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Mini-Review

COPPER IN THE HUMAN ORGANISM

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ABSTRACT

Copper is an essential micronutrient for human bodies, whose distribution in different organs and tissues, metabolism and physiological impact is not thoroughly explained yet. Further issues which need clarification are the exact composition and constitution of its combinations with other elements, the physiological impact of copper and copper-enzymes in sickness and health. Biomarkers for identification of copper status are still being defined. They are necessary in order to determine the prophylactics and treatment of diseases and conditions, which are related to changes of copper concentrations in human bodies. Researches continue in an attempt to develop analytical methods for determination of copper in serum and urine, which are used in clinical laboratory diagnostics. These current developments justify the purpose of our research: to make a brief review of some issues related to studying the trace element copper. In the present paper, we have reviewed a part of the essential copper enzymes – cytochrome c oxidase, lysyl oxidase, feroxidase, monoamine oxygenase, superoxide dismutase, etc., e.g the physiological functions, which depend on the presence of these essential copper enzymes in the human organism. The issues of copper metabolism and the interactions of copper with other micronutrients (zinc, iron, vitamins A, C, E) are briefly discussed. An overview of the influence of copper on several diseases: Menkes syndrome, Wilson's disease, cardiovascular diseases, oxidative stress, osteoporosis, diabetes, chronic diarrhea, cystic fibrosis, is performed as well. The results from our study in children, aged 1 ÷ 3 years, with anemia, cystic fibrosis and chronic diarrhea and a healthy control group, showed significantly lower levels of copper in blood serum of patients as compared to controls. In conclusion , based on our brief review we might state that the results obtained from different researchers in respect of the influence of copper in different diseases and conditions are conflicting. There is a necessity of accurate and accessible methods for determination of serum copper in research and clinical biomedical laboratories. It is obvious that further research work is needed in order to clarify the contradictions and determine the role of copper in the human organism.

Key words: serum copper and diseases, copper enzymes, copper status.

INTRODUCTION

Copper (Cu) is an essential trace element for humans and animals. In the human organism, copper exists in two forms – the first and second oxidation form, as most of the copper in the human organism is in the second form. The ability of copper to easily attach and accept electrons explains its importance in oxidative reduction processes and in disposing and removing free radicals from the organism (1). Although scientists identified copper compounds to treat diseases in 400 BC (during Hippocrates) (2), researchers still discover new information regarding the biochemistry, physiology, toxicology, many clinical, laboratory and other indicators of the impact of copper in the organism. In this respect, the exact composition and structure of copper compounds, generated in the organism, are not yet fully elucidated. The overall and inter-organ distribution of copper is not quite determined (3-11). The role and the participation of copper and copper enzymes in

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the metabolism, as well as the interaction of copper with other micronutrients are not clearly specified (1-3, 10, 12-22). Changes in copper concentrations in body fluids and tissues are observed in different diseases and conditions. There are indications of serious diseases, caused by disorders of the metabolism of copper in the organism, but the role of copper in most of them is not completely clarified (11, 19-20, 23-49). Investigation of copper functions in human bodies requires accurate, affordable, informative, low-detection-limit methods for determination of trace copper in biological samples (7, 51-60). Although a considerable number of methods for copper investigation exists, research continues in search of more sophisticated analytical approaches, for its determination in serum and urine, applicable in clinical laboratory diagnostics. These current developments justify the purpose of our research; to make a brief review of some issues related to studying the trace element copper.

COPPER STATUS AND PHYSIOLOGICAL IMPACT

The human organism contains about 70 ÷ 80 mg of copper (3). There is evidence that its content varies over the year and depends on gender and age (4-9). With age significant differences are observed in the concentrations of ceruloplasmin (the main carrier of copper in the blood) (5). Regarding gender, some studies have found no statistically significant differences in the copper content of blood serum in healthy children (10), but other studies discovered that the concentration of serum copper has significantly higher levels in women ($p < 0.05$) than in men (7). There can not be distinguished clear trends in serum copper of subjects with drinking and smoking habits. Differences in serum copper are reported in people from different regions. According to some authors, the demographic differences are due to variations in copper content of the soil and / or food habits of the population. Increased physical activity is also found to reduce the concentration of serum copper ($p < 0.05$) (7). A strong positive correlation is established between serum copper and body mass index (BMI) ($R = 0.85$, $p < 0.001$) in large-scale sample of 2233 subjects, aged 15-65 years (11).

There is evidence for a link between copper levels in children and their parents. In a study of serum copper in 66 healthy children, aged 3-14 years, a positive correlation is found between levels of trace elements in children and their parents. It is believed that this is due to identical dietary habits of children and their parents (8).

Copper is a trace element which can be found in almost every cell of the human organism. The highest concentrations of copper are discovered in the brain and the liver; the central nervous system and the heart have high concentrations of copper as well. About 50% of copper content is stored in bones and muscles (in skeletal muscle it is about 25%), 15% in skin, 15% in bone marrow, 8 to 15% in the liver and 8% in the brain (3).

Copper is a functional component of several essential enzymes, known as copper enzymes – cytochrome c oxidase, lysyl oxidase, feroxidase, 2-furoate-CoA dehydrogenase, amine oxidase, catechol oxidase, tyrosinase, dopamine beta-monooxygenase, D-galaktozo oxidase, D-hexozo oxidoreductase, indole 2.3-dioxygenase, L-ascorbatoxidase, nitratreductase, peptidylglycine monooxygenase, flavonol 2,4-dioxygenase, superoxide dismutase, PHM (peptidylglycine monooxygenase hydroxylation) and others. (1, 2, 12-18)

Some physiological functions, dependent on the presence these enzymes in the organism, are described below.

Cytochrome oxidase plays an essential role in cellular energy. As catalyzing the reduction of molecular oxygen (O_2) to water (H_2O), cytochrome c oxidase generates an electrical gradient, which is used by mitochondria to create vital energy for the organism and stored in molecules of ATP (1).

Another copper enzyme, lysyl oxidase, participates in cross-linking of collagen and elastin, which form the connective tissue. The effects of lysyl oxidase helps maintain the integrity and elasticity of connective tissue in the heart and blood vessels, but also plays a role in bone formation (2).

Two copper-containing enzymes, ceruloplasmin (feroxidase I) and (feroxidase II) have the ability to oxidize iron (Fe^{2+}) to iron (Fe^{3+}), which are connected to the protein transferrin for transportation to the red blood cells and blood formation. Although feroxidase activity of these two copper enzymes is still not thoroughly understood, the physiological significance and the involvement of copper in

iron metabolism has been clearly demonstrated (2, 12).

Many enzymatic reactions, which are essential for the proper functioning of the brain and the nervous system, are catalyzed by copper enzymes. Dopamine-beta-mono oxidase catalyses the conversion of the neurotransmitter dopamine into norepinephrine (12). Monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine and dopamine. MAO functions in the breakdown of the neurotransmitter serotonin, which justifies the use of MAO inhibitors as antidepressants (13). Myelin sheath is made of phospholipids whose synthesis depends on the activity of the cytochrome c oxidase copper enzyme (2).
Copper enzyme tyrosinase is required for the formation of melanin pigment. Melanin is produced in cells, called melanocytes, and plays a role in the pigmentation of hair, skin and eyes (2).

Superoxide dismutase (SOD) functions as an antioxidant, which catalyses the conversion of superoxide radicals (free radicals) in hydrogen peroxide, that can subsequently be reduced to water by other antioxidant enzymes (14). Two forms of SOD contain copper: 1) copper / zinc SOD is found in most cells of the organism, including red blood cells, and 2) extracellular SOD is a copper-containing enzyme, located in large quantities in the lungs and in low levels – in plasma (2).

Ceruloplasmin can function as an antioxidant in two different ways. Copper and iron ions are powerful catalysts in neutralizing of free radicals. Ceruloplasmin facilitates the catalyzation oxidative processes of the disposal of free radicals. In feroxidase, the activity of ceruloplasmin facilitates iron binding and the degradation of the transport protein transferrin, and may also prevent free ions (Fe^{2+}) from participating in the generation of harmful free radicals (15).

Copper-dependent enzymes are involved in the regulation of gene expression. Cellular copper levels may affect the synthesis of proteins in the organism by enhancing or inhibiting the transcription of specific genes. Enzymes, which are involved in the regulation of gene expression, are copper / zinc superoxide dismutase (Cu/Zn SOD), catalase (another antioxidant enzyme); proteins, associated with cell storage of copper, can be included here as well (1).

There are two main forms of copper in serum, one form is tightly associated with the plasma protein ceruloplasmin, and the other is bound reversibly to serum albumin. Serum also contains copper enzymes – cytochrome c oxidase and monoamine oxidase. (3)

There is still no consensus on the best biomarker for determination of copper status. Serum copper or serum ceruloplasmin are most commonly used in order to investigate copper levels. According to some authors, these markers should be combined with more sensitive, specific and functional markers such as cytochrome c oxidase, platelets and superoxide dismutase in erythrocytes. In the future it is possible to use as functional indicators the antioxidant status, the changes in immune function, in combination with other biochemical markers. (3)

METABOLISM AND INTERACTIONS WITH MICRONUTRIENTS

Common daily intake of copper is 2 ÷ 5 mg, which exceeds the required amount. Copper is carried by the blood protein ceruloplasmin. Once absorbed from the digestive system, it is transported to the liver. Copper is absorbed in the duodenum and upper sections of the small intestine. It is believed that it is mainly absorbed in the small intestine, although there is evidence for its absorption in the stomach. In circulation, copper ions are connected to proteins: ceruloplasmin by 95% and the remainder – to albumin and amino acids. Small quantities of copper are excreted in the urine. The main part of the copper intake in the organism is excreted in bile juice. The proper amount of copper which an adult person requires per day is about 0.9 mg. There is no evidence that high dietary intake of copper is a problem for human health. In the USA, the recommended intake of copper is: for adults ≥ 19.10 mg/d.; for pregnant and lactating women and adolescents – 8 mg/d (2001). (3)

Copper is necessary in human nutrition for normal iron metabolism and the formation of red blood cells. Anemia is a clinical sign of deficiency of both iron and copper. Infants, who receive food with high iron content, absorb less copper than babies who take food with low iron content. This suggests that high doses of iron can interfere with copper absorption in infants (15). There are

contradictive data on the copper content of blood serum in anemia. The results of some studies in children with IDA (19, 20) showed increased serum concentrations of copper. The authors concluded that high levels of copper reduced the absorption of iron and adversely affected haematological indices.

High additional intake of zinc - 50 mg/d or more for an extended period of time can lead to copper deficiency. Zinc supplemented diet increases the intestinal synthesis of cellular proteins, called metallothioneins. They bind metals and do not allow their absorption by intestinal cells. Metallothioneins have a stronger affinity for copper than for zinc, so high levels of metallothioneins due to increased zinc can cause reduced absorption of copper. On the other hand, it is found that high doses of copper affect zinc nutritional status (2, 15).

The effect of supplements of vitamin C and copper on nutritional status of humans is not clear. Two studies in healthy men showed that the activity of ceruloplasmin oxidase may be impaired by relatively high doses of supplementary vitamin C (21, 22). Adverse effects of vitamin C supplements on copper nutritional status are not reported in any of these studies.

In another study, the correlation between serum content of copper, zinc, iron and fat soluble vitamin A and E in healthy pre-school children is examined. A strong correlation between serum zinc and serum copper and iron is discovered. Serum levels of vitamin A are found to be significantly correlated to serum zinc and vitamin E as well. The authors believe that in order to investigate thoroughly the trace elements and fat-soluble vitamins, they should include in the study nutritional surveys, metabolic balance and correlations between micronutrient levels and anthropometric variables (stature, weight, body mass index) (10).

CHANGES IN SERUM COPPER IN VARIOUS DISEASES AND CONDITIONS

Deficiency or excess of copper in the organism is observed in metabolic disturbances and in various diseases and conditions.

In several studies, the authors concluded that medical conditions in which low concentrations (abnormal) of copper are found are: Menkes syndrome, Parkinson's disease, impaired intestinal resorption, parenteral nutrition, protein loss (nephrosis syndrome, exudative enteropathy and others.

Increased concentration of copper is observed in: pregnancy, cholestasis, increased ceruloplasmin – inflammation, tumors, lymphomas, liver cirrhosis, myeloid leukosis; Wilson's disease. Hypercupremia is considered to be related to several acute and chronic infections and malignancies – leukemia, Hodgkin's disease, severe anemia hemohromatosys, myocardial infarction, hyperthyroidism, etc. Serum levels of copper are higher in patients who use contraceptives or estrogens.

Two genetic diseases, which are caused by impaired metabolism of copper, are well studied.

Menkes syndrome is an acquired condition and a recessive disease which leads to copper deficiency (23). Intake and transport of copper is changed, whereby mineral substances in cells and organs are allocated abnormally. Symptoms include sparse and coarse hair, weak muscle tone (hypotonia), sagging facial features, seizures, mental retardation. Menkes disease is characterized as a recessive disorder with retardation of growth, brittle hair and focal cerebral and cerebellar degeneration (24). The neurodegenerative processes change the grey matter of the brain – impaired twisted cerebral arteries (25). This can lead to rupture or blockage of arteries. Weakened bones (osteoporosis) may result in fractures. Symptoms appear during infancy and are largely due to abnormal intestinal absorption of copper with an average deficit in mitochondrial copper-dependent enzymes. Reduced supply of copper decreases the activity of copper enzymes (e.g. lysine oxidase), which are necessary for structuring and functioning of bones, skin, hair, blood vessels and nervous system. (26, 27). In rare cases, symptoms appear later in childhood and are less severe. Early treatment with subcutaneous or intravenous injection of copper supplements (in the form of acetate salts) may be applied in the treatment of this disease. (28) Wilson's disease is an autosomal recessive disorder, which causes copper overload and toxicity in the organism. This impairment of copper metabolism causes copper accumulation and toxic damage to cells primarily in the liver and the brain tissue, but also in the kidneys, eyes,

joints and other organs. In some cases, large quantities of copper can destruct many red blood cells which results in severe anemia. (29) Symptoms of Wilson's disease usually appear at the age of 5-6 years. Kayser-Fleischer ring is a significant diagnostic indicator, because of the accumulation of copper in the eye. Neurological symptoms of the disease include behavioral abnormalities, tremor of the hands, unclear speech, mask expression on the face. Once the symptoms emerge, they usually quickly progress.

Disease diagnosis is confirmed by low levels of the protein ceruloplasmin, increased excretion of copper in the urine, high levels of copper in the liver or through confirmation of the genetic defect.

A retrospective study was performed in Bulgaria, which analyzed the clinical and laboratory profile of 13 patients with Wilson's disease for a period of 12 years. The onset of clinical symptoms includes predominantly neurological manifestations (61.5%). In 54% of the patients there are clinical and laboratory evidence of liver cirrhosis. The ring of K-F is observed in 69% of the cases. Serum concentration of ceruloplasmin was reduced in 92% of the patients, urine excretion of copper in 24-hour urine was increased in 92% and copper in serum was increased in all patients (30). It was discovered that the level of copper in the liver in Wilson's disease is about 25 times higher than in healthy subjects. A content of 89.8 μg/g copper in the liver is reported in a 22-year-old woman who was in the subclinical stage of the disease and suddenly died. (31)

Wilson's disease has a specific treatment. If the treatment is not implemented on time, it can lead to death after the age of 30.

Cardiovascular diseases are widely spread and their diagnostics and treatment are essential for human life and health. Studies on atherosclerosis have shown contradictory results. Some scientists suggest that elevated levels of copper can increase the risk of atherosclerosis (32) and others that copper deficiency rather than copper excess holds an increasing risk of cardiovascular disease (33). Contradictory results were discovered in epidemiological studies on serum copper and copper enzymes in cardiovascular diseases – coronary heart disease (34), chronic heart failure and coronary artery disease (34), rheumatic heart disease (35), atherosclerosis (35) , myocardial infarction (36). It is believed that these results are due to a lack of a reliable biomarker of copper status and it is not clear how copper is associated with cardiovascular diseases.

Contradictory results were obtained in experimental studies on oxidative stress. When the dietary intake of copper was low, adverse changes had been observed in blood cholesterol, including increased total and LDL-cholesterol and decreased HDL-cholesterol (37). Results from other studies did not confirm these findings (38). High copper supplements for four to six weeks did not lead to clinically significant changes in cholesterol levels (33), e.g. it was not proved that increased intake of copper lead to increase of oxidative stress.

It is known that copper plays an important role in the development and maintenance of immune system function, whose exact mechanism is not revealed yet. Neutropenia (abnormally low number of white blood cells – neutrophils) is a clinical sign of copper deficiency in the human organism. Adverse effects of copper deficiency on immune function is most pronounced in infants. Infants with Menkes disease, which causes severe copper deficiency, suffer from frequent and severe infections (39). In a study of 11 malnourished children with copper deficiency, the ability of white blood cells to absorb pathogens has increased significantly one month after administration of copper supplementations (40).

Osteoporosis is a socially significant disease of the bones. Copper-containing enzyme lysyl oxidase is required for the development (cross-linking) of collagen, which is a key element in the organic matrix of bone. Osteoporosis occurs in children and adults with severe copper deficiency, but it is not clear whether copper deficiency contributes to the manifestation and development of the disease. Serum levels of copper in elderly patients with hip fractures were found to be significantly lower than these of controls (41). Studies in healthy adult men and women showed that copper supplements significantly increased bone density (42). However, in another study, the addition of copper did not have effect on biochemical markers of bone resorption and

bone formation in healthy adult men and women (43).

The role of copper in glucose homeostasis in diabetic patients has not been thoroughly determined. In a study of plasma selenium, zinc and copper in patients with diabetes Type 1and healthy controls, there were not found any significant differences in plasma copper concentrations of males and females – controls and patients with diabetes. The reduction in plasma copper concentration in patients with poor metabolic control is less strong in women than in men with diabetes. (44)

Concentrations of copper in blood serum in type 2 diabetes were significantly higher than those in controls. In recent years, many experiments with animals are performed in order to discover a treatment for impaired copper status in diabetes. A copper chelating agent was used in these experiments. As a result, serum copper was reduced to levels consistent with those of controls, treatment with copper-chelating agent decreased insulin resistance and improved glucose intolerance in diabetic patients. In addition, the treatment reduced triglyceride levels in blood serum. In conclusion, the results show that copper was related to the manifestation of type 2 diabetes and should be applied in the treatment of diabetic patients. (45)

A research on patients with obesity and hypertension showed statistically significant higher serum levels of copper as compared to healthy subjects ($p < 0.001$). (11)

Studies in children with chronic diarrhea investigated zinc and copper status (46). The level of both trace elements in serum was reduced. The authors have found deficit of serum copper in chronic diarrhea (47).

Best K et al, 2004 (48) found moderate copper deficiency in cystic fibrosis patients. Other authors (49) found that patients with cystic fibrosis were at risk of nutritional deficiency due to the malabsorption syndrome, associated with endocrine pancreatic insufficiency. According to them, serum levels of copper and zinc demonstrate deficiency of these micronutrients in cystic fibrosis patients.

Trace elements alone or in combination can be used as additives to the treatment of different diseases. Copper supplements are used in the form of copper oxide, copper gluconate, copper sulphate and copper amino acid chelates (50). Often they are combined with vitamins and other micronutrients. These supplements are produced by renowned pharmaceutical companies and could be found under different brand names – Suppravit, Doppelherz aktiv products, etc.

In patients with Wilson's disease penicillamine is used to bind with copper and enhance its elimination from the organism. Since penicillamine dramatically increases urine excretion of copper, the dosing should be very precise (2).

It is known that antacids can interfere with copper absorption when applied in very large quantities (2).

CLINICAL LABORATORY METHODS AND BENCHMARKS (REFFERENCE VALUES) FOR DETERMINATION OF COPPER

The analytical methods which are applied most often for determination of copper are: atomic absorption spectroscopy – flame and electrotermal (FAAS and ETAAS) atomic emission spectroscopy (AES) and some photometric kinetic methods. Reference values for copper concentrations in human body fluids and tissues of healthy people are listed in **Table 1.**

Rükgauer et al. (3) have published reference values for serum copper in healthy children aged 1 to 18 years who had not received any vitamin and mineral supplements – **Table 2.**

Copper content in serum and urine are often used to determine Cu status in clinical laboratory tests. In some of these methods, preliminary preparation of samples for analysis – such as extraction, salinity and others, is required, in order to obtain lower limits of detection and / or to improve their accuracy and precision.

Recommended analytical methods, applied in clinical and chemical determination of trace elements (Al, Cu, Zn, As, Cd, Co, Cr, Hg, Mn, Ni, Pb, Se, etc.), are flame or electrothermal atomic absorption spectrophotometry. They have been adopted by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the Institute of Clinical and Laboratory Standards (CLSI) and the International Committee for Standardization and Haematology (ICSH).

Table 1. *Current reference values for copper concentrations in human body fluids and tissues of healthy people*

Sample	Units	Values, X ± SD or values range from – to**	Analytical method	Reference list
Blood serum	mg/l	1.23 ± 0.16	FAAS	51
	mg/l	1.16 ± 0.52	AAS	7
	μg/dl (15-19 years)*	from 64 to160	AAS	52
	μg/dl (18–60 years)*	77.11 ± 17.67	AAS	53
Urine	μg/l	26.2 ± 10.2	AAS	54
	μg/24 h	< 51	AAS	31
Liver	μg/g	3.58 ± 1.71	FAAS	31
Total blood	μg/ml	0.88	AAS	31
count	mg/l	0.989 ± 0.254	AAS	54
Hair	μg/g	from 22.9 to 46.6	AAS	54
	μg/g (9-10 год.)*	12.0 ± 10.3	ICP-AES	56
	mg/kg (3-15 год.)*	22.1	ICP-AES	6
	μg/g	from 3.2 to 32.8	FAAS	55
Nails	μg/g	2.6 ± 1.4	AAS	54

*Age of investigated patients

** X – average value; SD – standard deviation

Table 2. *Plasma copper concentrations in children according to age (X ± SD)*

Age of healthy subjects (years)	n	Copper concentrations in blood serum (μmol/l)
1-2	15	21.1 ± 4.5
2-4	23	21.5 ± 3.9
4-6	19	19.4 ± 5.3
6-10	25	23.4 ± 2.5
10-14	21	21.1 ± 3.7
14-18	17	20.5 ± 4.4

FAAS method are applied in linear correlation analysis to examine the correlation between serum copper, amniotic copper, lysyl oxidase and collagen III (57). For toxicological diagnostic purposes, atomic emission spectrometry with inductively coupled plasma (ICP-AES) can be used as a method for determining Cu in the urine, because of its low detection limit (below 10 μg/l) (58).

Detection limit of the spectrophotometric methods may be used for determining copper in biological matrices. The method should be applied after all interfering or masking components are pre-concentrated and separated from the samples. Copper in serum could be determined spectrophotometrically through different reagents: 4-(2-quinolylazo)phenol (59), disodium salt batokuproin disulfonat (BCDS) (60), etc.

The development of tools and methodology has significantly facilitated the application of spectrophotometric copper tests for biological fluids and tissues. Therefore, many clinical laboratories utilise the spectrophotometric methods. The manual application of tests usually requires using one or two reagent as some of the methods are direct – without prior protein separation; they only require the application of masking agents, which are often included in the composition of reagents. These methods are automated and do not require expensive and technologically sophisticated equipment. Modern large and small clinical laboratories have at their disposal spectrophotometers and autoanalysers with spectrophotometric indications.

ORIGINAL ANALYTICAL RESEARCH OF SERUM COPPER

In our study, we investigated copper in serum of children with anemia, cystic fibrosis and chronic diarrhea. Spectrophotometric method – tests AUDIT diagnostics (Ireland) was applied. The reagent, used in this method, is 4-(3,5-dibromo-2pyridylazo)-N-ethyl-N-sulfopropy-lanine, which forms a chelated copper complex and measures the absorption at a wavelength of 580 nm. The absorption is proportional to the

concentration of copper in the sample. We conducted a study of the copper status of children aged 1 ÷ 3 years – patients with anemia, cystic fibrosis and chronic diarrhea, and a group of healthy controls.

The obtained results show significantly lower copper content – 12.80 ± 2.30 μmol/l, in serum of patients with cystic fibrosis and chronic diarrhea (n = 11) and low content of copper – 12.8 ± 2.1 μmol/l, in serum of 25 children with anemia.

CONCLUSION

High serum levels of copper are associated with increased cardiovascular risk, but the significance of these findings is still unclear because of the effect of ceruloplasmin and inflammatory conditions on copper serum. Further research is required in order to clarify the relationship between copper nutritional status, ceruloplasmin levels and cardiovascular diseases.

While unbound copper and ceruloplasmin can lead to LDL oxidation in a tube, there is little evidence that the increase of copper in the dietary intake could increase the oxidative stress in the human organism. The results from experimental studies on oxidative stress and serum copper are contradictory.

All studies indicate that severe copper deficiency has an adverse effect on the immune system, but the effect and the activity mechanism of copper deficiency in humans has not yet been thoroughly elucidated.

Although severe copper deficiency is known for its adverse effects on bone health status, the effects of copper deficiency on bone metabolism and osteoporosis, associated with age, requires further investigation before any conclusions are drawn.

The impairments of Zn, Cu and Fe metabolism can lead to chronic pathogenesis, such as diabetes or diabetic complications. Fe and Cu chelating medicines can be applied to control diabetes and diabetic complications. The toxicity and the role of these minerals in the pathogenesis of diabetes and diabetic complications are still being discussed.

In various diseases and conditions, oral treatment with micronutrients is usually applied after the disease is clinically manifested. The investigation of micronutrient status of patients before and after micronutrient supplementation and the use of appropriate supplements can lead to rapid relief of clinical symptoms. Application of current findings on the synergistic correlation between clinical indicators and micronutrient levels can reduce the burden of disease and mortality rates. Further research is needed in order to clarify the metabolism of trace elements in sickness and health, and enlarge the scope of knowledge about micronutrient status. Copper is an essential micronutrient for different biochemical and physiological processes in health and disease conditions. Copper can be used in diagnosis, therapy and prevention of many diseases and conditions. The availability of accurate and accessible methods for determination of serum copper in biomedical research and clinical laboratories is very important, because it provides possibilities for performing studies that will clarify the role and participation of copper in metabolism of healthy people and patients.

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