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ESSENTIAL TRACE ELEMENTS IN HUMAN HEALTH: A PHYSICIAN'S VIEW

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Tomsk
Publishing House of Tomsk State University
2018

UDK 612:577.1
LBC 52.57
S66

Skalnaya Margarita G., Skalny Anatoly V.
S66 Essential trace elements in human health: a physician's view. – Tomsk : Publishing House of Tomsk State University, 2018. – 224 p.

ISBN 978-5-94621-683-8

Disturbances in trace element homeostasis may result in the development of pathologic states and diseases. The most characteristic patterns of a modern human being are deficiency of essential and excess of toxic trace elements. Such a deficiency frequently occurs due to insufficient trace element content in diets or increased requirements of an organism. All these changes of trace element homeostasis form an individual trace element portrait of a person. Consequently, impaired balance of every trace element should be analyzed in the view of other patterns of trace element portrait. Only personalized approach to diagnosis can meet these requirements and result in successful treatment. Effective management and timely diagnosis of trace element deficiency and toxicity may occur only in the case of adequate assessment of trace element status of every individual based on recent data on trace element metabolism. Therefore, the most recent basic data on participation of essential trace elements in physiological processes, metabolism, routes and volumes of entering to the body, relation to various diseases, medical applications with a special focus on iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), selenium (Se), iodine (I), cobalt (Co), chromium, and molybdenum (Mo) are reviewed.

The monograph may be of interest for physicians, nutritionists, researchers working in the field of trace elements in medicine and biology, as well as medical students.

UDK 612:577.1
LBC 52.57

ISBN 978-5-94621-683-8

FOREWORD

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Trace elements play a significant role in maintenance of a healthy state of an organism. Consequently, disturbances in trace element homeostasis may result in the development of pathologic states and diseases. The most characteristic patterns of a modern human being are deficiency of essential and excess of toxic trace elements. Such a deficiency frequently occurs due to insufficient trace element content in diets or increased requirements of an organism. Oppositely, excessive entrance of toxic metals into the organism is directly associated with unfavorable ecological conditions. All these changes of trace element homeostasis form an individual trace element portrait of a person. Consequently, impaired balance of every trace element should be analyzed in the view of other patterns of trace element portrait. Only personalized approach to diagnosis can meet these requirements and result in successful treatment [1].

Effective management of trace element deficiency and toxicity may occur only in the case of adequate assessment of trace element status of every individual. Timely diagnosis of impaired trace

element homeostasis would help to perform personalized approach to diseases treatment in each individual patient.

Moreover, the use of complex and modern techniques for estimation of trace element metabolism may not be effective for diagnosis without the appropriate reference values [2]. Taking into account the influence of various factors like geographical location, climate, occupation, dietary habits, the parameters of trace element exchange may differ between populations. Consequently, in the context of personalized approach to diagnosis of nutritive disturbances, the reference ranges of trace element metabolism parameters for every specific population should be estimated [3].

At the same time, a novel personalized approach to diagnosis and treatment of trace element-related disturbances should include the estimation of complex trace element portrait of the patient [4]. Such a portrait should indicate the levels of essential and toxic trace elements and macroelements in the organism. Taking into account possible synergistic and antagonistic interactions between trace elements, administration of the similar doses of trace elements (in the case of deficiency) in persons with different trace element status may have distinct effects.

Generally, personalized approach to treatment of trace element related disturbances through complex investigation of the individual will result in effective diagnosis and treatment. In particular, such an approach may help to increase the efficacy of treatment and reduce possible risks of drug supplementation.

Despite a large body of data, it should be noted that the problem of impaired essential trace elements homeostasis is not closed, as novel data on their role in numerous diseases appear regularly. Even the well-studied trace element “changes” its biological significance due to the change of the human lifestyle. Consequently, trace element metabolism should receive special attention of diagnostic services, nutritionists and physicians.

The most recent fundamental data on participation of trace elements in physiological processes, metabolism, routes and volumes of entering to the body, relation to various diseases, medical applications are reviewed.

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GENERAL INTRODUCTION

Essential trace elements in human health: basic concepts

Chemical elements in both free state and a variety of chemical compounds are included in all cells and tissues of the human body. They are the so-called "building blocks", the most important catalysts for various biochemical reactions, the essential and indispensable participants in the processes of growth and development, metabolism, adaptation to changing environmental conditions [1].

Physiological effects of elements depend on their dose. Therefore, toxic elements (arsenic, mercury, antimony, cadmium etc.) in low concentrations can act in the body as a medicine (producing sanogenetic effect), whereas sodium, potassium, calcium, iron, magnesium, and some other elements in high concentrations can cause severe toxic effects.

For each element there is an optimum range of concentrations to perform vital functions. At deficiency or excessive accumulation of elements, serious changes can occur in the body, violating activities of enzymes, directly or indirectly dependent on them.

In the body, chemical elements exist mainly in the form of coordination compounds, and their excessive formation or destruction can lead to a disturbance of the so-called metal-ligand homeostasis, and further to development of pathological changes. Metals and their ligands (e.g. glutamate, aspartate, picolinate or ascorbate etc.) can act as activators or inhibitors of various enzymes

that determines their essential role in the development and treatment of various diseases.

To systematize information about the content and physiological role of chemical elements in the body, several classifications has been proposed recently. Without examining them in detail, let us review just some fundamental points.

One of the principles of classification is division of chemical elements into groups, depending on their contents in the organism of mammals and humans.

The first group in this classification is "macro elements", whose concentration in the body exceeds 0.01%. They are: O, C, H, N, Ca, P, K, Na, S, Cl, Mg. The absolute body content of these elements (scaled to average human weight of 70 kg) ranges from a few grams (magnesium) to over forty kilograms (oxygen). Some elements of this group are called "organogens" (O, H, C, N, P, S) due to their leading role in formation of tissues and organs.

The second group is "trace elements", and their concentration ranges from 0.00001% to 0.01%. This group includes: Fe, Zn, F, Sr, Mo, Cu, Br, Si, Cs, I, Mn, Al, Pb, Cd, B, Rb. These elements are contained in the body in amounts from hundreds of milligrams to several grams. However, despite the "small" content, trace elements are not random ingredients of biological substances, but components of a complicated physiological system involved in the regulation of vital functions at all stages of development of the living organism.

The third group is "ultratrace elements", and their concentration is lower than 0.000001%. These are Se, Co, V, Cr, As, Ni, Li, Ba, Ti, Ag, Sn, Be, Ga, Ge, Hg, Sc, Zr, Bi, Sb, U, Th, Rh. Their content in the body is measured in milligrams or micrograms. At present many elements of this group, e.g. selenium, cobalt, chromium etc., are found to be essential to the organism.

The biological (most important) classification is based on ideas about the physiological role of chemical elements in the body. According to this classification, macro elements, constituting the bulk of cells and tissues, are "structural" elements. Fe, I, Cu, Zn, Co, Cr, Mo, Se, Mn are "essential" (vital) trace elements. For all of these trace elements (except chromium) the specific element-containing or -depending enzymes and other biologically active molecules were found, explaining their biological and medical significance. Some authors include fluorine (as fluoride) in the list of essential trace elements instead of chromium or cobalt [2-4]. Chromium and cobalt do not directly influence enzymatic activity, they just are components of chromodulin or vitamin B_{12}. As, B, Br, F, Li, Ni, Si, V are "conditionally-essential". The vital necessity, or essentiality, is the most important property of chemical elements for the life of living organisms. A chemical element is considered essential, if its absence or insufficient intake to the organism is accompanied by disturbance of normal life, delayed development, inability to reproduction. Replenishment of the missing amount of the element eliminates clinical manifestations of its deficiency and restores vitality of the organism.

The current definition of trace element essentiality states that an element is considered essential to an organism when reduction of its exposure below certain limit results consistently in a reduction in a physiologically important function, or when the element is integral part of an organic structure, preforming a vital function in the organism [5-6].

Al, Cd, Pb, Hg, Be, Ba, Bi, Tl are called "toxic" elements; Ag, Au, In, Ge, Rb, Ti, Te, U, W, Sn, Zr etc. are "potentially toxic" ones. Exposure of the organism to these elements results in the development of intoxication syndromes (toxicopathies).

T a b l e 1

Dietary reference intakes for essential trace elements (units/day) [10]

Life stage	Cr (μg)	Cu (mg)	F^- (mg)	I (mg)	Fe (mg)	Mn (mg)	Mo (mg)	Se (μg)	Zn (mg)
Children									
0–6 mo	0.2[a]	0.20[a]	0.01[a] {0.7}	0.11[a]	0.27[a] {40}	0.003[a]	0.002[a]	15[a] {45}	2[a] {4}
7–12 mo	5.5[a]	0.22[a]	0.5[a] {0.9}	0.13[a]	11[b] {40}	0.6[a]	0.003[a]	20[a] {60}	3[b] {5}
1–3 yr	11[a]	0.34[b] {1}	0.7[a] {1.3}	0.09[b] {0.2}	7[b] {40}	1.2[a] {2}	0.017[b] {0.3}	20[b] {90}	3[b] {7}
4–8 yr	15[a]	0.44[b] {3}	1[a] {2.2}	0.09[b] {0.3}	10[b] {40}	1.5[a] {3}	0.022[b] {0.6}	30[b] {150}	5[b] {12}
9–13 yr	25[a] (m) 21[a] (f)	0.7[b] {5}	2[a] {10}	0.12[b] {0.6}	8[b] {40}	1.9[a] (m) 1.6[a] (f) {6}	0.034[b] {1.1}	40[b] {280}	8[b] {23}
14–18 yr	35[a] (m) 24[a] (f)	0.89[b] {8}	3[a] {10}	0.15[b] {0.9}	11[b] (m) 15[b] (f) {45}	2.2[a] (m) 1.6[a] (f) {9}	0.043[b] {1.7}	55[b] {400}	11[b] (m) 9[b] (f) {34}
Adults									
19–50 yr	35[a] (m) 25[a] (f)	0.9[b] {10}	4[a] (m) 3[a] (f) {10}	0.15[b] {1.1}	8[b] (m) 18[b] (f) {45}	2.3[a] (m) 1.8[a] (f) {11}	0.045[b] {2}	55[b] {400}	11[b] (m) 8[b] (f) {40}
51+ yr	30[a] (m) 20[a] (f)				8[b] {45}				
Pregnancy									
14–18 yr	29[a]	1[b] {8}	3[a] {10}	0.22[b] {0.9}	27[b] {45}	2[a] {9}	0.05[b] {1.7}	60[b] {400}	12[b] {34}
19–50 yr	30[a]	1[b] {10}		0.22[b] {1.1}		2[a] {11}	0.05[b] {2}		11[b] {40}
Lactation									
14–18 yr	44[a]	1.3[b] {8}	3[a] {10}	0.29[b] {0.9}	10[b] {45}	2.6[a] {9}	0.05[b] {1.7}	70[b] {400}	13[b] {34}
19–50 yr	45[a]	1.3[b] {10}		0.29[b] {1.1}	9[b] {45}	2.6[a] {11}	0.05[b] {2}		12[b] {40}

a = Average Intake (AI) value
b = Recommended dietary allowance (RDA) value
{..} = Tolerable upper intake level (UL) value

It should be noted that all the above mentioned classifications of trace elements are, of course, conditional and can differ in different sources [7-8].

According to U. Lindh [9], the concept of essentiality has the practical consequence that it is necessary to supply an organism with adequate amounts of the concerned elements. For most of trace elements ranges of dietary recommendations and upper tolerable intakes (U.S. National Academy of Science, Food and Nutrition Board) have been defined (see Table 1). These ranges are very similar in different countries and reflect physiological needs of modern population in the world. In very general terms, the range may be presented as sinusoidal curve (Fig. 1).

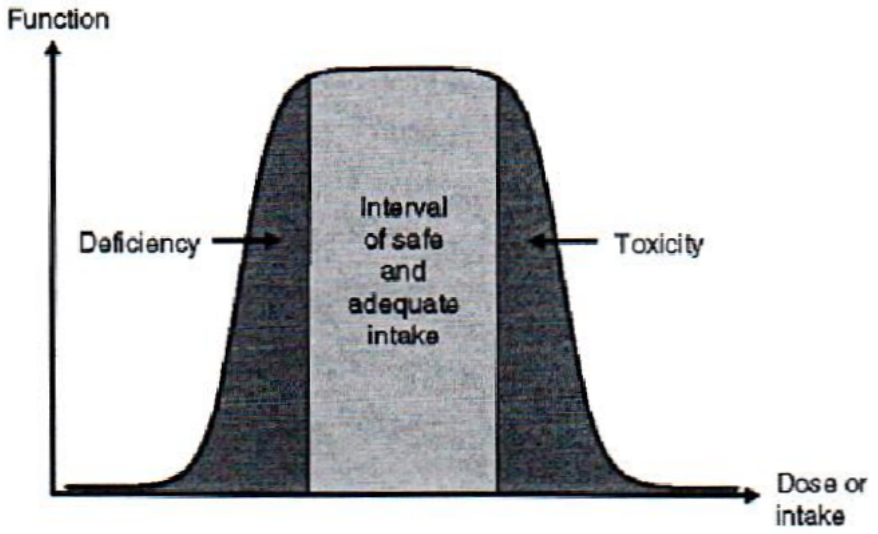

Fig. 1. Dose-response of essential trace elements [9]

Stability of chemical composition is one of the most important and essential conditions of organisms normal functioning [11]. Deviations in chemical elements concentration, caused by environmental, climatic, geographical factors, or diseases lead to

different disturbances of human health [12]. It should be also pointed out that many metals, abundant in the environment, belong to trace elements, necessary for normal functioning of human organism. Trace elements play a great role in formation of many important adaptive mechanisms including functioning of all vital systems of the organism. That is why sufficient content of essential elements in the organism and minimal concentration of toxic and relatively toxic elements, not threatening frustration of adaptive mechanisms, is one of the most important requirement of modern human [13].

Human health depends on the ability to receive trace elements, which cannot be synthesized in humans' body, from the environment, including foods. Thus, essential trace elements are called "micronutrients" (the definition includes also some amino acids, fatty acids and vitamins) which are required in small (μg-to-mg per kg of diet). Trace elements and other mineral elements are obtained mostly from foods-soils-rocks chain. Therefore, good mineral nutrition is, at least in part, a geological issue [14]. So, nutritionally important trace elements occur in soils as silicates (Mn, Zn, Se), sulfides (Cu, Fe, Zn, Se) and as the native elemental form (Fe); water-borne mineral elements of the greatest health importance include, besides macroelements Ca, Mg, K, Na, also Fe, Mn, Cu and Se [14]. Monotonous, non-diverse, grain-based diets accessible to the poor population in the developing world are likely to provide insufficient energy, protein and minerals [14]. On the other hand, non-diverse eating habits and fast food, refined products in industrialized countries also can cause nutritional deficiencies of some essential trace elements. This is why manufacturers propose fortified foods and supplements for customers. In fact, for providing the optimal nutrition, humans have to use mixed diets based on a diverse selection of foods, including the fortified ones.

According to Russian scientists A. P. Avtsyn and A. A. Zhavoronkov [15], there is a sufficient evidence to suggest

existence of a common biological system of TE homeostasis, which consists from: (1) entry routes; (2) metabolic (utilization) routes; (3) elimination routes; (4) regulatory influences of these biological systems.

The concept of microelementoses (an original synthetic approach in modern medical elementology), proposed by A. P. Avtsyn and co-workers [15], was used as the theoretical basis of our study. Its common epidemiological classification is present in Figure 2. According to the proposed classification, the following main groups of microelementoses have been defined:

- Natural endogenous (congenital and inherited);
- Natural exogenous (endemic);
- Technogenic (occupational, neighborourgh, transagressive), and, finally,
- Iatrogenic.

The diseases are generally divided into hypo(micro)elementoses, hyper(micro)elementoses and (trace) element imbalances.

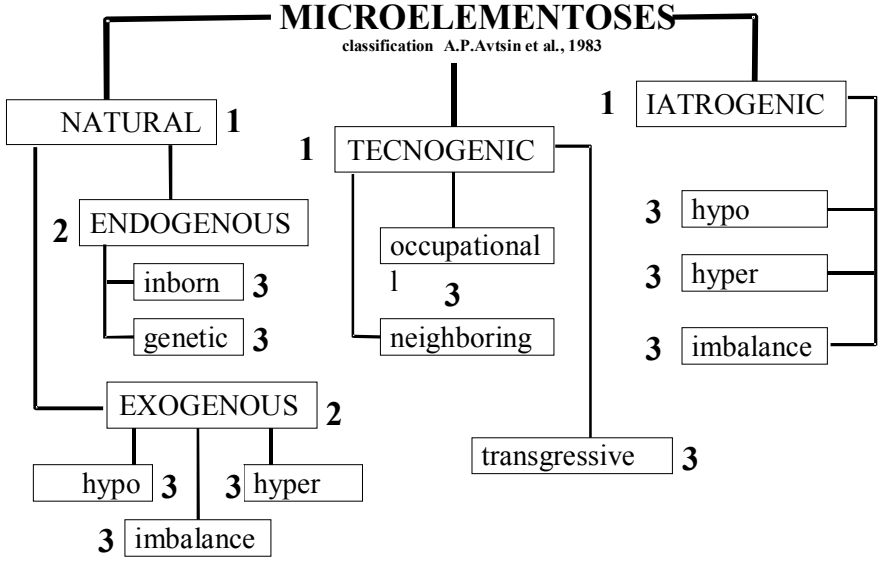

Fig. 2. Human microelementoses [15]

Naturally, in the cases of multielemental investigations the absolute majority of diseases are due to the element imbalances because of the numerous specific inter-element interactions, physiological, nutritional and ecological reasons, increased liability of main biochemical processes [16].

Recently, particular attention is given to correlations between concentration of toxic substances, including metal compounds, in blood, urine, hair and other human tissues on the one hand and rate of their negative influence on human organism on the other hand. At that, concentration of a substance in human tissues or excretions is used as indicator of both its influence on the organism and its concentration in the environment [17]. Hair is a biosubstance, which adequately reflects elemental balance in the organism [18]. Multielement hair analysis allows to determine reliably enough the risk groups of hyper- and hypoelementoses for their further investigation and forehanded prophylaxis.

The chronic deficiency of the essential trace elements in organism results in a pathology, accompanied by considerable metabolic disturbances and distinct clinical and morphological changes. The chronic trace elements deficiency can cause two separate types of changes: different metabolic disturbances on the one hand, and distinct immune disturbances, accompanied by decrease of general immune resistance, on the other hand. Both processes result in endocrinopathies, inflammations and neoplastic diseases.

The immune system is the main target for the influence of trace elements imbalance and various ecological pathogens (radionuclides, xenobiotics, allergens etc.) [19].

Thus, in present-day conditions the decrease of organism resistance to infective and non-infective factors is probably related to rapid reduction of phylogenetic elements-based mechanisms of the resistance, in conjunction with slow formation of new mechanisms.

Even non-specific increase of general intake of major and trace elements into the human organism, especially in the regions with low life standards, characterized by relatively low elemental body content, is capable to significantly increase the individual and population health.

For practitioners the therapeutic dosages are very important. Usually they are few times higher as dietary daily requirements. The amounts of essential trace elements administrated as preventive or curative agents are dependent on the route of administration, forms ("species") of trace element compounds and combinations with other biologically active agents. And, of course, on data of laboratory tests and clinical picture in each case.

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IRON

Introduction

Iron is being used for medical purposes since ancient times. It is an essential trace element and its role in human life cannot be underestimated. Primary functions of iron in biology are associated with its cofactor role in multiple proteins involved in oxygen transport, cellular metabolism, energy metabolism, mitochondrial respiration, DNA synthesis, cellular growth and differentiation [1-2]. Iron is present in the organism in ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms. Iron-mediated redox processes are involved in electron transport in mitochondrial respiration. However, the range of concentrations in which iron performs its essential functions is rather short. Along with participation in physiologic processes the role of iron in free radical generation is well known [3-4]. Free non-transferrin bound iron is extremely toxic. Excessive amounts of iron cause toxic damage of liver, heart and other organs. Despite a great importance of iron in human life, some aspects of its transport and metabolism are insufficiently studied and remain unknown to a wide circle of scientists and physicians [5].

Disruption of iron metabolism, as assessed by hair metal levels, may be considered as unfavorable prognostic sign in relation to both morbidity and demography in a number of regions [6].

Adult human organism contains approximately 3-5 g of iron and nearly 2/3 of total iron is incorporated in hemoglobin. Red blood cells, liver, muscles and bones are rich in iron [7].

Optimal daily intake of iron is 10-20 mg, whereas its toxic threshold is 200 mg and lethal dose is 7-35 g [7]. Resorption is 10% (35% from "animal" products), excretion via feces (ca. 15 mg, sweat (0.5 mg), urine (0.25 mg). Certain loss of iron occurs through desquamating epithelia and menstrual blood. Overall iron half-life in the body is 2000 days.

Calcium facilitates iron absorption, except the cases when doses of calcium are extremely high. Phosphates from eggs, cheese and milk; oxalates, phytates and tannins contained in black tea, bran or coffee, decrease iron absorption. Vitamin E and zinc in high concentrations also reduce iron absorption. Vitamins C, B_{12}, gastric acid, pepsin, copper promote absorption of iron, particularly when they come from animal sources. Decreased gastric juice acidity resulting from prolonged taking of antacids or acidity-reducing drugs (such as Zantac, Tagamet, etc.) is accompanied by a decrease in the absorption of iron. Coffee, tea, milk, dark green vegetables, and the deficiency of vitamin A can also reduce the body's ability to absorb iron. In turn, an excess of iron decreases the body's ability to absorb copper and zinc [8].

Transport, metabolism and homeostatic regulation of iron

A short range of iron concentrations in the human organism is controlled by interaction of transport proteins, involved in regulation of iron bioavailability, balance between its export and import. Iron homeostasis may be impaired due to its prolonged insufficient dietary intake, chronic and acute inflammatory diseases, obesity and other pathologic states.

Iron enters human organism with diet and drinking water. Animal products contain iron in the most available heme form

(bioavailability 3.5%), whereas plant foods may be rich in iron in forms with lower bioavailability.

Iron kinetics is tightly associated with gastrointestinal hormone gastrin that is the main regulator of gastric juice production. Relationship between circulating gastrin and iron is still insufficiently studied. Earlier data indicate that gastrin gene knock-out mice with gastrin deficiency are characterized by a high rate of iron absorption and divalent metal transporter 1 (DMT-1) mRNA expression in comparison to control animals. However, decreased gastric juice acidity as a result of vagotomy and administration of H_2-histamine receptor antagonists is accompanied by diminished iron absorption in gastrointestinal tract. At the same time, prolonged use of proton pump inhibitors (like omeprazole) did not alter iron status [9]. Gastrin binds 2 atoms of iron and delivers it into duodenum. Non-heme ferric iron is reduced by duodenal cytochrome b (DcytB) to Fe^{2+}. DMT-1 takes part in iron transport through apical membrane of enterocyte. It is known that DMT1 expression is increased in iron deficiency and decreased in iron overload [9]. It is interesting that goat's milk consumption increases liver DMT1 expression leading to elevated hepatocyte iron stores [10]. Entrance of heme iron into the cell occurs with the help of heme carrier protein (HCP-1). Iron is released from the complex with HCP-1 by heme oxigenase (HO-1) inside the cell. Data indicating gastrin participation in transferrin saturation also exist. In particular, the rate of transferrin saturation is decreased in the case of low gastrin levels. Oppositely, multiple endocrine neoplasia type 1 (MEN-1) syndrome patients are characterized by increased gastrin levels and transferrin saturation.

Transferrin takes part in iron homeostasis as a protein carrying iron to target cells. Iron binds transferrin and is transported in the form of Fe^{3+}-transferrin-Fe^{3+} complex. On the surface of the cell transferrin-iron complex contacts with the respective receptor (TFR) and enters the cell by endocytosis [11]. Iron reduction from Fe^{3+} to

Fe^{2+} occurs in acidic medium of endosomes. After the entrance into the cell iron is transported into labile iron pool. Iron is delivered to iron-containing proteins from labile iron pool. It is also notable that transferrin levels affect liver hepcidin expression [12-13].

Plasma iron has three main sources: 1) iron absorbed by enterocytes in proximal duodenum (dietary); 2) iron released from hepatocytes (deposited); 3) metal released from macrophages and reticuloendothelial cells (reutilization). Iron releasing from enterocyte into the bloodstream is oxidized by ceruloplasmin (hephestin) [14].

Iron elimination from cells is mediated by ferroportin (FPN). FPN activity depends on hepcidin that is produced in liver under a number of stimuli. Hepcidin binds FPN that leads to degradation of the latter and decreased iron export. An experimental study using hepcidin-deficient mice has indicated excessive iron deposition, whereas increased hepcidin production is accompanied by anemia. Complex influence of hepcidin on iron metabolism is characterized not only by depression of metal absorption but also by a decrease in recirculating iron from macrophages and hepatocyte iron mobilization [15]. Inflammation, hypoxia, erythroid factors, HFE gene, hemojuvelin, transferrin receptor-2 and total transferrin concentration influence hepcidin levels. Under inflammatory conditions and excessive IL-6 secretion increased hepcidin production occurs, leading to anemia development [2]. Impaired iron metabolism in acute and chronic inflammatory processes is considered to be a protective reaction decreasing bacterial growth. Anemia of inflammation is in general similar to iron-deficient anemia. However, iron therapy is not efficient in this case. It is supposed that hepcidin production mainly occurs in liver; however significant production is observed in macrophages, adipocytes, cardiomyocytes, placenta and kidneys. Increased activity of erythroid marrow and decreased blood glucose are negative stimuli for

hepcidin activity. Low hepcidin level is observed in hemochromatosis and other hypoferrogenic states as well as in inefficient hematopoiesis. Analysis of the protein level is used for screening, monitoring and hemochromatosis treatment and prognosis.

Table 1

Genes and transporters involved in iron metabolism

Genes and proteins	Function
Transferrin (TFR gene)	Iron transport
Hepcidin gene (HFE)	Regulates iron absorption in gastrointestinal tract, transport protein expression and translocation
Transferrin receptors 1 and 2 (TFR1,2)	Iron transport into the cell
DMT-1	Iron transport into the cell
Lipocalin-2 (LCN2)	Alternative transport of iron into the cell in LCN2-catechol complex
Iron regulatory proteins (IRP-1, IRP-2)	Intracellular iron homeostasis control
Hemojuvelin (HJV gene)	Regulates hepcidin absorption
Ferritin	Intracellular iron pool
Ferroportin	Iron excretion from the cell
Hephestin (Ceruloplasmin)	Ferrooxidase, oxidation of iron from Fe^{2+} to Fe^{3+}

Two iron regulatory proteins (IRP-1 and IRP-2) take part in intracellular iron homeostasis regulation. In the conditions of changing iron status these proteins regulate the activity of iron transport proteins like TFR1, TFR2, ferritin, etc. [2].

Intracellular iron accumulation is accompanied by increased ferritin synthesis. However, when excessive accumulation occurs, iron is involved in hemosiderin formation. This process takes place in normal conditions. Further iron overload and genetic impairment of iron metabolism causes formation of toxic non-transferrin bound

iron (NTBI). This compound takes part in free radical generating Fenton reaction leading to tissue oxidative damage. Accumulation of free iron is observed in secondary iron overload, hemolytic anemia, hemochromatosis, terminal stages of kidney failure [2].

It is estimated that cancer cells increase their iron stores. Accumulated ferritin activates IRP1 and IRP2 leading to elevated expression of ferritin and TFR1. Lipocalin2 in the complex with catechol takes part in alternative way of iron uptake by binding siderophores (low molecular weight iron ligands). Its expression is activated in various cancer types. Experimental studies have demonstrated that LCN2 inhibition results in decreased proliferation and angiogenesis. Oppositely, increased LCN2 levels are associated with worse prognosis in breast cancer and hepatocellular carcinoma [11]. Excessive iron accumulation in cancer cells is associated with impaired "hepcidin-FPN" axis and is necessary for high metabolic activity and proliferation of these cells. Observation involving breast cancer patients with estrogen positive receptors (ER^+) obtaining tamoxifen has indicated the highest survival prognosis in women with high ferroportin and low hepcidin levels being indicative of successive iron elimination from cells. Iron exchange reprogramming in cancer cells may be used for development of novel strategies of cancer treatment using ferritin, FPN, hepcidin and other iron transport proteins as target molecules.

It is known that iron homeostasis is tightly interrelated with manganese. In particular, manganese (III) has a high affinity to transferrin receptors (TfR) even in comparison with iron (III). Consequently, impaired iron homeostasis may be accompanied with altered manganese content and vice versa [16]. Experimental studies have indicated that FPN causes cellular excretion of ^{65}Zn и ^{57}Co along with iron. At the same time FPN activity did not affect intracellular manganese, cadmium and copper content [17].

Iron as a cofactor and clinical manifestations of its deficiency

The most significant function of iron is oxygen transport in the structure of hemoglobin and myoglobin. Being an integral component of prosthetic group of cytochromes, iron-sulfur clusters of mitochondrial respiration iron takes part in energy homeostasis. It is important to note the role of iron as a transcription factor in various signaling pathways involved in neurotransmitter metabolism, cellular growth and inflammation [2].

Table 2

Iron containing proteins

Enzyme	Functions
NADPH-oxidase	Free radical generation, immunity
Succinic acid dehydrogenase	Tissue respiration
Hemoglobin and myoglobin	
NADH-cytochrome c reductase, succinyl cytochrome c reductase	
Peroxidase and catalase	Hydrogen peroxide detoxication
Ribonucleotide reductase	Formation of deoxyribonucleotides from ribonucleotides
Prolylhydroxylase	Proline hydroxylation (collagen maturation) Regulation of immune response (involving HIF)

Low dietary iron levels and chronic bleeding cause microcytic hypochromic anemia. The latter is characterized by impaired hemoglobin synthesis leading to decreased erythrocyte size (microcytosis) and lowered cellular hemoglobin content (hypochromia). Development of tissue hypoxia is accompanied by the following symptoms: weakness, reduced work capacity, restless legs syndrome, pale skin, dysgeusia, Plummer-Vinson syndrome, as well as impaired immunity (glossitis, stomatitis). Hepcidin levels in

iron deficient-anemia are very low. This mechanism is compensatory as it helps to restore organism iron stores with increased iron absorption, liberation from depots and reutilization [5].

Increased iron requirements lead to the so-called chronic disease anemia. It is independent of dietary iron. Chronic disease anemia is observed in patients with chronic or acute infections, parasitic diseases, inflammation, cancer, traumas and terminal states. The observed impairment of iron homeostasis is a response to increased proinflammatory cytokine production (IL-6, IL-1 and TNF-α). Moreover, IL-6 increases liver hepcidin production leading to decreased FPN synthesis and impaired gastrointestinal iron absorption. Consequently, attempts to treatment of chronic disease anemia using iron-containing drugs are useless. Chronic diseases are accompanied with decreased iron release from macrophages due to impaired FRP expression under TNFα, interferon-γ and lipopolysaccharide (LPS) stimuli. Consequently, iron accumulates in reticuloendothelial system decreasing its availability for hematopoiesis [2]. At the same time, proinflammatory cytokines decrease trasferrin receptor activity of the cellular surface leading to decreased iron availability for bacteria [18].

The problem of anemia has a high importance in pregnant women. Relative decrease in iron levels in pregnancy is a normal physiological state accompanied by a depletion of maternal iron stores by a growing fetus. It is supposed that in the case of hepcidin overproduction the latter binds ferroportin. This iron transporter is present in macrophages, enterocytes, and placenta. Both maternal hepcidin and maternal and fetal iron levels take part in regulation of iron homeostasis in pregnancy. Placenta is capable of utilization of both heme and non-heme iron. It is supposed that maternal hepcidin production is decreased in late periods of pregnancy [18]. It is interesting that no correlations between maternal and fetal iron status biomarkers were found. However, a case of severe anemia in

pregnancy may be an exclusion, when decreased iron stores are observed both in maternal and fetal organisms. Extracellular iron transport is performed by two glycoproteins: transferrin and lactoferrin. It has been shown that total amount of transferrin in plasma exceeded the one of lactoferrin. However, lactoferrin levels significantly increase in the first and third trimesters of pregnancy [18]. Pregnancy is also accompanied by a relative decrease in transferrin saturation when compared to non-pregnant women [19]. Estrogens play a significant role in iron metabolism regulation both in pregnancy and during the use of replacement therapy with sex hormones. In particular, high doses of estrogen decrease liver hepcidin production leading to increased iron absorption in gastrointestinal tract and elevated organisms iron stores [19].

Increased iron deficiency risk is observed in obese persons [20]. A significant association between decreased serum iron levels and obesity severity was detected in the mid-60th of the twentieth century. Even in obese children and adolescents the incidence of anemia is 2-fold higher than in ones with normal body mass index. Increased hepcidin expression in obesity as a result of chronic low grade inflammation is supposed to be the main mechanism of obesity-related iron deficient anemia development. Increased hepcidin mRNA expression is observed both in liver and visceral adipose tissue [20-21].

An epidemiologic study involving population living in Central federal district of Russian Federation [6,22] demonstrated an increase in diabetes mellitus incidence in adults with low hair iron content ($r=-0,72$; $p<0,001$), whereas the frequency of congenital anomalies is associated with elevation of hair trace element content ($r=0,54$, $p<0,05$).

Metabolic disturbances associated with iron overload

Iron overload is associated with increased intracellular ferritin levels. The level of this protein may be increased from 10 to

20 times. This situation is observed in secondary iron overload in thalassemia, sideroblastic anemia, chronic liver disease, porphyria cutanea tarda, hemosiderosis, hemochromatosis, hemotransfusion [23]. Excessive iron accumulation may result in liver and pancreatic dysfunction (diabetes mellitus 2 type) and defective functioning of cardiovascular system (cardiomyopathy, heart failure) [24].

A number of epidemiological studies indicated that age-related increased ferritin deposition is associated with cardiovascular disease risk. In particular, it has been shown that in adult men with ferritin levels exceeding 200 ng/ml, the risk of cardiovascular disease is 2-fold higher in comparison to control subjects [2]. NTBI increases monocyte adhesion to vascular endothelium, production of selectins, intracellular adhesion molecule 1 (IAM-1) resulting in atherosclerosis development [2].

In particular, iron deposition in cardiomyocytes and epi- or endocardium cause hypoferremic cardiomyopathy. This type of cardiomyopathy is accompanied by systolic and diastolic dysfunction that is asymptomatic for a long period of time but finally leads to heart failure. Normally iron homeostasis is regulated through transferrin-mediated mechanism. In the case of iron overload transferrin is highly saturated with iron and further accumulation of metal is associated with an increase in NTBI. NTBI enters cardiomyocyte through L-type calcium channels, possibly, involving endosomes. Subsequently iron binds ferritin and transports to lysosomes. Moreover, iron accumulation may be observed as a result of increased dietary iron absorption as in porphyria cutanea tarda, chronic liver diseases like non-alcoholic steatohepatitis, viral hepatitis B and C, ineffective hematopoiesis (sideroblastic anemia) and thalassemia.

Chronic liver diseases (alcoholic hepatitis and steatohepatitis) and diabetes mellitus 2 type are accompanied by decreased hepcidin levels. Low hepcidin expression results in increased FPN activity and iron accumulation. It has been demonstrated that testosterone may increase hepcidin levels and normalize iron metabolism [25].

A number of investigators have revealed an association between iron overload and insulin resistance in metabolic syndrome [26].

Flataxin is a mitochondrial protein and impairment of its function is associated with neurodegeneration. One of the forms of iron hyperaccumulation is Friedreich ataxia. Flataxin defect is observed in neurodegenerative diseases. It also takes part in initial stages of Fe-S cluster synthesis and its dysfunction results in mitochondrial iron accumulation and oxidative stress development [11].

Iron takes part in regulation of hypoxia inducible factor-α (HIF-α). In normoxic conditions HIF-α protein is degraded whereas hypoxia induces its stabilization. HIF-α is involved in regulation of enzymatic activity.

Genetic diseases associated with impaired iron metabolism

Iron deficiency. Heterogenic genetic disease characterized by accumulation of sideroblasts in bone marrow cause sideroblastic anemia development. It may occur due to mutations of genes involved in heme, Fe-S clusters and mitochondrial proteins biosynthesis. X-linked sideroblastic anemia characterized by δ-aminolevulinate metabolism gene mutation is observed most frequently [27]. Mutations in specific erythroid mitochondrial transporter (SLC25A38), ATP synthesis (ABCB7), glutaredoxin (GLRX5), thiamine transporter (SLC19A2) mutations may also be involved.

Iron overload. Two types of iron overload like primary hemochromatosis and secondary iron overload may be defined. The first is related to genetic diseases whereas the second type is observed in various states that will be described further. Excessive iron is accumulated in liver, spleen, heart, bone marrow, adrenal

glands and CNS causing their damage. It has been shown that tissue iron induces oxidative stress.

Hemochromatosis is an autosomal genetic disturbance in HFE gene that regulates gastrointestinal iron absorption. The incidence of hemochromatosis is 25-fold higher in men than in women [28]. Iron accumulation in liver, pancreas, heart and other organs is observed as a result of its mutation. The symptoms like weakness, arthralgia, impotency, lethargy are not specific in the early periods of the disease. Osteoporosis, cirrhosis, hepatocellular cancer, cardiomyopathy, arrhythmia, diabetes mellitus and hypogonadism are observed at later stages of the diseases.

The diseases related to mtDNA mutation are Leber's hereditary orbital neuropathy, Kearns-Sayre syndrome, stroke-like episodes (MELAS syndrome), Leigh syndrome, as well as impaired exercise tolerance and cardiomyopathy [29].

Laboratory criteria for impaired iron metabolism diagnostics

It is well-known that nearly 70% of iron are incorporated in hemoglobin. Criteria for iron-deficient anemia diagnosis are described in clinical laboratory diagnostics manuals [30]. At the same time, criteria for anemia in pregnant women should be reviewed. In particular, in the first and third trimesters hemoglobin and ferritin levels lower than 11 g/dl and 15 μg/l, respectively, indicate the presence of anemia. At the same time, critical values for blood hemoglobin in the second trimester are 10.5 g/dl. However, a number of works propose that anemia should be diagnosed at hemoglobin levels of 9.5 g/dl in pregnant women [18]. Non-iron deficiency anemia is characterized by lowered hemoglobin level (< 11 g/dl) and increased ferritin levels (> μg/l). This type of anemia

may be caused by thalassemia, hemoglobinopathy, B_{12} and folic acid-deficient anemia, anemia of inflammation including the one in HIV-positive women [31].

Biochemical markers of circulating and deposited iron are ferritin, serum iron, total iron binding capacity, transferrin saturation, soluble transferrin receptors (sTfR). The latter is used for estimation of iron status at the early stages of iron deficiency.

Detection of iron overload is performed using the following criteria: transferrin saturation higher than 55%, increased serum ferritin levels (higher than 200 and 300 ng/ml for women and men, respectively). It should be kept in mind that ferritin is an acute-phase reactant that can be elevated in a number of inflammatory diseases. The use of ferritin for assessment of hypoferremic state may be uninformative and not correlate with tissue iron deposition.

The reference values of human hair iron content in Russia calculated in accordance with IUPAC guide in men and women were 11.1-40.5 and 8.9-25.6 μg/g [32].

Table 3

Laboratory criteria for impaired iron exchange diagnostics

Parameter	Iron-deficient anemia	Anemia of inflammation	Iron overload
Hemoglobin	Decreased < 12.5 mg/dl in men; < 11.5 mg/dl in women	Low or normal	Normal or increased
Transferrin saturation	< 20%	Low level	> 45%
Ferritin	<20 ng/ml	> 20 ng/ml	Increased >200 ng/ml in women; >300 ng/ml in men
Serum iron	Low	Low	Low or normal
Erythrocytes	Microcytic	Normocytic	Normocytic

Table 4

Laboratory parameters of iron status in certain diseases

Disease	Iron		Hb	Tf saturation, %	Ferritin	sTfR	Hepcidin
	Serum	Hair					
Iron deficient anemia (various forms)	↓ [33]	↓ [34]	↓ [35]	↓ [36]	↓ [37]	↑ [33]	↓ [38]
Hemochromatosis (various forms)	↑ [39]		↑ [40]	↑ [41]	↑ [41]	↓ [42]	
Obesity	↓ [43]	↓ [44]		↓ [43]	↑ [45]	↑ [46]	↑ [47]
Diabetes mellitus 2 type	↑ [48]	↓ [49]			↑ [50]		↓ [51]
Breast cancer	↑ [52]	↓ [53]			↑ [54]		↑ [55]
Colorectal cancer	↓ [56]			↓ [56]	↓ [57]		
Lung cancer	↓ [58]				↑ [59-60]		↑ [61]
Prostate cancer	↓ [62]			↑ [63]	↓ [64]	↑ [64]	↑ [65]
Hypothyroidism	↓ [66]		↓ [66]			↑ [67]	
Hyperthyroidism			↓ [68]	↓ [68]	↑ [69]		
Coronary artery disease (atherosclerosis)	↓ [70]				↑ [71]	↑ [72]	
Hypertension	↑ [73]		↑ [73]		↑ [74]		
Polycystic ovaries syndrome					↑ [75]		↑ [76]
Porphyria cutanea tarda	↑ [77]			↑ [78]	↑ [79]		↑ [80]
Thalassemia	↑ [81]				↑ [82]	↑ [83]	
Down syndrome			↑ [84]	↓ [85]	↓ [85]		
Alzheimer disease	↑ [86]			↑ [87]			
Alcoholism	↑ [88]			↑ [89]	↑ [90]		↓ [91]
Hepatocellular carcinoma	↑ [92]	↓ [93]	↓ [94]		↑ [95]	↑ [96]	
Preeclampsia	↑ [97]			↑ [98]	↑ [99]		↑ [100]
Idiopathic pulmonary hypertension	↓ [101]			↓ [101]	↓ [101]	↑ [101]	↑ [101]

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ZINC

Introduction

Zinc is an essential trace element. Since ancient times zinc is widely used in medicine as a remedy for wound healing, treatment of skin diseases, and in last decades as an immuno-enhancing, insulin-sensitizing and testosterone enhancing agent [1-3]. Adequate zinc balance is critical for growth, development, differentiation, DNA synthesis, RNA transcription, and cellular apoptosis [4]. Being a cofactor, zinc is a structural part of many enzymes. Zn^{2+} ion binds to more than 3000 proteins. The latter represents nearly 10-20% of the whole proteome [5]. More than 2000 signal molecules and transcription factors depend on intracellular zinc levels [6]. In particular, zinc fingers are structural components of transcription factors, hormonal receptors.

Epidemiological data indicate a high incidence of zinc deficiency, especially in the developing countries with more than 2 billion people affected [1, 7]. Clinical symptoms of zinc deficiency may include loss of appetite, anemia, immune deficient states, etc. Zinc deficiency is also associated with various pathological states like alopecia, impaired wound healing, delayed physical development, hypogonadism, neurologic diseases. Zinc deficient state may occur in malabsorption, chronic liver and kidney diseases, alcoholism, chronic pancreatitis, sickle-cell anemia and a number of other pathologies. The risk of infectious diseases and the period of rehabilitation are increased in zinc deficient persons [1]. The most

critical periods for zinc deficiency are embryogenesis, early childhood, and the elderly [8].

The total level of zinc in human organism is 2-4 g. High zinc content is observed in brain, muscles, bones, liver, and kidney. However, the maximal level is observed in prostate and eye [6]. At the same time, excessive zinc levels may be hazardous and the labile pool of zinc is strictly controlled. Optimal daily requirement in zinc is 10-15 mg\day, the toxic level is above 600 mg\day (chronic exposure). Lethal dose is 6000 mg. Zinc resorption is 20-30%; it is absorbed mainly in intestine; excreted with feces and less with urine (20-30%). The half-life in the organism is 245 days [9].

Copper, cadmium, lead are functional antagonists of zinc, especially against the background of protein deficiency. Increased intake of phytates, phosphates, calcium, intake of corticoids, oral contraceptives, anabolic steroids, anti-metabolites, diuretics, alcohol, immunosuppressive agents can lead to zinc deficiency in the body [2].

Transport, metabolism and regulation of zinc homeostasis

A number of transport proteins like albumin, transferrin, α2-macroglobulin, and immunoglobulin G take part in zinc transport in the bloodstream [6]. Consequently, zinc is delivered into liver for subsequent deposition. In accordance with the organism's requirement, zinc may be released from liver into the bloodstream and transported to the target-cells.

The special feature of zinc is the maximal concentration in nucleus and cytoplasm in comparison to the other trace elements. The structural role of zinc in the cell is mediated through stabilization of filaments, organization and stabilization of chromosomes, and DNA regulation.

Currently, it is known that zinc homeostasis is regulated by 14 transporters from ZIP family and 10 ZnT-family transporters [10]. The main function of ZnT transporters is to decrease cytoplasmic zinc content both by its elimination from the cell and accumulation in cellular compartments. At the same time, ZIP-family transporters act oppositely resulting in increased cytosolic zinc concentration through its excessive flux from the respective compartments. It is supposed that the activity of ZIP-family transporters is regulated by both dietary zinc and endocrine regulation [11].

The localization of zinc transporters and their primary functions are presented in Table 1. The entrance of zinc into the cell with the help of ZIP1 and 2 is energy-independent process. Expression of both transporters is increased after decrease of intracellular zinc concentrations [4]. The alternative route is Zn^{2+}/HCO_3^- symport [11]. ZIP1 expression is observed almost in all tissues of the organism. During pregnancy ZnT1, 2, and 3 play the key role in regulation of zinc homeostasis [11].

Zinc enters enterocytes in the proximal intestine by ZIP4 action. At high zinc concentration degradation of ZIP4 can occur. At the same time, ZIP4 is also regulated by Kruppel-like factor (KLF4) that is present in intestine. ZIP5 expression was observed at the basolateral membrane of enterocyte and acinar cells. In the case of zinc deficiency, its activity is decreased [11].

ZIP8 transporter is present in lysosomes of T-cells. Experimental studies involving knock-out mice have indicated that decreased activity of this transporter results in impaired interferon γ (IFN-γ) production that is required for immune defense. Oppositely, increased ZIP8 activity is associated with T-cell activation [11].

Another transporter ZIP14 is structurally similar to ZIP8 and also takes part in inflammatory response regulation. ZIP14 is located at hepatocyte cellular membrane and takes part in development of hypozincemia and hypoferremia under the influence of acute phase

proteins [11]. It has been also indicated that ZIP14 takes part in the transport of both transferrin-bound and non-transferrin-bound iron (NTBI). Earlier studies have also demonstrated that HFE gene expression in hemochromatosis is associated with decreased ZIP14 activity [11].

Table 1

Zinc transporters and their functions

Transporters	Localization in cellular compartments, specialized cells, tissues	Elimination from the cell
ZnT1	nucleus	from membranes
ZnT2	lysosomes	
ZnT3	synaptic vesicles	
ZnT4	endosomes of intestinal cells and mammary glands	
ZnT5,6 и 7	Golgi apparatus	
ZnT8	insulin vesicles	
ZIP 1, 3	all cells, prostate, mammary glands, central neural system, pancreatic α-cells	from lysosomes, vesicles
ZIP 2 and 6	all cells	
ZIP4	pancreatic β-cells, intestine, kidney, hippocampus	from lysosomes
ZIP5	pancreas, liver, kidney, stomach, intestine	
ZIP7	all cells	from nucleus, endoplasmic reticulum, Golgi apparatus, vesicles
ZIP 8	T-lymphocytes, erythrocytes, testes	from lysosomes, mitochondria
ZIP9		from Golgi apparatus
ZIP10	central neural system, liver, kidney, erythrocytes, thyroid	
ZIP12	central neural system, lungs, retina, testes	
ZIP 13	all cells	from Golgi apparatus, vesicles
ZIP14	all cells, liver	

Lysosomes cumulate the majority of divalent cations like Ca^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, etc. Intensification of red/ox processes is accompanied by increased zinc levels in the cellular compartments [12-13]. At the same time, their impaired biogenesis results in increased cytoplasmic zinc levels [14].

The regulation of zinc homeostasis is also mediated by the family of metallothioneins. Metallothioneins are cysteine-rich proteins that chelate zinc and other metals (copper, cadmium). Saturation of MT with zinc results in its storage in the protein-bound form and regulates the level of free zinc fraction. Metallothioneins are multifunctional proteins. As an antioxidant MT suppresses allergic inflammation through decreased production of proinflammatory cytokines like IL-1β and lowered histamine release [6, 14].

ZnT1 regulates zinc flux from the cell and, consequently, regulates its intracellular concentration. At the same time, ZnT2 delivers zinc to lysosomes, whereas ZnT3 mediates its entrance into synaptic vesicles where it can modulate neurotransmission. Expression of ZnT8 is almost exclusively observed in pancreas and subcutaneous adipose tissue. The presence of this transporter is associated with the storage of "zinc-insulin" complex and its postprandial release. Impaired ZnT8 expression or the presence of anti-ZnT8 antibodies is associated with diabetes mellitus 1 and 2 type development.

Various factors like the presence of phytates, calcium, iron, copper, and selenium may affect zinc absorption. It has been estimated that high bioavailability will be observed if the ratio between dietary phytates and calcium and zinc is lower than 200. Increased levels of phytates decrease zinc absorption. At the same time, elevated dietary calcium along with normal phytates levels may not affect zinc balance [15-16].

Consumption of additional quantities of iron may affect zinc absorption. The experimental study using Caco-2 cells has indicated

that in the presence of iron ^{65}Zn transport through the apical membrane was decreased. Possible mechanisms involve competition for divalent metal transporter 1 (DMT1) and ZIP14 that also takes part in iron absorption [8].

It is well known that zinc activates metallothionein synthesis. However, copper also stimulates MT expression and its affinity to MT is higher than for zinc. In the case of copper overload concurrent binding to MT results in impaired zinc homeostasis [17]. Oppositely, excessive zinc consumption may be associated with copper deficiency [18]. Zinc homeostasis is also interrelated with selenium. In particular, selenium-dependent glutathione peroxidase reduces oxidized MT with the subsequent release of zinc. The latter may exert antioxidant properties through activation of superoxide dismutase [8].

Multiple studies highlight the negative effect of cadmium on zinc balance. In particular, zinc deficiency results in increased cadmium accumulation and toxicity. At the same time, administration of zinc-containing preparations decreased absorption, accumulation, and toxicity of cadmium [19]. It has been estimated that MT are involved in heavy metal detoxification. Moreover, MT binds Cd(II) taking part in zinc and copper metabolism [20]. Experimental studies have also demonstrated that zinc transporters take part in cadmium transport. Thus, ZIP8 and ZIP14 expression was accompanied by enhanced cadmium accumulation [21]. ZIP8 and ZIP14 gene polymorphism also results in elevated blood cadmium concentration [22].

Zinc as a cofactor and clinical manifestations of its deficiency

It is supposed that more than 2 billion people worldwide suffer from zinc deficiency. The highest incidence is observed in the

developing countries [1,7]. Clinical manifestations of zinc deficiency include impaired growth, loss of appetite, impaired immunity (both innate and acquired) and hemostasis, altered bone mineralization, decreased sperm production, cognitive dysfunction, hair loss, skin diseases, impaired wound healing, eye pathology [26]. Age is associated with a decrease in the organism's zinc content. Particularly, less than a half of aged persons receive the required amount of zinc that is equal to the Recommended Dietary Allowance (RDA) [25].

Prof. Prasad [1] has proposed to classify zinc deficiency in accordance with the intensity of symptoms. In particular, marked deficiency is observed in acrodermatitis enteropathica, prolonged parenteral nutrition without zinc supplementation, alcohol consumption, and D-penicillamine therapy in Wilson disease. The prevailing clinical manifestations are bullous dermatitis on the extremities, development of alopecia, paronychia, diarrhea, weight loss, emotional and neurosensory disorders, intercurrent infections, blepharitis and conjunctivitis, hypogonadism, impaired wound healing [23]. Critically low level of zinc requires immediate pharmacological correction.

In moderate zinc deficiency the development of hypogonadism in teenagers, impaired growth, decreased appetite, rough skin, slow wound healing, apathy, immune and neurosensory disorders.

Moderate zinc deficiency is also characterized by decreased testosterone levels, oligospermia, decreased IL-2 and NK-cells production, impaired sense of taste, decreased crepuscular adaptation, decreased body mass [23].

Zinc acts as a cofactor in all classes of enzymes. Table 2 provides the list of some zinc-dependent enzymes, proteins, and hormones.

More than 300 enzymes contain zinc finger. In particular, RING (Really Interesting New Gene) fingers consist of 40-60 amino acids and two zinc atoms. Lim domains also contain two Zn atoms

and play a significant role in the formation of cytoskeleton, as well as in organ development and oncogenesis [6].

Table 2

Certain zinc-containing enzymes, proteins, and hormones

Compound	Function
Insulin	Regulation of carbohydrate, protein, and lipid metabolism The main anabolic hormone in the organism
S-100 proteins, calprotectin	Cellular adhesion
Carboanhydrase (EC 4.2.1.1)	Catalyze reversible hydration of carbon dioxide Regulate acid-base balance in tissues
Superoxide dismutase 1 (EC 1.15.1.1)	Antioxidant defense
Carboxypeptidase A (EC 3.4.17.1) and B (EC 3.4.17.2)	Hydrolytic cleavage of C-end amino acid from proteins and polypeptides
Alcohol dehydrogenase (EC 1.1.1.1)	Oxidation of alcohols and reduction of aldehydes
Metallothioneins	Antioxidant defense
Alkaline phosphatase (EC 3.1.3.1)	Hydrolytic cleavage of phosphate residue from various organic compounds
Alanine aminopeptidase (EC 3.4.11.2)	Hydrolytic cleavage of N-end animo acid from peptide, anide or acrylamide
Proteinkinase C (EC 2.7.11.13)	Phosphorylation of proteins and subsequent induction of signal transduction
RNA/DNA polymerases	Nucleic acid synthesis
Zn-fingers	Transcription and interaction factors
δ-aminolevulinate dehydratese (EC 4.2.1.24)	Synthesis of porphobilinogen, an intermediate metabolite of heme biosynthesis
Calcineurin	Activation of transcription of various structural and regulatory genes
Nucleases	Hydrolysis of phosphodiester bonds
NADPH-oxidase (EC 1.6.99.6)	Reduces oxygen to superoxide anion radical

Special attention is given to the proteins of extracellular matrix, matrix metalloproteinases (MMP). MMPs – a family of 23 matrixins,

Zn^{2+}-dependent endopeptidases. The primary function of matrixins is degradation of extracellular matrix proteins (collagen, proteoglycan, fibronectin). The other important function is the release of biologically active molecules like cytokines, growth factors, and chemokines. α2-macroglobulin depresses MMP production. 4 groups of matrixins are known to date: collagenases, gelatinases, stromelysins and heterogenous group [24]. The primary functions and localization of MMPs are presented in Table 3.

Table 3

Localization and function of some matrix metalloproteinases

Matrixins	Localization	Pathologies associated with impaired MMPs functions
MMP-1	lungs, skin, musculoskeletal system	lung emphysema, hyperkeratosis, arthritis, cancer
MMP-2	blood vessels	neovascularization and metastases
MMP-3	lungs, musculoskeletal system	bronchial asthma, arthritis, impaired wound healing
MMP-7	skin	impaired epitelization
MMP-8	neutrophils	impaired embryogenesis, cancer, increased susceptibility to infections, atherosclerosis
MMP-9	blood vessels, neutrophils, lymphocytes, dendritic cells	impaired embryo implantation, endometriosis, bronchial asthma, respiratory distress syndrome, frequent infections, cancer
MMP-10	skin	impaired wound healing
MMP-11	embryo, female reproductive system, adipose tissue	cancer
MMP-12	macrophages, placenta	bronchial asthma, impaired embryogenesis
MMP-13	bones	rheumatoid arthritis, chondrosarcoma, breast cancer, melanoma
MMP-14	blood vessels, musculoskeletal system	impaired growth, dysplasia, cancer
MMP-16 and 24	female reproductive system	endometriosis

MMPs are involved in the regulation of proteolytic and antiproteolytic balance in the human organism. Impairment of this balance results in chronic infections, impaired tissue remodeling, angiogenesis, tumorigenesis [24]. For example, the incidence of cardiovascular diseases is increased in persons with matrix metalloproteinase gene polymorphism, especially MMP-3 and MMP-9 [25]. Experimental studies have indicated increased MMP-2, 9, and 13 expression in allergic rhinitis and bronchial asthma patients [26]. However, the existing data does not provide information on the possible influence of zinc levels on matrixin expression. It is not clear if zinc treatment may be used for modulation of matrixin production in different pathologies.

Earlier data indicate that zinc may act as a signaling molecule [14]. Intracellular zinc activates secondary calcium messengers (Ca/calmodulin-dependent proteinkinase II), as well as cAMP-dependent kinase, tyrosine phosphatase, caspase 3 [27]. Moreover, zinc also may affect activation of ATP-dependent K^+ and Ca^{2+}-dependent K^+-channels. Intracellular zinc level is rapidly changes due to the presence of such mechanism. This phenomenon in mast cells was termed "zinc wave". In addition, zinc also activates kinases, phosphatases, and transcription factors (NF-kB) [1].

Zinc deficiency alters both innate and adapted immunity. The influence of zinc deficiency on immune system includes thymus atrophy, impaired lymphocyte differentiation and maturation, decreased timulin production, lymphopenia, and alteration of both T- and B-cells [1, 6]. Zn-deficient state results in increased glucocorticoid production that aggravates thymus atrophy, increased apoptosis in thymocytes and decreased lymphopoiesis [6]. At the same time, myelopoiesis is not affected by zinc deficiency [1]. Moreover, mature T-cells ($CD4^+$ and $CD8^+$) are resistant to zinc deficiency due to the high level of antiapoptotic protein Bcl-2. Patients with zinc deficiency are predisposed to infection, autoimmune diseases, and neoplastic processes [8, 28-29].

Zinc deficiency is also accompanied by decreased lytic activity of NK-cells, macrophages, mast cells, granulocytes, and complement system. At the same time, neuroendocrine-immune interaction is also impaired. Zinc treatment stimulates innate immunity in children through increased complement levels, phagocytosis, and functional activity of T-cells [6]. In the elderly zinc deficiency is accompanied by decreased NK-cell activity resulting in impaired elimination of cancerous and virus-infected cells. Consequently, the incidence of viral infections and cancer is increased [8]. Administration of 15 mg/day zinc by the aged persons results in increased NK-cell cytotoxicity, normalization of IFN-γ production, and, finally, decreased frequency of viral infections by 50% [30].

The ratio of T-helpers (Th1/Th2) is impaired in zinc deficiency. In particular, elevated IFN levels depress Th2 generation. Increased IL-10 production, oppositely, affects Th1. It has been noted that cytokine production (IL-4, 6) is not changed in zinc deficiency [1].

Decreased Th1 production along with increased Th2 generation is indicative of chronic low-grade inflammation that has been termed as “inflammaging” [1,8]. Increasing age on the background of zinc deficiency results in impaired Th1/Th2 ratio with the decrease in the latter. Moreover, impaired CD4/CD8 ratio, decreased T-cells and $CD45RA^+$ cells proliferation, lowered expression of antiapoptotic proteins Bcl-2 and p53 is also observed. At the same time, an increase in memory T-cells (CD45RO+) is also observed [8].

Zinc plays an important role in endocrine and exocrine function of pancreas including insulin and glucagon synthesis, storage and secretion as well as regulation of digestive enzymes activity. Nearly 2 mg zinc daily enters pancreatic acinar cells. Zinc binding with insulin is required for crystallization and hormone storage with formation of hexameric structure containing two zinc atoms [5]. Zinc-insulin complex is excreted from vesicles by

exocytosis. Consequently, zinc is released into the extracellular space and may be transported back into the cell or in other cells [31]. Moreover, zinc also takes part in regulation of insulin signaling. In particular, zinc decreases activity of tyrosine phosphatases resulting in increased insulin receptor b-subunit phosphorylation [23].

The mechanisms of zinc transport depend on blood glucose concentration. Particularly, ZnT transporters are activated at low glucose concentrations whereas in the hyperglycemic conditions zinc is transported into the cell through L-type voltage-gated Ca^{2+} channels [31]. Zinc deficiency is associated with obesity and diabetes mellitus (types 1 and 2) development [10, 14]. SLC30A8 gene polymorphism is associated with impaired zinc transport in pancreas (ZnT8) and results in impaired endocrine activity of pancreatic β-cells [10]. Epidemiologic studies have demonstrated that in 50-60% of patients with acute manifestation of type 1 diabetes mellitus anti-ZnT8 antibodies were detected [32]. Genetic polymorphism ZnT8/SLC30A8 also resulted in type 2 diabetes mellitus development [5, 10].

Experimental studies have indicated that Zn^{2+} depresses glucagon production by α-cells [31]. Zn^{2+} release from β-cells is obviously a signal to decreased glucagon secretion. Moreover, some investigators propose the possibility of glucose-independent regulation of glucagon content by zinc [31]. The mechanisms of action of zinc on carbohydrate metabolism are mediated not only by modulation of insulin and glucagon production and secretion but also involve glucose transporters and insulin receptors [10]. Increased urinary zinc losses were detected in type 2 diabetes mellitus patients. Positive correlation between hyperzincuria and glycosylated hemoglobin levels was also revealed [23]. In addition, hyperglycemia is one of the causes of oxidative stress that is associated with mitochondrial and cytoplasmic reticulum dysfunction in β-cells. Persisting oxidative stress is accompanied by impaired

production of antioxidant enzymes like SOD1, glutathione peroxidase and catalase. Moreover, experimental in vitro studies have also indicated that zinc may affect xanthine oxidase (molybdenum-containing enzyme) activity decreasing the intensity of oxidative stress. Zinc administration also influences antiapoptotic protein A20 production that is expressed in response to proinflammatory cytokines. A20 decreases IL-1β, IL-8, and TNF-α production in β-cells [10]. Therefore, zinc acts as a regulatory factor of apoptosis in pancreas. Multiple experimental studies have confirmed the role of ZIP8 in insulin production by β-cells.

Calcineurin is a Ca^{2+}/calmodulin-dependent serine/threonine phosphatase containing Zn^{2+} in the active center. This enzyme takes part in development of ischemia, immune response, and eye pathology [33]. It has been estimated that zinc decreases nickel-induced activity of calcineurin [34-35]. Under the neural or hormonal stimulation cytoplasmic calcium concentration is increased leading to Ca^{2+} calmodulin binding. Formation of zinc-calmodulin complex affects calcineurin activity [34]. Moreover, experimental study has indicated an inhibitory action of Cu,Zn-SOD on calcineurin activity in neurons [36].

Calprotectin is Ca^{2+}- and Zn^{2+}-binding protein from S100 protein family. Calprotectin possesses antibacterial and chemotactic activity. It takes part in the interaction of leukocytes and endothelium in the sites of inflammation. In laboratory diagnostics calprotectin is considered as an inflammatory marker. Plasma calprotectin levels are increased in response to zinc deficiency [37].

Zinc dependent processes

Cancer cells are characterized by zinc dysbalance due to impaired functioning of zinc transporters. Further, this process may

result in increased intracellular zinc levels leading to increased viability. At the same time, blood zinc levels are decreased in patients with cancer of various localizations. In particular, serum zinc concentration in colon, prostatic, and skin cancer is decreased. Oppositely, increased metal levels are observed in patients with breast and lung cancer [6, 11]. Moreover, breast cancer patients are characterized by increased ZIP6, 7, and 10 expression [11, 38]. Increased intracellular zinc and its transporter ZIP4 are observed in pancreatic cancer [38].

Apoptosis is an active genetically determined and strictly regulated process of cellular death. Mitochondria play a significant role in induction and coordination of this process through modulation of signaling pathways that involve antiapoptotic (Bcl-2) and proapoptotic (Bax) mechanisms. Impaired apoptosis is the pathogenetic mechanism of various disorders including neurodegenerative diseases, AIDS, diabetes mellitus, autoimmune diseases and neoplastic processes. Zinc is supposed to be one of the regulators of apoptosis. From the one hand, zinc protects the cell from oxidative stress through formation of zinc-thiolate complexes that prevent oxidative damage of proteins. This process stabilizes tubulin that is required for microtubules polymerization. Moreover, zinc is capable of caspase 3 inhibition due to formation of complexes with sulfhydryl group [6]. From the other hand, under the influence of zinc MT reduces apoptosis.

Earlier studies indicated that prolactin, growth hormone, and placental lactogen have similar structure. Zinc takes part in binding of these hormones with prolactin receptor. The most probable mechanism is conformational changes in hormonal structure in the presence of zinc. During zinc deficiency the affinity of growth hormone to its receptor is decreased, whereas its binding to prolactin receptor is increased [39]. At the same time, prolactin also affects zinc homeostasis. It has been demonstrated that prolactin increases

ZnT2 expression through Jak2/STAT5 signaling pathway resulting in increased zinc accumulation in secretory vesicles [40].

One of the markers of zinc deficiency in man and animals is hypogonadism. Severe zinc deficiency results in impaired spermatogenesis and steroidogenesis [41]. Multiple studies indicated decreased testosterone production in zinc deficiency [41-42]. The proposed mechanisms of such decrease may involve activation of apoptosis in Leydig cells [41]. Decreased zinc consumption is accompanied by increased cortisol levels also leading to impaired spermatogenesis. Zinc administration decreases FSH concentration and increases levels of LH and testosterone in patients including those at hemodialysis [43-44].

It has been estimated that low serum zinc is observed in hypothyroid patients, whereas hyperthyroidism is characterized by zinc excess [45]. Decreased thyroid hormones levels result in decreased zinc absorption and increased its urinary excretion in experimental animals [46].

Zinc also takes part in estrogen metabolism. In normal menstrual cycle the level of zinc in serum was directly associated with estrogen concentration. At the same time, its correlation with 17β-estradiol and copper was inverse [47]. It has been estimated that zinc finger protein 131 (ZNF131) interacts with estrogen receptor (ERα) decreasing the impact of estrogens [48]. In turn, estrogens may also influence zinc homeostasis. In particular, 17β-estradiol administration in ovariectomized animals resulted in increased hippocampal zinc content, improved memory and learning ability [49].

Bone zinc content decreases with age. Zinc stimulates osteoblasts activity and bone tissue mineralization. Moreover, it also decreases formation of osteoclasts. The influence of zinc on osteoclastogenesis is mediated through activation of NF-kB and induction of apoptosis in mature osteoclasts [50]. Additional zinc administration in phytoestrogen therapy potentiates antiresorbtive effect [51].

Zinc plays an important role in growth hormone metabolism in somatotrophic cells of the anterior pituitary [52]. Zinc increases production of insulin-like growth factor 1 (IGF1, somatomedin) [52]. Somatomedin is produced in liver in response to growth hormone signaling. Intracellular zinc levels regulate somatotropin production and storage in secretory vesicles [53]. Somatomedin deficiency manifests with impaired growth in children [18, 52]. A strong direct association was revealed between blood zinc, growth hormone, and IGF-1 concentration has been revealed [54–55]. Zinc deficiency results in decreased IGF-1 independently of total energy intake [23]. It has been also estimated that growth hormone affects Zn-α2-glycoprotein activity. As a result, children with low growth hormone levels are also characterized by adipocyte hypertrophy [56]. Zinc administration increased GH levels in children [54]. The mechanisms involved in the association between zinc and some signaling molecules are indicated in Table 4.

Table 4

The participation of zinc in hormone production

Hormone	Zinc-mediated functions
Insulin	Hormone accumulation
Angiotensin	Zn-mediated regulation of angiotensin converting enzyme
Enkephalin	Zn-mediated regulation of enkephalinase
Neurotensin	Zn-mediated degradation
Thyroxin	Amino and carboxypeptidases, endopeptidases, and metallomatrixins
Growth hormone	
Sex hormone binding globulin	Zn-dependent regulation of transcription factors
Glucocorticoid, estrogen, progesterone, thyroxin, and vitamin A receptors	Zinc fingers

Thymulin is a zinc-dependent hormone secreted by epithelial thymus cells. Thymulin is required for T-cell differentiation, cytotoxic properties and IL-2 production by T-lymphocytes [1]. It

has been demonstrated that zinc deficiency is accompanied by lymphopenia, impaired T- and B-cell immunity and allergic reactions. Increased ZIP2 expression is observed in leukocytes of children suffering from asthma. Zinc administration decreases histamine release and cytokine production [6].

Moreover, zinc affects mast cells degranulation. Under the influence of external stimuli (cytokines) intracellular zinc levels are sharply increased in mast cells, being caused by the active Zn transport from endoplasmic reticulum into cytoplasm. The process is mediated through ZIP7. Zinc is also released from MT by nitrosylation. An increase in zinc levels is required for depression of allergic reactions. Insufficient dietary zinc intake is associated with increased incidence of allergic diseases (atopic dermatitis, bronchial asthma, etc.). Children with borderline zinc deficiency have more frequent infections of the upper respiratory tract with prolonged rehabilitation period [14].

It has been estimated that zinc can suppress autoimmune diseases through inhibition of T-cell activation. However, the intimate mechanisms of this process are still unknown. It is supposed that the element affects T-helper 17 (Th17) production, whose function is impaired in polyarthritis and autoimmune encephalomyelitis patients. Zinc suppresses Th17 production activated by IL-6/STAT signaling pathway and ultimately results in decreased autoimmunity risk [14].

In physiological concentrations zinc possesses neuroprotective properties, whereas the high doses may be neurotoxic. It has been shown that both low and high concentrations of zinc increase oxidative and nitrosative stress that are accompanied by elevated NADPH-oxidase and NO-synthase activity [57]. Zinc imbalance is observed in depression, schizophrenia, Alzheimer and Parkinson diseases, lateral amyotrophic sclerosis, and ageing [57]. Zinc is accumulated in synaptic vesicles and is released with glutamate activating ionic channels. Consequently, zinc may be considered as an “atypical neurotransmitter”

[14]. Zinc delivery to synaptic vesicles is mediated through ZnT3 [4]. Moreover, a specific Zn^{2+}-sensitive receptor GPR39 was identified [57]. Due to interaction with glutamate receptor, zinc takes part in the formation of long-term memory. In an experimental study young ZnT3-KO mice were characterized by impaired memory and learning difficulties. Along with increasing age the animals demonstrated altered memory and cognitive functions due to hippocampal lesions and behavioral disorders [14]. The most probable mechanisms of zinc-induced include alteration of calmodulin, calmodulin -dependent protein kinase II, and cAMP-responsive element binding protein signaling [57]. Even borderline zinc deficiency manifests with decreased attention, motor and cognitive disturbances. Thus, low zinc levels were demonstrated in depressive patients [57]. Depression is a functional disorder associated with increased risk of neurodegenerative processes and impaired neurogenesis. At the same time, treatment with tricyclic neurodepressants, selective inhibitors of serotonin reuptake along with zinc administration or zinc alone resulted in a better clinical outcome. Moreover, prolonged zinc administration improved the efficiency of antidepressants [57].

Zinc deficiency may be observed in malabsorption syndrome, Krohn disease and steatorrhea. Persons with liver cirrhosis are characterized by decreased serum zinc content and its increase in liver tissue [23]. In 2004 WHO recommended zinc administration for treatment and prophylaxis of diarrhea in children. It has been noted that zinc deficient children are more sensitive to gut pathogens and subsequent infections [23].

Zinc deficiency is also observed in alcoholism [58], fetal alcohol syndrome [58-59], alcoholic liver disease [60]. It is very interesting that chronic alcohol consumption in females before and during pregnancy causes zinc deficiency in newborns and decreased zinc content in cerebral cortex even in adult offspring [60-61]. Also, low cortex zinc was found in mice and rats, genetically predisposed

to voluntary alcohol solution drinking, and zinc administration can protect alcohol abuse [58, 62-63].

Experimental studies have indicated that metallothionein transgenic mice are resistant to alcohol. Oppositely, MT-KO animals were prone to alcoholic liver diseases due to decreased liver zinc content [64]. At the first stage, ethanol is metabolized into acetaldehyde with the help of alcohol dehydrogenase and CYP2E1 isoform. In chronic alcohol abuse the activity of CYP2E1 is increased in relation to alcohol dehydrogenase. Increased requirements in alcohol dehydrogenase result in elevated zinc expenses as the enzyme is zinc-dependent. Moreover, in zinc-deficient state the activity of NADPH-oxidase is increased, whereas SOD1 is depressed being indicative of prooxidant conditions [65]. Finally, chronic alcohol intake increases adipose tissue lipolysis and triglyceride deposition in liver. Decreased adipose tissue adiponectin production also aggravates fatty liver [65].

Decreased hair and blood zinc in cerebral palsy (spastic dyplegy) [60, 66], autism [67-68] was described. Administration of zinc sulfate improved immunity and general clinical picture in cerebral palsy children [66].

Inherited hemoglobinopathies like sickle cell disease or thalassemia are accompanied by zinc deficiency. Zinc deficient state may be associated with hyperzincuria, kidney dysfunction, and iron metabolism dysregulation. At the same time zinc treatment results in increased linear growth and weight normalization in children [69-70].

Metabolic disorders associated with zinc accumulation in the organism

It is known that high doses of zinc are toxic for the human organism. Despite the presence of numerous studies devoted to zinc

deficiency, toxic effects of this metal are insufficiently studied. In particular, the workers of zinc-smelting industry were characterized by myeloneuropathy development. Clinical findings included paresthesia, sensory disorders, tactile allodynia and tactile hallucinations in the lower extremities resulting in formation of spastic-ataxic gait [71]. At the same time, the symptoms of zinc excess may also refer to those of copper deficiency. As indicated earlier, high doses of zinc decrease organisms copper content and vice versa [18].

Intracellular zinc levels are strictly regulated by specific transporters like Zip and ZnT. In particular, ZnT2 and 4 are responsible for a decrease in intracellular metal concentration and its transport into lysosomes [12]. Zn^{2+} export into the cellular organelles supposes its temporary storage and protection from zinc-induced toxicity. However, further accumulation of zinc in lysosomes results in altered membrane permeability, release of lysosomal enzymes and initiation of apoptosis [12]. Zinc exposition impairs intracellular signaling pathways through various mechanisms involving metal interaction with thiol groups of regulatory proteins. Increased intracellular H_2O_2 production is also observed in zinc overload. Further, Zn^{2+} exposure decreases α-ketoglutarate dehydrogenase and cytochrome c oxidase activity and alters mitochondrial membrane potential [13].

Multiple studies indicate the role of zinc in Alzheimer disease (AD) development. It is known that β-amyloid accumulation is the leading cause of neurodegeneration in AD. The participation of zinc in β-amyloid precursor (APP) synthesis through modification of transcription factors (NF-kB and sp1) and activation of α-secretase has been demonstrated [57]. Moreover, excessive intracellular zinc accumulation and MT expression result in mitochondrial dysfunction and ROS generation. Recent studies have indicated a significant increase in hyppoccampal and neocortex ZnT1, 3, 4, 6, and 7 levels

in transgenic mice with early AD development [72]. On the other hand, a number of investigators propose that increased zinc levels associated with its deposition in amyloid plaques cannot be associated with zinc toxicity. Oppositely, zinc accumulation in plaques results in its decreased bioavailability to neurons. AD patients are characterized by lower blood zinc levels in comparison to the respective healthy persons of the same age. Zinc administration in the dose of 150 mg/day during 6 months by these patients resulted in increased blood zinc level, decreased free copper concentration and improved cognitive functions [23, 73].

Along with AD excessive zinc accumulation is observed in another type of senile dementia – vascular dementia that is associated with short periods of ischemia. It is known that Zn^{2+} plays a significant role in synaptic plasticity, learning and memory [23]. It has been estimated that significant amounts of zinc are released into the synaptic cleft with glutamate resulting in its depolarization [74]. Ca-EDTA administration decreases zinc deposition in CNS structures preventing hippocampal neuronal death [74].

Genetic disorders associated with impaired zinc metabolism

ZIP4 gene mutation resulting in impaired gastrointestinal zinc absorption is the cause of acrodermatitis enteropathica [11]. ZIP4 coding gene is located at 8q24 chromosome [23]. The disease is characterized by autosomal recessive inheritance and frequently occurs in children from consanguineous marriages. The disease manifests soon after the ending of breast feeding. The main pathogenetic factor is the development of systemic zinc deficiency and the subsequent enzymopathy. Clinical symptoms include rash in skin folds and the joining secondary infections. Moreover, alopecia (both focal and total)

and diarrhea are also frequently diagnosed. Children are characterized by apathy, delayed intellectual and sexual development [14]. Zinc deficiency is associated with impaired proteinkinase phosphorylation, decreased myosin formation, alteration of enterocyte size and their increased permeability. The absence of adequate treatment with zinc preparations may be lethal [23].

ZIP13 gene mutation or polymorphism is associated with osteochondrodysplasia including Ehlers-Danlos syndrome [14]. Spondylochondrodysplastic form of Ehlers-Danlos syndrome is accompanied by dysplasia of spine and the upper limbs. The primary cause is collagen hydroxylation, however, the activity of lysil- and prolyloxidase is usually unaffected [11].

A rare genetic disease with autosomal-recessive inheritance is observed in mutation of gene coding zinc finger transcription protein (ZNF407). The gene is located at 18q23 chromosome. Clinically this metabolic disorder manifests with cognitive dysfunction, hypotension, dysmorphia (bilateral ptosis, epicantus, face hypoplasia), impaired skeleton formation (limited knee joint mobility, flexion contracture of fingers) [75].

Laboratory criteria of impaired zinc metabolism

It has been noted that plasma zinc levels remain stable during 4-5 months after a decrease of its intake [1]. The most frequently organism's zinc status is assessed using its concentration in serum/plasma. The value of 85-90 μg/dl is considered to be normal [30]. Zinc levels lower than 8.2 μmol/l is supposed to be prognostically unfavorable [18]. The level of intracellular zinc is estimated by the ability of zinc release from MT under NO donor stimulation [76]. Erythrocyte and neutrophil zinc content are also used in certain investigation. However, in routine medical practice

this approach is not widespread. Hair and nail zinc levels are also used as biomarkers of low zinc provision of the body [77]. In our opinion, estimation of zinc status is more reliable when analyzing zinc at least in two different biosubstrates (e.g. serum and hair). The reference values of hair zinc levels in adult men and women were estimated to be 125.7–262.8 and 140.0–315.1 μg/g [78].

It is known that zinc is excreted with feces. Urinary elimination is additional and does not exceed 300-600 μg/l. Hyperzincuria in the case of low serum zinc concentration is observed in patients with liver cirrhosis, increased catabolic processes (hyperthyreosis), and neoplastic processes. Increased zinc elimination in persons with normal and increased serum levels may be indicative of uncontrolled use of multivitamin and multielement preparations. Decreased zinc content in both substrates is observed in burn patients and gastrointestinal disorders associated with impaired zinc absorption [79].

In iron deficiency and lead intoxication impaired heme synthesis occurs. As a result, zinc-protoporphirin is cumulated in erythrocytes. The level of 70 μmol of Zn-protoporphyrin per mol of heme is an indicator of impaired heme synthesis including the cases of lead intoxication and iron-deficiency anemia [80].

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COPPER

Introduction

Copper is an essential trace element that is a structural part of a number of enzymes, being involved in regulation of metabolism, tissue respiration, biosynthesis of hormones and cofactors that promote iron absorption [1-2]. In turn, disturbances in Cu metabolism has numerous adverse health effects. Consequently, maintenance of adequate copper status is required for health management of an individual.

The metal has been widely used in medicine for a long time. Copper sulfate is used as an antimicrobial agent and cauterant. In the 60^{th}-80^{th} copper sulfate was used for treatment of anemia, hypotrophy (nowadays, copper chelates are used more widely). Preparations of various copper salts are used externally for irrigation, syringing, in the form of liniments and in physiotherapy. In association with iron, copper is used for treatment of hypochromic anemia, hypotrophy and other diseases. Copper-containing drugs and food supplements are also used in treatment and prophylaxis of musculoskeletal diseases, hypothyroidism. Lippes loop made from copper is widely used as a contraceptive device. ^{64}Cu isotope is used in radioisotope diagnostics of brain tumors, in radiobiology [3].

The total content of copper in an average adult human body is 100–150 mg. Brain, liver and heart muscle are rich in copper. The optimal daily intake of copper is 2–3 mg. Nutritional deficiency can develop when intake is lower than 1 mg/day, toxicity level – 200–

250 mg/day. Copper is absorbed mainly (90%) in stomach; excreted by bile (80–85%) and less by urine. The copper half-life in the organism is 12–61 hours [4].

Enhanced consumption of molybdenum and zinc can lead to copper deficiency. Cadmium, manganese, iron, antacids, tannins, ascorbic acid can reduce copper absorption. Zinc, iron, cobalt (in moderate physiological doses) increase the assimilation of copper. In turn, copper can inhibit the absorption of iron, cobalt, zinc, molybdenum and vitamin A in the body. Oral contraceptives, hormonal agents, cortisone preparations contribute to enhanced removal of copper from the body [5].

Transport, metabolism and regulation of copper homeostasis

Copper originating from foods is imported into enterocyte with the help of copper transport protein 1 (Ctr1). For its further transport into a bloodstream ATP7A protein is required. This protein is capable of transporting copper from the enterocyte. It is notable that copper export from cells is an energy-requiring process. Copper enters the bloodstream where it is transported in a complex with albumin and transcuprein. Copper transported to the liver enters hepatocytes with the help of Ctr1 [6]. This protein is widespread in a number of tissues, however, highest rates of expression are observed in cells of vascular plexus of brain ventricles, renal tubules, connective tissues of an eye, testes and ovaries, gastrointestinal tract. It is estimated that the presence of oxygen is a rate-limiting factor for Ctr1 expression [7]. Recent studies involving cell cultures have indicated two main Ctr1 localizations: cellular membrane and vesicles [8]. It has been demonstrated that silver ions may act as potential inhibitors of Ctr1 [9]. Moreover, its activity may be reduced

in the presence of excessive levels of Cd^{2+}, Mn^{2+}, Zn^{2+} и Co^{2+} ions. Recently discovered homologue Ctr2 is located predominantly in the cytoplasm (lysosomes and endsomes). It is supposed that Ctr2 may also play a role in intracellular copper homeostasis. However, this transporter has lower affinity to copper in comparison to Ctr1. Divalent metal transporter 1 (DMT1) may also perform copper transport inside the cell. It is demonstrated that copper transport inside the cell is ATP-independent process.

Further copper transport inside the cell occurs with the help of chaperones. Their role is delivery of copper to target proteins – copper-containing enzymes.

CCS is a chaperone that delivers copper to Cu, Zn-SOD (SOD1 and 3). SOD biosynthesis occurs in granular cytoplasmic reticulum. Various isoforms of SOD are present: cytoplasmic, nuclear and extracellular [10].

Cox17 delivers copper to mitochondria, where copper is included in the active center of cytochrome c oxidase (CCO). CCO activation also requires the presence of zinc, magnesium and iron. CCO1 and 2 (Cox1 and Cox2) subunits have centers for copper binding CuA and CuB, respectively. Sco1 and Sco2 chaperones are required for transport and binding of copper with CCO2 subunit. At least 4 chaperones take part in copper transport to cytochrome c oxidase: Cox17, Sco1, Sco2, Cox11. Cox11 delivers copper to CCO CuB subunit.

Atox1 chaperone transports copper to Golgi network for binding with transport ATPases. These chaperones perform excretory functions. Thus, dietary copper accumulates in liver and then is excreted with bile. It is supposed that Atox1 also delivers copper inside Golgi network to dopamine-β-monooxygenase, peptidylglycine α-amidating monooxygenase (PAM), lysiloxidase, tyrosinase, and hephestin. Mechanisms of copper delivery to extracellular SOD, aminooxidase, diaminooxidase and vascular adhesion protein 1 are still insufficiently studied.

After the entrance and distribution of copper inside the cell a number of mechanisms of copper elimination start to work. ATP7A/B family is related to copper-eliminating proteins. It is supposed that ATP7B takes part in copper binding to ceruloplasmin and copper release from hepatocytes with its subsequent excretion with bile. The primary function of ATP7A is excretion of copper from enterocytes, neurons and astrocytes into the bloodstream.

Copper-binding protein COMMD1 (MURR1 domain) and X-linked inhibitor of apoptosis protein (XIAP) are also involved in regulation of copper excretion. In the conditions of copper overload liver increases XIAP catabolism. The latter takes part in COMMD1 degradation that prevents excessive copper accumulation in hepatocytes.

Copper-containing enzymes and clinical manifestations of copper deficiency

Copper, being a metal with high redox potential, performs essential functions in biological systems. Copper plays an important role in cellular respiration, iron metabolism, neurotransmitter and pigment synthesis, connective tissue biology, and immunity [11]. However, in the case of overload copper may act as a toxic compound [12, 13]. Copper homeostasis in the human organism is associated with a rapid binding of copper by organic molecules, whereas the amount of unbound copper is very low. Efficiency of homeostasis is defined by the system of copper transport proteins. More than ten enzymes contain copper atoms in complex with amino acids in their active sites. The latter explains the variety of clinical manifestations of copper deficiency.

Nowadays more than 10 enzymes using copper as a cofactor are known. For example, diamine oxidase (DAO) performs

inactivation of histamine releasing in allergic reactions. Copper-containing aminooxidases also take part in putrescin, 1-phenylethylamine, serotonine and spermine detoxication [14]. In gastrointestinal diseases the consumption of foods rich in histamine or in the case of using DAO inhibitors histamine intolerance occurs [15, 16]. An impaired balance between histamine production and transformation causes allergic reactions accompanied by headache, diarrhea, nasal and conjunctival symptoms, asthma, hypertension, arrhythmia, skin rash and reddening [17]. Histamine-restricted diet is used for treatment of such diseases [16]. It is supposed that drug allergy, for example, to non-steroid anti-inflammatory drugs is based on DAO gene polymorphism [18].

A list of copper-containing enzymes and their functions is indicated lower (Table 1).

Table 1

Copper-containing enzymes

Name	Functions
Cytochrome c oxidase	Electron transfer from cytochrome c to molecular oxygen in tissue respiration
Dopanime-β-monooxygenase	Noradrenaline synthesis
Cu,Zn-superoxidedismutase	Dismutation of superoxide anion radical to hydrogen peroxide
Lysiloxidase	Maturation of collagen and elastin
Aminooxidases	Oxidative deamination of biogenic amines
Tyrosinase	Melanin biosynthesis
Peptidylglycine α-amidating monooxygenase	Neuropeptide transformation
Ceruloplasmin	Dismutation of superoxide anion radical to hydrogen peroxide; iron metabolism
Glycosylphosphatidylinositol (GLI)-ceruloplasmin	Elimination of iron from macrophages
Hephestin	Elimination of iron from enterocytes
Zyklopen	Elimination of iron from placenta

Monoamine oxidase (MAO) takes part in dopamine metabolism and oxidative deamination of serotonin (5-hydroxyriptamine) to the respective aldehyde and hydrogen peroxide. MAOs are enzymes bound to the outer mitochondrial membrane and having two forms: A and B [19]. MAO-B is active only in brain tissues, whereas MAO-A is present both in nervous and cardiovascular systems. It is supposed that MAO-A has higher affinity to serotonin and noradrenaline in comparison to MAO-B [20]. Decreased renalase (renal MAO) activity results in catecholamine accumulation, being accompanied by hypertension, impaired filtrating activity and insulin resistance [21]. Earlier studies have indicated that angiotensin II stimulates MAO activity leading to free radical generation and, consequently, endothelial dysfunction [22], hemodynamic stress and myoblast apoptosis [23]. Impaired behavior [24, 25], autism [26-29], schizophrenia [30, 31] and Down's syndrome [32] are associated with decreased MAO activity. Moreover, altered catecholamine and neuropeptide biotransformation is accompanied by epilepsy, emotional lability, anxiety [33-35]. We have observed increased hair and serum copper content in alcoholics and their children [36-40].

Amino oxidase (AOC3) also known as vascular adhesion protein 1 (VAP-1) is related to copper-containing proteins being secreted by vascular smooth muscle cells, adipocytes, endothelial cells [41]. This enzyme is a multifunctional molecule possessing both adhesive and enzymatic properties [42]. VAP-1 and copper take part in rheumatoid arthritis, psoriasis, systemic sclerosis, respiratory diseases, diabetes and its complications development [41,43-46].

Dopamine-β-hydroxylase converts dopamine to noradrenaline both in CNS and adrenal glands. Clinical manifestations of its deficiency include hypotension and decreased physical activity tolerance [47].

Tyrosinase takes part both in melanin synthesis in skin and hair and neuromelanin in CNS. This enzyme catalyzes two stages of

melanogenesis: tyrosine hydroxylation to DOPA and conversion of DOPA to dopaquinone [48, 49]. It is noted that tyrosinase activity is decreased in vitiligo patients [50-52].

Lysiloxidases (LOX and LOX1-4) family oxidizes lysine to hydroxylysine residues that are required for collagen and elastin maturation in bone tissue, lungs, cardiovascular system. This enzyme is required for covalent cross-linking in order to increase elasticity and structural integrity of the extracellular matrix [53]. Impaired transversal cross-links in collagen and elastin result in impaired connective tissue formation and musculoskeletal diseases. A number of researchers have proposed that LOX has a number of both intra- and extracellular functions. The involvement of this enzyme in pathologic process is observed in cancer, degenerative disorders, impaired chemotaxis [54], and fibrosis [55].

Cytochrome c oxidase is located on the inner mitochondrial membrane and performs the transport of electrons on molecular oxygen accompanied by the formation of endogenous water and increasing in proton gradient and the process of oxidative phosphorylation (ATP synthesis). Cytochrome c oxidase-coding gene mutation is accompanied by seizures, amaurosis fugax, muscle weakness, deafness, cataract, and cognitive disorders [56]. Decreased enzyme activity in muscle tissue bioptate is also observed in Leigh syndrome [57]. Impaired electron transport and oxidative phosphorylation in mitochondria may also result in diseases of CNS with defective myelination, including lateral amyotrophic sclerosis (LAS) [58]. In hypoxic conditions (stroke) synthesis of SOD1 and 3 and CCO is decreased in brain.

Peptidylglycine α-amidating monooxygenase (PAM) takes part in conversion of a number of peptide hormones including thyrotropin, neuropeptide Y, and vasopressin. Amidation increases the efficiency of interaction between peptide hormones and their receptors [59]. However, impaired copper metabolism is observed in hyper- and hypothyroidism [60-62].

In Cu, Zn-SOD enzyme two copper atoms play a significant role in superoxide dismutation with the formation of two hydrogen peroxide molecules. Zinc atom plays a conformational function in this enzyme. Development of systemic sclerosis is autoimmune pathology of the connective tissue being characterized by proliferation of fibrous tissue in skin, skeletal muscles, blood vessels and viscera. A significant increase in SOD3 production is observed in patients with this pathology [63]. Cu, Zn-SOD coding gene mutation is most frequently observed in lateral amyotrophic sclerosis being accompanied by dysphagia and dysartria [64].

It has been known since the middle of the 19^{th} century that the level of copper consumption and hemoglobin metabolism are tightly associated. Copper deficiency is accompanied by impaired iron homeostasis leading to anemia and excessive iron accumulation in adipocytes [65-67]. A group of plasmatic ferrooxidases take part in iron oxidation (from Fe^{2+} to Fe^{3+}) that is required for iron binding to transferrin. Localization and functions of these ferrooxidases are presented in Table 1. Main functions of ferrooxidases are antioxidant defense and iron elimination. Ceruloplasmin is a protein synthetized in liver and containing up to 95% of plasmatic copper [68]. Impaired ceruloplasmin production is not always related to anemia as it was supposed earlier [69]. However, the decrease in ceruloplasmin is accompanied by alteration of iron transport and utilization leading to its excessive accumulation in liver [70]. Under the influence of γ-interferon and other cytokines ceruloplasmin production is increased, as observed in traumas and inflammatory reaction [71]. Ceruloplasmin oxidizes iron and consequently helps its binding to transferrin. It also inhibits free radical oxidation. This antioxidant effect of ceruloplasmin and its rapid increase in inflammatory reaction have provided the basis for its consideration as acute phase protein [72, 73]. Ceruloplasmin also takes part in neuromediator metabolism associated with noradrenaline and serotonin oxidation [74]. Ceruloplasmin-mediated

inhibition of NO-synthase was also noted [75]. This fact is confirmed by increased frequency of cardiovascular diseases in persons with increased blood copper levels.

Accumulation of free (not bound to ceruloplasmin) copper is characteristic of a number of neurodegenerative diseases like Alzheimer and Parkinson diseases, lateral amyotrophic sclerosis, prion disease, and Huntington's chorea [76, 77]. Despite the presence of complex pathogenetic mechanisms of these diseases accumulation of copper is common for all states. Tissue copper overload leads to induction of free radical oxidation, impaired cytochrome c oxidase and SOD1 synthesis, overproduction of inflammatory cytokines (IL-1α, IL-6, IL-12, etc.), induction of apoptosis, and neural degeneration [78, 79]. It is supposed that Alzheimer disease is characterized by increased deposition of amyloid-β-protein plaques in intracellular space leading to synaptic dysfunction [80, 81]. At the same time, degenerative changes in dopaminergic neurons and substantia nigra are observed in Parkinson disease. Local deposition of copper in brain tissue is accompanied by decreased blood ceruloplasmin and copper concentration [79]. Lower ceruloplasmin level results in free copper accumulation [77]. Some investigators indicate that the level of blood ceruloplasmin is inversely associated with the age of Parkinson disease manifestation [82].

Copper deficiency is frequently observed in prematurely born children as copper accumulates in liver directly before the childbirth in order to provide a reserve of this essential trace element. The risk of copper deficiency is also increased in infants consuming cow milk or cow milk-based mixtures, as its copper content is relatively low [83-84].

Copper deficiency is a relatively widespread dysbalance both in developed and developing societies. In accordance with Biesalski et al. [85] and Oberleas et al. [2] up to several billion people worldwide may suffer from copper deficiency. Decreased dietary copper intake in hunger or due to consumption of highly-refined food

is not the only cause of copper deficiency. Chronic bleeding, alcoholism, the use of non-steroid anti-inflammatory drugs and contraceptives ("iatrogenic elementoses" according to A. P. Avtsyn [86]), increased incidence of genetic diseases accompanied by impaired copper metabolism also contribute to total incidence of copper deficiency.

Copper deficiency is related to atherogenic dyslipidemia, metabolic syndrome and impaired glucose tolerance [87-88]. In particular, the examinees with non-alcoholic steatohepatitis and diabetes mellitus are characterized by decreased copper content both in liver and blood [70, 89-90]. Lower dietary copper level is associated with increased cholesterol, lipid synthesis and hypertension in the liver [2, 91-93]. Oppositely, high level of copper was observed in patients with chronic cholelithiasis [94-95].

Decreased copper status is often associated with immune deficiency, decreased leukocyte and neutrophil count, antioxidant system depression. At the same time, serum copper and ceruloplasmin is increased in various inflammatory processes, myocardial infarction, liver diseases, pregnancy etc. [96-103]. It is supposed that these states may mask copper deficiency and complicate its diagnostics [104].

Glycosylphosphatidylinositol-ceruloplasmin (GPI-CP) is located in CNS, retina, kidneys and spleen. It is nearly identical to ceruloplasmin in its structure. Moreover, this ferrooxidase is involved in iron transport in macrophages, NK-cells, leptomeningeal and Sertoli cells [8].

The main function of hephestin is control of iron transport [105]. Hephestin in association with ferroportin takes part in the release of iron from enterocytes, hepatocytes, and macrophages. Possible explanation of copper-deficiency related anemia is decreased production of hephestin that regulates iron absorption in gastrointestinal tract and its retention by enterocytes. This

mechanism is in agreement with the observation that treatment of anemia with iron compounds without compensation of copper deficiency does not lead to complete anemia liquidation [106].

Zyklopen, a protein recently discovered in placenta, plays a significant role in placental iron transport. Zyklopen is capable of elimination of placental copper and iron transport from mother to fetus during pregnancy [107]. Activity of this ferrooxidase is increased in response to lowered intracellular copper content [71]. It is shown that zyklopen is also observed in other organs like retina, testes, kidneys, and brain. However, this enzyme does not possess biological activity neither in liver, nor in enterocytes. It is closely related to hephestin in its structure. It is interesting that GPI-CP, hephestin and zyklopen are all present in astrocytes, whereas the biological significance of these proteins in brain tissue is not completely studied.

Other copper-regulated processes

Metallothionein (MT-1 and MT-2), amyloid precursor protein (APP) and prion protein (PrPc) also possess copper-binding activity. Metallothioneins play a significant role not only in transport, but also in intracellular copper accumulation [108-109]. Such mechanism of accumulation of excessive amounts of intracellular copper under zinc treatment is used in Wilson-Konovalov disease patients. It is established that zinc treatment increased metallothionein levels [110-111]. It has been noted that copper is bound to metallothionein in infants due to immature biliary system and low ceruloplasmin synthesis. APP plays a significant role in copper transport in central nervous system. Decreased production of APP is accompanied by increased copper levels in brain [79, 112-113].

Endocrine regulation plays a significant role in copper metabolism. It has been estimated that estrogens and insulin increase

ATP7A expression [114]. Increased blood ceruloplasmin levels are observed during pregnancy and lactation [115]. Administration of hormonal drugs (estrogens, progesterone, dexamethasone, insulin, etc.) induces copper excretion from hepatocytes by increasing its binding with ceruloplasmin with the help of ATP7B. Earlier an association between androgenetic alopecia, alopecia areata and low hair and serum copper levels have indicated [35, 116-117].

Copper regulates NO synthesis in CNS microglia. Excessive production of this neurotransmitter dislocates redox potential of the medium towards more prooxidant conditions. The latter has a principal role in development of neurodegenerative diseases [74, 112, 118].

Adequate organism's copper status results in better fibrinolytic activity of blood resulting in decreased cardiovascular risk. In particular, copper-containing drugs administration decreases plasminogen activator inhibitor 1 (PAI-1) levels by 30-50% [119]. Moreover, copper is an important participant in angiogenesis process. Copper excess accompanies the development of multiple neoplastic processes like prostate, breast, lung, intestine and brain cancers [120-122]. Consequently, chelation therapy in neurodegenerative diseases and various cancer forms may be efficient [84, 104].

Vascular endothelial growth factor (VEGF) in the absence of copper stimulates cellular proliferation and differentiation. Copper administration was accompanied by regression in cardiomyocyte hypertrophy that occurred through the change of ratio between vascular endothelial growth factor receptors (VEGFR-1/VEGFR-2) [123]. An increase in serum copper concentration and VEGF activity resulting in impaired angiogenesis and excessive development of endometrium was detected in postmenopausal women with metrorrhagia [124].

We found that copper levels in blood and hair are elevated in alcoholics, alcohol chronically intoxicated rats and mice, and their offspring, even matured (adults) [39], as opposed to decreased zinc

content. It was concluded that alcohol consumption alters zinc/copper balance [40].

Genetic diseases of copper metabolism

The most frequent causes of copper deficiency are its low dietary intake and development of genetic diseases. Genetic defects leading to impaired copper metabolism are Wilson-Konovalov disease, Menkes disease and aceruloplasminemia [125].

Proteins ATP7A/B being capable of copper excretion from the cell are 60% identical. It is noted that ATP7B expression is observed in liver, kidney, eye, epithelial cells and CNS. At the same time, the primary function of ATP7A is regulation of copper absorption in gastrointestinal tract, its transport into cerebrospinal fluid, macrophages, etc. [113, 126]. Participation in neurotransmission and synaptogenesis are also proposed as possible functions for ATP7A [127].

ATP7A protein is coded by X-chromosome and gene mutation causes the development of Menkes disease ("kinky hair disease") [128]. The symptoms of marked copper deficiency occur, as ATP7A delivers metal to a number of copper-containing proteins (dopamine-β-monooxygenase, PAM, lysiloxidase, tyrosinase, etc.) [110, 129]. At the same time, impaired synthesis of this protein alters copper excretion from enterocytes and neurons. Menkes disease patients are characterized by delayed growth and development, abnormalities in connective tissue development, skin and hair pathology, convulsive syndrome impaired thermoregulation [130]. The disease has negative prognosis and untreated children usually die in early age.

ATP7B protein is coded by 13 chromosome and has similar structure as ATP7A. The principal role of ATP7B is regulation of copper homeostasis in liver. Expression of ATP7B is absent in liver of newborns and infants that are characterized by ATP7A expression instead.

Moreover, ATP7B is related to transport proteins in CNS, placenta, mammary glands, and kidneys. The result of gene mutation is accumulation of toxic amounts of copper in liver and brain [131]. This type of hepatocerebral insufficiency is called Wilson-Konovalov disease. This disease is accompanied by increased copper accumulation in astrocytes, hepatocytes and other cells, while serum copper levels remain low [132]. Golden brown or green Kayser–Fleischer rings appearing on eye cornea are pathognomonic symptoms of the disease. ATP7B mutation is also accompanied by impaired copper binding to ceruloplasmin resulting in low levels of the latter in blood serum [129, 133].

Calculation of the difference between total copper levels and protein-bound fraction allows to estimate the concentration of free copper. Toxic effect of copper is mainly mediated through this free fraction. Development of gene mutations in Wilson-Konovalov and Menkes diseases are accompanied by extreme copper accumulation in one organs (liver, brain, gut and kidneys) and its decrease in the others [134].

Mutation in ATP7A gene is observed in patients with occipital horn syndrome that is characterized by motor dysfunction, impaired connective tissue synthesis. As in Menkes disease, timely treatment of occipital horn syndrome with copper-containing drugs results in better disease control [135].

Aceruloplasminemia is autosomal-recessive disorder characterized by ceruloplasmin gene mutation [136]. The absence of ceruloplasmin is accompanied by increased copper accumulation in liver and other organs leading to fibrosis and liver cirrhosis, retinal degeneration, motor and mental dysfunction [65, 137].

Gene mutation of copper-containing protein is also observed in Down syndrome accompanied by SOD1 hyperproduction [129]. Its mutation is also observed in lateral amyotrophic sclerosis.

Genetic defect in tyrosinase results in vitiligo and albinism [138-139]. Impaired transport of copper to tyrosinase is also observed in genetic Hermansky–Pudlak syndrome that is

characterized by eyelid hypopigmentation and nystagmus [140]. Moreover, the development of hemorrhagic diathesis, granulomatous colitis and restrictive lung fibrosis is also present in clinical course of the disease. Mutation of tyrosinase coding gene results in oculocutaneous albinism (OCAs). OCA1A is characterized by a complete loss of functions, whereas OCA1B by a partial impairment. In OCA2 tyrosinase transport is impaired [139].

Decreased cytochrome c oxidase production is characteristic of inherited mitochondrial myopathy in newborns [141]. A number of other neurodegenerative disorders like lateral amyotrophic sclerosis, mitochondrial encephalomyopathy and progressive myoclonic epilepsy may also be referred to copper-dependent. Kearns-Sayre syndrome is also related to mitochondrial encephalomyopathy and is characterized by external eye muscles myopathy combined with ptosis, pigment degeneration and damaged conducting system of the heart. These diseases are also accompanied by the loss of myelin-associated glycoprotein, motor neuron defects, impaired mitochondrial respiration and cytochrome c oxidase synthesis [142]. Leigh's syndrome (subacute necrotizing encephalomyopathy) is a rare inherited disease from the group of mitochondrial encephalopathies also associated with impairment of copper-containing cytochrome c oxidase synthesis [57].

Impaired lysyl oxidase synthesis accompanies the appearance of X-linked form of cutis laxa - Ehlers-Danlos syndrome [143-144]. Possibly, the defects in copper metabolism may be observed in Von Willebrand disease (antihemophilic factor VIII deficiency) being characterized by impaired cytochrome c production in platelets [129].

Dopamine-β-hydroxylase deficiency is a rare genetic disease with autosomal-dominant inheritance pattern. Pathogenetic mechanisms include the absence of noradrenaline in the bloodstream and CNS with normal functioning of parasympathetic and cholinergic systems [145]. Clinical manifestations include hypotension, decreased physical activity tolerance, blepharoptosis and stuffiness in the nose [47].

Indian childhood cirrhosis is a chronic liver disease resulting in cirrhosis and observed in children (1-3 years old) [146]. This process is accompanied by deposition of Mallory hyaline and accumulation of both copper and zinc in liver [147-148].

The aforementioned data indicate a high variety of copper-dependent states, especially in persons with genetic diseases affecting CNS, chronic inflammatory diseases (including rheumatoid processes), anemias, musculoskeletal diseases, etc [2, 44, 149]. Unfortunately, the estimation of copper status is rarely performed in our country. It decreases the efficiency of treatment and prophylaxis of various diseases. The characteristics of copper status in different states are indicated in Table 2.

Table 2

Diseases and states associated with impaired copper metabolism

Disease	Copper content			CP
	Blood	Urine	Hair	
Acute and chronic inflammatory diseases	↑ [72,149]		↑	↑ [73]
Myocardial infarction	↑ [96]		↑↓	↑ [93]
Cancers	↑ [14,104,121]	↑ [122]	↑ [120]	↑ [84]
Rheumatoid arthritis	↑ [97]	↑ [44]	↑ [43]	↑ [97]
Iron-deficient anemia (various types)	↑[66] ↓[65, 67, 105]		↓ [67]	↓ [69]
Biliary cirrhosis	↑ [150]	↑	↑ [99]	↑ [99]
Nonalcoholic fatty liver disease	↓ [70,69]			
Cholelithiasis	↑ [94]	↑ [95]	↑	
Epilepsy	↑ [33,34]		↑ [151]	↑ [34]
Autistic spectrum disorders	↑ [28,29]		↑ [27]	↓ [25]
Alcoholism, alcoholic liver disease	↑ [36] ↓ [39]	↑ [38]	↑↓	↑ [37]
Atherosclerosis (coronary artery disease)	↑ [91]		↓	↑ [98]

Disease	Copper content			CP
	Blood	Urine	Hair	
Hypothyroidism	↓ [60] ↑ [61]	↑↓	↓	
Hyperthyroidism	↑ [60, 61]	↑	↑	↑ [62]
Periodontitis	↑ [101]			↑ [102]
Diabetes mellitus 2 type	↑ [87, 89]		↑ [88]	↓ [90]
Psoriasis	↑ [45, 46]			↑ [46]
Alopecia	↓ [116, 117]		↓ [35, 116]	
Albinism, vitiligo	↓ [50,51,139]	↓	↓	↓ [52] ↑ [49]
Copper occupational exposure	↑	↑	↑	↑ [13]
Renal Insufficiency Treated by Dialysis	↑ [152]			↑ [152]
Wilson-Konovalov disease	↓ [111, 127, 129, 130, 132, 134]	↑ [153]	↓ [131]	↓ [132]
Menkes disease	↓ [128-130, 135]	↓ [128]	↓ [128]	↓ [128]
Aceruloplasminemia	↓ [65, 137]	N [136]		↓ [137]
Occipital horn syndrome	↓ [125, 139]			↓ [125]
Down syndrome	↑ [32]		↑ [154]	
The third trimester of pregnancy	↑ [103,155]			↑ [103]
Occupational exposure to heavy metals		↑ [156, 157]	↑↓	↓ [158]
Ehlers-Danlos syndrome	↓ [138, 143-144]			
Indian childhood cirrhosis	↑ [148]	↑ [125]	↑ [125]	↑ [146]
Hypertension	↑ [3, 119]	↑ [92]		↑ [159]
Schizophrenia	↑ [30, 31]		↑ [12]	
Eclampsia	↑ [160] ↓ [161]	↓ [162]	↑ [102]	↑ [163]
Allergy, histamine intolerance	↓ [16-18, 164]			
Alzheimer and Parkinson diseases	↑ [76, 77, 79] ↓ [81, 82]			

Note: ↑ - increased values; ↑↓ - contradictory data; ↓ - decreased values of the parameter; CP – ceruloplasmin.

Copper-drug interactions

Impaired copper metabolism may occur as a side-effect of drug treatment or even dietary supplementations. Avtsyn et al [86] have originally called these states "iatrogenic microelementoses (trace element disturbances)". Previously published work has indicated iatrogenic lupus syndrome associated with impaired copper exchange [165]. Taking into account high prevalence of administration of such drugs as non-steroid anti-inflammatory drugs, D-penicillamine, antacids, anticonvulsants, contraceptives without doctor's advice, the risk of iatrogenic copper dysbalance may eventually be high but hard to diagnose [150].

Table 3

Copper interaction with drugs

Drug	Effect
Azidothymidine	Decreased tissue copper levels
Non-steroid anti-inflammatory drugs	High risk of impaired copper metabolism; Simultaneous administration with copper preparations increases therapeutic effect and decreases ulceration risk
Aspirin	Copper chelates increase aspirin efficiency and decrease toxicity
Nizatidine (histamine H2-receptor antagonist)	Prolonged administration results in copper deficiency
Ciprofloxacin (fluoroquinolone, antibiotic)	Inactivation by copper
Antacid (Al- and Mg-containing)	Decreased tissue copper levels
Anticonvulsants	Decreased serum and tissue copper levels
Oral contraceptives	Increased serum and tissue copper levels
Estrogens	Increased serum and tissue copper levels
Prolonged administration of copper-containing drugs	Increased serum and tissue copper levels
Prolonged administration of iron, zinc, manganese, molybdenum-containing drugs	Copper displacement

Laboratory criteria of impaired copper metabolism

Estimation of copper status is performed based on analysis of blood, urine and hair copper content, serum ceruloplasmin levels and activity of copper-dependent enzymes.

The most of copper in human organism is located in tissues (80 mg). Erythrocytes contain up to 60% of the whole copper in the form of Cu,Zn-SOD (formerly known as "erythrocuprein"). Plasma ceruloplasmin binds 80% of the whole plasmatic copper content with a ratio of 7 copper atoms per 1 protein molecule. The rest of copper in plasma is bound to transcuprein and albumin. It is supposed that plasma copper level does not reflect its intake. However, it may be used as an early marker of copper deficiency because tissue copper content is more stable. Minor portion of copper is present in the free form. It is the difference between total copper and protein-bound metal content. Normal values of this parameter should not exceed 25μg/dl. At the same time, this test may be used only in diagnosis of Wilson disease, but not for estimation of copper deficiency. Copper deficiency is accompanied by decreased ceruloplasmin levels in plasma. In particular, if the decrease exceeds 30%, a significant copper deficiency may be proposed. Normal ceruloplasmin levels are 200-600 μg/dl.

Scholarly articles present contradictory data on reference values of copper content in the human organism. In accordance with Linder and Hazegh-Azam [166] who have estimated copper content using atomic absorption spectrometry, normal values of plasma copper for men and women are 0.91-1.0 and 1.07-1.23 mg/l, respectively. Administration of contraceptives increased plasma copper to 2.16-3.0 mg/l ("estrogen effect"). Our data obtained using ICP-MS indicate that reference values for serum copper in males and females are 0.7-1.4 and 0.8-1.55 mg/l, whereas the reference values for urinary and hair copper both for men and women are 15-30 μg/l

and 8-15 μg/g, respectively [83, 167]. Normally, the highest levels of plasma copper are observed in pregnant women at the 38th week [155, 163]. The values of plasma copper concentration return to normal to the second week postpartum. Free copper concentration is supposed to be a more informative indicator of copper status as compared to protein-bound Cu. 1 mg of ceruloplasmin contain up to 3.3 mg copper [166]. Free copper increases the values of this parameter. Oppositely, in the case of Wilson disease (hepatolenticular degeneration), uremia, nephrosis, proteinic starvation the level of plasma ceruloplasmin is decreased. In particular, the ceruloplsmin level in Wilson disease is lower than 23 μg/dl [111, 168], whereas liver copper exceeds 250 μg/g.

Urinary copper excretion is 0.01-0.06 mg daily. Copper excretion slightly depends on copper intake. At the same time, it successfully reflects tissue free copper and albumin-bound copper. This parameter does not indicate copper status but may be useful in the study of copper metabolism. An increase in tissue free copper in Wilson-Konovalov disease is accompanied by a significant elevation of urinary copper excretion (> 1.5 mg/day) [153]. It is supposed that copper excretion lower than 50 μg/day excludes the diagnosis of hepatolenticular degeneration.

Estimation of hair copper levels is additional test. However, we suppose that this parameter may be informative in copper status analysis that frequently corresponds to serum copper values [169]. Hair analysis may provide additional information on copper status for a rather long period of time, whereas blood and urinary copper levels are indicative only of present condition.

In accordance with A. V. Skalny et al. data [167, 170-171], low copper status as defined by low hair copper content may be observed in 20-30% people living in different regions in Russia. Predominantly children suffer from copper deficiency (34-40%), whereas copper excess is observed in 15% of children and 15-30% of

adults [83, 167]. Low hair copper content is associated with various diseases of the respiratory tract, musculoskeletal system in adults, and urogenital system in children. Higher hair copper content corresponds to skin diseases in adults and traumas in children [11].

An association between low hair copper and the incidence of chronic bronchitis and bronchitis with undefined etiology, emphysema, blood diseases, and immune disturbances was demonstrated in the examinees living in the central region of Russia. In accordance with the same report [167], higher hair copper content is associated with increased life duration in females. The reference ranges for hair copper in Russian men and women are 10.4–22.6 and 12.1–44.5 μg/g, respectively [172].

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MANGANESE

Introduction

Manganese is one of the so-called bioelements that performs a cofactor function in enzymatic processes [1]. Manganese is also an essential trace element for humans. A tight association between manganese and iron transport has been detected. However, intimate mechanisms of manganese uptake are still unknown. It is a structural part of enzymatic systems in the brain, takes part in the functioning of antioxidant, musculoskeletal, immune and reproductive systems as well as in detoxification processes. At the same time, excessive quantities of manganese cause toxic effects, especially in CNS resulting in neurological diseases.

Manganese is a metal with high redox activity and variability of oxidation states from -3 to +7. In biological systems the most stable compound is Mn(II). However, in complex with transferrin Mn(III) is more stable than Mn(II). In gastrointestinal tract the absorption of manganese (Mn^{2+}, Mn^{3+}) is relatively low and amounts to 3-5% [2]. Minimal requirement in manganese is 0.74 mg/day or 10.8 μg/kg weight. After administration of labelled ^{54}Mn in the dose of 800 μg/day 50% of absorbed compound was excreted in 5 days whereas the latter in 40 days [3].

Total amount of manganese in human body is 10–20 mg. Optimal daily requirement is 3–5 mg. Manganese is poorly absorbed in gastrointestinal tract (1–4%), but the majority of inhaled manganese can enter to the organism [4]. Absorbed manganese accumulates mostly in liver and consequently excreted mostly with

bile (3.6 mg/day) being indicative of the key role of liver in manganese metabolism. Bones, pancreas, kidney and adrenal glands are also rich in manganese. Toxic amounts of manganese (per os) are ≥40 mg/day. The manganese half-life in the body is 4–40 days [5].

In physiological state manganese and iron compete for transferrin binding. In iron deficiency anemia manganese absorption is also impaired due to competition for transporter protein. Vitamins B_1, E, phosphorus and calcium (in moderate amounts) facilitate manganese absorption in the gastrointestinal tract while excessive intake of calcium and phosphorus impede it [6].

Transport, metabolism and homeostatic regulation of manganese

Existing data on manganese transporter proteins are presented in Table 1. Protein DMT1 performs absorption of manganese in gastrointestinal tract [7]. It is also notable that DMT1 performs transport of at least 8 metals including iron, manganese, nickel, cobalt, copper, zinc. Previous studies have demonstrated that iron (II) and manganese (II) are more dependent on DMT1 activity in comparison to copper and zinc [8].

It is known that homeostasis of iron and manganese is tightly interrelated. In particular, manganese possesses a high affinity to transferrin receptors (TfR) even in comparison with iron (III) [9]. Consequently, iron dyshomeostasis may be accompanied by impaired manganese balance and vice versa.

Ferroportin is also considered to be a possible manganese transporter. Experimental studies have indicated the influence of Fpn activity on cytoplasm manganese content being especially marked in the case of manganese exposure [10]. Iron deficiency, especially in vegetarians, may increase manganese absorption leading to increased

Mn content. Liver diseases and impaired biliary system also result in manganese accumulation [11].

Intracellular manganese binds another transporter protein SPCA1 or SPCA2 that transport the metal into Golgi network. Along with manganese SPCA1 also takes part in calcium transport [12]. Activity of SPCA1 has been detected in all cells of the organism, whereas SPCA2 expression was observed only in CNS. Recent studies have indicated that SPCA2 is involved in manganese elimination from brain cells in the case of its increased concentration [13-14].

ZIP8 and ZIP14, being Zn and Cd transporters, may also mediate manganese uptake. It has been shown that low ZIP8 and ZIP14 expression results in decreased manganese absorption [15].

T a b l e 1

Manganese transporters and their functions

	Manganese uptake	Manganese excretion
DMT1	+	
TfR	+	
ZIP8, ZIP14	+	
Fpn		+
SPCA1	+	
SPCA2		+
ACDP1		+
ATP13A2		+
SLCA30A10		+
Mtm1p	+	

Intimate mechanisms of manganese delivery to mitochondria are unknown. It is supposed that Mtm1p protein, a mitochondrial transporter, may take part in manganese uptake in *Saccharomyces cerevisiae*. This protein delivers manganese to mitochondria for further synthesis of SOD2 [14]. It is proposed that similar proteins may act in the human organism.

In the case of excessive administration of manganese its transport, deposition and excretion are changed. A number of studies using *Saccharomyces cerevisiae* have shown that phosphorus transporter Pho84p may serve as manganese transporter in the case of manganese excess. However, this protein has low affinity to manganese [14].

It is known that lysosomes play a significant role in ionic homeostasis including deposition and detoxication. Experiments using *S. cerevisiae* have demonstrated that manganese through Mam3p protein is accumulated in vacuoles resulting in impairment of their metabolic activity [14]. The presence of ACDP1 protein, that is homologous to Mam3p, in human allows to propose the presence of similar mechanism in the human organism.

Experimental studies have also shown that ATP13A2 gene mutation results in impaired manganese homeostasis in cells. This protein has a significant role in prevention of manganese toxicity. Consequently, manganese elimination is mediated by P-type ATPases that regulate cellular copper level [16]. An interrelation between copper and manganese has been demonstrated in experimental studies. An in vivo study has demonstrated an increase in peripheral blood and CNS copper content after manganese intoxication. The result of this process is Ctr1 and DMT1 dysregulation leading to accumulation of manganese and copper in CNS [17].

SLC30A10 that was considered as zinc transporter is also related to manganese transporters. Its expression is mainly observed in liver and brain tissue. Mutation of gene coding the protein results in manganese accumulation in liver and CNS [18].

Generally, a high variety and complexity of mechanisms of manganese uptake is indicative of high importance of the metal to the organism.

Manganese as a cofactor and clinical signs of its deficiency

Even 20 years ago in the classic monograph by Avtsyn et al. [19] it was pointed that manganese deficiency in humans was practically not described. However, since then in the scientific literature quite a large number of proven clinical manifestations of manganese deficiency became reported.

It has been shown that manganese deficiency is associated with impaired growth and development, defects in bone and cartilage formation, adipose tissue dysfunction, impaired glucose tolerance, and infertility. Suboptimal levels of manganese in the human organism are observed in epilepsy, osteoporosis, Pertes disease, impaired exocrine function of the pancreas, phenylketonuria, and hemodialysis patients [20]. A number of manganese-containing enzymes is present in the human organism (Table 2).

Arginase is a key enzyme in urea cycle that converts L-arginine to L-ornitine and urea. In macrophages, arginase activity is tightly associated with NO-synthase and prevents NP-mediated cytotoxicity [21]. Oxidative stress risk is increased in the case of excessive NO production. It is known that NO initiates and increases cascade reactions of free radical formation by binding to superoxide anion radical and formation of peroxynitrite. Impaired arginine metabolism is observed in a number of diseases like asthma, cardiovascular diseases and erectile dysfunction.

A number of diseases have demonstrated the role of manganese in smooth muscle cell contraction. This function is associated with the influence of manganese on α1-adrenoreceptors [22]. Potentiation of cardiomyocyte relaxation under the influence of manganese and the participation of slow calcium channels in manganese uptake have been demonstrated [23].

Table 2

Manganese-containing enzymes

Enzyme	Function
Mn-superoxide dismutase	Superoxide anion dismutation to hydrogen peroxide
Arginase	Urea cycle
Glutamine synthetase	Glutamine formation
Glycosyltransferase and xylosyltransferase	Protein glycosylation, glycosaminoglycans biosynthesis
Pyruvate carboxylase, phosphoenolpyruvate carboxykinase;	
Isocitrate dehydrogenase	Gluconeogenesis, maintenance of physiological oxaloacetate concentration
Formation of α-ketoglutarate (Krebs cycle)	
Serine/threonine phosphatase	Cell cycle, apoptosis

Pertes disease is characterized by aseptic necrosis of femoral head in children and is associated with manganese deficiency. One of the possible reasons for this disease is impaired vascularization, arterioles spasm, and, consequently, ischemia and necrosis [24]. An investigation involving volunteers consuming low-manganese diet has demonstrated a decrease in organism's calcium and phosphorus content as well as alkaline phosphatase activity [3].

Manganese deficiency may play a significant role in cancerogenesis and in predisposition to breast cancer in particular [25]. Experimental studies have demonstrated an association between manganese and p53 expression [26]. p53 activation is associated with mitochondria-induced cellular apoptosis, whereas Mn and Mn-SOD play a significant role in antioxidant defense in cancer intervention [27]. Breast cancer risk is increased in perimenopausal women with Mn-SOD gene polymorphism. At the same time, the risk has increased from 2 to 3-fold in excessive weight gain, smoking, and

hormonal replacement therapy [28-29]. Excessive expression of Mn-SOD is indicative of cancer progression. It has led to consideration of Mn-SOD/MAPK axis to regulatory mechanisms in tumor cells [30]. At the same time, the use of Mn-SOD inhibitors and H_2O_2 scavengers resulted in a significant decrease in tumor growth and formation of new cellular populations [31].

Gestagens also affect manganese content and Mn-SOD activity. Thus, an increased Mn-SOD production has been observed under progesterone stimulation [32]. Maximal levels of manganese and Mn-SOD were observed in the second phase of menstrual cycle that is associated with increased secretion of progesterone [33]. Experimental studies have demonstrated an association between prolactin and Mn-SOD synthesis. Prolactin treatment after hypophysectomy improved Mn-SOD activity [34]. Replacement hormonal therapy in postmenopausal women resulted in increased Mn-SOD activity and elevation of total antioxidant capacity of plasma [35]. However, such an increase especially in the presence of risk factors (obesity, smoking, decreased consumption of fruits and vegetables) may be an initiating agent in breast cancer.

Manganese content is also decreased in a number of chronic inflammatory diseases. Physiological response to infection includes a number of signaling cascades that result in sequestration of manganese and other metals (iron, copper, zinc). Decreased availability of manganese to bacterial cells is achieved due to secretion of calprotectin that chelates extracellular manganese and zinc [36]. Decreased manganese levels in bacterial cell are associated with oxidative stress resulting in impaired virulence and viability. Oppositely, increased manganese content is associated with frequent infectious diseases [37]. We found the association of low manganese status and predisposition to allergic diseases such as rhinitis, sinusitis, obstructive bronchitis and asthma in children [6].

Manganese takes part in energy metabolism. In dehydration-induced anorexia an increase in hypothalamic glutamine content associated with manganese accumulation was demonstrated [38]. Along with increased glutamine synthetase activity anorexia was characterized by increased serotonin production and decreased secretion of dopamine and neuropeptide Y.

Manganese deficiency is associated with impaired BMI, insulin and glucose values. In particular, in 8-13-year-old schoolgirls low dietary manganese intake was associated with increased serum insulin and HOMA-IR values [39]. Manganese deficiency is also accompanied by decreased activity of regulatory enzymes of gluconeogenesis, pyruvate carboxylase and phosphoenolpyruvate carboxykinase, finally leading to impaired glucose homeostasis [40]. Chronic hyperglycemia results in oxidative stress in skeletal muscles and sympathetic nervous system dysfunction. Thus, persons with uncontrolled diabetes mellitus type 2 (HbA1c > 9.0) were characterized by a significantly lower Mn-SOD activity in comparison to the ones with controlled diabetes mellitus and without diabetes [41].

Manganese-containing enzymes, glycosyltransferase and xylosyltransferase, take part in glycosaminoglycan synthesis in bones and cartilages. Moreover, manganese cannot be replaced by other metals in these enzymes. Manganese also takes part in activation of prolidase and prolinase that are required for proline and hydroxyproline-containing peptide hydrolysis. These enzymes take part in extracellular matrix metabolism. Genetic defects in prolidase are associated with impaired connective tissue functions, angiogenesis, carcinogenesis and mutagenesis, result in altered wound healing and inflammation [42].

Dopamine is a neurotransmitter that regulates cognitive functions, memory and attention. Dopamine is capable of decreasing thyrotropin production acting through dopamine receptors [20]. We

observed the high incidence of low hair and blood manganese levels in autistic children [43]. At the same time, manganese may regulate thyroid function via activation of deiodinases. Oppositely, thyroid gland (thyrotropin, thyroid hormones) also takes part in manganese balance regulation. Manganese is also known to influence male fertility and its deficiency is associated with decreased testosterone production and sperm mitochondrial dysfunction [44-45].

Manganese is also required for electrophysiological activity of neurons. From one hand, manganese is an activator of glutamine synthetase in astrocytes. From the other hand, it has been shown that manganese being secreted into the synaptic gap may influence neurotransmission [46]. Low blood manganese in human and experimental animals is associated with epilepsy development and increased convulsive activity of brain. Moreover, experimental study has indicated decreased liver arginase and brain glutamine synthetase activity in rats with experimental epilepsy [47].

Manganese-dependent enzymes and their functions

Generally, manganese is important for enzymes of all classes. For example, manganese takes part in production of phosphatidylinositol that is a substrate for phosphatidylinositol-3-kinase family playing a significant role in intracellular signal transduction. Kinases are enzymes that catalyze transfer of phosphate group from ATP molecule to various substrates. Dehydrogenases are related to a class of oxidoreductases. Lipoxygenase is also an oxidoreductase catalyzing polyunsaturated fatty acid oxidation [48]. A group of manganese-dependent enzymes where Mn may be replaced by other metals should be specially noted.

Deficiency of galactosyltransferase I that is activated by manganese is characterized by impaired protein-chondroitin sulfate

complex formation. Impaired enzyme activity was detected in a number of Ehlers-Dunlos subtypes, idiopathic anemia, and various certain forms of thrombocytopenia [49].

A tight association between manganese and magnesium in the human organism exists. In particular, manganese may replace magnesium in a number of biochemical processes and vice versa. It has been shown that magnesium deficiency is associated with impaired liver manganese metabolism. Experimental animals supplemented with magnesium-deficient diet were characterized by decreased manganese content in blood plasma, organs except adrenal glands and low liver pyruvate carboxylase activity [50].

An interrelationship between iron and manganese metabolism is well known. In iron deficiency and decreased serum ferritin levels manganese concentration was significantly increased [51]. Oppositely, manganese is higher in menopausal women in comparison to the premenopausal ones and is associated with elevated ferritin levels [52]. Significant manganese accumulation in pregnancy occurs due to its increased absorption and is related to iron metabolism dysregulation [53].

Metabolic disturbances associated with increased manganese content in the human organism

Excessive manganese intake influences hypothalamo-hypophysial system resulting in increased prolactin production along with decreased dopamine secretion [20]. It has been shown that manganese accumulation may depress dopamine production in dopaminergic brain structures [54]. Excessive manganese accumulation in brain as a consequence of occupational exposure in welders results in manganism development [55]. Phenotypically this state is similar to Parkinson disease. However, a number of significant differences are observed. First of all, different brain

regions are affected. Manganism is associated with disturbances in *globus pallidus*. This structure is the one of the regions rich with GABA. Moreover, increased brain manganese content results in impaired glutamate metabolism that is associated with glutamate excitotoxicity [16]. Oppositely, Parkinson disease is characterized by a defect in dopaminergic nuclei of *substantia nigra*. Consequently, manganese may affect all three neurotransmitters [2, 55]. It has been noted that hepatobiliary insufficiency, cholestasis and a number of other liver diseases may cause impaired manganese homeostasis and result in its accumulation in brain tissue. Taking into account different pathophysiologic pathways of Parkinson disease and manganism, treatment with L-DOPA is less effective in manganism [56]. Clinical manifestations of manganism are compulsive development, aggression, emotional instability, hallucinations. Weakness, headache, decreased appetite, apathy, muscular spasms, and decreased libido may be related to the early symptoms. Disease progression is associated by the appearance of muscular dystonia, tremor, and rigidity.

Excessive manganese stimulates melanin formation from DOPA via mediating DOPA oxidation. Consequently, manganese may regulate melanin metabolism and manganese dyshomeostasis may be accompanied by skin depigmentation [57].

Data on manganese accumulation in brain tissue and consequent degeneration of basal ganglia in Huntington disease exist [58]. This disease is an autosomal dominant neurodegenerative disease accompanied by impaired glutamine synthetase activity in *corpus striatum*, where manganese is required for glutamate conversion to glutamine. These patients are also characterized by decreased arginase and SOD2 activity. A number of researchers hypothesize that iron dyshomeostasis induced by ferritin receptors and ferroportin plays a key role. Along with impaired iron exchange manganese export is also affected resulting in accumulation of the latter in the CNS [59].

Potential role of impaired manganese metabolism in pathogenesis of Alzheimer disease, lateral amyotrophic sclerosis, prion disease is discussed in scholarly literature [58]. We described elevated manganese and decreased copper in patients with a neurodegenerative endemic disease, Vilyui encephalomyelitis (Yakutia, the Russian North-East) [60-61].

Genetic diseases associated with manganese imbalance

Manganese deficiency. Argininemia is characterized by a genetic deficiency of arginase (Arg1 and Arg2) that is responsible for arginine hydrolysis with the formation of ornithine and urea. Such a metabolic defect is clinically manifested in spastic diplegia, epileptic seizures, delayed psychomotor development [62]. The obtained data indicate that arginase deficiency may result in liver cholestasis. This rare metabolic disturbance is inherited in autosomal recessive pattern. The disease manifests in the childhood (2-4 years) and eventually leads to hepatosplenomegaly, cirrhosis and spastic paraparesis [63].

Rare genetic disease characterized by prolidase and prolinase deficiency is inherited in autosomal recessive pattern. This disturbance is accompanied by skin ulcers, mental retardation, splenomegaly, increased urinary proline and hydroxyproline content, and is associated with impaired manganese metabolism [64]. It has been demonstrated that treatment with manganese and ascorbate both being prolidase and prolinase cofactors results in decreased iminopeptiduria and frequency of inflammatory diseases [65].

Excessive manganese accumulation. Familial benign chronic pemphigus (Hailey-Hailey disease) is associated with manganese transporter (SPCA1) gene mutation and manifests with skin blister formation and impaired glycosaminoglycan synthesis [14]. A number

of studies have detected a mutation in ATP2C1 gene associated with impaired manganese elimination [66].

ATP132A gene mutation is observed in various parkinsonism types and Kufor-Rakeb syndrome. This gene codes P5-type ATPases [67].

Rare genetic disease related to ACDP1 results in urofacial syndrome (facial grimacing and urinary tract failure) [14].

Autosomal-recessive disease associated with manganese transporter SLC30A10 gene mutation results in liver and brain manganese accumulation. Clinical manifestations are characterized by liver cirrhosis, muscular dystonia, polycythemia [18, 68]. Moreover, mutation of this gene may be observed in patients suffering from Parkinson-like syndromes [58].

Laboratory criteria of impaired manganese homeostasis

Manganese status assessment is not a simple task. Usually, the manganese is assayed in plasma, whole blood, urine and hair. Reference values for blood plasma are 0.002–0.009 mg/l, urine – 0.0002–0.005 mg/l [69]. Normal urine excretion is 0.03 mg/day. Reference ranges for hair are 0.29–1.76 μg/g [70]. Workers with hair manganese above 30 μg/g can be observed at risk of manganism. Also, the detection of Mn-SOD and other manganese-dependent biochemical parameters can be useful for diagnostics of manganese status.

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SELENIUM

Introduction

Selenium is an essential trace elements with high metabolic activity. The main functions of selenium in the organism are antioxidant defense, regulation of immunity, thyroid functioning and reproductive system. Severe selenium deficiency was detected in 1980s in China and manifests with cardiomyopathy in children that was termed Keshan disease as well as Kashin-Beck disease that is also accompanied by osteochondropathy [1].

Biological effects of selenium in the organism are associated with various selenoproteins where selenium acts as central ion and cysteine residues are ligands. Nearly 25 selenoproteins were identified in humans, whereas only 24 of them were found in rodents [2]. Optimal daily intake is 20–70 μg/day, toxic level is 5 mg/day, the half-life in the body is 50–60 days [3]. Adequate level of dietary selenium intake and optimal selenoprotein expression guarantees protection from free-radical oxidation, that is observed in neurodegenerative, cardiovascular, and some forms of cancer [4].

The main dietary sources of selenium are grain, meat and fish. In the majority of products selenium is present in the form of selenomethionine. It is supposed that selenium absorption is not regulated [5]. However, experimental studies have indicated that the presence of glutathione and cysteine selenium absorption is increased [6]. The absorption of selenomethionine occurs through active transport. Selenium of dietary origin in the form of selenite and selenate is absorbed by passive and active diffusion, respectively.

The existing data indicate that in humans the absorption rate of selenomethionine is 98%, whereas that of selenite is 84%. Consequently, the absorbed forms of selenium are used for selenocysteine formation that is incorporated into selenoprotein structure [7]. The highest concentrations of selenium are observed in liver, kidneys, pancreas, skeletal muscle and myocardium.

Selenium excretion is performed by kidneys. In particular, in several days 15-20% of all ingested selenium is excreted with urine. The main urinary form of selenium is 1β-methylseleno-N-acetyl-d-galactosamine. Increased dietary selenium intake results in its increased urinary excretion. However, urinary excretion of selenium may be indicative of its total organism's burden and the availability of methyl donors like S-adenosylmethionine. Fecal selenium excretion is insignificant and is presented by unabsorbed dietary selenium fraction [7].

At selenium deficiency, there is an increased accumulation of arsenic, cadmium and mercury in the body. Selenium is an antagonist of mercury and arsenic, it is able to protect the body against cadmium, lead, thallium and silver. Vitamin E promotes assimilation of selenium. Excessive intake of mercury, copper, arsenic, sulphates, paracetamol, phenacetin, and antimalarials may cause selenium deficiency in the organism [8].

Transport, metabolism and homeostatic regulation of selenium

Absorbed selenomethionine is transported into liver. Finally, the obtained selenomethionine is transformed by hepatocytes into selenoprotein P (SEPP1) where selenium is bound to cysteine residues. SEPP1 is the primary selenium transporter and 90% of its plasmatic content is originated from liver [9]. It is supposed that the level of SEPP1 reflects selenium status better than that of other

selenoproteins. SEPP1 expression was detected in various tissues. At the same time, selenomethionine may be non-specifically incorporated into proteins [7]. Pancreatic SEPP1 expression is 50% of that in liver. At the same time, SEPP1 level in insulin-producing β-cells is higher than in glucagon-secreting α-cells being indicative of its biological function [10-11].

Transport of essential molecules through cellular membranes is one of the fundamental characteristics of the living organisms. However, limited data exist on selenium transport into the cell [6]. It is known that selenium transport depends on its chemical form (organic and inorganic). It has been estimated that selenite enters the cell using anion exchanger 1 (AE1) that acts as a transporter for phosphates, sulfites, and hydrocarbonates. This family includes SLC13 and SLC26 transporters. The recent study has also indicated the mechanism of selenium transport through the cellular membrane using monocarboxylate transporters (SLC16) [6].

Table 1

Selenium-containing proteins and their functions

Protein	Localization	Functions
Glutathione peroxidase 1 (GPx1)	cytoplasm	decomposition of lipid hydroperoxides and hydrogen peroxide
Glutathione peroxidase 2 (GPx2)	epithelium of the gastrointestinal tract	decomposition of lipid hydroperoxides and hydrogen peroxide
Glutathione peroxidase 3 (GPx3)	extracellular (plasma)	decomposition of lipid hydroperoxides and hydrogen peroxide
Glutathione peroxidase 4 (GPx4)	cellular membrane	decomposition of lipid hydroperoxides and hydrogen peroxide
Glutathione peroxidase 6 (GPx6)	olfactory	decomposition of lipid hydroperoxides and hydrogen peroxide

Protein	Localization	Functions
Thioredoxin reductase 1 (TrxR1)	Cytoplasm	Reduction of thioredoxin, regulation of intracellular redox balance
Thioredoxin reductase 2 (TrxR2)	Testes	Reduction of thioredoxin, regulation of intracellular redox balance
Thioredoxin reductase 3 (TrxR3)	Mitochondria	Reduction of thioredoxin and glutaredoxin
Methionine-R-sulfoxide reductase (MsrB1, SelR, SelX)	Cytoplasm, mitochondria, endoplasmic reticulum	Reduction of methionine residues, melanin metabolism
Iodothyronine deiodinase 1 (DI 1, Dio1)		Conversion of T4 to T3, thyroid hormone metabolism
Iodothyronine deiodinase 2 (DI 2, Dio2)		Thyroid hormone metabolism
Iodothyronine deiodinase 3 (DI 3, Dio3)		Thyroid hormone metabolism
Selenoprotein P (SEPP1)	Liver	Selenium transport and homeostasis
Selenoprotein 15 kDa (Sep15)	Endoplasmic reticulum	Quality control of protein folding
Selenoprotein N, W (SelN, SelW,)	Skeletal muscles	Muscle development
Selenoprotein S (SelS)	Endoplasmic reticulum	ER-associated degradation
Selenoprotein K (SelK)	Endoplasmic reticulum	ER-associated degradation
Selenoprotein V (SelV)	Testes	
Selenium binding protein (SBP1)	Golgi apparatus	Intracellular transport
Selenium binding protein 2 (SBP2)	Ribosomes	Intracellular transport
Selenophosphate synthetase 2 (SPS2)		Selenoprotein biosynthesis
Selenocysteine lyase (SCL)	Liver, kidneys	Selenium recirculation
Selenoprotein M, O, T, H, I (SelM,SelO, SelT, SelH, SelI)		Unknown functions

Experimental studies have demonstrated an inhibitory effect of Hg^{2+} on selenium transport in hepatocytes and enterocytes. One of the possible mechanisms of interaction between these trace elements may include formation of mercury selenite ($HgSeO_3$) with low solubility [6]. The role of aquaglyceroporines (AQP7 and AQP9) in intracellular mercury and selenium transport is also discussed [12]. It is also notable that methymercury exposure results in altered intracellular redox balance also affecting selenium status [13].

Further SEPP1 transport occurs through receptor-mediated endocytosis. In particular, SEPP1 is bound to low density lipoprotein receptor apoER2 [14]. With the help of megalin in proximal renal tubules endocytosis of SEPP1 occurs for subsequent renal synthesis of extracellular glutathione peroxidase (GPx3) [9].

Intracellular selenium transport is performed with the help of selenium-binding protein (SBP1). Protein coding gene is located on chromosome 1 at q21-22 [15]. SBP1 is detected in nucleus and cytoplasm of various cells of heart, lungs, gastrointestinal tract. Selenium transport into Golgi apparatus and participation in proteosomal degradation are supposed to be the functions of SBP1. Significant decrease in SBP1 levels was observed in ovarian, uterine, lung, stomach, intestinal and liver cancers [15]. A number of investigators indicate the association between low SBP1 levels and worse survival in these cancer types. An inverse association between SBP1 and GPx1 levels was also demonstrated [15].

The process of selenocysteine incorporation into selenoprotein structure is mediated by selenium binding protein 2 (SBP2) in ribosomes. Moreover, ribosomal protein L30, eIF4a3 and nucleoline also take part in this process [1]. SEPP1 is involved in retention of selenoproteins in endoplasmic reticulum, where complex formation under the influence of glycosyl transferase (UGT) occurs.

It has been estimated that approximately 40-60% of plasma selenium is presented by SEPP1. SEPP1 contains 10 selenium atoms

being the primary transporter of selenium to peripheral tissues [7,16]. At the same time, GPx3 refers to 10-25% of total blood selenium [7]. The family of thioredoxin reductases (TrxRs) is also related to selenoproteins that reduce thioredoxin using NADPH as a proton donor [16]. A protective role of thioredoxin reductase 1 in chemically-induced hepatocarcinogenesis via the control of redox potential was demonstrated [17]. Moreover, TrxR1 is an activator of p53 protein that suppresses of tumor growth [1].

The family of iodothyronine deiodinases (DIs) takes part in conversion of T_4 into more active T_3. During this process one iodine atom is cleaved from T_4 that is subsequently used for formation of new thyroid hormones. Low levels of selenium are observed in thyroid autoimmune diseases [18]. Selenophosphate synthetase 2 (SPS2) takes part in ATP-dependent selenophospate synthesis that acts as selenium donor for selenoprotein synthesis [1]. Selenoprotein K plays a significant role in T-cell differentiation, neutrophil and T-cell migration. In particular, selenoprotein K deficient transgene mice were characterized by decreased T-cell, neutrophil, and macrophage numbers [19]. At the same time, the functions of other selenoproteins remain poorly studied [20].

Altered selenium levels and SEPP1 and GPx activity may occur under the influence of hormonal stimuli. Prticularly, it has been estimated that estradiol treatment is accompanied by increased selenium concentrations and SEPP1 activity in plasma and liver [21]. Glucocorticoids oppositely suppress SEPP1 expression resulting in impaired selenium transport in the organism [22].

Selenium as a cofactor and clinical manifestations of its deficiency

Organism's selenium content is decreased with ageing, smoking, imflammation, and some types of cancer. A significant role

of selenium in protection from cardiovascular diseases and increase in latent period in HIV patients was demonstrated. A positive experience of selenium treatment in some types of cancer, muscular disorders, and autoimmune thyroiditis exist [1].

In selenium deficiency impairment of both innate and adapted immunity is observed [19]. Persons with insufficient selenium intake are characterized by impaired antiviral defense, immune response, increased risk of autoimmunity [23]. In particular, selenium deficiency is associated with the development of systemic connective tissue diseases like sclerodermia, lupus, rheumatoid arthritis, and Raynaud's syndrome [24]. Moreover, a significant relationship between selenium deficiency and allergic reactions and infective allergic asthma was demonstrated [20]. It is interesting that selenium treatment was protective against hepatitis B virus [25]. Selenoprotein K deficiency also results in ineffective immune response affecting activation, proliferation and differentiation of immune cells. Data on the influence of selenoprotein K on Ca^{2+}-dependent functions in immune cells also exist [19]. Moreover, a significant inverse correlation was observed between plasma selenium levels and C-reactive protein concentration. At the same time, Se/albumin×100 coefficient decreased proportionally to increase in C-reactive protein concentration [26]. Moreover, selenoprotein SelK coding gene polymorphism results in increased production of proinflammatory cytokines (TNFα, IL-1β) and increases the risk of inflammatory diseases [23].

As an anti-cancer compound selenium is involved in regulation of proliferation, migration and cancer cell apoptosis. It has been estimated that anticancer effect may be observed when dietary selenium is not less than 1.5 mg/kg. At the same time, efficient dietary level required for activation of the main selenoproteins is 0.1-0.2 mg/kg [7]. Previous investigation has demonstrated that administration of 200 μg selenium a day results in decreased

incidence of lung, gall bladder, prostate and colon cancer as well as mortality. High-dose selenium administration decreases cell proliferation, results in DNA fragmentation and induction of cancer cell apoptosis [27]. NCP (Nutritional Prevention of Cancer) and SELECT (Selenium and Vitamin E Cancer Prevention Trial) studies have demonstrated a decrease in total mortality and cancer mortality in comparison to the placebo group. The better results were obtained in the group where selenium levels did not exceed 121.6 ng/ml [28]. Methylselenol use (CH_3SeH) resulted in alteration of p53 activity leading to decreased protease activity and the availability of angiogenic factors required for tumor expansion [7]. A number of investigations propose that selenium may exert cytotoxic properties in cancer cells due to reduced expression of SBP1. Decreased SBP1 activity results in accumulation of hydroperoxides and reactive oxygen species finally triggering development of oxidative stress and apoptosis. In particular, decreased SBP1 is associated with increased intracellular glutathione concentrations [29].

Azoospermia and impairment of the later stages of spermatogenesis are associated with decreased selenoprotein activity in male reproductive system. It is known that decreased mitochondrial GPx4 activity is one of the causes of male infertility and correlates with oligospermia development. It has been indicated that impaired sperm morphology is observed in decreased SEPP1 activity [20]. SEPP1 performs selenium transport into Sertoli cells where GPx4 synthesis occurs [30]. Experimental studies have demonstrated that SEPP1 levels in seminal plasma is indicative of its quality (morphology and sperm motility) in contrast to plasma SEPP1 content [30].

The existing data indicate protective function of selenium in osteoarthritis. The use of selenomethionine suppresses IL-1β expression in chondrocytes. Moreover, selenomethionine also decreases both NO and prostaglandin (PGE) production. Selenium-

containing deiodinase (DIO2) also takes part in osteoarthritis development [31]. The main hypothetic mechanism of participation of this protein is its involvement in bone remodeling. As a result of prolonged selenium deficiency occurs the formation of skeletal abnormalities and osteochondrosis development that clinically manifests with Kashin-Beck disease. In addition, the main antioxidant enzyme of osteoclasts is GPx1 that is increased in estrogen treatment. Accordingly, estrogens are osteoclastogenesis inhibitors. Positive association between plasma SEPP1 concentrations and bone mineral density was revealed in postmenopausal women [32].

Thyroid gland is the one of the organs with high selenium content. Low dietary selenium intake increases the risk of diffuse enlargement of thyroid and multinodular goiter [33]. T_4/T_3 ratio is the one of the parameters that may be used for assessment of selenium status. Normally, the ratio does not exceed 20:1 whereas in selenium deficiency it is increased [7]. Indications of the influence of selenium preparations on the number of autoantibodies characteristic for Hashimoto disease and improvement of ultrasound parameters. Even in pregnant women administration of selenium preparations resulted in decreased subacute thyroiditis and hypothyreosis risk [34]. In Graves' disease additional administration of selenium was also accompanied by process normalization and euthyroid state. Analogical regression was observed in the case of endocrine ophthalmopathy after selenium treatment. It is concluded that these effects of selenium are associated with its influence on immune system and ROS production. Scholarly data indicate that improvement of thyroid functions is observed after administration of 400 μg/day selenomethionine [35]. It is supposed that impaired binding of selenium to SBP2 may result in its decreased incorporation into selenoproteins [18]. At the same time, data indicating the influence of thyroid hormones on selenium

homeostasis were obtained. In particular, critically ill patients with low T_3 syndrome are also characterized by decreased selenium content. Simultaneous decrease in selenium and T_3 levels is considered to be a negative prognostic marker [36]. Experimental studies have indicated that selenium levels increase after T_3 treatment. The mechanism of this effect may be mediated through activation of thyroid receptor α1 (TRα1). It has been indicated that TRα1 plays an important role in selenoprotein mRNA expression in liver [37].

It is interesting that SelT is involved in peptide hormone release in hypophysis [23]. SelT selenoprotein is related to thioredoxin-like proteins. SelT expression is detected in endocrine tissues like hypophysis, thyroid gland, pancreas, and testes [38]. Experimental studies with knock-out mice have indicated that the absence of SelT decreases insulin production by β-cells. It has been estimated that SElT is regulated by pituitary adenylate cyclase activating peptide (PACAP) [39]. One of the possible mechanisms of SelT action is regulation of PACAP-induced increase in intracellular Ca2+ [40].

Experimental studies indicate that SelM activity is maximal in paraventricular and arcuate nuclei of hypothalamus that are involved in regulation of energy metabolism [41]. SelM is related to the proteins associated with endoplasmic reticulum. In particular, it is known that obesity development is associated with hypothalamic ER-stress [42]. Decreased SelM activity associated with increased leptin levels, metabolic syndrome, and leptin resistance in hypothalamus was demonstrated [43].

Certain selenoproteins like GPx, SelM, S, SEPP1 and SBP1 are also active in intestine. Selenium deficiency is observed in Crohn diseases patients and in other inflammatory states [44]. Experimental studies have demonstrated that consumption of selenium-depleted diet is associated with increased incidence of colitis. The proposed mechanism involves participation of selenium in formation of 15-

hydroxyprostaglandin dehydrogenase (15-PGDH) in macrophages and their infiltration in the focus of inflammation. In particular, the activity of 15-PGDH is increased in selenium deficiency [45].

Decreased selenium content in brain tissue is associated with different neurological disturbances including epilepsy, ataxia, etc. [23, 46]. The maximal selenium concentration is observed in brain cortex, hippocampus, cerebellum, and olfactory bulb [47]. It is known that glutathione (GSH) is one of the main intracellular antioxidants. At the same time, increased oxidized glutathione (GSSG) and decreased GSH/GSSG ratio is indicative of altered cellular redox potential. In particular, decreased cysteine, GSH and GSH/GSSG ratio values and GPx activity are observed in autistic children [48]. Moreover, autistic children are also characterized by impaired methylation processes. Mehionine synthetase (MS) that is activated by folic acid and B_{12} also is decreased by oxidative stress. Altered MS activity results in decreased methyl donor-to-methylation inhibitors ratio. Consequently, reciprocal relationship between impaired antioxidant defense and methylation processes was observed in autistic children [48]. It is estimated that the formation of MS in neurons is stimulated by dopamine and growth factors whereas its depression is observed under the influence of neurotoxins including mercury [4].

Severe selenium deficiency is the cause of muscular diseases in human and animals. In particular, selenium deficiency manifests with myotonic dystrophy, muscular weakness and pain. Decreased SelW activity is accompanied by skeletal and heart muscle alterations [23]. Retrospective studies have revealed from 2- to 3-fold increase in cardiovascular mortality in persons with plasma selenium lower than 45 μg/l [49]. Moreover, selenium deficiency is also associated with Keshan and Chagas disease. Reduced antiviral defense associated with Coxsackie-B3-virus infection was observed in dietary selenium deficiency in Keshan disease [50]. At the same time, the specific

feature of Chagas disease is the decrease in antitubercular immunity resulting in *Trypanosoma cruzi* invasion [51]. Both diseases ultimately lead to the development of heart failure [23].

Selenium deficiency-associated oxidative stress results in alteration of vascular endothelium and development of atherosclerosis, hypertension, and progressive heart failure [23]. Selenium administration increases GPx1, 4 and TrxR1 activity in vascular endothelium and smooth muscles and decreases apoptosis stimulated by LDL and thiols [52]. Experimental studies indicate that GPx1 suppresses ischemia-induced apoptosis [53]. Oppositely, decreased GPx3 activity is associated with increased risk of ischemic stroke. In particular, ROS overproduction decreases GPx3 activity that is accompanied by increased NO levels and thrombocyte dysfunction and, finally, increased risk of arterial thrombosis [54]. In addition, GPx4 also decreases atherosclerosis risk through prevention of lipid peroxidation in lipoproteins [55]. It is known that urinary selenium excretion reflects not only its content in the organism but also the availability of methyl donors S-adenosylmethionine (SAM). Moreover, an inverse association was observed between urinary selenium content and plasmatic homocysteine, whereas folic acid and B_{12} levels were characterized by positive correlation with metalloid level [7].

Obese patients (BMI > 30) are characterized by decreased selenium, GPx, and SEPP1 as compared to the lean persons. The possible mechanism may involve decreased SEPP1 expression under the influence of proinflammatory cytokines and increased activity of gluconeogenesis enzymes [7]. Selenocysteine lyase (SCL) is required for selenium release and its use for selenoprotein biosynthesis. Both SCL and SEPP1 are synthetized in liver. Experimental studies on knockout animals have indicated the absence of SCL impairs glucose and lipid metabolism in liver. In particular, SCL-deficient animals maintained at selenium-adequate diet were characterized by the development of hyperinsulinemia, hyperlipidemia, impaired carbohydrate tolerance, liver

steatosis without significant alteration of selenium and selenoproteins. These metabolic disturbances are aggravated after administration of low-selenium diet in SCL-KO-mice. The investigators have proposed that impaired SCL formation may result in decreased SEPP1 and SelS turnover that is accompanied by obesity and metabolic syndrome development [56]. Therefore, impaired SCL generation may lead to carbohydrate and lipid metabolism disturbances without elevation of SEPP1 levels being an independent factor.

Metabolic disturbances associated with excessive selenium accumulation

Initially, selenium was studied as toxic element. In 1930s the development of neuropathy was detected in cattle feeding on the pastures with high soil selenium content [7]. To date, chronic selenosis is detected in the East of China where dietary selenium consumption exceeds the hundreds of mg/kg of food. Clinical manifestations include the development of alopecia, nail dystrophy, impaired skin sensitivity. These symptoms may be reversed after decreased selenium levels [57]. The majority of studies failed to reveal any adverse health effects of selenium when its plasma content is lower than 1000 ng/ml [7]. Along with the positive influence of selenium on the human organism the increasing amount of data is indicating of the adverse health effects of selenium. It is supposed that selenium status may be indicated with a U-shaped curve where both deficient and excessive content is accompanied by pathological states with the presence of a small safe limit of its concentrations [58]. Therefore, positive influence of selenium preparations may be observed only in persons with selenium deficiency. In adequate selenium state selenium supplementation may have negative effects. Multiple studies have indicated that selenium excess is associated with diabetes mellitus type 2 and obesity.

Multiple indications of the association between selenium and glucose metabolism exist. However, the existing data are contradictory. In particular, some studies have indicated that obese and diabetic are characterized by selenium deficiency [59] and increased levels of adiponectin, a recognized predictor of diabetes mellitus type 2 [60]. It has been also revealed that selenium and fibroblast growth factor-23 (FGF-23) levels were lower in children with metabolic syndrome as compared to those in healthy children [61]. Oppositely, in the Nutritional Prevention of Cancer study it has been shown that selenium administration in the dose of 200 μg/day and plasma selenium levels higher than 121.6 ng/ml resulted in a significant increase in the number of persons suffering from diabetes and obesity as compared to the control group. Moreover, SEPP1 level was significantly higher in persons with type 2 diabetes mellitus than in those with normal glucose tolerance [10]. Experimental studies have demonstrated that increased dietary selenium (0.4-3.0 mg/kg) result in overproduction of selenoproteins (GPx1, SelS, SEPP1) and clinically manifests with diabetes-like phenotype [62]. As SEPP1 is produced by liver, hypothetically its generation may be affected by gluconeogenesis enzymes like glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. Consequently, activation of gluconeogenesis and increased SEPP1 production is observed in glucotoxicity [63]. Some authors indicate that increased SEPP1 production by liver is the consequence of impaired glucose metabolism than its cause [64]. Scholarly data indicate that 3-months administration of selenium administration of selenium 200 μg/day in diabetic patients resulted in alteration of glucose and cholesterol metabolism parameters [65]. The other investigators have not observed diabetogenic effect of selenium in the same doses [60]. In experimental studies maintenance of animals on high-selenium diet (3 mg/kg) resulted in hyperinsulinemia and impaired glucose tolerance [7]. It is also notable that selenocysteine

lyase involved in regulation of selenium recirculation also influences energy metabolism. In particular, transgenic mice fed a selenium-adequate diet were characterized by increased levels of insulin, leptin, impaired glucose tolerance and liver steatosis [10]. Moreover, liver steatosis and metabolic syndrome was associated with increased levels of SEPP1, fetuin-A, and FGF-21. Production of these molecules results in impaired glucose utilization (GLUT-1), insulin resistance, elevated LDL and decreased HDL levels [66]. The obtained experimental and clinical data underline the necessity of further investigation of the role of selenium and SEPP1 in glucose metabolism. It is should be mentioned that uncontrolled high-dose selenium administration should be limited despite its anticarcinogenic effect. The use of these preparations should be based on the results of laboratory diagnostics.

We also found elevated hair selenium in children with both childhood (ICD-10: F84.0) and atypical autism (ICD-10: F84.1). At the same time, assessment of serum Se levels revealed lower concentrations of selenium. Therefore, autism spectrum disorders are associated with an inversion of hair and serum Se concentration in children with a shift to hair content.

Genetic diseases associated with impaired selenium metabolism

SBP2 coding gene mutation results in alteration of selenoprotein synthesis including deiodinase. This defect is associated with increased T_4, RT3, and decreased T_3 levels without alteration of TSH levels [67]. Clinical deficiency includes myopathy, delayed physical and psychical development, immune deficiency, azoospermia and infertility, hearing disorders and increased skin photosensibility [35]. It is also interesting that despite increased

insulin signaling this genetic disease is associated with elevated amount of adipose tissue [20].

Ryanodine receptor and SelN mutation results in inherited muscular dystrophy (multiminicore disease). The result of this mutation is disorganization of muscle fibers, Mallory's body formations, intracellular calcium accumulation associated with progressive scoliosis in children [23].

Laboratory diagnostics of impaired selenium metabolism

Estimation of selenium concentration in blood plasma (serum) is one of the widely used tests for selenium status assessment. Selenocystein is also incorporated into SEPP1 and GPx3 and their plasma concentration may be also used as markers of selenium status. It is supposed that total SEPP1 and GPx3 plasma selenium levels is not less than 70 μg/l. Therefore, if plasma selenium content is less, it may be indicative of impaired selenoprotein synthesis. Optimal SEPP1 level in blood serum is associated with 70 μg of dietary selenium a day [68].

Along with selenoproteins selenium may non-specifically bound to albumin. It has been estimated that after consumption of 300 μg/day of dietary selenium the level of non-specifically bound selenium is 73% of its total plasma content. Plasma selenium content higher than 70 μg/l is indicative of non-specifically bound pool like albumin [7].

Noninvasive markers of selenium status include its estimation in buccal epithelium, hair and nails [7]. However, selenium content in these substrates may be affected by the use of selenium-containing antiseborrheic shampoos, selenium mesotherapy, and insufficient pre-analytic hair washing.

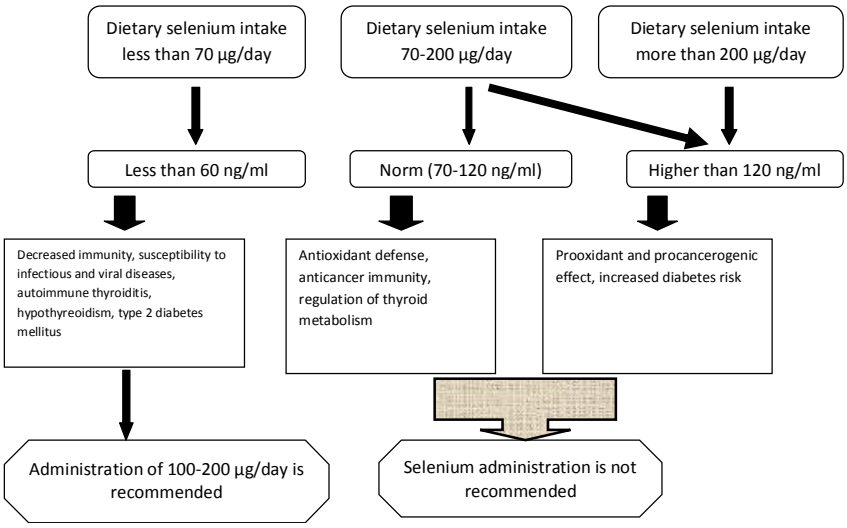

Fig. 1. The sceme of molybdenum-containing cofactor Moco biosynthesis

The reference values for hair Se levels in adult men and women are 0.089 - 0.480 and 0.094 - 0.504 µg/g, respectively

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IODINE

Introduction

Iodine is an essential trace element involved in regulation of protein, lipid, and carbohydrates metabolism as well as balance between catabolic and anabolic processes. Iodine deficiency is widespread in the world; more than 1 billion of the population can suffer from it [1]. Mainly the iodine deficient areas are situated far from seas and oceans, in mountains [2-3]. Iodine deficiency and the related thyroid diseases are known from ancient times. The most characteristic disease is goiter that is characterized by visual increase in thyroid volume. Hypothyreosis and goiter are detected in all age groups caused by inadequate dietary iodine consumption [4-6]. Even moderate iodine deficiency results in reproductive disorders in females that are associated with increased incidence of miscarriage and stillbirth [7]. Iodine deficiency is also critical for fetal and newborn development. The most dramatic manifestation is the development of neurologic cretinism associated with cognitive disorders [8]. Thyroxin (T4), a thyroid hormone with four atoms of iodine in its structure, plays the most important role in brain development. Under the influence of brain isoform of deiodinase thyroxin is transformed into the more active triiodothyronine. It is involved in nerve fiber myelinization, somatogenesis, and neuron differentiation. In the first trimester of pregnancy thyroxin takes part in development of brain cortex, inner ear, basal ganglia, whereas in the third trimester its primary role is participation in growth and differentiation of all brain regions [9-10].

The total content of iodine in human body is 15–25 mg, 50% is concentrated in thyroid gland. Much less content is in liver, ovaries, lungs. The average body abundance is 0.19 μg/g. Nutritional deficiency is observed at iodine intake <10 μg/day, toxicity – at >2 mg/day. The lethal dose is 35–350 g. Intake with food is ca. 0.1–0.2 mg/day with the resorption level 95–100%. Absolute majority of iodine is excreted with urine. The half-life in the body is 138 days.

Optimal daily requirement in iodine is 150 μg/day that provides thyroid hormone synthesis [11]. The necessity of iodine for humans is increasing in adolescence, pregnancy and lactation (250 μg/day) [12]. Also, there are a lot of so-called strumogenic agents, which can impair iodine metabolism and cause thyroid gland dysfunction [13]. Overloading by cobalt, manganese, toxic metals like lead, cadmium, and deficiencies of copper, zinc and especially selenium in food and in the body are factors affecting iodine metabolism and thyroid functions [1,13-14]. We found that the endemic goiter treatment in adolescents was less effective in presence of low hair copper, selenium, zinc, phosphorus and elevated chromium, cobalt, manganese [4].

Thyroid hormone production and iodine reuptake decrease with age, whereas the level of thyrotropin and reverse T3 tend to increase [15].

Excessive dietary iodine consumption influences trace element balance and is able to block synthesis of thyroid hormones and iodine organification. This effect is termed Wolff-Chaikoff effect. However, the inhibitory effect of high concentrations of iodine lasts for 2 days. After that period the level of hormones is restored being indicative of adaptive reaction. As a result, decreased iodine transport and NIS activity occur [16-18].

One should not simultaneously take supplements containing iodine and lithium carbonate. Lithium reduces activity of thyroid gland while iodine enhances manifestation of lithium side effects. Excessive amounts of Co, Mn, Pb, Ca, Br, Cl, F are antagonists of

iodine. A strengthening of the goitrogenic effect in human is observed at Se, Zn, Cu deficiency. In all these cases there may develop a disturbance of iodine metabolism and its utilization by the thyroid gland [14].

Transport, metabolism and homeostatic regulation of iodine

Iodine absorption occurs in the jejunum. The transporter (Na+/I- symporter, NIS) is located at the apical membrane of epithelial cells and regulates iodine absorption. In particular, in terms of excessive dietary iodine intake NIS expression is decreased. Intracellular iodine concentration also serves as a regulator of NIS activity that is confirmed by the test with perchlorate. The latter is the selective inhibitor of NIS [19].

Ghrelin levels also affect iodine absorption. Ghrelin is a multifunctional peptide regulating energy homeostasis [20]. Experimental studies demonstrated a significant increase in its levels in hypothyreosis and, oppositely, decreased concentrations in hyperthyreosis. The observed correlation between thyrotropin and ghrelin may indicate the development of adaptive mechanisms in terms of metabolic disorders [21].

NIS is critical for iodine transport from the bloodstream into thyrocytes and is located at the basolateral cellular membrane. SLC5A gene coding NIS is located at 19p12-13,2 chromosome. All transporters of SLC5A family depend on electrochemical gradient and performs transmembrane iodine transport [22-23]. Two sodium cations are transported into the cell per one iodine atom [19]. Iodine transport into the cell is energy-dependent. Na+/K+ ATPase takes part in this process that allows to cumulate iodine inside the cell in 10-fold higher quantities than those in blood [24]. Thyrotropin

stimulates intracellular iodine accumulation due to increased NIS activity. Experimental hypophysectomized animals were characterized by decreased thyroid levels being associated with decreased NIS activity [25]. At the same time, later studies demonstrated that thyrotropin does not affect NIS biosynthesis but its activity by modification of posttranscription mechanisms [26]. High NIS activity is observed in salivary glands, stomach, plexus vasorum, and mammary glands. NIS expression in these tissues does not depend on thyrotropin levels [27].

Table 1

Iodine transporters in cells

Transporter	Localization	Functions
Na+/I- symporter	Thyrocyte basolateral membrane	Iodine transport into the cell
Pendrin	Thyrocyte apical membrane	Iodine release into the follicle, regulation of intracellular iodine pool
Cl-/ HCO3- exchanger	Thyrocyte apical membrane	Iodine release from the cell
Cystic fibrosis transmembrane conductance regulator (CFTR)	Thyrocyte apical membrane	Iodine release from the cell
H+/ Cl- antiporter	Thyrocyte apical membrane	Iodine release from the cell

Further release of iodine from thyrocytes into the follicle is mediated by pendrin. This protein is related to anion transporters (Cl^- and I^-) that is located on the apical membrane. The importance of pendrin is increased in iodine deficient state. In particular, increased pendrin activity is associated with diminished intracellular iodine levels [28]. Consequently, pendrin regulates not only the release of iodine into the follicle but also intracellular iodine homeostasis. It is notable that thyrotropin and iodine themselves do not affect pendrin activity, whereas thyroglobulin (Tg) increases its expression [29, 30].

Pendrin expression was also detected in the inner ear. In kidneys pendrin functions as Cl^-/HCO_3^- exchanger resulting in chlorine and hydrocarbonate retention. In the inner ear it supports the transport of anions and endocochlear potential [30-31].

Chloride channels are permeable for iodine and may be considered as I^- transporter through the thyrocyte apical membrane (Cl^-/HCO_3^- transporter). Therefore, pendrin is not the only iodine transporter [28]. Cystic fibrosis transmembrane conductance regulator (CFTR) is the other candidate for iodine transporter and its expression is also observed in thyroid. In has been noted that the present transporter is involved in development of subclinical hypothyreosis [24]. Experimental studies have demonstrated the role of H^+/Cl^- antiporter, CLC-5. In particular, iodine transport refers to 70% of the total amount of Cl^- transported [24]. Experimental knockout animals (CIC-5 KO mice) are characterized by euthyroid goiter and pendrin expression was decreased by 60% [7]. Moreover, a tight relationship between pendrin and CLC-5 activity was detected. It is supposed that the decrease or loss of pendrin activity may be compensated by CLC-5 hyperproduction [32].

It is known that perchlorate suppresses the entrance of iodine into the thyroid and decreases hormone production [33]. Perchlorate may be used in hyperthyreosis therapy. In addition, this compound is used in clinical practice for assessment of iodine organification [24]. In euthyroid patients perchlorate blocks iodine accumulation but not radioactive iodine (I^{123}) due to its rapid organification. Oppositely, in impaired organification under the influence of perchlorate the release of I^{123} is increased that may be considered as a positive test result [24, 34].

The majority of uptaken iodine is used for thyroid hormone biosynthesis. The massive moiety of iodine in T_4 molecule is 65% [35]. Sufficient intake of iodine, effective transport and regulatory system are required for effective hormone production.

In the follicle ionized form of iodine (I^-) is oxidized to I_2 and subsequently bound to tyrosine residues of Tg. In this colloid form iodine is accumulated in follicles. Formation of two key proteins, thyroperoxidase and thyroglobulin, occurs in endoplasmic reticulum of thyrocytes [35]. At the requirement, monoiodothyronine (MIT) and diiodothyronine (DIT) are formed at the apical membrane of thyrocytes with the help of thyroperoxidase. Further, these compounds are used for formation of T3 and T4 (thyroxin and triiodthyronine) [24]. Dual oxidase (Duox2), a NADPH-oxidase, is essential for iodine organification. This process also requires the presence of hydrogen peroxide. It is notable that in contrast to other proteins involved in thyroid hormone synthesis Duox2 activity is not affected by thyrotropin concentration. The intracellular level of calcium is the positive regulator of Duox2 activity [7]. Exopeptidases and endopeptidases like cathepsins B, L, and D also take part in thyroglobulin synthesis. After the release of thyroid hormones into the follicle thyroglobulin is transported back for repeated use with the help of iodotyrosine dehalogenase 1 (DEHAL1). Tg production and lysosomal activity of thyrocyte are regulated by thyrotropin [35-36].

DEHAL1 also mediates the extraction of iodine from MIT and DIT for repeated use in the cycle of iodine organification. Decreased DEHAL1 activity is accompanied by increased MIT and DIT blood concentrations, as well as increased urinary excretion of organic iodine. It is also associated with the development of congenital hypothyreosis and goiter. The special characteristic of this state is characterized by the possibility of metabolic compensation by administration of increased doses of iodine [35].

Accumulation of iodine is also observed in salivary and mammary glands, uterus and ovaries, stomach and jejunum, vascular plexus, ciliary body and depends on NIS expression [19]. In these tissues NIS expression is not regulated by thyrotropin and iodine levels. Decreased NIS expression in some types of gastrointestinal

cancers is considered as diagnostic and prognostic disease marker. Moreover, a positive influence of radioactive iodine (^{131}I) on breast, ovarian, intestinal, liver, pancreatic and prostatic tumor regression has been demonstrated both in vivo and in vitro [37]. NIS expression is also affected by hormonal therapy. In particular, oxytocin and prolactin administration in experimental animals was associated with increased NIS activity in mammary glands [38-39].

The association between potassium channels (KCNQ/KCNE2) and thyroid function was also detected in experimental studies. Thus, in animals with impaired channel function hypothyreosis was observed along with the expected cardiac symptoms. This process was accompanied by decreased iodine uptake by NIS. Perchlorate test confirmed the absence of impaired iodine organification in laboratory animals [7]. Therefore, it is supposed that NIS may take part in regulation of KCNQ/KCNE2 activity.

Iodine excretion occurs through urine. Epidemiologic studies have revealed that the median of ioduria of 100-300 μg/l are characteristic for normal iodine content in the organism [12].

Clinical signs of iodine deficiency

Thyroid gland is an endocrine gland with the early start of functioning. In particular, fetal thyroid starts to synthetize hormones at 10-12 weeks of gestation. Before this period the fetal development depends on maternal thyroid hormone levels. Both hormones (T_3 and T_4) are detected in fetal brain cortex starting from the 12 week of pregnancy. These hormones play an important role in migration and differentiation of neurons, synaptogenesis, and myelinization. The fetal levels of T_3 are comparable to those in adults being associated with increased activity of type 2 deiodinase in brain. Severe thyroid hormone deficiency results in cretinism development. However, even moderate maternal T_4 deficiency in pregnancy may result in decreased IQ in children and other cognitive disorders [9-10]. The importance of thyroid hormones for children development is

confirmed by the fact that chorionic gonadotropin contributes to increased NIS expression and I^- transport. Moreover, chorionic gonadotropin may act on thyrotropin receptors leading to increased maternal thyroid hormone synthesis [8-9].

The incidence of congenital hypothyreosis is 1:2000-1:4000 of newborns. Clinical symptoms include decreased physical activity, drowsiness, impaired breast feeding, prolonged jaundice. Physical examination reveals myxidematous face, macroglossia, omphalocele, and hypotension [40].

T a b l e 2

Biological effects of iodine deficiency and thyroid hormones in mother and fetus

I and T_4 content	Clinical manifestation	Correction methods
Severe iodine deficiency and hypothyreosis in mother	Neurological cretinism	Iodine supply till birth and thyroxin treatment
Decreased T_4 levels in mother (hypothyroxinemia)	Decreased IQ in child	Thyroxin treatment during and after pregnancy
Fetal hypothyreosis (from 20 week of gestation) along with insufficient iodine status of mother	Myxedematous cretinism (mental disorders that are less expressed in neurologic forms)	Thyroxin treatment of the newborn
Congenital hypothyreosis	Impaired hormone synthesis in the newborn, neurological and mental disorders	Thyroxin treatment of the newborn

The risk of thyroid diseases depends on iodine intake and is characterized by U-shaped curve where both excess and deficiency exert negative effect [41]. Iodine deficiency is associated with goiter, hypothyroidism, increased risk of miscarriage, preterm birth, congenital fetal abnormalities, and elevated incidence of neonatal death [9, 42]. In hypothyreosis the development of decreased blood sodium is observed. Moreover, its incidence is higher in women, elderly, and after thiaside diuretics treatment [43].

A direct correlation was observed between maternal and fetal thyroid hormone levels. Consequently, even subclinical maternal hypothyreosis requires timely correction as it is a danger for fetal hypothyreosis. Fetal hypothyreosis is the cause of myxedematous form of cretinism that develops from the last trimester of pregnancy [8].

Thyrotropin consists of 2 subunits where α-subunit is absolutely identical to the other hormones like luteinizing hormone (LH), follicle stimulating hormone (FSH), and human chorionic gonadotropin (hCG), whereas β-subunit is unique. The functioning of thyroid gland is regulated by thyrotropin that acts through the respective receptors.

The development of thyroid cancer is observed both in regions with iodine deficiency and its excessive intake [41]. However, the increased ratio of differentiated papillary cancer to follicular in the regions with iodine excess as compared to the ones with normal iodine status and deficiency [44]. Papillary and follicular thyroid cancers refer to 5% of all malformations in women. The number of persons suffering from thyroid cancer has been multiplied by two in comparison to the 1970s [7]. The level of iodine and NIS activity in thyroid of these patients is one of the lowest. As the result of decreased NIS expression the patients may need higher cumulative dose of radioactive iodine for treatment and achievement of better prognosis [37, 45].

The development of benignant and malignant tumors in thyroid gland in women occurs more frequently as compared to men. It has been estimated that the number of estrogen receptors (ERα, ERβ) in undifferentiated stem cells is 8-fold higher than in differentiated thyrocytes [46]. However, thyrotropin-induced thyrocytes differentiation as well as NIS expression may be suppressed by estradiol. This fact is confirmed by the prevalence of nodular thyroid neoplasma in females as compared to males and their association with hyperestrogenia. Moreover, a positive correlation between NIS expression and estrogen receptors in women was demonstrated [47].

Clinical studies have revealed a significant increase in serum copper levels along with decreased iron concentration, free T_3 and T_4 in women with nodular endemic goiter [48].

Iodine excess

Thyrotoxic states are observed in Graves' disease, autonomous toxic adenoma. The most frequently these diseases are associated with thyrotropin receptor mutation and G-protein α-subunit stimulation. Despite the fact that autonomous toxic adenoma manifests in thyrotoxicosis and its development is associated with increased iodine consumption in the past [49]. Moderate and expressed iodine deficiency launches the compensatory mechanisms aimed at maintenance of thyroid function. Unfortunately, the negative consequence of such stimulation may be associated with the development of thyrotoxic nodular goiter with increasing risk with age [50]. Moreover, the transitory gestational thyrotoxicosis is developed under the influence of hCG-induced stimulation of thyrotropin receptors. Iatrogenic thyrotoxicosis may include administration of moderate and increased doses of iodine, antiarrhythmic preparations (amyodaron), contrast agents, antiseptics, phenobarbital, dopamine antagonists, lithium carbonate, glucocortocoidα-interferon, metformin, dietary preservatives [49,51-53]. In particular, the daily dose of amyodaron (300 mg) contains 9 mg of iodine. Amyodaron-induced impairment of thyroid function results in development of type I and II thyrotoxicosis [54-55]. Type I thyrotoxicosis is developed in patients with preexisting iodine deficiency (hyperplasia or goiter), whereas type II is characteristic for patients with normal iodine status. In the latter amyodaron administration is accompanied by destruction of follicles and the release of hormones into the bloodstream [11]. Administration of

high doses of propranolol (β-blocker) is accompanied by decreased peripheral conversion of T3 and T4. Amphetamine treatment and administration of a number of antipsychotics result in hyperthyroxinemia [51]. Excessive uptake of iodine by thyroid results in increased free radical production, oxidative stress, thyrocyte damage (apoptosis and necrosis), proinflammatory cytokine production, thyroid tissue infiltration with immune cells and anti-Tg antibodies production [41, 56].

Genetic diseases associated with impaired iodine metabolism

Pendred syndrome is an autosomal-recessive genetic disorder associated with impaired iodine organification. SLC26A4 gene mutation results in impaired pendrin production. As it has been indicated earlier, pendrin expression is observed not only in thyroid, but also in the inner ear. Therefore, this disorder results in decreased thyroid hormone production, development of goiter and hypothyreosis, as well as neurosensory hearing loss, and vestibular disorders [28, 31, 57].

Duox2 and DuoxA2 coding gene mutation results in impaired thyroid hormone synthesis and manifests with congenital hypothyreosis [7, 58]. Congenital I^- transport defect (ITD) is associated with MIS coding gene mutation. This autosomal-recessive disorder manifests with hypothyreosis, goiter, decreased uptake of radioactive iodine, and low saliva-to-plasma iodine ratio [59].

Laboratory criteria of iodine metabolism

In epidemiologic studies the normal value of ioduria is more than 100 μg/l [12]. Excessive levels of iodine levels correspond to

ioduria median of more than 300 μg/l for the general population and 500 μg/l in pregnant women (WHO, 7/20). American thyroid association offers to consider the values of ioduria of more than 500 μg/l as excessive corresponding to the daily intake of 1100 μg [60]. The level of thyroglobulin is supposed to be an alternative marker. The values of thyroglobulin not less than 13 μg/l are associated with adequate iodine status. The level of Tg less than 13 μg/l corresponds to the median of ioduria of more than 100 μg/l and more than 150 μg/l for pregnant [61].

Our investigations demonstrated the usefulness of hair iodine content as a biomarker of iodine status in humans [2-4,62-63]. In one report the better indicative ability of hair as compared to urine regarding the iodine status of iodine-sufficient and iodine-insufficient groups of population was observed [3]. So, urinary iodine concentration is the "epidemiological" indicator of iodine provision of population, but hair iodine level can be usable for individual iodine status testing [2].

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COBALT

Introduction

Cobalt is an essential trace element that is cumulated predominantly in liver, kidneys, pancreas, heart, skeletal muscle, and bones. In the bloodstream Co(II) exists in complex with albumin. 5-12% of total circulating cobalt in the bloodstream may be present in ionized free form [1]. Possibly, ionized cobalt may exert toxic properties. It is supposed that this mechanism takes place in sportsmen treated with erythropoietin [1]. The majority of cobalt in the organism is present in the structure of vitamin B_{12} [2]. Cobalt is an essential metal for B_{12}, which is involved in various methyl transfer reactions. Complex system of dietary vitamin intake includes transport proteins like haptocorrin, Castle intrinsic factor and transcobalamine [3].

The average cobalt content in human body is around 15 mg. Its abundance in the human body by mass is 0.021 μg/g [4]. Liver, kidney, spleen are rich in cobalt. The toxic dose for humans is 500 mg. Optimal daily intake of cobalt is above 0.3 mg, the resorption is 20%. Cobalt is excreted with urine (0.2 mg), feces (0.1 mg), sweat (0.004 mg); the half-time is 30–40 years [5].

Elevated content of protein and iron in the diet slows cobalt absorption in the gastrointestinal tract; in contrast, copper and zinc enhance this process. An excess of cobalt can lead to disturbance of iodine metabolism in the thyroid gland [6].

Transport, metabolism and homeostatic regulation of cobalt

Cobalt intake is mediated by divalent metal transporter 1 (DMT1). In turn, this transporter is also used for transport of iron and other metals [7]. Experimental studies have indicated that intake of iron is suppressed in the presence of both manganese (II) and cobalt (II). DMT1 was detected in intestine, upper respiratory tract and lungs. Moreover, ferroportin, a transport protein that is responsible for iron absorption also increases Co^{57} release from the cell [8]. Therefore, intake and metabolism of iron and cobalt are interdependent.

In cells with low ATP content like erythrocytes Co^{57} intake is dependent on electrochemical potential. It has been estimated that calcium channel does not take part in cobalt transport. At the same time, calcium channel blockers (nifedipine group) influence both calcium and cobalt transport into the cell [9]. It is supposed that cobalt is able to bind hemoglobin molecule, however, the exact mechanisms are not studied.

Cobalt excretion is ATP-dependent process as that for calcium, copper, manganese and other trace elements. A particular family of ATPases may be involved in this process. In particular, P1a-ATP-ase is involved in K+ transport, P2-ATPase regulates Ca^{2+}, Na+/K+, H+/K+ flux, and P3 and 4-ATPases are required for H+ transport, whereas P1B-ATPase performs excretion of heavy metals (Cd^{2+}, Pb^{2+}) as well as Zn^{2+}, Cu^{2+}, Cu+ and Co^{2+} [10].

It has been estimated that Co^{2+} is capable of stabilization of hypoxia inducible factor (HIF) that is involved in metabolism regulation in low oxygen levels [1]. Cobalt suppresses hypoxia-induced production of erythropoietin through HIF-1 activation [11].

It is known that cobalt plays a structural role in B_{12} vitamin. This vitamin is not synthesized in the organism and humans require

its intake from the environment where it is produced by bacteria. In acidic medium of the stomach cobalamine is released from foods. Parietal stomach cells release Castle intrinsic factor that binds cobalamine. The formed complex "Vitamin B_{12}-intrinsic factor" is transported into small intestine where it is absorbed in the terminal part. Cobalamin is bound to two proteins in blood serum. Approximately 70-90% of cobalamin is not active and bound to haptocorrine (transcobalamin I) [12]. Consequently, active fraction of cobalamin is bound to transcobalamin II and is transferred through the organism [13].

Transcobalamin complex with B_{12} has been termed as holotranscobalamin. Approximately 20% of total B12 is located in holotranscobalamin [14].

In the organism B_{12} undergoes further transformation with the formation of metabolically active derivatives: methylcobalamin and adenosylcobalamin (AdoCbl) [15]. It is known that the human organism is unable to synthetize B_{12} *de novo* and requires its nutritional intake. Cytosolic transporter of cobalamin (MMACHC) is the transporter protein. Methylcobalamin acts as a cofactor of methyonine synthetase, whereas adenosylcobalamin activates methylmalonyl-CoA-mutase [16].

Cobalt-containing enzymes and clinical signs of its deficiency

In contrast to other trace elements like zinc, copper, selenium, and manganese, cobalt takes part in the functioning of a limited number of enzymes.

Methylmalonyl-CoA-mutase is an AdoCbl-dependent enzyme, whereas methionine synthetase uses methylcobalamin (MeCbl) as a cofactor [17-18]. Methionine synthase transforms homocysteine to

methionine by remethylation with the help of B_{12} and B_3 [2, 19]. Therefore, B_{12} and B_3 deficiency results in homocysteine accumulation. Increased activity of this enzyme is observed in cytoplasm [20]. Hyperhomocysteinemia is an independent risk factor for atherosclerosis [13]. Moreover, B_{12} deficiency is associated with endothelial dysfunction. Therefore, deficiency is associated with increased carotid intima thickness, increased incidence of stroke, thrombosis and myocardial infarction [19, 21]. Based on these observations, it is supposed that B_{12} has cardioprotective functions. It is interesting that in folic acid deficiency the possibility of false reactions on vitamin B12 deficiency occurs.

Mythylmalonyl-CoA-mutase plays a significant role in degradation of amino acids taking part in formation of succinyl-CoA from methylmalonyl-CoA [15, 22]. Moreover, methylmalonyl-CoA-mutase is involved in purine metabolism and pyrimidine synthesis [23]. The enzyme is located in mitochondria [20]. Even partial decrease in enzyme activity results in accumulation of methylmalonic acid associated with mitochondrial dysfunction [24]. Clinical picture is characterized by methylmalonic aciduria, delayed children development, muscle hypotension, metabolic acidosis, hypoglycemia, nitrogenemia, pancytopenia [23, 25].

Methionine is transformed to S-adenosinmethionine (SAM) under the influence of methionine adenosyltransferase. This universal donor of methyl group is required for stabilization of proteins, DNA, myelin, melatonin, and xenobiotic conjugation [19]. Moreover, cobalt is involved in DNA synthesis through folic acid metabolism. In particular, cobalt takes part is transformation of N_5-methyltetrahydrofolate to tetrahydrofolate that is used as a cofactor of homocysteinmethyltransferase transforming homocysteine to methionine [25-26]. Inhibition of deoxyuridine monophosphate transformation to deoxythymidine monophosphate. The resulting increase in deoxyuridine triphosphophate levels lead to

misincorporation of deoxyuridine triphosphophate into DNA with subsequent fragmentation of the latter [12]. As a result, methylcobalamine deficiency is associated with megaloblast anemia development, whereas isolated AdoCbl deficiency does not result in its development. However, methylmalonic acid stimulates formation of bone marrow cells and its deficiency results in pancytopenia [25]. Therefore, cobalt deficiency affects both DNA methylation, synthesis and reparation [27-29].

B_{12} deficiency is accompanied by impaired erythrocyte and myelin formation, results in growth development, decrease in osteocalcin levels, neurological and psychical disorders [30]. Scholarly data indicate that the incidence of B_{12} deficiency rates to 45% in children. We have found that low cobalt level in hair is associated with low level of physical development in children. Low cobalt levels in hair of children and adults are typical for a majority of Russian population [31]. At the same time, the elderly is the most susceptible group of people where the incidence of B_{12} deficiency may achieve 80% [32]. Another study has demonstrated that only 15% of people older than 65 years were characterized by B_{12} deficiency as assessed by blood vitamin analysis [13, 33]. The leading cause of B_{12} deficiency is its insufficient dietary intake. Therefore, the incidence of B_{12} deficiency is higher in vegetarians, alcoholics, and the elderly consuming "butter-bread-tea" diet. Typical symptoms of B_{12} deficiency include weight loss, irritability, decreased appetite, glossitis and susceptibility to infections [34]. Clinical manifestations of deficiency may be observed in 2-5 years after the beginning of the deficient state formation [35].

Development of pernicious anemia is associated with the formation of antibodies to parietal cells responsible for dietary B_{12} fixation. As a result of atrophic gastritis associated with hypoacidic state the vitamin absorption is impaired. Moreover, B_{12} deficiency development is observed in gastrointestinal diseases accompanied by

dyspepsia, peptic ulcers, diarrhea (Zollinger-Ellison syndrome, Crohn disease, Whipple disease, etc.) as well as in gastric and intestinal surgery [13, 23]. The iatrogenic causes of B_{12} deficiency may include prolonged administration of H_2-histamine receptors blockers, proton pump blockers, biguanides (metformin), resulting in vitamin stores depletion [13, 36]. Despite the presence of antibodies to parietal cells in 90% of patients with pernicious anemia the specific of this test is rather low and may be observed in atrophic gastrointestinal states without anemia. More specific but less sensitive test (60%) is the estimation of antibodies to Castle intrinsic factor [37].

Vitamin B_{12} deficiency is associated with hematological, neurological and psychical disorders. One of the most studied disease associated with the vitamin deficiency is macrocytic (megaloblast) anemia. B_{12} deficiency is the cause of impaired erythrocyte maturation accompanied by cell lysis and hyperbilirubinemia [38]. Megaloblast anemia is associated with ineffective DNA synthesis in hematopoietic precursor cells. Ultimately, this results in desynchronosis in nucleus and cytoplasm development with the formation of enlarged nucleus. Despite the presence of cytopenia in peripheral blood, bone marrow is characterized by erythroid hyperplasia with the impaired myeloid-to-erythroid germ [12]. Neurological disorders include paresthesia, peripheral neuropathy, corticospinal tract demyelination. Psychical disorders like impaired memory, irritability, depression, dementia, and psychosis may also be associated with vitamin B_{12} deficiency development [13, 24]. The usage of B_{12} was effective in schizophrenic patients [39]. It is known that methylcobalamin is widely used in treatment of autistic spectrum disorders.

B_{12} deficiency development also affects bone mineral density, results in increased fractures, and alters osteocalcin levels. However, the intimate mechanisms are not estimated. It is supposed that B_{12} takes part in taurine synthesis in liver. Consequently, taurine increases growth hormone-dependent synthesis of insulin-like

growth factor 1 (IGF1) ultimately leading to osteoblast activation and improved parameters of bone mineral density [30].

Maternal B_{12} deficiency resulted in hyperbilirubinemia due to elevation of indirect bilirubin and newborn jaundice [38]. Severe maternal B_{12} deficiency (< 200 pmol/l) is associated with impaired nerve fiber myelination, nerve tube defects, and impaired growth and development [40]. Congenital abnormalities, preeclampsia, and increased rate of spontaneous abortions and the number of newborns with low body mass (< 2500 g) may be observed when the level of B_{12} is less than 148 pmol/l [41].

Epithelial manifestations of B_{12} deficiency include glossitis, aphthous stomatitis, hair loss, nail dystrophy, hyperpigmentation, and atopic dermatitis [42]. Hyperpigmentation in B_{12} deficiency is similar to that in Addison's disease and is associated with increased melanin synthesis [37, 43].

Heme oxygenase 1 (HO-1) plays a significant role in oxidative stress protection in diabetes mellitus and myocardial infarction. HO-1 activation is associated with decreased lactate dehydrogenase and creatine kinase activity. Experimental studies have indicated that cobalt protoporphyrin administration improved cardiac function through increased HO-1 production and decreased plasma TNF-α levels preventing apoptosis by alteration of Bcl-2/Bax ratio [44].

Cobalt stabilizes HIF-1 resulting in erythropoietin expression. Clinical efficiency of cobalt chloride was demonstrated in anemia of both renal and extrarenal genesis [45].

Metabolic disturbances associated with excessive cobalt accumulation

Excessive doses of cobalt are toxic to the organism. In particular, cobalt overexposure stimulates apoptosis, results in DNA damage by preventing its reparation [1].

Excessive cobalt occupational exposure or prolonged cobalt chloride treatment are accompanied by allergic rhinitis, lung disease, and, hypothetically, increase lung cancer risk. Moreover, the development of bilateral optic atrophy, retinopathy, hear loss, sensomotory polyneuropathy, and thyroid dysfunction may also be observed [46].

Increased serum cobalt, and especially ionized cobalt, content is observed in patients with artificial joint implants. Consequently, it is supposed that persons with joint implants should be examined for subclinical hypothyreosis and polycythemia especially in the case of high cobalt concentration [47]. However, gender differences in absorption, metabolism and excretion of cobalt should be taken into account. In particular, women are characterized by higher blood content as compared to men; whereas its excretion in females is lower [48]. The use of cobalt-chromium alloys in production of various implants results in corrosion of the surfaces of artificial joints, release of cobalt nanoparticles and its accumulation in the surrounding tissues [49]. Moreover, systemic dissemination of cobalt is realized through the lymphatic and cardiovascular system. Frequently, the manifestation of toxicity occurs in a few years. Three types of complications may be observed: neuro-ophthalmic, cardiotoxic, and thyreotoxic [50]. Nerve system disorders include the development of peripheral neuropathy, impaired vision and hearing, cognitive disorders [50-51]. Cobalt overload also results in cardiomyopathy, and reversible formation of hypothyroidism and polycythemia [52].

Impaired cobalt utilization that is associated with its intracellular accumulation is observed in lysosomal dysfunction. Impaired oxidation and proteolysis in lysosomes frequently occurs in Alzheimer's disease. In an experimental Alzheimer's disease model it has been demonstrated that increased lysosomal Co^{57} content is associated with β-amyloid deposition [53].

Genetic diseases associated with impaired cobalt metabolism

Methylmalonic aciduria and homocysteinuria are associated with MMACHC mutation that manifests with inactivation or decreased activity of B_{12}-dependent enzymes [54]. Genetic disorders associated with impaired methylcobalamin formation are characterized by the development of megaloblastic anemia, leuko- and thrombocytopenia, and neurological disorders [20]. At the same time, impaired adenosylcobalamin production results in methylmalonic academia and metabolic acidosis [20].

Methylmalonic acidemia is a rare autosomal recessive metabolic disorder caused by deficiency of methylmalonyl-CoA-mutase or its cofactor, a B12 derivative. Decreased activity or complete inactivation of the enzyme results in accumulation of toxic amounts of methylmalonic acid in urine and blood [55]. Clinical manifestation include exhaustion, respiratory distress, muscular hypotension, cardiomyopathy, pancreatitis and lethargy. However, the cases of normal pregnancy and birth in women with methylmalonic acidemia have also been observed [55].

Familiar homocysteinuria is associated with methionine synthetase coding gene mutation. The disease is characterized by delayed development and megaloblastic anemia [56]. Moreover, homocysteine accumulation results in increased cardiovascular risk (acute coronary insufficiency, myocardial infarction, stroke) especially in young age [13]. The use of hydroxycobalamin treatment with subsequent administration of folic acid decreased blood homocysteine levels in these patients [56].

Congenital insufficiency of Castle intrinsic factor or transcobalamin II production is a rare genetic disorder resulting in development of megaloblastic anemia and neurological deficiency. Imerslund-Grasbeck syndrome is also related to genetic disorders

associated with impaired B_{12} absorption in the intestine and its transport. Clinical manifestations include development of megaloblastic anemia, albuminuria, impaired growth and development [25].

Laboratory diagnostics of impaired cobalt homeostasis

Diagnostics of B_{12} deficiency should be based not only on estimation of the vitamin levels in blood, but also should include assessment of metabolites like homocysteine and methylmalonic acid. A number of specialists suppose that homocysteine and methylmalonic acid are more sensitive and specific laboratory markers [17-18]. However, different states and diseases (B_6 and folate deficiency, hypovolemia, hypothyroidism, psoriasis, neurodegenerative disorders) and drugs (methotrexate, theophylline, phenytoine, hydrochlorothiazides, L-DOPA) may increase the level of homocysteine [12]. To date the best indicator of cobalamin deficiency is the concentration of its biologically active form, holotranscobalamine [12].

Wide use of implants and the possibility of excessive cobalt accumulation resulted in reestimation of the standards of its concentration in biosubstrates. In particular, a number of clinics have accepted the elevation of serum cobalt content higher than 7-10 μg/l as a criterion of cobalt excess [51]. A number of studies have indicated that the use of cobalt preparations in medicine and its increase up to 300 μg/l was not associated with toxicity. The presence of hypoalbuminemia may increase cobalt toxicity even in low metal concentrations [51]. Therefore, this reference range may be used only for persons with orthoplastics taking into account the symptoms like polycythemia, hypothyroidism, neurological disorders and cardiovascular diseases.

B_{12} deficiency is developed when serum vitamin content is lower than 300 pmol/l [38]. Laboratory criteria of B_{12} deficient megaloblastic anemia include macrocytic anemia with ankylo- and poykilocytosis, decreased reticulocyte count, neutrophil hypersegmentation, leuko- and thrombopenia. Moreover, increased indirect bilirubin and homocysteine levels, as well as lactate dehydrogenase activity [12]. It should be taken into account that low B_{12} levels are observed in multiple myeloma, AIDS, pregnancy, and contraceptive use, that is associated with decrease in cobalamin-binding proteins (transcobalamin I, II) [12].

Hair also can be used for assessment of the organism Co status. The reference values of hair Co content in adult men and women were estimated to be 0.007-0.045 and 0.011-0.085 μg/g [57].

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CHROMIUM

Introduction

In 1950s Mertz and Schwarz have revealed a novel dietary factor. The absence of this factor in animals' diet resulted in increased serum glucose levels. It has been named "glucose-tolerance factor" [1]. However, its structure was unknown. The possible essential role of chromium in the human organism has been first observed in prolonged parenteral nutrition. Such patients were characterized by diabetes-like symptoms, whereas chromium supplementation resulted in improved serum glucose and insulin levels.

The total chromium content in human body is around 6 mg. The highest chromium levels are in kidney, liver, intestine, thyroid gland, bones and cartilages. Optimal daily requirement in chromium is 50–200 μg/day. The dose of chromium that can induce toxic effects in animals is 50 mg (Cr^{3+}) [2]. In humans, ingestion of 1–5 g of "chromate" results in severe acute toxic effects such as gastrointestinal disorders, hemorrhagic diathesis, and convulsions [3].

Despite the essentiality of chromium (III) for human beings (which is discussing now [4-5]) and its use in therapy for improvement of insulin resistance and diabetes complications, the intimate mechanisms of chromium action are poorly understood.

Scientific literature does not report any influence of other substances on chromium absorption in gastrointestinal tract. Zinc in chelating compounds can serve as a chromium synergist [6].

Transport, metabolism and homeostatic regulation of chromium

Chromium transport and metabolism is not completely studied to date. The existing difficulties occur due to differences in chromium forms, its chemical inertness and low tissue concentration. In particular, up to 1980s the estimation of tissue chromium was inaccurate due to sample contamination and the absence of international standards [7].

Dietary chromium is present both in inorganic and organic forms. Chromium absorption in gastrointestinal tract is rather low and amounts for 0.5-2% [8]. Chromium is absorbed as Cr(III) by passive diffusion. In the bloodstream it binds to transferrin and transported to target tissues in the form of Cr-transferrin. Approximately 50% of absorbed chromium is detected in tissues (liver, kidneys, testes, brain) in 30 minutes [9]. Maximal levels of chromium in tissues (liver and kidneys) are observed in 1-2 hours after metal administration [10]. Chromium may transfer through fetoplacental barrier. The ratio between tissue and blood chromium during pregnancy is increased by 70-300%. Increased fetal chromium utilization in pregnancy may result in depleted maternal chromium depots [11].

Chromium is present in the human organism in the form of chromodulin (earlier termed as glucose-tolerance factor or low-molecular-weight chromium-binding substance). Being a signal transductor, chromodulin modulates insulin receptor activity [8]. Chromium is present in the cytosol in the form of apo-chromodulin [12]. Maximal activity of chromodulin is observed in the presence of four chromium atoms. It is supposed that chromium is excreted by kidneys in this form [13]. It is notable that insulin affects chromium excretion. In particular, an experimental study has demonstrated that 50% of absorbed chromium appears in urine in 360 minutes, whereas

nearly 80% of chromium (^{51}Cr) appears in urine in twofold shorter period after insulin treatment [9].

The most probable chromium transporters are transferrin (80 kDa) and chromodulin (1.5 kDa) [14]. However, serum albumin also may serve as chromium transporter [15].

It is estimated that transferrin saturation with iron is nearly 25-30%. This allows transferrin to transport other metals (chromium, manganese, vanadium, aluminium). Experimental study has demonstrated the ability of chromium (III) to decrease transferrin saturation with iron by 13% [16-17]. Equal binding of iron (III) and chromium (III) by apotransferrin was observed in the case of chromium excess when its concentration was 5-fold higher than that of iron [17]. Oppositely, iron overload in hemochromatosis decreases the ability of transferrin to bind chromium [16]. It has been demonstrated that insulin stimulates chromium transport to target tissues (liver, kidneys, etc.) [18]. Moreover, experimental studies have shown that insulin affects iron transport. In particular, insulin activates transferrin receptors and stimulates their translocation to the membrane [19].

It is believed that chromium-transferrin complex transports chromium from bloodstream to insulin-sensitive cells. Further, transferrin molecule is bound to cellular receptors and initiates endocytosis with release of chromium into the cell. Under insulin stimulation the metal is bound to amino acids forming apochromodulin and then chromodulin [20]. Chromodulin contains 4 amino acid residues (Gly, Cys, Glu, Asp) and 4 chromium atoms. A molecule saturated with chromium possesses maximal activity.

Chromodulin stimulates insulin receptor tyrosine kinase activity [21-22]. A molecule is bound to β-subunit of insulin receptor containing active kinase center. Therefore, the primary function of chromodulin is modification of insulin receptors and amplification of insulin signaling [8]. Increased insulin signaling results in urinary chromodulin excretion [23].

Chromium-containing enzymes and clinical signs of its deficiency

Chromium modulates carbohydrate, protein and lipid metabolism. Impaired chromium transport and metabolism is associated with insulin signaling in insulin-dependent tissues.

Diabetes mellitus type 2 is the most widespread metabolic disease both in developed and developing countries. DM2 pathogenesis is based on two mechanisms: formation of insulin resistance (liver, muscles) and relative decrease in insulin production by pancreatic β-cells.

The interaction between chromium and insulin has been investigated in numerous studies starting from 1950s [2]. Insulin is bound to α-subunit of insulin receptor. Subsequently, autophosphorylation of β-subunit results in its transformation [24]. Chromodulin increases β-subunit tyrosin kinase activity and stimulates PI3-kinase and protein kinase B (Akt) resulting in GluT4 translocation to the cellular membrane. This signaling cascade stimulates glucose uptake [25-26]. Experimental studies have shown that chromium reduced tyrosine phosphatase 1B (PTP1B) activity by 21-33% [27]. Moreover, chromium decreased endoplasmic reticulum stress, oxidative stress, proinflammatory cytokine production (TNF-α, IL-6), and glutathione oxidation [24, 28-29]. Chromium increases 5'-AMP-activated protein kinase (AMPK) leading to cellular accumulation of AMP and regulation of energy metabolism [30].

It has been shown that insulin resistance is associated with decreased redox-enzymes activity in muscles and impaired quantity and quality of mitochondria. 5'AMP-activated proteinkinase (AMPK) plays a significant role in blood glucose regulation, gluconeogenesis and energy metabolism [8, 31]. Activation of glucose transporters (GluT2 and GluT4) is observed in skeletal muscle, myocardium and liver in response to increased AMPK levels [32]. Chromium increases the number of insulin receptors, modulates phosphorylation

and dephosphorylation, improves cellular insulin sensitivity. By means of AMPK activation chromium increases glucose uptake in muscles and adipose tissue [32-33].

Increased dietary fat and insulin resistance are associated with elevated production of proinflammatory cytokines, hepatocyte proliferation factors and apoptosis activation. Nuclear factor E2 (Nrf2) and heme oxygenase (HO-1) are activated by oxidative stress and regulate multiple genes involved in detoxification and cellular defense. At the same time, another nuclear factor (NF-kB p65) is involved in inflammatory and regenerative processes. Chromium supplementation (chromium histidinate) decreased blood glucose, NF-kB p65 and increased Nrf2 and HO-1 expression [33-36]. Experimental studies involving animals with diabetes mellitus have demonstrated that 400 μg/kg chromium dinicocysteinate supplementation for 6 weeks significantly lowered fasting blood glucose and glycosylated hemoglobin levels while increasing blood adiponectin and vitamin C concentrations [37]. A direct correlation between fasting glucose levels and hair chromium has been revealed [38]. Experimental studies using ^{51}Cr-labelled transferrin have demonstrated that chromium transport to peripheral tissues and especially to skeletal muscles is increased in diabetes. Moreover, diabetes is associated with increased urinary chromium excretion and decreased serum chromium levels [39-41]. It is also notable that increased excretion was accompanied by increased chromium absorption in diabetic animals [40, 42].

Chromium deficiency is characterized by decreased high-density lipoprotein level leading to increased incidence of cardiovascular diseases [43]. Earlier it has been noted that Cr(III) suppresses cholesterol synthesis [26, 33].

Overconsumption of dietary fats results in metabolic impairments like increased body mass index, elevation of blood glucose and insulin, decreased chromium depots in liver, impaired glucose transport, alteration of cellular signaling. However, these

changes may be reversed by chromium histidinate treatment [35]. Experimental studies have demonstrated that chromium histidinate supplementation is associated with chromium, zinc, selenium and manganese content in tissues (liver, kidneys) of diabetic animals. At the same time, copper levels decreased in response to chromium treatment, whereas iron metabolism was not affected by supplementation [44]. In turn, diet-induced obesity and insulin resistance was associated with a significant decrease in adipose tissue chromium content, being associated with metabolic parameters [45].

Oppositely, the frequency of diabetes mellitus and impaired lipid metabolism is increased in hemochromatosis patients with increased liver iron stores and transferrin saturation with iron [46]. One can explain such fact by concurrence between chromium and iron for transport protein, transferrin [16-17].

Alzheimer disease is also characterized by insulin resistance. Chromium supplementation improves glucose utilization, memory and decreases cognitive impairments [47]. It has been noted that chromium may possess antidepressant activity acting on glutamine and serotonin receptors [48]. Chromium-containing preparations may be used in treatment of atypical depression [49].

Amyloidosis is characterized by deposition of abnormal insoluble fibrils in tissues resulting in structural and functional disturbances. A previous investigation has demonstrated a protective role of chromium (III) in prevention of development and deposition of amyloid in tissues [50].

Metabolic disturbances associated with excessive chromium accumulation

Most frequently chromium excess is associated with Cr^{6+} overexposure. The main sources of Cr(VI) are water and air of the

industrial working area [51]. It is indicated that Cr^{6+} ion and sulfate ion have similar structure that allows chromium to enter into the cell through sulfate channels [52]. The allergic effect of Cr(VI) is well known [20]. Cr^{6+} contamination is accompanied by mutagenic and carcinogenic effects through DNA damage [53]. During Cr^{6+} overexposure from the environment (water, air) the risk of lung and stomach cancer is increased [54-55]. Particularly, the increase in lung cancer risk was observed when Cr^{6+} content in the working area air exceeded 100 $\mu g/m^3$. In response to such metal levels lungs were characterized by irritation, inflammation, excessive cell proliferation and cytotoxicity [56]. Carcinogenic effect was characterized by increased reactive oxygen species production, impaired DNA methylation, apoptosis, and chromosomal aberrations [57].

It is interesting that Cr^{6+} exposure decreases metallothionein and metal-response element binding transcription factor-1 (MTF-1) production that is induced by zinc and cadmium [58]. Such situation may impair intracellular zinc homeostasis, alter detoxification processes and attenuate heavy metal toxicity.

Another important source of chromium in the organism is the use of articular implants. It is supposed that the majority of chromium enters the surrounding tissues from the artificial joint in 1 year after arthroplasty. Increased chromium levels are associated with inflammatory response, altered immune reaction, sensibilization and cellular transformation [59-60]. It has been indicated that hexavalent chromium entering the tissues from the implant possesses higher oxidizing capability and cytotoxicity in comparison to trivalent chromium [61].

Laboratory criteria for impaired chromium metabolism diagnostics

Chromium concentration in blood (both whole blood and serum), hair (nails) and urine is assessed during laboratory estimation

of chromium status. It is notable that chromium excretion with urine is regulated by insulin and may be increased in diabetes mellitus 2 type [42, 46]. Reference values for chromium concentration in the whole blood and serum are 0.5-2.5 μg/l and 0.8-5.1 μg/l, respectively [62]; for hair – 0.009-0.073 μg/g [4]. These values may be corrected due to national dietary patterns and laboratory equipment quality.

After arthroplasty the level of chromium in the organism may be increased. Such a situation resulted in elevation of the upper limit of chromium in the whole blood to 2.56 μg/l [63]. Moreover, arthroplasty also resulted in hair chromium accumulation [64]. The level of chromium in the whole blood of workers occupationally exposed to chromium should not exceed 20 μg/l [65]. Chromium concentration in blood serum/plasma and urine has toxic reference range values: 0.5 μg/l for serum/plasma and <1: μg/g of creatinine for urine [3].

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MOLYBDENUM

Introduction

Molybdenum is an essential trace element being toxic in high doses. Foodstuffs containing high amounts of molybdenum are beans and green vegetables [1]. It is supposed that molybdenum enters the organism in the form of MoO_4^{2-} [2]. Molybdenum deficiency is observed in persons maintained at parenteral nutrition for a long period of time [3]. The recommended level of molybdenum consumption is 75-250 μg/day [4]. At the same time, minimal daily requirement in molybdenum is 25 μg/day [3, 5]. A total of 59-94% of dietary molybdenum is absorbed in gastrointestinal tract depending on the ingested dose [3, 6]. Increased molybdenum consumption results in increased absorption and is decreased at lower molybdenum doses [1, 7]. However, excessive dietary molybdenum intake is associated with increased urinary element excretion [5]. The main molybdenum-containing organs are liver and kidneys. The majority of absorbed molybdenum is excreted with urine, whereas fecal excretion is minimal and accounts to 0.2-0.4% [3].

It is believed that tungsten, lead and sodium act as antagonists of molybdenum and cause its deficiency in the body. Copper sulphate enhances excretion of molybdenum with bile. Ammonium thiomolybdate (a soluble salt of molybdenum) is an antagonist of copper; it impairs its utilization in the body. Copper and iron deficiency increases the content of molybdenum in the body [8].

Molybdenum transport, metabolism and homeostasis regulation

Molybdenum is transported into the cell by Mot1 and Mot2. Mot1 is localized at the cell and endoplasmic reticulum membrane, whereas Mot2 performs molybdenum transport from cellular compartments into the cytoplasm [9]. Inside the cell molybdate forms a bond with tricyclic pterin. The formed molecule is molybdopterin or Moco cofactor. In this form molybdenum performs catalytic function in active site of the enzymes [2]. Moco cofactor biosynthesis is performed in 4 stages [10] (Figure 1).

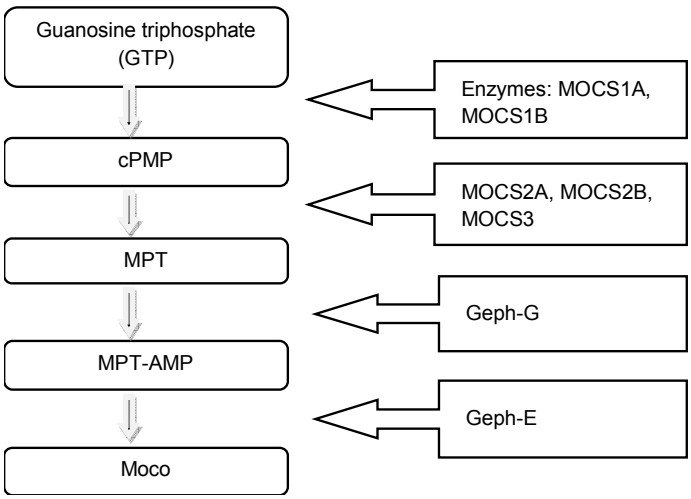

Fig. 1. The scheme of molybdenum-containing cofactor Moco biosynthesis

At the first stage of biosynthesis guanosine-5-triphosphate (GTP) is transformed into cyclic pyranopterin monosphosphate (cPMP). The conversion of these compounds requires two enzymes. One of them, MOCS1A is S-Adenosyl-L-methionine-dependent radical enzyme. MOCS1A contains Fe-S clusters connected to cysteine residues [11]. This family of proteins takes part in different cellular reactions like unusual methylations, isomerization, sulfur

insertion, ring formation, anaerobic oxidation, and protein radical formation [12]. MOCS1B expression is observed in the case of decreased MOCS1A activity [12]. Both enzymes are present in mitochondria. Further, cPMP is transported from mitochondria into the cytoplasm. This transport is performed by ATP-binding cassette transporters (ABC) like ABCB7 [2]. cPMP is later transformed into MPT, molybdopterin or metal-binding tetrahydropyranopterin enedithiolate [13]. The rate of the reaction depends on MOCS2A and MOCS2B activity. At this stage sulfur is used for thiocarboxylate formation [2]. MPT-synthetase sulfurase takes part in repeated sulfuration (MOCS3) [14]. Cysteine desulfurase Nfs1 is used as sulfur donor for MOCS3-dependent resulfuration [15]. At the third stage MPT adenylation with the formation of MPT-AMP formation occurs. Synthesis of this complex is performed in the presence of ATP and Mg^{2+} and Mo-insertase (Gephyrin-G; Geph-G) [16]. At the final stage of synthesis molybdenum is introduced into MPT-AMP molecule in the presence of Mg^{2+} and under the action of Gephyrin-E (Geph-E) [2]. Gephyrin plays a significant role not only in Moco synthesis, but also in neuromediator exchange. In particular, experimental studies using knockout mice have indicated that the absence of gephyrin results in Mo-dependent sulfite oxidase (SO) depression and alteration of nerve impulse transduction (hyperekplexia) [17]. Taking into account a high sensitivity of Moco cofactor to oxidation it is found in complex with protein [18]. This complex is termed Moco-binding protein (MoBP).

Molybdenum-containing enzymes and clinical manifestations of its deficiency

It is known that Mo-containing enzymes take part in nitrogen, carbon and sulfur metabolism. More than 50 Mo-dependent enzymes

are studied to date. However, the most of them are observed in bacteria, fungi, and plants [19-20]. Three of these enzymes are present in the human organism: xanthine dehydrogenase (XDH), sulfite oxidase (SO) and aldehyde oxidase (AO). In these enzymes molybdenum is present in the form of molybdopterin (Moco cofactor) [7]. In human molybdenum deficiency is rather rare and is associated with impaired reproductive functions and growth retardation [21]. Molybdenum deficiency is accompanied by decreased blood and urinary uric acid concentration, increased xanthine and hypoxanthine excretion [3].

SO takes part in conversion of sulfite to sulfate being indicative of its significance in detoxication processes. In humans SO is a mitochondrial enzyme that uses cytochrome C as an electron acceptor. The enzyme protects the organism and especially brain from sulfite toxicity taking part in degradation of sulfur-containing aminoacids and sulfolipids [2, 19].

Xanthine oxidoreductase is presented by two forms: XDH and xanthine oxidase (XO). XDH may be converted into XO as a result of proteolysis and cysteine residue oxidation [22]. XDH is the key enzyme of purine catabolism that utilizes NAD and oxygen. It takes part in transformation of hypoxanthine into xanthine and xanthine into uric acid. Each of two enzymatic subunits bind 2Fe-2S cluster, FAD and Moco [2, 23]. Enzymatic activity is observed in liver, mammary glands, stomach, brain, lungs, kidney and vascular endothelium [24]. It is supposed that the enzyme's activity is 20% higher in men than in women [25]. Despite the fact that the enzyme was discovered more than 100 years ago, its physiological and pathophysiological role is not fully estimated. In particular, in ischemic damage XDH is involved in ROS generation [19]. Low level of uric acid in the organism is associated with impaired antioxidant defense [22]. In ischemic conditions XDH also takes part in NO production that ultimately leads to vascular dilatation [26].

Conversion of XDH into oxidase is associated with generation of significant amounts of H_2O_2 and O^{2-}. Formation of peroxynitrite being a toxic compound decreases NO levels [27].

AO is an enzyme that takes part in conversion of aromatic and aliphatic heterocycles, aldehydes, purines and pterydines [23,28] being similar to XDH in its structure. The highest AO activity is observed in liver and lungs. It is supposed that OA takes part in neurotransmitter exchange and vitamin A metabolism (conversion of retinal into retinoic acid) [29-30]. AO and XDH also play a significant role in alcoholic liver damage [31].

Mitochondrial amidoxime reducing component (MARC) is also related to Mo-containing enzymes. It is supposed that this component takes part in NO formation and detoxication of drugs and xenobiotics [32-33].

A tight association between molybdenum and iron exists. It is known that Fe-S clusters are essential components for Moco synthesis. Moreover, this cluster is a structural component of XDH and AO [2,19]. Experimental studies have indicated that the level of ferritin is decreased in the presence of hypoxanthine and xanthine. Similarly, administration of uric acid lowers blood iron concentration. It is supposed that ferritin-xanthine oxidase system takes part in regulation of iron level and its cellular stores especially in hypoxic conditions [34].

The existing data indicate the role of copper in regulation of molybdenum homeostasis. In particular, antagonism between molybdenum and copper was detected in diabetic patients and its rate was increased after the development of complications [35]. An inverse correlation between serum testosterone, copper and molybdenum levels was observed in men [36]. After excessive dietary molybdenum consumption, the risk of toxicity is increased in copper-deficient persons [6]. Moreover, experimental studies have indicated that molybdenum increases urinary copper excretion [34].

Metabolic disorders of excessive molybdenum accumulation

High amounts of molybdenum are toxic. In regions with high molybdenum content in soil animals were characterized by gout development [37]. Increased XDH activity results in accumulation of uric acid, gout development, and reactive oxygen species-related diseases [22].

Increased XDH activity and hyperuricemia is observed in ischemia, cardiovascular diseases, metabolic syndrome, and diabetes complications [38]. It is supposed that XDH also take part in adipogenesis [39]. Hypoxia, increased IL-1, IL-6, TNF-a, lipopolysacharides and steroid administration results in increased XDH expression [40]. Significant increase of the enzyme's activity is also observed in ischemic conditions, massive surgery, hypertension, atherosclerosis and heart failure [41]. Thus, XDH activity and uric acid level in atherosclerotic plaques is 6-fold higher than in intact vessels [42]. Administration of XDH inhibitor, allopurinol, decreased the risk of endothelial dysfunction in metabolic syndrome and diabetic patients [43-44]. Some investigators suppose that high doses of allopurinol (50 mg/kg) act as an effective antioxidant [45]. Moreover, allopuronol possesses chelating properties in relation to copper, prevents low density lipoproteins oxidation and decreases leukocyte adhesion [46-47].

It is supposed that western-type diet rich in lipids and fructose is associated with uric acid elevation. In turn, XDH-induced hyperuricemia is associated with the development of left ventricle hypertrophy and diastolic dysfunction. The use of allopurinol for 16 weeks was accompanied by decreased enzymatic activity in heart muscle, normalization of uricemia and reduced oxidative stress that is associated with myocardial fibrosis and impaired diastolic relaxation [48]. However, short-term decrease on uric acid levels in

diabetic patients did not result in improvement of endothelial dysfunction and decreased cardiovascular risk [49].

It has been reported that the development of multiple sclerosis is accompanied by oligodendrocyte death and is associated with XDH-mediated ROS generation. In turn, the use of febuxostat, XDH inhibitor, slows down the process of demyelinization and axonal damage [45].

A clinical case of molybdenum intoxication after the administration of molybdenum-containing drug for thirty years was reported. However, afterwards the patient has increased the daily dose to 300-800 μg/day. Finally, at the 18th day of high-dose molybdenum consumption acute psychosis with visual hallucinations and seizures developed. In a year after termination of administration toxic encephalopathy, cognitive disorders and depression were diagnosed [50].

Genetic diseases associated with molybdenum dyshomeostasis

Moco cofactor deficiency is an autosomal recessive genetic disorder with the incidence of 1:100000 [10]. The most frequent disorder of its synthesis is associated with four genes coding MOCS1, 2, 3 and gephyrin [10, 51-52]. At the same time, more than 2/3 of cases are associated with impaired 1st stage of Moco and MOCS1 formation [53]. Altered Moco synthesis is associated with the loss of Mo-dependent enzymes activity and especially sulfite oxidase [10]. This disorder is associated with toxin accumulation in the organism and brain in particular resulting in neurological and dysmorphic disorders, seizures [2]. It has been also reported that cPMP administration in children resulted in disappearance of seizures and the levels of sulfite oxidase and XDH in urine were

nearly normal [54]. Analysis of polymorphism of genes responsible for XDH synthesis has indicated frequent disorders, however, the majority of them are asymptomatic [22].

Isolated SO deficiency is a rare genetic disease that is lethal in early childhood. Accumulation of sulfite and/or decreased sulfate results in toxic brain damage, impaired formation of sphingolipids, a main component of myelin [55]. In particular, neurons of a child who died from genetic deficiency of Moco deficiency were characterized by abnormally high sulfur and magnesium content. Impaired processes of excretion resulted in toxic neuronal damage and death [56].

Isolated XDH deficiency (type 1 and 2 xanthinuria) is an autosomal recessive genetic disorder. This deficiency results in tissue hypoxanthine accumulation [57]. Clinical manifestations include the development of urolithiasis and inflammatory diseases of genitourinary tract [58]. The level of uric acid in these patients is rather low (< 1 mg/dl) [59]. The development of type 1 xanthinuria is associated with impaired conversion of hypoxanthin into xanthine, whereas type 2 is characterized by genetic deficiency of molybdopterin synthesis. The performance of loading test with allopurinol in type 1 xanthinuria patients demonstrated increased oxypurinol concentrations in serum and urine. At the same time, oxypurinol is not detected in type 2 xanthinuria [60].

Gephyrin is a multifunctional protein. Along with participation in Moco synthesis it takes part in regulation of interaction of certain neurotransmitters (GABA and glycine) with their receptors [61]. Therefore, gephyrin is involved in regulation of synaptic transmission. Genetic disorders associated with gephyrin synthesis result in impaired CNS functions [10, 53, 61].

Laboratory diagnosis of impaired molybdenum homeostasis

Serum molybdenum concentration reflects its dietary intake. Isotope investigation of healthy males has demonstrated that that maintenance on molybdenum deficient diet for 24 and 120 days resulted in more than 20% decrease in blood levels [62].

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AFTERWORDS

Essential trace elements as "blocks of life" or bioelements: from fundamental basis to novel paradigm of diagnostics and treatment of trace element related diseases

It is known that the chemical element exists in the organism not by itself, but in close interaction with other compounds. There are no any particular elements in cell, which are typical of only living nature. On the atomic level, there are no differences between chemical composition of organic and inorganic matter. The differences are found on higher, molecular level of organization.

Thus, the position and classification of the chemical elements in the Periodic System of Elements (PSE) does not permit any statement to be made about their functional essentiality or their acute or chronic toxicity for living organisms. This is due to the fact that the Periodic System is based on purely physicochemical aspects [1]. Therefore B. Markert developed an idea about a Biological System of Elements (BSE), which primarily considers aspects of basic biochemical and physiological research. As the author said, "Biological processes on the molecular level are frequently based on physical and chemical conditions… However, these physical and chemical regularities are frequently modified in biological systems". The BSE of B. Markert is based on data on correlation analysis, physiological function of individual elements in the living organism, evolutional development out of the inorganic environment and with respect to their uptake by the living organism as a neutral molecule or charged ion.

Despite the biological role of chemical elements is intensively studied for the last decades, the “lack of multidisciplinary approach has been the Achilles heel of biological trace element research” [2]. The desire to integrate the "organic" and "inorganic" approach in studying the biological role of chemical elements is observed in a number of fundamental works. Since 2003 we put forward and develop the concept of bioelements and bioelementology as an integrative scientific direction [3-7]. From our point of view, bioelement is the elemental functioning unit of living matter, which acts as a biologically active complex of chemical elements like atoms, ions and nanoparticles with organic compounds of exogenous (primary) or biogenous (secondary) origin. Bioelements include any chemical structures found in living nature, but which do not have a set of fundamental properties of living things: metabolism, variability, reproduction and heredity. The complex of bioelements can be called “bioelementome”. As presented in Table 1, we propose to subdivide bioelements into simple (atoms, ions, among them structural elements C, H, N, O, P, S, Si, Ca, electrolytic K, Na, Ca, Cl, Mg, enzymatic Mg, Fe, Zn, Cu, Mn, Co, Cr, Mo, Se, Sn, F, I, Ni, V, B, and water as the universal solvent), and complex ones, consisting of the above-mentioned 68 molecules (8 of them are nucleosides, including DNA and RNA, 20 are essential amino acids necessary for protein synthesis, at least 32 glycans, 8 types of lipids) [8]. Bioelements can be also subdivided into primary, i.e. those existing before the origin of life, and secondary, i.e. those which have formed as production of living organisms [1].

Atoms, atomic nuclei, elementary particles and fields that bind them, which have independent significance at the physicochemical stage of evolution, after being included in biological molecules lose this self-importance and play their role in the ensemble, called by us bioelement [4-5], where everything is interdependent, more complicated and at the same time more vulnerable to external influence.

Table 1

Classification of bioelements [5]

Primary	Simple	C, H, N, O, P, S, Si, Ca (structural)
		K, Na, Ca, Cl, Mg (electrolytic)
		Mg, Fe, Zn, Cu, Mn, Co, Cr, Mo, Se*, Sn*, F*, I*, Ni*, V*, B** (enzymatic)
		H2O, O2, N2 etc.
	Complex	Nucleic acids (deoxyadenosine, deoxycytidine, deoxyguanosine, deoxythymidine, adenosine, cytidine, guanosine, uridine)
		Glycans (Fucose, galactose, glucose, glucuronic acid, mannose, N-acetylgalactosamine, N-acetylglucosamine, neuraminic acid, xylose, nononic acid, octulosonic acid, arabinose, arabinofuranose, colitose, fructose, galactofuranose, galacturonic acid, glucolactilic acid, heptose, legionaminic acid, mannuronic acid, N-acetylfucosamine, N-acetylgalacturonic acid, N-acetylmannosamine, N-acetylmannosaminuronic acid, N-acetylmuramic acid, N-acetylperosamine, N-acetylquinovosamine, perosamine, pseudaminic acid, rhamnose, talose)
		Proteins (Alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine)
		Lipids (Fatty acyls, glycerolipids, glycerophospholipids, polyketides, prenol lipids, saccharolipids, sphingolipids, sterol lipids)
Secondary	Complex (components of bioelemental systems, “omes”)	Metabolome (components)
		Metallome
		Lipidome
		Proteome
		Genome
		Transcriptome
		(…?)

Generally, bioelements include any chemical structures found in living nature, but which do not have a set of fundamental

properties of living things: metabolism, variability, reproduction and heredity.

Bioelement is not a chemical element inside a molecular compound, but it is temporarily formed biocomplex, where the chemical element is bound by covalent (chelate) bond to the organic molecule. They should not be considered separately, because, interacting, together they produce biological effect of new quality [9].

Bioelements including essential trace elements (enzymatic bioelements) can continuously form from ionic compounds when they enter the cell. Inside the cell, biopolymers and their complexes create a complicated, coordinated and regulated system for transformation of substances. Cell is the main place of natural birth of secondary bioelements and their destruction. Biosphere is an assembly of bioelements and living organisms existing under permanent regulatory influence of physico-chemical factors of terrestrial and cosmic origin [4, 5]. The scientific discipline, which study bioelements, is proposed to be called bioelementology [5]. This discipline could lay the foundation for the integration of bioorganic chemistry, bioinorganic chemistry, biophysics, molecular biology and other parts of life sciences [5, 9].

According to the modern view, the life processes cannot occur outside the cell. Therefore, the cell is considered as the smallest quantum of life, which, for managing its internal parameters and performing cell-cell interactions, use information, energy and substances, including bioelements, obtaining them from the environment. Bioelement is still a substance, whereas a cell (organism) is already a being [4, 5].

In our opinion (Figure 1), bioelements are precursors of the living matter, a successful combination of which, particularly of polymer-ion reactions running autocatalytically, led to the formation of cells.

In the recent years, along with the evolution of our knowledge and understanding of bioelements, the definitions of bioelementology evolved [3-4, 11]. Currently, we suppose that the most appropriate

definition of bioelementology is the following: "Bioelementology a fundamental discipline studying the transition state of the matter (evolution from biologically inert to living), formation and change of bioelements, which are vital or conditionally essential for the living matter, under the influence of various physical interactions and matrix effect of water."

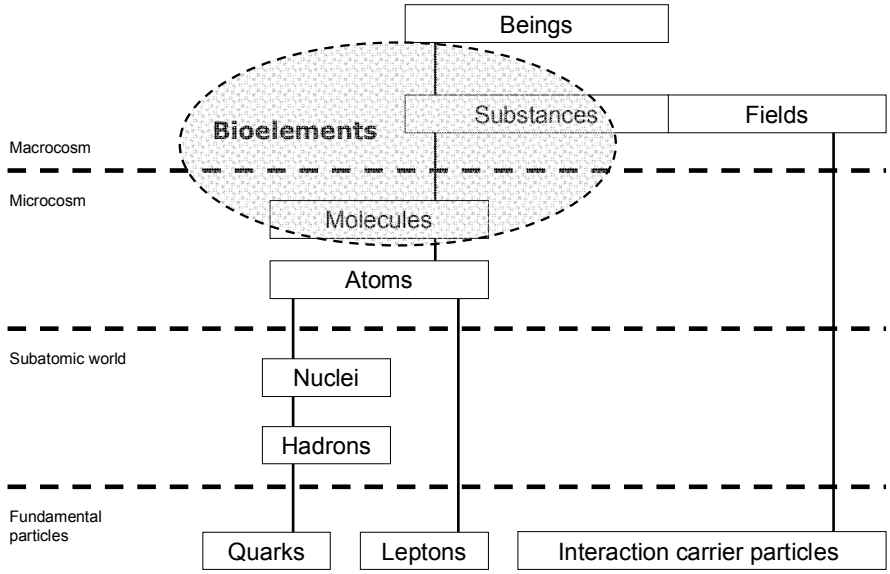

Fig. 1. Structural levels of the matter (by Yu. N. Orlov [10], expanded by A.V.Skalny [5])

Our opinion is that the bioelementology combines the systemic and integrative approaches in life science and is a possible precursor to systemic biology [5].

Essential trace elements as simple primary bioelements

The so-called "simple bioelements" produced four fundamental components of cellular life, which, according to J. D. Marth [8],

divided into 68 molecular building blocks ("building blocks of life"). I.e., the simplest bioelements formed more complex, macromolecular bioelements.

Thus, bioelements can be subdivided into primary, i.e. those existing before the origin of life, and secondary, i.e. those which have formed as production of living organisms. This division is necessary for a better understanding of the nature and role of bioelements. For example, the fact that life is a self-sustaining process that can produce "raw material" for new living structures. This agrees with the theory of natural self-organization of pre-biological processes by M. Eigen [12] and ideas of I. Prigogine [13] about self-organization in open systems.

Novel paradigm of the nutrition and pharmacology

It should be noted that a set of bioelements is a necessary but not sufficient condition for the formation of life. In many cases in medicine, in our opinion, it is possible to use bioelements for maintaining organs and tissues instead of using cell cultures and tissues, because it is not always necessary or possible (including financial reasons) to recover the function by a substance, organ or tissue, completely identical to the living one (e.g., in transplantology, orthopedics, in treatment of osteoporosis, diseases of skin, hair, etc.).

The development of bioelementology may lead to appearance of modified cells or technologies for creation of new cells which can be used for medical purposes. Without going into details, we only note that this tale may sooner become a reality with the correct formulation of tasks, based on the correct understanding of the hierarchy of “pre-living” processes and of the life itself, on the formation of new methodological approaches on its basis, on the proper division of essential substances in necessary and sufficient,

primary and secondary, with a better understanding of the boundary between "pre-living" and "living", between the set of bioelements and life.

It is reasonable that the deficiency of only one primary bioelement can result in the extinction of certain living organisms on the Earth. According to the US Department of Energy (2010), in the nearest future (~50 years) chemical (elemental) diversity may be sacrificed (Table 2). The lack of rare earths can stop the "Clean green future", but deficiency of only one trace element (cobalt) can have the dramatic effect on a lot of species, for which this element and the vitamin B_{12} are essential. Also, the lack of lithium as a pharmacologically active and conditionally essential trace element can cause a lot of problems in CNS, immunity and other very important human functions [14].

Table 2

Risk of supply disruption by 2015 (US Department of Energy, 2010)

Level	Elements
Critical	Y, Nd, Eu, Tb, Dy, In
Near-critical	La, Ce, Te
Non-critical (but not far future)	Co, Li, Ga

As it is shown in Figure 2, for the effective prevention and treatment of human disease it is essential to control and correct the initial imbalances of bioelements – essential trace elements, caused by suboptimal nutrition, ecological influence, stress etc. We can do this for relatively healthy people to prevent their disease. If we miss the possibility of the correction of human health by bioelements in stages I-II, we need to start the treatment but also with the restoration of basic defects of metabolism (stable deficiency of bioelements) [15].

So, there are few directions to serve and develop the "life set" of bioelements. As demonstrated in Figure 3, the mankind can use medical and pharmacological therapy for individual and populational

provision by bioelements (primary and secondary). This is the most expensive way, but realistic for rich countries, which needs to develop the technological diagnostics ("omics"), personalized healthy nutrition and treatment.

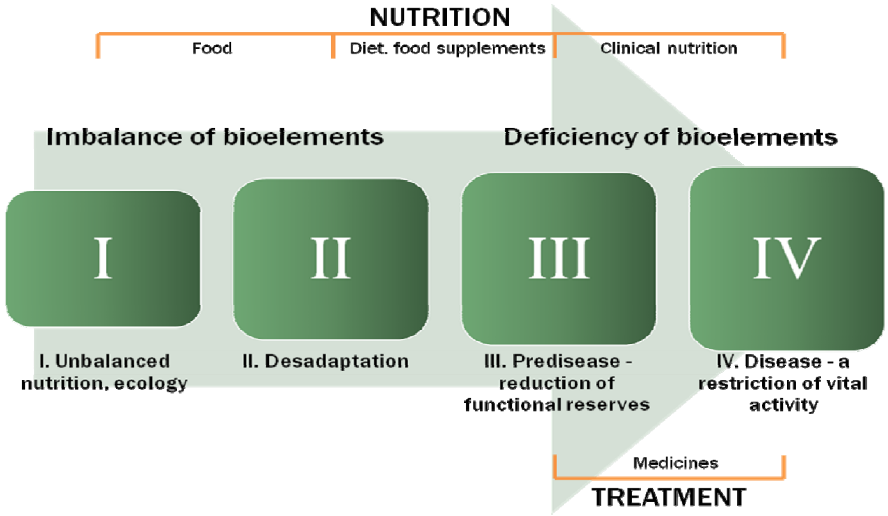

Fig. 2. Levels of bioelement imbalances and their correction

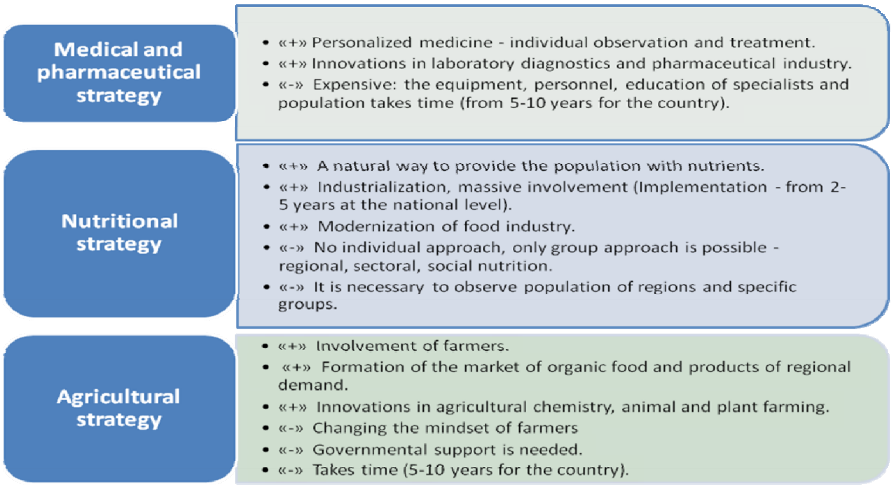

Fig. 3. Approaches to solving the problem of bioelements provision for human health [15]

The second direction is more natural – improvement of nutrition on the basis of fortified foods, "high food density" products. It is appreciable for almost all countries and layers of population, if the governments and international organizations (FAO, IMF, UNIDO etc.) will support the development of food industry and support authorities to manage the social nutrition, for example. The last strategy is most natural, but needs a lot of efforts in agricultural sector of industry and science. More detailed description of the strategies is presented in our review [15].

1. Medical and pharmaceutical strategy

• Development of personalized medicine and medical geography, epidemiology and ecology

• Improving methods of laboratory and clinical diagnostics of micronutrient deficiencies and intoxications

• Creation (reconstruction and development) of resource base for food and pharmaceutical industry

• Creation and production of adapted agents for nutraceutical and pharmaceutical correction of deficiency/excess of micronutrients (based on physiological, regional and professional requirements)

• Creation and production of new drugs for treatment of genetic and other rare diseases related to metabolic disorders of micronutrient exchange

2. Nutritional strategy

• Fortification (enrichment) of food with micronutrients at the production stage - focused and strictly bound to the elemental status of the population (massive involvement)

• Choice among existing products the required (enriched) ones to complete rations

• Exchange of products between the regions

• Dietary supplements - an individual approach - 10% of the population, in developed countries - up to 80%

• Functional foods

• Forming assortment of food products - information policy

• Creation of functional beverages, artificial water mineralization

• Exchange of products between regions according to regional needs in micronutrient

• Improving the quality of tap water

• Social nutrition (water consumption) on the basis of physiological and professional groups of the population

Food fortification as a long-term regional program "Development of a regional system for detection and prevention of natural and technogenic deficiencies and surpluses of macro and trace elements in the population"

Project

• Formation of scientific and methodological basis for monitoring of elemental status of the population and correction of deviations

Regional program for monitoring elemental status of the population

• Determination of deficiencies and surpluses of macro and trace elements

• Development of recommendations for correction of deviations

Formation of regional regulations based on governmental policy in the field of healthy nutrition

• Increasing the proportion of production for mass consumption, rich in vitamins and minerals, including mass varieties of bakery products, and dairy products - up to 40% of total production

Consolidated ordering of fortification ingredients for the regional food producers

• Formation of joint supply of fortification ingredients for the regional producers of products for social and mass feeding in accordance with results of the monitoring

Sectoral nutrition

• Scientific and methodological support of diets for different groups of people in the region: pupils, students, workers etc.

• Regional standards for food products

• System of targeted subsidies for fortification (enrichment of food with micronutrients)

3. Agricultural strategy

• Agrochemical service: targeted introduction of microfertilizers

• Variety zonation

• Breeding

• Specialized mixed feeds, premixes for animals, mineralized water

• Development of industry: fertilizers, animal feed, etc.

In conclusion, the progress in trace element research and application in clinical medicine will occur due to the interdisciplinary approach such as bioelementology. It requires changing the educational programs for high school students of biological, chemical and physical specialties, creation of special programs for biotechnologists, medical researchers, ecologists and pharmacists. And this will demand united efforts of scientists and specialists from adjacent fields. Integration of scientific researches without division into separate parts, studied by only one of the "omics", though this will demand deeper and more global planning of scientific investigations on the basis of the multidisciplinary concept. Also, it is necessary to change the paradigm of diseases' prevention and treatment from mainly symptomatic to basics to prevent and normalize the balance of bioelements by nutrition and use of sources of bioelements in pharmacotherapy.

The development of bioelementological approach can open the new perspectives in creation of integrative diagnostics of the health status and provision of the humans by "blocks of life" including essential trace elements as simple primary bioelements, and provide the real personalized nutrition and therapy of diseases at etiological niveau – imbalance of bioelements.

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CONTENT

Monograph

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ESSENTIAL TRACE ELEMENTS IN HUMAN HEALTH: A PHYSICIAN'S VIEW

Published in the author's edition

The original layout A.I. Lelour
Cover design by L.D. Krivtsova

Signed for printing on 13.03.2018. Format 70 × $100^{1}/_{16}$.
Paper for office equipment. Headset Times.
Usl. printer. l. 18,5.
Printed 250 copies. Order No. 3014.

Printed on equipment
Publishing House of Tomsk State University
634050, Tomsk, Lenin Ave., 36
Tel. 8+ (382-2)-52-98-49
Website: http://publish.tsu.ru
E-mail: rio.tsu@mail.ru

ISBN 978-5-94621-683-8