Abstract

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Originally published in the journal Cell Biology and Toxicology, Volume 24, Nos 4–5, 1–40.
DOI: 10.1007/s10565-008-9082-x

Trace elements in human diets, nutrition, and health: essentiality and toxicity

Preface

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The need to advance the field of trace element essentiality and toxicity requires high-quality, evidence-based research. The present supplement represents a compilation of review manuscripts presented at an international joint conference in Hersonissos, Crete-Greece, in October 2007 on the role of trace elements in diets, nutrition, and health in humans. The conference (ISTERH/NTES/HTES '07) constituted the VIIIth Conference of the International Society for Trace Element Research in Humans (ISTERH), the IXth Conference of the Nordic Trace Element Society (NTES), and the VIth Conference of the Hellenic Trace Element Society (HTES). The aim of the conference was to determine the current state of knowledge and gaps in experimental evidence related to the physiologic role and toxicity of trace elements.

Morbidity and mortality related to trace element deficiencies or toxicities affect more than half of the world's population. Etiologies may be related to insufficient food supply, inadequate diet quality, poor bioavailability, and physiological factors including impairments in absorption, digestion, utilization, and excretion, as well as mitigating conditions such as parasites, diseases, and inborn errors of metabolism. It is important that knowledge of trace elements is accessible to researchers, nutritionists, physicians, other health professionals, agricultural providers, and policymakers, so it can be integrated into research, and food, agriculture, and health policies. For example, although the pathogenesis and effects of iron, zinc, iodine, and selenium deficiencies are known, the difficulties in preventing the deficiencies emphasize the need for new approaches. In both transitional and affluent countries, risk of chronic diseases associated with food choices is increasing. Thus, it is imperative to understand the interrelationships of trace elements in foods and diets.

The limited understanding of the essentiality of some trace elements and incomplete knowledge of risks from environmental toxic trace elements is a major problem in trace element nutrition and toxicology. Basic knowledge of chemical mechanisms whereby trace elements affect protein structure, enzyme functions, and receptor and channel functions of membranes is essential for understanding nutritional problems and their prevention. Limited resources impede acquisition and application of new knowledge. This conference facilitated this process through face-to-face meetings of scientists who represented 41 different countries.

The conference and this supplement were sponsored by a variety of institutions, organizations, and industrial partners interested in improving human trace element nutrition and limiting trace element toxicity. The organizers of the conference wish to express appreciation for their support and encouragement in this endeavor.

An invited paper presented as the keynote address as part of ISTERH/NTES/HTES '07

Mediterranean diet, traditional foods and health: critical components and mediating mechanisms

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The traditional Mediterranean diet

The traditional Mediterranean diet is the dietary pattern found in the olive-growing areas of the Mediterranean region in the 1960s. Although different regions in the Mediterranean basin have their own diets, several common characteristics can be identified, most of which stem from the fact that olive oil occupies a central position in all of them. It is therefore legitimate to consider these diets as variants of a single entity, the Mediterranean diet. Olive oil is important not only because of its several beneficial properties but also because it allows the consumption of large quantities of vegetables and legumes in the form of salads and cooked foods. Mediterranean diet is characterized by high consumption of olive oil, vegetables, legumes, fruits, and unrefined cereals; moderate consumption of fish; low consumption of meat; and low to moderate intake of dairy products. It is also characterized by regular but moderate wine intake, mostly during meals, if this is accepted by religion and social norms (Trichopoulou 2007). The Greek variant of the traditional Mediterranean diet is depicted in Fig. 1 and expresses the official Greek nutritional guidelines.

Mediterranean diet and health: epidemiological evidence

The European Prospective Investigation into Cancer and Nutrition (EPIC) and the related EPIC—Elderly studies were designed to assess the impact of diet on the etiology of cancer and other chronic diseases in the adult population and in elderly Europeans, respectively. The Greek component of these studies has focused on the association between either the degree of adherence to the traditional Greek-Mediterranean diet or individual food groups and total mortality. A higher degree of adherence to the Greek version of the Mediterranean diet was associated with a significant reduction in total mortality (adjusted mortality ratio, 0.75), coronary heart disease (adjusted mortality ratio, 0.67), and cancer (adjusted mortality ratio 0.76; Trichopoulou et al. 2003). The adherence to the Mediterranean diet was further investigated in relation to survival from coronary heart disease, and a higher adherence to the Mediterranean diet was associated with a 27% reduction in overall fatality among individuals diagnosed as having coronary heart disease at enrolment (adjusted fatality ratio, 0.73). The reduced fatality was more evident and amounted to 31% (adjusted ratio, 0.69) when only cardiac deaths were considered as the relevant outcome (Trichopoulou et al. 2005a). Adherence to a modified Mediterranean diet, in which unsaturates were substituted for monounsaturates, was also associated with longer life expectancy among elderly Europeans. The reduction in overall mortality observed was more evident in Mediterranean countries (Trichopoulou et al. 2005b). The association of adherence to the modified Mediterranean diet, with survival among elderly with previous myocardial infarction was also investigated, and again, increased adherence was associated with 18% lower overall mortality rate (Trichopoulou et al. 2007). Individuals at high cardiovascular risk who improved their diet toward a traditional Mediterranean diet pattern showed significant reductions in cellular lipid levels and LDL oxidation (Fito et al. 2007)

Although the Mediterranean diet is characterised by high consumption of olive oil, no important association was found with body mass index (BMI) and *W*/*H* ratio (Trichopoulou et al. 2005c). In fact, data has suggested that the traditional Mediterranean dietary pattern could be inversely associated with BMI and obesity (Schröder et al. 2004). Compared with a low-fat diet, Mediterranean diets supplemented with olive oil or nuts have been reported to have beneficial effects on cardiovascular risk factors (Estruch et al. 2006).

Mediterranean diet and health: biochemical studies

The composition of the traditional Mediterranean diet includes several foods with antioxidant potential, but

the overall diet includes other cardio-protective components, such as reduced saturated fats and greater use of unsaturated lipids, particularly from olive oil. Ongoing research aims to elucidate the role of dietary antioxidants in disease prevention. The main approach has been based on the hypothesis that the chronic disorders common in many societies are related to cumulative oxidative damage to DNA, proteins, and lipids in body tissues. Natural Mediterranean diet antioxidants, which are present in olive oil and red wine, inhibit endothelial activation, suggesting a beneficial role in homocysteine-induced vascular damage and a potential protective role on early atherogenesis prevention (Carluccio et al. 2007, 2003). In vivo effects of wine consumption (400 ml/day) on antioxidant status and oxidative stress in the circulation imply that red wine provides general oxidative protection to lipid systems in circulation via the increase in antioxidant status and decrease in oxidative stress (Micallef et al. 2007). In vivo effects of olive oil consumption (25 ml/day) imply that olive oil is more than a monosaturated fat and its phenolic content can also provide benefits against oxidative damage (Covas et al. 2006).

Traditional foods: analytical data

The traditional Mediterranean diet is associated with longer survival. This could be partly attributed to Mediterranean traditional foods that this diet contains. Rather than based on single foods or nutrients, the combination of different types of food with healthy characteristics might be necessary to express their protective potential. The diet that the Mediterranean populations developed many years ago, without any scientific input, appears to meet existing dietary recommendations (Commission of the European Communities 1993) with respect to macronutrients and certain micronutrients, such as inorganic constituents (Trichopoulou et al. 2005d), as depicted in Fig. 2. Moreover, compared to northern European and American diets, the traditional Greek menu has a higher antioxidant content (Dilis et al. 2007).

The Mediterranean diet has two basic characteristics that distinguish it from other prudent diets. The first stresses the pattern rather than individual components, and the second imposes no restriction on lipids so long as they are not saturated and are preferably in the form of olive oil (specifically, extra virgin olive oil, which is a source of polyphenols). Currently, the market offers consumers a large variety of "functional foods," dietary supplements, and foods enriched with dietary fiber and inorganic constituents. The health claims of these products are generally based on short-term studies conducted with doses that exceed the amounts consumed in common diets, while the real effects of long-term consumption are unknown. The last three sentences can be modified to be made clearer. It is prudent to not exceed the amounts historically ingested. The natural ingredients and the processing methods used for centuries result in traditional foods with a high nutritional value, minimizing the needs for fortification. As the father of medicine, Hippocrates, wisely stated many centuries ago, "Let food be thy medicine and medicine be thy food."

Acknowledgment

The work for this review was supported by the Hellenic Health Foundation.

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Figure captions

Fig. 1 The traditional Mediterranean diet pyramid depicting dietary guidelines for adults in Greece

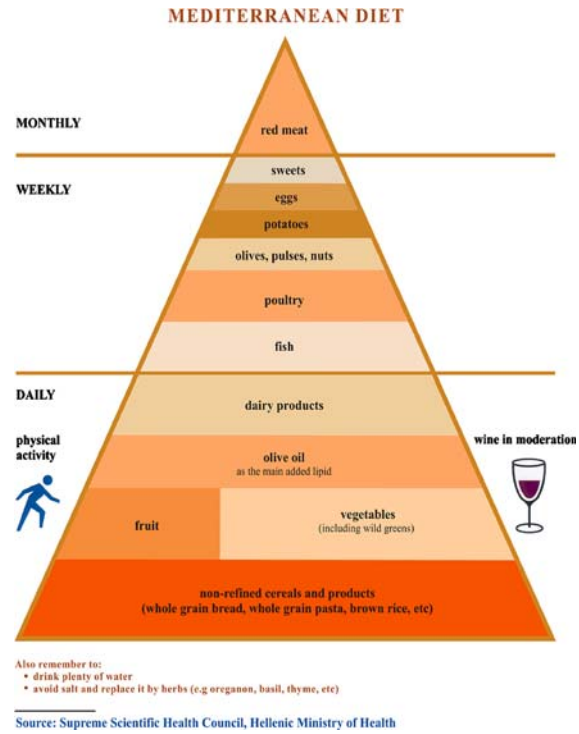
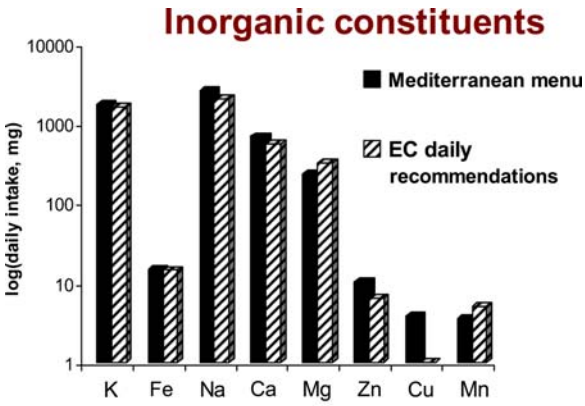


Fig. 2 Comparison of the daily intakes in inorganic constituents of a typical Mediterranean menu with existing EC daily recommendations



An invited paper presented in the plenary session "Trace Minerals: Modulators of Arterial Function"

Manganese: modulator of arterial function and metabolism

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Manganese as an essential trace element

Manganese (Mn) is ubiquitous in nature; it is found in high amounts in the skeleton (25%) and exists in high concentrations in tissues rich in mitochondria. Only 3–4% of dietary manganese is absorbed, and its absorption, which is not well regulated, is independent of intake or its concentration in the body (Klimis-Tavantzis 1994). The reported clinical symptoms of experimental Mn deficiency in humans have been dermatitis (miliaria crystallina), hypocholesterolemia, depressed vitamin K-dependent clotting factors, and reddening of hair (Doisy 1972; Freidman et al. 1987). Suboptimal Mn status has been reported in epileptics (Carl et al. 1993), patients with Down's syndrome (Burlow et al. 1981), osteoporosis (Freeland-Graves et al. 1988; Strause et al. 1994), congestive heart failure (Gorelic et al. 2003), atherosclerosis (Volkov et al. 1962), exocrine pancreatic insufficiency (Aggett et al. 1979), and rheumatoid arthritis (Cerhan et al. 2003). Additionally, increased consumption of processed food, refined carbohydrates, high fiber, and phytate diets and routine use of iron and calcium supplements (Temple 1983) may compromise manganese bioavailability and utilization. Manganese deficiency has also been documented in several animal models and involves skeletal deformities such as chondrodystrophy and chondrodysplasia (Leach 1971; McLaren et al. 2007), abnormal otoliths accompanied by ataxia and ultrastructural abnormalities in mitochondria (Hurley et al. 1970). Recent studies on Mn deficiency documented ocular abnormalities such as loss of photoreceptor cells in the retina (Gong and Amemiya 1996) and optic nerve changes, i.e., fewer myelinated optic nerve axons and decreased diameter and lamellae (Gong and Amemiya 1999). Additionally, Mn deficiency alters lipid and lipoprotein metabolism in animals, as it leads to a reduction in total and high-density lipoprotein (HDL) cholesterol (Klimis-Tavantzis et al. 1983; Kawano et al. 1987; Davis and Feng 1999) and alterations in HDL1 and HDL2 structure and composition (Taylor et al. 1997).

Manganese and glycosaminoglycan structure and metabolism

Manganese has been reported to influence the genesis and development of cardiovascular disease (CVD) by participating as an essential component of metalloenzymes that protect against oxidative stress, such as manganese superoxide dismutase (Greger 1998) and as a cofactor of metal-activating enzymes such as glycosyltransferases (Leach 1971) and sulfonases (Gundlach and Conrad 1985) that aid in the synthesis and maintenance of connective tissue extracellular matrix and structure.

Glycosaminoglycans (GAGs) are functionally important macromolecules of the extracellular matrix of the arterial wall. They are linear polysaccharides composed of alternating disaccharide units of hexosamine and uronic acid, and with the exception of hyaluronan, they are covalently bound to proteins forming proteoglycans (PGs). As multifunctional cell regulators, they play crucial roles as components of cell membrane receptors, influencing ligand-receptor complex function and cell signaling (Schriver et al. 2002), regulating

endothelial cell permeability and migration of vascular smooth muscle cells (Koyama et al. 1998), and altering lipoprotein binding and retention (Pentikainen et al. 2000). Chondroitin sulfate (CS), heparan sulfate (HS), and dermatan sulfate (DS) are the major PGs that exist in blood vessels. Vascular endothelial cells synthesize predominantly heparan sulfate PGs, whereas vascular smooth muscle cells synthesize and secrete principally chondroitin sulfate/dermatan sulfate PGs. We now know that the degree of GAG sulfation is biosynthetically regulated and confers great structural complexity, enabling them to interact in divergent ways with biologically effective molecules, such as enzymes, cytokines, growth factors, and proteins (Turnbull et al. 2001), modulating key events in the process of atherosclerosis (Theocharis et al. 2002). We also know that the expression and distribution of HSPGs is significantly altered during disease conditions, such as vascular injury (Han et al. 1997) inflammation (Hoff and Wagner 1986), atherosclerosis and hypertension (Risler et al. 2002), thus affecting vascular response.

Manganese affects PG and GAG metabolism (Leach 1971; Yang and Klimis-Tavantzis 1998a; Yang and Klimis-Tavantzis 1998b) and is a specific activator of glycosyltransferases, enzymes that are involved in the elongation and polymerization of GAG chains in connective tissue (Leach et al. 1969). Manganese also effectively activates sulfotransferases, enzymes involved in GAG sulfation and synthesis (Gundlach and Conrad 1985). Manganese deficiency affects the biosynthesis of GAGs and decreases total and individual GAG concentrations, especially chondroitin sulfate (CS) in chick cartilage and rat skin (Leach 1969; Bolze et al. 1985; Shetlar and Shetlar 1994). We reported in the past that Mn affects arterial glycosaminoglycan (GAG) metabolism by altering the total proteoglycan (PG) content of the rat aorta and the molecular weight and sulfation pattern of CS, thus predisposing the vessel to lipid deposition, lipoprotein oxidation, and cardiovascular disease (Klimis-Tavantzis et al. 1983; Taylor et al. 1997; Yang and Klimis-Tavantzis 1998a). Additionally, suboptimal in vivo activity of arterial galactosyltransferase-I has been documented in Mn deficiency (Yang and Klimis-Tavanzis 1998b). Transmission electron microscopy of the arterial wall revealed less dense extracellular matrix surrounding smooth muscle cells, especially in the medial layers of Mn deficient rats, suggesting possible changes in the endothelial and/or vascular smooth muscle cells (Ekanayake and Klimis-Tavantzis 1995). Recently, we examined the effect of dietary Mn on the composition and structure of rat aortic GAGs (Kalea et al. 2006a) fed a Mn-deficient (MnD), adequate (MnA), or supplemented (MnS) diet (Mn<1, 10–15, and 45–50 ppm Mn, respectively) for 15 weeks. We observed increased concentration of total GalAGs and decreased concentrations of HS and HA in the MnS aorta compared to the MnA and MnD. Aortas from animals fed the MnS diet contained higher concentration (41%) of non-sulfated units of HS chains, while tri- and di-sulfated HS units were not detectable. Overexpression of HSPGs in Mn deficiency might indicate normal endothelium repair during early stages of inflammation and wound healing, whereas oversulfated HS chains may enhance cell membrane binding and retention to a great variety of extracellular ligands including lipoprotein particles (Kaplan et al. 1998; Llorente-Cortes et al. 2002) and thus affect signal transduction pathways and functional properties of the vascular wall. The potential role of manganese in atheroprotection needs to be further investigated.

Manganese and vascular function

The vascular endothelium is crucial in regulating vasomotor tone by balancing the release of endothelium-derived contracting factors (EDCFs) such as endothelin and eicosanoids (TXA_2) and endothelium-derived relaxing factors (EDRFs) such as nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (Luscher and Vanhoutte 1990). Nitric oxide induces vasodilation through the activation of the cGMP pathway (Lucas et al. 2000), while PGI_2, a product of cyclo-oxygenase (COX), acts synergistically with NO to induce vasodilation (Salvemini et al. 1996). Endothelial dysfunction is a result of an imbalance between EDRFs and EDCFs (Shimokawa 1999), and its role on the pathophysiology of vascular disease has been repeatedly documented (John and Schmieder 2000). Alterations in the biomechanical properties of the vascular system might not only affect blood flow but also platelet aggregation and vessel permeability, processes associated with early stages of atherosclerosis, hypertension, and several cardiovascular disorders (van Popele et al. 2001).

The putative role of Mn as a modulator of vascular biomechanical properties may stem from its involvement as a cofactor of enzymes such as MnSOD, arginase, CaM-dependent phosphatase, adenylate and guanylate

cyclase (Korc 1993), as well as its role in receptor structure (Wedler 1994). Furthermore, Mn increases the accumulation of second messengers (cAMP, cGMP) that activate proteins for cell signaling (Korc 1993), modulates in vitro cell-surface receptor binding and adhesion (Wedler 1994), and functions as a Ca^{+2} ion channel entry blocker, affecting vascular function (Kasten et al. 1995; Yan et al. 1998). We examined the effect of dietary Mn on phenylephrine-induced vasoconstriction and acetylcholine (Ach)- and sodium nitroprusside (SNP)-induced vasodilation in rat aorta. Sprague–Dawley rats were fed either a manganese-deficient (MnD) or adequate (MnA; <1 and 10–15 ppm Mn, respectively) for 15 weeks. The maximal force (F_{max}) of contraction and relaxation, as well as vessel sensitivity (pD_2), were determined in rat intact and endothelium-disrupted aortic rings. We observed that, in the presence of endothelium, aortic rings from MnD animals developed higher vessel sensitivity (pD_2) compared to controls (MnA), an effect that was abolished when the endothelium was disrupted. Thus, manganese modifies vascular response to an a1-adrenergic receptor stimulus, which is modified by the presence of the endothelium. In other experiments we have documented (Kalea et al. 2005) that the presence of dietary manganese at 45–50 ppm affects the arterial contractile machinery by reducing maximal vessel contraction and vascular sensitivity, thus influencing signaling pathways.

We investigated ED- and endothelium-independent vasodilation (Kalea et al. 2006b) in rings precontracted with phenylephrine in the presence or absence of inhibitors of NO synthase and COX. We observed a significant decrease both in Ach-induced (endothelium-dependent) and SNP-induced (endothelium-independent) vasodilation in MnD aortas when compared to MnA (controls) but found no effect on vessel sensitivity. Inhibition of NO synthase blunted Ach-mediated vasorelaxation to the same degree for both diet groups, but inhibition of COX enhanced both Ach- and SNP-induced vasodilation of MnD rings compared to controls. Thus, Mn affects endothelium-dependent and endothelium-independent vasodilation, and seems to alter the synthesis or activity of a prostanoid-derived vasoconstrictor present at basal and stimulated levels. Furthermore, it modifies vascular response to an a1-adrenergic receptor stimulus influencing membrane-related events. Our results provide further information on the critical role of Mn on maintenance of vasomotor tone with implications on CVD.

Acknowledgment

This review was partially supported by the USDA (project no. 35483), the Maine Agriculture and Forestry Experiment Station (Scientific Contribution no. 2994), Harokopio University, Department of Nutrition and Dietetics, Athens, Greece, and the Aristidis Daskalopoulos Foundation, Athens, Greece.

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An invited paper presented in the plenary session "Trace Minerals: Modulators of Arterial Function"

The role of copper in nitric oxide-mediated vasodilation

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Introduction

The role of copper as an essential nutrient for both structure and function in the cardiovascular system is well known. Dietary Cu deficiency in experimental animals causes a variety of vascular defects (for review, see Saari and Schuschke 1999). These defects include altered contractile responses of blood vessels. Kitano (1980) reported an increased rat aortic contraction to norepinephrine, and Allen and Saari (1994) demonstrated an augmented vasoconstriction to angiotensin II in isolated rat lungs. In addition to exaggerated constrictor responses, dilation is also altered. In separate studies, Saari (1992) and Lynch et al. (1997) reported the inhibition of nitric oxide (NO)-mediated vascular smooth muscle relaxation in aortic rings from Cu-deficient (CuD) rats. Similar inhibition was found when Cu was chelated before functional testing of rat aortic rings (Omar et al. 1991; Plane et al. 1997).

Our studies have addressed the role of dietary Cu in the vasoreactivity of microvascular arterioles.

These vessels, which have an endothelium and one or two layers of smooth muscle cells, regulate local tissue blood flow and are the primary contributors to total peripheral vascular resistance. Altered vasoreactivity of these resistance vessels may be an important factor in the altered blood pressure that has been associated with inadequate Cu nutrition. For example, rats fed CuD diets can be either hypertensive (Medeiros 1987) or hypotensive (Fields et al. 1984), depending on the age when Cu restriction begins. In humans, stress-induced elevation of blood pressure occurs during short-term Cu deficiency (Lukaski et al. 1988).

Vasoreactivity in the Cu-deficient microcirculation

In initial studies, the in vivo microcirculation was examined in copper-adequate (CuA) and CuD rats. These experiments did not demonstrate a difference in the response of small arterioles (10- to 25-μm diameter) to norepinephrine-induced vasoconstriction between groups (Schuschke et al. 1995a). However, NO-mediated vasodilation was inhibited in the CuD group (Schuschke et al. 1992; 1995a). Similar results were also seen in an adult model of marginal Cu deficiency (Falcone et al. 2005). This inhibition occurred when the endothelium-dependent NO signal transduction pathway was stimulated by several different agonists including receptor-dependent acetylcholine (Ach) and receptor-independent calcium ionophore A23187. Vasodilation was also depressed in CuD rats when the vasculature was stimulated by the endothelium-independent NO donor, sodium nitroprusside (Schuschke et al. 1992). Similar results have been reported in aortic rings from CuD rats (Saari 1992).

Because the vasodilator response was attenuated in the CuD rat, relaxation mechanisms of the vascular smooth muscle were examined. Relaxation in response to the dibutyryl analogs of the second messengers, cGMP and cAMP, was not different between CuD and CuA groups (Schuschke et al. 1995a). Also, maximal dilation in response to the phosphodiesterase inhibitor papaverine did not differ between dietary groups in either the microcirculation (Schuschke et al. 1992) or aortic rings (Saari 1992). These results demonstrated that the ability of vascular smooth muscle to relax is not altered by dietary Cu deficiency but that the NO-mediated dilation pathway is specifically inhibited.

Possible mechanisms of attenuation

In the endothelial cell, NO is synthesized from the amino acid L-arginine in the presence of increased intracellular free calcium and the endothelial isoform of NO synthase (eNOS). The NO diffuses to the vascular smooth muscle and stimulates soluble guanylate cyclase (GC-S), which increases the second messenger cyclic GMP and causes relaxation. We have recently shown that arteriolar endothelium from CuD rats release significantly less NO than controls (Schuschke et al. 2007).

Cu,Zn superoxide dismutase (Cu,Zn–SOD) and soluble guanylate cyclase (GC–S) are two Cu-containing enzymes that are either directly or indirectly involved in the NO–cGMP signaling pathway. Cu,Zn–SOD is an antioxidant responsible for the dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). GC–S is stimulated by NO to produce cGMP in the vascular smooth muscle cells. We hypothesized that inactivation or attenuation of these enzymes by the removal of Cu may result in the loss of NO-mediated dilation.

One possible mechanism of attenuation is the direct inactivation of NO by oxygen-derived free radicals that are by-products of cellular metabolic reactions. Dietary Cu deficiency has been shown to increase free radical activity because of the reduced activity of Cu-dependent antioxidant enzymes including Cu,Zn–SOD (Johnson and Saari 1991). Superoxide anion is known to inactivate NO and has been shown to inhibit cGMP-mediated relaxation of vascular smooth muscle in rats (Cherry et al. 1990). Superoxide dismutase (SOD) is the metabolizing enzyme of O_2^-, but because the activity of cytoplasmic Cu,Zn–SOD is reduced by restricting dietary Cu, O_2^- concentrations should be increased in CuD animals. Elevated O_2^- would lead to enhanced destruction of NO, resulting in loss of the diffusion gradient for NO between the endothelium and the underlying smooth muscle.

In the in vivo microcirculation, the dilator response of small arterioles to Ach is significantly attenuated during dietary Cu deficiency. However, this attenuation disappears when the antioxidant tempol is added to the drinking water (unpublished results) or after exposure to exogenous Cu,Zn–SOD (Schuschke et al. 1995a). These data suggest that during Cu deficiency, excess O_2^- degrades NO, directly decreasing NO dilator capability.

In addition to a direct inactivation of NO, the interaction of NO and O_2^- may indirectly inhibit the

synthesis of additional NO by the endothelial cell. NO and O_2^- combine to produce peroxynitrite ($ONOO^-$), which causes oxidative damage including the inhibition of endothelial cell Ca^{2+} signaling (Elloitt 1996). We have shown in CuD rats that, when Cu,Zn–SOD activity is depressed, plasma $ONOO^-$ is increased and agonist-induced endothelial cell Ca^{2+} mobilization is decreased (Schuschke et al. 2000). These results support the hypothesis that excess O_2^- and the subsequent production of $ONOO^-$ inhibits endothelial cell Ca^{2+} signaling and causes attenuation of NO-mediated vascular dilation.

Another possible effect of Cu deficiency on NO-mediated dilation involves the interaction of NO with GC–S. Cu and Fe are transition metals that are components of this enzyme (Gerzer et al. 1981), which converts GTP to cGMP. As Cu is a functional cofactor in the NO-heme-binding site, NO may not be able to activate the GC–S when Cu concentrations are inadequate. Alternatively, iron metabolism is known to be altered in dietary Cu deficiency and may be a mechanism by which both the NO-heme binding is prevented, and the NO activation of GC-S is depressed in CuD animals.

Aside from the effects on NO binding, Cu depletion may also depress the activity of the GC–S enzyme independently of heme content. H_2O_2, which activates GC–S by a NO-independent mechanism, was used to test the activity of the GC–S during Cu deficiency (Schuschke et al. 1995a). In these studies, the microvessel dilation to H_2O_2 was not different between the CuD and the CuA groups. These data suggest that the general activity of the GC–S is not affected by dietary Cu deficiency. Therefore, our results indicate that, if Cu is a functional component of GC–S, its role is likely at the NO-binding site, but it is not a requisite for the basal activity of the enzyme. However, because the administration of Cu,Zn–SOD restored the dilation to Ach (Schuschke et al. 1995a), it is unlikely that altered NO-heme binding is the primary mechanism for the depressed vasodilation.

Other studies have examined the generation of NO in endothelial cells. Western blot analysis of eNOS did not demonstrate a difference between CuD and CuA groups, and pretreatment with the eNOS substrate L-arginine did not alter the attenuated dilation to Ach in CuD arterioles (Schuschke et al. 2000). Therefore, inactivation of Cu,Zn–SOD by inadequate Cu intake appears to be the primary mechanism by which NO-mediated dilation is reduced.

Compensatory mechanisms

While the attenuation of NO-dependent vasodilation is consistent among models of Cu deficiency, the effect on blood pressure is less predictable. As noted in the introduction, Cu deficiency may cause either hypertension or hypotension. However, other studies report no difference in blood pressure associated with dietary Cu restriction (Schuschke et al. 1997). The lack of an effect on blood pressure when NO-mediated vascular relaxation is decreased in resistance vessels suggests that compensatory mechanisms are involved. This idea is supported by recent data showing that inhibition of NO synthesis has less of an effect on blood pressure in normotensive CuD rats than in CuA controls (Saari 2002).

One possible compensatory mechanism is the up-regulation of the inducible isoform of NO-synthase (iNOS) during Cu deficiency. Although eNOS is the prevailing isoform of NOS in the vascular system, Saari and Bode (1999) report that iNOS and NO production are elevated in hearts of CuD rats. These results suggest that iNOS is up-regulated at a time when endothelial-derived NO may be inhibited.

Another compensatory mechanism may involve the up-regulation of the prostacyclin (PGI_2)–cAMP vasodilation pathway. We have shown that the sensitivity to carbacyclin (a stable analog of PGI_2) is increased in the in vivo microcirculation of CuD rats when NO-mediated dilation is depressed (Schuschke et al. 1997). This change in sensitivity may indicate an up-regulation of receptors on the vascular smooth muscle that maintains vasodilator input.

Cu requirement

The relationship between dietary Cu concentration and NO-mediated vasorelaxation was studied to determine the minimal dietary Cu intake necessary to prevent attenuation of the signaling pathway. By using Ach as the NO-dependent agonist, we have shown that the dilator response decreases when liver Cu concentration is less than 5 μg/g dry weight (Schuschke et al. 1999). The sensitivity of this dilation pathway to dietary Cu restriction is similar to that reported previously in a study on the role of Cu in hemostatic mechanisms (Schuschke et al. 1995b). Based on a study by Klevay and Saari (1993), dietary Cu intakes of greater than 1 μg/g diet are required to maintain liver Cu concentration above 5 μg/g in rats.

Conclusions

Several groups using various models of Cu deficiency have demonstrated that NO-mediated vasodilation is Cu-dependent. These data suggest that inactivation of cytosolic Cu,Zn–SOD by Cu restriction or chelation results in the depression of NO.

We have proposed that this depression of the NO response is caused by the buildup of O_2^- in the microcirculation, which then inhibits the NO pathway by direct and indirect mechanisms. O_2^- reacts with NO to produce $ONOO^-$, which reduces the NO available to diffuse to the smooth muscle. The $ONOO^-$ also reduces the requisite increase in intracellular Ca^{2+} for the further synthesis of NO from L-arginine. This hypothesis is supported by data showing that $ONOO^-$ is increased and agonist-stimulated endothelial Ca^{2+} mobilization is depressed in the vasculature of Cu deficient rats.

Acknowledgment

This work was supported in part by NIH DK55030.

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An invited paper presented in the plenary session "Trace Minerals: Modulators of Arterial Function"

Selenium status and regulation of vascular homeostasis

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This review was partially supported by a grant from USDA 2007-35200-18235.

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Introduction

Evidence suggests that there may be an inverse relationship between selenium (Se) nutrition and cardiovascular disorders. Several intervention studies are currently underway to assess the benefits of Se supplementation to control a wide variety of condition in which oxidant stress and inflammation are the predominant pathological features, including atherosclerosis. Earlier epidemiological and ecological studies that examined the potential benefit of Se administration to prevent or treat cardiovascular disease however have proven to be inconclusive (Alissa et al. 2003; Huttunen 1997; May 2002). An underlying factor to explain some of the equivocal finding from these prospective studies includes the inability to accurately assess Se status within different tissue microenvironments. The metabolism of Se in mammals is determined largely by the dietary sources, and it is known that the level of bioavailability will vary considerably in the body (Stadtman 2000). Therefore, to design diets that will maximize the benefits of Se on human health, it will be necessary to not only identify the specific cellular processes that are affected by Se status but also determine the specific Se-dependent bioactive components that are responsible for the desired response in targeted cellular environments.

The importance of Se to human health may be related to selenoprotein activities, such as glutathione peroxidase and thioredoxin reductase, that have diverse biological roles. These enzymes can function as potent antioxidants by directly reducing pro-atherogenic reactive oxygen species (ROS) and fatty acid hydroperoxides (FAHP) to less reactive water and alcohols, respectively. However, more recent evidence suggests that certain selenoproteins also are capable of modifying cellular responses to oxidant challenge by controlling the balanced expression of cytoprotective, apoptotic, and pro-inflammatory factors. This paper will describe some of the regulatory roles of individual selenoproteins in orchestrating vascular homeostasis during oxidant stress. The long-term implications for this area of research will be the ability to identify foods that can provide the optimal amount of specific biological active selenoproteins needed to control the development of atherosclerosis and other cardiovascular disorders.

Selenoproteins and endothelial anti-oxidant defense

Since the recent discovery of selenocysteine as the 21st amino acid in proteins, the field of Se biology has expanded rapidly. Indeed, many of the beneficial effects of this micronutrient are thought to be mediated by selenoproteins, which have selenocysteine residues incorporated into their active sites. There are at least 25 mammalian selenoproteins that have been identified, and some have important enzymatic functions (Papp et al. 2007). Recent studies showed that cytosolic glutathione peroxidases (GPX1), phospholipid hydroperoxide GPX (GPX4), and thioredoxin reductase 1 (TrxR1) are the major selenoproteins expressed by endothelial cells (Brigelius-Flohe et al. 2003; Hara et al. 2001). An important regulatory phenomenon that may be of particular importance to the development of cardiovascular disease is the antioxidant capabilities of these selenoproteins. Antioxidant potential is catalyzed either directly or indirectly by these selenoproteins based upon

their ability to reduce many different forms of hydroperoxides, including H2O2 and FAHP, to less reactive water and alcohols. For example, 15-hydroperoxyeicosatetraenoic acid (15HPETE) is a FAHP that represents the immediate oxygenated product formed from arachidonic acid metabolism via the 15-lipoxygenase (15LOX1) pathway. Several studies have documented the significance of this FAHP in vascular dysfunction (Cornicelli and Trivedi 1999; Cyrus et al. 2001). Indeed, research from our laboratory suggests that 15HPETE can directly affect vascular homeostasis by inducing apoptosis and enhancing the expression of pro-inflammatory mediators (Sordillo et al. 2008; Sordillo et al. 2005). Both GPX1 and TrxR1 have the capacity to reduce 15HPETE to a less toxic form, 15-hydroxyeicosatetraenoic acid. Therefore, selenoproteins clearly can help maintain cellular integrity by combating the accumulation of toxic hydroperoxides and reducing the oxidative modification of membrane lipids in artery walls that is associated with cardiovascular disease (Neve 2002).

Selenoproteins and eicosanoid biosynthesis

By controlling the accumulation of hydroperoxides within endothelial cells, selenoproteins also play a role in regulating the activities of enzymes involved in eicosanoid metabolism. Both prostaglandins and leukotrienes are essential for the regulation of vascular tone. For example, prostacyclin (PGI_2) causes vasodilation and inhibits platelet aggregation, while thromboxane A_2 (TXA_2) promotes aggregation and vasoconstriction. The peroxide tone of endothelial cells can directly affect the activity of enzymes involved in arachidonic acid metabolism such as cyclooxygenase, prostacyclin synthatase, and the lipoxygenases (Cao et al. 2000). Recent research showed that high peroxide levels resulting from the accumulation of the primary 15LOX product, 15HPETE, can reduce PGI2 production by the inactivation of prostacyclin synthase while having no effect on thromboxane synthase activity (Mayer et al. 1986; Weaver et al. 2001). As both PGI2 and TXA2 are derived from the same cyclooxygenase product (prostaglandin G2), the end result of elevated peroxide tone is reduced PGI2 synthesis and increased TXA2 levels during oxidant stress (Schilling and Elliott 1992). Therefore, the ability of selenoproteins to control the accumulation of intracellular lipid hydroperoxides can impact eicosanoid biosynthesis and vascular health.

Selenoproteins and cell signaling

Beyond the well-characterized hydroperoxide scavenging role, selenoproteins also can impact the activities of several enzymes involved in cell signaling cascade by controlling the intracellular redox environment (Brigelius-Flohe et al. 2003; McKenzie et al. 2002). Cellular redox state is a consequence of the balance between oxidizing and reducing equivalents, the levels of which can be controlled by both thioredoxin (Trx)/TrxR1 and glutathione (GSH)/GPX redox couples. There is a growing body of evidence to suggest that hydroperoxides can influence endothelial cell signaling cascades by redox modification of various protein kinases, protein phosphatases, and transcription factor activities. For example, the mitogen-activated protein kinase (MAPK) are a family of serine/theronine kinases that can control cellular physiologic responses through several redox-sensitive pathways including the extracellular signal-regulated kinase (ERK1/2), cJun N-terminal kinase (JNK), and p38. Previous studies showed that hydroperoxide-induced apoptosis of EC occurs through activation of the apoptosis signal-regulating kinase 1 (ASK1) and its downstream molecules JNK and p38 (Griendling et al. 2000). However, phosphorylation of the ERK1/2 pathway protects endothelial cells from oxidant stress. Treatment of cells with Se can suppress hydroperoxide-induced apoptosis by inhibiting ASK1 activation of JNK and p38 through a thiol-dependent mechanism. It was shown that ASK1 is inactivated when complexed with reduced Trx. Exposure to hydroperoxides can oxidizes Trx and results in its dissociated from ASK1, thereby enabling ASK1 to become active to promote the apoptotic process (Sarker et al. 2003; Yoon et al. 2002a; Yoon et al. 2002b). As TrxR1 can regenerate reduced Trx, the Trx/TrxR1 system can effectively modulate cell death by regulating the intensity of signaling cascades.

Further downstream in the signaling cascade, there are several transcription factors that are modified during oxidant stress including AP1 and NFκB (Papp et al. 2007). These redox-sensitive transcription factors are thought to control vascular homeostasis by modifying the expression of genes that are under the control of antioxidant-responsive elements. Se was shown to regulate some of these transcription factors by several different mechanisms. One mechanism involves the modification of the binding strength of transcription factors to DNA that involves a redox-sensitive regulator,

Ref1. For example, the transcription factor AP1 is a dimer complex composed of either Jun family (c-jun, JunB, and JunD) homodimers or c-jun/Fos family (c-fos, fosB, Fra-1, and Fra-2) heterodimers. These proteins are joined by a leucine zipper domain that utilizes a conserved cysteine to bind DNA and are therefore subject to redox control. Studies have shown that Trx can increase AP1 activity indirectly by its ability to translocate to the nucleus and bind Ref1. The Trx-bound Ref1 then associates transiently with AP1 and reduces the conserved cysteines in Fos and Jun family members, thus enhancing their binding activity (Hirota et al. 1999).

Another mechanism by which Se can regulate transcription factor activity is by changing the activation state of transcription factor regulatory subunits. For example, the NFκB/IκB complex resides in the cytoplasm and requires the release of phosphorylated IκB from this core complex for activation of NFκB subunits (p50 and p65). Considerable evidence suggests that addition of Se to cells in culture or overexpression of GPX1 and GPX4 can decrease NFκB activation by blocking IκB phosphorylation (Brigelius-Flohe et al. 2003; Brigelius-Flohe et al. 1997). This would prevent the migration of NFκB subunits to the nucleus where they can bind to DNA (Barchowsky et al. 1995). The impact of Se or selenoproteins on NF_kB activation was suggested to be from the breakdown of excess hydroperoxides that are responsible for oxidant-induced phosphorylation of IκB (Brigelius-Flohe et al. 2003). Through the actions of selenoproteins, Se can regulate intracellular signaling and transcription factor activation during oxidant stress. Therefore, it follows that this micronutrient also may impact the repertoire of gene expression that determines whether a cell is able to return to a state of homeostasis after oxidant challenge.

Conclusions

Considerable evidence suggests that low Se intake can cause adverse health effects while supra-nutritional levels may provide added protection from disease. Despite the clear relationship between Se status and optimal health, the mechanisms responsible for the beneficial effects remain elusive. Se is thought to mediate many of its beneficial effects through the antioxidant capabilities of selenoproteins. The ability of selenoproteins to control the redox environment of the cell also is likely to have an impact on the expression of genes that will determine the survival of endothelial cells during oxidant stress. It is not known whether the optimal health benefits of Se depends upon maximization of one or more of the selenoproteins within a localized tissue area. Further characterization of the role of selenoproteins in endothelial cell metabolism may provide new insights as to how Se may function as a nutraceutical and produce specific health benefits in combating cardiovascular disease.

Acknowledgment

The project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant 2006-35200-17190. This review was partially supported by a grant from USDA 2007-35200-18235.

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An invited paper presented as the Raulin Award Lecture as part of ISTERH/NTES/HTES '07'

The ISTERH Raulin Award lecture: zinc nutriture and the fetal origins of disease

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Adverse effects of gestational malnutrition on progeny have long been known (Ebbs et al. 1941; Smith 1916). In recent times, birth weight was shown to be inversely and linearly related to risk of coronary heart disease (Barker et al. 1989), type-2 diabetes mellitus, and hypertension (Osmond and Barker 2000), and gestational malnutrition was discovered to increase the risk of adult diseases, such as atherosclerotic cardiovascular disease and schizophrenia, in progeny of Dutch women who conceived during the famine of 1944–1945 (Roseboom et al. 2006; Susser et al. 1998).

Current theory suggests that malnutrition affects risk of later diseases through epigenetic mechanisms that