Peer-reviewed version available at Nutrients 2018, 10, 1591; doi:10.3390/nu10111591

Review

Copper metabolism of newborns is adapted to milk ceruloplasmin as a nutritive source of copper

Ludmila V. Puchkova[1,2,3*], Polina S. Babich[4], Yulia A. Zatulovskaia[3#], Ekaterina Y. Ilyechova[1], Francesca Di Sole[5]

[1] Laboratory of Trace elements metabolism, ITMO University, Kronverksky av., 49, St.-Petersburg, 197101, Russia; puchkovalv@yandex.ru (L.V.P.); ikaterina2705@yandex.ru (E.Y.I.)
[2] Department of Molecular Genetics, Research Institute of Experimental Medicine, Acad. Pavlov str., 12, 197376 St.-Petersburg, Russia; puchkovalv@yandex.ru (L.V.P.)
[3] Department of Biophysics, Peter the Great St. Petersburg Polytechnic University, Politekhnicheskaya str., 29, St.-Petersburg, 195251 Russia; puchkovalv@yandex.ru (L.V.P.); yuliaz@stanford.edu (Y.A.Z.)
[4] Department of Zoology, Herzen State Pedagogical University of Russia, Kazanskaya str., 6, St.-Petersburg, 191186, Russia; babich.polina@gmail.com (P.S.B.)
[5] Physiology and Pharmacology Department, Des Moines University, Des Moines, IA USA; francesca.disole@dmu.edu (F.D.S)
[#] Present address: Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305, USA. E-mail: yuliaz@stanford.edu (Y.A.Z.)
[*] Correspondence: puchkovalv@yandex.ru; Tel.: +7-921-881-8470

Abstract: Copper, which can potentially be a highly toxic agent, is an essential nutrient due to its role as a co-factor for cuproenzymes and participation in signaling pathways. In mammals, the liver is a central organ that controls copper turnover throughout the body: copper absorption, distribution, and excretion. In ontogenesis, there are two types of copper metabolism: embryonic and adult, which maintain the balance of copper in each of these periods, respectively. In the liver cells, these types are characterized by specific expression patterns and activity levels of the genes encoding ceruloplasmin, which is the main extracellular ferroxidase and copper transporter and proteins mediating ceruloplasmin metalation. In newborns, the molecular-genetic mechanisms responsible for copper homeostasis and the ontogenetic switch from embryonic to adult copper metabolism are highly adapted to milk ceruloplasmin as a dietary source of copper. In the mammary gland cells, the level of ceruloplasmin gene expression and the alternative splicing of its pre-mRNA govern the amount of ceruloplasmin in milk, and thus, the amount of copper absorbed by the newborn is controlled. In the newborns, absorption, distribution, and accumulation copper are adapted to milk ceruloplasmin. In the newborns, which are not breast-fed at the early stages of postnatal development, the control for alimentary copper balance is absent. We tried to focus on the neonatal consequences of a violation of the balance of copper in the mother / newborn system. Although there is still much to be learned, the time to pay attention to this problem came because the neonatal misbalance of copper may provoke the development of copper related disorders for future life.

Keywords: embryonic type copper metabolism; milk ceruloplasmin; baby formula

1. Introduction

According to the modern scientific concept of nutrition, the suboptimal content of micronutrients in a mother's diet during pregnancy and lactation may be a cause of developmental defects in newborns. This concept is fully supported by data showing that severe neonatal defects can be prevented by correction of a mother's diet, e.g. iodine, folate, and iron supplementation [1-8].

Less clear effects of deficiency/excess of copper, one of the most important micronutrients, in fetus/neonate have been report [9]. In mammals, about two dozen enzymes, which control the basic cellular processes: respiration, antioxidant defense, formation of connective tissue, neurotransmitter synthesis, neuropeptide processing, iron transport and others, require copper as a co-factor for their activity [10,11]. Moreover, copper operates as a secondary messenger in some signaling ways [12]. In adult mammals, copper deficiency or excess of copper from dietary factors are rarely observed. However, it can be developed from surgical removal of the small intestine, genetic disease, diseases which alter micronutrient metabolism [13,14]. The inherited defects in copper transport cause the development of both copper deficiency and accumulation simultaneously because the loss of copper from transporting proteins results in copper accumulation in inappropriate cellular compartments and the deficiency of bioavailable copper. As a result, copper dyshomeostasis is developed [15]. The liver and the brain, the organs with the most intensive copper metabolism, are affected first by the defects of copper transport. In these organs, signs of toxic copper accumulation and cuproenzyme deficiency manifest earlier compared to other organs.

In newborns, the mechanisms for excreting copper through bile and controlling copper absorption in the small intestine do not operate [16]. Therefore, dietary factors may cause copper misbalance in the early stages of postnatal development. Copper deficiency in the food of pregnant and nursing females caused a lack of cuproenzyme activity, multiple developmental aberrations, teratoma formation, and death among fetuses or offspring during the early postnatal stages [17,18]. In babies, fed cow's milk that has much lower concentration of copper than human milk, copper-dependent anemia almost inevitably develops [19]. On the other hand, there are two inherited diseases (childhood copper-associated cirrhosis [20] and Indian childhood cirrhosis [21]), in which even a small excess of copper in the food is fatal for infants [22].

The aim of the present review is to emphasize the dependence of copper homeostasis in newborns on food sources and pay attention to possible long-term consequences of copper imbalances in neonates.

2. Biological roles of copper

Copper is an essential component of the cells of almost all modern organisms. It was involved in metabolic processes in the early stages of Earth's evolution, possibly at the beginning of the Great Oxygenation Event when it became bioavailable due to the oxidation of insoluble sulfides. In aqueous media, copper ions have two stable oxidation states Cu(I)↔Cu(II); and both forms can have high toxicity [23]. Because copper ions have strong coordination properties, they can readily form coordination compounds with ligands that contain carboxyl, amine, pyrrole (imidazole, indole), pyridine, hydroxyl, nitrile, and, especially, thiol donor groups. Depending on the chemical nature of ligands and the geometry of the coordination sphere, copper displays a wide range of redox potentials. A combination of these properties makes copper a valuable cofactor for enzymes catalyzing redox reactions. Cuproenzymes catalyze different redox reactions in which copper is cycled in Cu(I)↔Cu(II) states and serve both as an electron donor and an electron acceptor depending on the stage of the catalytic cycle. The most abundant enzymes are the cuproenzymes that catalyze electron transfer to dioxygen. In active centers of cuproenzymes, copper is typically tightly bound by 4, 5 or 6 ligand groups and cannot be easily released or removed from the enzyme under physiological conditions [23]. The largest fraction of copper, which is present in mammals, is contained in the active centers of cuproenzymes and does not possess toxic properties. The physiological role of major mammalian cuproenzymes, their structure, function, localization in the cell and in the organism, and their gene expression has been actively studied for many decades. The established data on these cuproenzymes are briefly summarized in Table 1.

93 **Table 1.** The main mammalian cuproenzymes.

Cuproenzyme	Localization (the main place)	The main functions
SOD1 (Cu(II)/Zn(II)-superoxide dismutase)	Cytosol, nuclear matrix, lysosomes, peroxisomes, mitochondria	Disproportionation of superoxide anions to oxygen and hydrogen peroxide [24]
SOD3 (Cu(II)/Zn(II)-superoxide dismutase)	Extracellular liquids (blood serum, lymph, sclera, etc.)	Antioxidant functions, signaling, stimulation of cell proliferation, decrease of apoptosis and inflammation [24]
COX (cytochrome-c-oxidase)	Mitochondrial inner membrane	Transfer of electrons from the respiration chain to molecular oxygen [25]
Protein-lysine 6-oxidase (lysyl oxidase)	Extracellular matrix	Oxidation of lysine residues to aldehydes in collagen and elastin precursors [26]
PAM (peptidylglycine α-hydroxylating monooxygenase)	Vesicles, membrane-bound and soluble forms	Pro-neuropeptide processing by converting to the corresponding amide [27]
DBH (dopamine-β-hydroxylase)	Vesicles, membrane-bound and soluble forms	Conversion of dihydroxyphenylalanine (DOPA) to noradrenaline [28,29]
Tyrosinase (phenol oxidase)	Melanosomes	Synthesis of melanin from tyrosine [30]
Soluble ceruloplasmin (Cp)	Blood serum, milk, cerebrospinal fluid, and other extracellular liquids	Oxidation of Fe(II) to Fe(III), oxidation of aromatic amines, copper transporter [31,32]
GPI-Cp[1] (splice isoform Cp)	Plasma membrane, brain	Oxidation of Fe(II) to Fe(III) [33]
Hephaestin	Plasma membrane, enterocytes	Oxidation of Fe(II) to Fe(III) [34]
Zyklopen	Plasma membranes, placenta	Oxidation of Fe(II) to Fe(III) [35]

94 [1] - glycosylphosphatidylinositol anchor.

95 The native tertiary structure of cuproenzymes is formed during successful metabolic insertion
96 of copper ion(s). The removal of copper from holoenzymes in vitro, or defects in the processes of
97 metalation of apo-enzymes in vivo can cause disruption of the tertiary structure and loss of catalytic
98 functions [36]. Therefore, copper is considered both as a catalytic and structural cofactor of
99 cuproenzymes.

100 In the past few years, evidence has been accumulated for the existence of regulatory role of
101 copper, and this role remained elusive for a long time although the first evidence for the involvement
102 of copper in the regulation of endothelial cell growth was obtained almost 40 years ago [37]. Recently,
103 it was shown that intracellular and local extracellular changes in copper concentration influence the
104 activity of transcription factors NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells
105 [38], and HIF1, hypoxia-inducible factor 1 [39]. These factors regulate the expression of several dozens
106 of genes, including genes, whose products take part in the reprogramming of energy metabolism in
107 tumor cells. XIAP protein (X-linked inhibitor of apoptosis protein), which is a common member of
108 several signaling pathways, can bind copper. This binding leads to the release of caspase 3, which
109 triggers XIAP-mediated apoptosis [40,41]. Copper also takes part in the Ras/MAP-kinase [42] and
110 BRAF-dependent [43] signaling pathways, modulates the function of growth factor receptors [44,45],
111 γ-aminobutyric acid [46] and glutamate receptors [47], regulates cyclic-AMP-dependent lipolysis
112 [48], and induces Golgi-complex independent secretion of interleukins and cytokines [49]. It has been
113 shown that copper controls organogenesis and cell differentiation in embryo development [50].

Two prerequisites are implied from the existence of copper regulatory functions: a pool, in which copper is accumulated and from which it is liberated on demand and copper-regulated sensors should exist. In the cell, two copper pools that may take part in rapid local change of copper concentrations exist (Table 2).

Table 2. Mammalian proteins and substances for controlling copper homeodynamics or participating in copper-dependent signaling.

Protein/substance	Localization	Function
Metallothionein (a large set of isoforms)	Cytosol, mitochondrial matrix, nucleoplasm, blood serum	Detoxification of heavy metals, maintenance of copper and zinc balance, control of apoptosis, cell protection from death and neoplasia [51,52]
COMMD1 (Copper Metabolism gene MURR Domain 1; previously named MURR1)	Cytosol, nucleoplasm	Excretion of Cu(II) through bile, stabilization of the ATP7B[1] structure, participation in copper-dependent signaling with NF-κB [53]
XIAP (X-linked inhibitor of apoptosis protein)	Cytosol	The inhibitor of caspase 3, ubiquitin-ligase related to COMMD1, and a copper level regulator in the cell [40]
SCO1/SCO2 (assembly of cytochrome c oxidase 1/2)	Inner mitochondrial membrane	Incorporation of copper ions into COX[2], assembly of the COX complex, control of copper balance in the cells [54,55]
SCC (small copper carrier)	Circulation, urine	Removal of copper from the liver to the bloodstream [56]
Copper ligand (CuL)	Cytosol, mitochondrial matrix	Transfer of copper between the mitochondrial matrix, mitochondrial intermembrane space and cytosol [57-58]
LOXL (1-4) (lysyl-oxidase-like proteins) transcription factors	Cytosol, nucleus	Copper-dependent suppressors and activators of tumor growth and metastasis [59,60]
Sp1 (specificity protein 1)	Cytosol, nucleus	Multifunctional transcription factor; regulator of CTR1 gene activity [61,62]
MAC1* (Copper-sensing transcription factor)	Cytosol, nucleus	Transcription factor involved in regulation of CTR1 gene [63]
ACE1* (transcription factor)	Cytosol, nucleus	Regulation of expression of the metallothionein gene [63]

1 – copper transporting ATPase, 2 - cytochrome c oxidase, *- not found in mammals.

The first one is the system that includes metallothionein-Cu(I), glutathione (Cu(I)/Cu(II)) and COMMD1 (Cu(II)), which possibly controls the changes of copper concentration in cytosol [64]. The members of this system can bind copper, change its oxidation state and share it on demand with cuproenzyme metalation, signaling pathways and excretion routes. The second pool is associated with mitochondria, which play an important role in copper homeodynamics and may be viewed as an intracellular copper depot [65]. It has been shown that in yeast and mammals, mitochondria accumulate copper from cytosol and release it back with the help of copper ligand, which is a low molecular weight substance (~1 kDa) whose structure has not been identified yet. Therefore, there are means for rapid changes in the copper concentration in the cytosol. The existence of a kinetically labile copper pool, which is predominantly localized in the mitochondria and the Golgi apparatus were shown by synchrotron X-ray fluorescence microscopy [66]. The local changes in the extracellular

space near the cell membrane may occur when copper is secreted in complexes with interleukins, cytokines, growth factors and metallothioneins under stress conditions. Copper-regulated sensors have been characterized in yeast, insects and mammals (Table 2). In mammals, they include lysyl-oxidase-like proteins (LOXL1-4), and an imbalance in LOXL levels promotes tumor and metastasis growth. Another example is Sp1 (specificity protein 1), which is a transcription factor regulating the activity of the CTR1 (copper transporter 1) gene (see Table 3). Studies of copper's regulatory role are relatively novel, and to date, there are insufficient data to examine the consequences of impaired copper-dependent of the gene's regulation.

Table 3. Mammalian copper transporting proteins involved in intracellular copper routes.

Protein	Localization	Function
CTR1 (high affinity copper importer 1)	Plasma membrane homotrimeric integral protein, universal copper importer	Transfer of Cu(I) from extracellular space to cytosol (ATP-independent); Cu^{1+}/K^{1+}-exchanger; control of morphogenesis [69]; stabilization of the CTR2 structure [70]
CCS (Cu(I)-chaperon for SOD1)	Cytosol	Transportation of Cu(I) from CTR1 to apo-SOD1 [76]
COX17 (Cu(I)-chaperon for COX)	Mitochondrial intermembrane space	Transportation of Cu(I) from CTR1 to SCO1/SCO2 [77]
SCO1/SCO2	Inner mitochondrial membrane	Insertion of copper to COX; control of copper balance in the cell, implementation of the Cu(II)→Cu(I) redox cycle [54,55]
ATOX1 (Antioxidant 1, Cu(I)-chaperon for ATP7A/B)	Cytosol	Transportation of Cu(I) from CTR1 to copper-binding motifs of the ATP7A/B; a component of the cytosolic antioxidant system, transcription factor [78,79]
Menkes ATPase (ATP7A, Cu(I)/Cu(II)-transporting ATPase P1 type)	Membranes of trans-network Golgi complex (except for hepatocytes of adult mammals)	Acceptance of Cu(I) from ATOX1 and its ATP-dependent transfer to the lumen of Golgi complex; oxidation of Cu(I) to Cu(II) and insertion of copper into extracellular cuproenzymes [80]
Wilson ATPase (ATP7B, Cu(I)/Cu(II)-transporting ATPase P1 type)	Membranes of trans-network Golgi complex and plasma membrane of the liver, mammary gland, brain cells	Acceptance of Cu(I) from ATOX1 and its ATP-dependent transfer to the lumen of Golgi complex; oxidation of Cu(I) to Cu(II) and insertion of copper into ceruloplasmin, copper excretion through bile [80]
CTR2 (low affinity copper transporter 2)	Membranes of the endolysosomes, plasma membrane	Transfer of Cu(I) from lysosomes to cytosol; regulation of copper import [81,82]
DMT1 (divalent metal transporter 1)	Apical domain of the plasma membrane of the enterocytes and other cells	Transfer of Cu(II) from GIT into enterocytes [84]; compensation of CTR1 deficiency [85]

3. Transport of copper during cuproenzyme formation in adult mammals

The formation of holo-cuproenzymes completely is depended on copper traffic, which delivers copper from extracellular space to the locations of apo-cuproenzyme metalation in the cell (i.e., the cytosol, mitochondria, lumen of Golgi complex). The extracellular part of the pathway involves the absorption of food copper ions from the gastrointestinal tract and the delivery of them to the liver. On it, copper is transferred in the oxidation state of Cu (II). The intracellular pathway starts at the surface of the hepatocytes; it requires the reduction of Cu(II) to Cu (I), followed by their transfer to the cell. Copper ions with random coordination spheres, which are usually called 'free' copper ions,

are highly toxic agents. They can initiate Fenton-type reactions and produce reactive oxygen species, which produce the same effects as ionizing radiation [67].

Inside the cells, the problem of safe copper transport is solved by the system of transporter proteins, which bind copper in a Cu(I) state [68]. Briefly in general, this system is highly conserved in the evolution of eukaryotes. In mammals, the **c**opper **t**ransport **s**ystem (CTS) contains the largest number of components, and their patterns and expression levels are specific to tissues and stages of ontogenesis. These proteins share a common trait, which is a copper binding domain that typically contains a motif with two cysteine residues (CXC or CXXC). This domain is capable of bidentate Cu(I) coordination. The total length of the domain with the cysteine motif is comprised of dozens of amino acid residues. Their composition and sequence tune the affinity of the protein to copper and its abilities to accept or deliver copper ions. The proteins, which are also known as Cu(I)-chaperones, form transport chains and pass copper to each other in direct protein-protein contacts cycling between the holo-form and apo-form. The direction of transportation is determined by the increasing affinity to copper ions along the chain, which provides delivery of copper from the extracellular space to various cell compartments. Transporters have two domains for interaction with their respective partners. One domain is characteristic of the apo-form and provides binding to copper donors, and the other domain provides recognition of a copper recipient in its apo-form. Cu(I)-chaperones that insert copper into active centers of cuproenzymes have domains for interaction with apo-forms of these enzymes. While all transporter proteins share the same principles and mechanisms of copper transfer, they can be naturally classified into soluble and integral transmembrane proteins (pore-like transporters or active pumps). Detailed, copper is transported into the cell by the CTR1 protein, which is a universal high affinity copper importer (Table 3). The transport does not require ATP, and this protein has a highly selective Cu(I) pore [69,70]. Knockout of the *Ctr1*$^{-/-}$ gene in mice is lethal, and the embryos die in the first half of gestation as well as display globally impaired morphogenesis [71]. The extracellular copper donors for mammalian CTR1 can be ceruloplasmin [72,73], albumin, and alfa-2-macroglobulin [74]. Cu(I), which crosses the membrane through the CTR1 pore, is bound by the cytosolic domain of this protein [75]. Then, the ion is passed to cytosolic chaperones (CCS, COX17, ATOX1) that deliver copper to SOD1, mitochondria and Cu(I)/Cu(II)-ATPases, respectively [76-80]. In mammals, there are two P1-type copper transporting ATPases: ATP7A (Menkes ATPase) and ATP7B (Wilson ATPase) [80]. These proteins were named according to the hereditary diseases (Menkes disease and Wilson disease) that are associated with the loss of the respective functions of each protein. The translocation of copper from the cytosol to the lumen of Golgi complex is ATP-dependent and coupled with copper oxidation to Cu(II).

The CTR2 protein (low affinity copper transporter 2) is homologous to CTR1 through its primary structure and channel-forming domain architecture, and it is localized to the membranes of endosomes and lysosomes basically [81,82]. The proteins of the STEAP (Six-Transmembrane Epithelial Antigen of Prostate) metalloreductase family are also present in those locations, including cupric reductase STEAP4, which reduces Cu(II) to Cu(I) in endosomes [83]. Therefore, STEAP4/CTR2 activity may recycle copper from cuproenzymes that gets into the endolysosomal space during endocytosis or macroautophagy and returns copper to a bioavailable pool. Perhaps, CTR2 controls the entry of copper into cells, since copper is accumulated in *Ctr2*$^{-/-}$ strains [82]. It is possible that part of the copper is transported by DMT1 (the divalent metal transporter 1) [84], which can play role of the compensatory mechanism for CTR1 deficiency in *Ctr1*$^{-/-}$ cells [85]. One of the unresolved questions in copper import mechanisms is how Cu(II) is converted to Cu(I) near cellular surface. The reductase reducing copper prior to binding by the N-terminal domain of CTR1 was found in unicellular eukaryotes but was not identified in mammals. STEAP2, localized on the plasma membrane, is suspected as a copper reductase [73]. Maybe in high eukaryotes, specific reductase is not required, and copper reduction is realized by CTR1 ectodomain, which resembles bacterial CopK protein capable of intramolecular reduction of Cu(II) [86].

Copper is not accumulated by the proteins of the transport system, and these proteins may be viewed as a temporary package for safe delivery. Therefore, the physiological role of CTS comprises copper import, metalation of cuproenzymes, copper recycling and copper excretion from the cell.

Because of CTS, there are no 'free' copper ions in the cell [87]. However, copper disbalance can result in the appearance of 'free' copper, the generation of ROS and oxidative stress as well as in the decrease of bioavailable copper leading to cuproenzyme deficiency. As a result, neurodegeneration, oncological and cardiovascular disorders develop [14]. The increase of 'free' copper levels also poses a risk of zinc-copper displacement in zinc-finger transcription factors, which may cause global changes in the regulation of gene expression [88]. "Free" copper ions were also shown to be able to disrupt active sites of [Fe-S]-metalloproteins [89], which control electron transport, DNA synthesis and repair, regulation of gene expression, iron metabolism *etc.* [90-92].

4. Copper turnover in body of adult mammals

In the extracellular fluids of multicellular organisms, like intracellular space, copper is coordinated by various carriers. Copper is found in ceruloplasmin (Cp), albumin, α2-macroglobulin, and the bis-histidine complex [93-96], but more than 95% of it is bound to Cp [93]. Experiments with radioactive copper have shown that copper is absorbed in the intestine, passed through the intestinal cells to the bloodstream, where it is bound by albumin and transported to the liver. Near the plasma membranes of hepatocytes, copper from albumin is converted to [$His_2Cu(II)$] and hepatocytes absorb copper from the latter complex [95-97]. Inside these cells, copper is inserted in Cp and intracellular cuproenzymes, bound by metallothionein, or excreted in bile [97]. The copper, which was inserted in Cp, is secreted back to the bloodstream and distributed to organs. Cp is an N-glycoprotein that binds 6-9 copper atoms, and six of them are tightly bound in active centers, while the others are weakly associated with the peptide chain [98]. In ceruloplasmin, the labile copper atoms can be replaced by zinc atoms [99]. Cp has many functions, and it is classified as a "moonlighting" protein [31]. Cp belongs to the family of blue multicopper (ferr)oxidases [32]; and the major function of Cp is the facilitation of iron redox transitions, which are required for transferrin/transferrin receptor mediated iron transport through membranes. *In vivo* Cp oxidizes dopamine, serotonin, epinephrine, and norepinephrine and thus inactivates these hormones. Cp is an acute phase protein. Its level increases several times in inflammation, ovulation, pregnancy, lactation, etc. There is a weak antioxidant activity of Cp towards ROS. Cp is possibly the strongest regulator of neutrophil oxidative status and apoptosis [100]. Cp can also serve as a copper donor for non-hepatocyte cells. In pulse-chase experiments, it has been shown that copper atoms associated with Cp are transferred to the cytosol of non-hepatic cells following the binding of Cp by a protein of the cell membrane. Candidate proteins for Cp binding include the Cp receptor [101-103], CTR1 [72] or STEAP2 [73]. The protein part of Cp, which has given part of its copper atoms (apo-Cp), is absorbed by endocytosis, desyalated in endolysosomal vesicles and then returned to circulation. This processed Cp is then captured by endocytosis in hepatocytes through the group-specific receptor of acidic desalted glycoproteins [104,105]. Cp, containing copper atoms that do not dissociate at low pH, is secreted to bile [106]. Thus, in adult mammals, Cp plays important role in supporting copper balance outside cells.

5. Ontogenetic changes in copper metabolism in mammals

Ontogenetic development of mammals relies on two systems of cuproenzyme metalation, which successively operate in the liver. The first system corresponds to the **e**mbryonic **t**ype of **c**opper **m**etabolism (ETCM). It is active in prenatal and early postnatal stages of development, and then it is superseded by the **a**dult **t**ype of **c**opper **m**etabolism (ATCM). The phenotypic traits of ETCM include the absence of regulated copper absorption in the small intestine (copper freely passes through the wall of the intestine), low copper and Cp levels in blood serum (3-4 times lower compared to the adults), the absence of copper excretion through bile, when copper is excreted with urine, and copper is accumulated in the liver [16,107-109]. The concentration of metallothionein-associated copper in blood serum of the newborns is 2 times higher than in adults [108]. The distribution of copper between the liver and blood in ETCM and the pathways of its excretion corresponds to that observed in Wilson's disease [14].

In ETCM, blood serum copper status correlates with hepatic copper metabolism [110,111]. So, hepatic Cp-gene expression is low; the expression of *Atp7b* is practically absent. The translocation of

copper to the Golgi complex is implemented only by ATP7A, consequently, Cp is metallated by ATP7A, but not ATP7B. The activity of the *Sod1* gene and the level of holo-SOD1 are decreased by 30% compared to adults. However, the activity of the *Ccs* gene is decreased by a factor of 10 [108]. It may be suggested that holo-SOD1 formation in newborns is performed with the help of the MT/glutathione pair in cytosol, or in the intermembrane space of mitochondria [64,65]. Despite the rapid, almost exponential, accumulation of copper in liver cells, the activity of the *Ctr1* gene coding for the major universal copper importer is low, and its expression level comprises ~10% of that in the adults. The low expression level of *Ctr1* in the liver of newborns is accompanied by high expression levels of *Ctr2* [108]. It is possible that CTR1 is not the main route of copper import to the liver during the stage of development, which is characterized by ETCM. During the transition from ETCM to ATCM copper concentration in the liver drops abruptly, while concentrations of Cp and Cp-associated copper in serum increase. The change in copper metabolism type accompanies profound changes in the expression pattern of copper transporters in the liver: the *Atp7a* gene is repressed, the *Mt* gene is downregulated, while the *Sp1*, *Atp7b*, *Cp*, *Ctr1*, *Atox1*, and *Ccs* genes are activated [108,110,111]. In intestinal, the transition from ETCM to ATCM occurs through increased abundance and altered localization of Ctr1, Atp7A, and Atp7B [110], as a result the mechanism controlling copper absorption is formed [113].

In adult mammals, the brain, like the liver, is organ with the high copper concentrations [114]. This fact is related to high concentrations of cuproenzymes in the brain cells. In addition to ubiquitous cuproenzymes (COX and SOD1), the brain contains copper-dependent enzymes that take part in iron transport, processing of the pro-neuropeptides and metabolism of neurotransmitters and synthesis of melanin (Table 1). The distribution of the enzymes and the respective copper content in brain tissue is not uniform [114,115]. The primary stages of copper delivery to the brain are unknown, and the specific ontogenetic features are not known either. During development, the concentration of copper in brain does not change dramatically (in contrast to the liver), but significantly increases in some regions (cortex, hippocampus, cerebellum) [116], possibly in accordance with the synthesis of brain-specific cuproenzymes during differentiation as shown on the PC12 cells [117]. Also, the differentiation of PC12 cells into neurons induces metallothionein-3 expression, thereby resulting in intracellular copper accumulation [118]. The cerebrospinal fluid contains copper and Cp, and their concentrations are approximately 100 times lower compared to blood serum and they do not change during ontogenetic development [119]. So, in the mammalian brain, intimate changes occur in the copper metabolism during transition from ETCM to ATCM, which have not yet been evaluated.

In many regions of the brain, the synthesis of Cp splice-isoforms (secretory Cp and membrane-associated GPI-Cp), which are formed by the alternative splicing of the primary transcript, occurs [120]. GPI-anchored Cp is required for iron efflux from cells in the central nervous system [121]. The synthesis of secretory Cp is stimulated by adjacent endothelial cells, which form the blood-brain barrier [122] through synthesis of interleukin-6 activating transcription of the *Cp* gene [123]. In some brain regions, secretory Cp is used as a ferroxidase [124]. *In vivo* the formation of Cp splice forms is shifted towards GPI-Cp during neuronal differentiation [116]. Generally, the brain copper metabolism are unrelated to the changes that occur in the liver during the early stages of development. However, the facts are enough to consider that ontogenetic changes in the liver and brain are controled by transcriptional and posttranscriptional regulation of the genes for extracellular and intracellular copper transporters.

6. Copper metabolism in the mammary gland through milk ceruloplasmin production

In adult mammals, copper assimilated in the small intestine is typically absorbed completely by the liver in several minutes. In approximately 90 minutes, copper is returned to the bloodstream as a component of serum Cp [125]. However, in lactating females, ~30% of assimilated copper (the total amount of which is 9-10 times higher compared to non-pregnant animals) bypasses the liver and is absorbed by the cells of the mammary gland. In 30 minutes, this copper can be found in milk [126]. The dynamics of copper transfer in lactating females perfectly coincides with the dynamics of the secretion of [^{14}C]Cp to the milk [127]. The mRNA coding the secretory form of Cp is found in the

transcriptome of cells of the lactating mammary glands. The length of milk Cp-mRNA and the molecular mass of milk Cp do not differ from hepatic Cp-mRNA and plasma Cp correspondingly [127,128]. Milk Cp possesses oxidase and ferroxidase activities [129-131]; however, the structure of glycan chains in milk Cp are different from serum Cp, as indicated by 2D-immunoelectrophoresis with lectins [131]. It is likely that milk Cp glycan moiety has no N-acetyl neuraminic acid residues. Concentration of milk Cp is the highest in colostrum and decreases during lactation [129-131].

In mammary gland cells, strong and rapid upregulation of the *Cp* gene as well as the *Ctr1* and *Atp7b* genes, which provide Cp metalation, is observed shortly before the end of gestation [132]. During lactation, the expression levels of *Cp*, *Ctr1* and *Atp7b* genes gradually decrease according to change in Cp concentration in milk. The same pattern of Cp gene expression was reproduced *in vitro* in the PMC42-LA mammary epithelial cell culture models [133]. The activity level of the Cp gene expression in the cells of the mammary gland does not depend on the availability of copper in the female's diet or Cp levels in the blood [134,135]. Thus, the cells of the mammary gland produce a tissue-specific molecular form of soluble Cp, and the concentration of that Cp is strictly regulated at the transcription and splice levels in during pregnancy and lactation.

7. Milk Cp is a source of copper, which adapted to ETCM of the newborns

In milk, copper is present in a nondialyzable fraction and approximately 75-80% of copper is found in Cp [136,137]. In colostrum, the Cp molecule binds more labile copper atoms, which can be removed by the copper-specific resin Chelex-100, than in mature milk [137]. The concentrations of Cp and copper in milk decrease proportionally during lactation. In human colostrum, Cp and copper concentrations are 150±30 mg/L and 600±200 μg/L, respectively, and they drop to 40±20 mg/L and 150±20 μg/L in transitional milk and decrease up to ~10% of the initial values in mature milk. The decrease of the copper concentration follows the increase of the consumed milk volume, so the total quantity of copper in the daily diet of the newborn remains practically invariable. Up to an age of 6 months, the infant typically consumes approximately 1 L of milk, and the concentration of copper is 10 times lower than in the colostrum. We measured milk Cp and copper levels in more than 200 women during the first ten days of lactation, and only in one case their concentrations did not decrease to the 10th day of lactation. In this patient, an A→C point mutation was found in the promoter region of the *Cp* gene (at position –1966). This nucleotide is part of the *cis*-element for transcription factor C/EBPβ, which may potentially take part in gradual suppression of the Cp gene activity [137]. The copper concentration in breast milk decreases approximately the same manner in Turkish women [138]. In all mammalian species (rats, pigs, dogs, mares, cats, human), in which milk copper status indexes were assessed, Cp and copper concentrations decrease during lactation, and this process does not depend on the copper concentration in blood [136-142]. Due to, in breastfeeding, the copper content in the infant's food can be approximately maintained at a constant level. Therefore, the decrease of Cp and the Cp-associated copper concentration in milk is a trait that is conserved between species and inside the species. Looks like that the decrease in the activity of the *Cp* gene in breast cells during lactation is preserved by natural selection.

The high biological importance of milk Cp is indicated by the following facts. Copper atoms that are associated with Cp molecules are assimilated by newborns more easily [130]. Knockout of the *Cp*$^{-/-}$ gene in mice causes a decrease in the copper concentration in milk of the females and copper misbalance in the pups [143]. The same effect is produced by mutations of the *Atp7b* gene in the *toxic milk* line of mice [144]. The progeny of *tx*/*tx* mice dies because of the copper deficiency in mother's milk, but it completely survives if fed from the first day of life by a wild type 'nurse' [145]. In the gastrointestinal tract of newborns, milk Cp molecule is not degraded and retains oxidase activity [146], because in stomachs of newborns the pH levels are close to neutral. From the gastrointestinal tract to bloodstream milk Cp is transferred without any modifications by transcytosis. A specific endocytic Cp receptor takes part in this process. The same receptor facilitates the capture of milk Cp by the membranes of hepatocytes. The Cp receptor is synthesized in the cells of the small intestine and the liver only at the ETCM stage [146,147]. It is likely that copper is released from milk Cp in the acidic medium of endolysosomes, then it is reduced to Cu(I) by STEAP4 and transported to the

cytosol by CTR2 [107]. Thus, the copper transport system of newborns is adapted to milk Cp as the source of copper nutrients (Figure 1).

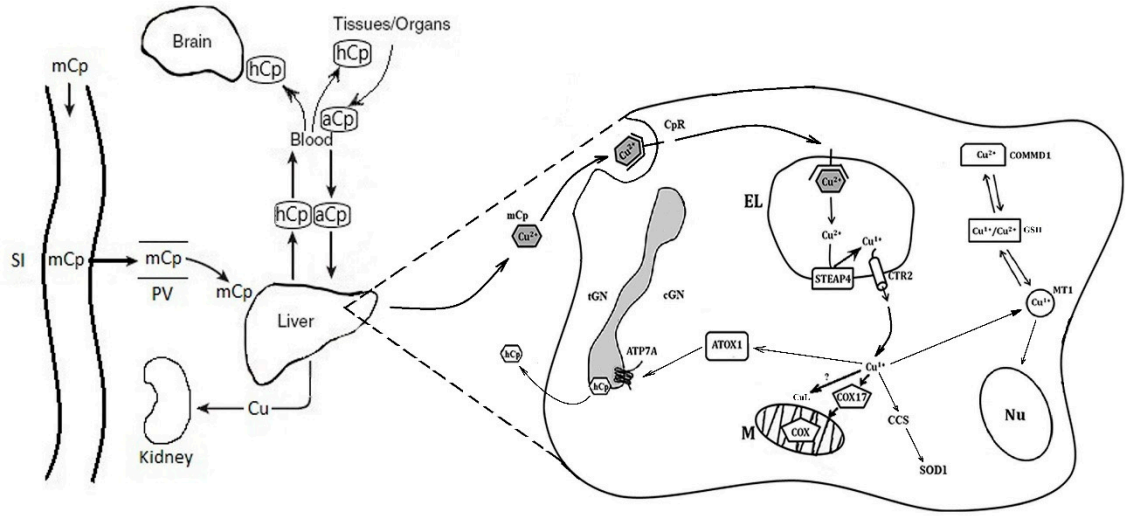

Figure 1. Cartoon scheme of the copper turnover in the hepatocytes of newborns. In newborns, milk Cp enters to gastrointestinal tract and due to transcytosis transfers into bloodstream without modification, and then it binds with hepatic Cp receptor and proceeds into endolysosomes. At pH > 5, Cu(II) ions are dissociated from milk Cp molecule, Cu(II) is reduced to Cu(I) by STEAP4 and imported by CTR2 in cytosol. Here, Cu(I) is redistributed between Cu(I)-chaperons to be delivered to COX, SOD1 and Cp. In cytosol, copper is bound with MT1, involved to redox cycle MT/GSH and can be excreted by COMMD1. CuL bind Cu(I) ions and transported their into mitochondria as well as exported to extracellular space. Because MT1 is found in mitochondria and nucleus, possibly, it transfers copper to the nucleus and mitochondria (or brings copper to their cytosolic surface). Abbreviations on the scema: mCp – milk ceruloplasmin; SI – small intestine; PV – portal vein; hCp – holo-ceruloplasmin; aCp – desialic ceruloplasmin; CpR – ceruloplasmin receptor; Nu – nucleus; M – mitochondria; EL – endolysosome; cGN – cis-Golgi; tGN – trans-Golgi; GSH – glutathione; MT1 – metallothionein 1; ATP7A – Cu(I)/Cu(II)-transporting ATPase; CTR2 – low affinity copper transporter 2; ATOX1, CCS, and COX17 – cytosolic Cu(I)-chaperons for ATP7A, superoxide dismutase 1 (SOD1), and cytochrome-c-oxidase (COX) respectively; STEAP4 – six-transmembrane epithelial antigen of prostate; CuL – low molecular weight carrier of copper; Cu – copper ions.

8. Specific features of copper metabolism in newborns fed infant formulas

The children fed exclusively with cow milk, which contains copper and milk Cp, but at concentrations that are lower compared human milk, at an age of 6 months develop severe anemia, neutropenia, hypocupremia, display malformation of their bones, defects in erythrocyte maturation *etc* [18]. These symptoms can be avoided by adding copper salts to the infant formula. The adapted infant formulas contain approximately 600 μg of copper per 1 L of the consumed liquid, and that amount roughly corresponds to the concentration of copper in colostrum. Copper ions are added as inorganic salts, as simple coordination compounds, or as a [$His_2Cu(II)$] complex. In infant formula, copper is dialyzable, it is not "packed" into Cp and its concentration in the diet from birth up to 6 months of age does not change. So, the newborns fed with such formulas receive copper in a highly mobile form, which easily gets to the bloodstream but is not delivered to the endosomes of hepatocytes. Additionally, the daily copper supply progressively increases along with the volume of consumed food. By the end of the first month, copper consumption with the milk formula exceeds the normal copper consumption of breastfed infants many times [136]. In rats, which were fed

analogous milk formula during 1-9 days of life, the transition from ETCM to ATCM occurred on the fifth day of life, that is, seven days earlier than in nursed rats (Table 4, borrowed from our article [136]). The transition manifested as an abrupt drop of copper concentration in the liver, an increase in blood serum copper levels and preterm activation of the *Cp* gene at the transcription and translation levels. Copper status was also affected in the cerebrospinal fluid. The concentration of copper and Cp were increased by a factor of 7. The specific content of copper in the brain cells did not change. In newborn rats with ETCM and ATCM, which were supplemented with copper ions, Ctr1, Atp7b, and metallothionein mRNA levels in the liver increase [148]. Simultaneously, alanine aminotransferase levels elevated suggesting a risk of copper toxicity with supplementation during infancy. Both experiments with baby formula [136] and copper oral supplementation [148] show that suckling rat pups are ability to adapt higher amounts of nutrition copper due to changes in copper transporters expression. But the data also demonstrate that a high concentration of 'free' copper ions in the food strongly disturbs physiological copper balance in newborns. And brain copper dyshomeostasis is one of the traits of neurodegenerative diseases [149].

9. Conclusion

The reviewed data suggest that the copper imbalance in early postnatal period, which is induced by feeding infant formulas, influences various aspects of copper metabolism. This is primarily an increase in the Cp and copper concentration in the blood serum and cerebrospinal fluid, but there are no grounds that it can affect the cuproenzymes formation. It is possible that, in individuals that carry no latent inherited defects in copper homeostasis, the nutrient copper excess is compensated in further development and has no significant impact on health. But the individuals carrying heterozygous mutations in genes related to copper homeostasis (e.g., heterozygous carriers of Wilson's disease) may be especially sensitive to copper imbalance in early childhood [150]. However, the disturbance of regulatory pool copper can influence on the signaling, activity of the transcription factors (HIF1, p53, nuclear hormone receptors), and [Fe-S]-dependent enzymes. Such effects can be very significant, but now their identification is difficult. Because some participants of CTS are still unknown while some copper-binding proteins are 'moonlighting' and their activities dependent on copper level (e.g. Cp, CTR1, ATOX1, COMMD1) [31,151-155], we may suggest that the differences between breastmilk and infant formulas with respect to copper concentrations and copper 'packaging' by the Cp protein may be one of the factors that contribute to the negative effects of bottle feeding on the cognitive abilities of children [156,157].

The delayed effects of impairments of copper homeostasis in early infancy remain poorly studied. If this problem is ignored, there is a risk that it will impact the development of intellectual abilities, physical and mental health. Although many aspects of copper metabolism need further thorough investigation, it may be stated that for ideal development of the intellectual and physical qualities of the individual significant attention should be given to the balanced content of copper in the infancy diet.

Author Contributions: Author Contributions: Conceptualization: L.V.P.; First draft of the manuscript: L.V.P., P.S.B., Y.A.Z., E.Y.I., F.D.S.; Investigation: P.S.B., Y.A.Z., E.Y.I.; Resources: E.Y.I.; Cartoon scheme of the copper turnover in the hepatocytes of newborns: P.S.B., Y.A.Z.; Co-author of **c**onceptualization and critical revision of the manuscript for important intellectual content: F.D.S.

Funding: The work was supported by grants: RFBR 16-34-60219, 18-015-00481 and MK 2718.2018.4, 6.7509.2017/8.9

Acknowledgments: The authors thank Tatiaina Sankova and Alexey Skvortsov for their constructive discussion of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dunn, J.T. Iodine supplementation and the prevention of cretinism. *Ann. N. Y. Acad. Sci.* **1993**, 678, 158–168. DOI: 10.1111/j.1749-6632.1993.tb26119.x

2. Pearce, E.N.; Lazarus, J.H.; Moreno-Reyes, R.; Zimmermann, M.B. Consequences of iodine deficiency and excess in pregnant women: an overview of current knowns and unknowns. *Am. J. Clin. Nutr.* **2016**, 104 Suppl 3, 918S-923S. DOI: 10.3945/ajcn.115.110429.
3. Steegers-Theunissen, R.P. Folate metabolism and neural tube defects: a review. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **1995**, 61, 39–48. DOI: 10.1016/0028-2243(95)02151-H
4. Ebara, S. Nutritional role of folate. *Congenit Anom (Kyoto)* **2017**, 57, 138-141. DOI: 10.1111/cga.12233.
5. Taylor, R.M.; Fealy, S.M.; Bisquera, A.; Smith, R.; Collins, C.E.; Evans T.J.; Hure, A.J. Effects of nutritional interventions during pregnancy on infant and child cognitive outcomes: a systematic review and meta-analysis. *Nutrients* **2017**, 9, pii: E1265. DOI: 10.3390/nu9111265.
6. Fowler, J.R.; Jack, B.W. Pregnancy, *StatPearls Publishing [Internet], Treasure Island (FL)* **2017**. Available from: https://www.ncbi.nlm.nih.gov/books/NBK448166/
7. Beard, J.L. Why iron deficiency is important in infant development. *J. Nutr.* **2008**, 138, 2534-2536. DOI: 10.1093/jn/138.12.2534.
8. Zhao, G.; Xu, G.; Zhou, M.; Jiang Y.; Richards, B.; Clark, K.M.; Kaciroti, N.; Georgieff, M.K.; Zhang, Z.; Tardif, T.; Li, M.; Lozoff, B. Prenatal iron supplementation reduces maternal anemia, iron deficiency, and iron deficiency anemia in a randomized clinical trial in rural China, but iron deficiency remains widespread in mothers and neonates. *J. Nutr*. **2015**, 145, 1916-1923. DOI: 10.3945/jn.114.208678.
9. Darnton-Hill, I.; Mkparu, U.C. Micronutrients in pregnancy in low- and middle-income countries. *Nutrients* **2015**, 7, 1744-1768. DOI: 10.3390/nu7031744.
10. Tapiero, H.; Townsend, D.M.; Tew, K.D. Trace elements in human physiology and pathology. *Copper. Biomed. Pharmacother.* **2003**. 57, 386–398. DOI: 10.1016/S0753-3322(03)00012-X.
11. Ridge, P.G.; Zhang, Y.; Gladyshev, V.N. Comparative genomic analyses of copper transporters and cuproproteomes reveal evolutionary dynamics of copper utilization and its link to oxygen. *PLoS One* **2008**, 3, e1378. DOI: 10.1371/journal.pone.0001378.
12. Ackerman, C.M.; Chang, C.J. Copper signaling in the brain and beyond. *J. Biol. Chem.* **2018**, 293, 4628-4635. DOI: 10.1074/jbc.R117.000176
13. Via, M.A.; Mechanick, J.I. Nutritional and Micronutrient Care of Bariatric Surgery Patients: Current Evidence Update. *Curr. Obes. Rep.* **2017**, 6, 286-296. DOI: 10.1007/s13679-017-0271-x.
14. Polishchuk, R.; Lutsenko, S. Golgi in copper homeostasis: a view from the membrane trafficking field. *Histochem. Cell Biol.* **2013**, 140, 285-295. DOI: 10.1007/s00418-013-1123-8.
15. Kozlowski, H.; Kolkowska, P.; Watly, J.; Krzywoszynska, K.; Potocki, S. General aspects of metal toxicity. *Curr. Med. Chem.* **2014**, 21, 3721–3740. DOI: 10.2174/0929867321666140716093838.
16. Hurley, L.S.; Keen, C.L.; Lönnerdal, B. Copper in fetal and neonatal development. *Ciba Found. Symp.* **1980**, 79, 227–245. DOI: 10.1002/9780470720622.ch12.
17. Shavlovski, M.M.; Chebotar, N.A.; Konopistseva, L.A.; Zakharova, E.T.; Kachourin, A.M.; Vassiliev, V.B.; Gaitskhoki, V.S. Embryotoxicity of silver ions is diminished by ceruloplasmin – further evidence for its role in the transport of copper. *Biometals* **1995**, 8, 122–128. DOI: 10.1007/BF00142011.
18. Prohaska, J.R. Long-term functional consequences of malnutrition during brain development: copper. *Nutrition* **2000**, 16 502-504. DOI: 10.1016/S0899-9007(00)00308-7.
19. Naveh, Y.; Hazani, A.; Berant, M. Copper deficiency with cow's milk diet. *Pediatrics* **1981**, 68, 397–400.
20. Muller, T.; Muller, W.; Feichtinger, H. Idiopathic copper toxicosis. *Am. J. Clin. Nutr.* **1998**, 67, 1082S–1086S. DOI: 10.1093/ajcn/67.5.1082S.
21. Tanner, M.S. Role of copper in Indian childhood cirrhosis. *Am. J. Clin. Nutr.* **1998**, 67, 1074S–1081S. DOI: 10.1093/ajcn/67.5.1074S.
22. Betard, C.; Rasquin-Weber, A.; Brewer, A.; Drouin, E.; Clark, S.; Verner, A.; Darmond-Zwaig, A.; Fortin, J.; Mercier, J.; Chagnon, P.; Fujiwara, T.M.; Morgan, K.; Richter, A.; Hudson, T.J.; Mitchell, G.A. Localization of a recessive gene for North American Indian childhood cirrhosis to chromosome region 16q22 and identification of a shared haplotype. *Am. J. Hum. Genet.* **2000**, 67, 222–228. DOI: 10.1086/302993.
23. Rubino, J.T.; Franz, K.J. Coordination chemistry of copper proteins: how nature handles a toxic cargo for essential function. *J. Inorg. Biochem.* **2012**, 107, 129–143. DOI: 10.1016/j.jinorgbio.2011.11.024.
24. Zelko, I.N.; Mariani, T.J.; Folz, R.J. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* **2002**, 33, 337–349. DOI: 10.1016/S0891-5849(02)00905-X.

25. Timón-Gómez, A.; Nývltová, E.; Abriata, L.A.; Vila, A.J.; Hosler, J.; Barrientos, A. Mitochondrial cytochrome c oxidase biogenesis: Recent developments. *Semin. Cell. Dev. Biol.* **2017**, pii: S1084-9521(17)30275-6. DOI: 10.1016/j.semcdb.2017.08.055.
26. Kumari, S.; Panda, T.K.; Pradhan, T. Lysyl oxidase: its diversity in health and diseases. *Indian J. Clin. Biochem.* **2017**, 32, 134–141. DOI: 10.1007/s12291-016-0576-7.
27. Kumar, D.; Mains, R.E.; Eipper, B.A. 60 YEARS OF POMC: From POMC and αMSH to PAM, molecular oxygen, copper and vitamin *C. J. Mol. Endocrinol.* **2016**, 56, T63–T76. DOI: 10.1530/JME-15-0266.
28. Brazeau, B.J.; Johnson, B.J.; Wilmot, C.M. Copper-containing amine oxidases. Biogenesis and catalysis; a structural perspective. *Arch. Biochem. Biophys.* **2004**, 428, 22–31. DOI: 10.1016/j.abb.2004.03.034.
29. Finney, J.; Moon, H.J.; Ronnebaum, T.; Lantz, M.; Mure, M. Human copper-dependent amine oxidases. *Arch. Biochem. Biophys.* **2014**,546, 19–32. DOI: 10.1016/j.abb.2013.12.022.
30. Solano, F. On the Metal Cofactor in the Tyrosinase Family. *Int. J. Mol. Sci.* **2018**, 19, pii: E633. DOI: 10.3390/ijms19020633.
31. Bielli, P.; Calabrese, L. Structure to function relationships in ceruloplasmin: a 'moonlighting' protein. *Cell. Mol. Life Sci.* **2002**, 59, 1413–1427. DOI: 10.1007/s00018-002-8519-2.
32. Vashchenko, G.; MacGillivray, R.T.A. Multi-copper oxidases and human iron metabolism. *Nutrients* **2013**, 5, 2289–2313. DOI: 10.3390/nu5072289.
33. Jeong, S.Y.; David, S. Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. *J Biol Chem.* **2003**, 278, 27144-27148. DOI: 10.1074/jbc.M301988200.
34. Anderson, G.J.; Frazer, D.M. Recent advances in intestinal iron transport. *Curr. Gastroenterol. Rep.* **2005**, 7, 365-372. DOI: 10.1007/s11894-005-0005-1.
35. Chen, H.; Attieh, Z.K.; Syed, B,A,; Kuo, Y.M.; Stevens, V.; Fuqua, B.K.; Andersen, H.S.; Naylor, C.E.; Evans, R.W.; Gambling, L.; Danzeisen, R.; Bacouri-Haidar, M.; Usta, J.; Vulpe, C.D.; McArdle, H.J. Identification of zyklopen, a new member of the vertebrate multicopper ferroxidase family, and characterization in rodents and human cells. *J. Nutr.* **2010**, 140, 1728-1735. DOI: 10.3945/jn.109.117531.
36. Palm-Espling, M.E.; Niemiec, M.S.; Wittung-Stafshede, P. Role of metal in folding and stability of copper proteins in vitro. *Biochim. Biophys. Acta.* **2012**, 1823, 1594–1603. DOI: 10.1016/j.bbamcr.2012.01.013.
37. Raju, K.S.; Alessandri, G.; Ziche, M.; Gullino, M.N. Ceruloplasmin, copper ions, and angiogenesis. *J. Natl. Cancer Inst.* **1982**, 69, 1183–1188. DOI: 10.1093/jnci/69.5.1183.
38. Bartuzi, P.; Hofker, M.H.; van de Sluis, B. Tuning NF-κB activity: a touch of COMMD proteins. *Biochim. Biophys. Acta.* **2013**, 1832, 2315–2321. DOI: 10.1016/j.bbadis.2013.09.014.
39. Li, S.; Zhang, J.; Yang, H.; Wu, C.; Dang, X.; Liu, Y. Copper depletion inhibits CoCl2-induced aggressive phenotype of MCF-7 cells via downregulation of HIF-1 and inhibition of Snail/Twist-mediated epithelial-mesenchymal transition. *Sci. Rep.* **2015**, 5, 12410. DOI: 10.1038/srep12410.
40. Mufti, A.R.; Burstein, E.; Duckett, C.S. XIAP: cell death regulation meets copper homeostasis. *Arch. Biochem. Biophys.* **2007**, 463, 168–174. DOI: 10.1016/j.abb.2007.01.033.
41. Hou, M.M.; Polykretis, P.; Luchinat, E.; Wang, X.; Chen, S.N.; Zuo, H.H.; Yang, Y.; Chen, J.L.; Ye, Y.; Li, C., Banci, L.; Su, X.C. Solution structure and interaction with copper in vitro and in living cells of the first BIR domain of XIAP. *Sci. Rep.* **2017**, 7, 16630. DOI: 10.1038/s41598-017-16723-5.
42. Turski, M.L.; Brady, D.C.; Kim, H.J.; Kim, B.E.; Nose, Y.; Counter, C.M.; Winge, D.R.; Thiele, D.J. A novel role for copper in Ras/mitogen-activated protein kinase signaling. *Mol. Cell. Biol.* **2012**, 32, 1284–1295. DOI: 10.1128/MCB.05722-11.
43. Brady, D.C.; Crowe, M.S.; Turski, M.L.; Hobbs, G.A.; Yao, X.; Chaikuad, A.; Knapp, S.; Xiao, K.; Campbell, S.L.; Thiele, D.J.; Counter C.M. Copper is required for oncogenic BRAF signalling and tumorigenesis. *Nature* **2014**, 509, 492-496. DOI: 10.1038/nature13180.
44. Finney, L.; Mandava, S.; Ursos, L.; Zhang, W.; Rodi, D.; Vogt, S.; Legnini, D.; Maser, J.; Ikpatt, F.; Olopade, O.I.; Glesne, D. X-ray fluorescence microscopy reveals large-scale relocalization and extracellular translocation of cellular copper during angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, 104, 2247–2252. DOI: 10.1073/pnas.0607238104.
45. Grasso, G.; Santoro, A.M.; Magrì, A., La Mendola, D.; Tomasello, M.F.; Zimbone, S.; Rizzarelli, E. The inorganic perspective of VEGF: interactions of Cu(2+) with peptides encompassing a recognition domain of the VEGF receptor. *J. Inorg. Biochem.* **2016**, 159, 149–158. DOI: 10.1016/j.jinorgbio.2016.03.004.
46. D'Ambrosi, N.; Rossi, L. Copper at synapse: Release, binding and modulation of neurotransmission. *Neurochem. Int.* **2015**, 90, 36-45. DOI: 10.1016/j.neuint.2015.07.006.

47. Christensen, P.C.; Welch, N.C.; Brideau, C.; Stys, P.K. Functional ionotropic glutamate receptors on peripheral axons and myelin. *Muscle Nerve.* **2016**, 54, 451-459. DOI: 10.1002/mus.25078.
48. Krishnamoorthy, L.; Cotruvo Jr., J.A.; Chan, j.; Kaluarachchi, H.; Muchenditsi, A.; Pendyala, V.S.; Jia, S.; Aron, A.T.; Ackerman, C.M.; Wal, M.N.; Guan, T.; Smaga, L.P.; Farhi, S.L.; New, E.J.; Lutsenko, S.; Chang, C.J. Copper regulates cyclic-AMP-dependent lipolysis. *Nat. Chem. Biol.* **2016**, 12, 586–592. DOI: 10.1038/nchembio.2098.
49. Prudovsky, I. Nonclassically secreted regulators of angiogenesis. *Angiol. Open Access.* **2013**, 1, 1000101. DOI: 10.4172/2329-9495.1000101.
50. Haremaki, T.; Fraser, S.T.; Kuo, Y.M.; Baron, M.H.; Weinstein, D.C. Vertebrate Ctr1 coordinates morphogenesis and progenitor cell fate and regulates embryonic stem cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, 104, 12029–12034. DOI: 10.1073/pnas.0701413104.
51. Vašák, M.; Meloni, G. Chemistry and biology of mammalian metallothioneins. *J. Biol. Inorg. Chem.* **2011**, 16, 1067–1078. DOI: 10.1007/s00775-011-0799-2.
52. Kang, Y.J. Metallothionein redox cycle and function. *Exp. Biol. Med. (Maywood)* **2006**, 231, 1459–1467. DOI: 10.1177/153537020623100903.
53. Maine, G.N.; Burstein, E. COMMD proteins: COMMing to the scene. *Cell. Mol. Life Sci.* **2007**, 64, 1997–2005. DOI: 10.1007/s00018-007-7078-y.
54. Leary, S.C.; Cobine, P.A.; Kaufman, B.A.; Guercin, G.-H.; Mattman, A.; Palaty, J., Lockitch, G.; Winge, D.R.; Rustin, P.; Horvath, R.; Shoubridge, E.A. The human cytochrome c oxidase assembly factors SCO1 and SCO2 have regulatory roles in the maintenance of cellular copper homeostasis. *Cell Met.* **2007**, 5, 9–20. DOI: 10.1016/j.cmet.2006.12.001.
55. Leary, S.C. Redox regulation of SCO protein function: controlling copper at a mitochondrial crossroad. *Antioxid. Redox. Signal.* **2010**, 13, 1403–1416. DOI: 10.1089/ars.2010.3116.
56. Gray, L.W.; Peng, F.; Molloy, S.A.; Pendyala, V.S.; Muchenditsi, A.; Muzik, O.; Lee, J.; Kaplan, J.H.; Lutsenko, S. Urinary copper elevation in a mouse model of Wilson's disease is a regulated process to specifically decrease the hepatic copper load. *PLoS One* **2012**, 7, e38327. DOI: 10.1371/journal.pone.0038327.
57. Cobine, P.A.; Pierrel, F.; Bestwick, M.L.; Winge, D.R. Mitochondrial matrix copper complex used in metallation of cytochrome oxidase and superoxide dismutase. *J. Biol. Chem.* **2006**, 281, 36552–36559. DOI: 10.1074/jbc.M606839200.
58. Boulet, A.; Vest, K.E.; Maynard, M.K.; Gammon, M.G.; Russell, A.C.; Mathews, A.T.; Cole, S.E.; Zhu, X.; Phillips, C.B.; Kwong, J.Q.; Dodani, S.C.; Leary, S.C.; Cobine, P.A. The mammalian phosphate carrier SLC25A3 is a mitochondrial copper transporter required for cytochrome c oxidase biogenesis. *J. Biol. Chem.* **2018**, 293, 1887-1896. DOI: 10.1074/jbc.RA117.000265.
59. Nishioka, T.; Eustace, A.; West, C. Lysyl oxidase: from basic science to future cancer treatment. *Cell Struct. Funct.* **2012**, 37, 75–80. DOI: 10.1247/csf.11015.
60. Payne, S.L.; Hendrix, M.J.; Kirschmann, D.A. Paradoxical roles for lysyl oxidases in cancer - a prospect. *J. Cell. Biochem.* **2007**, 101, 1338–1354. DOI: 10.1002/jcb.21371.
61. Liang, Z.D.; Tsai, W.B.; Lee, M.Y.; Savaraj, N.; Kuo, M.T. Specificity protein 1 (Sp1) oscillation is involved in copper homeostasis maintenance by regulating human high-affinity copper transporter 1 expression. *Mol. Pharmacol.* **2012**, 81, 455–464. DOI: 10.1124/mol.111.076422.
62. Yuan, S.; Chen, S.; Xi, Z.; Liu, Y. Copper-finger protein of Sp1: the molecular basis of copper sensing. *Metallomics* **2017**, 9, 1169–1175. DOI: 10.1039/c7mt00184c.
63. Wegner, S.V.; Sun, F.; Hernandez, N.; He, C. The tightly regulated copper window in yeast. *Chem. Commun. (Camb.)* **2011**, 47, 2571–2573. DOI: 10.1039/c0cc04292g.
64. Bhattacharjee, A.; Chakraborty, K.; Shukla, A. Cellular copper homeostasis: current concepts on its interplay with glutathione homeostasis and its implication in physiology and human diseases. *Metallomics* **2017**, 9, 1376-1388. DOI: 10.1039/c7mt00066a.
65. Baker, Z.N.; Cobine, P.A.; Leary, S.C. The mitochondrion: a central architect of copper homeostasis. *Metallomics* **2017**, 9, 1501–1512. DOI: 10.1039/c7mt00221a.
66. Yang, L.; McRae, R.; Henary, M.M.; Patel, R.; Lai, B.; Vogt, S.; Fahrni, C.J. Imaging of the intracellular topography of copper with a fluorescent sensor and by synchrotron X-ray fluorescence microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, 102, 11179–11184. DOI: 10.1073/pnas.0406547102.
67. Linder, M.C. The relationship of copper to DNA damage and damage prevention in humans. *Mutat. Res.* **2012**, 733, 83-91. DOI: 10.1016/j.mrfmmm.2012.03.010.

68. Kaplan, J.H.; Maryon, E.B. How mammalian cells acquire copper: An essential but potentially toxic metal. *Biophys. J.* **2016**, 110, 7-13. DOI: 10.1016/j.bpj.2015.11.025.
69. Wittung-Stafshede, P. Unresolved questions in human copper pump mechanisms. *Q. Rev. Biophys.* **2015**, 48, 471–478. DOI: 10.1017/S0033583515000128.
70. Tsai, C.Y.; Liebig, J.K.; Tsigelny, I.F.; Howell, S.B. The copper transporter 1 (CTR1) is required to maintain the stability of copper transporter 2 (CTR2). *Metallomics* **2015**, 7, 1477–1487. DOI: 10.1039/c5mt00131e.
71. Sharp, P.A. Ctr1 and its role in body copper homeostasis. *Int. J. Biochem. Cell. Biol.* **2003**, 35, 288-291. DOI: 10.1016/S1357-2725(02)00134-6.
72. Zatulovskiy, E.; Samsonov, S.; Skvortsov, A. Doking study on mammalian CTR1 copper importer motifs. *BMC Syst. Biol.* **2007**, 1, 54. DOI: 10.1186/1752-0509-1-S1-P54.
73. Ramos, D.; Mar, D.; Ishida, M.; Vargas, R.; Gaite, M.; Montgomery, A.; Linder, M.C. Mechanism of copper uptake from blood plasma ceruloplasmin by mammalian cells. *PLoS One* **2016**, 11, e0149516. DOI: 10.1371/journal.pone.0149516.
74. Moriya, M.; Ho, Y.H.; Grana, A.; Nguyen, L.; Alvarez, A.; Jamil, R.; Ackland, M.L.; Michalczyk, A.; Hamer, P.; Ramos, D.; Kim, S.; Mercer, J.F.; Linder, M.C. Copper is taken up efficiently from albumin and alpha2-macroglobulin by cultured human cells by more than one mechanism. *Am. J. Physiol. Cell Physiol.* **2008**, 295, C708-C721. DOI: 10.1152/ajpcell.00029.2008.
75. Field, L.S.; Luk, E.; Culotta, V.C. Copper chaperones: personal escorts for metal ions. *J. Bioenerg. Biomembr.* **2002**, 34, 373–379. DOI: 10.1023/A:1021202119942.
76. Kawamata, H.; Manfredi, G. Import, maturation, and function of SOD1 and its copper chaperone CCS in the mitochondrial intermembrane space. *Antioxid. Redox. Signal.* **2010**, 13, 1375–1384. DOI: 10.1089/ars.2010.3212.
77. Takahashi, Y.; Kako, K.; Ohmura, K.; Tsumori, K.; Ohmasa, Y.; Kashiwabara, S.; Baba, T.; Munekatat, E. Genomic structure of mouse copper chaperone, COX17. *DNA Seq.* **2001**, 12, 305–318. DOI: 10.3109/10425170109084454.
78. Yu, C.H.; Yang, N.; Bothe, J.; Tonelli, M.; Nokhrin, S.; Dolgova, N.V.; Braiterman, L.; Lutsenko, S.; Dmitriev O.Y. The metal chaperone Atox1 regulates the activity of the human copper transporter ATP7B by modulating domain dynamics. *J. Biol. Chem.* **2017**, 292, 18169–18177. DOI: 10.1074/jbc.M117.811752.
79. Hatori, Y.; Lutsenko, S. The role of copper chaperone ATOX1 in coupling redox homeostasis to intracellular copper distribution. *Antioxidants (Basel)* **2016**, 5, pii: E25. DOI: 10.3390/antiox5030025.
80. Lutsenko, S.; Barnes, N.L.; Bartee, M.Y.; Dmitriev, O.Y. Function and regulation of human copper-transporting ATPases. *Physiol. Rev.* **2007**, 87, 1011–1046. DOI: 10.1152/physrev.00004.2006.
81. Öhrvik, H.; Thiele, D.J. The role of Ctr1 and Ctr2 in mammalian copper homeostasis and platinum-based chemotherapy. *J. Trace Elem. Med. Biol.* **2015**, 31, 178–182. DOI: 10.1016/j.jtemb.2014.03.006.
82. Öhrvik, H.; Logeman, B.; Noguchi, G.; Eriksson, I.; Kjellén, L.; Thiele, D.J.; Pejler, G. Ctr2 regulates mast cell maturation by affecting the storage and expression of tryptase and proteoglycans. *J. Immunol.* **2015**, 195, 3654–3564. DOI: 10.4049/jimmunol.1500283.
83. Knutson, M.D. Steap proteins: implications for iron and copper metabolism. *Nutr. Rev.* **2007**, 65, 335–340. DOI: 10.1111/j.1753-4887.2007.tb00311.x.
84. Shawki, A.; Anthony, S.R.; Nose, Y.; Engevik, M.A.; Niespodzany, E.J.; Barrientos, T.; Öhrvik, H.; Worrell, R.T.; Thiele, D.J.; Mackenzie, B. Intestinal DMT1 is critical for iron absorption in the mouse but is not required for the absorption of copper or manganese. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2015**, 309, G635–G647. DOI: 10.1152/ajpgi.00160.2015.
85. Lin, C.; Zhang, Z.; Wang, T.; Chen, C.; Kang, Y.J. Copper uptake by DMT1: a compensatory mechanism for CTR1 deficiency in human umbilical vein endothelial cells. *Metallomics* **2015**, 7, 1285–1289. DOI: 10.1039/c5mt00097a.
86. Ash, M.R.; Chong, L.X.; Maher, M.J.; Hinds, M.G.; Xiao, Z.; Wedd, A.G. Molecular basis of the cooperative binding of Cu(I) and Cu(II) to the CopK protein from Cupriavidus metallidurans CH34. *Biochemistry* **2011**, 50, 9237–9247. DOI: 10.1021/bi200841f.
87. Rae, T.D.; Schmidt, P.J.; Pufahl, R.A.; Culotta, V.C.; O'Halloran, T.V. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* **1999**, 284, 805–808. DOI: 10.1126/science.284.5415.805.

88. Shimberg, G.D.; Ok, K.; Neu, H.M.; Splan, K.E.; Michel, S.L.J. Cu(I) disrupts the structure and function of the nonclassical zinc finger protein tristetraprolin (TTP). *Inorg. Chem.* **2017**, 56, 6838–6848. DOI: 10.1021/acs.inorgchem.7b00125.
89. Brancaccio, D.; Gallo, A.; Piccioli, M.; Novellino, E.; Ciofi-Baffoni, S.; Banci, L. [4Fe-4S] cluster assembly in mitochondria and its impairment by copper. *J. Am. Chem. Soc.* **2017**, 139, 719–730. DOI: 10.1021/jacs.6b09567.
90. Gnandt, E.; Dörner, K.; Strampraad, M.F.J.; de Vries, S.; Friedrich, T. The multitude of iron-sulfur clusters in respiratory complex I. *Biochim. Biophys. Acta.* **2016**, 1857, 1068–1072. DOI: 10.1016/j.bbabio.2016.02.018.
91. Fuss, J.O.; Tsai, S.L.; Ishida, J.P.; Tainer, G.A. Emerging critical roles of Fe-S clusters in DNA replication and repair. *Biochim. Biophys. Acta.* **2015**, 1853, 1253–1271. DOI: 10.1016/j.bbamcr.2015.01.018.
92. Pain, D.; Dancis, A. Roles of Fe-S proteins: from cofactor synthesis to iron homeostasis to protein synthesis. *Curr. Opin. Genet. Dev.* **2016**, 38, 45–51. DOI: 10.1016/j.gde.2016.03.006.
93. Bernevic, B.; El-Khatib, A.H.; Jakubowski, N.; Weller, M.G. Online immunocapture ICP-MS for the determination of the metalloprotein ceruloplasmin in human serum. *BMC Res. Notes.* **2018**, 11, 213. DOI: 10.1186/s13104-018-3324-7.
94. Linder, M.C. Ceruloplasmin and other copper binding components of blood plasma and their functions: an update. *Metallomics* **2016**, 8, 887–905. DOI: 10.1039/c6mt00103c.
95. Waldrop, G.L.; Ettinger, M.J. Effects of albumin and histidine on kinetics of copper transport by fibroblasts. *Am. J Physiol.* **1990**, 259, G212-G218. DOI: 10.1152/ajpgi.1990.259.2.G212.
96. Harris, E.D. Copper transport: an overview. *Proc. Soc. Exp. Biol. Med.* **1991**, 196, 130–140. DOI: 10.3181/00379727-196-43171B.
97. Kim, B.E.; Nevitt, T.; Thiele, D.J. Mechanisms for copper acquisition, distribution and regulation. *Nat. Chem. Biol.* **2008**, 4, 176–185. DOI: 10.1038/nchembio.72.
98. Zaitsev, V.N.; Zaitseva, I.; Papiz, M.; Lindley, P.F. An X-ray crystallographic study of the binding sites of the azide inhibitor and organic substrates to ceruloplasmin, a multi-copper oxidase in the plasma. *J. Biol. Inorg. Chem.* **1999**, 4, 579-587. DOI: 10.1007/s007750050380.
99. Samygina, V.R.; Sokolov, A.V.; Bourenkov, G.; Schneider, T.R.; Anashkin, V.A.; Kozlov, S.O.; Kolmakov, N.N.; Vasilyev, V.B. Rat ceruloplasmin: a new labile copper binding site and zinc/copper mosaic. *Metallomics* **2017**, 9, 1828-1838. DOI: 10.1039/c7mt00157f.
100. Golenkina, E.A.; Viryasova, G.M.; Galkina, S.I.; Gaponova, T.V.; Sud'ina, G.F.; Sokolov, A.V. Fine regulation of neutrophil oxidative status and apoptosis by ceruloplasmin and its derivatives. *Cells* **2018**, 7, pii: E8. DOI: 10.3390/cells7010008.
101. Stern, R.V.; Frieden, E. Partial purification of the rat erythrocyte ceruloplasmin receptor monitored by an electrophoresis mobility shift assay. *Anal. Biochem.* **1993**, 212, 221–228. DOI: 10.1006/abio.1993.1315.
102. Puchkova, L.V.; Verbina, I.A.; Denezhkina, V.V.; Vakharlovskii, V.G., Voitsekhovskii, B.L; Gaitskhoki, V.S.; Neifakh, S.A. Molecular forms of ceruloplasmin in hepatolenticular degeneration and their interaction with human erythrocyte ceruloplasmin receptor. *Biomed. Sci.* **1990**, 1, 460–466.
103. Hilton, M.; Spenser, D.C.; Ross, P.; Ramsey, A.; McArdle, H.J. Characterisation of the copper uptake mechanism and isolation of the ceruloplasmin receptor/copper transporter in human placental vesicles. *Biochim. Biophys. Acta.* **1995**, 1245, 153–160. DOI: 10.1016/0304-4165(95)00084-O.
104. Puchkova, L.V.; Sasina, L.K.; Aleinikova, T.D.; Zakharova, E.T.; Gaitskhoki, V.S. Reconstitution of the intercellular transfer pathway of the peptide moiety of ceruloplasmin in mammals. *Biochemistry (Mosc.)* **1997**, 62, 697–703.
105. Gregoriadis, G.; Morell, A.G.; Sternlieb, I.; Scheinberg, I.H. Catabolism of desialylated ceruloplasmin in the liver. *J. Biol. Chem.* **1970**, 245, 5833–5837.
106. Verbina, I.A.; Puchkova, L.V.; Gaitskhoki, V.S.; Neifakh, S.A. Isolation and partial characterization of molecular forms of ceruloplasmin from human bile. *FEBS Lett.* **1992**, 298, 105–108. DOI: 10.1016/0014-5793(92)80032-C.
107. Bingle, C.D.; Epstein, O.; Srai, S.K.; Gitlin, J.D. Hepatic ceruloplasmin-gene expression during development in the guinea-pig. Correlation with changes in hepatic copper metabolism. *Biochem. J.* **1991**, 276, 771–775. DOI: 10.1042/bj2760771.
108. Zatulovskaia, Y.A.; Ilyechova, E.Y.; Puchkova, L.V. The features of copper metabolism in the rat liver during development. *PLoS One* **2015**, 10, e0140797. DOI: 10.1371/journal.pone.0140797.
109. Luza, S.C.; Speisky, H.C. Liver copper storage and transport during development: implications for cytotoxicity. *Am. J. Clin. Nutr.* **1996**, 63, 812S–820S. DOI: 10.1093/ajcn/63.5.812.

110. Lenartowicz, M.; Kennedy, C.; Hayes, H.; McArdle, H.J. Transcriptional regulation of copper metabolism genes in the liver of fetal and neonatal control and iron-deficient rats. *Biometals* **2015**, 28, 51–59. DOI: 10.1007/s10534-014-9802-z.
111. Gyulikhandanova, N.E.; Tsymbalenko, N.V.; Platonova, N.A.; Babich, V.S.; Puchkova, L.V. Regulation of ceruloplasmin gene in mammals. *Bull. Exp. Biol. Med.* **2004**, 137, 485–489. DOI: 10.1023/B:BEBM.0000038160.60211.6f.
112. Lönnerdal, B. Intestinal regulation of copper homeostasis: a developmental perspective. *Am. J. Clin. Nutr.* **2008**, 88, 846S-850S. DOI: 10.1093/ajcn/88.3.846S.
113. van den Berghe, P.V.; Klomp, L.W. New developments in the regulation of intestinal copper absorption. *Nutr. Rev.* **2009**, 67, 658-672. DOI: 10.1111/j.1753-4887.2009.00250.x.
114. Mason, K.E. A conspectus of research on copper metabolism and requirements of man. *J. Nutr.* **1979**, 109, 1979–2066. DOI: 10.1093/jn/109.11.1979.
115. Platonova, N.A.; Barabanova, S.V.; Povalikhin, R.G.; Tsymbalenko, N.V.; Danilovskii, M.A.; Voronina, O.V.; Dorokhova, I.I.; Puchkova, L.V. In Vivo expression of copper-transporting proteins in rat brain regions. *Biol. Bull.* **2005**, 32,108–120.
116. Babich, P.S.; Ilyechova, E.Y.; Skomorokhova, E.A.; Puchkova, L.V. The changes of copper concentration and ceruloplasmin gene expression level in the brain departments during development of the rats. (unpublished; manuscript in preparation).
117. Hatori, Y.; Yan, Y.; Schmidt, K.; Furukawa, E.; Hasan, N.M.; Yang, N.; Liu, C.N.; Sockanathan, S.; Lutsenko, S. Neuronal differentiation is associated with a redox-regulated increase of copper flow to the secretory pathway. *Nat. Commun.* **2016**, 7, 10640. DOI: 10.1038/ncomms10640.
118. Ogra, Y.; Tejima, A.; Hatakeyama, N.; Shiraiwa, M.; Wu, S.; Ishikawa, T.; Yawata, A.; Anan, Y.; Suzuki, N. Changes in intracellular copper concentration and copper-regulating gene expression after PC12 differentiation into neurons. *Sci. Rep.* **2016**, 6, 33007. DOI: 10.1038/srep33007.
119. Platonova, N.A.; Zhiguleva, E.A.; Tsymbalenko, N.V.; Mishchenko, B.S.; Vasin, A.V.; Zhivul'ko, T.V.; Puchkova L.V. Age-related features of ceruloplasmin biosynthesis and distribution in rats. *Ontogenez* **2004**, 35, 171-182.
120. Patel, B.N.; Dunn, R.J.; David, S. Alternative RNA splicing generates a glycosylphosphatidylinositol-anchored form of ceruloplasmin in mammalian brain. *J. Biol. Chem.* **2000**, 275, 4305–4310. DOI: 10.1074/jbc.275.6.4305.
121. Jeong, S.Y.; David, S. Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. *J. Biol. Chem.* **2003**, 278, 27144–27148. DOI: 10.1074/jbc.M301988200.
122. McCarthy, R.C.; Kosman, D.J. Activation of C6 glioblastoma cell ceruloplasmin expression by neighboring human brain endothelia-derived interleukins in an in vitro blood-brain barrier model system. *Cell. Commun. Signal.* **2014**, 12, 65. DOI: 10.1186/s12964-014-0065-7.
123. Conley, L.; Geurs, T.L.; Levin, L.A. Transcriptional regulation of ceruloplasmin by an IL-6 response element pathway. *Brain Res. Mol. Brain Res.* **2005**, 139, 235–241. DOI: 10.1016/j.molbrainres.2005.05.027.
124. Ke, Y.; Ho, K.; Du, J.; Zhu, L.; Xu, Y.; Wang, Q.; Wang, C.Y.; Li, L.; Ge, X.; Chang, Y.; Qian, Z.M. Role of soluble ceruloplasmin in iron uptake by midbrain and hippocampus neurons. *J. Cell. Biochem.* **2006**, 98, 912–919. DOI: 10.1002/jcb.20740
125. Ilyechova, E., Skvortsov, A., Zatulovsky, E.; Tsymbalenko, N.; Shavlovsky, M.; Broggini, M.; Puchkova, L. Experimental switching of copper status in laboratory rodents. *J. Trace Elem. Med. Biol.* **2011**, 25 27-35. DOI: 10.1016/j.jtemb.2010.08.002.
126. Donley, S.A.; Ilagan, B.J.; Rim, H.; Linder, M.C. Copper transport to mammary gland and milk during lactation in rats. *Am. J. Physiol. Endocrinol. Metab.* **2002**, 283, E667-E675. DOI: 10.1152/ajpendo.00115.2002.
127. Puchkova, L.V.; Aleinikova, T.D.; Tsymbalenko, N.V.; Zakharova, E.T.; Konopistseva, L.A.; Chebotar', N.A;. Gaitskhoki, V.S. Biosynthesis and secretion of ceruloplasmin by rat mammary cells during lactation. *Biokhimiia* **1994**, 59, 296–303.
128. Jaeger, J.L.; Shimizu, N.; Gitlin, J.D. Tissue-specific ceruloplasmin gene expression in the mammary gland. *Biochem. J.* **1991**, 280, 671–677. DOI: 10.1042/bj2800671.
129. Kiyosawa, I.; Matsuyama, J.; Nyui, S.; Fukuda, A. Ceruloplasmin concentration in human colostrum and mature milk. *Biosci. Biotechnol. Biochem.* **1995**;59, 713–714. DOI: 10.1271/bbb.59.713.

130. Wooten, L.; Shulze, R.A.; Lancey, R.W.; Leitsow, M.; Linder, M.C. Ceruloplasmin is found in milk and amniotic fluid and may have a nutritional role. *J. Nutr. Biochem.* **1996**, 7, 632–639. DOI: 10.1016/S0955-2863(96)00127-1.
131. Puchkova, L.V.; Zakharova, E.T.; Aleinikova, T.D.; Mokshina, S.V.; Tsymbalenko, N.V.; Sasina, L.K.; Shirmanova, M.R.; Rogacheva, N.P.; Gaitskhoki, V.S. Comparative analysis of the molecular heterogeneity of ceruloplasmin from human blood and breast milk. *Biochemistry (Mosc)* **1997**, 62, 928–930.
132. Platonova, N.A.; Orlov, I.A.; Klotchenko, S.A.; Babich, V.S.; Ilyechova, E.Y.; Babich, P.S.; Garmai, Y.P.; Vasin, A.V.; Tsymbalenko, N.V.; Puchkova, L.V. Ceruloplasmin gene expression profile changes in the rat mammary gland during pregnancy, lactation and involution. *J. Trace Elem. Med. Biol.* **2017**, 43, 126–134. DOI: 10.1016/j.jtemb.2016.12.013.
133. Freestone, D.; Denoyer, D.; Jakab, M.; Leigh Ackland, M.; Cater, M.A.; Michalczyk, A. Ceruloplasmin is regulated by copper and lactational hormones in PMC42-LA mammary epithelial cell culture models. *Metallomics* **2016**, 8, 941-950. DOI: 10.1039/c6mt00086j.
134. Choi, Y.K.; Kim, J.V.; Lee, J.T.; Cho, M.S.; Kang, B.S.; Choi, H.; Kim, Y. Association of maternal diet with zinc, copper, and iron concentrations in transitional human milk produced by Korean mothers. *Clin. Nutr. Res.* **2016**; 5, 15–25. DOI: 10.7762/cnr.2016.5.1.15.
135. Zavaleta, N.; Lanata, C.; Butron, B.; Peerson, J.M.; Brown, K.H.; Lönnerdal, B. Effect of acute maternal infection on quantity and composition of breast milk. *Am. J. Clin. Nutr.* **1995**, 62, 559–563. DOI: 10.1093/ajcn/62.3.559
136. Platonova, N.; Guolikhandanova, N.; Tsymbalenko, N.; Zhiguleva, E.; Zhivulko, T.; Vasin, A.; Evsukova, I.; Puchkova, L. Milk ceruloplasmin is a valuable source of nutrient copper ions for mammalian newborns. *J. Trace Elem. Med. Biol.* **2007**, 21, 184–193. DOI: 10.1016/j.jtemb.2007.04.003.
137. Tsymbalenko, N.V.; Giulikhandanova, N.E.; Platononova, N.A.; Babich, V.S.; Evsiukova, I.I.; Puchkova, L.V. Regulation of ceruloplasmin gene activity in mammary gland cells. *Genetika* **2009**, 45, 390–400. DOI: 10.1134/S1022795409030132.
138. Kılıç Altun, S.; Dinç, H.; Temamoğulları, F.K.; Paksoy, N. Analyses of essential elements and heavy metals by using ICP-MS in maternal breast milk from Şanlıurfa, *Turkey. Int. J. Anal. Chem.* **2018**, 2018, 1784073. DOI: 10.1155/2018/1784073.
139. Adkins, Y.; Lepine, A.J.; Lönnerdal, B. Changes in protein and nutrient composition of milk throughout lactation in dogs. *Am. J. Vet. Res.* **2001**, 62, 1266–1272. DOI: 10.2460/ajvr.2001.62.1266.
140. Cerveza, P.J.; Mehrbod, F.; Cotton, S.J.; Lomeli, N.; Linder, M.C.; Fonda, E.G.; Wickler, S.J. Milk ceruloplasmin and its expression by mammary gland and liver in pigs. *Arch. Biochem. Biophys.* **2000**, 373, 451–461. DOI: 10.1006/abbi.1999.1572.
141. Grace, N.D.; Pearce, S.G.; Firth, E.C.; Fennessy, P.F. Concentrations of macro- and micro-elements in the milk of pasture-fed thoroughbred mares. *Aust. Vet. J.* **1999**, 77, 177–180. DOI: 10.1111/j.1751-0813.1999.tb11230.x.
142. Keen, C.L.; Lonnerdal, B.; Clegg, M.S.; Hurley, L.S.; Morris, J.G.; Rogers, Q.R.; Rucker, R.B. Developmental changes in composition of cats milk: trace elements, minerals, protein, carbohydrate and fat. *J. Nutr.* **1982**, 112, 1763–1769. DOI: 10.1093/jn/112.9.1763.
143. Chu, Y.L.; Sauble, E.N.; Cabrera, A.; Roth, A.; Ackland, M.L.; Mercer, J.F.; Linder, M.C. Lack of ceruloplasmin expression alters aspects of copper transport to the fetus and newborn, as determined in mice. *Biometals* **2012**, 25, 373–382. DOI: 10.1007/s10534-011-9509-3.
144. Theophilos, M.B.; Cox, D.W.; Mercer, J.F. The toxic milk mouse is a murine model of Wilson disease. *Hum. Mol. Genet.* **1996**, 5, 1619–1624. DOI: 10.1016/S0168-9525(97)81397-2.
145. Coronado, V.; Nanji, M.; Cox, D.W. The Jackson toxic milk mouse as a model for copper loading. *Mamm. Genome.* **2001**, 12, 793–795. DOI: 10.1007/s00335-001-3021-y.
146. Puchkova, L.V.; Aleinikova, T.D.; Bichevaia, N.K.; Mokshina, S.A.; Platonova, N.A.; Sasina, L.K.; Skvortsova, N.N.; Tsymbalenko, N.V.; Chebotar', N.A.; Gaitskhoki, V.S. A comparative study of the transport dynamics of the peptide moiety of the milk ceruloplasmin molecule in the body of rats with embryonic and adult types of copper metabolism. *Ontogenez* **1999**, 30, 31–39.
147. Tavassoli, M.; Kishimoto, T.; Kataoka, M. Liver endothelium mediates the hepatocyte's uptake of ceruloplasmin. *J. Cell Biol.* **1986**, 102, 1298-1303. DOI: 10.1083/jcb.102.4.1298.

148. Bauerly, K.A.; Kelleher, S.L.; Lönnerdal, B. Effects of copper supplementation on copper absorption, tissue distribution, and copper transporter expression in an infant rat model. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2005**, 288, G1007–G1014. DOI: 10.1152/ajpgi.00210.2004

149. Ferreira, C.R.; Gahl, W.A. Disorders of metal metabolism. *Transl. Sci. Rare Dis.* **2017**, 18, 101–139. DOI: 10.3233/TRD-170015.

150. Cheah, D.M.; Deal, Y.J.; Wright, P.F.; Buck, N.E.; Chow, C.W.; Mercer, J.F.; Allen, K.J. Heterozygous tx mice have an increased sensitivity to copper loading: implications for Wilson's disease carriers. *Biometals* **2007**, 20, 751–757. DOI: 10.1007/s10534-006-9038-7.

151. Celauro, E.; Muka, A.; Fierro-González, J.C.; Wittung-Stafshed, P. Copper chaperone ATOX1 regulates pluripotency factor OCT4 in preimplantation mouse embryos. *Biochem. Biophys. Res. Commun.* **2017**, 491, 147–153. DOI: 10.1016/j.bbrc.2017.07.064.

152. Ozumi, K.; Sudhahar, V.; Kim, H.W.; Chen, G.F.; Kohno, T.; Finney, L.; Vogt, S.; McKinney, R.D.; Ushio-Fukai, M.; Fukai, T. Role of copper transport protein antioxidant 1 in angiotensin II-induced hypertension: a key regulator of extracellular superoxide dismutase. *Hypertension* **2012**, 60, 476–486. DOI: 10.1161/HYPERTENSIONAHA.111.189571.

153. Murata, K.; Fang, C.; Terao, C.; Giannopoulou, E.G.; Lee, Y.J.; Lee, M.J.; Mun, S.H.; Bae, S.; Qiao, Y.; Yuan, R.; Furu, M.; Ito, H.; Ohmura, K.; Matsuda, S.; Mimori, T.; Matsuda, F.; Park-Min, K.H.; Ivashkiv, L.B. Hypoxia-sensitive COMMD1 integrates signaling and cellular metabolism in human macrophages and suppresses osteoclastogenesis. *Immunity* **2017**, 57, 66–79. DOI: 10.1016/j.immuni.2017.06.018.

154. Mu, P.; Akashi, T.; Lu, F.; Kishida, S.; Kadomatsu, K. A novel nuclear complex of DRR1, F-actin and COMMD1 involved in NF-κB degradation and cell growth suppression in neuroblastoma. *Oncogene* **2017**, 36, 5745–5756. DOI: 10.1038/onc.2017.181.

155. Riera-Romo, M. COMMD1: A multifunctional regulatory protein. *J. Cell. Biochem.* **2018**, 119, 34–51. DOI: 10.1002/jcb.26151.

156. Rao, M.R.; Hediger, M.L.; Levine, R.J.; Naficy, A.B.; Vik, T. Effect of breastfeeding on cognitive development of infants born small for gestational age. *Acta Pediatr.* **2002**, 91, 267–274. DOI: 10.1111/j.1651-2227.2002.tb01713.x.

157. Zhou, G.; Ji, X.; Cui, N.; Cao, S.; Liu, C.; Liu, J. Association between serum copper status and working memory in schoolchildren. *Nutrients* **2015**, 7, 7185-7196. DOI: 10.3390/nu7095331.