

Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus

Abdul Hamid Zargar, Nissar Ahmad Shah, Shariq Rashid Masoodi, Bashir Ahmad Laway, Farooq Ahmad Dar, Abdul Rashid Khan, Fayaz Ahmad Sofi, Arshad Iqbal Wani

Summary

A relationship has been reported between trace elements and diabetes mellitus. This study evaluated the role of such a relationship in 83 patients with non-insulin dependent diabetes mellitus (40 men and 43 women), with a mean duration of diabetes of 3.9 ± 3.6 years. Patients with nephropathy were excluded. Thirty healthy non-diabetic subjects were studied for comparative analysis. Subjects were subdivided into obese and non-obese. Diabetic subjects were also subdivided into controlled and uncontrolled groups; control was based on fasting blood glucose and serum fructosamine levels. Plasma copper, zinc and magnesium levels were analysed using a GBC 902 double beam atomic absorption spectrophotometer. Plasma zinc and magnesium levels were comparable between diabetic and non-diabetic subjects, while copper levels were significantly elevated ($p < 0.01$) in diabetic patients. Age, sex, duration and control of diabetes did not influence copper, zinc, or magnesium concentrations. We conclude that zinc and magnesium levels are not altered in diabetes mellitus, but the increased copper levels found in diabetics in our study may merit further investigation of the relationship between copper and non-insulin dependent diabetes mellitus.

Keywords: copper; magnesium; diabetes mellitus; zinc

Interest in trace elements has been steadily increasing over the last 25 years. Trace elements are accepted as essential for optimum human health, because of their diverse metabolic characteristics and functions. They serve a variety of catalytic, structural and regulatory functions, in which they interact with macromolecules such as enzymes, pro-hormones, pre-secretory granules and biological membranes.

Copper is one of the essential trace elements, and has a particular role in cytochrome oxidase function at the terminal end of the mitochondrial electron transport chain. The loss of this activity may contribute to the characteristic swelling and distortion of mitochondria which can be observed in copper deficiency, particularly in metabolically active tissues such as pancreatic acinar cells, enterocytes, and hepatocytes.¹ In subjects with insulin-

dependent diabetes mellitus (IDDM), zinc concentrations have been demonstrated to be lower in leucocytes and erythrocytes than in serum, while no such alteration has been found with copper.² No definite relationship has been described between copper concentrations and the clinical status of patients with diabetes mellitus.³

Zinc, another essential trace element, is a component of many enzymes, and plays an important role in the maintenance of several tissue functions,⁴ including the synthesis, storage and release of insulin.⁵ Zinc has been found to enhance the effectiveness of insulin *in vitro*, and it has been postulated that zinc deficiency may aggravate the insulin resistance in non-insulin dependent diabetes mellitus (NIDDM).⁶ The development of glucose intolerance in rats after dietary zinc deprivation, together with the occurrence of zinc deficiency in diabetes mellitus, suggest a role for zinc deficiency in the pathogenesis of diabetes mellitus.⁷ It has been suggested that zinc repletion could improve insulin sensitivity in patients with NIDDM.⁸

Magnesium is an essential ion involved in glucose homeostasis at multiple levels. A complex interplay exists between magnesium and glucose metabolism. Hypomagnesaemia has been reported in both IDDM and NIDDM patients.^{9,10} Magnesium plays an important role in the activities of various enzymes involved in glucose oxidation, and may play a role in the release of insulin.^{11,12} Magnesium has been reported to be mainly intracellular, and its intracellular uptake is stimulated by insulin, although the cellular physiology of magnesium metabolism is not fully understood.¹³ The role of magnesium in diabetes mellitus has not been clearly defined and still remains a controversial issue.

In the present study, plasma levels of copper, zinc and magnesium were measured in patients with NIDDM and the influence of body weight and control of diabetes on serum concentration of these elements was determined. The primary aim was to ascertain whether or not patients with NIDDM had a zinc deficiency, as zinc has been implicated in the pathogenesis of insulin resistance. In trace element metabolism the best known interaction is the reported antagonism between zinc and copper.¹⁴ We therefore sought to investigate the existence of any such antagonism in subjects with NIDDM.

Institute of Medical Sciences, Soura, Srinagar, Kashmir, India

Department of Endocrinology

A H Zargar
N A Shah
S R Masoodi
B A Laway
F A Dar
F A Sofi
A I Wani

Department of Biochemistry
A R Khan

Correspondence to
Professor AH Zargar, PO
Box 1098, GPO
Srinagar-190001, Kashmir,
India

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Materials and methods

One hundred randomly selected patients with NIDDM on oral hypoglycaemic agents who were attending the diabetes clinic of this Institute and 30 healthy non-diabetic subjects (with normal glucose tolerance test) constituted the study population. Patients receiving insulin and patients with nephropathy were excluded. Clinical and biochemical analysis was carried out in NIDDM patients to document nephropathy, neuropathy or retinopathy. Nephropathy was defined as 24-hour urinary protein excretion of more than 500 mg, in the absence of urinary tract infection or severe hypertension, on two separate occasions.¹⁵ All the diabetic patients were receiving glibenclamide in variable doses (2.5–15 mg/day), in addition to a diet appropriate for their ideal weight. None of the subjects had a history of zinc supplementation.

ANTHROPOMETRY

Subjects were divided into obese and non-obese according to weight and body mass index (BMI). Subjects were considered obese if body weight was more than 20% of the desirable weight. Subjects whose weight was within the desirable weight range with a BMI less than 27.8 in males and 27.3 in females were categorised as non-obese.¹⁶

DIABETIC CONTROL

Control was assessed by overnight fasting blood glucose and serum fructosamine levels. Patients were considered to be well controlled if their fasting blood glucose levels were 3.3–7.2 mmol/l, with serum fructosamine levels $\leq 285 \mu\text{mol/l}$.¹⁷ If fasting glucose levels were beyond the acceptable range with elevated serum fructosamine, the subjects were considered uncontrolled. Patients who had a disparity between fasting blood glucose and fructosamine values were excluded. A final study cohort of 83 diabetic subjects was obtained.

LABORATORY SAMPLES

Venous blood was collected in the morning after an overnight fast. Samples were analysed for haemogram, glucose, urea, creatinine and fructosamine. In addition, a 12-lead electrocardiogram, chest X-ray, urine examination, 24-hour urinary protein and creatinine clearance were done in the diabetic patients.

SERUM FRUCTOSAMINE ESTIMATION

Serum fructosamine levels were estimated by reduction test with nitroblue tetrazolium

(NBT) based on the principle of conversion of NBT to formazan by fructosamine; the colour changes thus observed are proportional to serum fructosamine concentration in alkaline medium. Carbonate buffer was used to attain a pH of 10.3 and the reaction was catalysed by uricase. Measurements were made against a standard using quality control by Precinorm fructosamine and Precipath fructosamine (Germany) for normal and pathological ranges, respectively. Values were considered normal up to $285 \mu\text{mol/l}$.¹⁸

COPPER, ZINC, AND MAGNESIUM ESTIMATION

Five ml venous blood was collected in heparinized, zinc- and copper-free, polypropylene tubes in the fasting state from all subjects. Heparinized samples were centrifuged at 1500 g for 10 min to separate the plasma for estimation of copper and zinc. Plasma was diluted with an equal volume of trichloroacetic acid to precipitate proteins. The precipitate was kept at 0°C for 10 min. The supernatant was directly aspirated into GBC 902 double beam atomic absorption spectrophotometer (Victoria, Australia). The instrument was calibrated using standards from Sigma (St Louis, MO, USA). For magnesium estimation, plasma samples were diluted 1 in 200 with distilled water and the diluted samples aspirated into the atomic spectrophotometer for analysis. Analytical reliability was determined by quality control sera obtained from Boehringer Mannheim Diagnostica (Germany).

STATISTICAL METHODS

Statistical analysis included standard methods for comparison among variables. A two-tailed p-value was used for calculating statistical significance. A p-value of < 0.05 was taken as statistically significant.

Results

Eighty-three patients with NIDDM (40 men, 43 women; mean age 51.6 ± 8.8 years) and 30 healthy non-diabetic subjects (21 men, 9 women; mean age 36.6 ± 7.7 years) comprised the study group. The mean BMI was $24.5 \pm 3.8 \text{ kg/m}^2$ in the diabetic group and $22.8 \pm 3.8 \text{ kg/m}^2$ in the controls. The mean duration of diabetes was 3.9 ± 3.6 years. Mean fasting blood glucose levels were $9.3 \pm 4.2 \text{ mmol/l}$ in the diabetic group and $4.5 \pm 0.83 \text{ mmol/l}$ in controls, while the mean serum fructosamine was $318 \pm 116 \mu\text{mol/l}$ in the diabetics compared with 178 ± 43 in controls.

Table 1 Clinical and biochemical characteristics of control and diabetic subjects (figures given as means \pm SD)

	Control (n=30)	Controlled diabetics			Uncontrolled diabetics		
		Obese (n=13)	Non-obese (n=27)	Total (n=40)	Obese (n=25)	Non-obese (n=18)	Total (n=43)
Age (years)	36.3 \pm 7.71	51.9 \pm 9.12 *	50.2 \pm 8.26	50.7 \pm 8.47	52.7 \pm 9.35	51.9 \pm 9.12	52.4 \pm 9.15
Sex (M/F)	21/9	4/9	19/8	23/17	8/17	9/9	17/26
Weight (kg)	—	66.1 \pm 9.61	57.2 \pm 8.14	60.8 \pm 10.4	63.4 \pm 9.77	55.6 \pm 6.74	60.1 \pm 10.5
BMI (kg/m ²)	22.79 \pm 3.75	28.9 \pm 2.79	21.6 \pm 2.31	24.0 \pm 3.75	27.5 \pm 2.43	22.2 \pm 2.79	24.9 \pm 3.75
Fasting blood glucose (mmol/l)	4.46 \pm 0.83	5.9 \pm 0.99	5.9 \pm 1.05	5.9 \pm 1.02	9.8 \pm 2.63	12.0 \pm 5.02	10.7 \pm 3.92
Fructosamine ($\mu\text{mol/l}$)	178 \pm 43	218 \pm 28.2	229 \pm 35.6	225 \pm 34.8	413 \pm 97.0	407 \pm 96.7	407 \pm 95.2

Table 2 Plasma copper concentrations ($\mu\text{mol/l}$, mean \pm SD): effect of obesity and diabetic control

Group	Total	Obese	Non-obese	p-value
Non-diabetic	13.91 \pm 3.02 (n=30)	14.72 \pm 1.83 (n=4)	13.79 \pm 3.18 (n=26)	>0.5 (NS)
All NIDDM	16.87 \pm 4.69* (n=83)	17.13 \pm 5.34 (n=38)	16.64 \pm 4.12 (n=45)	>0.5 (NS)
Controlled NIDDM	16.02 \pm 3.97† (n=40)	16.13 \pm 4.95 (n=13)	15.97 \pm 3.47 (n=27)	>0.5 (NS)
Uncontrolled NIDDM	17.67 \pm 5.25†† (n=43)	17.65 \pm 5.54 (n=25)	17.56 \pm 4.84 (n=18)	>0.5 (NS)

*p<0.01, †p<0.02, ††p<0.01 vs non-diabetics; p>0.1 in controlled vs uncontrolled group; NS: not significant

Table 3 Plasma zinc concentration ($\mu\text{mol/l}$, mean \pm SD): effect of obesity and diabetic control

Group	Total	Obese	Non-obese	p-value
Non-diabetic	15.80 \pm 4.12 (n=30)	18.58 \pm 3.96 (n=4)	15.37 \pm 4.05 (n=26)	>0.1 (NS)
All NIDDM	17.19 \pm 4.92* (n=83)	18.03 \pm 3.94 (n=38)	16.47 \pm 5.54 (n=45)	>0.1 (NS)
Controlled NIDDM	16.20 \pm 3.22† (n=40)	17.48 \pm 3.32 (n=13)	15.58 \pm 3.04 (n=27)	>0.05 (NS)
Uncontrolled NIDDM	17.31 \pm 4.58†† (n=43)	18.31 \pm 4.32 (n=25)	17.79 \pm 7.88 (n=18)	>0.5 (NS)

*p>0.1, †p>0.5, ††p>0.1 vs non-diabetics; p>0.2 in controlled vs uncontrolled group

NIDDM patients were subdivided into controlled and uncontrolled groups; the comparability of the study groups with respect to age, weight, BMI, duration of diabetes, fasting blood glucose levels and serum fructosamine is shown in table 1. Fasting blood glucose was 5.9 \pm 1.0 mmol/l in the controlled group and 10.7 \pm 3.9 mmol/l in uncontrolled group (p<0.001).

COPPER, ZINC AND MAGNESIUM LEVELS

Table 2 shows mean plasma copper concentration in non-diabetic subjects and NIDDM patients. Plasma copper was significantly elevated in diabetic patients (p<0.01). In diabetic patients, control of diabetes did not influence plasma copper levels. Similarly, obesity did not influence plasma copper levels in diabetic patients.

Table 3 shows plasma zinc concentrations in the various groups. Diabetes did not significantly influence plasma zinc concentration, and nor did the control of diabetes among NIDDM patients. A subtle elevation in plasma zinc level was observed among obese compared with non-obese NIDDM patients but this difference was not statistically significant.

A detailed statistical analysis of magnesium levels failed to reveal any statistically significant differences between the different subgroups. Magnesium levels were comparable between NIDDM patients and non-diabetic subjects (p>0.2) and control of diabetes did not influence magnesium levels. There was no difference in magnesium levels in obese and non-obese NIDDM patients.

No difference was found between males and females with respect to copper, zinc, or magnesium levels, and there was no relationship between duration of diabetes and levels of any of the trace elements.

Discussion

Trace elements have long been accepted as essential for optimum health.¹ The clinical significance of trace elements is still somewhat controversial, however. Among the trace elements, copper and zinc are of particular interest.^{19, 20} Copper levels have been found to be elevated in IDDM subjects, while urinary

excretion of copper has been found to be affected by diabetes mellitus.^{21, 22} Our study showed that copper concentrations were significantly higher in NIDDM patients than non-diabetic subjects (p<0.01). In a study by Schlienger *et al* on the effect of diabetes on trace elements, elevated levels of serum copper were found in patients with IDDM and NIDDM; glycaemic control did not affect copper levels.²³ This is consistent with our study, although another study failed to identify any such a relationship.⁴ Increases in copper levels have also been implicated as an additional factor for atherogenicity.

In our study zinc concentrations were similar in NIDDM patients and controls. Control of diabetes did not influence zinc concentration, although when obese NIDDM patients were compared to non-obese NIDDM patients, zinc concentrations were slightly elevated. These results are similar to those of Niewoehner *et al*.²⁴ In other studies, however, many different types of relationships have been reported between diabetes mellitus and serum zinc levels. These differences could be at least partly due to heterogeneity in patient selection and study design. Decreased serum zinc concentration has been reported in NIDDM patients and this depletion was shown to be a consequence of excessive urinary losses; urinary zinc loss was found to be greater when patients had proteinuria.⁸ (In our study we excluded patients with nephropathy.) In another study, plasma zinc concentrations were found to be reduced in patients with diabetes mellitus; no association was found between lower levels of zinc and age, sex, diabetic control and anthropometric characteristics.²³ On the other hand, increased zinc levels have been found in patients with diabetes mellitus previously treated with insulin.³ Our study did not include NIDDM patients who were receiving insulin therapy.

The best known interaction in trace element metabolism is the reported antagonism between copper and zinc.¹⁴ Excessive dietary zinc is reported to induce copper deficiency by several mechanisms, all involving induced synthesis of an intracellular binding protein, metallothionein. Excessive intake of zinc is thought to

induce synthesis of the protein, resulting in sequestering of both metals, with subsequent excretion when cells are sloughed into the intestinal lumen. Thus, the protective mechanism preventing zinc toxicity also results in copper deficiency. Hormonal influences may also lead to apparently antagonistic zinc-copper interactions. Both carbohydrate-active steroids and a mononuclear phagocyte-produced hormone, interleukin-1, enhance intracellular zinc accumulation while increasing intracellular copper efflux as caeruloplasmin.²⁵ The net result of these effects is a decreased plasma concentration of zinc and an increased concentration of copper. We wonder whether any such interaction exists between zinc and copper in subjects with NIDDM which would explain the elevated plasma copper levels found in this study.

The cellular physiology of magnesium metabolism is not fully understood. Reduced mean plasma magnesium levels have been reported in NIDDM patients.^{26,27} IDDM subjects with microalbuminuria and clinical proteinuria have been found to have hypomagnesaemia which has been implicated to increase cardiovascular morbidity and mortality in such patients.²⁸ Magnesium levels in our study did not differ significantly between NIDDM and control subjects. Resnick *et al* reported intracellular and extracellular magnesium depletion in NIDDM patients.²⁷ Schlienger *et al* studied the influence of glycaemic control on various trace elements and reported significantly reduced plasma magnesium levels in IDDM patients with poor con-

Summary points

- a complex interplay exists between micro-nutrients and diabetes mellitus
- plasma zinc and magnesium levels are not altered in NIDDM patients
- plasma copper levels are significantly elevated in NIDDM patients; this may be incidental or a result of the NIDDM

trol of diabetes (glycaemic control evaluated by glycosylated haemoglobin).²⁵ In another study, reduced mean plasma magnesium levels were reported in patients with diabetes compared with non-diabetic subjects.^{26,29} It is, however, unclear whether chronic stable diabetes mellitus, especially NIDDM, is also a state of magnesium depletion.³⁰ Our patients with stable diabetes also had normal magnesium levels.

Diabetes as such has been reported to alter copper, zinc and magnesium status, although differences in trace element levels occurring as a result of diabetes have not been confirmed.²² We conclude that plasma zinc and magnesium levels are not significantly affected in NIDDM patients. Copper was significantly elevated in our patient population, irrespective of control of diabetes. It may be worth exploring further the interaction between zinc and copper levels in diabetic patients.

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