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Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance

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Introduction

The human body has an elaborate system for managing and regulating the amount of key trace metals circulating in blood and stored in cells. Nutrient metals from our diet are incorporated into blood if blood levels are depleted, transported into cells if cellular levels are inadequate, or excreted if blood and cell levels are sufficient or overloaded. When this system fails to function properly, abnormal levels and ratios of trace metals can develop. One of the most common trace-metal imbalances is elevated copper and depressed zinc. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals [1].

There are 2-4 grams of Zn distributed throughout the human body [2]. Most zinc is in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye [3]. It is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes [2,4].

Copper is also a vital dietary nutrient, although only small amounts of the metal are needed for well-being [5]. Although copper is the third most abundant trace metal in the body [behind iron and zinc], the total amount of copper in the body is only 75-100 milligrams [6]. Copper is present in every tissue of the body, but is stored primarily in the liver, with fewer amounts found in the brain, heart, kidney, and muscles [7].

The Path and the Role of Zinc [Zn] in Organisms

Biological role of Zn

Zinc is involved in numerous aspects of cellular metabolism [8]. It was estimated that about 10% of human proteins potentially bind zinc, in addition to hundreds which transport and traffic zinc. It is required for the catalytic activity of more than 200 enzymes [9,10] and it plays a role in immune function [10,11], wound healing [10], protein synthesis, DNA synthesis and cell division [12]. Zinc is required for proper sense of taste and smell [13,14] and supports normal growth and development during pregnancy, childhood, and adolescence [15-18]. It is believed to possess antioxidant properties, which may protect against accelerated aging and helps speed up the healing process after an injury; however, studies differ as to its effectiveness [16,19]. Zinc ions are effective antimicrobial agents even at low concentrations. Gastroenteritis is strongly attenuated by ingestion of zinc and this effect could be due to direct antimicrobial action of the zinc ions in the gastrointestinal tract, or to the absorption of the zinc and re-release from immune cells [all granulocytes secrete zinc], or both [20,21,22]. Cells in the salivary gland, prostate, immune system and intestine use Zn signaling as one way to communicate with other cells [23]. In the brain, zinc is stored in specific synaptic vesicles by glutamatergic neurons and can modulate brain excitability [24]. It plays a key role in synaptic plasticity and so in learning [24-26]. It also can be a neurotoxin, suggesting zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system [24].

Zn metabolism

In blood plasma, Zn is bound to and transported by albumin (60%, low-affinity) and transferrin (10%) [27]. Since transferrin also transports iron, excessive iron can reduce zinc absorption, and vice-versa [28]. The concentration of zinc in blood plasma stays relatively constant regardless of zinc intake [29]. Zinc may be held in metallothionein reserves and also transferred in metal transporters of ZIP and ZnT family transporter proteins [30]. Metallothioneins in intestinal cells are capable of adjusting absorption of zinc by 15–40% [31]. Excess zinc particularly impairs copper absorption because metallothioneins absorb both metals [32].

Zn enzymes

Two examples of zinc-containing enzymes are carbonic anhydrase and carboxypeptidase, which are vital to the processes of carbon dioxide (CO_2)regulation and digestion of proteins, respectively [33,34]. In vertebrate blood, carbonic anhydrase (Figure 1) converts CO_2 into bicarbonate and the same enzyme transforms the bicarbonate back into CO_2 for exhalation through the lungs [35]. Without this enzyme, this conversion would occur about one million times slower at the normal blood pH of 7 or would require a pH of 10 or more [36]. Carboxypeptidase cleaves peptide linkages during digestion of proteins. A coordinate covalent bond is formed between the terminal peptide and a C=O group attached to zinc, which gives the carbon a positive charge. This helps to create a hydrophobic pocket on the enzyme near the Zn, which attracts the non-polar part of the protein being digested [34].

Zn serves a purely structural role in zinc fingers (Figure 2) [37]. Zinc fingers form parts of some transcription factors, which are proteins that recognize DNA base sequences during the replication and transcription of DNA. Each of the nine or ten Zn^{2+} ions in a zinc finger helps maintain the finger's structure by coordinately binding to four amino acids in the transcription factor [38]. The transcription factor wraps around the DNA helix and uses its fingers to accurately bind to the DNA sequence [39,40]. Zn ions are coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine [41]. The metal also has a flexible coordination geometry, which allows proteins using it to rapidly shift conformations to perform biological reactions [42].

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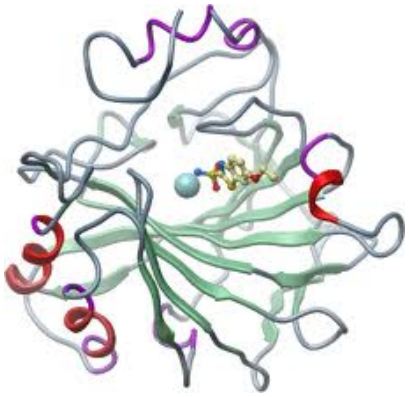

Figure 1: Diagram of human carbonic anhydrase, with Zn atom visible in the center.

Figure 2: The Zn ion (green), coordinated by two histidine side chains in Zn fingers.

Zn transport system

Zinc functions in biology are numerous, but can be separated into three main categories: catalytic, regulatory, and structural roles [42]. Greater than ten percent of the human genome codes for zinc-containing proteins [43]. Zn homeostasis is controlled by the coordinated actions of Zn transporters, which are responsible for Zn influx and efflux, and regulate the intracellular and extracellular Zn concentration and distribution. Zn transporters contribute to cellular events at the molecular, biochemical, and genetic level, with recent progress uncovering the roles of Zn transporters in physiology and pathogenesis [42,43].

Tight-control of cellular zinc homeostasis is maintained by proteins that can affect the amount of available zinc. Metal transporters of ZIP family and zinc transporters of ZnT family, as well as zinc-binding by metallothioneins; maintain control of intracellular zinc levels. Currently, 10 ZnT and 14 ZIP transporters have been identified. The ZnT proteins function in cellular zinc efflux or vesicular storage. ZnT1 was the first zinc transporter to be characterized, and is expressed in all tissues, localizing to the plasma membrane of cells [43]. Subsequent studies revealed zinc-regulated expression of ZnT1, and that zinc regulates expression of ZnT1 through activation of the metal response element – metal binding transcription factor MTF-1. Already known metal-inducible genes regulated by MTF-1 include the metallothioneins, glutamate–cysteine ligase heavy chain (which codes for an oxidative stress-related protein), and already mentioned ZnT1. MTF-1 responds to changes in intracellular zinc and other heavy-metals (cadmium and copper), where upon metal-occupancy there is translocation of MTF-1 from the cytosol to the nucleus. Subsequently this transcription factor binds to metal response elements located in the proximal promoters of metal responsive genes, resulting in an increased rate of transcription [43]. The importance of MTF-1 to zinc homeostasis and mammalian physiology is accentuated by the fact that ablation of the gene leads to severe liver degeneration and embryonic lethality in mice. Subsequent investigation of a liver-specific conditional knockout of the MTF-1 gene in mice revealed another MTF-1 regulated zinc transporter gene – ZIP10: The experiment on isolated mice hepatocytes showed that MTF-1 is an integral part of ZIP10 related cellular zinc homeostasis in the liver both during zinc restriction and zinc excess. The results showed that MTF-1 has physiologic significance and can act as a repressor of ZIP10 under normal cellular levels of zinc. Upon reducing cellular zinc, repression is lost and MTF-1 is not translocated to the nucleus, allowing enhanced ZIP10 transcriptional activation. The results also show that ZIP10 is a new target to investigate dietary influences on zinc metabolism by the liver and within specific regions of the brain. Zinc is believed to have second messenger functions in brain and the ZIP transporters are believed to be a key to cellular Zn homeostasis [43].

The Path and the Role of Copper [Cu] in Organisms

Biological role of Cu

Copper plays an important role in our metabolism, largely because it allows many critical enzymes to function properly [44]. Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Cu plays a role in the production of hemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally [7,44,45]. Copper can act as both an antioxidant and a pro-oxidant. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and contribute to the development of a number of health problems and diseases. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause [5,46-48]. When copper acts as a pro-oxidant at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease [49,50]. Maintaining the proper dietary balance of Cu, along with other minerals such as zinc and manganese, is important [5].

Cu metabolism

Copper is absorbed in the gut and transported to the liver bound to albumin. It enters the bloodstream via the plasma protein called ceruloplasmin, where its metabolism is controlled, and is excreted in bile [51]. A transporter protein on the cells of the small bowel, copper membrane transporter 1 - CMT1, carries copper inside the cells, where some is bound to metallothioneins and part is carried by copper transport protein - ATOX1 (copper chaperone for ATP7A and ATP7B) to the trans-Golgi network. Here, in response to rising concentrations of copper, an enzyme called ATP7A releases copper into the portal vein to the liver. Liver cells also carry the CMT1 protein, and metallothioneins and ATOX1 bind Cu inside the liver cells, but here it is ATP7B that links copper to ceruloplasmin and releases it into the bloodstream, as well as removing excess copper by secreting it into bile [52]. Approximately 90% of the copper in the blood is incorporated into ceruloplasmin, which is responsible for carrying copper to tissues that need the mineral [44,45]. Since excretion of copper is so slow (10% in 72 hours) an excessive dose of Cu is a lingering problem [51]. Proper absorption and metabolism of copper requires an appropriate balance with the minerals zinc and manganese. Because zinc can compete with

copper in the small intestine and interfere with its absorption, persons who supplement with inappropriately high levels of zinc and lower levels of copper may increase their risk of copper deficiency [44,45]. In addition to the role of ceruloplasmin as a transport protein, it also acts as an enzyme; catalyzing the oxidation of minerals, most notably iron [51]. The oxidation of iron by ceruloplasmin is necessary for iron to be bound to its transport protein- transferrin, so iron deficiency anemias may be a symptom of copper deficiency [5,45]. Mutations in copper transport proteins, ATP7B and ATP7A, can disable these transport systems and leading to Wilson's disease and Menkes disease, respectively [53,54].

Cu enzymes

Copper proteins have diverse roles in biological electron transport and oxygen transportation, processes that exploit the easy interconversion of Cu(I) and Cu(II) [55]. In cytochrome c oxidase, which is required for aerobic respiration, copper and iron cooperate in the reduction of oxygen. Copper is also found in Cu/Zn superoxide dismutase, an enzyme that detoxify superoxides, by converting it to oxygen and hydrogen peroxide: $2\ HO_2 \rightarrow H_2O_2 + O_2$ [7,55]. Copper is also a component of lysyl oxidase, an enzyme that participates in the synthesis of collagen and elastin, two important structural proteins found in bone and connective tissue. As a part of the enzymes cytochrome c oxidase copper plays a role in energy production, as part of dopamine ß-hydroxylase a role in conversion of dopamine to norepinephrine, and with Factor IV helps in blood clotting. Copper is also important for the production of the thyroid hormone thyroxine. A copper- containing enzyme tyrosinase, converts tyrosine to melanin. Cu is also necessary for the synthesis of phospholipids found in myelin sheaths in peripheral nerves [5,7,44,45]. Several Cu protein do not interact directly with substrates, hence they are not enzymes. These proteins relay electrons by the process called electron transfer [55].

Cu/Zn Superoxide dismutase1 [Cu/ZnSOD1]

Cu/ZnSOD1 is one of the three human superoxide dismutases identified and characterized in mammals (SOD2-manganese superoxide dismutase and SOD3-extracellular superoxide dismutase) [56,57,58]. Cu/ZnSOD1 is a homodimeric detoxifying metalloenzyme localized mostly in the cytosol and also in nucleus, peroxisomes, and mitochondria [56]. When SOD1 was isolated for the first time, it was thought to be a copper storage protein; the catalytic function of SOD1 was discovered in 1969 and it was clear that SOD1 acts as a scavenger of superoxide, through a two-step reaction involving reduction and reoxidation of the copper ion in its active site (Figure 3) [59]. Primarily, this reaction occurs in the cytoplasm where SOD1 is highly expressed. In the 1990s, the scientific community focused their studies on the genetic and biochemical characterization of SOD1, demonstrating that SOD1 is linked to diseases as inflammatory bowel disease [60], cancer [61], hypertension and cardiovascular diseases [62,63], Down's syndrome [64] and amyotrophic lateral sclerosis [65,66,67]. In 1993, the first SOD1 gene mutations associated with ALS were described [56]. The human SOD1 gene is located on chromosome 21q22.11, and it codes for the monomeric SOD1 polypeptide (153 amino acids, molecular weight 16 kDa). The coding region consists of five exons interrupted by four introns. Several polymorphisms have been identified in SOD1 gene, mainly distributed in the regulatory regions, including promoter and introns [68].

Superoxide radicals are generated during normal metabolism, as well as when white blood cells attack invading bacteria and viruses (phagocytosis). If not eliminated quickly, superoxide radicals cause damage to cell membranes [5,48]. When copper is not present in sufficient quantities, the activity of superoxide dismutase is diminished, and the damage to cell membranes caused by superoxide radicals is increased. When functioning in this enzyme, copper works together with the mineral zinc, and it is actually the ratio of copper to zinc, rather than the absolute amount of copper or zinc alone, that helps the enzyme function properly (Figure 4) [44,45].

How coordination of metal ions modulates protein structures is not only important for elucidating biological function but has also emerged as a key determinant in protein turnover and protein-misfolding diseases [69]. The study of folding catalysis by transient coordination of Zn to the Cu ligands of the Cu/Zn superoxide dismutase showed that the coordination of Zn to the enzyme Cu/Zn superoxide dismutase is directly controlled by the protein's folding pathway. Zn first catalyzes the folding reaction by coordinating transiently to the Cu ligands of SOD1. Then, after the global folding transition has commenced, the Zn^{2+} ion transfers to the higher affinity Zn site, which structures only very late in the process. The relatively rapid equilibration of metals in and out of the SOD1 structure [$10^{-5}s^{-1}$] provided an explanation: if a dissociated Zn^{2+} ion is prevented from rebinding to the SOD1 structure then the lifetime of the encyme is reduced to a just a few hours [69].

The maturation and activation of the Cu/Zn superoxide dismutase (SOD1) are highly regulated processes that require several post-translational modifications. The maturation of SOD1 is initiated by incorporation of zinc and copper ions followed by disulfide oxidation leading to the formation of enzymatically active homodimers [70,71]. Present data indicate that homodimer formation is a regulated final step in SOD1 maturation and implicate the recently characterized copper homeostasis protein COMMD1 in this process. COMMD1 interacts with SOD1, and this interaction requires copper incorporation into SOD1. COMMD1 does not regulate disulfide oxidation of SOD1 but reduces the level of enzymatically active SOD1 homodimers. RNAi-

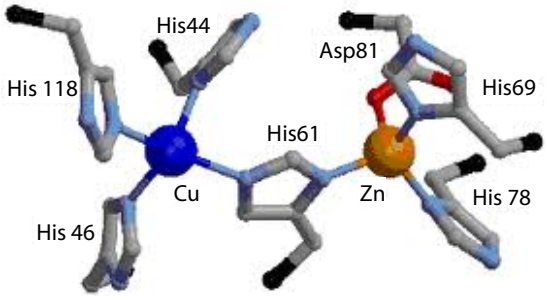

Figure 3: Active site of Cu/Zn Superoxide dismutase1.

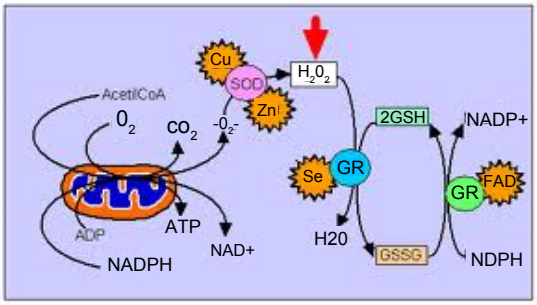

Figure 4: Cu/Zn SOD mostly presented in the cytoplasm.

mediated knockdown of COMMD1 expression results in a significant induction of SOD1 activity and a consequent decrease in superoxide anion concentrations, whereas overexpression of COMMD1 exerts exactly the opposite effects. COMMD1 is a novel protein regulating SOD1 activation and associate COMMD1 function with the production of free radicals [70,71].

Cu chaperones

The intracellular trafficking of Cu to copper-dependent proteins is fundamental to normal cellular metabolism. The copper chaperones perform the dual functions of trafficking and the prevention of cytoplasmic exposure to copper ions in transit. Only a small number of copper chaperones have been identified at this time but their conservation across plant, bacterial and animal species suggests that the majority of living systems utilise these proteins for copper routing [72]. The available data suggest that each copper-dependent protein in the cell is served by a specific copper chaperone. Specificity for a given copper-dependent protein appears to be mediated by the residues surrounding the copper-binding motif [73].

Copper is needed within mitochondria to supply the Cu proteins and intramembrane Cu sites of cytochrome oxidase, within the trans-Golgi network to supply secreted cuproproteins and within the cytosol to supply Cu/Zn superoxide dismutase1 (SOD1). Subpopulations of copper-zinc superoxide dismutase also localize to mitochondria, the secretory system and the nucleus [74]. Copper metallochaperones assist copper in reaching vital destinations without inflicting damage or becoming trapped in adventitious binding sites [72]. Copper ions are specifically released from copper metallochaperones upon contact with their cognate cuproproteins, so metal transfer is thought to proceed by ligand substitution [73].

Copper chaperone for SOD1 specifically delivers copper to SOD1 in cytoplasm of mammalian cells. In the present study, small interfering RNA targeting copper chaperone for SOD1 (CCS) was introduced into metallothionein-knockout mouse fibroblasts (MT-KO cells) and their wild type cells (MT-WT cells) to reveal the interactive role of CCS with other Cu-regulating proteins. CCS knockdown significantly decreased the level of Cu influx transporter (Ctr1). On the other hand, mRNA expression for a Cu efflux transporter (ATP7A) was increased and was 3.0 - 2.5 times higher than those of the control. The experiment also showed that the ATP7A knockdown significantly increased the intracellular Cu concentration. The activity of SOD1 were maintained in both MT-WT and MT-KO cells even when copper chaperone for SOD1 protein expression was reduced to 0.30-0.35 of control. This suggests that the amount of CCS protein exceeds that required to supply Cu to SOD1 in the cells. Further, the CCS knockdown induces Cu accumulation in cells. The results showed that Cu accumulation is ameliorated by the metallothionein induction, the decrease of Ctr1 expression and the increase of ATP7A expression to maintain Cu homeostasis [76].

Sources of Copper and Zinc and Dietary Intakes

Food sources

A daily intakes of Cu and Zn is required to maintain a steady state because the body has no specialized storage system of these two elements [5,12,77]. A wide variety of foods contain zinc [18]. Oysters contain more zinc per serving than any other food, but red meat, especially beef, lamb and liver have some of the highest concentrations of zinc in food [78]. Other good food sources include beans, nuts, other types of seafood (such as crab and lobster), whole grains, cereals, almonds, pumpkin seeds, sunflower seeds [18,78]. Rich sources of copper include oysters and other sea food, beef and organ meats (especially liver), dark green leafy vegetables, enriched cereals, nuts and sunflower seeds, green olives, avocados, dried legumes, chocolate, cocoa, and black pepper [5,45].

Dietary supplements

Supplements contain several forms of zinc, including zinc gluconate, zinc sulfate, and zinc acetate. The percentage of elemental zinc varies by form of supplement [79].Scientists has not determined whether differences exist among forms of zinc in absorption, bioavailability, or tolerability [17].

Multivitamins that include minerals generally provide copper. It is also available as an individual oral supplement. Cu supplements should not be given to children, at children it should be obtained from food [5,7].

Recommended intakes for Zn

Intake recommendations for Zn are provided in the Dietary Reference Intakes developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies. The current Recommended Dietary Allowances [RDA] for zinc is listed in Table 1 [18]. For infants aged 0-6 months, the FNB established an Adequate Intake * for Zn (= intake of Zn in breastfed infants) [80,81]. U.S. National Research Council set a Tolerable Upper Intake for adults of 40 mg/day [82,83].

Recommended intakes for Cu

The 10th edition of Recommended Dietary Allowances (RDA) did not include an RDA for copper; rather a safe and adequate daily intake was suggested. Some nutritionists think, committees that establish RDAs should return to the traditions of the first nine editions and make recommendations that meet functional needs, because lack of a recommended dietary allowance for copper may be hazardous to health [81,84]. The following Table 2 provide the Recommended daily dietary intake (RDI) of copper for children and adults and Tolerable upper intake levels for copper [83,85].

Interactions of Cu and Zn with micronutrients

Fortification of foods with iron in iron-deficiency anemia does not significantly affect zinc absorption. But large amounts of supplemental iron, greater than 25 mg, might decrease zinc absorption [86,87]. Taking iron supplements between meals helps decrease its effect on zinc absorption [87]. Several laboratory and human studies have found that high levels of supplemental zinc taken over extended periods of time may result in decreased copper absorption in the intestine, and copper deficiency with associated anemia [88]. But some studies in humans suggest that high dietary Zn may not interfere with the actual tissue or plasma concentrations of Cu [7]. Some experts believe that elevated copper levels, especially when zinc levels are also low,

Age	Male [mg]	Female [mg]	Pregnancy [mg]	Lactation [mg]
0–6 months	2 *	2 *		
7–12 months	3	3		
1–3 years	3	3		
4–8 years	5	5		
9–13 years	8	8		
14–18 years	11	9	12	13
19+ years	11	8	11	12

Table 1: Recommended Dietary Allowances [RDAs] for Zn /per day.

Age	Male [mcg]	Female [mcg]	Tolerable Upper Intake Levels [mcg]
0–6 months	200	200	Not possible to establish a TUL, sources of Cu must be from food and formula only.
7–12 months	220	220	
1–3 years	340	340	1000
4–8 years	440	440	1000
9–13 years	700	700	5000
14–18 years	890	890	8000
19+ years	900	900	10000
Pregnancy		1000	8000 [14-18 years], 10000 [19 + years]
Lactation		1300	8000 [14-18 years], 10000 [19 + years]

Table 2: Recommended Daily Intakes [RDIs] and Tolerable Intake Levels [TULs] for Cu.

may be a contributing factor in many medical conditions including schizophrenia, hypertension, autism, fatigue, muscle and joint pain, headaches, childhood hyperactivity, depression, insomnia, senility, and premenstrual syndrome [1]. Adults taking copper supplements should also take zinc supplements (8 - 15 mg of zinc for every 1 mg of copper), because of an imbalance of these two minerals [89,90]. Copper is known to react with a variety of nutrients, including iron, zinc, molybdenum, sulfur, selenium and vitamin C [5]. There's also some evidence, not conclusive, that high supplemental doses of vitamin C in a range approaching 1 gram or more-may decrease Cu availability [91]. There is also some evidence that in the formula feeding of infants, too much iron in a formula can lower absorption of copper from that formula [45,92].

Zinc deficiency

Nearly two billion people in the developing world are deficient in zinc [93]. Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function [94,95,96]. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions. Weight loss and impaired appetite, delayed healing of wounds, taste abnormalities, and altered cognition can also occur [13,17,94,97-99]. When zinc deficiency does occur, it is usually due to inadequate zinc intake or absorption, increased losses of zinc from the body, or increased requirements for zinc [94,98]. It can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses [97-102]. In children it causes an increase in infection and diarrhea, contributing to the death of about 800,000 children worldwide per year [94,101]. The WHO advocates zinc supplementation for severe malnutrition and diarrhea [103].

Severe Zn deficiency depresses immune function [104], and even mild to moderate degrees of zinc deficiency can impair macrophage and neutrophil functions, natural killer cell activity, and complement activity [105,106]. The body requires zinc to develop and activate T-lymphocytes [107]. Individuals with low zinc levels have shown reduced lymphocyte proliferation response to mitogens and other adverse alterations in immunity that can be corrected by zinc supplementation [104,108]. These alterations in immune function might explain why low zinc status has been associated with increased susceptibility to pneumonia and other infections in children in developing countries and the elderly [94, 109-111]. However, zinc supplements should not be administered alone, since many in the developing world have several deficiencies, and zinc also interacts with other micronutrients [112].

Groups at risk of Zn inadequacy

Pregnant and lactating women: Pregnant women, particularly those starting their pregnancy with marginal zinc status, are at increased risk of becoming zinc insufficient due, in part, to high fetal requirements for zinc [113]. Lactation can also deplete maternal zinc stores [114]. For these reasons, the RDA for zinc is higher for pregnant and lactating women than for other women [80]. The following study was performed, to analyse the inter-relationship among trace elements: iron, copper and zinc of blood sample in pregnant women. The level of copper was found to be significantly higher in iron deficiency anaemia, when compared to non-iron deficiency anaemia [$p<0.05$], and it was also higher in non-anaemic pregnant women, compared to non-anaemic non-pregnant women [controls]. The level of zinc was significantly lower in iron deficiency anaemic pregnancy, when compared to the other groups. There is evidence of influence of pregnancy on the level of trace elements in blood. This could be a result of competitive inhibition in the absorption of trace elements in the intestine, or an effect of hormonal changes [insulin, estrogen], during pregnancy [115].

Breast-fed infants: Zinc deficiency in breast-fed infants is a rare disease caused by a low level of zinc in their mother's milk [116]. Premature infants are more vulnerable to develop zinc deficiency than full-term infants because, despite their high zinc requirements, they have insufficient body stores of zinc and a poor capability to absorb zinc from the gut. Breast milk provides sufficient zinc [2 mg/day] for the first 4–6 months of life but does not provide recommended amounts of zinc for infants aged 7–12 months, who need 3 mg/day. In addition to breast milk, infants aged 7–12 months should consume age-appropriate foods or formula containing zinc [80,116,117]. Zinc supplementation has improved the growth rate in some children who demonstrate mild-to-moderate growth failure and who have a zinc deficiency [94,117].

People with gastrointestinal diseases: Gastrointestinal surgery and digestive disorders, such as ulcerative colitis, Crohn's disease, and short bowel syndrome can decrease zinc absorption and increase endogenous zinc losses primarily from the gastrointestinal tract [100,102,118].

Vegetarians: Phytates, which are present in whole-grain breads, cereals, legumes, and other foods, bind Zn and inhibit its absorption. Thus, the bioavailability of zinc from grains and plant foods is lower than that from animal foods, although many grain- and plant-based foods are still good sources of zinc [119-121].

People with sickle cell disease: Results from a large cross-sectional survey suggest that 44% of children with sickle cell disease have a low plasma zinc concentration [122], possibly due to increased nutrient requirements and/or poor nutritional status [123]. Zinc deficiency also affects approximately 60%–70% of adults with sickle cell disease [124]. Zinc supplementation has been shown to improve growth in children with sickle cell disease [123].

Diarrhea: Chronic diarrhea also leads to excessive loss of zinc [94]. Pooled analysis of randomized controlled trials show that malnourished children in India, Africa, South America, and Southeast Asia experience shorter and less severe courses of infectious diarrhea after taking zinc supplements [112]. The children in these studies received 4–40 mg of zinc a day in the form of zinc acetate, zinc gluconate, or zinc sulfate [109,112]. Similar findings were reported in a meta-analysis published in 2008 and a 2007 review of zinc supplementation for preventing and treating diarrhea [125,126]. But the effects of zinc supplementation on

diarrhea in children with adequate zinc status, such as most children, are not clear. The WHO and UNICEF now recommend short-term zinc supplementation (20 mg of zinc per day, or 10 mg for infants under 6 months, for 10–14 days) to treat acute childhood diarrhea [103].

Alcoholics: Approximately 30%–50% of alcoholics have low zinc status because ethanol consumption decreases intestinal absorption of zinc and increases urinary zinc excretion [127]. In addition, the variety and amount of food consumed by many alcoholics is limited, leading to inadequate zinc intake [128,129].

Acrodermatitis enteropathica

Zinc supplementation is an effective treatment for acrodermatitis enteropathica, an inborn error of zinc metabolism that is inherited as an autosomal recessive disorder. The lack of zinc presents, characteristically, as: pustular dermatitis, diarrhea, and nail dystrophy. Irritability and emotional disturbances are due to atrophy of the brain cortex. The severity of the disease is proportional to the zinc level. Before zinc supplementations acrodermatitis enteropathica was fatal to babies born with it [97,98].

Cooper Deficiency

Because copper is involved in many functions of the body, copper deficiency can produce an extensive range of symptoms [130]. Deficiencies of copper can result in hernias, aneurysms, blood vessel breakage manifesting as bruising or nosebleeds [5], iron deficiency anemia [45], osteoporosis and joint problems [131], brain disturbances [54], abnormalities in glucose and cholesterol metabolism [132], increased susceptibility to infections due to poor immune function [neutropenia] [133], loss of pigment, weakness, fatigue, skin sores, poor thyroid function [5], irregular heart beat [134] and low body temperature [46]. If copper is important in cellular membrane structure, then a copper deficiency could seriously alter the movement of nutrients through cell walls [5].

Groups at risk of Cu inadequacy

Despite the fact that most people consume less than recommended amounts of copper in their diet, these symptoms of copper deficiency are relatively rare [5]. However, certain medical conditions including chronic diarrhea, celiac sprue, Crohn's disease and GIT surgery result in decreased absorption of copper and may increase the risk of developing a copper deficiency. Inflammatory bowel disease (IBD) may be related to oxidation or damage caused by free radicals. In fact, copper levels may be low in the inflamed tissue of those with IBD, particularly Crohn's disease. When treating IBD, clinicians often recommend multivitamin containing essential minerals [135]. In addition, copper requires sufficient stomach acid for absorption, so people who consume antacids regularly may increase the risk of developing a copper deficiency [7]. Inadequate copper status is also observed in children with low protein intake and in infants fed only cow's milk without supplemental copper (they might have poor feeding habits and lack of proper gowth) [5,45].

Menkes disease

Menkes disease is an X-linked recessive disorder characterized by copper deficiency resulting in a diminished function of copper-dependent enzymes. A diversity of mutations in the gene encoding of the copper-transporting ATPase, ATP7A (located on chromosome Xq12-q13), underlies Menkes disease [68]. Signs and symptoms of this disorder include weak muscle tone, sagging facial features, seizures, mental retardation, developmental delay and kinky, colorless hair. There can be an extensive neurodegeneration in the gray matter of the brain. Arteries in the brain can also be twisted with split inner walls. This can lead to rupture or blockage of the arteries. Osteoporosis may result in fractures. Most patients die in early childhood, although mild forms have also been described [53,54].

Diagnosis of Copper and Zinc Deficiency

The diagnosis of Cu and Zn deficiency is based on four main criteria, namely anamnesis, symptomatology, belonging to well-defined risk groups and the determination of biomarkers [5,7,8]. The optimal plasma or serum ratio between these two elements is 0.70 - 1.00 [1].

Diagnosing zinc deficiency is a persistent challenge. Zinc nutritional status is difficult to measure adequately using laboratory tests due to its distribution throughout the body as a component of various proteins and nucleic acids [18,136]. Plasma or serum zinc levels are the most commonly used indices for evaluating zinc deficiency, but plasma zinc, has poor sensitivity and specificity - these levels do not necessarily reflect cellular zinc status due to tight homeostatic control mechanisms. So clinical effects of zinc deficiency can be present in the absence of abnormal laboratory indices [17,25,47].

Severe Cu deficiency can be found by testing for low plasma or serum copper levels, low ceruloplasmin, and low red blood cell superoxide dismutase levels; but these are not sensitive at a state of marginal copper status [46,137]. The cytochrome c oxidase activity of leucocytes and platelets has been stated as another factor in deficiency, but in the studies this hypotesis has not been confirmed yet [138].

Copper and Zinc Toxicity

Health risks from excessive zinc

Although zinc is an essential requirement for good health, excess zinc can be harmful [9]. Excessive absorption of zinc suppresses copper and iron absorption [32]. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches [9]. One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate [139]. Intakes of 150–450 mg of zinc per day have been associated with such chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins. Reductions in the superoxide dismutase levels, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks [140,141].

Zn poisoning

In 1982, pennies were made primarily of zinc covered with copper. With the new zinc pennies, there was the potential for zinc toxicosis, which can be fatal. One reported case of ingestion of 425 pennies (over 1 kg of zinc) resulted in death due to gastrointestinal bacterial and fungal sepsis, while another patient, who ingested 12 grams of zinc, showed lethargy and ataxia [142,143].

Copper toxicity

Excessive copper intake can cause nausea, vomiting, abdominal pain and cramps, headache, dizziness, weakness, diarrhea, and a metallic taste in the mouth (assosiated with water containing copper concentrations greater than 6 mg/L) [5]. Chronic copper toxicity does not normally occur in humans because of transport systems that regulate absorption and excretion [144]. Since excess copper is excreted through bile, copper toxicity is most likely to occur in individuals with liver disease or other medical conditions in which the excretion of bile is compromised [5]. Whether copper is carcinogenic has not been

determined yet [145-147]. Postpartum depression has also been linked to high levels of copper. This is because copper concentrations increase throughout pregnancy to approximately twice normal values, and it may take up to three months after delivery for copper concentrations to normalize [148]. There are scientific articles that indicate a link between long-term exposure to high concentrations of copper and a decline in intelligence with young adolescents [149]. Whether this should be of concern is a topic for further investigation.

Cu poisoning

In recent years, nutritionists have been more concerned about copper toxicity than copper deficiency. One explanation for this was the increase in the amount of copper found in drinking water due to the switch in some areas from galvanized water pipes to copper water pipes. Cooking with copper cookware can also increase the copper content of foods. Industrial exposure to copper fumes, dusts or mists may result in metal fume fever with atrophic changes in nasal mucous membranes [5]. Intentionally high uptakes of copper may cause liver and kidney damage and even death. Gram quantities of various copper salts have been taken in suicide attempts and produced copper toxicity in humans, possibly due to redox cycling and the generation of reactive oxygen species that damage DNA [150]. Corresponding amounts of copper salts (30 mg/kg) are toxic in animals [151].

Wilson's disease or hepatolenticular degeneration

Wilson's disease is a rare, progressive, autosomal recessive disorder characterised by impaired transport and excessive accumulation of copper in the liver, brain, and other tissues [152]. The condition is due to mutations in the Wilson disease protein of ATP7B gene (located on chromosome 13q14.3) [68], by impaired copper incorporation to ceruloplasmin and biliary copper excretion. It is characterisied by a hepatic cirrhosis, neurological manifestations (dystonia, dysarthria, muscle weakness, vertigo), psychiatric manifestations, renal disease, and copper deposition in the cornea – Kayser-Fleischer ring (Figure 5) [53,54,152]. The treatment of Wilson's disease involves avoidance of foods rich in copper and any supplements containing copper and drug treatment with chelating agents that remove the excess copper from the body (D-penicillamine, Zn acetate)[153].

In the study of copper toxicosic in humans, lipid peroxidation and copper content were significantly increased ($P < 0.05$) in hepatic mitochondria from patients with Wilson's disease. More modest increases in lipid peroxidation were present in microsomes from patients with Wilson's disease. Mitochondrial copper concentrations correlated strongly with the severity of mitochondrial lipid peroxidation. These data suggest that the hepatic mitochondria are an important target in hepatic copper toxicity and that oxidant damage to the liver may be involved in the pathogenesis of copper-induced injury. A significant decrease (37%) in the vitamin E/lipid ratio was also detectable in patients with Wilson's disease showing high free serum copper (>10 micrograms/dl). The data support a role for free radicals in the pathogenesis of active liver diseases [154].Another study revealed that impaired conversion of 25(OH)D to 1,25(OH)2D occurs in copper intoxication and suggests that altered vitamin D metabolism is a potential factor in the development of bone and mineral abnormalities in Wilson's disease [155].

Figure 5: Kayser-Fleischer's ring (the deposition of copper on Descemet's membrane and the anterior and posterior lens capsule).

The Studies about the Role of Copper and Zinc in Diseases

Immunological disorders

The common cold: Researchers have hypothesized that zinc could reduce the severity and duration of cold symptoms by directly inhibiting rhinovirus binding and replication in the nasal mucosa and suppressing inflammation [156]. Although studies examining the effect of zinc treatment on cold symptoms have had somewhat conflicting results, overall zinc appears to be beneficial under certain circumstances. In a randomized, double-blind, placebo-controlled clinical trial, 50 subjects (within 24 hours of developing the common cold) took a zinc acetate lozenge (13.3 mg zinc) or placebo every 2–3 wakeful hours. Compared with placebo, zinc lozenges significantly reduced the duration of cold symptoms (cough, nasal discharge, and muscle aches) [157]. In another clinical trial involving 273 participants with experimentally induced colds, zinc gluconate lozenges significantly reduced the duration of illness compared with placebo but had no effect on symptom severity [158]. In 2007, Caruso and colleagues published a structured review of the effects of zinc lozenges, nasal sprays, and nasal gels on the common cold. Of the 14 randomized, placebo-controlled studies included, 7 showed that the zinc treatment had a beneficial effect and 7 showed no effect [159]. A Cochrane review concluded that zinc (lozenges or syrup) is beneficial in reducing the duration and severity of the common cold in healthy people, when taken within 24 hours of onset of symptoms [160]. Recently (in August 2011), in the meta-analysis all of the identified placebo-controlled trials, included in MEDLINE, Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL) databases, were analysed together. The study shows strong evidence that the zinc lozenge effect on common cold duration is heterogeneous so that benefit is observed with high doses of zinc but not with low doses: a total daily zinc dose of less than 75 mg found no effect on common cold duration, whereas using zinc acetate in daily doses exceeding 75 mg showed a 42% reduction in the duration of colds and using zinc salts other than acetate in daily doses exceeding 75 mg showed a 20% reduction in the duration of colds. The effects of zinc lozenges should be further studied to determine the optimal lozenge compositions and treatment strategies [161]. Numerous case reports of anosmia, in some cases long-lasting or permanent, from the use of zinc-containing nasal gels or sprays (but only in these forms) raise questions about the safety of intranasal zinc [162, 163]. In June 2009, the FDA warned consumers to stop using three zinc-containing intranasal products because they might cause anosmia [164]. On the basis of the long-term studies with high zinc doses, mentioned before, there does not seem to be any basis for assuming that treating the common cold for a week with high doses of zinc in the form of lozenges would cause unanticipated harm. A patient suffering from acute adverse effects such as bad taste can simply stop taking the lozenges [161].

Arterial and venous leg ulcers: Zinc helps maintain the integrity of skin and mucosal membranes [105]. Patients with chronic leg ulcers have abnormal zinc metabolism and low serum zinc levels [165]. Clinicians frequently treat skin ulcers with zinc supplements [166]. The authors of a systematic review concluded that zinc sulfate might be effective for treating leg ulcers in some patients who have low serum

zinc level, but it is not effective in the general use in patients with chronic leg ulcers, arterial or venous ulcers [167,168].

Wound healing: Copper plays a major role in wound healing. Scientists think that introducing copper into wound dressings would not only reduce the risk of wound and dressing contamination, but also stimulate faster healing. Releasing copper from the dressings directly onto the wound promotes skin regeneration [169].

Burns: When skin is burned, a substantial percentage of micronutrients, such as copper, selenium, and zinc may be lost. This increases the risk for infection, slows the healing process, prolongs the hospital stay, and even increases the risk of death. However, people with major burns tend to lose copper more rapidly than other minerals. Although it is unclear which micronutrients are most beneficial for people with burns, many clinical studies suggest that a multivitamin including copper and other minerals may aid in the recovery process [170].

Immunological disorders on animal models: Another study shows that copper deficiency in mice impairs immune system function. Dietary copper was restricted in mice during five discrete intervals over a 9 week period of perinatal development: gestation only (G), lactation only (L), 3 week postlactation (PL), 1 week after birth through postlactation (2/3L + PL), and lactation plus postlactation (L + PL). Signs of severe copper deficiency, such as low liver copper levels, and significant reductions in activity of plasma ceruloplasmin and splenocyte Cu/Zn superoxide dismutase were most evident in 6-week-old mice from two groups, -Cu 2/3L + PL and -Cu L + PL. Mice in these groups were anemic and had small thymuses and enlarged spleens compared to controls receiving +Cu treatment. The -Cu mice demonstrated impaired antibody - plaque-forming cells response (PFC) to sheep erythrocytes, and the attenuation was proportional to copper deficiency, as judged by liver copper levels. Total plasma IgM levels were not greatly altered by -Cu treatment except in model L + PL. Total IgG levels were markedly reduced in this group and in the –Cu 2/3L + PL group. The PFC response of mice in the -Cu PL group was normal even though signs of copper deficiency were evident; however, the PFC response was reduced when -Cu treatment was extended to 5 weeks, and was reversible by switching to +Cu treatment. It is evident that severity of copper deficiency in mice is related to degree of impaired immunity. Furthermore, severity of copper deficiency is dependent on duration and time of initiation of dietary copper restriction [171].

In the next study, the effects of severe, moderate, and mild copper deficiencies on cellular and humoral immunity of fifty male rats were studied. All rats were immunized once with sheep red blood cells. Mean plasma-copper concentration reflected the dietary levels of copper, and ceruloplasmin activity correlated highly to plasma copper. Rats consuming suboptimal levels of copper responded differently to the deficiencies, so copper status varied among those animals. After 8 weeks, cell proliferation, when stimulated by phytohemagglutinin, was dependent on the copper status of the animal. Severely deficient rats had consistently lower lymphocyte stimulation indexes for phytohemagglutinin, but specific antibody response was not reduced. ImmunoglobulinG concentrations were variable for all rats, and immunoglobulin M concentrations were lower for the severely deficient rats [172].

The role of Cu/ZnSOD1 in oxidative stress

Detailed studies in the past two decades have shown that redox active metals like iron, copper, chromium, cobalt and other metals undergo redox cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radical and nitric oxide in biological systems. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders, chronic inflammation and others. A special position among metals is occupied by the redox inert metal zinc. Zn is an essential component of numerous proteins involved in the defense against oxidative stress. It has been shown, that depletion of Zn may enhance DNA damage via impairments of DNA repair mechanisms. In addition, Zn has an impact on the immune system and possesses neuroprotective properties. The mechanism of metal-induced formation of free radicals is tightly influenced by the action of cellular antioxidants. Besides the encymes (SOD, catalase), many low-molecular weight antioxidants (ascorbic acid, alpha-tocopherol, glutathione, carotenoids, flavonoids, and other antioxidants) are capable of chelating metal ions reducing their catalytic activity to form reactive oxygen species [173]. In the study designed to assess the antioxidant status and erythrocyte oxidative injuries in Iranian fat-tailed sheep that suffered from malignant theileriosis, blood samples were taken and hematological parameters, the activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase, erythrocyte osmotic fragility, and serum concentrations of some trace elements (copper, iron, zinc, manganese, and selenium), were measured. As an index of lipid peroxidation, the level of malondialdehyde (MDA) was also determined. According to the results, a significant decrease in red blood cell count, packed cell volume, the activities of SOD, GPX, and catalase ($p<0.001$), and also serum concentrations of Cu, Zn, Mn, and Se ($p<0.05$) were evident in the infected sheep. Significantly increased levels of MDA and erythrocyte osmotic fragility ($p<0.001$) as well as serum concentration of iron ($p<0.05$) were recorded in the infected animals. The significant decrease in antioxidant enzyme activities and substantial elevated levels of lipid peroxidation and erythrocyte osmotic fragility associated with the increase in parasitemia indicate increased exposure of red blood cells to oxidative stress. It appeared that disturbed antioxidant defense mechanisms can promote the development of anemia in ovine theileriosis [174].

In another study, of Cu/Zn imbalance in rats, the scientists determined the content of copper in blood, the activity of superoxide dismutase (SOD), and the content of malondialdehyde (MDA) to analyse the relations among the copper concentration, the copper-zinc ratio (Cu/Zn) and the biomarkers of lipid peroxidation (MDA), by controlling the level of copper intake in copper deficiency rats. In the status of copper deficiency, the SOD activity was lower than the normal level, and then presented a rise trend with the increased of copper intake that is, the ratio of copper-zinc (Cu/Zn). The content of MDA was higher than the normal level in Cu deficiency rats and the content of MDA decreased with the increasing of copper intake and the Cu/Zn ratio, and maintained at a relatively low level [175].

Developmental disorders

The studies show that zinc deficiency during early development can result in multiple brain abnormalities and altered neuronal functions [25,96]. In rats, a gestational deficit of Zn can affect the rat fetal brain cytoskeleton and signaling cascades involved in cellular processes that are central to brain development. Scientists tested the hypothesis that oxidative stress is involved in Zn deficiency-induced altered tubulin dynamics and the associated dysregulation of transcription factor

[NF-κB] in rat cortical neurons in animal model of Zn deficiency. A low rate of in vitro tubulin polymerization, an increase in tubulin oligomers, and a higher protein cysteine oxidation were observed in the Zn-deficient neuronal cells. In this animal model of Zn deficiency the conclusion was that a deficit in Zn viability could affect early brain development through: an induction of oxidative stress, tubulin oxidation, altered tubulin dynamics and deregulation of signals (NF-κB) involved in critical developmental events [176]. In another study, rat embryos were fed with Cu-adequate (8 micrograms Cu/g) or Cu-deficient (< 0.5 micrograms Cu/g) diet and were cultured in Cu-adequate (16.2 microM) or Cu-deficient (1.0 microM) rat serum. Control embryos cultured in control serum were morphologically normal. Cu-deficient embryos developed abnormally when cultured in Cu-deficient serum; the abnormalities included distended hind brains, blisters, blood pooling, and cardiac defects. Control embryos cultured in Cu-deficient serum and Cu-deficient embryos cultured in control serum also showed abnormal development, but to a lesser degree than that of the Cu-deficient embryos cultured in Cu-deficient serum. To test the idea that the above abnormalities were due in part to free radical induced damage occurring secondary to an impaired oxidant defense system, a chemiluminescence assay was used to detect superoxide dismutase (SOD) activity in the cultured embryos. SOD activity was lowest in embryos cultured in Cu-deficient serum. When the Cu-deficient serum was supplemented with antioxidants (Cu/ZnSOD or glutathione peroxidase), its teratogenicity was reduced. These data support the idea that an impaired oxidant defense system contributes to the dysmorphology associated with Cu deficiency. However, the Cu-deficient embryos also had low cytochrome c oxidase activity compared to control embryos - thus, multiple factors are likely contributing to Cu deficiency induced abnormalities [177].

The Studies of Cu/Zn imbalance in neurological disorders

The highest levels of zinc are found in the hippocampus in synaptic vesicles, boutons, and mossy fibers. Zinc is also found in large concentrations in the choroid layer of the retina. Zinc plays an important role in axonal and synaptic transmission and is necessary for nucleic acid metabolism and brain tubulin growth and phosphorylation. Lack of zinc has been implicated in impaired DNA, RNA, and protein synthesis during brain development. For these reasons, deficiency of zinc during pregnancy and lactation has been shown to be related to many congenital abnormalities of the nervous system. Furthermore, in children insufficient levels of zinc have been associated with lowered learning ability, apathy, lethargy, and mental retardation. Children with attention deficit disorder may be deficient in zinc and vitamin B-6 and have an excess of lead and copper. Alcoholism, schizophrenia, Wilson's disease, and Pick's disease are brain disorders dynamically related to zinc levels. Zinc has been employed with success to treat Wilson's disease, achrodermatitis enteropathica, and specific types of schizophrenia [3].

Also, copper and zinc are regarded as neurotransmitters and are in high concentrations in brain hippocampus. As a result elevated copper and depressed zinc have been associated with hyperactivity, attention deficit disorders, behavior disorders, and depression. Also, many of those labeled with autism and paranoid schizophrenia have elevated blood copper levels in addition to other biochemical imbalances [1]. Elevated copper/zinc ratios can be especially serious for persons with low blood histamine. This combination of imbalances has been associated with anxiety, panic disorders, paranoia, and, in severe cases, hallucinations [1]. Low histamine patients are typically overstimulated with thoughts racing through their minds making normal ideation difficult. Low histamine children are hyperactive while often healthy in other respects. Serum Cu levels in these patients are abnormally high. Since Cu is a brain stimulant and destroys histamine (over-methylation), the elevated serum (and presumably brain) Cu level probably accounts for many symptoms, including the low blood histamine level. If the laboratory tests showed a high copper: low zinc ratio and low histamine levels, the treatment should consist of the administration of zinc, manganese, vitamin C, niacin, vitamin B-12, and folic acid. Folic acid in conjunction with B-12 injections raises blood histamine while lowering the degree of symptomatology. Zn allows for the normal storage of histamine in both the blood cells and the brain. Zn and Mn increase the urinary excretion of Cu [178].

On the other hand in the research of Effect of a deficiency of ceruloplasmin copper in blood plasma on copper metabolism in the adult rat brain, the copper deficiency in adult rats was induced by addition of silver chloride to the feed [179]. The concentrations of silver, copper, iron, and zinc and relative activity of genes for copper transporting proteins and copper enzymes were measured in the cortex, cerebellum, hippocampus, amygdala, pituitary gland, and hypothalamus. Silver was accumulated only in the hypothalamic-pituitary system. These changes were accompanied by a decrease in the concentration of copper and increase in the contents of iron and zinc. Activity of genes for copper transport enzymes (high-affinity copper transporter; and two copper transport ATPases, ATP7A and ATP7B) and copper enzymes that were formed in the intracellular secretory pathway did not decrease in the brain of rats with copper deficiency. Relative activity of genes for intracellular copper enzymes (Cu/Zn superoxide dismutase and subunit IV of cytochrome c oxidase), concentration of immunoreactive polypeptides of superoxide dismutase, and enzymatic activity of superoxide dismutase remained unchanged under these conditions [179].

Hippocampal neuronal injury: An experiment was conducted to investigate whether intracellular zinc depletion can actually change expression of voltage-dependent anion channel VDAC1 and VDAC2 in cultured hippocampal neurons of rats. Hippocampal neurons were obtained by primary culture from hippocampus of newborn rats. Cultured hippocampal neurons were exposed to a cell membrane permeable zinc chelator – ethylenediamine. The results demonstrated that exposure of hippocampal neurons to chelator for 24 hours induced notably neuronal injury, significantly increased the number of apoptotic nuclei, up-regulated the expression of VDAC1 protein level and down-regulated the expression of VDAC2 protein level. Significant down-regulation of mRNA levels for both, VDAC1 and VDAC2 were observed. Co-addition of zinc almost completely reversed chelator induced neuronal injury and above alterations in VDAC1 and VDAC2 protein levels and mRNA levels. Present results implicate a possibility that up-regulation of VDAC1 and down-regulation of VDAC2 may participate in hippocampal neuron injury induced by zinc deficiency [180].

Traumatic brain injury: Depression, anxiety, and impairments in learning and memory are all associated with traumatic brain injury (TBI). Because of the strong link between zinc deficiency, depression, and anxiety, in both humans and rodent models, scientists hypothesized that dietary zinc supplementation prior to injury could provide behavioral resiliency to lessen the severity of these outcomes after TBI. Rats were fed with marginal zinc deficient, zinc adequate, or zinc supplemented diet for 4weeks followed by a moderately-severe TBI. While moderate zinc deficiency did not worsen outcomes following TBI, rats that were fed with the zinc supplemented diet for 4weeks showed significantly attenuated increases in adrenal weight

($p<0.05$) as well as reduced depression-like behaviors ($p<0.001$). Supplementation prior to injury improved resilience such that there was significant improvements in cognitive behavior compared to injured rats fed an adequate diet ($p<0.01$). These data suggest a role for supplemental zinc in preventing cognitive and behavioral deficits associated with TBI [181].

Brain ischemia : To determine whether the mitochondria or cytoplasm produces superoxides during ischemia - reperfusion of the brain, experts analyzed lucigenine-enhanced chemiluminescence emission in slices of mouse brain tissue prepared from manganese-superoxide dismutase (MnSOD2)-deficient and copper/zinc-superoxide dismutase [Cu/ZnSOD1]-deficient mice during oxygenation and hypoxia-reoxygenation. The steady-state level of chemiluminescence under oxygenated conditions was significantly enhanced by a lack of either SOD. They hypothesized that the enhanced chemiluminescence produced by SOD2 and SOD1 deficiency reflects in situ superoxide generation in the mitochondria and cytoplasm, respectively. The study also indicated that the major site of intracellular superoxide generation in the brain during oxygenation is the cytoplasm, whereas it is the mitochondria during reoxygenation [182].

Autism: Autism is a severe developmental disorder with poorly understood etiology. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipid peroxidation, detoxifying agents, such as glutathione, and other antioxidants involved in the defense system against reactive oxygen species. Lipid peroxidation markers are elevated in autism, indicating that oxidative stress is increased in this disease. Levels of major antioxidant serum proteins, namely transferrin and ceruloplasmin are also decreased in children with autism. There is a positive correlation between reduced levels of these proteins and loss of previously acquired language skills [183,184]. The alterations in ceruloplasmin and transferrin levels may lead to abnormal iron and copper metabolism [185]. The membrane phospholipids, the prime target of reactive oxygen species, are also altered [186]. Several studies have suggested alterations in the Cu/Zn ratio, the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, altered glutathione levels and homocysteine/methionine metabolism in autism [183-187]. One study has hypothesised that there is a significant difference in the copper/zinc ratio between young children who have autism and their typically developing peers and trying to test the hypothesis could correction of elevated copper to zinc ratios in children with autism be accomplished by oral supplementation with zinc and vitamin C, and if these children show measurable changes in improvement in receptive or expressive language or behavioral parameters associated with autism after supplementations with some of trace elements. Anyway, the results about this research topic have not yet been revealed [188]. Additionally, increased inflammation, excitotoxicity, as well as mitochondrial and immune dysfunction have been suggested in autism. Furthermore, environmental and genetic factors may increase vulnerability to oxidative stress in autism [188].

Amyotrophic lateral sclerosis: Amyotrophic lateral sclerosis disease (ALS) is a multifactor and multigenic disorder with still unknown aetiology and pathogenesis. The pathological characteristics of ALS include protein aggregation, proteasome inhibition, impaired axonal transport, mitochondria damage and apoptosis, oxidative stress, glutamate induced excitotoxicity, neuroinflammation and transcriptional dysfunction. Many compounds targeted to one or more of these mechanisms have been tested in multiple clinical trials. Nonetheless, nowadays only one drug, riluzole, has demonstrated a positive effect in the disease progression, but a number of recent compounds are promising in ALS therapy [189]. The discovery that in approximately 10% of ALS patients mutations in SOD1 gene cause a subset of familial amyotrophic lateral sclerosis has attracted great attention, and studies have been mainly focused on discovering mutations in the coding region and investigation at protein level. Considering that changes in SOD1 mRNA levels have been associated with sporadic ALS, a molecular understanding of the processes involved in the regulation of SOD1 gene expression could unravel novel regulatory pathways that may govern cellular phenotypes and changes in diseases. The progress in understanding the mechanisms of transcriptional and post-transcriptional control could offer hope for the development of new-generation drugs or medical treatment strategies [190].

Because alteration of the activity of SOD1 leads to an oxidative stress imbalance, which damages the structure of lipids and proteins in the CNS, the membrane fluidity was monitored in the spinal cord and the brain in a widely used animal model of ALS, the SOD)(G93A) mouse, which develops symptoms similar to ALS with an accelerated course. The results show that the membrane fluidity of the spinal cord in this animal model significantly decreased in symptomatic animals compared with age-matched controls. Changes in membrane fluidity likely contribute substantially to alterations in cell membrane functions in the nervous tissue from SOD(G93A) mice [191]. Also, the spinal cord and brain of SOD(G93A) mice showed increased lipid peroxidation after 100 or 130 days compared to age-matched controls. The CNS was most affected, but lipid peroxidation was also detected in the skeletal muscle and liver on day 130. Thus, oxidative stress represents a potential biomarker that might be useful in developing new therapeutic strategies for ALS [192]. When replete with zinc and copper, amyotrophic lateral sclerosis (ALS)-associated mutant SOD proteins can protect motor neurons in culture from trophic factor deprivation as efficiently as wild-type SOD. However, the removal of zinc from either mutant or wild-type SOD results in apoptosis of motor neurons through a copper- and peroxynitrite-dependent mechanism. It has also been shown that motor neurons isolated from transgenic mice expressing mutant SODs survive well in culture but undergo apoptosis when exposed to nitric oxide via a Fas-dependent mechanism. It was found that zinc-deficient SOD-induced motor neuron death required Fas activation, whereas the nitric oxide-dependent death of SOD(G93A)-expressing motor neurons required copper and involved peroxynitrite formation. Surprisingly, motor neuron death doubled when Cu/Zn-SOD protein was either delivered intracellularly to SOD(G93A)-expressing motor neurons or co-delivered with zinc-deficient SOD to nontransgenic motor neurons. These results could be rationalized by biophysical data showing that heterodimer formation of Cu/Zn-SOD with zinc-deficient SOD prevented the monomerization (the active form of the enzyme) and subsequent aggregation of zinc-deficient SOD. Taken together, these results are consistent with copper-containing zinc-deficient SOD being responsible for the toxic gain of function conferred by mutant SOD [193].

Alzheimer's disease: Alzheimer's disease (AD) is a highly heterogeneous and progressive dementia which is characterised by a progressive decline in cognitive functioning, selective neuronal atrophy, and loss of cortical volume in areas involved in learning and memory. Recent study has indicated that the AD-affected brain is also besieged by increases in oxidative stress as well as perturbations to the homeostasis of biometals, such as copper and iron. These metals are known to interact with the neuropathological hallmark of AD, the β-amyloid peptide (Aβ), in a manner which increases Aβ's neurotoxic effects. The reported results suggest that zinc competes with copper for Aβ binding and inhibits copper-mediated Aβ redox chemistry [194].

In another study dyshomeostasis of extracellular zinc and copper has been implicated in β-amyloid aggregation, the major pathology associated with Alzheimer disease. Presenilin mediates the proteolytic cleavage of the β-amyloid precursor protein to release β-amyloid, and mutations in presenilin can cause familial Alzheimer disease. In the recent study there was tested, whether presenilin expression affects copper and zinc transport in murine embryonic fibroblasts from presenilin knock-out mice. They observed a marked decrease in saturable uptake of radiolabeled copper and zinc in several tissues, including brain. Copper/zinc superoxide dismutase activity was significantly decreased and copper chaperone of SOD1 levels was also decreased. These data indicate that presenilins are important for cellular copper and zinc turnover, influencing SOD1 activity, and having the potential to indirectly impact β-amyloid aggregation through metal ion clearance [195].

Despite the crucial role of redox active metals like copper and iron in central biological reactions, their elevated levels are involved in the pathogenesis of Alzheimer's disease. Similarly reactive oxygen/nitrogen species (ROS/RNS) produced during normal metabolic activities, specifically oxidative phosphorylation of the cell, are scavenged by superoxide dismutase, catalase, but impaired metabolic pathways tend to generate elevated levels of these ROS/RNS. Alterations in trace elements as iron, copper, and zinc may intensify this process and contribute for the pathogenesis of Alzheimer's disease [196].

The aim of the present study was to evaluate the status of plasma essential trace elements magnesium, copper, zinc, iron and selenium concentrations and their some related antioxidant enzyme activities, erythrocyte glutathione peroxidase (GPX), superoxide dismutase, and catalase activities in patients with Alzheimer's disease. Fifty patients with AD and fifty healthy control subjects were included in this study. Plasma Cu and Zn concentrations by atomic absorption spectrometry (AAS), plasma Mg and Fe concentrations by spectrophotometric methods and plasma Se concentrations by graphite furnace AAS were determined. Erythrocyte GPX, SOD and catalase activities were measured by spectrophotometric methods. Plasma Mg, Cu, Zn, Fe and Se levels and erythrocyte GPX, SOD and catalase activities were found to be significantly lower in patients with AD compared with controls. These results suggest that alterations in essential trace elements and their related enzymes may play a role in the etiopathogenesis of AD. Also, there is a defect in the antioxidant defense system, which may lead to oxidative damage in patients with AD. The changes in antioxidant enzyme activities may be secondary to the alterations in their cofactor concentrations [197].

Parkinsonism: The biometals iron, manganese and copper have been associated to Parkinson's disease and Parkinsonism. In recent work, it was reported for the first time that acute or chronic Fe, Mn and Cu exposure significantly reduced life span and locomotor activity in Drosophila melanogaster. It was shown that the concentration of those biometals dramatically increase in Drosophila's brain acutely or chronically fed with metal, and that the metal accumulation in the fly's head is associated with the neurodegeneration of several dopaminergic neuronal clusters. Furthermore, they found that the chelator desferoxamine, ethylenediaminetetraacetic acid, and D-penicillamine were able to protect but not rescue D. melanogaster against metal intoxication. Taken together these data suggest that iron, manganese and copper are capable to destroy dopaminergic neurons in the fly's brain, thereby impairing their movement capabilities [198].

Malignant glioma: The scientists assessed relevance of mineral trace element and heavy metal levels in patients with malignant gliomas. In the study, erythrocyte catalase, and carbonic anhydrase, serum copper, zinc, lead, iron, cadmium, cobalt, manganese, and magnesium levels were measured in plasma of 22 healthy humans and 22 malignant glioma patients. The Cd, Fe, Mg, Mn, Pb and Zn levels were significantly elevated in the patients as a whole compared to controls ($P<0.05$), while copper was decreased and cobalt demonstrated no change. Although mean erythrocyte activity were significantly lowered, carbonic anhydrase exhibited significant increase. The results of the current study indicate that antioxidant enzymes may have a role in the genesis of considerable oxidative stress in patients with malignant glioma [199].

Serum copper and zinc concentrations and copper/zinc ratios have been shown to be increased in several types of human malignancies, including human brain tumors. In this study, copper and zinc levels and copper/zinc ratios were determined by atomic absorption analysis in tissue and serum from 29 primary and metastatic brain tumor patients. Metastatic carcinomas and malignant gliomas revealed significantly higher tissue copper concentrations than control tissues and meningiomas. Malignant gliomas demonstrated significantly higher tissue copper/zinc ratios. Also in serum, both serum copper and copper/zinc ratio were significantly higher in the metastatic carcinoma group than control. There were no differences both in the serum and the tissue concentrations of these trace elements in meningiomas and controls. These data suggested that copper, an important angiogenic factors, is accumulated within the malignant tissues of metastatic carcinoma and malignant glioma, but not meningiomas. These findings may have implications regarding Cu in angiogenesis in these tumors [200].

Age-related macular degeneration: Some studies have suggested that both zinc and antioxidants delay the progression of age-related macular degeneration (AMD) and vision loss, possibly by preventing cellular damage in the retina [89,90]. In a population-based cohort study in the Netherlands, high dietary intake of zinc as well as beta carotene, vitamin C, and vitamin E was associated with reduced risk of AMD [201]. Participants also received 2 mg of copper to prevent the copper deficiency associated with high zinc intakes. After an average follow-up period of 6.3 years, supplementation with antioxidants plus zinc (but not antioxidants alone) significantly reduced the risk of developing advanced AMD and reduced visual acuity loss. Zinc supplementation alone significantly reduced the risk of developing advanced AMD in subjects at higher risk but not in the total study population [201,202]. However, the authors of a systematic review and meta-analysis published in 2007 concluded that zinc is not effective for the primary prevention of early AMD, although zinc might reduce the risk of progression to advanced AMD [203].

Auditory system disorders: Copper is a trace element known to be critical for normal brain function, and abnormal copper metabolism in rats has been associated with some disorders involving the auditory system. The scientists examined effects of copper deficiency on metabolism in major structures of the auditory system. Cochlea, cochlear nucleus and inferior colliculus of rats, as well as whole brain, were assayed for activities of enzymes of oxidative and glycolytic energy metabolism - malate and lactate dehydrogenase, enzymes of acetylcholine metabolism -choline acetyltransferase and acetylcholinesterase, and concentrations of amino acids. Whole brain was also assayed for activity of superoxide dismutase, and concentrations of minerals. The significant differences between copper-deficient and copper-adequate rats were: decreased copper and magnesium and increased potassium concentrations in whole brain of copper-deficient rats and an elevation of glutamine concentration

in inferior colliculus and whole brain of copper-deficient rats. The elevated glutamine could not be related to any change in activity of glutamine synthetase or glutaminase, major enzymes of glutamine metabolism. It is speculated that the increase in glutamine might result from a net increase in ammonia accumulation in the brains of copper-deficient rats [204].

Metabolic and endocrinological disorders

Disorders of thyroid gland: The aim of the present study was to investigate the effect of copper deficiency on thyroid hormone metabolism in rats. Therefore, an experiment with growing male rats was carried out, consisting of two groups of rats fed either a copper-deficient (0.06 mg Cu/kg) or a copper-adequate diet (16 mg Cu/kg). Copper deficiency decreased the final body weight of the rats by 5% compared to copper-adequate control rats. A severe copper-deficient state in the rats fed the copper-deficient diet was proved by a large decrease of ceruloplasmin activity in serum (by 97%) and hematological changes. Copper-deficient rats had an increased concentration of T3 in serum, whereas the concentrations of total and free thyroxine were not different compared with copper-adequate control rats [205].

In the subsequent study the intracellular localization of Cu/Zn- and Mn-superoxide dismutase was studied in the thyroid tissue of various thyroid disorders by an immunohistochemical technique. The concentrations of both SODs in those tissues were measured also by a sandwich enzyme immunoassay technique. Copper/zinc-SOD in thyroid tissues was identified by immunocytochemical staining in most cases of papillary carcinoma and in some cases of other thyroid disorders. In normal follicular cells this enzyme is localized in the perinuclear cytoplasm, whereas in thyroid tumor or hyperplastic follicular cells it exists homogeneously in cytoplasm. Manganese-SOD stained strongly in papillary carcinoma and papillary-growing cells in the thyroid tissue of adenoma and Graves' disease. The concentrations of Cu/Zn-and Mn-SOD in thyroid tumor tissues and hyperplastic follicular disorders were significantly higher than those in normal thyroid tissue. In conclusion, SOD seems to be related to cell proliferation and differentiation in the thyroid follicular cell because Cu/Zn-SOD changes its localization in tumor and hyperplastic follicular cells and because the Mn-SOD concentration is increased in papillary carcinoma or papillary-growing cells [206].

Diabetes: A laboratory animal study found that copper-deficient rats tend to have elevated blood sugar levels over time, indicating a possible connection between low copper and diabetes [132]. But a clinical study including people with diabetes, however, found very different results. Copper levels were higher in people with diabetes compared to those without. In fact, the higher the copper level, the more likely the person was to have complications from diabetes, including retinopathy, high blood pressure, or vascular disease [207].

Digestive system: In the next study, a growing rat model of zinc deficiency was established to investigate the effect of zinc deficiency on intestinal mucosal morphology and digestive enzyme activity. Symptoms of zinc deficiency, such as anorexia, diarrhea, dermatitis, and growth retardation, were observed. Zinc deficiency can cause loss of appetite, weight loss, and decreased activity of peptidase in the jejunal mucosal brush border. Zinc deficiency has little effect on the height ratio of the villus and crypt and lactase activity, thereby indicating that zinc deficiency may first affect protein digestion and absorption [208].

Osteoporosis: Trace elements are essential for normal growth and development of skeletons in humans and animals. Although they are minor building components in teeth and bone, they play important functional roles in bone metabolism and bone turnover. Zinc regulates secretion of calcitonin from thyroid grand and influences on bone turnover. Copper induces low bone turnover by both suppressions of osteoblastic and osteoclastic functions. Among the trace elements in bone and hair, significant differences were found in the contents of zinc, copper and manganese between normal subjects and osteoporotic patients. However, exact involvements of the trace elements in osteoporosis have not yet been clarified [209].

Zinc is implicated as an activator for bone formation. The following study examined how zinc regulates the bone matrix calcification in osteoblasts. The findings suggest that zinc deprivation inhibits extracellular matrix calcification in osteoblasts by decreasing the synthesis and activity of matrix proteins, type I collagen and alkaline phosphatase, and decreasing Ca and P accumulation. Therefore zinc deficiency can be considered as risk factor for poor extracellular matrix calcification [210].

Testosterone deficiency: Testosterone deficiency is associated with late-onset hypogonadism. Micronutrients including copper and zinc have influence on testosterone synthesis. The association between micronutrient concentrations in hair tissue and serum testosterone was studied in Korean men. Subjects with normal testosterone group had a significantly higher Zn level compared to low testosterone group (P=0.003). Significant negative correlations were evident between total testosterone and Cu level (P=0.022), and the Cu/Zn ratio (P=0.008). Normal testosterone is associated with a higher Zn level. Decreased serum testosterone is significantly associated with a high level of Cu and elevated Cu/Zn ratio in hair tissue [211].

The role of Zn in fertility: Semen is particulary rich in Zn, which is a key factor in prostate gland function and reproductive organ growth [78]. In the present study on rats, scientists observed changes in the testes after dietary zinc deficiency. Ultrastructural studies revealed several apoptotic features such as wavy basement membrane, displaced nuclei, chromatin condensation, plasma membrane blebbing, nuclear membrane dissolution, loss of inter-Sertoli cell junctional complexes, and intercellular bridges and deformed mitochondria. Increased apoptotic degeneration in testes may cause irreversible changes in the germ cells associated with decreased epididymal sperm concentration, motility, and fertility index which contributes to the low efficiency of spermatogenesis thereby indicating a possible role of zinc in fertility [212].

Cardiovascular system disorders

Little is known about the selective toxicity to the heart. Therefore, the following study demonstrated the relationship between the severity of copper deficiency-induced oxidative damage and the capacity of antioxidant defense in heart and liver to investigate a possible mechanism for the selective cardiotoxicity in rats. Copper deficiency induced a 2-fold increase in lipid peroxidation in the heart (thiobarbituric assay) but did not alter peroxidation in the liver. The antioxidant enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase were, respectively, 3-, 50- and 1.5-fold lower in the heart than in the liver, although these enzymatic activities were depressed in both organs by copper deficiency. In addition, the activity of glutathione reductase was 4 times lower in the heart than in the liver. The data suggest that a weak antioxidant defense system in the heart is responsible for the relatively high degree of oxidative damage in copper-deficient hearts [213].

Several research groups have demonstrated that essential trace elements play important roles in states of cardiovascular diseases. The

aim was to investigate whether there is a relationship between trace elements, Zn and Cu and the degree of atherosclerosis. The serum levels of zinc and copper were found to be significantly lower in patients with atherosclerosis than in the control group, but there were no significant differences in the serum levels of Cu and Zn between severe atherosclerosis and mild atherosclerosis. The present study revealed a relationship between the serum levels of zinc and copper and atherosclerosis, but not between these levels and the severity of the disease [214].

To understand the role of Cu and Zn in human blood both in controls as well as in cardiovascular (CVD) patients, whole blood samples of 181 CVD patients and 185 controls between the ages of 20-66 years were investigated. The mean blood-Cu levels (1.50 mg/l) were found as enhanced, whereas Zn levels (5.88 mg/l) were reduced in cardiovascular patients group. Cu/Zn ratios for CVD patients are also higher than in control subjects. However, when the CVD patients were checked for their systolic and diastolic pressure it was found that copper concentrations in these patients was significantly increased ($p < 0.001$) with the rise of blood systolic pressure, so a positive correlation was observed between copper and systolic pressure. Zn on the other hand has an inverse relation with systolic as well as diastolic pressure ($p < 0.001$). Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, and triglyceride (TG) in blood samples have also been determined and their probable role in the CVD complication has been observed. A positive correlation of blood Cu with TC, TG, and LDL-C indicates that rise in blood Cu levels may initiate the development of CVD. An increase in Cu/Zn ratio can instigate the cardiovascular risk factor. The findings from this study can definitely update our knowledge of the role of Cu and Zn in the development of CVD risk in humans [215,216].

Cu and Zn Interactions with Medications

Zn/Antibiotics

Both quinolone antibiotics and tetracycline antibiotics interact with zinc in the gastrointestinal tract, inhibiting the absorption of both zinc and the antibiotic. Taking the antibiotic at least 2 hours before or 4–6 hours after taking a zinc supplement minimizes this interaction [217,218].

Zn/Penicillamine

Zinc can reduce the absorption and action of penicillamine. To minimize this interaction, individuals should take zinc supplements at least 2 hours before or after taking penicillamine [86].

Zn/Diuretics

Thiazide diuretics such as chlorthalidone and hydrochlorothiazide increase urinary zinc excretion by as much as 60 %. Prolonged use of thiazide diuretics could deplete zinc tissue levels, so clinicians should monitor zinc status in patients taking these medications [219].

Cu/Nonsteroidal anti-inflammatory drugs [NSAIDs]

Copper binds to NSAIDs and appears to enhance their anti-inflammatory activity [220].

Cu/Penicillamine

Penicillamine reduces copper levels that may be the intended use - as a helating agent in the case of Wilson's disease to reduce toxic copper deposits [153].

Cu/Antacids

Antacids may reduce copper absorption by decreasing the amount of hydrochloric acid in the stomach [221].

Cu/Allopurinol

The studies suggest that allopurinol may reduce copper levels [222].

Cu/Oral contraceptives

Oral contraceptives increase the absorption of copper. Estrogen replacement for post-menopausal women can increase blood levels of copper. Estrogen enhances the absorption and half-life of copper which in turn inhibits the absorption of zinc [223].

Superoxide Dismutase/Valproic acid

The recent study evaluated changes in antioxidant status in blood during valproate monotherapy of adult patients with epilepsy. Significant differences between the study group and controls were found. The activity of erythrocyte SOD was higher in patients treated with valproate for a longer period (7-14 years) in comparison to controls ($p=0.001$) and patients with a short period of VPA treatment ($p<0.001$). Patients with uncontrolled epilepsy exhibited higher serum Zn than seizure-free patients ($p=0.041$). The antioxidant status of epileptic patients was modified by valproate monotherapy. The frequency of seizures and duration of VPA therapy were associated with changes of oxidative/antioxidative balance [224].

Conclusion/Summary

The human body has an elaborate system for managing and regulating the amount of key trace metals circulating in blood and stored in cells. When this system fails to function properly, abnormal levels and ratios of trace metals can develop. One of the most common trace-metal imbalances is elevated copper and depressed zinc. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals. Zn is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes, while copper is present in every tissue of the body, but is stored primarily in the liver, with fewer amounts found in the brain, heart, kidney, and muscles.

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of more than 200 enzymes and it plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division. Zinc possesses antioxidant properties, which may protect against accelerated aging and helps speed up the healing process after an injury.

Copper plays an important role in our metabolism, largely because it allows many critical enzymes to function properly. Copper is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Cu plays a role in the production of hemoglobin, myelin, melanin and it also keeps thyroid gland functioning normally. Copper can act as both an antioxidant and a pro-oxidant. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause. When copper acts as a pro-oxidant at times, it promotes free radical damage.

More than the concentration of Zn or Cu in blood serum, it is important the balance between them. If the balance is changed several organic systems can be affected. Different diseases can be prevented when supplements are taken, and different drugs affect Cu and Zn concentrations what can cause the onset of different diseases.

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