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COPPER AND THE REGULATION OF LIPID AND CARBOHYDRATE METABOLISM, CARDIOVASCULAR SYSTEM FUNCTION AND PHYSICAL PERFORMANCE

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Abstract

Copper (Cu) plays a vital role in living systems as a co-factor for a number of enzymes. Inherited disturbances in copper homeostasis (Wilson's or Menkes diseases) have pronounced and deleterious effects on many body functions. At present it is well recognized that copper homeostasis is tightly regulated at the level of intestinal absorption, cellular influx and efflux and intracellular trafficking to prevent detrimental effects of both copper deficit and overload. Since dietary copper deficiency has been recognized in many populations there is a wealth of studies concerning the associations between human health and daily copper intake. It is well documented that dietary copper deficiency disturbs liver lipid and lipoprotein synthesis and possibly contributes to the development of atherosclerosis. Additionally, copper deficiency affects endothelium functions depressing nitric oxide production. There are also data suggesting an insulin-like effect of copper on tissue glucose uptake and insulin signaling and copper deficiency has been shown to disturb glucose tolerance. Furthermore, inadequate daily copper intake has been shwon to depress cytochrome c oxidase activity, and to shift substrate utilization from carbohydrate to fat. Moreover, plasma copper levels have been found to positively correlate with maximal oxygen uptake. On the other hand, in physically active subjects copper status may be compromised due to ion losses with sweat and urine but also to inadequate intake with diet. However, until now copper supplements are not recommended due to the lack of a reliable method of diagnosing marginal copper deficiency and hazardous effects of copper excess.

Key words: *copper homeostasis,copper deficiency,lipoproteins,carbohydrates cardiovascular system, performance*

Copper (Cu) plays a vital role in living systems as a co-factor for a number of enzymes involved in mitochondrial respiration, antioxidant protection, collagen and neurotransmitter synthesis (1, 2). The body of a healthy, 70 kg human adult contains 50 to 150 mg of copper with 40% found in the bone, 23% in the muscle, 8% in the brain and 5.5% in the blood with the remaining 23.5% almost equally distributed in other tissues (3).

It is well documented that the disturbances in copper homeostasis contribute to the deterioration in variety of metabolic processes (4-6).

Despite its pivotal role in metabolism copper is a transition metal and has the ability to exist in two distinct redox states – as Cu^{+1} or Cu^{+2} and can readily participate in oxygen radical formation (7). To prevent possible harmful effects of copper its homeostasis in the living systems is tightly regulated at the level of intestinal absorption, cellular influx, efflux and intracellular trafficking.

Copper homeostasis

Copper absorption and excretion

Isotope studies have indicated that copper from food is absorbed in the gastro-intestinal tract and transported into the circulation. After its entry into the blood copper is bound to albumin and transcuprein and taken up by different organs with the greatest amount taken up by the liver and subsequently by the brain, muscles, kidneys and heart (8). Copper taken by the liver is incorporated into ceruloplasmin and released into the circulation (9). Relatively little copper is stored in the body and most endogenous copper is lost via the bile into feces, with a small amount lost in the urine and sweat.

The magnitude of copper absorption, retention and excretion are adjusted to its availability in the diet. In young healthy volunteers copper absorption was highest (67%) during low copper intake (0.38 mg/day) and decreased to 54% and 44% with increasing intake (0.66 and 2.49 mg/day, respectively) (10). Concomitantly, for a daily intake of 0.38 mg for 24 days the mean copper excretion with feces was markedly lower than with 0.66 mg/day period (12% vs. 26%, respectively) and for daily intake of 0.66 mg – slightly lower than during the 2.49 mg/day period (26% vs. 34%). It is worth noting that changes in copper excretion in response to low dietary intake were more pronounced than the changes in absorption suggesting that copper excretion is a major point of the regulation of the body copper pool (11).

Copper transport

A wealth of animal and human studies have indicated that a high affinity copper transporter named Ctr1 is widely distributed in many tissues including the intestine, liver, and kidney and to lesser extent in the brain being responsible for dietary copper uptake by the tissues (12).

The Ctr1 mediated Cu transport is copper-specific, saturable, time-dependent, and energy-independent (13). The importance of Ctr1 in copper transport to the cells has been confirmed in mice completely deficient for Ctr1 which exhibit profound growth and developmental defects and mostly die *in utero* (14, 15). Additionally, Kuo et al. (16) have revealed that in mice fed Cu-deficient diet Ctr1 expression in the duodenum, kidney and brain was higher than in controls reflecting transporter adaptation to copper deficiency. It seems feasible that similar changes in Crt1 expression are observed in humans being responsible for the above mentioned greater Cu absorption from a low than from a high copper diet.

However, the divalent metal transporter 1 (DMT1) which probably does not respond to copper availability, but is highly responsive to iron which competes with copper for binding with DMT1 also contributes to copper intestinal absorption (17).

Copper taken up by the cells (eg. enterocytes, hepatocytes) is bound by intracellular peptides (chaperones) and transported between intracellular compartments. In the cells of the alimentary tract copper is bound by the HAH1 chaperone which enables its transport for binding with the ATP-7A transporter responsible for copper efflux into the circulation (18).

After entering the hepatocytes copper binds to different chaperones (CCS, COX17, HAH1) which provide copper for superoxide dismutase, cytochrome c oxidase and ATP-7B transporter, respectively (19, 20). ATP-7B-bound copper is transported into the secretory pathway of hepatocytes for incorporation into nascent cuproproteins (e.g. holoceruloplasmin) and subsequently excreted from the cells as ceruloplasmin.

In contrast to copper uptake by the cells, its efflux from the cells is ATP-dependent since both ATP-7A and ATP-7B transporters were recognized to have ATP-ase activity (21). Similarly to the influx-regulating Ctr1 transporter, ATP-7B transporter expression in the tissues is sensitive to copper availability in the diet and is higher in rats supplemented with copper than in control animals, thus protecting the organs against copper excess (22).

Studies concerning inherited defects in copper transport – Wilson and Menkes diseases confirmed the importance of both ATP-7A and ATP-7B transporters in copper homeostasis (23, 24).

Briefly, Menkes disease (or Menkes syndrome) is caused by a defect in copper transporter ATP-7A and symptoms result from impairment of copper export from the intestine where dietary copper is absorbed. In consequence, the disease reflects the deficiency of copper in the body, therefore plasma copper level is low and the activities of copper enzymes are depressed. In the severe, lethal form of Menkes disease skeletal and vascular abnormalities, hypothermia, diarrhea, hyperelastic skin, "kinky" hair, seizures, bladder diverticuli and delayed development have been noted.

On the contrary, in Wilson disease a defect of the copper transporter ATP-7B has been demonstrated resulting in reduced copper excretion from the liver into the bile and subsequently its accumulation in the liver and with progression of the disease also in other tissues (e.g. brain, heart).

Thus, Wilson disease reflects the state of copper excess in the body. The most common symptoms of Wilson disease are hepatic (cirrhosis in later stages of disease), and neurological (tremor, poor coordination, loss of motor control, rigidity, depression, and abnormal behavior).

The chaperones and ATP-7A/7B transporters are not the only proteins which handle copper inside the cells. High-cysteine proteins – metallothioneins (MT) are known to bind copper excess and probably serve as its source during a transient scarcity , but in contrast to ATP-related transporters, MT are not able to eliminate copper excess from the cells (25-27).

It should be stressed that inherited copper-related disorders such as Menkes and Wilson diseases are rare. On the contrary, dietary copper deficiency is common possibly affecting human health.

Dietary copper sources, intake and copper status

Data analyzing copper sources in the Western Europe have indicated that it originates mostly from cereals (32%), meat and eggs (27%) and to a lesser extent from vegetables (14%), fish and fruits (less than 10%) with minor contribution of diary products and other food stuffs (28). In the Polish diet 30% of copper is provided by grains, up to 22% by potatoes, approximately 13% by fish, up to 19% from fruits and vegetables (29).

Daily copper intake may differ substantially due to dietary habits of populations. In Western Europe it is in the range 0.8-1.8 mg/day (28). In Polish students an average daily copper intake in females ranged from 0.85 to 1.0 mg, in males from 1.34 to 1.40 mg (30, 31). USA diet provides daily 1.17-1.25 mg of copper in adult males and 0.86-0.97 mg in adult females (32). On the contrary, in Australian women the average daily copper intake was 1.56 mg and was markedly higher than in most Western countries (33).

However, daily copper intake calculated from food consumption does not provide reliable information concerning copper status since its bioavailability is

regulated by copper content itself and also by other dietary factors. As was mentioned earlier low copper content in the food induces Ctr1 transporter expression in enterocytes increasing copper absorption. Additionally, organ copper conservation has been noted in response to dietary copper restriction especially in the brain and heart responding to the loss of some copper (34). Moreover, it is well documented that copper absorption from the alimentary tract is adversely affected by excess of zinc, iron, fructose and ascorbic acid in the diet and stimulated by protein intake (35-39). Special attention has to be paid to adverse effects of zinc on copper homeostasis, since recently it has been demonstrated that zinc supplementation (12 weeks, 22 mg/day of zinc gluconate) decreases plasma copper levels (40)

Unfortunately, until now there is no reliable biomarker of copper status since neither plasma copper level nor cuproenzyme activities precisely reflect marginal states of copper nutrition and data concerning this topic are incomplete (41-43). For this reason recommended dietary allowances (RDA) for copper have not been established. So far in USA safe and adequate copper dietary intake was set at between 1.5 and 3.0 mg, in Poland the recommended copper daily intake ranges from 2.0 to 2.5 mg (29,44). Thus, dietary copper intake both in American and Polish populations is far below recommended and widespread marginal copper deficiency in Western countries has been postulated (44,45). Therefore, the effects of inadequate copper intake on metabolic processes have been carefully studied both in humans and animals.

Consequences of dietary copper deficiency

Iron homeostasis in copper deficiency

As was mentioned above there is a competition between copper and iron at the level of absorption since both metals compete for binding to divalent metal transporter 1 (DMT1) in the intestine (46, 47). In consequence, copper deficiency increases, but copper excess decreases iron transport into the enterocytes. On the other hand, in enterocytes copper deficiency brings about reduced synthesis of hephaestin in enterocytes, a multi-Cu-ferroxidase oxidizing divalent iron to enable its binding with ferroportin 1 (FPN1) exporting iron from enterocytes (48). Thus, in the copper deficient state iron efflux from enterocytes is depressed possibly leading to iron deficit, disturbed hematopoiesis and microcytic/hypochromic anemia (49-51). However, copper repletion in the diet restores intestinal hephaestin synthesis and iron efflux from the gut improving hematological parameters (52). Interestingly, signs of iron deficiency in copper-deficient rats are not affected by iron supplements because of persistent depression of iron in the face of copper deficiency (53).

Cardiovascular system in copper deficiency

There are numerous data indicating that in animals long term dietary copper deficiency induces cardiac hypertrophy, necrosis of myocytes, fragmentation of elastic fibers in the aorta and altered mechanical properties of the heart (54). In copper deficient rats cardiac hypertrophy has been shown to coexist with depressed cytochrome c oxidase activity and altered expression of Na/K– ATP-ase indicating disturbed myocyte respiration and contractility (55, 56). Low copper intake in mice induces marked changes in myocardial gene controlling expression of mRNA for Ca^{+2} channels, VEGF (vascular endothelial growth factor) and myosin heavy chains which are attenuated by copper repletion (57). Other studies have found altered collagen metabolism in the heart of copper-deficient rats together with other symptoms of cardiomyopathy (e.g. increased volume of mitochondria, disruption of crista structure, diastolic and systolic dysfunction and electrocardiograph alterations) and excessive fat accumulation in the myocytes (58-60). Moreover, copper deficiency in rats resulted in depressed fatty acid utilization by cardiomyocytes (61). Substantial importance of copper for cardiac function has been proved by Jiang et al. (62) who indicated that in mice copper supplementation reverses hypertrophic cardiomyopathy induced by chronic pressure overload.

Detrimental effects of copper deficiency in the cardiovascular system are not limited to morphology and function of cardiomyocytes (63). In copper-deficient rats *in vitro* nitric oxide-mediated arteriole dilatation was shown to be markedly attenuated due to suppressed nitric oxide formation and the effect is reversed by addition of cooper to the medium (64). Similarly, depressed aorta dilatation has been found in copper deficient rabbits (65). Furthermore, i*n vivo* studies have revealed that long term dietary copper deficiency and compromised body copper brings about depressed acetylcholine-induced arteriole dilatation (66).

The contribution of copper to the regulation of vascular function was supported by human studies indicating that mild copper depletion causes elevated systolic and diastolic blood pressure (67). Additionally, in hypertensive patients copper plasma levels were found to be lower than in healthy controls (68).

The mechanism of copper action in the cardiovascular system is not fully elucidated. However, it is well documented that vasculature is very sensitive to detrimental action of the reactive oxygen species (ROS), but especially to the superoxide anion generated even under physiological conditions (69). It is also known that dietary copper deficiency markedly depresses Cu/Zn superoxide dismutase (SOD) activity in the heart and vessels, thus decreasing the protection against superoxide (70). In consequence, redox balance is perturbed and oxidant stress is increased affecting

cadiovascular function. On the other hand, taking into account the previously mentioned copper contribution to lysyl oxidase activity and collagen synthesis but also to cytochrome c oxidase activity and respiration, copper scarcity has the potential to disturb a wide range of metabolic processes vital for cardiac muscle and vessel function.

It should be stressed that adverse effects of copper deficiency on the cardiovascular system are not limited to the changes in the cardiac structure and function, vascular activity or ROS production.

Lipids and lipoproteins in copper deficiency

At present it is well documented that inadequate copper dietary intake has a pronounced effect on lipoprotein metabolism. In copper-deficient rats increased apoB100 synthesis has been noted contributing to elevated plasma LDL-cholesterol concentrations (71). Additionally, both severe and marginal copper deficit in the diet (0.2 mg/kg and 0.6 mg/kg) has been shown to induce atherosclerotic lesions in rabbits (72).

The mechanism of the relationship between copper status and lipoproteins is due to enhanced activity of a key enzyme of cholesterol synthesis in the liver – 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) induced by glutathione which is markedly elevated in copper deficiency (73, 74). Moreover, in copper-deficient state increased expression of genes encoding fatty acid synthase and sterol regulatory element binding protein 1 (SREBP-1) in the liver have been found contributing to overall greater endogenous lipid synthesis (75, 76).

Despite the above mentioned copper effect on lipids and lipoproteins turnover, the proinflammatory effect of low copper intake in the vascular endothelium and neutrophils contributes to atherosclerosis progression (77).

On the contrary, the beneficial effects of copper on atherosclerosis have been confirmed in animal studies indicating that dietary copper supplementation reduces atherosclerotic lesions and inhibits apoptosis in the aortic intima in cholesterol-fed rabbits and decreases plasma total cholesterol and triacylglycerol concentrations in steers and rats (78-81).

Moreover, increased copper intake has a favorable effect on antioxidant protection increasing superoxide dismutase expression in the rabbit aorta and in this way reducing the interaction of nitric oxide with superoxide, and hence potentiating a nitric oxide-mediated pathway that potentially protects against atherosclerosis (82). Improved antioxidant protection has been also observed in rats supplemented with copper due to increased activity of glutathione peroxidase and depressed levels of lipid peroxides (83).

It is worth noting that the above-mentioned disturbances in cardiovascular function and lipid metabolism following copper deficit in the diet in animals are similar to that found in humans with ischemic heart disease (84, 85). Thus, copper inadequacy has been recognized as an important or even decisive factor contributing to the epidemic of cardiovascular disease.

Copper status and cardiovascular risk

At present data concerning the association of copper deficiency and cardiovascular disease are equivocal. Albala et al. (86) have found that in patients with acute myocardial infraction daily copper intake is similar to that found in healthy controls, although in both groups it was below the recommended intake. Neggers et al. (87) have not noticed any relationship between copper status and lipoprotein profile in adult males and females. On the other hand, Mielcarz et al. (88) have demonstrated that in patients with coronary artery disease (CAD) there is a linear and inverse relationship between leukocyte copper level, recognized as a marker of body copper status, and the angiogram score. Additionally, serum copper levels in patients with CAD were markedly lower than in healthy ones (89).

However, there are also data suggesting that the progress in atherogenesis, the risk of cardiovascular disease and cardiovascular mortality increase with increasing plasma copper levels (90-92). Moreover, in an apparently healthy population unfavorable changes in lipoprotein profile have been noted with increasing plasma copper levels (93). Furthermore, recent data have shown that in healthy men both dietary copper intake and its plasma levels were significantly and inversely associated with total and LDL-cholesterol levels but also significantly and positively correlated with highly-sensitivity C-reactive protein (hCPR) concentrations - the marker of inflammation and with nitrotyrosine-the marker of oxidative stress (94). The above data indicate a divergent effect of copper status on cardiovascular risk factors with higher copper intake reducing atherogenic LDL-cholesterol plasma levels but also promoting oxidative stress.

The data cited above clearly demonstrate that the role of copper in atherogenesis and cardiovascular disease in humans is far from being elucidated.

However, animal studies shed more light on the relationship between copper status and atherosclerosis indicating biphasic modulation of atherosclerosis by copper. In the rabbit fed high cholesterol both high copper intake (20 mg/day) and no copper in the diet were associated with increased aortic lesions compared to moderately supplemented animals (1 mg and 3 mg of copper daily) (95). The above data also suggest that there is an optimal copper intake with an increased susceptibility to aortic atherosclerosis in copper deficit and excess.

On the other hand, it is feasible that in humans low dietary copper intake and other false dietary habits contribute to the development of atherosclerosis. Recently Aliabaldi (96) has suggested that at least in the USA high consumption of fructose with processed foods together with low copper intake possibly lead to increasing incidents of cardiovascular disease. This seems feasible as in animals fructose has been found to exacerbate the effects of long-term marginal copper intake such as heart hypertrophy, hyperlipidemia and increased mortality (97).

Copper, insulin and carbohydrate metabolism

Despite the above mentioned stimulatory effects of copper deficiency on enzymes contributing to lipid metabolism it is well documented that copper scarcity also affects insulin action and in this way possibly alter both lipid and carbohydrate turnover.

Cohen et al. (98) have demonstrated that rats fed a copper-poor diet have a reduced insulin response to an oral glucose load in comparison with rats fed a copper supplemented diet. In addition, the increment in plasma glucose above the fasting level was significantly higher in rats maintained on a copper-poor diet suggesting impaired glucose tolerance in copper deficiency. Impaired glucose tolerance in copper-deficient rats has been confirmed by Hassel et al (99). Moreover, *in vitro* glucose incorporation into diaphragm glycogen and into adipose tissue was stimulated by the addition of copper chloride to the incubation medium (98).

On the contrary, in diabetic, copper-deficient rats the effect of copper plus insulin injection on glucose plasma levels after glucose load was markedly greater than that of insulin or copper alone (100). Furthermore, the stimulation of glucose incorporation into adipose tissue by combined insulin and copper injection was greater than by insulin or glucose alone suggesting that copper mimics insulin action on glucose turnover.

The mechanism of the insulin-like effect of copper action in adipocytes has been studied by Cohen et al (101). They have demonstrated that copper stimulates glucose incorporation into lipids even after removal of the insulin receptor by enzymatic treatment of adipocytes. Thus, the authors have postulated that copper either affects cell membrane permeability or exerts oxidant action such as e.g. hydrogen peroxide formation which is also known to stimulate glucose uptake

On the other hand, it has been demonstrated that copper, despite its influence on glucose tolerance and disposal, affects islet function stimulating insulin release from perfused rat pancreas and preventing the inhibitory action of interleukin-1 on insulin secretion (102, 103). Additionally, it should be pointed out that copper deficiency affects pancreas morphology. Fields and Lewis (104) have indicated reduced glandular mass, but Tosh et al. (105) have shown islet hyperplasia and disorganized α and β-cell distribution with copper deficiency.

Recent studies concerning molecular aspects of insulin-mimetic copper action have confirmed that copper ions, like insulin, strongly activate phosphoinositide-3-kinase/Akt pathway (106, 107). Since Akt is known to stimulate glycogen synthase, copper deficiency brings about a net depression of glycogen synthesis**.**

Whole-body consequences of insulin-like copper effects on carbohydrate metabolism have been noted in rats fed a low-copper diet which reduce body weight due to greater fat but less carbohydrate utilization as energy source under resting conditions (108). Additionally, it is worth noting that at least some copper effects on substrate utilization may be mediated by leptin, since positive and significant correlation has been noted between plasma copper and leptin levels (109).

Copper and physical activity

Taking into account the above mentioned effects of copper deficiency on cytochrome c oxidase activity (i.e. mitochondrial respiration) and substrate utilization (i.e. shift from carbohydrate to fat utilization) and the positive correlation between maximal oxygen uptake and plasma copper levels indicated by Savas et al. (110) adequate copper status in highly active subjects seems to be important for performance.

On the other hand, regular physical training, even in the face of adequate daily copper intakes, adversely affects plasma copper levels in young men and women and plasma copper concentrations lower than the physiological limit have been noted in 47% of physically active young females (111-113). Moreover, prolonged physical activity has been found to depress plasma ceruloplasmin activity both in humans and animals (114, 115).

As a reason for adverse effects of physical stress on plasma copper ion losses with sweat and urine have to be taken into consideration (115).

However, Koury et al. (116) have noted that in endurance and sprint athletes daily copper intake is highly differentiated and varied from 0.5 to 4 mg per day. Thus, at least in some athletes daily copper intake was below the recommended intake. In Polish male endurance athletes daily copper intake covered 87-108% of the safe intake level, but in female athletes it covered 59-76% of the safe intake level (117). Additionally, approximately 40% of male senior athletes and 75% of female senior athletes were at risk of copper deficiency by consuming less than 2/3 of the safe intake level. In regularly active Polish students daily copper intake covered 56% and 40% of the safe intake levels in males and females, respectively and the risk

of deficiency was found in 69.9% of male and 94.4% of female subjects (31).

Thus, at least in some athletes and active subjects low copper status is related to false dietary habits, which together with copper losses with sweat and urine, adversely affect plasma copper levels and performance. Additionally, poor plasma copper status in athletes decreases plasma ceruloplasmin concentration and antioxidant protection (118). Taking into account that strenuous physical exercise induces free radical production adequate copper status and appropriate antioxidant protection is of special importance in high performance athletes (119, 120).

Despite that until now copper supplements are not recommended due to the lack of reliable indices of marginal copper deficiency and hazardous for health effects of copper excess.

Summing up, due to the pronounced effect of copper on many metabolic processes such as mitochondrial respiration, collagen and elastin cross-linking, antioxidant protection, lipid and lipoprotein as well as carbohydrate metabolism, an adequate daily copper intake is important for both health and performance. However, in many populations copper intake is inadequate and possibly contributes to health problems including increased risk of atherosclerosis. On the other hand, copper excess is harmful adversely affecting iron status, lipoprotein metabolism and antioxidant protection.

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