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Association of Reduced Zinc Status With Angiographically Severe Coronary Atherosclerosis: A Pilot Study

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Abstract

In vitro studies attribute antiatherogenic and insulin-like properties to zinc (Zn). However, only a few conflicting clinical data exist concerning the relationship between Zn and coronary artery disease (CAD) as well as glycemic indices. We studied 72 patients without prior history of myocardial infarction or revascularization procedures, who underwent coronary angiography for evaluation of chest pain. Coronary artery disease severity was estimated using 3 angiographic scores. Zn in serum and 24-hour urine, as well as serum Zn/24-hour urine Zn ratio were determined. Serum Zn was not associated with CAD prevalence and severity. However, urinary Zn loss was significantly higher among patients with CAD and showed a positive association with CAD severity. Serum Zn/24-hour urine Zn ratio was inversely associated with CAD, as well as with diabetes mellitus prevalence, fasting glucose, and glycated hemoglobin levels. Low serum Zn/24-hour urine Zn ratio is associated with angiographically severe atherosclerosis and impaired glucose homeostasis.

Keywords

coronary artery disease, zinc, diabetes mellitus, impaired glucose homeostasis

Introduction

Zinc (Zn) is responsible for maintaining the structural and functional integrity of more than 2000 transcriptional factors[1] and 300 enzymes,[2] such that almost every signaling or metabolic pathway is influenced by Zn-containing proteins. Zn exerts antiatherosclerotic actions by participating in numerous intracellular signaling cascades, which are essential for endothelial cell integrity.[3] Zn deficiency is associated with susceptibility to oxidative stress,[4] greater interleukin 1 and tumor necrosis factor alpha expression,[5] and increased endothelial cell apoptosis.[6] These factors are involved in the process of atherosclerosis. It is, therefore, of interest that in a series of patients undergoing coronary angiography, serum Zn levels were significantly lower among patients with coronary artery disease (CAD) compared to those with minimal or no coronary lesions.[7] However, according to a systematic review of the effect of Zn supplements in humans, besides the demonstrated adverse effect on plasma high-density lipoprotein cholesterol (HDL-C) concentrations, the impact of Zn supplementation on heart disease risk, antioxidant status, and thrombogenesis is unclear.[8]

Compared with the general population, diabetic patients have been found to be Zn deficient as indicated by the ion's low serum levels.[9] Plasma 5′-nucleotidase activity, a Zn-containing enzyme, was also reduced in participants with type 2 diabetes mellitus, suggesting that these patients tend to be moderately Zn deficient.[10] Furthermore, in a cross-sectional study, obese children and adolescents had significantly lower plasma and erythrocyte Zn concentrations and greater urinary loss than normal weight children.[11] However, a 4-week Zn supplementation in 56 glucose-tolerant obese women did not influence insulin resistance, leptin and insulin concentration, lipid metabolism, and fasting plasma glucose compared with placebo.[12]

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The purpose of the current study was to test the hypothesis that reduced Zn status, as reflected by low serum levels and high urinary loss, is associated with the presence and severity of CAD as well as with impaired glucose metabolism.

Methods

Study Population

In all, 72 consecutive patients who underwent diagnostic coronary angiography for the evaluation of chest pain were studied. Participants with prior history of myocardial infarction or revascularization procedures (ie, percutaneous coronary angioplasty or coronary artery bypass grafting) were not included in the study. Zn deficiency may be a result of malabsorption (eg, Crohn disease, persistent diarrhea, short bowel syndrome, celiac sprue, or intestinal bypass surgery) or increased loss (eg, diabetes or alcoholism) or increased needs (eg, children or pregnancy).[2,3] None of these conditions were present in our participants. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Institutional Ethics Committee of the Aristotle University of Thessaloniki. Written informed consent was obtained from all patients.

A detailed medical history was obtained from each patient including current and past medical conditions, family history, lifestyle, socioeconomic status, and medications. Patients also had a thorough physical examination. Blood pressure measurements were made with the patient in a sitting position and the mean of 3 consecutive recordings 5 minutes apart was calculated. Participants who had a clinically well-documented history of hypertension (140/90 mm Hg or greater) or who were prescribed antihypertensive medication were considered as hypertensive. A family history of CAD was considered present if a participant had any first-degree relatives with a history of CAD or sudden cardiac death prior to the age of 55 years for males and 65 years for females.

Coronary Angiography

Coronary angiograms were evaluated by 2 interventional cardiologists who were blinded to the study plan and each other. Angiographic extent of disease was determined by counting the total number of major coronary arteries with >50% stenosis and categorized to 1-vessel, 2-vessel, or 3-vessel disease. Patients with $\geq$2 diseased arteries were combined into 1 group because of the small number of patients with 3-vessel disease. Left main coronary artery (LMCA) disease of >50% was considered a 2-vessel disease.

A more thorough description of CAD severity was made by using 3 different angiographic scoring systems: gensini score,[13] extent score,[14] and arbitrary index.[15] For the calculation of the Gensini score, the coronary artery tree was divided into 15 segments according to Austen's nomenclature[16]: left main coronary artery, right coronary artery (RCA) was divided into 4 segments (proximal, middle, distal, and posterior descending artery), left anterior descending (LAD) into 5 segments (proximal, middle, distal, first, and second diagonal branch), and left circumflex (LCx) into 5 segments (proximal, distal, first, second, and third obtuse marginal branch). An anatomical coefficient was assigned to each coronary artery segment depending on the functional significance of the area supplied by that segment. This coefficient was 5 for LMCA, 2.5 for the proximal segments of LAD and LCx, 1.5 for the middle segment of LAD, 1 for the distal segments of LAD and LCx as well as for all the RCA segments, posterior descending artery, first diagonal, and first obtuse marginal branch, and finally 0.5 for the second diagonal and the second obtuse marginal branch. Each stenosis was classified as grade of 1 for 1% to 49% reduction in lumen diameter, 2 for 50% to 74%, 3 for 75% to 99 %, and 4 for total occlusion (100%). The stenosis grade was multiplied with the respective anatomical coefficient in each segment and all segmental products were added together to give a total score out of the theoretical maximum of 82.

The extent score was defined as the percentage of analyzed segments with luminal narrowing of 25% or more. Extent score indicated the angiographically apparent extent of coronary artery wall abnormalities throughout the coronary bed ranging from 0 to 1.

Arbitrary index was calculated by adding all stenoses (in percentage), expressed in SI units (eg, 50% = 0.50).

Blood Tests

Participants were asked to fast for 12 hours, and blood samples were drawn approximately 30 minutes before catheterization. The samples were centrifuged at 3000*g* for 10 minutes at ambient temperature. Serum triglycerides (TG), total cholesterol (TC), HDL-C, and glucose were determined by standard enzymatic procedures. Serum low-density lipoprotein cholesterol (LDL-C) was calculated using the formula LDL-C = TC − (HDL-C + TG/5 in mg/dL). Patients were regarded diabetic if this was documented in their medical record, if fasting blood glucose levels were >125 mg/dL (6.9 mmol/L), and/or if hypoglycemic medication was prescribed.

Serum and 24-Hour Urine Zinc

Zinc levels were measured in serum and in 24-hour urine collection in all patients (n = 72). Results were expressed in μg/L. Twenty-four-hour urine collection was performed starting in the morning of the day following catheterization. Urine was collected in polyethylene containers and the total volume was recorded. A 10-mL sample was separated, centrifuged at 3000*g* for 10 minutes, and further analyzed. Zn concentrations were determined using atomic absorption spectroscopy (Varian, SpectrAA-300). The elements absorbance was measured at a wavelength of 213.9 nm, using an oxidizing air/acetylene flame. For analytical purposes, a calibration graph was plotted by measuring the absorbance of a series of aquatic standard Zn solutions which were freshly prepared from a stock solution containing Zn at a concentration of 1 g/L.

Table 1. Patient Demographic Data According to the Presence of CAD[a]

Variables	CAD (+) (n = 40)	CAD (−) (n = 32)	*P* Value
Age (years)	66 (43)	61 (51)	.087
Gender (men)	27 (67.5)	17 (53.1)	.214
BMI (kg/m^2)	28.7 ± 2.9	28.2 ± 3.0	.547
HbA1c (%)	5.3 (6.2)	4.8 (3.7)	**.003**
TC (mg/dL)	179 ± 42	186 ± 46	.555
TG (mg/dL)	141 ± 53	108 ± 44	**.007**
HDL-C (mg/dL)	47.1 ± 13.0	55.8 ± 14.8	**.011**
LDL-C (mg/dL)	105 ± 35	108 ± 41	.687
Non-HDL-C (mg/dL)	133 ± 37	130 ± 47	.760
Diabetes	22 (55.0)	7 (21.9)	**.006**
Family history of CAD	12 (30.0)	9 (28.1)	.753
Hypertension	35 (87.5)	26 (81.3)	.464
Smoking	24 (60.0)	18 (56.3)	.748
Thiazide Tx	25 (62.5)	14 (43.8)	.113
Serum Zn (μg/L)	626.5 (611.0)	628.5 (420.0)	.828
24h-urine Zn (μg/24h)	620.0 (2540.1)	469.4 (1164.0)	**.014**
Serum Zn/24h-urine Zn	1.0 (4.5)	1.3 (18.3)	**.024**

Abbreviations: BMI, body mass index; CAD, coronary artery disease; HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; Thiazide Tx, thiazide diuretic use; Zn, zinc.

[a] Data represent mean ± SD, median (range), or absolute numbers (%) of total patients in each column, boldface letters are used to highlight significant differences.

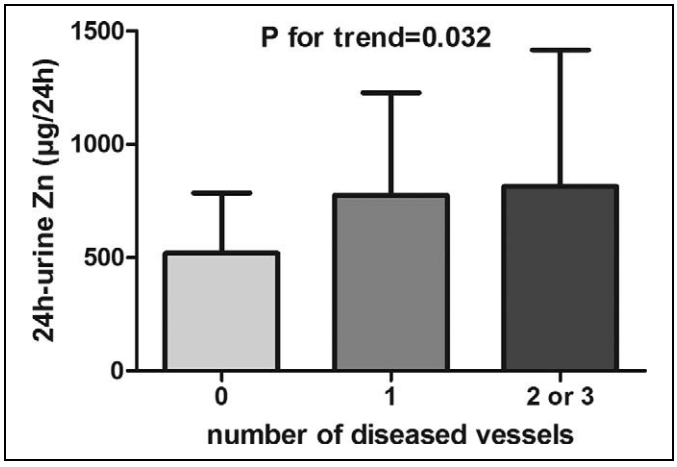

Figure 1. Twenty-four-hour urine Zn in relation to the number of diseased vessels. Bars represent mean values and error bars represent ± 1 SD. *P* value represents the overall trend.
Zn indicates zinc.

Statistical Analyses

Analysis of data was done with SPSS analysis software version 15.0 (SPSS Inc, Chicago, Illinois). Continuous variables are expressed as the mean ± SD or as median and range, whereas categorical variables are expressed as absolute numbers and percentages. Comparison of means between 2 groups was performed with Student *t* test or Mann-Whitney *U* test for normally and nonnormally distributed variables, respectively. Comparison of means among 3 or more groups was performed with 1-way analysis of variance (ANOVA) or Kruskal-Wallis *H* test for normally and nonnormally distributed variables, respectively. Comparison between proportions was performed using chi-square analysis. The association of continuous variables with the angiographic scores was assessed by using Pearson or Spearman correlation coefficients for normally and nonnormally distributed variables, respectively. A 2-sided $P < .05$ was considered significant.

Results

Among 72 individuals, 40 (55.6%) had angiographically severe CAD, whereas 32 (44.4%) had a nonobstructive CAD or normal coronary angiogram. Half of the patients with CAD (n = 19) had 1-vessel disease, while the rest of them (n = 21) had 2-vessel or 3-vessel disease. Diabetes mellitus was diagnosed in 30 (41.6%) patients. Table 1 summarizes the clinical and biochemical characteristics of CAD and non-CAD patients. Compared with participants with a normal angiogram, patients with CAD had greater values of TG and hemoglobin A1c (HbA1c) and lower values of HDL-C. Moreover, diabetes mellitus was more frequent among patients with CAD. Thiazide use was more prevalent among patients with CAD (62.5%) compared with non-CAD individuals (43.5%), but this difference was not significant ($P = .113$). Finally, neither serum Zn nor 24-hour urine Zn levels correlated significantly with age ($r = -.054$, $P = .651$ and $r = -.047$, $P = .695$, respectively).

Zn and Presence of CAD

There was no significant difference in serum Zn concentrations between patients with angiographically documented severe CAD and those with nonobstructive CAD or normal arteries (Table 1). Patients with CAD excreted significantly higher amounts of urinary Zn compared with non-CAD individuals (Table 1). Likewise, the serum Zn/24-hour urine Zn ratio was significantly lower in patients with angiographically severe CAD compared with patients with angiographically normal coronary arteries or nonobstructive CAD (Table 1).

Zn Status and Severity of CAD

There was no significant association between serum Zn levels and the number of diseased vessels ($P = .606$). In contrast, there was a significant rise in urinary Zn loss over the increasing number of diseased vessels (Figure 1). Serum Zn/24-hour urine Zn ratio, however, was not associated with the number of diseased vessels ($P = .103$).

For a more detailed description of the coronary artery atherosclerotic burden, the Gensini score, the extent score, and the arbitrary index were used. In Table 2, the correlation coefficients of Zn concentrations in serum and urine as well as the ratio of the above 2 with each angiographic index are shown.

Table 2. Zn Parameters in Relation to the 3 Angiographic Scores

	Gensini Score		Extent Score		Arbitrary Index	
Variables	*r*	*P*	*r*	*P*	*r*	*P*
Serum Zn	.119	.324	.075	.533	.088	.465
24-hour urine Zn	.262	**.027**	.303	**.010**	.308	**.009**
Serum Zn/24-hour urine Zn ratio	−.238	**.046**	−.302	**.010**	−.298	**.012**

Abbreviations: Zn, zinc; *r*, Pearson correlation coefficient; significant correlation coefficients are indicated in bold font.

Serum Zn levels revealed no significant correlation with any of the 3 angiographic scores. Nevertheless, the amount of urinary Zn excreted showed a significant positive association with all 3 angiographic indices. In addition, the serum Zn/24-hour urine Zn ratio showed a significant negative association with the angiographic scores, supporting the association of reduced Zn status with the severity of CAD.

Zn Status and Glucose Homeostasis

With regard to serum Zn levels, no significant difference between diabetic patients and normoglycemic individuals was observed ($P = .934$). However, diabetic patients excreted significantly greater quantities of Zn compared with the normoglycemic group (Figure 2A). In addition, diabetics had a significantly lower serum Zn/24-hour urine Zn ratio than the normoglycemic individuals (Figure 2B). Furthermore, daily urinary Zn loss showed a significantly positive while serum Zn/24-hour urine Zn ratio a significantly negative association with glucose and HbA1c levels, respectively. Our findings were not confounded by diabetic polyuria because 24-hour urine volume was not associated with the degree of glycemia. Finally, no significant association between serum Zn concentrations and glycemic control parameters (blood glucose and HbA1c) was observed (Table 3).

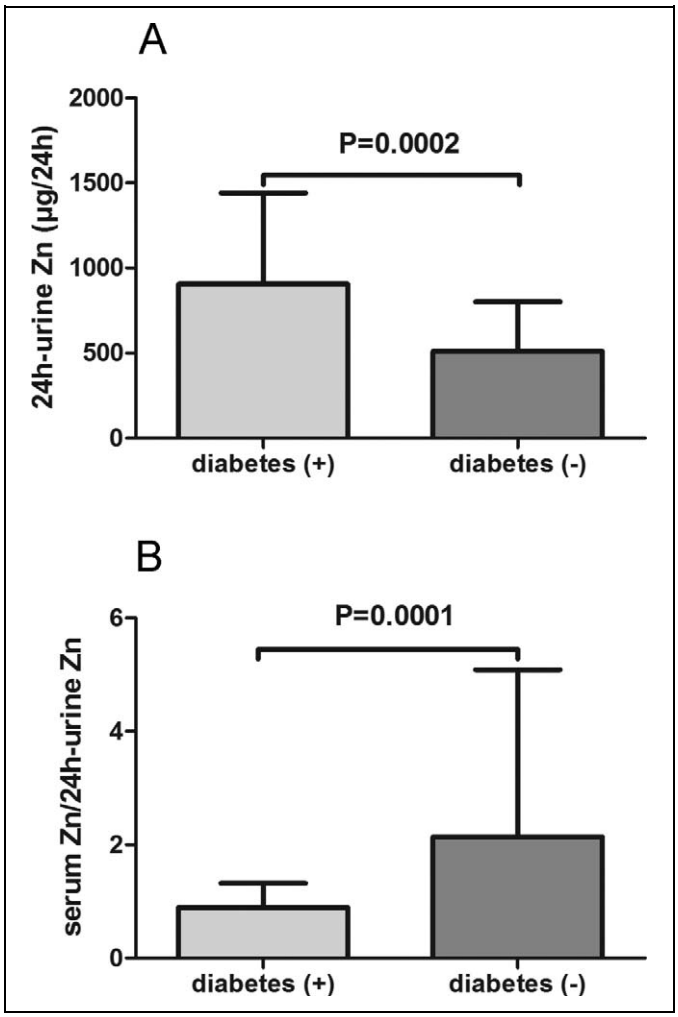

Figure 2. Twenty-four-hour urine Zn (A) and serum Zn/24-hour urine Zn ratio (B) in relation to diabetes mellitus. Bars represent mean values and error bars represent ± 1 SD. Zn indicates zinc.

Discussion

Zn Status and CAD

We demonstrated that serum Zn concentration is not associated with the presence and severity of CAD, whereas the amount of Zn excreted daily via urine as well as the serum Zn/24-hour urine Zn ratio are significantly associated with CAD prevalence and severity. The literature has been controversial concerning the relationship between serum Zn levels and CAD, with some authors detecting no relationship[17] and others reporting a negative association.[18] In the current study, our results of increased Zn in urine and normal levels of Zn in serum indicate that in individuals with severe CAD, there is an increased urinary Zn loss, which may shift Zn from the intracellular compartment to extracellular fluid (plasma) to maintain serum Zn levels within the physiological range. Reduced intracellular Zn could significantly affect Zn-dependent enzymes and subsequent intracellular signaling cascades, thereby activating nuclear factor κB,[19] reducing nitric oxide bioavailability,[20] promoting apoptosis,[21] inducing macrophage-mediated oxidative modification of LDL-C and its subsequent endocytosis,[22] and stimulating tumor necrosis factor−mediated endothelial injury,[23] all of which ultimately promote atherogenesis.

Our findings imply that an individual may be Zn deficient despite serum Zn concentrations remaining within the reference range. This interpretation is supported by studies which showed that plasma Zn decreases late in the course of Zn deficiency, after other laboratory indexes and physiologic functions become abnormal,[24] such as low plasma 5′-nucleotidase activity.[10] This is further supported by dietary Zn restriction studies, which showed that a fall in serum Zn concentration was a late finding.[25]

Zn Status and Glucose Homeostasis

We did not detect any significant difference in serum Zn concentrations between diabetic and nondiabetic individuals.

Table 3. Zn Indices in Relation to Glucose Metabolism Parameters

	Blood Glucose		HbA1c	
	r	*P*	*r*	*P*
Serum Zn	.021	.865	−.098	.430
24-hour urine Zn	.320	**.007**	.398	**.001**
Serum Zn/24 hour -urine Zn ratio	−.337	**.004**	−.443	**.0002**
24-hour urine volume	.141	.243	−.023	.856

Abbreviations: HbA1c, hemoglobin A1c; Zn, zinc; *r*, Pearson correlation coefficient; significant correlation coefficients are indicated in boldface.

However, we demonstrate that diabetics excrete greater quantities of urinary Zn compared with normoglycemic individuals and also have a lower serum Zn/24-hour urine Zn ratio. Likewise, we did not find any significant correlation between the serum Zn concentrations and the indices of glycemic control, such as fasting glucose and HbA1c, a finding which is in agreement with other studies.[9,26,27] However, urinary Zn concentrations as well as serum Zn/24-hour urine Zn ratio were both significantly associated with fasting glucose and HbA1c, independently of daily urine volume.

The association between serum Zn levels and diabetes mellitus prevalence is controversial because some studies support an inverse relationship[9,10,28,29] while others do not.[26,30,31] However, our findings of increased urinary Zn are complementary to other studies.[27,29,31] In our study, it is very likely that serum Zn levels were preserved provided there was an adequate dietary intake of the trace metal; however, circulating Zn levels could dramatically decrease in a setting of reduced external supply.[10] This hypothesis is supported by a study where a single dose of Zn was administered intravenously both in diabetic and normoglycemic individuals. Initially, Zn plasma levels were higher among diabetics but subsequent values exhibited a more rapid decline than in the controls.[32]

Whether Zn deficiency contributes directly to the pathophysiology of hyperglycemia or is just a consequence that remains to be elucidated. In vitro and animal studies have shown that Zn exerts an insulin-like effect in cultured adipocytes by stimulating lipogenesis as well as glucose transport.[33] Zn increases tyrosine phosphorylation of the insulin receptor β subunit of both preadipocytes and adipocytes, thus activating the signaling pathway for glucose endocytosis.[34] In keeping with that, diabetic rats fed with Zn and arachidonic acid showed increased insulin sensitivity and better glycemic control.[35] Acute gastric gavage of Zn significantly improved oral glucose tolerance both in normoglycemic and diabetic rats, probably by increasing skeletal muscle glucose uptake.[36] These results indicate that Zn depletion could contribute to glucose intolerance.

Serum Zn/24-Hour-Urine Zn Ratio and Total Body Zn Status

Serum Zn accounts for only 0.1% of total body Zn, while greater quantities are bound in tissues such as bones and liver expressing very low kinetics compared with plasma.[3] Serum Zn concentrations are therefore an insensitive marker of whole body Zn reserves. Because our main concern is atherosclerosis, the ideal approach would have been direct measurement of endothelial Zn concentrations. Unfortunately there are no studies involving direct measurements of endothelial Zn concentrations in humans and there is no evidence that Zn concentrations in other tissues, such as peripheral blood cells, correlate with endothelial cells levels. Therefore, we evaluated serum Zn/24-hour urine Zn ratio as a novel marker that may better reflect the risk of CAD than serum or urine Zn concentrations alone. The advantage of this marker is that it is relatively easily applied in everyday clinical practice. Indeed, in an epidemiological study, offsprings of parents with CAD had significantly higher Zn urine levels compared with descendants of healthy controls, suggesting that in genetically predisposed individuals with CAD, a long-term, gradual Zn loss takes place before the onset of clinically apparent symptoms.[37] Whether supplementation of Zn in individuals with Zn depletion could exert a beneficial effect is a hypothesis that needs to be tested in large clinical trials.

Study Limitations

Our pilot study is limited by the small number of patients enrolled. In addition, due to the cross-sectional design, it is not possible to assess any causal relationship between Zn levels and atherosclerosis or glycemic control. A significant proportion of the participants in our study were taking thiazide diuretics, which have been reported to increase urinary Zn excretion.[38] There was no record of Zn dietary intake. However, it is plausible to assume that there were no major differences concerning dietary Zn intake, because all participants were of comparable socioeconomic status and resided in urban areas in northern Greece, while none of them were taking any Zn supplements. Moreover, data from a subsample of 3128 participants evaluated in the SU.VI.MAX study, which is one of the largest nutritional studies performed ever, revealed that unlike β-carotene, vitamin C, and vitamin E serum concentrations, serum Zn levels were not influenced by participants' dietary intake.[39] In our study, CAD was assessed by coronary angiography, which does not provide information about the arterial wall and therefore may underestimate the portion of patients with a significant atherosclerotic burden and preserved lumen (expansive remodeling). However, we focused on patients with luminal obstructions of >50%, which are easily identifiable by conventional angiography. Serum Zn levels are likely to be influenced by nonfasting, diurnal variation of serum Zn, and protein binding of serum Zn. The assay procedure involved no sources of Zn contamination. All samples were collected, after at least 8 hours fasting, between 8 and 10 AM. About 70% of plasma labile Zn is bound to albumin while the rest is carried by α2-macroglobulin.[40] However, the concentrations of these protein carriers were not assessed, and this could confound our results. Finally, no serum copper measurements were included in our study. As suggested by

others,[7,17] Zn/copper ratio may be a more sensitive marker of CAD compared with any of these 2 trace elements assessed separately. This could be an interesting aspect for a future study.

Conclusions

In this angiographic study, we demonstrate for the first time that serum Zn/24-hour urine Zn ratio is significantly lower among patients with angiographically severe CAD compared with patients with angiographically normal or minimally stenotic coronary arteries, as well as among diabetic patients compared with normoglycemic individuals. A low serum Zn/24-hour urine Zn ratio could be related to reduced total body Zn status, a condition without clinically apparent symptoms but quite common in the general population. Whether reduced Zn status contributes to the pathophysiology of atherosclerosis and glucose intolerance remains to be elucidated. The serum Zn/24h-urine Zn ratio could make it easier to assess such a relationship when compared with measuring intracellular levels.

Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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