer (Cobas-Mira Plus, Switzerland). Other clinical variables were determined by using standard clinical laboratory methods: red blood count, white blood count, hemoglobin, hematocrit, 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. C-peptide, glucagon, and cortisol were measured by the radioimmunoassay (Diagnostic Products Corporation, USA), with an intraassay error of 3.1, 1.9, and 2.6%, and a sensitivity of 0.1 ng/mL, 5.3 pg/mL, and 0.2 μg/dL, respectively. All samples were processed in the same assay to avoid inter-assay variations.

Statistical analysis

Student's *t* test, linear regression test, and correlation analysis were used for statistical assessments.

Results

Daily intake of nutrients

Healthy and diabetic patients referred a diet with adequate caloric contents and they all presented a good clinical nutritional state. No patient showed signs of zinc deficiency as assessed by food intake, reported symptoms, and medical examination. Mean energy intake was in acceptable limits to the recommended dietary allowances in both groups: control-male = 2345.38 kcal; control-female = 1632.23 kcal; IDDM-male = 2357.60 kcal; IDDM-female = 1670.45 kcal. Mean protein intake was in the acceptable range in both groups with high intake of food from animal sources (more than 72%): control = 48.74 g; IDDM = 50.00 g. Mean zinc intake was more than 12.80 mg in both groups, 86% provide by animal protein. Mean vegetal fiber was more than 14.11 g, which did not affect zinc bioavailability.

Other clinical laboratory parameters were in the normal range for control and diabetic subjects, exception to cholesterol and triglycerides, which were high in some diabetics.

Serum zinc concentrations

Basal serum zinc (SZn^{++}) levels were in the usual range (0.7–1.2 μg/mL) and identical in the control and diabetic patients (1.04 ± 0.22 and 1.02 ± 0.19 μg/mL, respectively), and there were no conflicting results between sexes in these two groups. During the venous zinc tolerance test, SZn^{++} levels showed the same

Table 1. Values of glucose, C-peptide, glucagon, and cortisol obtained in 10 healthy and 10 IDDM patients following venous zinc tolerance test (25 mg Zn^{++})*

Time	Control			IDDM			Control			IDDM		
	Glucose						C-peptide					
Min	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n
0	86.20	6.94	10	208.10	137.07	10	1.51	0.76	10	0.44	0.79	10
3	85.30	6.70	10	206.50	137.17	10	1.42	0.65	10	0.36	0.65	10
30	86.60	8.72	10	204.00	134.50	10	1.62	0.85	10	0.33	0.61	10
60	85.30	8.00	10	197.60	130.36	10	1.38	0.73	10	0.41	0.79	10
90	85.00	8.98	10	193.70	125.98	10	1.52	0.55	10	0.30	0.54	10
120	85.20	8.01	10	192.10	124.02	10	1.32	0.52	10	0.37	0.71	10
	Glucagon						Cortisol					
0	49.70	24.48	10	73.75	25.42	10	17.09	3.18	10	6.15	2.12	10
3	65.81	32.20	10	68.13	27.23	10	15.83	2.72	10	5.86	2.41	10
30	44.88	19.06	10	59.90	26.96	10	13.19	2.65	10	5.58	2.71	10
60	51.42	15.51	10	66.14	30.78	10	11.19	3.23	10	3.96	1.61	10
90	55.54	29.73	10	65.71	26.98	10	8.40	2.71	10	3.95	2.47	10
120	42.55	13.04	10	83.63	30.39	10	7.32	2.37	10	2.83	1.93	10

*Values were obtained during 120 min after Zn^{++} injection. Values are means ± SD. Glucose: normal range: 70–110 mg%. C-peptide: normal range: 0.8–4.0 ng/mL. Glucagon: normal range: 40–130 pg/mL. Cortisol: normal range: 5.0–25.0 μg/dL.

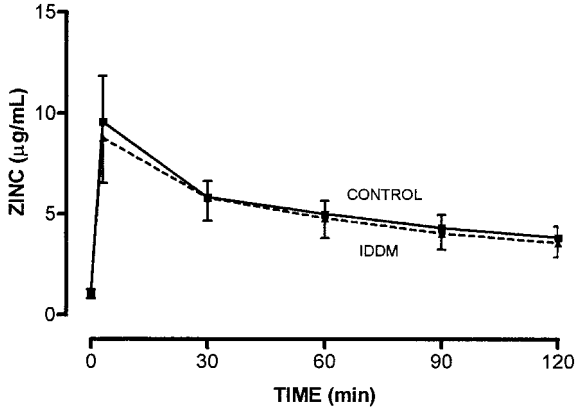

Figure 1. Changes in serum zinc levels following the venous zinc tolerance test (25 mg Zn^{++}). Values are expressed as means ± SD. The areas under the curves were similar in control ($n = 10$) and IDDM ($n = 10$) patients ($p > 0.05$).

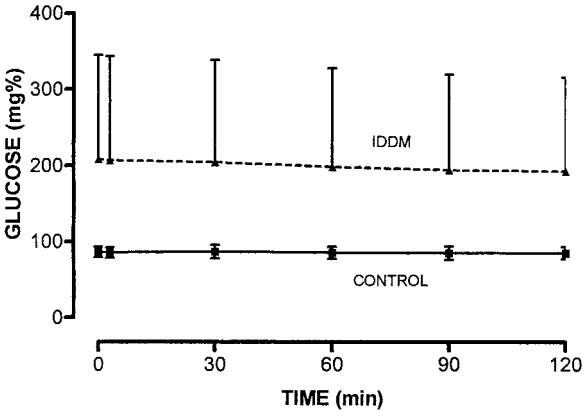

Figure 2. Plasma glucose following venous zinc tolerance test (25 mg Zn^{++}) in control ($n = 10$) and IDDM ($n = 10$) patients. Values are means ± SD. No significant differences among plasma levels were observed in both groups ($p > 0.05$).

profile in the two groups (Figure 1). The curves and the area under the curves were statistically not significant ($p>0.05$).

Plasma glucose concentrations

The plasma glucose profile was similar in both groups. Although the levels of glucose were more elevated in diabetic group than in control group, acute venous zinc administration was not capable to change blood glucose concentrations (Figure 2 and Table 1). These levels remain constant in both groups, throughout the 120 min of the study ($p>0.05$).

Plasma C-peptide concentrations

C-peptide levels did not change throughout the study in both groups ($p>0.05$). On the other hand, plasma

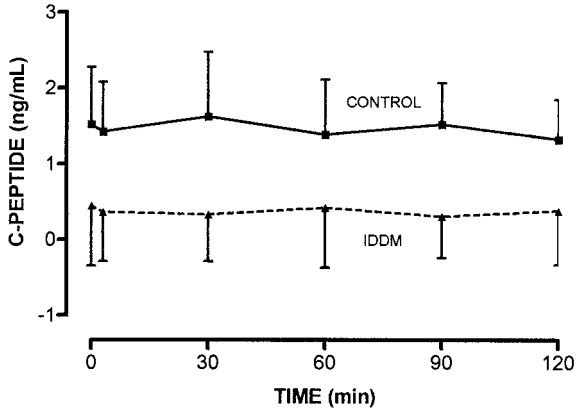

Figure 3. Plasma C-peptide following venous zinc tolerance test (25 mg Zn^{++}) in control ($n = 10$) and IDDM ($n = 10$) patients. Values are means ± SD. No significant differences among plasma levels were observed in both groups ($p > 0.05$).

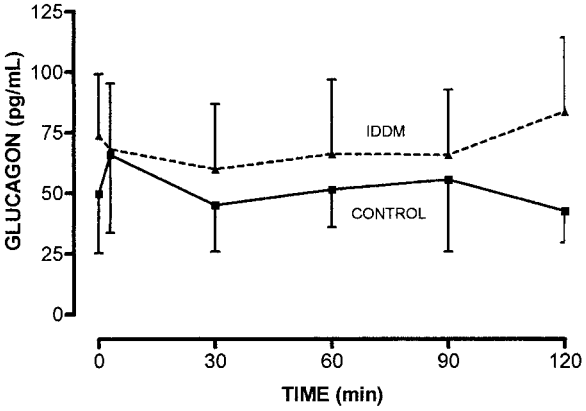

Figure 4. Plasma glucagon following venous zinc tolerance test (25 mg Zn^{++}) in control ($n = 10$) and IDDM ($n = 10$) patients. Values are means ± SD. No significant differences among plasma levels were observed in both groups ($p > 0.05$).

C-peptide levels was lower in IDDM patients than in healthy subjects (Figure 3 and Table 1).

Plasma glucagon concentrations

Venous Zn^{++} injection did not change the glucagon in both groups studied. Plasma glucagon values were constant and no statistical difference was detected between healthy and diabetic patients ($p>0.05$) (Figure 4 and Table 1).

Plasma cortisol concentrations

Zinc acutely decreased plasma cortisol levels in all groups studied, and more markedly in control group. IDDM group had hypocortisolemia in comparison to

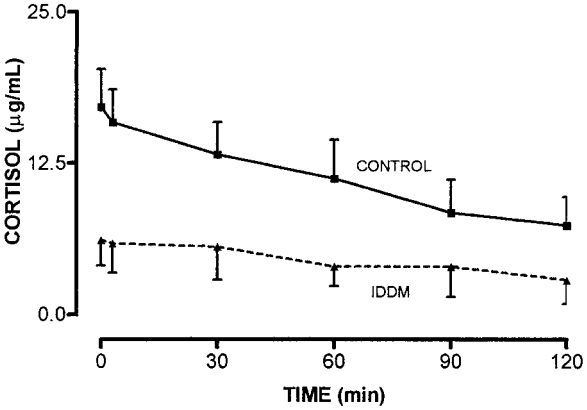

Figure 5. Plasma cortisol following venous zinc tolerance test (25 mg Zn^{++}) in control ($n = 10$) and IDDM ($n = 10$) patients. Values are means ± SD. Zinc administration modified the profile of cortisol and the fall of cortisol was statistically significant in both groups ($p < 0.05$). Hypocortisolemia was observed in IDDM patients in comparison to the controls ($p < 0.05$).

control group, and both curves were significantly different ($p<0.05$). Correlation analysis revealed the existence of a positive correlation between cortisol and Zn^{++} ($r = 0.92$, $p<0.05$) (Figure 5 and Table 1).

Discussion

In this study, we investigated the acute effect of Zn^{++} on plasma glucose, C-peptide, glucagon, and cortisol in healthy and IDDM patients. At present, there are no satisfactory hallmarks of zinc deficiency in humans. However, serum zinc is regarded as a reasonable indicator of zinc status. In our series, we observed normal serum zinc levels in healthy and diabetic patients. These patients had energy intake, protein intake, and zinc intake, mainly from animal sources, within the recommended dietary allowance (FAO/WWHO/UNO, 1985).

The results relate no acute action of Zn^{++} on glucose metabolism. Horak & Sunderman (1975) reported, in normal, adrenalectomized and hypophysectomized rats, hyperglycemic responses following parenteral injections of Zn^{++}. This effect was not observed in this experiment, since plasma glucose levels were constant throughout the 120 min of the study (Figure 2), corroborating preliminary studies with oral Zn^{++} administration (Brandão-Neto *et al.* 1990, 1991). On the other hand, Horak & Sunderman (1975) attributed to hyperglycemia the permissive actions of glucocorticoids and glucagon upon hepatic gluconeogenesis and glycogenolysis. To examine the

involvement of these hormones, we measure glucagon and cortisol. For instance, we did not observe plasma glucagon elevation (Figure 4). Moreover, rather than elevation on glucocorticoids, we detected a fall in plasma cortisol in control and diabetic patients (Figure 5). This fall should not be attributed only to the circadian rhythm, since correlation analysis revealed the existence of a positive correlation between cortisol and Zn^{++} (Table 1). Although, the cortisolemia was lower in diabetic patients than in controls, the decrease of glucocorticoid was also statistically significant in diabetic patients ($p<0.05$). Similar results were previously reported by Brandão-Neto *et al.* (1990a, b). Kinsley & Simonson (1996) have attributed the reason for this finding to an abnormal activity of the hypothalamic pituitary-adrenal axis in IDDM patients.

Also, contrary to our results, Etzel & Cousins (1983) investigating normal, adrenalectomized, and diabetic rats reported elevation of serum glucose within 15 min after intraperitoneal (or oral) administration of Zn^{++} (25 μmol), returning to normal ranges within 4 h. Plasma glucagon was significantly elevated within 15 min and insulin was significantly depressed within 30 min, both reverting to normal limits from 4 h later. Like Horak & Sunderman (1975), this rapid alteration in blood glucose was attributed to glucocorticoids and glucagon as a major factor in the hyperglycemic effect of Zn^{++}. In attention to insulin in this metabolic process, we determined the C-peptide and again we did not detect any variation in the levels of this hormone in control and IDDM patients (Figure 3). This is indicative that acute zinc injection was unable to modify the blood C-peptide, or insulin for inference, and also do not support the opinion of Ghafghazi *et al.* (1981) who have shown that Zn^{++} inhibits insulin release in vitro.

Finally, the pharmacological dose (Figure 1) administered in the present study demonstrate that acute hyperzincemia did not alter peripheral glucose, C-peptide, and glucagon in human beings, but depress cortisol. These results did not agree with that from experimental animals, probably indicating that Zn^{++} has different mechanism of action on glucose metabolism in humans.

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