

Newer aspects of micronutrients in chronic disease: copper

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The proliferation of citations on Cu appearing on computer databases such as MEDLINE 'Dialog Service' indicate the increasing interest in the metabolism of this micronutrient in chronic disease processes. A review based on a presentation given at a Nutrition Society meeting cannot hope to cover all aspects of the involvement of Cu in chronic disease. One pathological process, however, associated with a number of chronic diseases, is oxidative damage (Strain *et al.* 1991). The current review attempts to define the role of Cu in oxidative damage and then indicate how these roles might relate to mechanisms involved in ischaemic heart disease (IHD), the chronic disease which is the major cause of premature death in most Western societies. At the same time it should also become obvious that the versatility of Cu in metabolism gives this micronutrient potential roles which extend beyond those associated with pro-oxidant or antioxidant properties.

COPPER AS A PRO-OXIDANT

The most important reactants in free-radical biochemistry in aerobic organisms are reduced derivatives of oxygen (Cheeseman & Slater, 1993) categorized as reactive oxygen species (ROS). ROS are toxic as they can oxidize biomolecules leading to cell death and tissue injury (Fig. 1). Of these the hydroxyl radical is the most reactive and is generally the ROS favoured as the initiator of free-radical-mediated chain reactions involving biomolecules. Although all the major classes of biomolecules can be attacked by ROS, lipids, especially polyunsaturated fatty acids in cell membranes, are probably the most susceptible to initiation and propagation of chain reactions. The breakdown of lipid hydroperoxides formed in these chain reactions to yield lipid peroxy and alkoxy radicals and other toxic products can be catalysed by transition (Fe^{2+} and Cu^+) metal ions. Moreover, these transition metal ions are important catalysts for the initial generation of the hydroxyl radical in *in vitro* systems. There is currently much debate, however, on the ROS that initiate lipid peroxidation and the precise involvement of transition metals in these systems (Halliwell & Chirico, 1993).

The participation of transition metal ions in oxidative modification of low-density lipoprotein (LDL), suggested to play a causal role in atherosclerosis (Steinberg *et al.* 1989), has received much interest. There is a strict requirement for transition metal ions before LDL oxidation can occur *in vitro* and Cu^{2+} has been recognized as the most effective catalyst (Esterbauer *et al.* 1992). Fe salts can also oxidatively modify LDL but they require a source of superoxide to reduce Fe^{3+} to Fe^{2+} , whereas Cu^{2+} -mediated oxidation of LDL can occur without superoxide production and is also independent of H_2O_2 and aqueous hydroxyl radical (Lynch & Frei, 1993). Adding CuSO_4 to cell culture media or phosphate-buffered saline (9 g NaCl/l) is the usual method for preparing oxidized LDL for biological experiments in cell-free systems (Esterbauer *et al.* 1992).

Experiments *in vitro*, therefore, seem to indicate that Cu is a pro-oxidant and is involved in atherogenesis. These observations appear to be supported by epidemi-

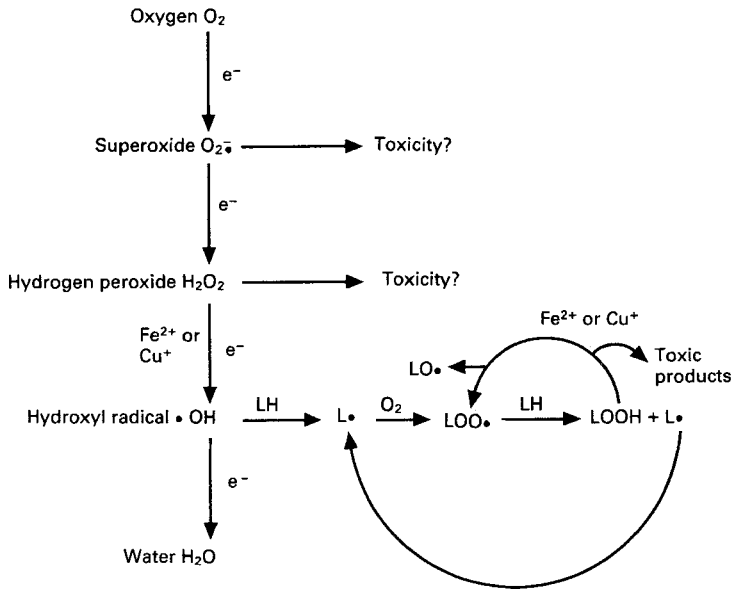


Fig. 1. Major reactive oxygen species in biological systems. LH, lipid; $L\bullet$, lipid radical, $LOO\bullet$ lipid peroxyl radical; $LO\bullet$ lipid alkoxyl radical; $LOOH$, lipid hydroperoxides.

ological data which have found increased serum Cu (Kok *et al.* 1988) or increased caeruloplasmin (*EC* 1.16.3.1), the major Cu-containing protein in serum (Reunanen *et al.* 1992), to be associated with increased risk of IHD in case-control studies. Serum Cu, also, has been identified as a risk factor for IHD in a Finnish prospective study (Salonen *et al.* 1991a). The latter workers have concluded that high Cu status, as measured by elevated serum Cu concentration is an independent risk factor for IHD (Salonen *et al.* 1991a) and that there is evidence of a synergistic effect of Cu (a pro-oxidant), a low serum concentration of Se (a cofactor of an enzyme that scavenges free radicals) and LDL concentrations in atherogenesis (Salonen *et al.* 1991b).

A major defect in the interpretation of these studies arises in the assumption that serum Cu is indicative of high nutritional Cu status. In human populations, particularly ageing populations, serum Cu may reflect neither Cu intake nor organ Cu (Klevay, 1992; Arnaud, 1994; Milne & Nielsen, 1994). This is because caeruloplasmin, which accounts for probably 95% or more of serum Cu, is an acute-phase protein which rises sharply in inflammatory conditions. For example, DiSilvestro (1990) has shown that marginally-Cu-deficient rats can respond to an inflammatory (turpentine) challenge by increasing caeruloplasmin activities (Cu is required for this enzymic activity) to levels greater than in healthy Cu-sufficient rats. Caeruloplasmin also acts as an antioxidant by oxidizing Fe^{2+} to Fe^{3+} (ferroxidase (*EC* 1.16.3.1) activity) without formation of ROS, by inhibiting lipid peroxidation and deoxyribose degradation stimulated by Fe or Cu ions, by slowly scavenging H_2O_2 , superoxide and hydroxyl radical and by inhibiting carbonyl formation during inflammation (Krssek-Staples & Webster, 1993).

COPPER AS AN ANTIOXIDANT

The antioxidant nature of caeruloplasmin is in keeping with the antioxidant and anti-inflammatory effects of other Cu complexes *in vivo* (Sorenson, 1989) but is in sharp contrast to the pro-oxidant effects of Cu ions found in the test-tube. Perhaps it is not surprising that the effects of Cu are so different *in vitro* and *in vivo*, as the great bulk of Cu in the body is bound to storage and transport proteins in forms which are unable to act as pro-oxidants. Moreover, there is now accumulating evidence that adequate Cu is required to maintain antioxidant defences within the body, and Cu deficiency in animals can have pro-oxidant effects (for review, see Johnson *et al.* 1992). Some observations which suggest increased susceptibility to oxidant stress *in vivo* during Cu deficiency include: (a) increased rat mortality after hyperoxia (Jenkinson *et al.* 1984), (b) enhanced breath ethane of rats after intraperitoneal injection of carbon tetrachloride (Lawrence & Jenkinson, 1987), (c) decreased body weight of mice after ozone treatment (Dubick *et al.* 1988), (d) increased breath ethane of rats in air and hyperoxia (Saari *et al.* 1990), (e) increased rat hepatic plasma membrane injury after intraperitoneal injection of carbon tetrachloride (DiSilvestro & Medeiros, 1992), (f) exacerbated rat cardiomyopathy with focal collagen accumulation after aerobic exercise training (Davidson *et al.* 1993), (g) exaggerated respiratory membrane defects in rat lung after hyperbaric hyperoxia (Akers & Saari, 1993). These studies are complemented by others where antioxidant supplementation during Cu deficiency in rats decreased putative measures of oxidant stress and include: (a) t-butylhydroquinone which ameliorated growth retardation, anaemia and cardiac hypertrophy (Johnson & Saari, 1989), (b) dimethyl sulphoxide which inhibited cardiovascular, but not renal, changes (Saari *et al.* 1990), (c) vitamin E which decreased hepatic lipid peroxidation but not cardiac lipid peroxidation or mortality (Silverman *et al.* 1990), (d) coenzyme Q₁₀ which decreased mortality and maintained the integrity of the heart but did not affect anaemia or cardiac enlargement (Lewis *et al.* 1993). Oxidant stress has also been demonstrated in cells and cellular fractions during Cu deficiency *in vitro* (for review, see Johnson *et al.* 1992).

Taken together these studies strongly suggest increased oxidant stress during Cu deficiency. Apart from caeruloplasmin, Cu deficiency is known to affect a number of other enzymes with antioxidant activities (Fig. 2). The alteration in Cu status of animals can result in changes in the activity of specific cuproenzymes, which can affect ROS production such as cytoplasmic, erythrocyte and extracellular CuZn-superoxide dismutase (*EC* 1.15.1.1; CuZn-SOD), which facilitates the disproportionation of superoxide, and cytochrome C oxidase (*EC* 1.9.3.1; CCO), which is involved in energy production via the electron chain. Decreased activity of the latter enzyme might encourage incomplete reduction of oxygen with the resulting enhancement of ROS production. Changes in Cu status can also affect many other enzymes not known to contain Cu (for review, see Prohaska, 1990). Those enzymes with antioxidant properties include Se-dependent glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) and the ferrous-enzyme, catalase (*EC* 1.11.1.6). Furthermore, Cu deficiency results in lower hepatic mRNA concentrations of GSH-Px (Prohaska *et al.* 1992), and catalase (Lai *et al.* 1994a). These results suggest that Cu status has a regulatory effect on these enzymes at the molecular level affecting gene expression.

Cu deficiency may also affect Se metabolism independently of the antioxidant defence system, as the other known selenoenzyme, Type I iodothyronine-5-deiodinase (*EC* 3.8.1.4), is also lowered (Olin *et al.* 1994). The effects of Cu deficiency on the regulation

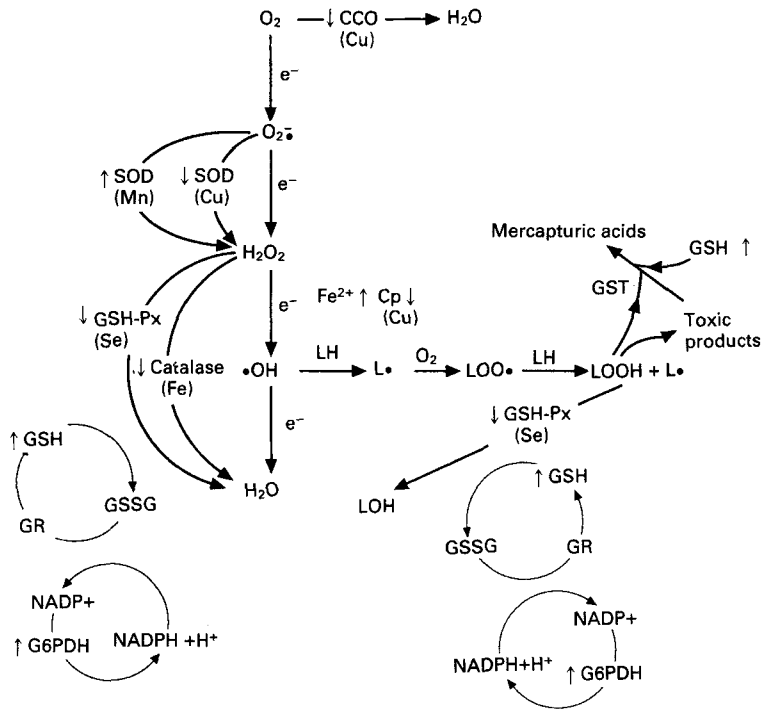


Fig. 2. Oxidant stress in copper deficiency. CCO, cytochrome C oxidase (*EC* 1.9.3.1); SOD, Mn- or CuZn-superoxide dismutase (*EC* 1.15.1.1); GSH-Px, glutathione peroxidase (*EC* 1.11.1.9); catalase (*EC* 1.11.1.6); GSH, reduced glutathione; GSSG, oxidized glutathione; GR, glutathione reductase (*EC* 1.6.4.2); GST, glutathione-S-transferase (*EC* 2.5.1.18); G6PDH, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) Cp, caeruloplasmin (*EC* 1.16.3.1); LH, lipid; $L\cdot$, lipid radical, $LOO\cdot$, lipid peroxyl radical, $LO\cdot$, lipid alkoxy radical; $LOOH$, lipid hydroperoxides; \uparrow , increased \downarrow , decreased activity or level.

of these enzymes are tissue specific with the brain generally being unaffected. Similarly, recent work indicates that CuZn-SOD activity, enzyme content and mRNA levels in the liver, and the activity and enzyme protein content of CuZn-SOD in the heart were decreased; in the brain dietary Cu deficiency had no effect (Lai *et al.* 1994b). In some studies (Lynch & Strain, 1989) but not others (Lynch & Strain, 1990) there is some compensatory increase in mitochondrial Mn-SOD (*EC* 1.15.1.1). Lai *et al.* (1994b) found that Mn-SOD activity, protein and mRNA levels increased in parallel (but were not fully compensatory) with decreases in CuZn-SOD in liver and heart. It appears, therefore, that the major antioxidant enzymes are, under conditions of oxidant stress imposed by dietary Cu deprivation, regulated at the molecular level and that different mechanisms (transcriptional, post-transcriptional or post-translational) may be involved in their regulation. Furthermore, apart from Mn-SOD, adaptations by inducement of the activities of these enzymes, which normally might occur under other conditions of oxidant stress, are prevented and/or repressed when there is an underlying dietary Cu deficiency.

Changes in the activities of other antioxidant enzymes during Cu deficiency are inconsistent. For example, glutathione-S-transferase (*EC* 2.5.1.18; GST) activities have been found to be increased (Lynch & Strain, 1990), decreased (Prohaska, 1991) or

unchanged (Arthur *et al.* 1987) in Cu deficiency under different dietary or physiological conditions. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PDH) activity is generally found to be increased in the tissues of Cu-deficient rats in our laboratory (Lynch & Strain, 1989) and this may be an adaptive response since the enzyme is known to be subject to tissue-specific regulation by oxidant stress (Kletzien *et al.* 1994). G6PDH is the rate-determining enzyme in the pentose-phosphate pathway, regenerating NADPH from NADP for use by glutathione reductase (EC 1.6.4.2) in reducing the oxidized (GSSG) form of glutathione to the reduced (GSH) form (White *et al.* 1994). Marked increases in hepatic GSH have also been found in Cu deficiency (Allen *et al.* 1988; Chao & Allen, 1992), most probably as another adaptive response to increased oxidant stress.

COPPER AND ATHEROSCLEROSIS

Adaptive responses to the increased oxidant stress of Cu deficiency may help to explain the occurrence of the associated hypercholesterolaemia, first reported by Klevay (1973) over 20 years ago. Yount *et al.* (1990) found that Cu deficiency increases the activity of the rate-controlling enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCoA reductase, EC 1.1.1.34). This enzyme is subject to multiple regulatory mechanisms (Fig. 3). One such regulatory control is by thiol-disulphide exchange (Cappel & Gilbert, 1988). GSH is the principal cellular reductant for protein sulphhydryls and increases in GSH can activate rat liver microsomal HMGCoA reductase by positive co-operativity in NADPH binding (Roitelman & Shechter, 1981). Kim *et al.* (1992) prevented the rise in GSH in Cu-deficient rats by administering L-buthionine sulphoxide (BSO), a specific inhibitor of γ -glutamyl-cysteine synthetase (EC 6.3.2.2); this enzyme, which catalyses the production of the dipeptide γ -glutamyl-cysteine from glutamate and cysteine is rate-limiting for GSH synthesis. BSO was found to prevent the hypercholesterolaemia in the Cu-deficient rats, suggesting that the increased GSH, probably resulting from increased oxidant stress, is responsible for the hypercholesterolaemia. Results also suggested that elevated hepatic GSH in Cu deficiency also either increased expression and/or decreased degradation of HMGCoA reductase, as dithiothreitol (DTT, a sulphhydryl reductant) increased the activity of the enzyme ninefold in the Cu-deficient rats without BSO but only sixfold when BSO had been consumed. Interestingly G6PDH activity, another possible candidate for regulation by the GSH:GSSG value for hepatic tissue (Zeigler, 1985), is also increased in Cu deficiency (Lynch & Strain, 1989).

Cu deficiency not only increases serum cholesterol but also increases the susceptibility of very-low-density lipoprotein (VLDL) and LDL to *in vitro* Cu-induced oxidation (Rayssiguier *et al.* 1993). These workers also found increased thiobarbituric acid-reactive substances in heart and liver tissues from Cu-deficient rats while Nelson *et al.* (1992) found increased aortic lipid peroxides (malondialdehyde equivalents) in rats fed on Cu-marginal and Cu-deficient diets, the latter increase being associated with depressed aortic CuZn-SOD activity.

The contribution of hypercholesterolaemia and lipoprotein oxidizability to atherosclerotic-like changes in Cu-deficient animals is controversial. Although arterial foam cells have been found in pigs deficient in Cu (Coulson & Carnes, 1962), arterial disease in animals and in humans with the Cu-deficiency genetic disorder, Menkes' disease, is

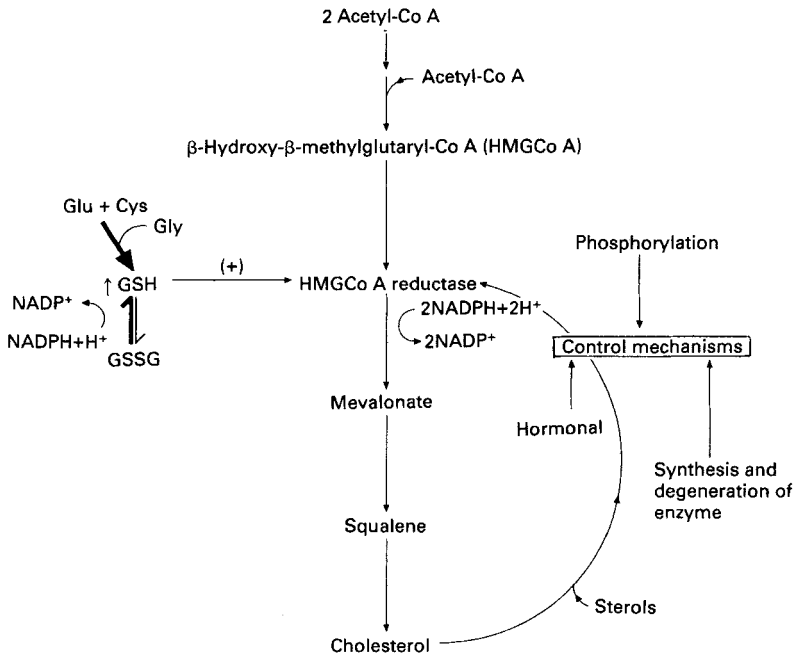


Fig. 3. Copper deficiency-induced hypercholesterolaemia. Glu, glutamate; Cys, cysteine; GSH, reduced glutathione; GSSG, oxidized glutathione; HMGCoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*EC* 1.1.1.34); ↑ increased level; (+), activation.

largely confined to the major arteries rather than the coronary arteries. In Menkes' disease the arterial abnormalities are elongation and dilation of major arteries, leading to rupture and haemorrhage and subintimal thickening with partial occlusion of major arteries (for review, see Danks, 1988). Changes in arterial walls in Menkes' disease, and in Cu deficiency in animals, principally involve large increases in soluble elastin and cross-linked mature fibrils (O'Dell, 1981), but the other two classes of connective tissue, collagen and ground substance, are also adversely affected by Cu deficiency in several species of animals (for review, see Klevay, 1990a). Klevay (1993) has recently reviewed the anatomical similarities between animals deficient in Cu and people with IHD and noted among the similarities: elastin degeneration, fibrosis, intramural haemorrhage, mucopolysaccharide increase, necrosis and smooth muscle proliferation. There are species differences in susceptibility to arterial disease in Cu deficiency, with pigs and poultry being particularly susceptible to aortic rupture and aneurysm while Cu deficiency in rats leads primarily to cardiomyopathy (Petering *et al.* 1986).

Abnormalities in the major arteries of animals with Cu deficiency are undoubtedly linked to lowered functional activity of the Cu-containing enzyme, lysyl oxidase (*EC* 1.4.3.14) which oxidatively deaminates lysine or hydroxylysine residues in the biosynthesis of cross-links in collagen and elastin (Reiser *et al.* 1992). Nevertheless, the loss of structural integrity together with decreases in antioxidant enzyme activities, may also lead to increased oxidant stress in aortic tissue. There are few findings linking the oxidant

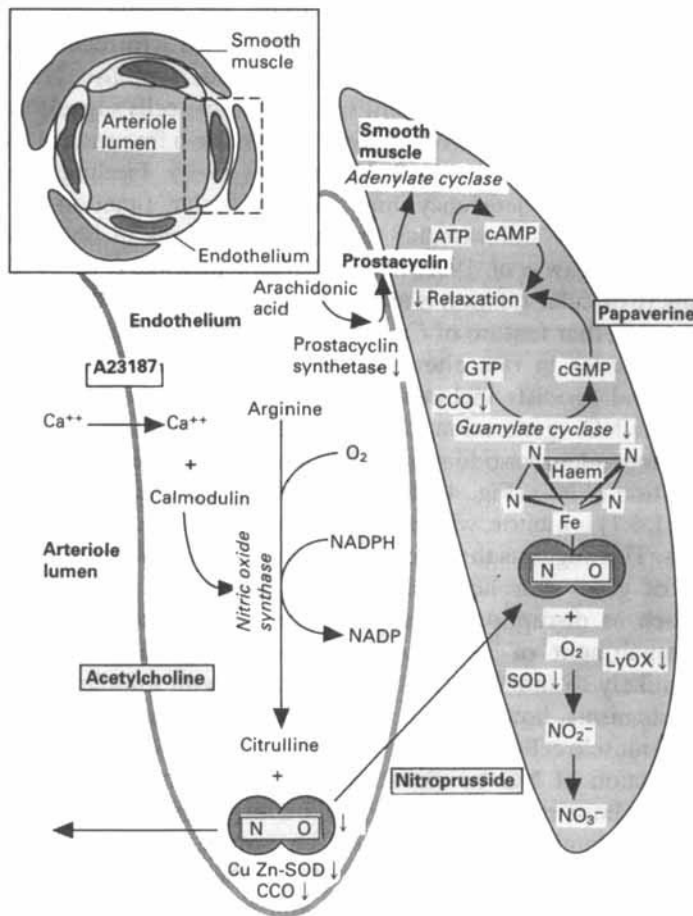


Fig. 4. Nitric oxide (NO) and vasodilation in copper deficiency. CCO, cytochrome C oxidase (EC 1.9.3.1); SOD, CuZn-superoxide dismutase (EC 1.15.1.1); LyOX, lysyl oxidase (EC 1.4.3.14); cAMP, cyclic AMP; cGMP, cyclic GMP; A23187, a calcium ionophore; ↑, increased ↓, decreased activity or level (adapted from Anonymous, 1992).

stress, arising from low Cu status, with atherosclerosis in humans, but Dubick *et al.* (1987) found lower activity of CuZn-SOD in both aneurysmal and occluded aortic tissue from humans. Moreover, patients with chronic arterial disease had significantly lower erythrocyte SOD compared with healthy persons (Belch *et al.* 1989) and Kinsman *et al.* (1990) found a significant correlation between Cu in leucocytes and the degree of patency of the coronary arteries of non-smoking, non-hypercholesterolaemic men.

COPPER AND ARTERIAL COMPLIANCE

Changes in endothelial function occur early in vascular disease and can result in abnormal vasoconstriction, enhanced interactions of blood cells with the vessel wall,

activation of coagulation mechanisms, and migration and proliferation of vascular smooth muscle cells (for review, see Lüscher, 1994). Fig. 4 highlights the involvement of one important endothelium-derived mediator, NO, which is formed from arginine by the action of a constitutive form of the enzyme, nitric oxide synthase. NO is a gas which is released into the lumen and into adjacent smooth muscle cells suggesting that it protects against both thrombosis and vasoconstriction. NO is also a free radical and can be swiftly inactivated by superoxide (Rubanyi & Vanhoutte, 1986). Findings from a number of studies suggest that Cu deficiency may alter vascular function by modifying the interaction between NO and vascular smooth muscle cells in both the aorta and the microvasculature (Megaw *et al.* 1992; Saari, 1992a; Schuschke *et al.* 1992). The observed reduction in microvascular dilation is also of interest because of the possible relationship to hypertension, another feature of Cu deficiency (Klevay, 1987).

Mild Cu deficiencies in rats alter relaxation of the aorta and microvasculature in response to several agonists including acetylcholine and the Ca ionophore, A23187, which stimulate NO generation from arginine, and sodium nitroprusside, an endothelium-independent vasodilator that is thought to cause smooth muscle relaxation by direct formation of NO (Fig. 4). Response to papaverine, a non-specific phosphodiesterase (*EC* 3.1.4.1) inhibitor, which prevents the hydrolysis of cGMP, was not affected by low-Cu diets. This suggests that the vasodilatory capacity was not altered and together with the lack of gross morphological changes (Schuschke *et al.* 1992) suggests that mechanisms such as disruption of collagen and elastin integrity (via decreased lysyl oxidase) or impairment of mitochondrial energy production (via decreased CCO activity) are unlikely to influence the function of smooth muscle or endothelium. The most likely mechanisms, however, for the altered interactions in Cu deficiency between vascular smooth muscle cells and NO in large blood vessels and the microvasculature are enhanced destruction of NO by elevated superoxide (via decreased CuZn-SOD and CCO activities) or impaired interaction of NO with guanylate cyclase (*EC* 4.6.1.2; lung guanylate cyclase contains Cu and a haem-Fe group; Gerzer *et al.* 1981) leading to decreased cGMP for smooth muscle relaxation. Hypercholesterolaemia, which is known to affect endothelium-dependent relaxation and is associated with Cu deficiency, or decreased prostacyclin release, may also be involved. With respect to the latter, Nelson *et al.* (1992) found that there was decreased prostacyclin production by aortic rings from Cu-deficient rats. Nevertheless, depressed endothelium-independent dilations suggested that there was some impairment of NO mediation.

COPPER AND THROMBOSIS

Continued synthesis of NO in vascular endothelium is required to maintain arterial blood vessels in an active state of vasodilation, but release of NO to the luminal surface also provides lubrication of the vascular wall and prevents platelets and leucocytes from adhering to the surface. Therefore, in both vasodilation and in providing a defence layer against the aggregation of activated platelets, NO may complement the actions of prostacyclin. If there is increased scavenging of NO by superoxide, a distinct possibility in Cu-deficient animals, then increased thrombotic activity might ensue. Also any impairment of prostacyclin production by arterial endothelium would give similar results. The observation by Nelson *et al.* (1992), that prostacyclin production by aortic rings taken from Cu-deficient rats is lower than those from control animals, is

particularly pertinent. Furthermore, decreases in aortic prostacyclin production in Cu-deficient rats were associated with CuZn-SOD depression and increases in aortic lipid peroxides. These workers suggested that increased levels of hydroperoxides were responsible for inhibiting prostaglandin H synthase (EC 1.14.99.1) the initial and rate-controlling step in the synthesis of prostaglandins and prostacyclin synthetase (EC 5.3.99.4) which converts prostaglandin H to prostacyclin. Both enzymes (Lands *et al.* 1984), but especially prostacyclin synthetase (Moncada *et al.* 1976), are inhibited by lipid hydroperoxides. The mechanism of increased oxidant stress, therefore, may explain decreased prostacyclin production in Cu deficiency and also in vitamin E (Okuma *et al.* 1980) and Se (Schoene *et al.* 1986) deficiencies.

Interference by ROS with NO and/or prostacyclin inhibition of platelet aggregation could explain the massive occlusive atrial thromboses observed by Klevay (1985) in Cu-deficient mice. Yet links between Cu status and thrombosis extend into coagulation pathways and are additional to effects postulated to arise from disturbances in oxidant-antioxidant balance. After platelet aggregation, the blood coagulation pathways encourage fusion of platelets and reinforce the stability of the evolving platelet plug. Platelets have procoagulant properties which can be classified into two groups: those which have collagen-induced coagulant activity and, thereby, activate the intrinsic pathway of blood coagulation, and those involving the formation of complexes between coagulation factors and platelet phospholipids. The two points in the cascade at which complexes between coagulation factors and platelet phospholipids occur involve participation of the factors VIII and V. Paradoxically these same clotting factors contain Cu, and Cu-deficient mice were found by Lynch & Klevay (1992) to have lower factor V and VIII activities than those fed on a Cu-supplemented diet. These workers concluded that the haemostatic defect responsible for the observed thrombosis in Cu-deficiency was not in the coagulation system. This seems a reasonable assumption as the bleeding disorders, haemophilia and van Willebrand's disease, are either caused by, or associated with, low factor VIII levels.

A further study by Lynch & Klevay (1993) demonstrated a defect in the fibrinolytic system with a highly significant prolongation of euglobulin clot lysis time in Cu-deficient mice. They also reported no significant differences in two important plasma coagulation inhibitors, anti-thrombin III and protein C, although there was a trend ($P=0.08$) towards lower plasma protein C activity in both male and female Cu-deficient mice. Protein C is a key component in the regulation of haemostasis. It circulates in plasma as an inactive precursor but, after contact with thrombin that is bound to the thrombomodulin receptor on vascular endothelial cells, it is rapidly converted to activated protein C (APC). Once generated, APC acts as an anticoagulant by inactivating the two activated Cu-dependent cofactors of the coagulation cascade, factors VIIIa and Va; therefore, a lowering of APC might be regarded as a homeostatic response which enables the Cu-deficient animal to maintain a functional coagulation system. APC also plays an important role in the fibrinolytic pathway by decreasing the level of fast-acting plasminogen activator inhibitor-1 (PAI-1), thus effectively promoting fibrinolysis. APC resistance or decreased levels of APC would be expected to promote thrombosis and inhibit fibrinolysis. Recent findings suggest that APC resistance may occur in one-third (Dahlbäck *et al.* 1993) to over half (Griffin *et al.* 1993) the patients presenting with venous thrombosis and Bauer (1994) has indicated that revisions in current recommendations for screening for hereditary thrombotic disorders (as well as those prone to arterial thrombosis) may be

necessary. Interestingly Dahlbäck & Hildebrand (1994) have identified defects in factor V as the one responsible for hereditary APC resistance. Although factor V (when activated) promotes thrombosis by accelerating the activation of prothrombin to thrombin, it may also be required for anticoagulant (and fibrinolytic) activity. This seemingly paradoxical behaviour of a procoagulant having anticoagulant function is also demonstrated by thrombin, which is responsible for fibrin and clot formation yet when bound to thrombomodulin converts protein C to APC. It is tempting to speculate that nutritional influences which decrease functional factor V levels may lead to thrombosis and, furthermore, that elevated levels of factor VII, an important risk factor for IHD, could be an adaptive response (for increasing factor Xa) to low factor VIII levels owing to low Cu status in human populations.

COPPER AND CARDIAC FUNCTION

Feeding low-Cu diets to a number of animal species produces cardiac lesions and hypertrophy and often results in death by haemothorax or aneurysms. The cardiomyopathy of Cu deficiency is associated with concurrent hypertrophy in rats fed on low-Cu diets from weaning for 5–8 weeks while Cu deficiency-induced postweaning can produce cardiomyopathy without any apparent hypertrophy (Davidson *et al.* 1992). There is currently some debate as to the mechanisms which lead to cardiac hypertrophy in Cu deficiency (for review, see Medeiros *et al.* 1993). Hearts from Cu-deficient rats show a predominantly concentric pattern (Medeiros *et al.* 1991a) characterized by increased left ventricular mass:chamber volume, but with normal ventricular function (Strauer, 1979). Concentric cardiac hypertrophy is usually associated with increased systolic blood pressure and/or increased blood viscosity whereas eccentric hypertrophy (inappropriately low mass:chamber volume, resulting in left ventricular dysfunction) is more often observed in pregnancy, renal failure, obesity and anaemia. Jenkins & Medeiros (1993) have shown that hearts in Cu-deficient rats fed on coconut oil with cholesterol have eccentric hypertrophy and ventricular dysfunction in contrast to the predominantly concentric nature of hearts in Cu-deficient animals fed on maize oil. There is also some eccentric pattern usually shown in the enlarged heart of Cu deficiency, but cardiac hypertrophy can occur before anaemia and in the absence of hypertension (Medeiros *et al.* 1991a).

Two components, an enlarged mitochondrial area and myofibrillar enlargement appear to contribute to cardiac hypertrophy in Cu deficiency (Medeiros *et al.* 1991a). There are also additional ultrastructural and morphological changes in the left ventricle associated with Cu deficiency in both pigs (Medeiros *et al.* 1991b; Vadlamudi *et al.* 1993) and rats (Davidson *et al.* 1992). These include increases in type III:type I collagen (leading to greater compliance and elasticity), decreased collagen cross-linking, increased distances between the capillary lumen and myocyte mitochondria, and distortion of the basal laminae underlying the sarcolemma. Possible initiating factors leading to the cardiac hypertrophy and/or cardiomyopathy must include decreased activities of Cu-dependent enzymes such as lysyl oxidase, CCO, CuZn-SOD and dopamine- β -hydroxylase (EC 1.14.17.1). The latter enzyme converts dopamine to noradrenaline and Seidel *et al.* (1991) have proposed that changes in the concentrations of noradrenaline and dopamine may contribute to the development of cardiac hypertrophy. Morphological observations indicate that dietary Cu deficiency results in structural

damage to cardiac mitochondria, which is associated with decreased mitochondrial respiration consistent with decreased CCO activity, but there is no difference in phosphorylation (amount of ADP phosphorylated per unit oxygen consumed) ability (Bode *et al.* 1992).

Undoubtedly increased oxidative stress must also be considered as a contributing factor in the cardiomyopathy. Decreased CuZn-SOD and caeruloplasmin may lower tissue antioxidant protection while increased leakage of ROS from the mitochondrial electron transport chain could be associated with decreased CCO activity. Dietary Cu deficiency reduces cardiac heart shock protein expression and this may decrease protection against oxidative or other stresses and contribute to the altered mitochondrial structure and function and the overall impairment of cardiac function known to accompany Cu deficiency (Matz *et al.* 1994).

It has been argued, with some justification, that the cardiac abnormalities seen in various animal models have more in common with the inherited cardiomyopathy, associated with CCO deficiency, in humans rather than IHD. Nevertheless there are many anatomical and electrophysiological similarities between animals deficient in Cu and humans with IHD (for review, see Klevay, 1993). Moreover electrocardiogram abnormalities have been reported in a male volunteer after 15 weeks on a diet supplying 0.8 mg Cu/d (Klevay *et al.* 1984), while the study of Reiser *et al.* (1985) on twenty-four men with Cu intakes of 1 mg/d was terminated at 11 weeks after one man developed myocardial infarction (4 weeks), another tachycardia (7 weeks) and a third type II heart block (11 weeks). More recently three of twelve postmenopausal women, aged 49–75 years, on a low Cu (0.6 mg Cu/8.4 MJ) diet exhibited changes in electrocardiograms during the 105 d depletion experiment and were supplemented early (Milne & Nielsen, 1994). Turnland (1988), however, found no difference in cardiac function in eleven men after 6 weeks on diets containing 0.8 mg Cu/d. Hypercholesterolaemia, decreased glucose tolerance (historically one of the earliest recognized signs of Cu deficiency in animals), and abnormal blood pressure response to sustained hand grip have also been found in these Cu-depletion experiments with human subjects.

COPPER AND HUMAN NUTRITION

Severe nutritional Cu deficiency has rarely been documented in human populations (Danks, 1988). Most diagnoses of Cu deficiency have been in elderly patients on parenteral nutrition and in young South American children with severe malnutrition and diarrhoea who had been premature babies. Cu deficiency, also, has been described in children undergoing chronic peritoneal dialysis and in patients given oral Zn supplements (for review, see Danks, 1988).

Although severe Cu deficiency in humans is not a public health problem, there is concern by some that a disparity may exist between putative Cu requirements and mean apparent intakes in Western populations, resulting in widespread mild, sub-clinical Cu deficiencies (Klevay, 1990b). Trying to evaluate the extent or consequences of a marginal Cu deficiency in humans, however, is problematic. First, there is currently no adequate way to diagnose mild Cu deficiency in human populations (Arnaud, 1994). Second, measurement of dietary intakes in populations is fraught with difficulty (Livingstone *et al.* 1990). Third, even if it were possible to measure Cu intakes accurately, functional Cu status of individuals will depend on interactions among Cu and a host of other dietary

and lifestyle factors. Fourth, a chronic mild Cu deficiency may have markedly different outcomes from acute deficiency. With respect to the latter, Saari (1992b) has shown that differences in affected variables between chronic marginally Cu-deficient rats and controls may be exaggerated, decreased or even reversed relative to differences between severely-deficient animals and controls. The need to examine balanced interactions within the whole diet rather than simply focusing on the amount of a particular trace element in a diet has been emphasized by Mertz (1993). Some examples of dietary interactions which might increase requirements for Cu in human diets are: sucrose or fructose (Schofield *et al.* 1990; O'Dell, 1993), animal proteins and S amino acids (Brown & Strain, 1990; Lynch & Strain, 1990; Fields *et al.* 1993; Kato *et al.* 1994), Zn and histidine (Wapnir *et al.* 1993). Beer (Klevay & Moore, 1990), a peptide in mushrooms (Shimaoka *et al.* 1993) and possibly other antioxidants may decrease requirements. Interestingly fruit and vegetables, rich sources of some chain-breaking antioxidants, can contribute up to 30% of total Cu intake in some Western populations (Levander, 1990). Particularly rich food sources of Cu include offal, seafood, nuts, seeds, wholegrain cereals and legumes.

SUMMARY

Cu ions are pro-oxidants when added to biological material *in vitro* and excessive levels of Cu in the body, such as in Wilson's Disease (Yarze *et al.* 1992) promote oxidant-related pathologies. In contrast there is now substantial evidence that an optimum level of Cu is required to maintain antioxidant defence and that Cu deficiency in animals increases oxidant stress. There are abundant mechanistic relationships linking Cu deficiency and processes associated with IHD, some of which do not directly involve oxidant damage. These mechanistic relationships, however, have mostly been demonstrated in animal models and more information is urgently required concerning possible chronic mild Cu deficiencies in human populations. A major hurdle to advances in this area is the lack of indices of Cu status which are sensitive enough to detect marginal Cu deficiency in humans. The question, therefore, of whether or not there is a role for mild Cu deficiency in the onset of chronic disease processes, including IHD, remains unanswered.

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