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Effect of copper supplementation on indices of copper status and certain CVD risk markers in young healthy women

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Western diets containing suboptimal Cu concentrations could be widespread. A link between marginal Cu deficiency and CVD has been suggested. The objective of the present study was to investigate the effect of Cu supplementation on both Cu status and CVD risk factors in healthy young women. Sixteen women with a mean age of 24 (SD 2) years participated in a randomised crossover study of three 4-week periods with 3-week washouts between periods. During each intervention period, subjects received 0, 3 or 6 mg elemental Cu/d as $CuSO_4$ in addition to their habitual diet. Blood samples were taken to assess the effect of supplementation on putative markers of Cu status. The content of plasma lipids, lipoprotein (a), apo and certain haemostatic factors, as putative indices of CVD, was also analysed. Daily supplementation with 3 mg Cu significantly increased ($P<0·05$) serum Cu concentration and the activity of erythrocyte superoxide dismutase, although there was no further significant increase after an intake of 6 mg Cu/d. The concentration of the fibrinolytic factor plasminogen activator inhibitor type 1 was significantly reduced ($P<0·05$) by about 30 % after supplementation with 6 mg Cu/d. No other marker of Cu status or CVD risk factor was affected by Cu supplementation. The results indicate that supplementation with 3 or 6 mg Cu/d may improve Cu status in these healthy young women. Increased Cu intake could reduce the risk of CVD and atherosclerosis in man by promoting improved fibrinolytic capacity.

Cu status: Cu supplementation: Marginal Cu deficiency: CVD

Clinically significant Cu deficiency is rare in man, suggesting that the current dietary intake is usually adequate for requirements. Cu deficiency has been reported mostly in infants recovering from malnutrition, in premature infants fed cows' milk diets or during total parenteral nutrition with inadequately supplemented preparations (Danks, 1988). Deficiency is associated with a low concentration of both serum Cu and caeruloplasmin (Cp), clinical features including anaemia, leucopenia and osteoporosis. A new RDA for Cu of 0.9 mg/d has recently been published (Food and Nutrition Board, 2001) based on an estimated average requirement of 0·7 mg/d, and it has been reported that 0·8 mg Cu/d is sufficient to maintain Cu status in men (Turnlund *et al.* 1990). The recommendation is, however, based on data from only three studies involving thirty-two men and women, and may therefore be inappropriate. More than 30 % of diets in North America and Europe contain less than 1·0 mg Cu/d (Klevay *et al.* 1993), and in a more recent study conducted in Maryland, USA, dietary Cu intake was below the current RDA in 60 % of the population (Pang *et al.* 2001). It has been suggested that marginal Cu deficiency could be linked to a number of degenerative and inflammatory conditions, including arthritis, cancer, osteoporosis and CVD (Strain, 1994; Klevay, 1998; Milne, 1998).

Many studies have reported adverse effects of Cu deficiency on risk factors for CVD in experimental animals. Cardiovascular abnormalities associated with such Cu deficiency have included hypercholesterolaemia (Klevay, 1975; Allen & Klevay, 1978; Lau & Klevay, 1981), increased susceptibility of VLDL and LDL to oxidation *in vitro* (Rayssiguier *et al.* 1993) and irregular electrocardiograms (Viestenz & Klevay, 1982; Medeiros *et al.* 1991*a*). A severe impairment of fibrinolysis has also been observed in Cu-deficient mice (Lynch & Klevay, 1993). A number of short-term human studies of experimental Cu deprivation have shown inconsistent results (reviewed in Milne, 1998). Adverse changes such as abnormal electrocardiograms (Reiser *et al.* 1985) and increased serum cholesterol (Klevay *et al.* 1984; Reiser *et al.* 1987; Nielsen *et al.* 1990) have again been reported, although usually in the absence of conventional biochemical signs of Cu deficiency. Some of the abnormalities in CVD risk factors were observed with Cu intakes of between 0·8 and 1·0 mg/d, which has tended to support the claim for an

Abbreviations: CCO, cytochrome C oxidase; Cp, caeruloplasmin; PAI-1, plasminogen activator inhibitor type 1; SOD, superoxide dismutase; t-PA, tissue plasminogen activator; TGF-β, transforming growth factor-β; WBC, leucocyte.

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association between low dietary Cu and CVD (Klevay, 1998) and may also suggest that the current RDA for Cu is too low.

Few studies have investigated the effect of dietary Cu intake on Cu status in women (Johnson *et al.* 1988; Milne *et al.* 1988; Johnson *et al.* 1992), despite evidence of some differences in Cu metabolism between men and women (Johnson *et al.* 1992). The aim of the present study was therefore to examine Cu status indicators and certain risk markers for CVD (including plasma lipids, lipoproteins and haemostatic factors) in young women during supplementation with Cu. The study was conducted as part of a multicentre European study (FOODCUE), which was undertaken to provide data on the biological effects of increased dietary Cu.

Subjects and methods

Subjects

Sixteen apparently healthy women aged 21–28 years were recruited for the study and were asked to maintain a normal lifestyle. The subjects' mean baseline characteristics were as follows: age 24 (SD 2) years, weight 60 (SD 7) kg, height 166 (SD 8) cm, BMI 21 (SD 2) kg/m^2. Exclusion criteria included smoking, the use of dietary supplements or oral contraceptives, pregnancy, breast-feeding and participation in competitive sports. Ethical approval was obtained from the Local Research Ethics Committee for Copenhagen and Frederiksberg (Journal no. 01-065/96), and all subjects signed an informed consent document.

Experimental design

The study was a double-blind, placebo-controlled randomised crossover trial, consisting of three 4-week periods, with a minimum 3-week washout between periods. Subjects received 0, 3 or 6 mg elemental Cu/d as $CuSO_4$ (Thomson and Joseph Ltd, Norwich, UK) during the 4-week intervention period in the form of a daily capsule in addition to their usual diet. The 4-week supplementation period was considered to be sufficient to enable the detection of any changes in indices of Cu status and CVD risk markers with the concentrations of Cu used. Previous studies of Cu depletion have shown a recovery of superoxide dismutase (SOD) activity after supplementation with at least 3 mg Cu/d for at least 30 d, but not with less than 2·6 mg Cu/d for up to 42 d (Milne, 1998). Another study has detected changes in certain CVD indices after 4 weeks with 2 or 3 mg Cu/d (Medeiros *et al.* 1991*b*). The 6 mg/d Cu dose was considered to be a suitably high concentration that was also below the tolerable upper intake level for Cu of around 10 mg/d (Food and Nutrition Board, 2001). The 3 mg/d dose of Cu was chosen because it was at the top end of the safe and adequate daily allowance of between 1·5 and 3·0 mg Cu recommended at the time (National Research Council, 1989).

Blood sampling and sample preparation

After an overnight fast, venous blood samples were taken on the morning of the last day of each 4-week supplementation period. Subjects abstained from alcohol for at least 24 h and from physical activity for at least 36 h before sampling. Blood was collected in trace element-free tubes (Vacutainer 606526; Becton Dickinson, Meylan-Cedex, France) for analysis of serum Cu and C-reactive protein, a marker of systemic inflammation. Blood samples were allowed to clot at room temperature for 1 h and were then centrifuged (3000 ***g***, 15 min, 18°C). Serum was transferred into plastic vials and stored at −20°C.

Blood was drawn into tubes containing citrate (Vacutainer 606608; Becton Dickinson) for determination of erythrocyte SOD and Hb activity, and into pre-cooled heparin tubes (Becton Dickin-son) for analysis of leucocyte (WBC) SOD, Cp and cytochrome C oxidase (CCO). The pre-cooled blood was centrifuged (1700 ***g***, 10 min, 4°C), and plasma for Cp analysis was stored at −80°C. The WBC were treated as described in Kehoe *et al.* (2000), and the WBC pellet subsequently obtained (which did not include platelets) was divided into two, centrifuged (2000 ***g***, 10 min, 25°C) and then resuspended in either PBS (for SOD analysis) or PBS-EDTA buffer (for CCO analysis) and stored at −80°C. Erythrocytes were washed three times in 154 mM-NaCl, with centrifugation (1700 ***g***, 10 min, 4°C), and stored at −80°C.

Blood was taken into EDTA tubes (Vacutainer 606457; Becton Dickinson) for analysis of total cholesterol, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol, total triacylglycerol, LDL-, HDL- and VLDL-triacylglycerol, and apo. Blood for lipoprotein analysis was stored at −80°C; blood for lipid analysis was immediately placed on ice and centrifuged (3000 ***g***, 15 min, 4°C) before storage at −20°C. Blood for analysis of vitamin E in the LDL fraction was drawn into vacutainer tubes (606430; Becton Dickin-son), EDTA (5 %) was added, and the sample was centrifuged (2000 ***g***, 30 min, 4°C). Plasma was removed and sucrose (0·6 %) added before samples were stored at −80°C, after overlaying with N.

Blood was drawn into citrate tubes (Vacutainer 606608; Becton Dickinson) for analysis of fibrinogen and plasma factor VIIc, into stabilyte tubes (Biopool, Umeå, Sweden) for determination of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1), and into diatubes (Becton Dickinson) for transforming growth factor β (TGF-β), platelet factor 4 and β-thromboglobulin analysis. Samples for analysis of TGF-β were cooled and centrifuged (700 ***g***, 5 min, 4°C) prior to storage at −80°C. Samples for PAI-1, t-PA and β-thromboglobulin analysis were centrifuged (3000 ***g***, 15 min, 4°C) and the plasma was transferred into plastic vials and stored at −80°C. Samples for platelet factor 4 analysis were centrifuged (2500 ***g***, 30 min, 4°C), and platelet-depleted plasma was carefully liberated and stored in plastic tubes at −80°C.

Analytical methods

Serum copper and C-reactive protein. Serum Cu was determined by atomic absorption spectrometry (Spectra AA-200; Varian, Victoria, Australia), under standard flame operating conditions as recommended by the manufacturer. A standard reference solution for Cu in serum (Seronorm Trace Element Serum; Nycomed Pharma AS, Oslo, Norway) was run simultaneously. The reference value was 19·8 μmol/l and the mean and standard deviation analysed values (n 5) were 18·23 (SD 0·59) μmol/l. Serum C-reactive protein levels were measured by an immunoassay (Roche, Basel, Switzerland) using a Cobas Mira automatic analyser (Roche).

Superoxide dismutase, caeruloplasmin and cytochrome C oxidase activity. Determination of erythrocyte SOD activity, serum Cp oxidase activity and Hb concentration was performed as described previously (Kehoe *et al.* 2000). The activity of

WBC SOD was measured on a Cobas Fara automatic analyser (Roche), by modification of the method of Jones & Suttle (1981), using a commercial kit (Ransod; Randox Laboratories, Co. Antrim, Northern Ireland). WBC SOD activity was expressed as units/g protein (Bio-Rad protein assay; Bio-Rad, Hemel Hempstead, Herfordshire, UK).

WBC CCO activity was determined as described previously (Kehoe *et al.* 2000). Results were expressed as units/g protein (Bio-Rad protein assay; Bio-Rad).

Analysis of plasma lipids and vitamin E. Total plasma cholesterol and triacylglycerol concentrations were analysed using an enzymatic procedure (Boehringer Mannheim GmbH, Mannheim, Germany) on the Cobas Mira analyser (Roche). Total HDL-cholesterol was measured enzymatically after precipitation with polyethylene glycol (Quantolip; Immuno AG, Vienna, Austria). The VLDL fraction was separated by ultracentrifugal flotation of fresh plasma (125 000 ***g***, 16 h, 4°C) at a density of 1·006 g/l in a Beckman 50 T:L rotor (Beckman Instruments, AL, USA). Accuracy was ensured by use of a control serum of known value (Precinorm L; Boehringer Mannheim). LDL-cholesterol was calculated as the difference between the HDL and VLDL fractions.

The vitamin E content of LDL was measured by HPLC as described previously (Rock *et al.* 2000).

Analysis of lipoprotein (a) and apo. The concentrations of lipoprotein (a), apo A-1 and apo B were determined by rate nephelometry using a Beckman Array 360 analyser (Global Medical Instrumentation, Inc., Minnesota, USA).

Measurement of haemostatic factors. The plasma concentration of clottable fibrinogen was determined by a modified Clauss assay (1957)*** using a commercial kit (Multifibren U; Dade Behring GmbH, Liederbach, Germany). A commercial ELISA kit was used for analysis of TGF-β (Quantikine; R&D Systems Inc., MN, USA), using an ELISA reader (SLT Rainbow; SLT LabInstruments Diagnostica GmbH, Salzburg, Austria). The total plasma antigen concentration of t-PA and PAI-1 was also determined by an ELISA method using commercial kits (Chromolize (t-PA) and TintElize (PAI-1); Biopool). Accuracy was ensured by use of a fibrinolysis reference plasma (Biopool). The values obtained were 1·10 (SD 0·13) IU/ml (*n* 9) and 22·78 (SD 2·54) ng/ml (*n* 10), compared with certified values of 0·9 (SD 0·04) IU/ml and 24·2 (SD 0·4) ng/ml, for t-PA and PAI-1, respectively.

Plasma factor VIIc was determined in a one-stage clotting assay using an ACL300 (Instrumentation Laboratory SpA, Milan, Italy), by incubation of 50 μl diluted test plasma (1:10 v/v in Tris buffer) with added human factor VII-deficient plasma (Biopool) and Tromborel S (Behring Diagnostics, Liederbach, Germany). Factor VIIc was expressed relative to an internal standard: CV % 3·69 (*n* 24).

Plasma platelet factor 4 and β-thromboglobulin were determined using commercial ELISA kits (Asserachrom; Diagnostica Stago, Asnieres-sur-Seine, France). Accuracy was verified by using appropriate reference material (Diagnostica Stago). Values obtained were 81·9 (SD 6·8) IU/ml (*n* 12) and 145·7 (SD 18·6) IU/ml (*n* 14) compared with certified values of 81 (range 68–94) IU/ml and 149 (127–171) IU/ml, for PF-4 and β-thromboglobulin, respectively.

Statistical analysis

Data are expressed as means and standard deviations. Data were analysed by the general linear model-repeated measures test using the Statistical Package for the Social Sciences version 8.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was analysed by ANOVA with repeated measures, after confirming that there were no significant carry-over effects. The paired *t* test was used to make comparisons between individual treatments. A significance level of $P<0{\cdot}05$ was used for all statistical tests (two-sided).

Results

The effect of Cu supplementation on putative indices of Cu status in young women is shown in Table 1. There was a significant increase ($P=0{\cdot}04$) of about 14 % compared with placebo in the concentration of serum Cu after daily supplementation with 3 mg Cu for 4 weeks. Supplementation with 6 mg Cu/d led to no further significant increase. Similarly, addition of 3 mg Cu/d to the habitual diet significantly increased ($P=0{\cdot}03$) erythrocyte SOD activity by about 11 % compared with placebo, whereas there was no further significant increase with 6 mg Cu/d supplementation.

The activity of both WBC SOD and serum Cp increased by about 12 % and 16 %, respectively, after supplementation with 3 mg Cu/d compared with placebo, but these increases were not significant ($P>0{\cdot}05$). The activity of WBC CCO was unaffected by Cu supplementation. C-reactive protein concentrations were measured in twelve samples for each subject. Levels were below 5 mg/l in all samples, except for one subject who had raised levels of 8 and 7 mg/l in the first and last sample, respectively. These values were only slightly above the normal C-reactive protein level of <6 mg/l and did not appear to be associated with any adverse effect on the results for that subject.

The effect of Cu supplementation on risk markers for CVD is shown in Tables 2 and 3. There was no significant effect of any Cu supplementation treatment on the total plasma concentrations of cholesterol or triacylglycerol, including the LDL, HDL and VLDL fractions of both these lipid components (Table 2).

Table 1. Putative indices of Cu status in healthy female subjects given 0 (placebo), 3 and 6 mg Cu/d, each for 4 weeks

	Placebo		3 mg Cu		6 mg Cu	
	Mean	SD	Mean	SD	Mean	SD
Serum Cu (μmol/l) (*n* 16)	14·7	3·9	16·7*	6·2	17·6*	6·9
Erythrocyte superoxide dismutase (U/g Hb) (*n* 15)	742	144	825*	236	816*	176
Leucocyte superoxide dismutase (U/g protein) (*n* 14)	1·62	0·4	1·81	0·9	1·91	1·3
Serum caeruloplasmin activity (U/l) (*n* 15)	586	195	677	298	623	215
Cytochrome C oxidase activity (U/g protein) (*n* 14)	3·32	1·5	3·15	1·9	3·69	1·4

Mean value was significantly different from placebo group, * $P<0{\cdot}05$.

Table 2. Plasma lipid, lipoprotein (a) and apo concentrations in female subjects (*n* 16) given 0 (placebo), 3 and 6 mg Cu/d, each for 4 weeks

	Placebo		3 mg Cu		6 mg Cu	
	Mean	SD	Mean	SD	Mean	SD
Total cholesterol (mmol/l)	4·5	0·9	4·5	0·9	4·4	0·7
VLDL-cholesterol	0·24	0·18	0·23	0·14	0·31	0·2
LDL-cholesterol	2·82	0·8	2·80	0·8	2·77	0·6
HDL-cholesterol	1·43	0·3	1·47	0·3	1·35	0·3
Total triacylglycerol (mmol/l)	0·96	0·47	0·95	0·38	1·06	0·51
VLDL-triacylglycerol	0·58	0·40	0·54	0·33	0·67	0·45
LDL- + HDL-triacylglycerol	0·39	0·11	0·41	0·11	0·39	0·11
Lipoprotein(a) (g/l)	0·20	0·37	0·16	0·26	0·19	0·31
Apo A-1 (g/l)	1·7	0·2	1·8	0·4	1·7	0·2
Apo B (g/l)	1·04	0·3	0·99	0·29	0·98	0·26

The content of vitamin E in the LDL fraction was similarly unaltered by Cu intake (with values of 2·4 (SD 1·1) μg/ml, 2·2 (SD 1·2) μg/ml and 2·2 (SD 0·8) μg/ml for supplementation with 0, 3 and 6 mg Cu/d, respectively).

There was no significant effect of any Cu supplementation regime on the plasma concentrations of lipoprotein (a), apo A-1 and apo B (Table 2).

The effect of Cu supplementation on a number of haemostatic factors associated with thrombosis was also investigated in the present study (Table 3). The concentrations of coagulation factors fibrinogen and factor VIIc were unaffected by increased Cu intake. In contrast, there was a significant decrease ($P=0·04$) in the concentration of the fibrinolytic factor PAI-1 of about 30 % after supplementation with 6 mg Cu/d compared with placebo. There was a similar decrease after an intake of 3 mg Cu/d (about 15 %), although this was not significant ($P>0·05$). The decrease in PAI-1 was accompanied by a simultaneous non-significant increase in the measured activity of t-PA.

There was no effect of Cu supplementation on the concentration of the inflammatory cytokine TGF-β in the present study (Table 3). The activity of platelet factor 4 and β-thromboglobulin was also unaffected by Cu intake. The standard deviations for platelet factor 4 were particularly high and were probably caused by artefacts introduced during phlebotomy.

Discussion

The improved Cu status observed after Cu supplementation in the present study, illustrated by significantly increased serum Cu and erythrocyte SOD activity and a tendency towards increased Cp and WBC SOD activity, indicates that these women may have been marginally Cu deficient. Studies of short-term Cu deprivation have shown variable results with regard to putative markers of Cu status. Plasma Cu and Cp are relatively insensitive indicators (Milne & Nielsen, 1996). Several studies have been unable to demonstrate significant changes in plasma Cu concentrations with low Cu intakes for varying periods (Reiser *et al.* 1985; Milne *et al.* 1988; Milne & Nielsen, 1996). Other studies have seen no increase in serum Cu after Cu supplementation (Salmenpera *et al.* 1989; Medeiros *et al.* 1991*b*). Previous studies have also shown variable results for Cp (Milne *et al.* 1988; Milne, 1998; Kehoe *et al.* 2000).

The Cu-dependent enzymes such as erythrocyte SOD and CCO are generally considered to be more reliable indicators of Cu status. Some studies have shown changes in erythrocyte SOD or CCO (erythrocyte, WBC or platelet) during Cu deprivation or supplementation, in the absence of changes in circulating Cu or Cp (Reiser *et al.* 1985; Milne *et al.* 1988; Milne & Nielsen, 1996). The increase in erythrocyte SOD observed in the study after supplementation with 3 or 6 mg Cu/d might therefore be a more reliable indicator that the subjects had a suboptimal Cu status. Studies of Cu deprivation resulting in a reduced activity of erythrocyte SOD have demonstrated that activity can be restored by the subsequent intake of at least 3 mg Cu/d (Klevay *et al.* 1984; Reiser *et al.* 1985; Milne *et al.* 1990), but not by amounts of $<2·7$ mg Cu/d (Milne *et al.* 1988; Milne & Nielsen, 1996). This is consistent with the present results. However, other short-term studies of Cu supplementation for up to 8 weeks in both men and women with the same intakes (3 or 6 mg Cu/d) have shown no effect on erythrocyte SOD activity

Table 3. Haemostatic factors measured in healthy female subjects (*n* 16) given 0 (placebo), 3 and 6 mg Cu/d, each for 4 weeks

	Placebo		3 mg Cu		6 mg Cu	
	Mean	SD	Mean	SD	Mean	SD
Fibrinogen (g/l)	3·0	1·4	2·7	1·0	2·9	1·1
Factor VIIc (IU/ml)	0·97	0·24	0·96	0·20	0·93	0·18
Tissue plasminogen activator (IU/ml)	0·65	0·4	0·68	0·4	0·73	0·3
Plasminogen activator inhibitor-1 (ng/ml)	7·9	4·5	6·7	2·8	5·5*	3·2
Transforming growth factor-β (ng)	65·5	24·5	65·7	26·8	61·4	25·2
Platelet factor 4 (IU/ml)	7·0	12·4	7·9	8·6	6·0	4·5
β-Thromboglobulin (IU/ml)	29	15	21	7·4	31	17

Mean value was significantly different from placebo group, * $P<0·05$.

(Baker *et al.* 1999; Kehoe *et al.* 2000; Rock *et al.* 2000; Harvey *et al.* 2003), whereas a recent study of long-term Cu supplementation showed increases in some indices of Cu status, including SOD, after high (7 mg/d) Cu intake for 5 months (Turnlund *et al.* 2004). We consider it most likely that the habitual diet of these young Danish women was inadequate compared with other groups with regard to Cu. This is supported by our overall findings of both increased erythrocyte SOD and serum Cu and non-significant increases in Cp and WBC SOD after supplementation. In addition, SOD activity in the placebo periods of the other short-term studies (Baker *et al.* 1999; Kehoe *et al.* 2000; Rock *et al.* 2000) was always higher than the placebo SOD activity in the present study, whereas SOD activity after 5 months' supplementation (Turnlund *et al.* 2004) increased well above the levels obtained in the current study. No published data are available regarding dietary Cu intake in Danish women. A study of young Danish men reported Cu intake to be 1·2 (SD 0·6) mg Cu/10 MJ (Bro *et al.* 1990), and intakes for women appear to be less than for men (Johnson *et al.* 1992).

Increases in serum cholesterol concentrations are a consistent consequence of Cu deficiency in animal studies (Klevay, 1975; Allen & Klevay, 1978; Lau & Klevay, 1981). Changes in cholesterol concentration during short-term studies of Cu deprivation in human subjects have been much less consistent. Only a few studies have reported increased total cholesterol with Cu depletion (Klevay *et al.* 1984; Reiser *et al.* 1987; Nielsen *et al.* 1990), whereas cholesterol concentrations in other studies were unchanged (Milne *et al.* 1988; Turnlund *et al.* 1990; Milne & Nielsen, 1996). In the present study, we found no significant changes in total cholesterol or triacylglycerol, including lipoprotein fractions, or in lipoprotein (a), apo A-1 and apo B. This was in agreement with other studies that found no effect on certain CVD risk factors in men after supplementation with between 0·7 and 6 mg Cu/d (Jones *et al.* 1997; Harvey *et al.* 2003). It is possible that effects are seen only during more severe Cu deprivation, over a longer supplementation period or in subjects already at risk.

Impairment of the haemostatic system is also associated with thrombosis and atherosclerosis, and there is some evidence that Cu deprivation may promote these events (Lynch & Klevay, 1993; Klevay, 1998). Cu is known to have a role in blood clotting through factors V and VIII, both of which increase during Cu depletion in human subjects (Milne & Nielsen, 1996). We found no effect of Cu supplementation on any haemostatic factor except PAI-1, which was significantly decreased after supplementation with 6 mg Cu/d. PAI-1 helps to regulate thrombogenesis via an inhibition of fibrinolysis, and high PAI-1 activity has been shown to be a risk factor for atherosclerosis and thrombosis (Aznar & Estelle, 1994). An effect of Cu could be mediated through activated protein C, as activated protein C decreases the level of PAI-1 and the Cu-binding protein Cp appears to play an important role in the regulation of this anticoagulant protein. Cp inhibits the activated protein C-catalysed inactivation of coagulation factors Va and VIII (Walker & Fay, 1990). It is thus possible that if activated protein C activity is reduced during Cu deficiency, via binding of Cp, a variety of atherogenic factors could be increased.

In conclusion, we found that the young female volunteers who participated in this study appeared to be marginally Cu deficient when consuming their habitual diet. Both SOD activity and serum Cu concentration increased after supplementation with 3 and 6 mg Cu/d for 28 d. It would be useful to determine the Cu content of the habitual diet for these women, since it could be representative of an inadequate intake in other (European) populations. Such information is especially relevant in view of the recent establishment of a RDA for Cu (Food and Nutrition Board, 2001). The only risk marker for CVD that was affected by Cu supplementation was PAI-1. PAI-1 concentration decreased after Cu supplementation, but all risk factors measured were within normal ranges for each group. Future studies should perhaps focus on high-risk groups. The effect of Cu supplementation on the activity of PAI-1 may warrant further investigation.

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