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Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus

Alena Viktorínová[a], Eva Tošerová[b], Marián Križko[b], Zdenka Ďuračková[a,*]

[a]Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Comenius University, 811 08 Bratislava, Slovak Republic
[b]Faculty of Medicine, 1st Department of Internal Medicine, Comenius University, 811 08 Bratislava, Slovak Republic

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Abstract

Diabetes mellitus (DM) is associated with the alterations in the metabolism of copper (Cu), zinc (Zn), and magnesium (Mg). The aim of the present study was to investigate plasma levels of these elements in patients with DM and in healthy subjects. Association between glycated hemoglobin and levels of metals was also evaluated. We studied 36 subjects with DM (type 1, 11; type 2, 25) and 34 healthy subjects matched for age, sex, and duration of diabetes. Plasma concentrations of Cu, Zn, and Mg were measured by atomic absorption spectrometry. An imbalance in the levels of studied metals was observed in both type 1 and type 2 DM. We found higher levels of Cu ($P < .001$) and Cu/Zn ratio ($P < .0001$) and decreased levels of Zn ($P < .01$) and Mg ($P < .0001$) in patients with DM when compared with controls. Negative correlation between Cu and Zn ($r = -0.626$, $P < .0001$) was found in patients with DM. Glycated hemoglobin levels were positively correlated with Cu ($r = 0.709$, $P < .001$) and Cu/Zn ratio ($r = 0.777$, $P < .001$) and inversely correlated with Zn ($r = -0.684$, $P < .001$) and Mg ($r = -0.646$, $P < .001$). In conclusion, patients with DM had altered metabolism of Cu, Zn, and Mg; and this may be related to increased values of glycated hemoglobin. We concluded that impaired metabolism of these elements may contribute to the progression of DM and diabetic complications.

1. Introduction

A relationship between diabetes mellitus (DM) and minerals is frequently reported [1,2]. Trace elements as a component of oxidative stress are suggested to be a good indicator for diagnosing various diseases. Oxidative stress is an important contributing factor in the pathogenesis of many diseases, including also DM [3-5]. Several studies have confirmed that hyperglycemia plays a key role in inducing oxidative stress in DM [6,7]. The contribution of other factors, such as the alternations in the homeostasis of some trace elements, to the development and progression of this disease is still under discussion in the literature [8-10].

Copper (Cu) and zinc (Zn) play a pivotal role in the oxidant/antioxidant mechanism, imbalance of which leads to increased susceptibility to oxidative damage of tissues, thereby leading to the pathogenesis of DM or diabetic complications [11,12]. Copper acts as a prooxidant and may participate in metal-catalyzed formation of free radicals. On the other hand, Cu and Zn act as structural and catalytic components of some metalloenzymes. Copper is necessary for the catalytic activity of enzymes such as Cu/Zn superoxide dismutase (SOD) that is involved in the protection of cells from superoxide radical. Zinc acts as an antioxidant by protecting the sulfhydryl groups of proteins and enzymes against free radical attack in the body [13].

The changes in the metabolism of Cu and Zn that occur during oxidative stress may be important in several processes where oxidative stress is implicated [14-17]. Both the essentiality and toxicity of these metals in the pathogenesis of DM and diabetic complications are often reported [6,18,19]. Some investigators have reported the hypothesis that glycated proteins bind transition metals such as Cu and iron and that such glycocholates play an important role in the etiology of peripheral vascular dysfunction and peripheral neuropathies in DM [2].

* Corresponding author. Tel.: +42 1 2 59357416; fax: +42 1 2 59357557.
E-mail address: zdenka.durackova@fmed.uniba.sk (Z. Ďuračková).

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It is well known that Zn plays a key role in the synthesis, storage, and secretion of insulin. Hyperglycemia causes the increased urinary losses of Zn and decreased Zn levels in the body. The decreased levels of Zn affect adversely the ability of the islet cell to produce and secrete insulin [8,9,11,20].

Magnesium (Mg) is an essential component in various enzymatic pathways involved in glucose homeostasis. The relationship between hypomagnesemia and insulin resistance, impaired glucose tolerance, as well as decreased insulin secretion has been suggested by recent studies [18,21,22]. Reduced plasma levels of Mg have been documented in both type 1 and type 2 DM (T1DM and T2DM, respectively), especially in poorly controlled DM [23]. The cause of hypomagnesemia was attributed to osmotic renal losses from glycosuria, decreased intestinal absorption, and redistribution of Mg from the plasma into blood cells caused by insulin effect [24,25]. Magnesium deficiency may have some effects on the development of diabetic complications with other risk factors [26].

The aim of the present study was to compare the status of some minerals of patients with DM with nondiabetic healthy subjects and also to assess the association between these elements and glycated hemoglobin (HbA_{1c}).

2. Subjects and methods

2.1. Study subjects and design

Thirty-six outpatients with DM (matched for age, sex, duration of diabetes, and type of diabetes) treated at the 1st Department of Internal Medicine, University Hospital, Comenius University, Bratislava, were included in the study. The metabolic control of diabetes was assessed by analysis of HbA_{1c} in the plasma.

Eleven patients with T1DM were treated with insulin (9 men and 2 women; age, 49.9 ± 9.4 years; duration of diabetes, 23.9 ± 12.9 years; $HbA_{1c,}$ 7.19% ± 0.6%). Of the patients with T2DM (10 men and 15 women), 8 were treated with insulin (age, 60.4 ± 12.5 years; duration of diabetes, 17.1 ± 12.2 years; HbA_{1c}, 7.66% ± 1.5%); and 17 were treated with oral antidiabetic agents (OAD) (age, 58.7 ± 10.1 years; duration of diabetes, 7.4 ± 5.4 years; HbA_{1c}, 7.12% ± 1.1%). Patients with DM respected diabetic diet and did not take nutritional supplements and any drugs that are known to interfere with metabolism of studied metals during the period of study. None of the patients had marked renal damage, chronic diarrhea, acute infection, or pregnancy or had taken hypotensive diuretics.

The control group consisted of 34 healthy blood volunteers (18 men and 16 women; age, 30.2 ± 9.7 years). The study was approved by the Ethical Committee of the University Hospital, Bratislava. Healthy blood volunteers gave written consent for participation in the study.

2.2. Measurements of Cu, Zn, Mg, and HbA_{1c}

Blood samples from subjects were taken after overnight fasting into commercial tubes for analysis of HbA_{1c} and into special metal-free tubes for analysis of Cu, Zn, and Mg using the standard venipuncture technique (Vacutainer Trace Element Tubes, Sarstedt, Nűmbrecht, Germany). After blood centrifugation, plasma was aliquoted into metal-free Eppendorf test tubes, shock frozen, and stored at −80°C until further analysis.

Flame technique of atomic absorption spectrometry (FAAS Techtron Pty., Ltd., Springvale, Australia) was used for determination of Cu, Zn, and Mg concentrations in the plasma according to the method by Qi Jian-Xin [27]. The accuracy of determination was evaluated by measuring the metal contents of certificated biological reference materials (Seronorm Trace Elements Serum; Nycomed Pharma, Oslo, Norway). Plasma levels of HbA_{1c} were measured by using standard biochemical procedures at the Biochemical Clinical Laboratory of the University Hospital, Comenius University, Bratislava; and the results were identified with the use of reference ranges in this laboratory.

2.3. Statistical analysis

Means ± SD are given for the normally distributed parameters; for data showing departures from normality, median and interquartile range are given. Data that showed no departures from normality were analyzed with the Student t test for independent or pair-matched samples. If the data were skewed but other criteria were met, nonparametric Mann-Whitney U test was used to detect differences between groups.

Spearman rank correlations were used to determine the associations between individual levels Cu, Zn, Mg, and Cu/Zn ratio and also between these metals and HbA_{1c}, duration of diabetes (years), age, and sex. A value of $P < .05$ was considered as significant in all statistical analyses.

For statistical analysis, we used the statistical program StatsDirect 2.3.7 (StatsDirect Sales, Sale, Cheshire, M33 3UY, United Kingdom). Graphical representation of data was made using the program Excel 2000 (Microsoft, Redmond, WA).

3. Results

Baseline characteristics of patients with DM (DM group) and control group as well as plasma concentrations of Cu, Zn, and Mg and the Cu/Zn ratio are shown in Table 1. We found significantly higher Cu levels ($P < .001$), lower Zn levels ($P < .01$), higher values of Cu/Zn ratio ($P < .0001$), and also lower Mg levels ($P < .0001$) in patients with DM in comparison with healthy subjects. Seventy-five percent of patients with DM had plasma levels of HbA_{1c} lower than 8% (6.1%-8%). Glycated hemoglobin levels higher than 8% (8%-10.1%) were present in 25% of diabetic subjects.

Table 1
Plasma concentrations of Cu, Zn, and Mg and Cu/Zn ratio in the DM group and the control group of healthy subjects

	DM group	Control group	*P* value
n	36	34	
Sex (M/F)	19/17	18/16	
Age (y)	56.4 ± 11.1 (35-77)	30.2 ± 9.7 (19-57)	<.0001
Duration of diabetes (y)	14.6 ± 12.0 (2-42)		
HbA_{1c} (%)	7.26 ± 1.1 (6.1-10.1)		
Cu (μmol/L)	18.73 ± 2.6 (16.4-21.5)	17.37 ± 2.4 (14.6-19.8)	<.001
Zn (μmol/L)	13.48 ± 2.2 (10.2-16.3)	14.41 ± 1.8 (12.2-16.9)	<.01
Cu/Zn ratio	1.42 ± 0.3 (1.08-2.03)	1.21 ± 0.1 (1.06-1.32)	<.0001
Mg (mmol/L)	0.77 ± 0.2 (0.62-0.93)	0.90 ± 0.1 (0.77-1.18)	<.0001

Data are given as a mean ± SD (range). n is the number of subjects per group. *P* value between DM group and control group.

Table 3
Plasma concentrations of Cu, Zn, and Mg and Cu/Zn ratio in patients with T2DM treated with insulin or OAD

	T2DM (insulin)	T2DM (OAD)	*P* value
n	8	17	
Sex (M/F)	2/6	8/9	
Age (y)	60.4 ± 12.5 (39-77)	58.7 ± 10.1 (35-73)	.72
Duration of diabetes (y)	17.1 ± 12.2 (3-38)	7.4 ± 5.4 (2-23)	<.01
HbA_{1c} (%)	7.66 ± 1.5 (6.1-10.1)	7.12 ± 1.1 (6.1-9.4)	.36
Cu (μmol/L)	18.93 ± 2.8 (16.4-21.4)	18.61 ± 2.5 (16.4-21.5)	.66
Zn (μmol/L)	13.20 ± 1.9 (10.5-14.8)	13.57 ± 2.1 (10.2-16.3)	.66
Cu/Zn ratio	1.47 ± 0.3 (1.13-2.03)	1.42 ± 0.3 (1.08-1.99)	.74
Mg (mmol/L)	0.74 ± 0.1 (0.62-0.83)	0.77 ± 0.09 (0.62-0.91)	.41

Data are given as mean ± SD (range). n is the number of subjects per group. *P* value between T2DM group treated with insulin and T2DM group treated with OAD.

We evaluated levels of Cu, Zn, Cu/Zn ratio, and Mg separately for reasonably controlled diabetes (HbA_{1c} levels <8%) and also for poorly controlled diabetes (HbA_{1c} levels >8%). The differences between the group with HbA_{1c} levels less than 8% and the group with HbA_{1c} greater than 8% were obtained. We found that the group with HbA_{1c} levels greater than 8% (n = 8) had significantly increased levels of Cu ($P < .001$) and Cu/Zn ratio ($P < .001$) and decreased levels of Zn ($P < .0001$) and Mg ($P < .001$) when compared with the group with HbA_{1c} less than 8% (n = 28).

We calculated associations between levels of HbA_{1c} and studied metals in both groups. Positive correlations between levels of HbA_{1c} and Cu ($r = 0.492$, $P = .004$) as well as Cu/Zn ratio ($r = 0.589$, $P = .0006$) and negative correlations between levels of HbA_{1c} and Zn ($r = -0.437$, $P = .01$) and also Mg ($r = -0.418$, $P = .014$) in both groups were obtained.

Table 2 shows the baseline characteristics and plasma concentrations of studied metals in patients with T1DM and T2DM. We found that T2DM patients with higher values of HbA_{1c} than those in T1DM patients are at increased risk for Zn deficiency (values <11.0 μmol/L). Zinc deficiency was detected in 13.9% of patients with T2DM. The deficiency of Zn was not found among patients with T1DM. Furthermore, reduced levels of Mg (values <0.7 mmol/L) were present in 24% of T2DM patients and in 9% of T1DM patients. We recorded no significant differences in the levels of Cu, Zn, Mg, Cu/Zn ratio, and HbA_{1c} ($P > .05$) between T1DM and T2DM groups (Table 2). Duration of diabetes (years) was significantly higher in T1DM group compared with T2DM group ($P < .001$).

The levels of Cu, Zn, Mg, and Cu/Zn ratio and the baseline characteristics of patients with T2DM treated with insulin or OAD are shown in Table 3. We recorded no significant differences in the levels of Cu, Zn, Cu/Zn ratio, and Mg ($P > .05$) between T2DM (insulin) and T2DM (OAD) groups (Table 3). There was significant difference in the duration of diabetes ($P < .01$) between T2DM (insulin) and T2DM (OAD) groups.

We calculated associations between individual concentrations of metals and Cu/Zn ratio. An imbalance in the levels of Cu and Zn was found in patients with DM when compared with control group. Statistical analysis showed a positive correlation between plasma levels of Cu and Zn in the group of healthy subjects ($r = 0.791$, $P < .0001$). In contrast, we found a negative correlation between these metals ($r = -0.626$, $P < .0001$) in patients with DM (Fig. 1).

Table 2
Plasma concentrations of Cu, Zn, and Mg and Cu/Zn ratio in patients with T1DM and T2DM

	T1DM	T2DM	[a]*P* value	[b]*P* value	[c]*P* value
n	11	25			
Sex (M/F)	9/2	10/15			
Age (y)	49.9 ± 9.4 (37-63)	59.2 ± 10.7 (35-77)	<.0001	<.0001	<.05
Duration of diabetes (y)	23.9 ± 12.9 (6-42)	10.5 ± 9.2 (2-38)			<.001
HbA_{1c} (%)	7.19 ± 0.6 (6.4-8.2)	7.29 ± 1.3 (6.1-10.1)			.79
Cu (μmol/L)	18.75 ± 2.4 (17.3-20.9)	18.72 ± 2.7 (16.4-21.5)	<.01	<.001	.95
Zn (μmol/L)	13.54 ± 2.3 (11.8-15.3)	13.46 ± 2.1 (10.2-16.3)	<.05	<.05	.88
Cu/Zn ratio	1.39 ± 0.2 (1.15-1.66)	1.43 ± 0.3 (1.08-2.03)	<.0001	<.001	.70
Mg (mmol/L)	0.79 ± 0.1 (0.66-0.93)	0.76 ± 0.2 (0.62-0.91)	<.01	<.0001	.31

Data are given as mean ± SD (range). n is the number of subjects per group.
[a] *P* value between T1DM group and control group.
[b] *P* value between T2DM group and control group.
[c] *P* value between T1DM group and T2DM group.

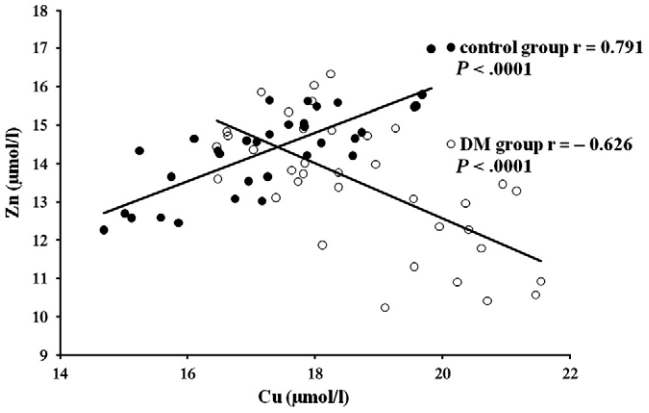

Fig. 1. Correlation between plasma levels of Cu and Zn in control group of healthy subjects and in patients with DM.

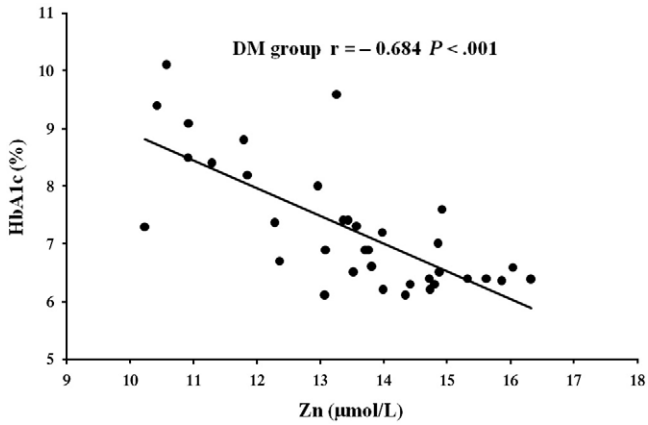

Fig. 3. Correlation between plasma levels of HbA_{1c} and Zn in patients with DM.

The associations between HbA_{1c}; baseline characteristics of patients with DM; and plasma concentrations of Cu, Zn, and Mg and the Cu/Zn ratio were also evaluated.

A positive correlation between plasma levels of HbA_{1c} and Cu in DM group ($r = 0.709$, $P < .001$) is shown in Fig. 2. There is also a positive correlation between HbA_{1c} and Cu/Zn ratio ($r = 0.777$, $P < .001$). We recorded a negative correlation between plasma levels of HbA_{1c} and Zn ($r = -0.684$, $P < .001$) as well as between HbA_{1c} and Mg ($r = -0.646$, $P < .001$) in patients with DM (Figs. 3 and 4).

We also found a positive correlation between values of HbA_{1c} and duration of diabetes (years) in patients with T2DM ($r = 0.598$, $P < .001$). However, no significant correlation between HbA_{1c} and duration of diabetes was detected in patients with T1DM ($P = .113$).

The limitation of our study was the difference in the age between healthy volunteers and patients with DM. Because healthy subjects were younger than those with diabetes, we determined the impact of the age on the metal levels. We evaluated the data separately for patients with DM as well as for controls in age-matched groups: age less than 50 years and age more than 50 years. We found no significant differences in the levels of Cu, Zn, Cu/Zn ratio, and Mg between age-matched groups of patients with DM and healthy subjects.

4. Discussion

In the present study, an imbalance in the levels of trace elements was observed in both T1DM and T2DM. We found significantly higher levels of Cu and Cu/Zn ratio and significantly reduced levels of Zn and Mg in patients with DM when compared with healthy subjects (Table 1). Plasma level of Cu was positively correlated with Zn in the group of healthy subjects (Fig. 1). In contrast, we found a negative correlation between levels of Cu and Zn in patients with DM. Consequently, we may suggest that the imbalance in the levels of studied metals may play an important role in the pathogenesis of DM.

Healthy subjects in our study were younger than patients with DM; therefore, we also evaluated the impact of the age

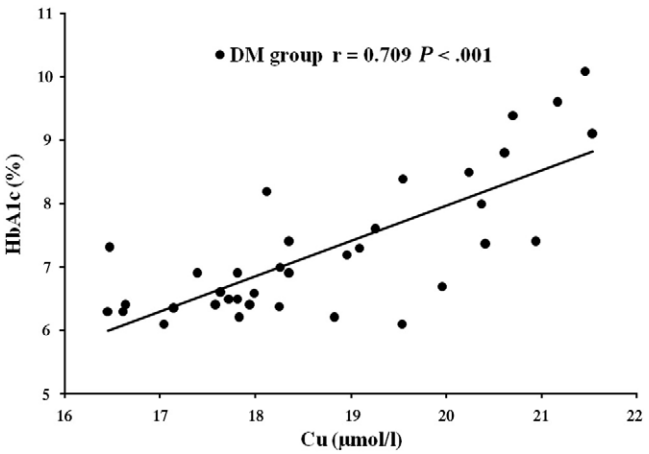

Fig. 2. Correlation between plasma levels of HbA_{1c} and Cu in patients with DM.

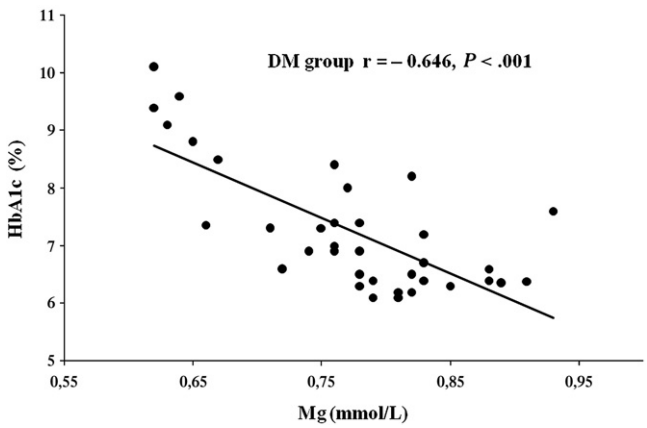

Fig. 4. Correlation between plasma levels of HbA_{1c} and Mg in patients with DM.

on the levels of Cu, Zn, Cu/Zn ratio, and Mg. No significant differences in the metal levels between age-matched groups of patients with DM as well as healthy subjects were found. There appears to be no significant impact of the age on the levels of these metals in our study.

Several previous studies have suggested that metabolic disturbances associated with insulin resistance and hyperglycemia can cause the deficiency of some minerals [1,21,22]. Deficiency of Zn and Mg has been frequently reported in DM as a contributing factor to the etiology of diabetic complications such as hypertension, retinopathy, and thrombosis [9,11,25]. In our study, decreased Zn levels were associated with increased Cu levels in patients with DM, as shown in Fig. 1. True prevalence of Zn and Mg deficiency among patients with DM is still under discussion in the literature [17,28,29]. Zinc and Mg deficiency might be related to differences in the important factors regulating homeostasis of these metals such as gastrointestinal absorption and urinary excretion.

Recent publications have established [18,25] that plasma levels of some minerals are lower in patients with DM than those in healthy subjects, especially in poorly controlled diabetes. Increased urinary excretion of Zn and Mg due to hyperglycemia and osmotic diuresis may contribute to reduced levels of these elements. Because deficiency of Zn and Mg may be attributed to the use of diuretics [21], diabetic patients taking hypotensive diuretics were excluded from the study. The results of our study indicate that the alterations in plasma levels of Cu, Zn and Mg and in Cu/Zn ratio in patients with DM are associated with increased levels of HbA_{1c}. We found that HbA_{1c} levels were positively correlated with Cu (Fig. 2) as well as with Cu/Zn ratio and inversely correlated with Zn and Mg (Figs. 3 and 4).

In our study, we also determined the impact of increased levels of HbA_{1c} on the levels of Cu, Zn, Cu/Zn ratio, and Mg. We found differences between the group with HbA_{1c} levels less than 8% and the group with HbA_{1c} levels greater than 8%. On the basis our results, we can suggest that in the patients with poorly controlled diabetes exists a strong relation between increased levels of HbA_{1c} and altered levels of studied metals. These patients are at increased risk for development of diabetic complications. Our findings also support several studies [9-11,13] based on the hypothesis that long-term hyperglycemia is considered to be a risk factor of diabetic complications and is associated with impaired status of some minerals.

It has been reported [9,20] that Zn deficiency is associated with reduced insulin secretion and increased tissue resistance to insulin action. Our study showed that reduced levels of Zn (values <11 μmol/L) were present in 13.9% of T2DM patients who had plasma levels of HbA_{1c} higher than 8% (8%-10.1%). The deficiency of Zn was not detected among patients with T1DM (Table 2). Consequently, we may suggest that suboptimal Zn status may be more prevalent in T2DM patients than T1DM patients. We also found that reduced Mg levels (values <0.7 mmol/L) were present in 24% of T2DM patients and 9% of T1DM patients. Furthermore, we found a positive correlation between HbA_{1c} and duration of diabetes (10.5 ± 9.2 years) in patients with T2DM. In contrast, no significant correlation between HbA_{1c} and duration of diabetes (23.9 ± 12.9 years) was recorded in patients with T1DM. In accordance with recent studies [1,11,25], we can speculate that patients with T2DM, especially those with poor metabolic control, are at increased risk of Zn and Mg deficiency. Poorly controlled DM may cause the alterations in the homeostasis of these metals.

Several reports have indicated [8,9] that the metabolism of some trace elements such as Cu and Zn is altered in DM and that these alterations might be a contributing factor in the pathogenesis of this disease. It has been also suggested [4,11] that hyperglycemia and hyperinsulinemia increase the production of free radicals and decrease the efficiency of antioxidant defense systems. It is well known that Cu and Zn play a vital role in oxidative stress [13]. Therefore, changes in the levels of Cu, Zn, and Cu/Zn ratio may influence the equilibrium in the antioxidant defense system and enhance the toxic effect of metal-dependent free radicals. These associations may in this way initiate and potentiate the pathogenetic processes leading to diabetic complications [6,18,30]. Copper and Zn are needed for essential activity of antioxidant enzyme Cu/Zn SOD. Superoxide dismutase catalyzes dismutation of superoxide radical into hydrogen peroxide. Therefore, abnormal metabolism of Cu and Zn may affect the function of SOD and result in decreased protection of cells from superoxide radical. Moreover, under conditions of hyperglycemia, glycated proteins exhibit increased affinity to Cu ions and may result in glycocholates formation. The glycocholates can be accumulated in the endothelium and participate in redox reactions. Therefore, alterations in the metabolism of Cu can be an important contributing factor for the progression of diabetic vascular complications [8,19].

We found no significant differences in the levels of Cu, Zn, Cu/Zn ratio, Mg, and HbA_{1c} between T1DM and T2DM groups (Table 2) and also between T2DM (insulin) and T2DM (OAD) groups (Table 3). It is not known whether the alterations in the metabolism of these metals are a consequence of DM or, alternatively, whether they contribute to the development of this disease [18,26]. However, the precise mechanism responsible for the alterations in the metabolism of Cu, Zn, and Mg in DM is unclear and requires further evaluation.

In conclusion, the present results demonstrate that there is an imbalance in the levels of some trace elements such as Cu and Zn among patients with DM in comparison with healthy subjects. These changes may play an important role in the pathogenesis of this disease by the participation of these elements in the oxidative stress. We have also shown that increased levels of Cu and Cu/Zn ratio and decreased levels of Zn and Mg are associated with increased values of HbA_{1c} in diabetic patients. These findings may contribute to explaining the role of impaired metabolism of some mineral

elements in the pathogenesis of diabetes. We conclude that impaired metabolism of Cu, Zn, and Mg may be suggested as a contributing factor in the progression of DM and also in the development of diabetic complications.

The present study provides significant evidence showing that altered metabolism of Cu, Zn, and Mg is strongly associated with increased levels of HbA_{1c}. These associations might represent a risk factor for the development of diabetic complications. Our findings indicate that it is necessary to take into consideration possible changes in the metabolism of these metals, mainly their associations with long-term hyperglycemia.

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