Original article

Determinants of serum copper, zinc and selenium in healthy subjects

M Ghayour-Mobarhan[1,4], A Taylor[1,3], SA New[2], DJ Lamb[1] and GAA Ferns[1,3]

Addresses
[1]Centre for Clinical Science & Measurement and [2]Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK
[3]Department of Clinical Biochemistry, The Royal Surrey County Hospital, Egerton Rd, Guildford, Surrey GU2 7XX, UK
[4]Faculty of Medicine, Mashad University of Medical Sciences, Mashad 9138813944, Iran

Correspondence
Professor GAA Ferns
Email: g.ferns@surrey.ac.uk

Abstract

Background We have investigated the association between serum copper, zinc and selenium concentrations, dietary intake, and demographic characteristics, including individual coronary risk factors, in healthy subjects.

Methods Serum copper, zinc and selenium were measured by atomic absorption spectrometry in 189 healthy subjects. Serum glutathione peroxidase and caeruloplasmin were also determined for each subject. A previously validated food frequency questionnaire was used to estimate the dietary trace element intake.

Results Male subjects had significantly lower serum copper ($P<0.001$) and caeruloplasmin ($P<0.001$), and higher serum zinc ($P<0.05$) and zinc:copper ratio ($P<0.001$) than female subjects. Significant differences were observed in serum copper and caeruloplasmin concentrations ($P<0.01$) with age. Weak but significant associations between dietary trace elements and their serum concentrations were observed for zinc ($r=0.18$, $P=0.02$), copper ($r=0.17$, $P=0.03$) and selenium ($r=0.19$, $P=0.02$). Obese subjects had significantly lower serum concentrations of zinc ($P<0.05$). In multifactorial analysis, dietary zinc ($P<0.05$), serum high-density lipoprotein-cholesterol (HDL-C) ($P<0.05$), diastolic blood pressure ($P<0.05$) and age ($P=0.05$) emerged as major predictors of serum zinc concentrations. The corresponding predictors for serum copper were C-reactive protein (CRP) ($P<0.001$), serum HDL-C ($P<0.001$), gender ($P=0.01$), physical activity levels ($P<0.05$) and dietary copper ($P<0.05$). Serum selenium concentrations were predicted by serum total cholesterol ($P<0.01$), serum CRP concentrations ($P<0.05$) and dietary selenium ($P<0.03$).

Conclusion Serum copper, zinc and selenium concentrations are influenced by physiological conditions such as age, diet and gender. Their serum concentrations are also associated with coronary risk factors, including body mass index, levels of physical activity, serum HDL-C and CRP.

Ann Clin Biochem 2005; **42**: 364–375

Introduction

Copper, zinc and selenium are essential trace elements that form part of the functional groups of several key enzymes.[1,2] Although serum concentrations of these trace elements on their own do not accurately reflect total body status,[3] their serum concentrations are frequently measured in combination with other indices, for example enzyme activities or tissue content, to assess deficiency, toxicity or inborn errors of metabolism.[3–6] Free radicals are thought to play an important role in the pathogenesis of coronary heart disease (CHD) and carcinogenesis.[7,8] Hence, the action of the antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase and catalase may play an important protective role in these diseases. The trace elements zinc, copper and selenium are essential components of these enzymes, but may also be involved in lipid oxidation.[1,9–12] There are several putative mechanisms by which selenium may protect against atherosclerosis.[13] Because of the effects of copper ions on the cellular elements of the arterial wall, leukocyte and platelet function, and lipoprotein metabolism, they clearly have the potential to play an important role in

atherogenesis in humans.[14] Furthermore, it has been proposed that zinc deficiency may predispose to glucose intolerance, diabetes mellitus, insulin resistance, atherosclerosis and coronary artery disease.[12] Several epidemiological studies relating measures of trace element status to disease susceptibility have been reported, and perturbations in trace element status have been variously shown to be associated with coronary[15,16] or cancer risks.[1,17] However, the interpretation of these studies may be confounded by variables that contribute to disease risk, while also affecting trace element status. Effects of age, gender, smoking habit and body mass index (BMI) on some trace elements have previously been reported, but the results of these studies have been inconsistent and few have also assessed the possible confounding effects of dietary trace element intake.[18–20]

In the present study, we aimed to clarify the relationship between a number of factors that have been reported or may be expected to affect trace element status and serum copper, zinc and selenium concentrations.

Materials and methods

Subjects

Approximately 200 healthy subjects were recruited from an unselected population of employees at the local university and hospital in Guildford, UK. Subjects with established CHD, diabetes mellitus and hypertension were excluded, leaving 189 subjects. Of these subjects, 41 were obese (BMI >30), six had metabolic syndrome as defined using NCEP-ATP III criteria,[21] and seven were taking aspirin.

Each subject gave informed written consent to participate in the study, which was approved by the South-West Surrey Research Ethics Committee and Surrey University's Advisory Committee on Ethics.

Estimation of dietary intake of micronutrients

Dietary intake over the previous 12 months was assessed using a food frequency questionnaire (FFQ), as previously detailed.[22] In brief, the FFQ was developed and validated against seven-day weighed records and biochemical markers of antioxidant status.[23] The short and long-term reproducibility of the FFQ have also been evaluated. Subjects were instructed on how to complete the FFQ during the initial interview, and completed FFQs were checked for inaccuracies and inconsistencies at subsequent interviews.

Blood sampling

Blood samples were collected between 08:30 and 10:30 h after a 12-h fast by venepuncture of the antecubital vein. Blood was collected into plain Vacutainer tubes (Becton-Dickenson, Cowley, Oxford, UK) and allowed to clot; the serum was then removed, taking care to avoid possible sources of trace element contamination.

Materials

All chemicals were obtained from Sigma (Sigma Chemical Co., Dorset, UK) unless stated otherwise.

Routine biochemical analyses

A full fasted lipid profile, comprising total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C), was determined for each patient. LDL cholesterol was calculated using the Friedewald equation, except for patients with triglyceride concentrations of >4.0 mmol/L. Serum lipid and blood glucose concentrations were measured by enzymatic methods, and C-reactive protein (CRP) concentrations were determined by polyethylene glycol-enhanced immunoturbidimetry using a Bayer Advia 1650 analyser (Bayer, Newbury, UK).

Serum trace element analysis

Copper and zinc concentrations in serum were measured by flame atomic absorption spectrometry following a one in four dilution with water,[24] and selenium was determined by electrothermal atomic absorption spectrometry with Zeeman background correction using a palladium chloride chemical modifier.[25] Typical between-batch precision (coefficients of variation [CVs]) for these assays were 3.9%, 2.27% and 3.65%, respectively.

GPx assay

Serum GPx was measured using a modification of the Paglia and Valentine method.[26] Briefly, 10 μL of serum, standard (0.1–0.3 U/mL purified GPx) or water (blank) was added in quadruplicate to a 96-well plate. In all, 290 μL of 0.1 mol/L phosphate buffer (containing 5 mmol/L EDTA disodium salt, 200 μmol/L sodium azide, 1 U/mL glutathione reductase, 0.86 mmol/L NADPH, 2 mmol/L reduced glutathione and 7.8 μmol/L *t*-butyl hydroperoxide) was added to each well. The reagents were mixed and the absorbance at 340 nm measured continuously for 5 min in an iEMS MF plate reader. The between-assay CV was typically 5.5%.

Caeruloplasmin measurement

Serum caeruloplasmin concentration was determined as ferrioxidase activity using Sunderman and Nomoto's method.[27] Briefly, 10 μL of serum was added to 200 μL of 0.1 mol/L acetate buffer (pH 5.2) and 100 μL of *p*-phenylene diamine in eight wells of a 96-well plate. Samples were incubated at 37°C and the reaction terminated in four wells after 5 min and in the remaining four wells after 35 min by adding 5 μL of sodium azide (1.5 mol/L). The absorbance was measured at 525 nm in an iEMS MF plate reader. The caeruloplasmin concentration was calculated using the formula:

$$\text{Caeruloplasmin concentration (g/L)} = 0.752\,(A_R - A_B)$$

where A_R is the absorbance after 35 min (sample reaction) and A_B is the absorbance after 5 min (sample background). The between-assay CV for the assay was typically 3.8%.

Statistical analysis

Statistical analysis was undertaken using Minitab (release 13, Minitab Inc, 2000, USA), with descriptive statistics (mean, median, standard error of the mean [SEM] and interquartile range) being determined for all variables. Data were assessed for normality using the Kolmogorov–Smirnov test. Between-group comparisons of dietary and biochemical parameters were assessed by ANOVA using a Bonferonni correction for multiple comparisons. Categorical data were compared using Fisher's exact or χ^2 tests. Values were expressed as mean $\pm$ SEM, or median and interquartile range for non-normally distributed data. Analysis of covariance (ANCOVA) was used to assess differences after adjustment for important confounding factors such as age and gender. The CRP concentrations were non-normally distributed and were therefore logarithmically transformed prior to parametric analysis.

Stepwise multiple regression analysis was used to predict whether serum trace elements were related to dietary trace elements and traditional coronary risk factors such as age, gender, BMI, smoking, fasting glucose and lipid profile, and systolic and diastolic blood pressure (DBP). A P value <0.05 was considered significant.

Results

Demographic characteristics and variation in serum trace element concentrations

Gender

Among the 189 healthy subjects, 95 were men and 94 were women (Table 1). Men and women did not differ with respect to age, smoking status, BMI, systolic blood pressure (SBP) and serum total cholesterol ($P>0.05$). However, men had significantly higher waist:hip ratio, DBP, fasting blood glucose, serum triglycerides and total cholesterol:HDL ratio, and lower serum HDL-C and percentage body fat than women (Table 1).

Men had significantly lower serum copper ($P<0.001$) and caeruloplasmin ($P<0.001$), and higher serum zinc ($P<0.05$) and zinc:copper ratio than women ($P<0.001$) (Table 2). However, serum selenium, GPx and copper:caeruloplasmin ratio did not differ significantly between men and women ($P>0.05$) (Table 2).

Men had a significantly higher dietary intake of zinc and zinc:copper ratio compared with women (Table 2).

Age

Men and women were divided into six subgroups based on their age (Table 3). Within the male subgroups, significant differences were observed in serum caeruloplasmin concentrations ($P<0.01$) with age, being significantly lower in the age group 20–29 years compared with the other age groups. Significant differences in serum copper:caeruloplasmin ratio ($P<0.001$) and borderline differences in serum GPx ($P=0.09$) were also observed comparing the youngest and oldest men (Table 3). The serum zinc:copper ratio was lowest in the oldest male subgroup (Table 3).

Within the female subgroups, significant differences were observed in serum copper concentrations ($P<0.05$), being higher in groups aged between 30–39, 40–49, 50–59 and 60–69 years compared with the group aged 70 years or more. Women aged between 50–59 years had significantly higher serum selenium compared with the group aged 70 years or more (Table 3).

No significant correlations between age and dietary trace element intake were observed in these subjects.

Smoking habit

Among the 189 subjects, 124 had never smoked and 65 were current or ex-smokers (32 current smokers and 33 ex-smokers). Subgroups of current, ex-smokers and non-smokers did not differ with respect to gender, BMI, SBP, DBP, fasting blood glucose, serum total cholesterol, HDL-C, and total cholesterol:HDL-C ratio ($P>0.05$) (Table 1). However, smokers had significantly higher waist:hip ratio and serum triglycerides, and were older than the non-smoking subjects ($P<0.05$) (Table 1). The dietary intake of trace elements did not differ between smokers and non-smoking subjects (Table 4).

Table 1 *Clinical and biochemical characteristics of subgroups of healthy subjects (e.g. gender, obesity, and smoking habits)*

Groups	*n*	Age (year)	% Body fat	WHR	SBP	DBP	TC	HDL-C	TG	FBG	CRP
Gender											
M	94	49.51 ± 1.55	20.98 ± 0.64*	0.93 ± 0.01*	127.3 ± 1.8	78.03 ± 0.94†	5.40 ± 0.11	1.49 ± 0.03*	1.24‡ (0.91–1.64)	5.26 ± 0.05‡	0.65† (0.18–1.56)
F	95	49.14 ± 1.46	31.45 ± 0.83	0.82 ± 0.01	128.7 ± 2.1	74.63 ± 1.05	5.45 ± 0.10	1.80 ± 0.05	1.05 (0.82–1.40)	4.94 ± 0.09	1.26 (0.23–2.64)
Obese											
Yes	41	51.29 ± 2.20*	31.09 ± 0.79*	0.95 ± 0.02*	137.1 ± 3.3‡	81.94 ± 1.73*	5.62 ± 0.16	1.43 ± 0.05‡	1.63* (1.71–4.5)	5.45 ± 0.15*	1.97* (1.14–5.96)
No	148	48.87 ± 1.26	26.56 ± 0.63	0.86 ± 0.01	125.2 ± 1.4	74.73 ± 0.79	5.41 ± 0.09	1.70 ± 0.04	1.06 (0.86–1.39)	4.97 ± 0.04	0.58 (0.17–1.42)
Smoker											
Yes	65	52.55 ± 1.92†	26.33 ± 1.36	0.91 ± 0.01‡	129.2 ± 2.3	76.8 ± 1.2	5.53 ± 0.13	1.59 ± 0.05	1.23† (0.96–1.70)	5.08 ± 0.09	0.94 (0.33–2.31)
No	124	47.63 ± 1.25	26.98 ± 0.87	0.86 ± 0.01	127.4 ± 1.7	76.1 ± 0.9	5.37 ± 0.09	1.68 ± 0.04	1.10 (0.83–1.44)	5.11 ± 0.07	0.66 (0.15–1.89)

Values are expressed as mean ± SEM, or median and interquartile range. Between-groups comparisons were assessed by Mann–Whitney test for non-normal distribution data (serum triglycerides) and by one-way ANOVA for normally distributed data. *$P<0.001$. †$P<0.05$. ‡$P<0.01$. WHR, waist:hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; FBG, fasting blood glucose; CRP, C-reactive protein.

Table 2 *Effects of gender on indices of dietary and serum trace element status in control subjects*

	Males	Females
Subjects (*n*)	95	94
Serum trace elements		
Zinc (μmol/L)	13.86 ± 0.23*	13.30 ± 0.24
Copper (μmol/L)	14.76 ± 0.32†	17.52 ± 0.34
Zn:Cu ratio	0.97 ± 0.02†	0.78 ± 0.02
Cp (g/L)	0.16 ± 0.01†	0.19 ± 0.01
Cu:Cp ratio (μmol/g)	100.50 ± 3.54	97.86 ± 3.12
Selenium (μmol/L)	1.05 ± 0.02	1.01 ± 0.02
GPx (U/mL)	0.35 ± 0.01	0.36 ± 0.01
Dietary trace elements		
Zinc (mg)	9.93 ± 0.29*	9.03 ± 0.28
Copper (mg)	1.63 ± 0.06	1.64 ± 0.07
Zn:Cu ratio	6.42 ± 0.20	5.88 ± 0.18
Selenium (μg)	92.45 ± 4.48	82.19 ± 4.14

Values are expressed as mean ± SEM. *$P < 0.05$. †$P < 0.001$; comparison between patients and controls using one-way ANOVA. Cp, caeruloplasmin; Cu, copper; GPx, glutathione peroxidase Zn, zinc.

Serum concentrations of zinc, copper, zinc:copper ratio, caeruloplasmin and copper:caeruloplasmin concentrations did not differ significantly between smokers and non-smokers ($P > 0.05$) (Table 4). Smokers had slightly lower mean serum concentrations of selenium ($P = 0.08$) and GPx ($P = 0.09$) compared with non-smokers (Table 4).

Obesity

Among the 189 control subjects, 41 were obese (BMI > 30), 64 were overweight (BMI 25–30), and 84 were normal weight (BMI < 25). Obese and non-obese subjects did not differ with respect to gender, current smoking status, or serum total cholesterol ($P > 0.05$). However, as might be expected, obese subjects had significantly higher measures of adiposity (BMI, percentage body fat and waist:hip ratio), higher SBP and DBP, higher serum triglycerides and fasting blood glucose, and lower HDL-C than non-obese subjects ($P < 0.01$). Obese subjects were also significantly older than non-obese subjects ($P < 0.001$) (Table 1).

Serum zinc concentrations fell with increasing body mass category in the group as a whole and among the male subgroup ($P < 0.05$) (Table 5). In the male subgroup, serum zinc:copper ratio ($P < 0.05$) and GPx ($P < 0.05$) also fell with body mass category, although these failed to reach statistical significance for the group as a whole, or in the female subgroup (Table 5).

Univariate analysis of factors associated with serum trace elements

Association between dietary trace elements and their serum concentrations

There were weak but significant associations between the dietary intake of specific trace elements and their respective serum concentrations: zinc ($r = 0.18$, $P = 0.02$), copper ($r = 0.17$, $P = 0.03$) and selenium ($r = 0.19$, $P = 0.02$). There were strong and significant correlations between dietary intakes of zinc, copper and selenium ($P < 0.0001$ in each case). The reciprocal effects of dietary zinc, copper and selenium on other serum trace element concentrations were generally weak and non-significant ($P > 0.05$). However, dietary copper was positively associated with serum selenium ($P < 0.04$).

Relationship between CHD risk factors and serum trace element status

Serum copper concentrations were positively associated with HDL, serum CRP concentrations and reported physical activity levels ($P < 0.001$), and negatively associated with waist:hip ratio ($P < 0.05$) (Table 6). The serum zinc:copper ratio was positively associated with fasting blood glucose ($P < 0.05$) and waist:hip ratio ($P < 0.01$), and negatively associated with HDL ($P < 0.05$), serum CRP ($P < 0.001$) and reported physical activity levels ($P < 0.001$) (Table 6). Serum caeruloplasmin concentrations were positively associated with age ($P < 0.05$), serum HDL ($P < 0.05$), serum total cholesterol ($P < 0.01$) and serum CRP ($P < 0.001$) (Table 6). Serum copper:caeruloplasmin ratio was negatively associated with age ($P < 0.01$), fasting blood glucose ($P < 0.05$), total cholesterol ($P < 0.05$) and SBP ($P < 0.05$) (Table 6).

Serum GPx was negatively associated with age ($P < 0.05$) and SBP ($P < 0.05$), and positively associated with HDL concentrations ($P < 0.001$) and DBP ($P < 0.05$) (Table 6).

There were no significant correlations between these coronary risk factors and serum concentrations of zinc and selenium ($P > 0.05$) (Table 6).

Multifactorial analysis of trace element status

In order to evaluate the relationship between serum trace element concentrations and individual CHD risk factors, stepwise multiple regression analysis was undertaken.

Serum zinc concentrations

A total of approximately 10.7% of the variation in serum zinc concentrations could be explained by factors

Table 3 *Effects of age on indices of dietary and serum trace element status in healthy subjects*

Age (years)	Gender	Number	Zinc (μmol/L)	Copper (μmol/L)	Zn:Cu ratio	Cp (g/L)	Cu:Cp ratio (μmol/g)	Selenium (μmol/L)	GPx (U/mL)
20–29	Male	13	14.0 ± 0.77	14.5 ± 0.86	0.99 ± 0.06	0.12 ± 0.02†	137.8 ± 11.0‡,§	1.08 ± 0.06	0.42 ± 0.03‡
	Female	13	13.4 ± 0.44	16.3 ± 0.77*	0.84 ± 0.04	0.18 ± 0.02	102.5 ± 11.9	1.00 ± 0.07	0.38 ± 0.03
30–39	Male	9	14.2 ± 0.93	14.7 ± 1.38	1.01 ± 0.10‡	0.14 ± 0.01	107.4 ± 13.3	1.01 ± 0.07	0.36 ± 0.05
	Female	9	13.8 ± 0.78	19.3 ± 1.082**,‡	0.76 ± 0.08	0.19 ± 0.02	103.5 ± 9.78	0.91 ± 0.04	0.38 ± 0.03
40–49	Male	24	14.3 ± 0.44	15.2 ± 0.68	0.97 ± 0.05‡	0.16 ± 0.01**	101.4 ± 6.96	1.04 ± 0.05	0.38 ± 0.03
	Female	23	13.3 ± 0.48	17.6 ± 0.61‡	0.78 ± 0.04	0.19 ± 0.01	98.06 ± 5.76	1.05 ± 0.05	0.37 ± 0.02
50–59	Male	28	13.4 ± 0.33	14.3 ± 0.58	0.96 ± 0.04	0.16 ± 0.01**	89.6 ± 4.24	1.08 ± 0.05	0.33 ± 0.02
	Female	31	13.1 ± 0.45	18.1 ± 0.51‡	0.75 ± 0.04	0.20 ± 0.01	93.4 ± 4.98	1.07 ± 0.06‡	0.36 ± 0.02
60–69	Male	14	14.1 ± 0.43	14.2 ± 0.60	1.02 ± 0.05‡	0.18 ± 0.01**	83.9 ± 6.15	1.06 ± 0.06	0.30 ± 0.03
	Female	11	14.1 ± 0.95	17.9 ± 0.93‡	0.80 ± 0.05	0.19 ± 0.02	99.3 ± 7.38	0.94 ± 0.05	0.29 ± 0.03
70+	Male	7	12.7 ± 1.17	16.9 ± 0.83	0.78 ± 0.10	0.19 ± 0.02	96.3 ± 12.9	0.90 ± 0.08	0.30 ± 0.04
	Female	7	12.0 ± 0.60	14.3 ± 0.98	0.85 ± 0.05	0.16 ± 0.02	98.6 ± 14.8	0.88 ± 0.09	0.29 ± 0.06

Values are expressed as mean ± SEM. One-way ANOVA used for comparison between groups. *$P<0.05$. $^{\dagger}P<0.01$. **$P<0.05$ compared with the group aged between 20 and 29 years. $P<0.05$ compared with the group aged 70 or above. $^{\S}P<0.001$ and Fisher's (*post-hoc*) test for determining differences within individual groups. Cp, caeruloplasmin; Cu, copper; GPx, glutathione peroxidase Zn, zinc.

Table 4 *Effects of smoking status on indices of dietary and serum trace element status in healthy subjects*

	Smokers	Non-smokers
Subjects (*n*)	65	124
Serum trace elements		
Zinc (μmol/L)	13.83 ± 0.28	13.45 ± 0.21
Copper (μmol/L)	16.25 ± 0.40	16.07 ± 0.33
Zn:Cu ratio	0.87 ± 0.03	0.88 ± 0.02
Cp (g/L)	0.18 ± 0.01	0.17 ± 0.01
Cu:Cp ratio (μmol/g)	100.25 ± 3.97	97.17 ± 2.93
Selenium (μmol/L)	0.99 ± 0.03	1.05 ± 0.02
GPx (U/mL)	0.33 ± 0.02	0.36 ± 0.01
Dietary trace elements		
Zinc (mg)	9.69 ± 0.36	9.32 ± 0.25
Copper (mg)	1.59 ± 0.08	1.66 ± 0.06
Zn:Cu ratio	6.46 ± 0.24	5.96 ± 0.16
Selenium (μg)	87.09 ± 4.76	87.09 ± 3.95

Values are expressed as mean ± SEM. One-way ANOVA used for comparison between smokers and non-smokers. Cp, caeruloplasmin; Cu, copper; GPx, glutathione peroxidase Zn, zinc.

entered into the best-fitting multi-factorial model. These factors included: dietary zinc ($P < 0.05$, +2.6%), serum HDL-C ($P < 0.05$, +1.9%), DBP ($P < 0.05$, +2.3%), age ($P = 0.05$, −1.7%), serum triglycerides ($P > 0.05$, +1.2%) and gender ($P > 0.05$, +1%).

Serum copper concentrations

The major predictors of serum copper concentrations accounted for approximately 35.9% of its variation; these included CRP ($P < 0.001$, +18.8%), serum HDL-C ($P < 0.001$, +10.7%), gender ($P = 0.01$, 4.6%), reported physical activity levels ($P < 0.05$, +1.6%) and dietary copper ($P < 0.05$, +2%). The major predictors of the total variation in zinc:copper ratio were gender ($P < 0.001$, +13.7%) and CRP ($P < 0.001$, −9.1%).

Serum caeruloplasmin concentrations

Approximately 21.6% of the total variation in caeruloplasmin concentrations could be explained by gender ($P < 0.001$, 6.3%), serum total cholesterol ($P = 0.001$, +5.2%), serum CRP ($P = 0.01$, +3.3%), dietary copper ($P < 0.01$, +4%), serum HDL-C ($P < 0.05$, +2%) and SBP ($P = 0.08$, +0.8%).

Serum selenium concentrations

Approximately 8.2% of the total variation in serum selenium concentrations could be explained by serum total cholesterol ($P < 0.01$, +4.2%), serum CRP concentrations ($P < 0.05$, −2%) and dietary selenium ($P < 0.03$, +2%).

Serum GPx concentrations

The best-fitting model showed that a total of approximately 19.2% of the variation in serum GPx concentrations could be explained by serum HDL-C ($P < 0.001$, +10.7%), age ($P < 0.001$, −7.4%) and fasting blood glucose ($P > 0.05$, +0.9%).

Discussion

Effects of gender on trace element status

We found that healthy women have significantly higher serum copper and caeruloplasmin concentrations and lower serum zinc concentrations and zinc:copper ratios than men. This is consistent with several previous reports.[18,19,28] Whether there is a gender difference in serum selenium concentrations remains controversial.[17,29–32] We did not find a significant difference in serum selenium concentrations related to gender.

It has previously been proposed that the gender-related differences in serum zinc and copper concentrations may be explained by differences in dietary intake, or efficiency of absorption.[19] In our sample, we found that women have a lower dietary intake of zinc than men ($P < 0.05$). Dietary copper and selenium were similar for men and women, and of a similar order, as previously reported.[19]

It has also been reported that treatment with exogenous oestrogens, for example the oral contraceptive pill or postmenopausal therapy, may be associated with increased concentrations of serum copper.[19] It is possible that this may explain some of the difference between the groups and may also explain the age-related differences in the female group discussed further below.

Previous studies have reported that serum GPx activity is not significantly related to gender,[33] although others have reported that young, healthy women have higher serum GPx activities than their male counterparts.[34] We found no significant gender difference in serum GPx activities. Similarly, erythrocyte GPx activity has been reported as being either slightly higher[33,35] or no different[36] in women compared with men.

Effects of age on trace element status

We found significant differences in serum copper and caeruloplasmin concentrations related to age in our study. These findings are similar to previous reports.[18,19,28] It has been suggested that these

Table 5 *Association between BMI and indices of serum trace element status in healthy subjects*

	Obese	Overweight	Normal weight
Serum trace elements			
Males			
Subjects (*n*)	23	40	33
Zinc (μmol/L)	12.94 ± 0.39*	14.06 ± 0.32	14.59 ± 0.42
Copper (μmol/L)	15.42 ± 0.70	14.91 ± 0.44	14.04 ± 0.61
Zn:Cu ratio	0.87 ± 0.05*	0.97 ± 0.03	1.05 ± 0.04
Cp (g/L)	0.171 ± 0.01	0.158 ± 0.01	0.147 ± 0.01
Cu:Cp ratio (μmol/g)	111.37 ± 2.18	100.63 ± 2.93	13.84 ± 0.20
Selenium (μmol/L)	1.06 ± 0.06	1.06 ± 0.05	1.04 ± 0.04
GPx (U/mL)	0.30 ± 0.03*	0.39 ± 0.02	0.34 ± 0.02
Females			
Subjects (*n*)	18	25	51
Zinc (μmol/L)	12.44 ± 0.52	13.30 ± 0.56	13.50 ± 0.35
Copper (μmol/L)	17.85 ± 0.76	17.96 ± 0.70	16.97 ± 0.41
Zn:Cu ratio	0.72 ± 0.05	0.76 ± 0.04	0.82 ± 0.03
Cp (g/L)	0.202 ± 0.01	0.216 ± 0.02	0.179 ± 0.01
Cu:Cp ratio (μmol/g)	89.65 ± 6.11	88.48 ± 4.60	102.50 ± 4.80
Selenium (μmol/L)	1.00 ± 0.06	1.08 ± 0.05	0.99 ± 0.03
GPx (U/mL)	0.32 ± 0.04	0.34 ± 0.02	0.37 ± 0.02
Males and females			
Subjects (*n*)	41	65	84
Zinc (μmol/L)	12.73 ± 0.35*	13.77 ± 0.29	13.93 ± 0.27
Copper (μmol/L)	16.45 ± 0.55	16.07 ± 0.43	15.81 ± 0.38
Zn:Cu ratio	0.81 ± 0.04	0.89 ± 0.03	0.91 ± 0.03
Cp (g/L)	0.184 ± 0.01	0.180 ± 0.01	0.166 ± 0.01
Cu:Cp ratio (μmol/g)	92.59 ± 4.25	100.13 ± 4.72	101.40 ± 3.71
Selenium (μmol/L)	1.03 ± 0.04	1.06 ± 0.03	1.01 ± 0.03
GPx (U/mL)	0.31 ± 0.02	0.37 ± 0.02	0.36 ± 0.01
Dietary trace elements			
Males and females			
Zinc (mg)	9.75 ± 0.50	9.04 ± 0.36	9.63 ± 0.28
Copper (mg)	1.55 ± 0.12	1.59 ± 0.09	1.69 ± 0.06
Zn:Cu ratio	6.78 ± 0.38	6.00 ± 0.21	6.01 ± 0.19
Selenium (μg)	87.09 ± 7.15	87.09 ± 5.27	69.27 ± 4.30

Values are expressed as mean ± SEM. One-way ANOVA used for comparison between obese, overweight and normal weight healthy subjects; *$P < 0.05$. Cp, caeruloplasmin; Cu, copper, GPx, glutathione peroxidase Zn, zinc.

age-related changes in biochemical indices of copper status are not due to altered absorption or excretion of copper.[19] In our study, the age-related changes in biochemical indices of copper status were not related to dietary copper intake. The variation with age in women may be related to use of the contraceptive pill in the younger age group. Menopausal status may further complicate interpretation, together with the greater use of hormone replacement therapy (HRT) in the women over 50 years old. We had incomplete data on forms of oral contraceptive and HRT to undertake this analysis, and further studies are needed to assess this formally. Caeruloplasmin is an acute-phase reactant and, as may be expected, serum concentrations of caeruloplasmin (and therefore copper) were strongly associated with serum CRP concentrations.[2]

A fall in plasma zinc with age has been reported and attributed to a decline in the rate of absorption and/or an accelerated clearance from the plasma.[37] There have been other reports of changes in zinc metabolism with age.[38] We did not find a consistent change in serum zinc concentrations with age in our healthy subjects.

Table 6 *Correlations (r) between indices of serum trace elements status and individual coronary risk factors*

	Zinc (μmol/L)	Copper (μmol/L)	Zn:Cu ratio	Cp (g/L)	Cu:Cp ratio (μmol/g)	Selenium (μmol/L)	GPx (U/mL)
Age	−0.10	0.05	−0.10	0.18*	−0.28**	−0.07	−0.28**
Fasting BSL	0.09	−0.14	0.20*	0.10	−0.18*	0.13	0.00
BMI	−0.01	0.01	−0.02	0.10	−0.07	0.13	−0.00
Waist:hip ratio	0.15	−0.22*	0.26**	−0.09	−0.07	−0.09	−0.07
HDL	0.09	0.35***	−0.22*	0.22*	−0.01	0.03	0.30***
Triglycerides	−0.03	−0.05	0.01	−0.05	0.04	0.02	−0.09
Total cholesterol	−0.02	0.11	−0.09	0.24**	−0.18*	−0.08	−0.11
Systolic BP	0.09	−0.01	0.08	0.15	−0.20*	−0.05	−0.17*
Diastolic BP	0.04	−0.09	0.10	−0.02	−0.06	−0.06	0.16*
CRP	0.03	0.49***	−0.33***	0.33***	−0.02	−0.13	−0.06
Physical activity level	−0.14	0.30***	−0.33***	0.18	−0.01	−0.01	0.05

Correlations were assessed using Pearson correlation coefficients; *$P<0.05$, **$P<0.01$, ***$P<0.001$. Non-normally distributed data such as serum CRP and triglycerides log transformed before using the Pearson correlations. Physical activity levels were associated with adiposity indices including BMI ($r=-0.36$, $P<0.001$) and waist/hip ratio ($r=-0.46$, $P<0.001$), which supported the validation of our questionnaire. Cp, caeruloplasmin; Cu, copper; GPx, glutathione peroxidase; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein Zn, zinc.

The relationship between age and serum selenium concentrations remains unclear.[2,19,39] In the present study, no significant correlations between age and serum selenium concentrations were observed.

Association between dietary intake of trace element and their serum concentrations

Due to our relatively large sample size and problems associated with weighed sampling methodology (e.g. under-reporting), we chose to use the FFQ as opposed to weighed records. Our FFQ tool for assessing trace element intake has limitations. The McCance and Widdowson Food Composition Tables, which were used to assess nutritional intake, are not complete with respect to trace element content for several foods. This is a universal problem with assessing dietary intake and would occur irrespective of dietary methodology used. Univariate analysis revealed a weak relationship between the dietary intake of copper, zinc or selenium and their respective serum concentrations. These associations remained following multifactorial analysis, though the contribution of diet to serum concentrations was small for all three elements. The reasons for this are likely to be complex, but may include the interaction between trace elements at the level of absorption and metabolism, as previously described for copper and zinc;[40] the form of dietary trace element consumed, which may effect the efficiency of absorption, and other confounding conditions such as menopausal status, low-grade inflammation, and perhaps recent intake. We looked for possible reciprocal interactions between dietary trace elements, for example between zinc and copper. There were no clear relationships, although we found a weak association between dietary copper and serum selenium.

Effects of smoking on trace element status

In the current study, we found no significant differences in serum concentrations of zinc, copper, zinc: copper ratio, caeruloplasmin, selenium or GPx between current or ex-smoker subjects and non-smokers. Some investigators have previously reported that serum zinc and copper concentrations decreased with increasing use of cigarettes, but no explanation was provided.[15,18] It has also been reported that smoking has no effect on serum selenium concentrations,[32,41] while other investigators have found lower serum selenium concentrations in smokers.[18,42] The effects of smoking are likely to be dependent on the type, quantity and manner by which the tobacco is smoked. We did not have these details, and it is likely that this lack of specific information is also responsible for the discrepancies in the literature.[5]

Trace element status in obesity

Consistent with some previous reports, we found lower serum zinc concentrations[16,43–45] and higher serum copper[16,46,47] and caeruloplasmin concentrations[16,48] in obese compared with non-obese subjects. However, Gjorup *et al.*[49] reported no significant differences of serum zinc concentrations between obese and non-obese subjects, and Yakinci *et al.*[47] and Taneja *et al.*[50] found that serum zinc concentrations in obese children were significantly higher than the control group.

It has also been reported that serum selenium concentrations are significantly lower in obese subjects compared to non-obese subjects.[49] Although we found

no significant differences between obese and non-obese subjects with regard to selenium, we did find lower serum GPx concentrations in obese men compared to non-obese subjects, consistent with previous reports.[44]

Trace elements and lipid metabolism

In our population, we found that serum copper concentrations were strongly positively related to HDL cholesterol and reported habitual physical activity and negatively related to waist:hip ratio. The associations with both HDL and physical activity remained significant on multifactorial analysis. The mechanism for this association is unclear, although there has been a previous report of higher concentrations of both copper and zinc in athletes[51] and the positive association between HDL and physical activity is well established. We, like Jiang *et al.*,[52] did not find a strong association between serum copper and LDL cholesterol, as previously reported for a Kuwaiti population in which it was also found that high environmental exposure to copper was associated with high serum LDL concentrations.[53] However, Jiang *et al.*[52] report a negative association between serum copper and HDL cholesterol concentrations in their sample from South China. According to the zinc/copper hypothesis of Klevay,[40] which was based on observational studies in man and experimental studies in rats, dietary deficiency of copper, either alone or in association with a high zinc intake, is associated with hypercholesterolaemia and increased risk of atherosclerosis. Several studies have reported that mild degrees of copper deficiency induced by dietary zinc excess may lead to raised cholesterol concentrations and increased coronary risk.[14,54,55] However, we have recently reported that the association between copper status and atherosclerosis is likely to be biphasic.[56] Nor, given the population sample and their indices of copper status, including their estimated dietary copper, is it likely that copper deficiency is likely to play a major part in determining serum LDL concentrations. Consistent with other reports,[48] we found a significant correlation between serum caeruloplasmin and serum cholesterol.

On univariate analysis, we found no significant correlations between serum zinc concentration and several coronary risk factors (Table 6). However, multivariate analysis revealed that serum zinc concentrations were weakly associated with serum HDL cholesterol and DBP, with lesser contributions from age, serum triglycerides and gender. Tully and colleagues[57] have previously shown that serum zinc concentrations are positively related to LDL cholesterol concentrations in a female population, and they have proposed that both zinc and cholesterol may be related to a third factor, possibly dietary intake of red meat.

Jossa *et al.*[58] have previously found no significant association between serum selenium and several coronary risk factors, including: triglycerides, HDL cholesterol, systolic and DBP, age and BMI. However, they did find a positive and significant correlation between serum selenium and serum cholesterol ($r = 0.12$; $P = 0.02$). Although we found no association between serum selenium and cholesterol on univariate analysis, multivariate analysis did show weak associations between serum selenium and total cholesterol and between serum GPx and HDL cholesterol concentrations.

In conclusion, serum copper, zinc and selenium concentrations, and other measures of trace element status appear to be significantly influenced by, or associated with, several demographic, physiological and pathophysiological factors, including age, gender, diet, physical activity and inflammatory state. Although some of these associations may be due to a causal relationship, others are likely to be due to unidentified confounding factors. Although these findings are not likely to alter the interpretation of trace element data when gross deficiency, or toxicity, is suspected, they may be important in the interpretation of epidemiological data on disease susceptibility.

References

1 Rayman MP. The importance of selenium to human health. *Lancet* 2000; **356**: 233–41

2 Tietz NW. *Clinical Guide to Laboratory Tests*. 3rd edn. Philadelphia: WB Saunders Company, 1999

3 Vitoux D, Arnaud J, Chappuis P. Are copper, zinc and selenium in erythrocytes valuable biological indexes of nutrition and pathology? *J Trace Elements Med Biol* 1999; **13**: 113–28

4 Taylor A. Detection and monitoring of disorders of essential trace elements. *Ann Clin Biochem* 1996; **33**: 486–510

5 Robberecht H, Deelstra H. Factors influencing blood selenium concentration values: a literature review. *J Trace Elem Electrolytes Health Dis* 1994; **8**: 129–43

6 Gibson R. Hair as a biopsy material for the assessment of trace element status in infancy. *J Hum Nutr* 1980; **34**: 405–16

7 Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem* 2004; **266**: 37–56

8 Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med* 2000; **28**: 1815–26

9 Craig WY, Poulin SE, Palomaki GE, Neveux LM, Ritchie RF, Ledue TB. Oxidation-related analytes and lipid and lipoprotein concentrations in healthy subjects. *Arterioscler Thromb Vasc Biol* 1995; **15**: 733–9

10 Ferns GA, Lamb DJ, Avades TY, Taylor A. Copper and atherosclerosis. *Metal Ions Biol Med* 2000; **6**: 622–4

11 Khassaf M, McArdle A, Esanu C, *et al.* Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J Physiol* 2003; **549**: 645–52

12 Roth H, Kirchgessner M. Zinc and insulin metabolism. *Biol Trace Element Res* 1991; **31**: 491–3
13 Alissa EM, Bahijri SM, Ferns GA. The controversy surrounding selenium and cardiovascular disease: a review of the evidence. *Med Sci Monitor* 2003; **9**: RA9–18
14 Ferns GA, Lamb DJ, Taylor A. The possible role of copper ions in atherogenesis: the Blue Janus. *Atherosclerosis* 1997; **133**: 139–52
15 Kim CH, Park JY, Kim JY, *et al.* Elevated serum ceruloplasmin levels in subjects with metabolic syndrome: a population-based study. *Metabolism* 2002; **51**: 838–42
16 Tungtrongchitr R, Pongpaew P, Phonrat B, *et al.* Serum copper, zinc, ceruloplasmin and superoxide dismutase in Thai overweight and obese. *J Med Assoc Thai* 2003; **86**: 543–51
17 Burguera JL, Burguera M, Gallignani M, Alarcon OM, Burguera JA. Blood serum selenium in the province of Merida, Venezuela, related to sex, cancer incidence and soil selenium content. *J Trace Elem Electrolytes Health Dis* 1990; **4**: 73–7
18 Grandjean P, Nielsen GD, Jorgensen PJ, Horder M. Reference intervals for trace elements in blood: significance of risk factors. *Scand J Clin Lab Invest* 1992; **52**: 321–37
19 Johnson PE, Milne DB, Lykken GI. Effects of age and sex on copper absorption, biological half-life, and status in humans. *Am J Clin Nutr* 1992; **56**: 917–25
20 Kim SH, Kim JS, Shin HS, Keen CL. Influence of smoking on markers of oxidative stress and serum mineral concentrations in teenage girls in Korea. *Nutrition* 2003; **19**: 240–3
21 NCEP. Executive summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486–97
22 New SA, Macdonald HM, Campbell MK, *et al.* Lower estimates of net endogenous non-carbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women. *Am J Clin Nutr* 2004; **79**: 131–8
23 Bodner C, Soutar A, New S, *et al.* Validation of a food frequency questionnaire for use in Scottish population: correlation of antioxidant vitamin intakes with biochemical measures. *J Hum Nutr Dietetics* 1998; **11**: 373–80
24 Taylor A. Measurement of zinc in clinical samples. *Ann Clin Biochem* 1997; **34**: 142–50
25 Campillo N, Vinas P, Lopez-Garcia I, Hernandez-Cordoba M. Selenium determination in biological fluids using Zeeman background correction electrothermal atomic absorption spectrometry. *Anal Biochem* 2000; **280**: 195–200
26 Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; **70**: 158–69
27 Sunderman FW, Nomoto S. Measurement of human serum ceruloplasmin by its *p*-phenylenediamine oxidase activity. *Clin Chem* 1970; **16**: 903–10
28 Milne DB, Johnson PE. Assessment of copper status: effect of age and gender on reference ranges in healthy adults. *Clin Chem* 1993; **39**: 883–7
29 Beaglehole R, Jackson R, Watkinson J, Scragg R, Yee RL. Decreased blood selenium and risk of myocardial infarction. *Int J Epidemiol* 1990; **19**: 918–22
30 Malvy DJ, Arnaud J, Burtschy B, *et al.* Reference values for serum zinc and selenium of French healthy children. *Eur J Epidemiol* 1993; **9**: 155–61
31 McMaster D, Bell N, Anderson P, Love AH. Automated measurement of two indicators of human selenium status, and applicability to population studies. *Clin Chem* 1990; **36**: 211–16
32 Thuluvath PJ, Triger DR. Selenium in chronic liver disease. *J Hepatol* 1992; **14**: 176–82
33 Guemouri L, Artur Y, Herbeth B, Jeandel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. *Clin Chem* 1991; **37**: 1932–7
34 Rush JW, Sandiford SD. Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity. *Clin Biochem* 2003; **36**: 345–51
35 Andersen HR, Nielsen JB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem* 1997; **43**: 562–8
36 Habif S, Mutaf I, Turgan N, *et al.* Age and gender dependent alterations in the activities of glutathione related enzymes in healthy subjects. *Clin Biochem* 2001; **34**: 667–71
37 Bales CW, Steinman LC, Freeland-Graves JH, Stone JM, Young RK. The effect of age on plasma zinc uptake and taste acuity. *Am J Clin Nutr* 1986; **44**: 664–9
38 Wastney ME, Ahmed S, Henkin RI. Changes in regulation of human zinc metabolism with age. *Am J Physiol* 1992; **263**: R1162–8
39 Kafai MR, Ganji V. Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988–1994. *J Trace Elem Med Biol* 2003; **17**: 13–18
40 Klevay LM. Coronary heart disease: the zinc/copper hypothesis. *Am J Clin Nutr* 1975; **28**: 764–74
41 Swanson CA, Longnecker MP, Veillon C, *et al.* Selenium intake, age, gender, and smoking in relation to indices of selenium status of adults residing in a seleniferous area. *Am J Clin Nutr* 1990; **52**: 858–62
42 Bukkens SG, de Vos N, Kok FJ, Schouten EG, de Bruijn AM, Hofman A. Selenium status and cardiovascular risk factors in healthy Dutch subjects. *J Am Coll Nutr* 1990; **9**: 128–35
43 Marreiro DN, Fisberg M, Cozzolino SM. Zinc nutritional status in obese children and adolescents. *Biol Trace Elem Res* 2002; **86**: 107–22
44 Ozata M, Mergen M, Oktenli C, *et al.* Increased oxidative stress and hypozincemia in male obesity. *Clin Biochem* 2002; **35**: 627–31
45 Singh RB, Beegom R, Rastogi SS, Gaoli Z, Shoumin Z. Association of low plasma concentrations of antioxidant vitamins, magnesium and zinc with high body fat per cent measured by bioelectrical impedance analysis in Indian men. *Magnes Res* 1998; **11**: 3–10
46 Omar S, Abdennebi M, Ben Mami F, *et al.* Serum copper levels in obesity: a study of 32 cases. *Tunis Med* 2001; **79**: 370–3
47 Yakinci C, Pac A, Kucukbay FZ, Tayfun M, Gul A. Serum zinc, copper, and magnesium levels in obese children. *Acta Paediatr Jpn* 1997; **39**: 339–41
48 Cignarelli M, DePergola G, Picca G, *et al.* Relationship of obesity and body fat distribution with ceruloplasmin serum levels. *Int J Obes Relat Metab Disord* 1996; **20**: 809–13
49 Gjorup I, Gjorup T, Andersen B. Serum selenium and zinc concentrations in morbid obesity. Comparison of controls and patients with jejunoileal bypass. *Scand J Gastroenterol* 1988; **23**: 1250–2
50 Taneja SK, Mahajan M, Arya P. Excess bioavailability of zinc may cause obesity in humans. *Experientia* 1996; **52**: 31–3

51 Tuya IR, Gil EP, Marino MM, Carra RMG, Misiego AS. Evaluation of the influence of physical activity on the plasma concentrations of several trace metals. *Eur J Appl Physiol Occupational Physiol* 1996; **73**: 299–303
52 Jiang H, Tell GS, Tang YC, Mo PS, He GQ. Relation of serum zinc and copper to lipids and lipoproteins – the YI people study. *J Am Coll Nut* 1992; **11**: 74–8
53 Adam B, Aslan S, Bedir A, Alvur M. The interaction between copper and coronary risk indicators. *Jpn Heart J* 2001; **42**: 281–6
54 Klevay LM. The influence of copper and zinc on the occurrence of ischemic heart disease. *J Environ Pathol Toxicol* 1980; **4**: 281–7
55 Klevay LM. Dietary copper and risk of coronary heart disease. *Am J Clin Nutr* 2000; **71**: 1213–14
56 Lamb DJ, Avades TY, Ferns GA. Biphasic modulation of atherosclerosis induced by graded dietary copper supplementation in the cholesterol-fed rabbit. *Int J Exp Pathol* 2001; **82**: 287–94
57 Tully C, Snowdon D, Belcher JD. Serum zinc and plasma lipoproteins in elderly women: findings from the nun study. *J Trace Elements Expl Med* 1995; **8**: 201–9
58 Jossa F, Trevisan M, Krogh V, *et al.* Serum selenium and coronary heart disease risk factors in southern Italian men. *Atherosclerosis* 1991; **87**: 129–34

Accepted for publication 16 June 2005