Serum Microminerals and the Indices of Lipid Metabolism in an Apparently Healthy Population

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Serum copper and zinc concentrations were measured in 560 apparently healthy Kuwaitis (238 males and 322 females) aged 15–80 years to assess micromineral effect on the indices of lipid metabolism. Following the recommended guidelines of the European Atherosclerosis Society (EAS) and the National Cholesterol Education Program Expert Panel (NCEPEP), the incidence of dyslipidemia was assessed from enzymatic assay data of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations. Males had significantly lower TC ($P=0.029$) and HDL-C ($P<0.0001$)

levels than females, while TG were significantly ($P=0.023$) lower in females. The prevalence of hypercholesterolemia, hypertriglyceridemia, elevated LDL-C, and low HDL-C levels were 35, 30, 22, and 13%, respectively. Copper did not correlate with zinc ($r = -0.067$, $P = 0.135$) but was positively associated with TC ($r=0.196$, $P<0.0001$), LDL-C ($r=0.134$, $P = 0.003$), TG ($r = 0.092$, $P=0.039$), and age ($r=0.281$, $P<0.0001$). It is concluded that unlike in animal studies, copper excess in humans is associated with hyperlipidemia and therefore will predispose to atherosclerosis. J. Clin. Lab. Anal. 17:61–65, 2003.

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INTRODUCTION

Elevated serum total cholesterol (TC) and triglycerides (TG) are risk factors for the development of coronary heart disease (1). It has been demonstrated that low-density lipoprotein cholesterol (LDL-C), also like TC, directly relates to the incidence of coronary heart disease, while high-density lipoprotein cholesterol (HDL-C) is indirectly related to it (2). The determination of the concentrations of these lipid indices in serum is therefore of relevance to identify the risk of a subject for dyslipidemia.

Experimental studies (3–5) have consistently reported that severe copper deficiency raises serum cholesterol, predisposing to cardiovascular abnormalities including aortic lesions in several animal species, and that high doses of zinc induce copper deficiency, adversely affecting lipid metabolism by decreasing apolipoprotein A-I and HDL-C and causing injury to the myocardium (5). However, reports of studies on the effect of copper and zinc on lipid metabolism in human subjects with cardiovascular disease were divergent (6–8). The present

study was therefore designed to investigate the effect of copper and zinc on the indices of lipid metabolism in apparently healthy humans in which the authors hypothesize that similar to the experimental studies (3–5), serum copper and zinc levels may affect lipid indices of metabolism.

MATERIALS AND METHODS

Subjects

Following house-to-house calls in various Kuwait Governorates, 560 subjects (238 males and 322 females, aged from 15 to 80 years), responded for the present

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study, and this constituted the number in the present study. The subjects were apparently healthy, had no infection, injury, or chronic inflammatory disease, and were not previously diagnosed for dyslipidemia nor was any one of them on lipid-lowering medications. The females were not pregnant, on oral contraceptives, or hormone replacement therapy. The study was approved by the Ethical Committee for Human Research of the Faculty of Medicine, Kuwait University. Informed consent was obtained from each subject at the beginning of the study.

Samples

Fasting blood samples were drawn from each respondent into plastic tubes (W. Sarstedt, Inc., Princeton, NJ) to avoid contamination of the trace metals. The blood collections were promptly transported in an icebox from the homes, to the clinical chemistry laboratory, Department of Pathology, Faculty of Medicine, Kuwait University. After clotting, the blood was centrifuged at 2,000 rpm for 10 min. Using disposable plastic pasture pipettes, serum was separated from the cells into Beckman microfuge tubes (Beckman Instruments) and frozen at -80°C . None of the samples was hemolysed. Analyses were carried out within 24 hr.

Chemicals

Reagent-grade glycerol ($\text{C}_3\text{H}_8\text{O}_3$) was from Panreac (Montpiet, Esteban, Madrid). Water was demineralized ultrapure (MilliQ plus reagent grade; Millipore).

Standard Solutions

Stock solutions of copper (15.9 mM) and zinc (15.1 mM) in 1% HCl were purchased from Aldrich (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin). Working standard solutions for copper and zinc of 1.6, 3.2, and 6.4 μM concentrations were suitably diluted from the stock in glycerol matrix (50 mL/L deionized water) to simulate viscosity of diluted serum.

Controls

The protein matrix control used was Utak Laboratories Trace Elements Control Level II (Utak Laboratories, Inc., Valencia, CA). Pooled sera were also prepared for use as controls. For the measurement of lipid indices, Notrol and Abtrol (SA Scientific, Inc., San Antonio, Texas) controls were run.

Atomic Absorption Spectrophotometer

A GBC 902 double beam atomic absorption spectrophotometer with an air-acetylene flame (GBC Scientific

Equipment, Dandenong, Australia) interfaced to a PC integrator and a printout system was used for the measurement of copper and zinc. The atomic absorption spectrophotometric method was a modification of that of Smith et al. (9). Copper and zinc calibrations of the instrument using the respective working standards were linear beyond the maximum standard concentrations. Controls and samples diluted 10-fold with water were aspirated into the flame and the signal from the diluted controls and samples were compared with that from the working standards. The concentrations of copper and zinc in controls and samples were calculated from the mean of three replicate absorbance readings. The maximum absorbance wavelengths of measurement were 324.7 and 213.9 nm for copper and zinc, respectively.

Assay of the Lipid Indices

The concentrations of TC, TG, and HDL-C were measured by routine enzymatic methods on the Beckman Synchron LX20 discrete random access clinical chemistry system (Beckman Instruments, Inc. Brea, CA). Together with TC and TG, the determination of HDL-C allows the calculation of LDL-C according to Friedewald et al. (10).

Precision and Accuracy

The commercial control, Utak Laboratories Trace Element Control Level II, was used for internal quality control (QC) of the study as well as pooled serum. These were repeatedly analyzed (20 times) for copper and zinc to obtain intra-assay coefficients of variation (CVs), which were below 3%. Inter-assay CVs were obtained by analyzing the internal QC and pooled serum for 20 sequential days and were less than 5%. For the TC, TG, and HDL-C, Notrol (normal level control) and Abtrol (abnormally high level control) were similarly analyzed 20 times to obtain intra-assay CVs, which were less than 4%. Inter-assay CVs were obtained by analyzing the internal QC serums for 20 sequential days and were less than 5%. The values obtained for the internal QC were on target in most analyses and occasionally within the acceptable range.

Statistical Analysis

Statistical analyses of data were performed using SPSS software (SPSS Inc., Chicago, IL). Differences between two groups were evaluated by independent samples *t*-test. Multiple comparison tests were performed using Scheffé post-hoc tests to determine differences between the means of three or more groups. The values are reported as mean $\pm$ standard deviation

TABLE 1. Values of lipids (mean $\pm$ SD and range) in males, females, and the total population

Parameter	Male (n = 238)	Female (n = 322)	Total (n = 560)
TC (mM) ^a	4.8 $\pm$ 1.1	5.0 $\pm$ 1.0	4.9 $\pm$ 1.0
Range	2.6–8.1	2.6–7.9	2.6–8.1
TG (mM) ^b	2.2 $\pm$ 1.5	1.9 $\pm$ 1.2	2.0 $\pm$ 1.3
Range	0.4–10.7	0.3–9.2	0.3–10.7
LDL-C (mM)	2.7 $\pm$ 0.9	2.8 $\pm$ 0.8	2.8 $\pm$ 0.8
Range	0.4–5.2	0.8–5.7	0.4–5.7
HDL-C (mM) ^c	1.1 $\pm$ 0.3	1.3 $\pm$ 0.4	1.2 $\pm$ 0.4
Range	0.3–2.2	0.4–3.0	0.3–3.0

Statistical significance was considered at $P < 0.05$.

^aTotal cholesterol values higher in females than in males; $P = 0.029$.

^bTriglyceride values higher in males than in females; $P = 0.023$.

^cHDL-C values higher in females than in males; $P = 0.0001$.

(SD) in the result tables. Correlation analyses were performed by linear regression. Probability (P) values were two-tailed and $P < 0.05$ was considered to be significant.

RESULTS

The values of lipids in males, females, and the total population expressed as mean $\pm$ SD and range are presented in Table 1. Total cholesterol level was significantly lower ($P = 0.029$) in males than in females. Triglyceride concentration was significantly higher ($P = 0.023$) in males than in females. High-density lipoprotein cholesterol level was significantly lower ($P < 0.0001$) in males than in females.

Table 2 represents the percent prevalence of dyslipidemia in the subjects. Hypercholesterolemia, hypertriglyceridemia and LDL-C hyperlipoproteinemia were more prevalent in females than in males: 21% vs. 14%, 16% vs. 14%, and 14% vs. 8%, respectively. Hyperlipoproteinemia due to low HDL-C was more prevalent in males (8%) than in females (5%) and had an incidence of 13% in the total population.

The associations between the analytes are depicted in Table 3. There was no correlation between copper and

TABLE 2. The prevalence of dyslipidemia in males, females, and the total population

Hyperlipidemia	Male (n = 238)	Female (n = 322)	Total (n = 560)
Hypercholesterolemia ^a	14%	21%	35%
Hypertriglyceridemia ^a	14%	16%	30%
Elevated LDL-C ^a	8%	14%	22%
Low HDL-C ^a	8%	5%	13%

^aPercentages were calculated for subjects with values for TC, TG, LDL-C greater than 5.2 mM, 2.3 mM, 3.3 mM, respectively, and values of HDL-C lower than 0.9 mM.

TABLE 3. Associations between analytes

Analytes		Correlation coefficient (r)	P values
Copper vs.	Zinc	−0.067	0.135
	TC	0.196	<0.0001
	TG	0.092	0.039
	LDL-C	0.134	0.003
Zinc vs.	HDL-C	0.077	0.087
	TC	0.034	0.451
	TG	−0.007	0.884
	LDL-C	0.071	0.115
	HDL-C	−0.018	0.685

Statistical significance was considered at $P < 0.05$.

zinc ($P = 0.135$). Copper significantly associated with TC ($P < 0.0001$), TG ($P = 0.039$), and LDL-C ($P = 0.003$).

Table 4 shows the relationship between age and the analytes. There were strong significant positive correlations between age and copper ($P < 0.0001$), TC ($P < 0.0001$), TG ($P < 0.0001$), LDL-C ($P = 0.003$), and HDL-C ($P = 0.008$).

DISCUSSION

The authors established reference ranges for serum concentrations of copper and zinc for the subjects in a previous study (11). They showed a normal distribution, were sex-specific, and corroborated with values reported for populations in the West (12–14). Apart from LDL-C, the lipid indices like the microminerals were sex-specific. The mean levels for HDL-C validated a previous Western study that reported sex-specific mean levels for HDL-C (1.15 mmol/L for men and 1.40 mmol/L for women) (15).

Dyslipidemia was based on whether serum TC and TG levels exceeded the EAS-defined optima for TC and TG of 5.2 mM and 2.3 mM, respectively (16). Low-density lipoprotein cholesterol level higher than 3.3 mM and the concentration of HDL-C below 0.9 mM were classified as hyperlipidemia in compliance with the NCEPEP recommendation (17). The prevalence of dyslipidemia due to raised cholesterol, TG, LDL-C, and low HDL-C levels was 35% (females 21% and

TABLE 4. Relationship between age and the analytes

Analytes	Correlation coefficient (r)	P values
Copper	0.281	<0.0001
Zinc	0.027	0.717
TC	0.409	<0.0001
TG	0.407	<0.0001
LDL-C	0.264	0.003
HDL-C	0.241	0.008

Statistical significance was considered at $P < 0.05$.

males 14%), 30% (females 16% and males 14%), 22% (females 14% and males 8%), and 13% (females 5% and males 8%), respectively.

That copper did not correlate with zinc in the present study (see Table 3) may be explained by the suggested zinc-copper antagonism; zinc and copper are similar and impair functions by competing for ligands (18,19). The report of an experimental study confirmed the antagonism (5). The present study observed that copper and not zinc strongly associated with all the indices of lipid metabolism except HDL-C; this would imply a positive link between hypercupremia and hyperlipidemia in humans. One cross-sectional study (20) has reported a positive correlation between levels of copper and TC in serum and an inverse association with HDL-C. A longitudinal study (21) reported that the adjusted risk of death from cardiovascular disease was four times higher for subjects in the highest quintile of serum copper level compared to those with normal level. An increased risk was also observed in the same study, for subjects in the lowest serum copper quintile, implying a U-shaped association (21). It has been suggested that the high serum copper levels seen in subjects with atherosclerotic heart disease could be a result rather than the cause of the disease as ceruloplasmin, the main carrier of copper in serum, is elevated in various acute and chronic diseases (22).

A mechanism by which copper affects lipid indices may involve free radical scavenging. Copper is found at the active site of copper zinc-superoxide dismutase (CuZn-SOD), an enzyme that deactivates O_2^- (23–25). To accomplish this reaction, the mechanism employs an alternating reduction/oxidation of copper associated with the enzyme. The zinc (Zn^{2+}), a nontransition metal, does not function in the catalytic cycle but appears to stabilize the enzyme (23–25), which may explain why copper and not zinc affected the lipid indices. Inflammation and tissue injury activate neutrophils to engulf foreign agents (phagocytosis) and rapidly consume molecular oxygen (respiratory burst) (26,27). The burst of respiratory activity is initiated by membrane-bound NADPH oxidase, which univalently reduces molecular oxygen to $O_2^{\cdot-}$ in increased amount, requiring equally increased activity of CuZn-SOD to deactivate the $O_2^{\cdot-}$ (26,27). Aortic lesions observed in copper-deficient experimental animals are assumed to result from the impaired function of lysyl oxidase, a copper-containing enzyme essential for the structural integrity of vascular connective tissue (4).

Evident in the data of the present study is the positive association of age with all the indices of lipid metabolism thus suggesting that like copper, their concentrations increased with age. The strong association of copper with age confirmed an earlier study that

demonstrated an increase in serum copper with age (13). It is concluded from these findings that, unlike in animal studies (3–5), copper excess in humans is associated with dyslipidemia and therefore may predispose to atherosclerosis.

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