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Review Article

Essential metals in health and disease

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ABSTRACT

In total, twenty elements appear to be essential for the correct functioning of the human body, half of which are metals and half are non-metals. Among those metals that are currently considered to be essential for normal biological functioning are four main group elements, sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca), and six d-block transition metal elements, manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn) and molybdenum (Mo). Cells have developed various metallo-regulatory mechanisms for maintaining a necessary homeostasis of metal-ions for diverse cellular processes, most importantly in the central nervous system. Since redox active transition metals (for example Fe and Cu) may participate in electron transfer reactions, their homeostasis must be carefully controlled. The catalytic behaviour of redox metals which have escaped control, e.g. via the Fenton reaction, results in the formation of reactive hydroxyl radicals, which may cause damage to DNA, proteins and membranes. Transition metals are integral parts of the active centers of numerous enzymes (e.g. Cu,Zn-SOD, Mn-SOD, Catalase) which catalyze chemical reactions at physiologically compatible rates. Either a deficiency, or an excess of essential metals may result in various disease states arising in an organism. Some typical ailments that are characterized by a disturbed homeostasis of redox active metals include neurological disorders (Alzheimer's, Parkinson's and Huntington's disorders), mental health problems, cardiovascular diseases, cancer, and diabetes. To comprehend more deeply the mechanisms by which essential metals, acting either alone or in combination, and/or through their interaction with non-essential metals (e.g. chromium) function in biological systems will require the application of a broader, more interdisciplinary approach than has mainly been used so far. It is clear that a stronger cooperation between bioinorganic chemists and biophysicists - who have already achieved great success in understanding the structure and role of metalloenzymes in living systems - with biologists, will access new avenues of research in the systems biology of metal ions. With this in mind, the present paper reviews selected chemical and biological aspects of metal ions and their possible interactions in living systems under normal and pathological conditions.

1. Introduction

The six elements, H, C, N, O, P and S, are used to build all of the important molecules and biomolecules present in living systems, including proteins, nucleic acids and biomembranes. If we add Ca to this list of elements, about 98% of the elemental mass of the human body is thus accounted for, and the total is brought up to almost 100% by adding another five elements, Na, Mg, S, Cl and K [1].

In total, twenty elements appear to be essential for humans: ten of which are metals and ten are non-metals. In addition to sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca), essential metallic elements also include manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn) and molybdenum (Mo), which are collectively known as trace elements (Fig. 1) [2].

Several other elements have been proposed as essential for humans, of which chromium has become popular as a nutritional supplement for

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weight loss, even though it has been the subject of extensive debate in regard to the actual roles it may play in biological systems [3]. While Cr (VI) has been classified from a number of studies as a carcinogen, trace amounts of Cr(III) are necessary for the metabolism of lipids and sugars [3]. Accordingly, the current state of knowledge holds that chromium is a beneficial, rather than an essential element in living organisms. Elements such as boron, silicon, vanadium, nickel and tungsten, have been found to be essential, but only in a restricted number of species.

Accepting that metal ions have vital biological roles, not surprisingly, if they are present in excess, they can produce toxic effects. Either a deficiency or an excess of essential metals is connected with many disease states of organisms and studies at the molecular level have been made to elucidate potentially relevant mechanisms for this. Thus the most significant results obtained from such investigations may serve in the design of drugs used to treat metal-related diseases [4]. The aim of the present paper is to consider the biological roles of essential metals in different aspects of health and disease.

2. Chemistry of essential metals

The biological functioning of ten essential metal elements is closely connected to their physico-chemical properties, of particular importance being their ligand binding strengths and mobilities (Table 1).

The mobility of metals is inversely correlated with their ligand binding strength (Table 1) [5]. The metal ions, Na^+, Mg^{2+}, K^+, and Ca^{2+}, have weak to moderate ligand binding strengths and possess high mobilities in biological systems. Conversely, Fe, Cu, Zn, Co, Mn and Mo are transition metal elements (containing *d*-electrons), which form discrete metal complexes in which ligands are strongly bound and adopt a wide range of coordination modes.

2.1. Sodium and potassium

Na^+ and K^+ are hard Lewis acids and can bind weakly to organic ligands, along with hydrogen and chlorine ions, and are able to generate electrochemical gradients across biological membranes which are responsible for their electrical excitability [6]. In solution, only single +1 oxidation states are presented by Na^+ and K^+, which have the electronic configurations of Neon and Argon, respectively. Both ions have relatively low electrophilicities due to their low charge/radius ratios. However, the physico-chemical properties of the channel walls maintain a relatively low dielectric environment, thus ensuring a high hydration number for the permeating K^+, and a sufficiently tight restriction to force the competing Na^+ to adopt an unfavourable 8-fold coordination. The interaction of both Na^+ and K^+ with ligands is weak and predominantly ionic in origin. In the solid state, the coordination numbers are in the range of 4–12 and the coordination geometry is variable, although with O-donor ligands such as hydroxyl and carboxylate it is predominantly octahedral [7]. As a result of the biological concentrations of Na^+ and K^+ and their ability to accommodate four or more ligand groups, these two cations are selected over H^+ within the same biological space, and finely tuned chelating groups of ionophores can provide specific binding sites for Na^+ and K^+. X-ray diffraction studies revealed that both these cations can act as cofactors for several proteins by binding at specific sites, thus confirming the specificity of the proteins for monovalent cations and their important role served in the biological activity of proteins [8,9].

2.2. Calcium and magnesium

Ca^{2+} and Mg^{2+} are hard Lewis acids and prefer binding to hard Lewis bases. Hence they both possess intermediate binding strengths towards organic ligands and have lower mobilities than Na^+ or K^+ [10,11]. In addition, Ca^{2+}-signalling is a significant factor in the mechanisms that underpin the life and death of a cell.

Many enzymes require Mg^{2+} as an essential cofactor, especially those which use ATP for various cellular activities. Mg^{2+} also acts as a regulator of K^+ and Ca^{2+} ion channels and can antagonize the function of calcium [12]. Under normal circumstances, the cytoplasmic concentration of calcium is very low; however, the binding of specific ligands to proteins of the plasmatic membrane results in calcium release from intracellular compartments which in turn activates a number of enzymes that are important for metabolic functions.

Ca^{2+} is most frequently coordinated by oxygen donors, which originate from glutamic and aspartic acids, carbonyl oxygens, peptide backbone and water. The coordination number of Ca^{2+} is in the range of five to eight, with the most common being seven. In addition to the coordination of donor atoms originating from proteins, Ca^{2+} is often coordinated by one or two water molecules, although there are some examples of three or four coordinated water molecules, for example in proteinase K [13]. The binding constants determined for Ca^{2+} vary from weak binders (e.g. EGF-like domains, log $K_a = 3$) to strong binders (e.g. thermolysin, log $K_a = 11$). From a detailed inspection of a variety of calcium-binding proteins, it has been demonstrated that there is no correlation between the calcium binding strength and the structural properties of coordinating ligands, such as their electrical-charge, the number of protein-based ligands or the number of coordinated water molecules. It appears that the key role in Ca^{2+} affinity is played by weak interactions, including the surface charge surrounding the binding site, the extent of conformational changes induced following calcium binding, and other factors.

Magnesium is one of the most abundant elements on Earth, typically found in the form of complex silicate minerals [10]. Metallic Mg oxidizes rapidly in air to form MgO in which the oxidation state of Mg is +2

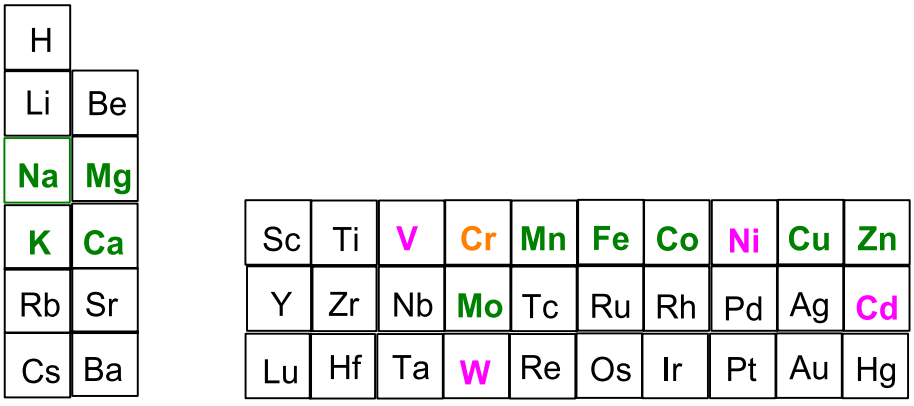

Fig. 1. Biologically relevant metals: Essential elements (green), elements that are essential only for some forms of life (pink) and one element – Cr, that is important only for certain biological functions (yellow).

Table 1
Essential metals and their oxidation states, ligand binding properties, mobility, function in biological systems, quantities occurring naturally in the human body, and daily dietary allowances.

Metal ion	Ligand binding properties	Mobility	Biological function	Mass of metals (g)	Daily dietary allowance for adults/infants (mg)
Na^{+}	Weak	High	Regulatory function: osmotic pressure, membrane potential,	65–115	1100-3300/260
K^{+}	Weak	High	enzyme activity Nerve action, osmotic pressure	155–195	2000-5500/530
Mg^{2+}	Intermediate	Semi-mobile	Energy metabolism ATP → ADP, chlorophyll	30	300-400/60
Ca^{2+}	Intermediate	Semi-mobile	Signalling, enzyme regulation, muscle contraction, skeletal system (Bones, teeth)	1100	800-1200/420
Zn^{2+}	Intermediate/Strong	Intermediate	Lewis acid (Carbonic anhydrase, carboxypeptidase), structural roles – zinc fingers, repair enzymes	2.5	15/5
$Co^{2+,3+}$	Strong	Low	Vitamin B12 coenzyme	0.003	0.2/0.001
$Cu^{+,2+}$	Strong	Low	Electron transfer (copper blue proteins), oxygen storage, transport proteins, ceruloplasmin	0.075	1.5–3.0/1.0
$Fe^{2+,3+}$	Strong	Low	Oxygen storage (Hemoglobin, hemerythrin,. Electron transfer Fe–S proteins, cytochromes)	4.2	10-20/7.0
$Mn^{2+,3+,4+}$	Strong	Low	Enzyme (phosphatase, mitochondrial Mn-SOD, photoredox activity PS II.	0.013	2-5/1.3
$Mo^{2+,3+,4+,5+,-6+}$	Strong	Low	Enzymes (nitrogenase, reductases, hydroxylases)	0.005	0.075–0.250/0.06

Adapted from: L. Virag, F. Erdodi, P. Gergely, Bioinorganic chemistry for medical students, University of Debrecen, Hungary, 2016, see ref. 5.

(Mg^{2+}), and the coordination number of Mg^{2+} is six with exclusively oxygen donor atoms. Mn^{2+} has been found to be a useful probe for ascertaining magnesium sites in proteins [14].

2.3. Manganese

All six essential transition metal elements contain electrons in *d*-shells and may therefore exist in several different oxidation states and participate in electron transfer reactions, along with the activation, storage and transport of molecular oxygen, and other important biological processes.

Manganese furnishes strongly oxidizing species in biological systems and exists in the oxidation states +2, +3, +4, adopting various coordination geometries. The coordination of N-donor and O-donor ligands to Mn results in a lowering of the reduction potential [7,15]. The most common ion, Mn(II), contains 5 d electrons (d^5) either in a high spin arrangement (S = 5/2) or in a low spin arrangement with just a single unpaired electron (S = ½). Hexa-aqua Mn(II) and Mn(III) complexes are not particularly stable, thermodynamically. Mn(IV) forms octahedral complexes, and also quite frequently forms multinuclear complexes with mixed Mn(III)/Mn(IV) valences. Divalent Mn is known to readily substitute for Zn(II), Fe(II), Co(II) in the metal active sites of enzymes, while frequently retaining their biological function [5].

2.4. Iron

Due to its electronic configuration, iron participates in single-electron transfer reactions, which makes this metal an active participant in the formation and metabolism of oxygen free radicals. In biological systems, iron is found in three oxidation states, Fe(II), Fe(III) and less frequently as Fe(IV) [7]. While Fe(II) is soluble at physiological pH, Fe(III) instead precipitates in the form of oxy- and hydroxy species. In biological systems Fe(II) is readily oxidized by molecular oxygen to Fe (III) and the superoxide radical anion [8]. In solution, the complex $[Fe(H_2O)_6]^{3+}$ hydrolyzes readily, especially as the pH increases; in contrast, the hexa-aqua Fe(II) complex $[Fe(H_2O)_6]^{2+}$ is relatively stable in solution. A very rich coordination and organometallic chemistry is known for iron [9], and Fe(III) complexes are found as high-spin or low-spin, depending on the ligand field strength. While high-spin Fe(III) complexes are the most stable, low-spin Fe(III) complexes, for example containing cyanide ligands, are also known. Divalent iron complexes are less stable than trivalent complexes and may undergo oxidation to form the latter.

2.5. Cobalt

The essential role of Cobalt is demonstrated by its integral functionality in the coenzyme vitamin B12, which is the only known naturally occurring organometallic compound containing a Co–C bond. The most common oxidation states of cobalt are +3 and + 2, in which it forms a variety of organic and inorganic salts [16,17], and typically gives rise to complexes with distorted octahedral coordination geometries. In behaviour, Co(III) is a hard Lewis acid, while Co(II) is a borderline Lewis acid. Co(II) forms stable complexes with many ligands, including water, chloride ion and ammonia. Trivalent cobalt(III) complexes can be prepared by the oxidation of Co(II) salts in the presence of a stabilizing auxiliary ligand.

2.6. Copper

Copper is central to many important biological processes, for example as a cofactor for a variety of enzymes, including photosynthesis, the transfer of electrons within biological molecules, iron metabolism, the scavenging of free radicals, and various neurological functions [7,8]. In biological systems, copper occurs in oxidation states of +1 (cuprous) and +2 (cupric), although it has been speculated that the +3 oxidation state may occur, transiently, under exceptional conditions [3,5]. Cu(I) is a soft Lewis acid and favours the soft coordination provided by S atoms, with coordination numbers of two, three or four. Cu(I) is a closed shell ion, with its d orbitals fully occupied by electrons: it is therefore diamagnetic, and not detectable by EPR spectroscopy, while also lacking characteristic features in optical spectra [5]. Accordingly, the majority of structural information regarding Cu(I) systems has been obtained from their luminescent properties, X-ray diffraction and EXAFS measurements. Cu(II) has nine electrons held in its *d*-orbitals and is, therefore, a paramagnetic ion active in the EPR spectroscopy. It is also a borderline Lewis acid and prefers harder N-donor atoms than is the case for Cu(I). The most common coordination numbers in Cu(II) compounds are four, five and six, but the actual coordination geometries around the Cu(II) ion are quite plastic. In addition to the results from X-ray diffraction measurements, EPR spectroscopy is very informative in regard to elucidating the coordination geometry of Cu(II) compounds both in the solid state and in fluid media. In addition, EPR spin trapping studies provide valuable information regarding the catalytic activities of Cu(II)/Cu(I) compounds in the formation of free radicals [18].

2.7. Zinc

In contrast to the compounds of other transition metals such as copper or iron, those of zinc are colourless due to its completely filled set of *d*-orbitals, both rendering the metal diamagnetic and preventing it from undergoing oxidation or reduction reactions. In common with divalent Fe^{2+}, Co^{2+} and Cu^{2+} ions, Zn^{2+} exhibits borderline Lewis acid properties, and is stable in biological environments. In its complexes, the coordination numbers of zinc vary from two to eight, but most frequently are found to be four, five and six. Due to the plasticity of the coordination polyhedrons of Zn complexes, the latter provide effective catalysts as a result of their ability to adopt transient coordination geometries. Application of ^{19}F NMR spectroscopy with the fluorinated chelator probe revealed that the level of free intracellular zinc is approximately 0.5 nM [19]. The values for the binding constants between Zn^{2+} and coordinating ligands are intermediate between those of magnesium and calcium and the other five essential transition metal elements. Zn^{2+} is an integral component of more than 200 different enzymes, the most important of which are involved in protein metabolism and immune function [20]. In addition, zinc is an important structural element in motifs known as zinc fingers, which occur in a variety of RNA and DNA binding proteins [21].

2.8. Molybdenum

Molybdenum occurs most frequently in oxidation states of +2 to +6, the most physiologically relevant of which are in the range of +4 to +6. Unusually, Mo is present in the zero oxidation state in $Mo(CO)_6$ [22]. The majority of Mo compounds are prepared from hexavalent Molybdenum trioxide, MoO_3, a compound that is produced in industrial quantities. While biologically important Mo compounds tend to exist in higher oxidation states, those formed in lower oxidation states, in aqueous solutions, tend to form dimeric, trimeric or even aggregated structures linked with -hydroxo or -oxo bridges and complemented with H_2O molecules. The aggregation of Mo compounds can be prevented by the coordination or chelation of ligands originating from protein residues, which results in the formation of "specific" coordination environments [7]. The redox potentials of physiologically relevant oxidation states of Mo (between +4 and + 6) are in the region of 0.35 V, with the molybdenum ion coordinated predominantly by negatively charged sulphur and oxygen donors such as sulphides, thiolates, and hydroxides. Nitrogen ligands are also known to coordinate to Mo, under physiological conditions [7].

3. Biology of essential metals in health and diseases

Essential metals are involved in many important biological functions, including catalytic action, the stabilizing ability of proteins, moving electrons from electron donors to electron acceptors, prevention of damage by ROS, building DNA, and the regulation of hormone levels, to name but a few [1,2]. The process of metal homeostasis ensures that the metals are distributed in the required quantities within cells, tissues and organs, on an appropriate time scale. Conversely, metal dyshomeostasis at various levels of an organism is a common denominator and cause of many illnesses, such as neurodegeneration, cancer, cardiovascular diseases, and diabetes. However, it should also be noted, that metal dyshomeostasis may be a consequence of disease. This section discusses predominantly the role of metals in health, and some causes of metal dyshomeostasis and the related incidence of human diseases.

3.1. Sodium and potassium

Sodium and potassium are key electrolytes for the maintenance of cellular homeostasis [6,23,24], and both are involved in the regulation of osmotic pressure, pH, the transport and distribution of water molecules in cellular compartments, cardiac development, energy metabolism, electron transfer reactions, and as cofactors for a variety of enzymes and their functions.

3.1.1. Sodium and potassium in health

The recommended daily intake of Sodium by the American Heart Association is no more than 2300 mg, while the physiological normal extracellular level of sodium lies in the rather narrow range 135–150 mmol/L, with the intracellular concentration of sodium being approximately 12 mmol/L [5].

Maintaining sodium homeostasis is very important and is achieved by a balance between its intake and excretion. The excretion of sodium is regulated by blood pressure, the antidiuretic hormone aldosterone and other factors [25], and organisms have evolved complex systems to maintain a critical sodium balance in case any of these mechanisms are disturbed, for example by xenobiotics or health disorders.

Potassium is necessary for the normal functioning of an organism at all levels [24]. The recommended daily intake of potassium in adults is 4700 mg, while breastfeeding women should increase this up to 5200 mg. Although sodium is a major cation in the extracellular fluid, potassium is the major metal present intracellularly, where the concentration is in the range of 140–150 mmol/L, which represents 98% of the total amount of potassium in the body; in contrast, the extracellular concentration of 3–5 mmol/L amounts to only 2% of the total pool [26]. A subtle regulation of the transmembrane chemical gradient for singly charged potassium ions is extremely important for maintaining the negative charge across the membrane, and is termed the Nernst equilibrium potential for K^+, as is necessary to oppose the transport of potassium ions along the concentration gradient. The equilibrium potential for potassium is −96 mV, corresponding to an extracellular concentration for this metal of 150 mmol/L and an intracellular concentration of 4 mmol/L [27]. The homeostasis of potassium is regulated by aldosterone, activation of β_2-adrenergic receptors stimulated for example by adrenaline, necrosis or cell death and heavy exercise. Rapid changes in potassium plasma levels may have fatal consequences.

Both sodium and potassium gradients across the membrane are maintained by the protein Sodium–Potassium-ATP-ase (known also as Na–K or sodium pump), which is responsible for the coupled antiport transport of Na^+ and K^+ ions across the plasma membrane using energy provided by ATP [24,25]. ATP hydrolysis ensures transport of three sodium cations out of the cell and two potassium cations into the cell. The release of potassium from the cell is driven by passive diffusion. The pump is located on the surface of cell as was discovered in 1957 by the Danish biochemist Jens C. Skou for which he was awarded the 1997 Nobel Prize [28]. It is essential for sodium to be pumped out of the cell, otherwise water is retained within which may cause the cell to swell or even burst.

Electrochemical gradients (gradients in both concentrations and charge) of sodium and potassium cations maintain many physiological processes in various organs. An unusually highly expressed Na–K pump is present in the kidney and is capable of reaching as much as 50 million pumps in a single kidney cell [29]. The removal of metabolic waste products and foreign toxic particles from blood, by a healthy kidney, is achieved by means of a sodium gradient. Other important kidney functions include regulation of the levels of electrolytes in the blood, and the tubular reabsorption of water, amino acids, glucose and other solutes back into the extracellular fluid and circulation.

Na,K-ATP ase (fourth Na,K-ATPase alpha isoform) plays an important role in human sperm motility, since the sperm need the pump to regulate its transmembrane potential, especially when penetrating into the egg [30].

Remarkably, to run the Na,K-pump accounts for the majority of the energy used by the brain [6]. Both isoforms, (the ubiquitous alpha1, and the more selectively expressed alpha3) of Na,K-ATPase are required for regulation of basal neuronal functions. Isoform alpha3 alone plays an important role in the restoration of the large intracellular concentration of sodium cations, and control of membrane potential. In astrocytes, a

Na,K-pump is used to achieve a transient decrease of the extracellular potassium concentration, which is a part of the mechanism for the modulation of neuronal activity, including control of neurotransmitter receptor functioning [31]. Brain grey matter consumes a large amount of energy to maintain neuronal cellular signalling, which amounts to approximately 30–50 μmol of ATP per gram per minute. Interestingly, if the Na,K-pump is blocked, the whole brain energy use is reduced by about 50% [32].

3.1.2. Disorders of sodium and potassium homeostasis

Hyponatremia is one of the most common electrolyte-related diseases and occurs when the sodium concentration in serum is abnormally low (less than 135 mmol/L). The condition arises when the intake of water exceeds its excretion rate (dilutional disorders) or a rapid depletion of sodium occurs (depletional disorder) [33].

Causes of dilutional disorders include: renal failure, a syndrome of inappropriate antidiuretic hormone secretion (SIADH), diseases of the neuroendocrine system, hyperglycemia, heart failure, hepatic disease - mainly cirrhosis, occasional, but fatal complications of diuretic therapy.

Another cause of hyponatremia is the very rare but inherited Bartter's syndrome (discovered in 1962 by Bartter and coworkers), which originates in genetic mutations of the Na^+–K^+–$2Cl^-$ cotransporter encoding gene [34]. This syndrome is manifested by salt reabsorption, which in turn causes a reduction in the volume of extracellular fluid.

Depletional disorders may arise from abnormal sweating or as a result of vomiting and diarrhea usually caused by bacterial enterotoxins or enteroviruses.

Another, less common, disorder related to sodium is hypernatremia [35], which may arise from either a loss of water (dehydration) or an excess intake of sodium. Loss of water can take place from the gastrointestinal tract by vomiting, sweating, or urination. Hypernatremia occurs mainly among individuals with a disordered sense of thirst (patients with mental disorders or who are elderly). Less frequently, hypernatremia arises from an intake of sodium in excess of compensating amounts of water, as may occur in patients who are supplied with large amounts of water, either in critical care or during the course of hypertonic therapy by sodium bicarbonate during a cardiac arrest, or as a result of salt ingestion [36].

Disruptions of potassium homeostasis are very common in clinical practice, and the term hypokalemia is used for when blood potassium levels are low (less than 3.5 mmol/L in adults) [37]. Should this fall below 2.5 mmol/L, immediate medical care is required. Potassium homeostasis depends on the ratio of intake/absorption to excretion (external balance) and the distribution of potassium in intracellular and extracellular fluids (internal balance) [38]. In addition to blood tests, hypokalemia can be diagnosed using changes in electrocardiograms. One of the most frequent causes of hypokalemia is loss of potassium via urine from patients, suffering from high blood pressure and heart conditions, who are treated with diuretics. Potassium loss also occurs as a result of vomiting, diarrhea, excessive sweating, folic acid deficiency, heavy alcohol consumption, and chronic kidney diseases. Low potassium levels may be diagnosed from an abnormal heartbeat [24].

Hypokalemia may also occur among people with eating disorders such as anorexia, bulimia and in cases of starvation. Refeeding and weight restoration in patients with anorexia may increase the secretion of insulin which in turn promotes the cellular uptake of potassium, removing it from the blood, and resulting in hypokalemia. The consequence is a disturbed balance of the membrane electrochemical potential, which manifests clinically as cardiac arrhythmias and even cardiac arrest [39]; neuromuscular dysfunction is also frequently observed.

Potassium is important in protein synthesis and accordingly, individuals, often children from a low social background on low protein diets, have a low muscle mass. Renal loss of potassium is a rather complex problem and may occur from the adverse effect of anticancer therapy (*cis*-platin) or use of antibiotics (gentamicin, penicillin) or diuretics. Hypokalemia occurs among patients with congestive heart failure as a result of altered of Na,K-pump activity, with an oxidative stress-modified distribution of potassium and neurohormonal activation [40]. Heart failure is known to stimulate metabolic changes such as insulin resistance, which may exacerbate the initial condition [41].

Although less common than hypokalemia, another pathological condition associated with abnormal potassium blood levels (>5 mmol/L), is hyperkalemia [42] whose symptoms include weakness, respiratory problems, paralysis, and may be signified by changes in the electrocardiogram. Hyperkalemia may originate from an imbalance between intake and excretion, or from the uneven distribution of potassium between intracellular and extracellular regions. Although it may occur in patients with normal renal function only after a very high intake of potassium, in patients whose renal function is impaired, even a small increase in potassium consumption may result in severe hyperkalemia [43]. A suppressed renal excretion of potassium may be caused by either acute or chronic renal insufficiency. There are also several drugs which may interfere with the urinary excretion of potassium by different mechanisms, for example Digoxin for heart failure treatment (Na, K-pump inhibitor), beta blockers (drugs used to treat cardiovascular diseases, mainly high blood pressure), heart failure and arrhythmias (renin secretion inhibitors), non-steroidal anti-inflammatory drugs used to treat pain and inflammation (renal prostaglandin synthesis inhibitors), Acetyl-cholinesterase inhibitors - drugs used to inhibit activity of acetylcholinesterase (suppressed aldosterone synthesis in the adrenal cortex), Amiloride-Midamor, used to treat hypertension (inhibits epithelial sodium channels), Spironolactone, used for the treatment of hypertension and heart failure (inhibits synthesis of adrenal aldosterone), Cyclosporine, used to suppress transplanted organ rejection (increases efflux of potassium from cells), Heparin, used as an anticoagulant to prevent formation of blood clots (inhibits synthesis of adrenal aldosterone) and several other drugs [44,45].

The correct treatment of hyperkalemia depends on the cause and severity of the disease, but requires frequent potassium measurements and monitoring of changes in the electrocardiogram. More intense treatment is required in cases of large and rapid increases in the potassium levels. Medical interventions provide temporary improvements based on the influx of potassium into the intracellular space. For patients with acute hyperkalemia and sufficient renal function, treatment is often effective, but where renal functions are impaired, severe hyperkalemia (potassium blood level >6.6 mmol/L) therapy is more difficult and renal replacement therapy (RRT) is often required also including kidney transplantation [46].

Patients suffering from hyperkalemia should fully eliminate their sources of potassium intake, suspend taking any drugs (see above) that aggravate the condition, and maintain the influx of potassium into the intracellular space using dextrose, insulin beta-adrenergic agonists or sodium bicarbonate administration. The excretion of potassium may be achieved by the administration of diuretics (furosemide), and RRT, including hemodialysis or hemofiltration [6].

3.2. Calcium and magnesium

Calcium is one of the most important nutritional elements, and is critical for healthy bones and many vital physiological functions. An adult of average weight needs to consume about 1000 mg calcium per day, and for women over 50 and men over 70 years of age the recommended daily amount is 1200 mg. During pregnancy, up to 1300 mg/day is advised, in order to achieve a proper physiological balance [11]. The plasma concentration of calcium in human fluids tends to range between 2.1 and 2.6 mmol/L, although there are exceptions, for example the extracellular concentration of calcium in the inner ear is in the micromolar range.

Magnesium is the second most abundant intracellular metal and plays important roles in the physiological functions of the cardiovascular and neurological systems and muscles [10,47]. This metal is a cofactor for several hundred enzymes and it acts as an activator for at

least 200 enzymes [48].

In contrast to magnesium, whose concentration gradient across the membrane is very low, that for calcium is about 20,000, with the intracellular free concentration of calcium being ca 100 nM, and a concentration in the extracellular space in the millimolar range [11]. The majority of calcium present in the human body is present in as Ca^{2+} ions, complexes, and inorganic salts. The ratio between the total and ionized concentrations of calcium varies from fluid to fluid and is not straightforward to determine.

3.2.1. Calcium and magnesium in health

To maintain the intracellular concentration of free calcium in the cytosol of cells at nanomolar levels, mechanisms exist to remove the excess of free calcium, transporting it either to the extracellular space or to intracellular calcium stores. This mechanism depends on calcium pumps at the plasma membrane or endoplasmic reticulum. In addition, calcium buffering, mediated by calcium-binding proteins, also serves to maintain the cytosolic concentration of free calcium, [Ca2+] down to nanomolar levels (ca 100 nM) [11].

Calcium signalling plays a fundamental role in both the life and death of cells. Positively charged calcium ions, together with negatively charged phosphate groups are key cellular signalling species. Cytosolic calcium ensures allosteric regulation of proteins and enzymes, and the activation of membrane ion channels as a second messenger. Accordingly, the low (nanomolar) cytoplasmic concentration of calcium is tightly controlled and time dependent, with temporary variations of intracellular calcium triggering a signal for transmission. The process of fertilization is a good example of this, during which a series of sharp temporary cytosolic intracellular calcium increases occurs in mammalian oocytes, for about 24 h, following their fusion with sperm. Such a sharp and temporary increase of calcium concentration is necessary for the release from the cell cycle arrest to occur, and for the stimulation of the fertilized oocyte-to-embryo transition [49].

The calcium concentration in human blood plasma is regulated by three hormones, namely parathyroid (responsible for increase of calcium concentration in the blood), then by calcitonin (the protein hormone which lowers plasma concentration of calcium by inhibiting the target cells of this hormone (osteoclast) and finally by the active form of vitamin D3 [11].

While calcium and sodium are principal cations that occur in extracellular space, the most abundant intracellular metal cations are magnesium and potassium (and also zinc). While all the potassium and the majority of sodium are present in cells as free cations, most of the magnesium, calcium (and zinc) are present as bound to organic and inorganic ligands [5,7]. Indeed, the concentration of free calcium in cells is negligible with respect to the total concentration of the metal. The calcium ion can coordinate 4–12 oxygen donor atoms from calcium binding proteins, where the carboxyl and carbonyl oxygens (and water) are the most frequent donor ligands. Calcium is known to bind to several thousands of various proteins in order to: (i) initiate necessary stereochemical changes of active sites, (ii) dimerize proteins, (iii) create a sufficiently large electrostatic potential that can, in turn, promote the association of proteins with anionic membrane leaflets, (iv) compensate for negatively charged protein loops to ensure penetration of negatively charged aromatic amino acids into the cell membrane bilayer, along with many other functions [50].

Magnesium is a natural calcium antagonist (calcium channel blocker) and has anti-inflammatory properties. While the recommended daily intake of magnesium is 420 mg for men and 330 mg for women, about 60% of people consume less than this. In healthy subjects, the serum concentration of manganese is in the range 0.7–1.1 mmol/L. Manganese homeostasis depends on the capacity of the intestines to absorb manganese from food, the storage of manganese in bones, and renal functions that regulate the urinary excretion of manganese. More than half of the total amount of magnesium in the body is located in bones, 30–40% in skeletal muscles and only 1% in extracellular fluid [51]. As present in the skeletal tissues, magnesium controls the amount of ATP available for muscle contraction and the uptake and release of calcium, while in the soft tissues it is a cofactor for many enzymes. It also serves an important role in transferring the electrical signal responsible for muscle contraction and heart beat.

The Transient Receptor Potential Cation Channel Subfamily M Member 6 (*TRPM6*) gene is expressed in the colon and kidney and encodes the first protein identified that is important in the active reabsorption of magnesium [52]. TRPM6 forms a hetero-tetramer with TRPM7 and has a permeability for magnesium that is several times higher than for calcium ions. The protein allows reabsorption of these cations via the apical plasma membrane of the epithelial cells. The exact structure of TRPM6 is still not known but the mutations in the TRPM6 gene have been shown to be linked with several diseases including hypomagnesemia (see below).

Magnesium is a component of the DNA and RNA ternary structures that electrostatically compensates the negative charges of oxygen and nitrogen. The majority of studies so far have been devoted to the interaction of magnesium with tRNA, although magnesium has also been shown to stabilize secondary and tertiary structures of B-DNA by binding to a minor groove.

Since magnesium is a physiological calcium antagonist, the Mg/Ca ratio is of great importance in the activity of Ca-ATP-ases and other proteins, for calcium transport. Even mild disturbances in the magnesium content of cells may in turn cause changes in calcium-signalling or calcium-induced toxicity [53].

The important role of magnesium in heart functions is well established, through influencing myocardial metabolism, calcium homeostasis, cardiac output, vascular resistance and other important factors. Magnesium is critical to regulating cardiac activity by maintaining the proper functioning of the ion channels, thus directly influencing the electric properties of membranes [54].

Based on the forced vital capacity and expiratory volume, an important role for dietary magnesium in lung function has been inferred. This connection is based on the influence of magnesium on (i) vasodilator and bronchodilator effects, (ii) regulation of acetylcholinesterase release and (iii) its anti-inflammatory properties.

3.2.2. Disorders of calcium and magnesium homeostasis

Disturbed calcium homeostasis can be either a cause or a consequence of various pathological dysfunctions. Hypercalcemia is a condition where blood calcium levels increase abnormally (>2.6 mM/L) [55].

Symptoms of hypercalcemia include: frequent urination and excessive thirst, as a result of aberrant kidney activity in filtering excess calcium, the occurrence of kidney stones, bone pain and weakness due to depleted calcium from bones, neurological and psychological disturbances including depression, altered heart functions, including arrythmias and other cardiovascular issues, and digestive problems such as nausea, vomiting, and constipation.

Causes hypercalcemia include various diseases, such as cancers (predominantly lung, breast and renal cancers), hyperparathyroidism, granulomatous disease, dehydration, and the effects of drugs (hydrochlorothiazide and diuretics), intoxication by vitamin D and vitamin A [56]. Causes of cancer-induced hypercalcemia are (i) overproduction of parathyroid hormone related peptide by cancer cells, (ii) aberrant conversion of vitamin D to 2-OH-vitamin D by lymphomas, resulting in enhanced absorption of calcium by gut mucosa, (iii) metastatic bone destruction, and much less commonly, (iv) release of parathyroid hormone by cancer cells [57].

Management of hypercalcemia depends on the level of serum calcium and the absence/presence of clinical manifestations. Mild hypercalcemia (<2.9 mmol/L) is characterized by mild symptoms, and treatments include the administration of diuretics. Patients with moderate levels of serum calcium (3–3.5 mmol/L) may develop symptoms and require treatment, while high levels of serum calcium (>3.5 mmol/

L) require intense treatments [58]. A first step is usually to administer fluids intravenously to restore the extracellular volume. Bis-phosphonates are well tolerated and used in cancer-induced hypercalcemia, as a first line therapy to reduce bone resorption [59]. Calcitonin reduces levels of calcium temporarily, and is used in combination with bi-phosphonate and intravenous fluids. For patients with both acute hypercalcemia and kidney injury, the most suitable treatment is hemodialysis using low concentrations of calcium dialysate (<1 mmol/L) [60].

Hypocalcemia (serum calcium <2.12 mM/L) is characterized by a net calcium efflux from extracellular fluid, often through the kidneys, and in greater amounts than can be replaced by the bones or intestines. About 80% of calcium is bound to serum albumin, and therefore patients with either very high (hyperalbuminemia) or very low (hypoalbuminemia) albumin levels may have incorrectly diagnosed (too high or too low) levels of serum calcium. To correct for these abnormal values of albumin is not an especially accurate procedure, and so a determination of Ca^{2+} should be made directly. Symptoms of hypocalcemia are muscle spasms, epileptic seizures, cramps, cognitive impairment, and cardiovascular problems.

One of the common causes of hypocalcemia is hypoparathyroidism, a disorder characterized by impaired production of parathyroid hormone or of vitamin D. A low level of calcium is also accompanied by high phosphorus levels (hyperphosphatemia) [11].

Patients with impaired renal function, an increased intake of phosphate-containing laxatives or tissue breakdown due to tumour lysis, may have increased levels of phosphorus (>1.46 mmol/L) and develop hyperphosphatemia. Abnormal phosphorus levels may induce hypocalcemia as the body struggles to maintain a balance between phosphorus and calcium.

Hypocalcemia can be triggered by certain anticancer drugs such as cisplatin, antiepileptics, diuretics and proton pump inhibitors, both hypo- and hyper-magnesemia, radiation therapy of the parathyroid glands, and renal or liver diseases which cause vitamin D deficiencies [56]. Since glucocorticoids inhibit intestinal vitamin D-driven absorption of calcium, patients taking these hormones should also increase their consumption of vitamin D. Antifungal agents are also known to inhibit vitamin D activity via hydroxylation blockage of the vitamin in the kidney.

Hypocalcemia may result from a massive blood transfusion, during which blood containing an anticoagulant - citrate phosphate dextrose with adenine - can form calcium-citrate complexes. Hypocalcemia can also be caused by magnesium deficiency (hypomagnesemia), since a low level of magnesium suppresses the secretion of parathyroid hormone (hypoparathyroidism) which brings on the condition.

Management of acute hypocalcemia is usually based on an intravenous supply of calcium. However, a rapid increase of serum calcium levels may cause arrythmias, and so it is necessary to simultaneously monitor cardiac functions, in particular for patients undergoing digoxin therapy. Chronic hypocalcemia is treated using an oral intake of calcium and vitamin D, in addition to hypomagnesemia treatment. It is found that calcium is absorbed most efficiently in the form of its carbonate and citrate. Treatment of hypocalcemia caused by hypomagnesemia with calcium and vitamin D is often not very successful; the best results have been achieved by magnesium supplementation alone [61].

Hypermagnesemia is the most commonly occurring disease related to magnesium homeostasis, along with hypomagnesemia which is less frequently observed [62]. Hypermagnesemia is characterized by abnormally elevated blood magnesium levels, and is usually accompanied by impaired renal function. In contrast, hypomagnesemia, is associated with abnormally low levels of magnesium, and since, in its early stages of development, it presents non specific symptoms, it is often not appropriately treated. It is thought that the condition may originate from a concurrent disease.

As described above, magnesium homeostasis involves an equilibrium between magnesium absorption in the gastrointestinal tract, storage, mainly in the bones, and excretion by the kidneys. Hypermagnesemia is diagnosed when the blood level of magnesium exceeds 1.1 mmol/L and occurs most often in patients with impaired renal function who have been prescribed drugs containing bound magnesium or magnesium salts, including those that can relieve constipation (laxatives) and proton pump inhibitors which neutralize stomach acid. Typical symptoms include problems such as cardiac arrythmia and an unusually slow heart rate – bradycardia, which occur as a result of disrupted electrical signals in the heart. Other symptoms include nausea and vomiting, muscle weakness, shortness of breath and impaired tendon reflexes [63].

Hypermagnesemia occurs among pregnant women who are suffering an abnormal rise in blood pressure (preeclampsia) which is treated by administering magnesium sulphate either intramuscularly or intravenously. The detailed etiology of hypermagnesemia is unknown, although it occurs in ca 10% of patients with diagnosed acute or chronic kidney disease, accompanied by alcoholism and malnourishment. Patients taking proton pump inhibitors also form a high risk group. Other causes of hypermagnesemia are an underactive thyroid gland (hypothyroidism) and a highly reactive parathyroid gland (hyperparathyroidism). When hyperparathyroidism is accompanied by a disturbed calcium metabolism, an increased magnesium absorption occurs [64]. Patients with bipolar disorder who are taking lithium compounds can also have a reduced excretion of magnesium, which results in hypermagnesemia.

The management of hypermagnesemia depends on the exact condition, and in its mild form is simply based on eliminating all external sources of magnesium; more severe cases require the intravenous administration of calcium gluconate, which acts as an antagonist of magnesium at the site of action. Increased renal excretion of magnesium can be achieved by diuretics and hemodialysis [65].

Hypomagnesemia occurs when the magnesium serum level drops below 0.7 mmol/L, as a result either of a low intake of magnesium and/or increased urinary excretion [66]. Since alcohol consumption increases the renal excretion of magnesium, it often occurs in patients with problems of alcohol abuse. The main clinical manifestations of the condition are nausea, weakness, and cardiological problems, while further symptoms include psychiatric and neurological disorders.

Particular attention should be paid to patients with inexplicable hypocalcemia whose serum concentration of magnesium is normal, and to those with inexplicable neurological problems and prolonged diarrhea or who have received metal-based chemotherapy. A temporary deficiency of magnesium occurs in patients with acute myocardial infarction [10].

The majority of symptoms can be cured by increasing the amount of magnesium in the diet or with oral supplements, while an increased intake of vitamin D3, selenium and vitamin B6 may also be beneficial. In severe hypomagnesemia, intravenous supplementation with magnesium is necessary [67].

Cardiovascular pathologies are primarily associated with low magnesium serum levels. As a natural blocker of calcium channels or as a sodium antagonist, magnesium plays a key role in maintaining the normal electrical activity of the heart. In accordance with this, low magnesium levels are linked with a variety of cardiac arrythmias, including tachycardia, fibrillation, and extra systoles. A low level of magnesium in cardiac patients is mainly due to increased urinary excretion as a result of therapy by digoxin and diuretics.

It is significant that epidemiological trials conducted with the intention of confirming a beneficial role of magnesium supplementation in myocardial infarction and coronary diseases have proved inconclusive [67,68]. The inconsistency of the results obtained may be related to differences in the bioavailability of magnesium in the patients or that the effect of magnesium supplementation is preventive rather than therapeutic. Conversely, a positive effect of magnesium intake on lowering blood pressure has been confirmed by various trials [69]. An even greater effect on blood pressure lowering has been observed when magnesium is co-administrated with normal/high doses of potassium,

low sodium, and taurine [69]. Together with magnesium, taurin is able to reduce intracellular levels of calcium and sodium [70]. While a positive effect of magnesium on lowering blood pressure has been confirmed, the exact mechanism of its antihypertensive effect is not fully understood. However, one of the proposed mechanisms involves the formation of ROS including nitric oxide (NO•) [71], which positively affects the contractile activity of vascular smooth muscle cells (vascular tone) and plays a significant role in the regulation of blood pressure [72].

While the detailed mechanism is not understood, insulin is a hormone known to increase the concentration of cellular magnesium and decrease that of cellular sodium [73]. In accord with this, diabetes is one of the common illnesses known to suppress magnesium levels in humans as well as in animals, and hence special attention should be paid to control the plasma level of magnesium in diabetic patients.

Hypomagnesemia is associated with increased proinflammatory cytokines, such as tumour necrosis factor (TNFα) and interleukin 1 (IL 1). In addition to other possible mechanisms, a deficiency of manganese results in an excessive formation of ROS, membrane oxidation and activation of Nuclear factor kappa B (NF-κB) [74].

Magnesium is an important element in cell proliferation and in the synthesis of proteins in all cell types [75]. A low level of magnesium was found to affect negatively the capacity of cells to synthesize proteins and to proceed effectively through all stages of mitosis [76]. As an extension of these findings, researchers have attempted to evaluate the role of magnesium on tumour growth and metastasis, the results from which have shown that tumour cells are independent of magnesium content; only extremely low levels of magnesium (lower that 0.2 mM) were found to retard the cell growth.

DNA studies revealed that the level of magnesium affects more than 30 genes, most of which control cell proliferation [77]. Consistent with this, mice with inoculated tumours that were fed a very low magnesium diet exhibited reduced tumour growth as compared with mice on a normal diet [78]. This observation accords with patients suffering from colon cancer who are receiving monoclonal antibodies, for which a reduction in tumour size was observed and a suppressed metastasis with concomitant hypomagnesemia along with an increased amount of magnesium excreted in their urine [79].

Further research efforts in the area of magnesium homeostasis will explore the physiological roles of magnesium and related mechanisms in greater detail.

3.3. Manganese

Manganese can donate up to seven electrons and may exist in several different oxidation states. In biological systems the metal occurs as Mn^{2+}, Mn^{3+} and possibly as Mn^{4+} Ions. Mn is an essential element that is necessary for many biological functions in living organisms, including their development and growth. The majority of Mn enters the body from dietary sources, along with a minor fraction from dermal intake and inhalation.

3.3.1. Manganese in health

Manganese homeostasis is tightly regulated in order to maintain the delicate balance between its essentiality and toxicity. Particular attention has been focused on the transport of manganese across the blood brain barrier (BBB), even though a complete identification of the manganese carriers has not been made satisfactorily [80]. It is known that the following major transporters are involved in the process: Divalent metal – ion transporter (DMT-1), ZIP8 (belonging to the ZIP family of Mn, Zn, Fe and Cd transporters), and Transferrin (Tf) - a major transport protein for Fe, Mn and Al, that mediates their transport through the cell surface of Tf receptors. DMPT-1 works as a symporter of hydrogen ions, and is primarily involved in the accumulation of Mn in the brain. An alternative transport route for manganese from the blood stream is via the voltage regulated, or glutamate activated, ionic channels, which are responsible for the transport of calcium into the cell [81]. Much less is known about the putative extracellular transport mechanism for manganese. The intestine ferroportin-1 (Fpn) principally exports iron but has been also implicated as a manganese exporter, in agreement with results obtained from studies using cell models which revealed that the expression of Fpn causes a suppression in the toxicity of manganese and in its accumulation [82]. Interestingly, the artificial radioisotope ^{54}Mn (half-life 312 days) has been shown to be readily transported into the brain without bearing any coordinating ligands [83].

Mn-SOD (SOD2) is the key mitochondrial antioxidant enzyme that dismutates superoxide radical anions according to reaction

$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{Mn-SOD} H_2O_2 + O_2$and is also considered to be the critical defence agent against oxidants in mitochondria. Mn-SOD increases the expression of various genes that play important roles in diverse functions related to radiation-induced adaptive responses [84], and in addition, the enzyme is a target for p53 which indicates its importance in the development of cancer and potential clinical applications for cancer treatment.

Mn is a known cofactor for many enzymes, such as hydrolases, transferases and lyases, while further manganese-dependent enzymes are currently being investigated. One of the most abundant Mn-based proteins, expressed in specialized glial cells (astrocytes), is glutamine synthetase which controls and converts toxic levels of glutamate to the less toxic glutamine [85]. Glutamine synthetase is an enzyme which uses either Mg^{2+} or Mn^{2+} in its active site, although the latter metal is more commonly found. Disorders in the functioning of glutamine synthetase may result in neurological disorders, including Alzheimer's disease, epilepsy, depression and the most aggressive and frequent brain tumour, glioblastoma multiforme.

Pyruvate carboxylase (PC) is a mitochondrial enzyme which catalyses the carboxylation of pyruvate to oxaloacetate according to the reaction [86].

$$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightarrow{\text{Pyruvate carboxylase}} \text{Oxaloacetate} + \text{ADP} + \text{P}$$

This enzyme contains a biotin prosthetic group along with magnesium or manganese ions and acetyl coenzyme A, and it is an important agent in the metabolism of glucose and in the synthesis of neurotransmitters.

Arginase is a hydrolase: specifically a Mn-containing terminal (sixth) enzyme in the urea cycle where it catalyses the catabolism of L-arginine to urea and L-ornithine [87].

$$\text{Arginine} + H_2O \xrightarrow{\text{Arginase}} \text{Ornithine} + \text{Urea}$$

X-ray diffraction measurements on human arginases I and II, revealed a homo-trimeric structure, containing a binuclear Mn(II) cluster, and that the structure of bacterial arginase is hexameric.

3.3.2. Manganese and diseases

Although manganese deficiency is not a very common condition, it may lead to various biochemical disorders and physiological defects [88]. An insufficient daily intake of manganese may affect the functioning of Mn-dependent enzymes (described above) which may in turn be associated with various health disorders [89].

3.3.2.1. Manganese and neurological disorders. The levels of manganese vary among different regions of the brain, and the accumulation of excess manganese in this organ may result in neurotoxicity. The abnormal accumulation of manganese in those areas of the brain where dopaminergic neurons are abundant is referred as manganism, a condition that is most commonly caused by an occupational exposure to manganese (employment in welding, mining etc.).

3.3.2.1.1. Manganism and Parkinson's disease. In excess, Mn can oxidise molecules in the catecholamine cascade, including dopamine, and hence perturb its level in various brain areas, as presented by a

biphasic syndrome which comprises two different components. Early stages of increased dopamine levels are manifested as psychiatric disturbances and in the latter stages of the disease's progression, by parkinsonian symptoms [90]. While early stages are reversible, upon interruption of Mn exposure, the later stages, characterized by motor symptom abnormalities are severe and irreversible [91].

Parkinson's disease (PD) is characterized by tremor, imbalance, slowness (bradykinesia) and stiffness of movement. Idiopathic Parkinson's (of unknown cause) is the most common form of the condition. About 15–20% of patients with symptoms suggesting PD have several disorders, which may involve multiple system atrophy, corticobasal syndrome, vascular parkinsonism other conditions. From the above, it follows that while Mn-induced parkinsonism and PD share some similar clinical manifestations such as motor symptoms, there are nonetheless pathological and clinical differences between the two diseases. The pathologic phenotype of manganism is different from idiopatic PD where dopaminergic neurons of the substantia nigra pars compata (SNc) are affected, since in manganism different brain areas such as cortex and hypothalamus are affected. In addition, manganism occurs as a result of acute exposure to Mn, whereas PD originates from long term exposure to low concentrations of Mn. Both diseases share mitochondrial dysfunction, Mn-induced oxidative stress and several common genetic factors [92,93].

3.3.2.1.2. Alzheimer's disease. A transcriptional target of p53, the Amyloid-Beta precursor-like protein 1 (APLP1), has been found to be upregulated in the frontal cortex of monkeys following chronic exposure to manganese. An accumulation of manganese correlates with the cognitive decline of animals, the expression of apoptotic markers and degeneration of neurons [94]. Typical features of AD among others (outlined above) are increased oxidative stress and an imbalance in the mitochondrial energy metabolism due to the suppression of Mn-SOD activity, despite an accumulation of manganese [95]. Moreover, manganese interferes with the cholinergic and dopaminergic systems, as well as the GABA systems, and thus affects the incidence and progression of AD.

3.3.2.1.3. Huntington disease. The symptoms of Huntington disease (HD) are motor impairment, cognitive and emotional decline and psychiatric problems. HD is inherited and is characterized by both a mutation in the huntingtin gene (HTT), and the accumulation and formation of toxic huntingtin protein clusters in neurons. It has been proposed that huntingtin proteins may play a role in the transport of manganese and the affected cells have lower levels of manganese than healthy cells [96]. One recent study provided evidence that the modulation of the transport of manganese in cells is linked with compensatory metabolic responses in the pathology of HD [97]. Both mouse models and human studies have shown that Mn-dependent signalling pathways were impaired in HD. While there is some evidence that manganese may play a role in the early stages of HD development, it is unclear whether it is involved in the actual onset of the disease.

3.3.2.1.4. Amyotrophic lateral sclerosis. Another neurodegenerative disease which is linked to manganese is Amyotrophic lateral sclerosis (ALS), which affects motor neurons in the spinal cord and cerebral cortex [98]. As the disease progresses, patients develop respiratory distress syndrome, lost of motor neurons and muscle weakness. Approximately 5–10% of ALS is hereditary in origin, due to mutations in the Cu,Zn-SOD (SOD1) gene, although recent studies have reported an association with mutations in extracellular SOD (EC, SOD3). Interestingly, no association between ALS and SOD2 has been found [99]. Increased oxidative stress caused by the mutations in SOD1 result in increased oxidative damage, an imbalance between magnesium and calcium, magnesium toxicity, mitochondrial dysfunction and excitotoxicity [100].

MRI scans of a large number patients suffering from ALS exhibit neurological changes that are typical for a manganese overload condition, even though an exact quantification of the levels of this metal has not yet been reported [101,102].

3.3.2.2. Manganese and mitochondrial dysfunction. Manganese is present in cells predominantly in its bivalent oxidation state (Mn^{2+}) and as bound to ATP [103].

Intracellular Mn^{2+} accumulates predominantly in mitochondria where it may interfere with the electron transport chain and the oxidative phosphorylation process, thus resulting in a significant formation of ROS. Manganese can readily switch between several oxidation states, e.g. between Mn^{2+} and Mn^{3+} thereby increasing the prooxidant potential of the metal, which in turn increases the degree of oxidative stress and mitochondrial damage [104]. The direct involvement of manganese in electron transport may lead to suppressed ATP production, an increased leaked electron flux, the formation of superoxide radical anions along with a variety of superoxide-derived radicals, for example highly reactive and damaging hydroxyl radicals. In addition, manganese has been found to interfere with the homeostasis of both calcium and magnesium [105].

3.4. Iron

Iron is an essential element in all living organisms [106]. Cells have evolved mechanisms to uptake iron in biologically beneficial forms, for example using bacterial siderophores as low molecular mass iron chelating compounds. Many mechanisms for iron uptake that are found in lower organisms also occur in humans, and in the human body, iron is contained in the form of heme proteins such as hemoglobin and myoglobin, or non-heme complexes such as transferrin and ferritin. More than 60% of the body's iron is incorporated in hemoglobin, about quarter is present in the form of a readily mobilizable iron store, and 15% is bound in myoglobin and in enzymes that participate in metabolic and cellular functions [107].

3.4.1. Iron and health

Iron in the diet stems from two sources, heme iron and non-heme iron. Heme-iron originates in the oxygen storage proteins, hemoglobin and myoglobin, and is abundant in meat, fish and poultry, while non-heme iron is predominantly present in vegetables, fruits and cereals. While the bioavailability of heme-iron is relatively high (20–40%) and is not affected by dietary factors, the bioavailability of iron from non-heme sources is relatively low (ca 15%), but is strongly affected by the properties of other food components [108]. The content of non-heme iron in the diet is much higher than that of heme iron originating from meat, and so the main proportion of dietary iron comes from vegetable and fruit sources. Absorption of iron is inhibited by calcium, zinc, flavonoids and certain proteins, but is assisted by vitamin C which can reduce trivalent iron to a more bioavailable divalent iron form, Fe(III) to Fe(II), according to Fig. 2 [108].

Generally, Fe(III) compounds do not dissolve well in water due to the formation of Fe(III)-hydroxy or oxy polymeric structures, which have a low solubility. Fe(II) salts are generally more soluble in water environments and therefore present a greater bioavailability [3]. Stomach acid has a low pH (around 1–2) which helps in the reduction of insoluble ferric iron to the more soluble ferrous form.

As has already been mentioned, the positive effect of vitamin C on iron absorption is a result of its reducing power, and this can overcome the suppressant effect of several iron absorption inhibitors. Thus, a healthy vegetarian diet should contain a relatively high intake of vitamin C to promote iron absorption from fruits and vegetable sources [109]. Industrial processing and cooking may degrade the content of vitamin C in food sources and thus affect the absorption of iron [110]. Iron absorption is suppressed by high amounts of calcium and zinc [111]. In the body, iron is held in ferritin, an iron binding protein, and is stored predominantly in the liver, bone marrow and spleen. The cellular level of iron is regulated by iron-responsive element binding proteins IRP1 and IRP2. Under physiological conditions, iron is highly conserved, and any loss of it occurs predominantly by menstrual bleeding. (Fig. 3).

To achieve iron homeostasis, an organism maintains a constant

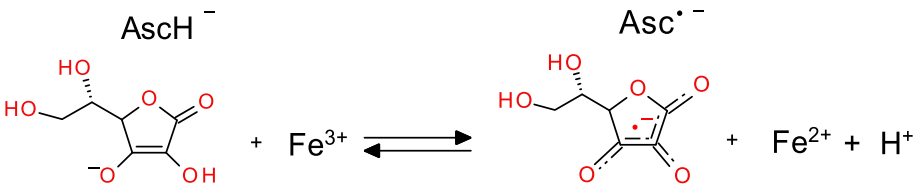

Fig. 2. Reduction of Fe^{3+} by ascorbate anion ($AscH^-$) resulting in the formation of ascorbate free radical anion ($Asc^{\bullet -}$) and Fe^{2+}.

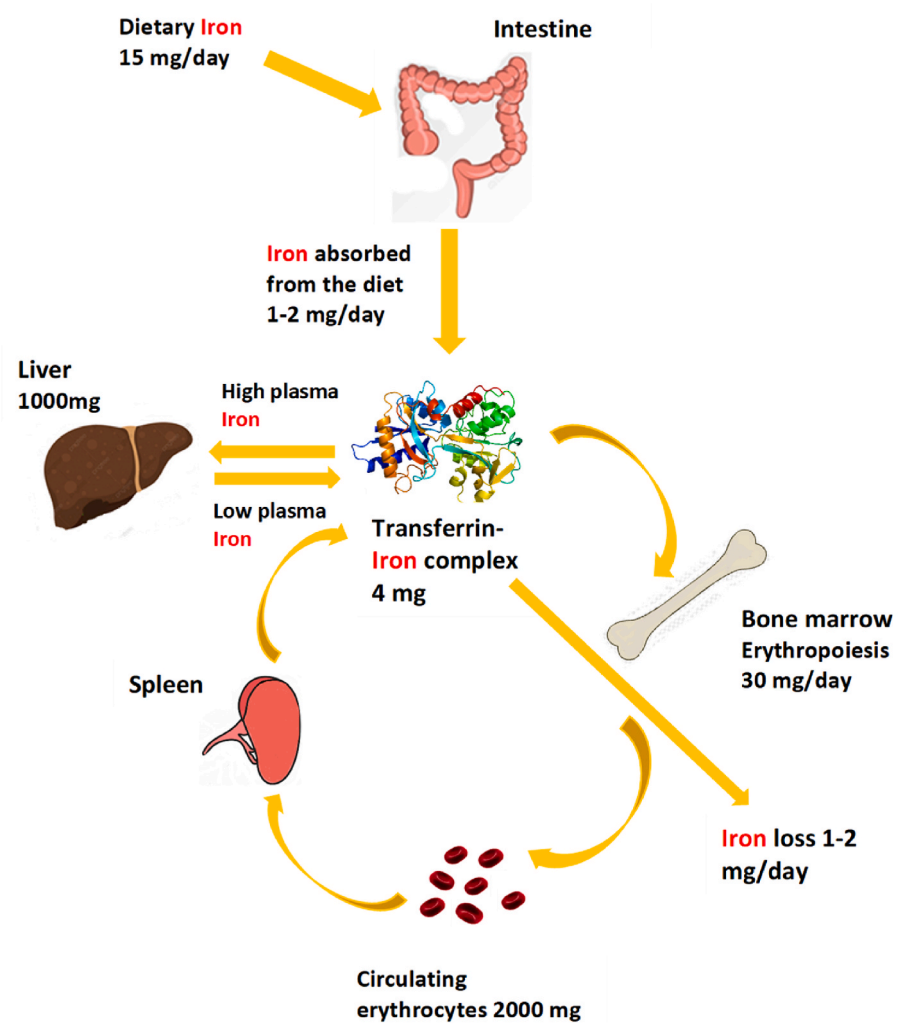

Fig. 3. A simple scheme of iron absorption, distribution and recycling in the body.

balance between the absorption, transport, storage and distribution of iron [112]. Iron, as present in the form of Fe(III) taken from digested food, is reduced to Fe(II) by ferrireductase duodental cytochrome *b* (DCYTB) and is transported by the divalent metal transporter (DMT1) (also see above) across the cell membrane of enterocytes and into the cell. Heme iron is transported by the heme carrier protein 1 (HCP1), and thus both DMT1 and HCP1 supply iron to the iron pool. In the enterocytes, iron is stored in the iron storage protein, ferritin, or released into the body via ferroportin-1 (FPN1) [113]. FPN-1 is a unique protein found in the basolateral membrane of enterocytes, post-translationally repressed by hepcidin with the assistance of a hephaestin, a transmembrane Cu-dependent ferroxidase that is capable of oxidizing Fe(II) to Fe(III). Deletion of the *FPN1* gene results in the blocking of iron export and leads to its accumulation within the enterocytes. Ferroxidases, located at the cell surface, support the stability of FPN1. The impaired cellular export of iron is associated with several diseases. The above mentioned HCP1, (a ferroxidase), also plays an important role in export of iron from intestinal enterocytes and its loading onto transferrin (Tf), the key protein involved in the transport of iron in plasma. Typically, fewer than half of the binding sites in Transferrin are saturated by Fe (III), and hence the degree of transferrin saturation is a good marker for iron deficiency and iron overload. Iron in the cytoplasm is toxic and therefore its intracellular transport is mediated by poly(rC)-binding proteins (PCBPs), which function as a gateway to ferritin [114]. One molecule of ferritin can accommodate up to 4500 atoms of iron, which it sequesters in the ferric form, and the protein also acts as a catalyst which reduces ferric to ferrous iron.

In conclusion, Iron is the critical metallic element for hemoglobin synthesis, but since free iron is very toxic, a sophisticated set of regulatory mechanisms has been developed by mammals to achieve the

proper intestinal absorption, transport, storage, utilization and excretion of the metal.

3.4.2. Iron related diseases

While iron is an indispensable metallic element for supporting life, it is also able to participate in the formation of ROS which may, in turn, cause severe damage to all biomolecules. A breakdown of iron homeostasis results in the development of iron-related disorders, which are classified as iron-deficiency or iron-overload diseases.

3.4.2.1. Iron deficiency. Despite the large quantities of iron that are present in the Earth' crust (ca 5%), iron deficiency is very common and represents the most common cause of anemia in humans [115]. Anemia occurs when there is an imbalance between the intake, circulation, store, and loss of iron, and consequently the formation of erythrocytes is impaired. Although iron anemia - often overlooked by physicians - is not a life threatening disease, it may nonetheless exert a significant impact on human health.

The causes of iron anemia are: (i) insufficient dietary intake of iron due to eating food that contains iron inhibitors, heavy antacid therapy, long periods of fasting, monotrophic diet, (ii) increased requirements for iron due to menstruation, pregnancy, trauma, therapies, (iii) increase iron loss mainly due to the bleeding from GI tract, heavy exercise, blood donation, (iv) suppressed iron absorption due to disorders of GI tract - Crohn's disease, Helicobacter pylori, celiac disease and (v) genetic diseases such as mutations in glutaredoxin and *DMT1* genes.

Iron deficiency manifests itself by fatigue and decreased physical activity, both related to decreased levels of hemoglobin, myoglobin and cytochromes, which in turn leads to restricted transport of oxygen and its supply into muscles. In addition, synthesis of iron-sulphur proteins is diminished due to the decreased oxidative capacity of mitochondria [116].

Since about a fifth of the total oxygen taken into the body is utilized by the brain, adverse effects of anemia are associated with mental health due to the decreased oxygen supply to this organ. In addition, iron is required for the synthesis of neurotransmitters such as dopamine and serotonin, as well as for mitochondrial functioning which also affects the brain. On the basis of intervention studies, iron deficiency has been linked with delays in development and performance [117].

Iron deficiency can be reversed by means of iron supplements such as Fe(II) sulphate, and gluconate. While iron salts administered orally have a good bioavailability, in about 40% of patients this is linked with various GI side effects, but these can be overcome by using less soluble ferric salts with a lower bioavailability than ferrous salts. Although a high solubility and effective bioavailability of Fe(III) maltol compounds has been reported, the effectiveness of these compounds has been called into question [118]. During therapy with iron salts, it should be born in mind that they can inhibit the action of various drugs, including antibiotics, thyroxide, and L-Dopa and others [119]. Furthermore, there is a definite need for the introduction of new, safer iron supplements to avoid the toxic effects of ferrous sulphate.

Anemia is an accompanying disorder of several chronic ailments, which include cancer, kidney diseases, diseases of the ear, autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease, and infections such as HIV and tuberculosis. The anemia associated with these diseases is usually mild to moderate. Anemia, under such pathological conditions is manifested by impaired iron recycling by macrophages, which is connected with suppressed intestinal iron absorption and the increased expression of an iron regulator, hepcidin. Clinical interventions in chronic anemia may involve the mobilization of iron macrophage using a suitable chelating agent and subsequent redistribution to the transferrin iron pool [120]. The effectiveness of oral supplementation therapy is not sufficient for many patients, who often require intravenous iron infusions.

3.4.2.2. Iron overload. Iron overload can be a life-threatening condition, and this occurs in two forms, one of which is characterized by increased levels of plasma iron and normal formation of erythrocytes (erythropoiesis) while the second type is associated with an increased catabolism of erythrocytes.

The former condition occurs when the iron binding capacity of transferrin is fully saturated and the residual non-transferrin bound iron is deposited in the liver, heart and endocrine systems [121]. When iron accumulates in organs it acts catalytically to promote the Fenton reaction, which results in the formation of hydroxyl radicals and other derived radical species. Serum contains predominantly ferric iron, Fe (III), which can be coordinated by several low molecular mass chelating agents such as citrate, phosphate, and acetate, most usually in the metal-to-ligand ratio of 1:2. Residual Fe(III) is also bound to albumin, which is present in blood plasma at high concentrations (500 μM).

Hereditary hemochromatosis describes a group of iron overload disorders that are characterized by gene mutations with the pathogenic feature of hepcidin deficiency. As a result, a significantly increased absorption of iron from the diet occurs, along with saturation of transferrin, and so the amount of non-transferrin bound iron increases, which becomes distributed to various tissues where damage occurs [122]. The liver is most affected, with the prognosis of cirrhosis and liver cancer, along with other conditions, such as diabetes, arthritis, and cardiomyopathy [123]. The blood brain barrier serves to protect the brain against iron overload.

Mutations in proteins involved in the synthesis of heme or iron-sulphur clusters result in the accumulation of iron in mitochondria and lead to pathologies such as Friedreich's ataxia, glutaredoxin-5 deficiency and sideroblastic anemias.

3.4.2.3. Iron and neurological disorders. Neurological disorders are multifactorial in origin, many of them are characterized by a disturbed iron metabolism [124]. One of the pathological features of neurological diseases is the accumulation of abnormal proteins, for example, Alzheimer's disease, which is characterized by accumulation of Amyloid-β - a main constituent of amyloid plaques. In the cases of Parkinson's disease and Huntington's disease, characteristic accumulations of Lewy bodies and mutant huntingtin proteins, occur, respectively. In addition, abnormalities in the mitochondrial electron transport are typical for neurological diseases.

One common denominator for all these diseases is a disrupted iron homeostasis, which causes an increased level of iron in the brain [125]. Neuronal iron levels are controlled via the concerted action of transmembrane fluxes in mitochondria, ferritin and lysosomes and also by neuromelanin, which acts as a storage site for iron. Neuromelanin is a black-brownish pigment and may be first formed in the cytosol and then taken up by lysosomes, resulting in the formation of neuromelanin granules.

As will be discussed in the section "Copper and human diseases" it has been observed that the level of copper in Alzheimer's tissues is almost three times that in normal tissues. Correspondingly enhanced iron levels are also found, with the concentration of Fe in cerebrospinal fluid determined to be in the range 0.2 and 1.1 μM, and well above the binding capacity of transferrin. Such increased levels of iron as are present in neurological diseases participate catalytically in the Fenton reaction, which leads to the formation of damaging hydroxyl radicals and additionally derived radical species formed via radical cascade processes [126]. In addition to elevated levels of oxidative stress markers, Alzheimer's disease is characterized by a deficiency of acetylcholine, caused by the disturbed activity of acetylcholinesterase and the occurrence of amyloid-β, a peptide which accumulates in amyloid plaques that occur in the brains of patients suffering from this condition.

Clinical manifestations of Parkinson's disease (PD) involve predominantly motor impairments caused by the progressive death of

dopaminergic neurons in the substantia nigra pars compacta [127]. It has been documented that the increased levels of iron in the midbrain contribute to neurodegeneration, while iron chelation by clioquinol appears to be effective in either preventing the disease or delaying its progression. Coordination of this ligand to iron to form a Fe-clioquinol complex suppresses the metal's catalytic activity in the Fenton reaction, and consequent radical reactions.

Iron (and also copper) has been found to be elevated in brain tissues taken from Huntington's disease (HD) patients, post mortem, and MRI examinations revealed an increased accumulation of iron in the basal ganglia and cortex, which correlated with the severity of the disease pathogenesis [128].

3.4.2.4. Iron chelation therapy. Iron chelators represent an important class of substances for the treatment of iron-related diseases [129], of which, historically, the first one used in clinical practice was desferrioxamine (Fig. 4). More recently, deferiprone and deferasirox have been introduced for clinical purposes (Fig. 4). Desferrioxamine is inactive when taken orally, due its bulky size and hydrophilicity which impedes its effective transport across biological membranes. In contrast, both deferiprone and deferasirox are orally active and, in addition to their use in the treatment of iron overload diseases, have been shown to be promising both in the treatment of Parkinson's disease and macular degeneration. In designing and developing suitable and safe iron chelators, it should be borne in mind that these must be redox inactive. This requirement is fulfilled by analogues of 2-pyridylcarboxaldehyde isonicotinoyl hydrazone, di-2-pyridylketone isonicotinoyl hydrazone, di-2-pyridylketone thiosemicarbazone, and the clinically trialled chelator 3-aminopyridine-2-carboxaldehyde thiosemicarbazone [130], all of which possess different modes of action.

3.5. Cobalt

As already mentioned, the most common oxidation numbers of cobalt are +2 and + 3. The essentiality of cobalt in the diet was confirmed in 1948 when vitamin B12, which contains 4% of the metal, was discovered [131].

3.5.1. Cobalt and health

Vitamin B12 is one of the eight B group vitamins, and is abundant in food derived from animals, mostly beef, lamb, chicken and eggs. Eggs have been confirmed as a rich source of B12 covering about 25% of the adult daily requirement [3].

The cobalt central ion in Vitamin B12 is coordinated by four equatorial nitrogen atoms in a square-planar arrangement, which originate from the pyrrole groups A-D of the corrin ring. A diversity exists in both the axially coordinated ligands, those at the base and at the apical coordinated sites, where cyano- (vitamin B12), methyl- (Methylcobalamin (MeCbl1), 5′-deoxyadenosyl- (AdoCbl or coenzyme B12) and hydroxy-(injected form of vitamin B12) groups are found. Methylcobalamin and deoxyadenosylcobalamin function as coenzymes in various metabolic reactions, while the cyano form, is that most frequently used in supplements.

Vitamin B12 is very soluble in water and if ingested in its free form it binds to the vitamin B12 binding protein R in the middle part of the throat and in the stomach, following the entry of the vitamin B12-R protein complex into the duodenum where the R binder is degraded and free vitamin B12 interacts with a 50-kDa glycoprotein (intrinsic factor) which is produced by parietal cells that is necessary for vitamin B12 absorption to take place in the small intestine [132].

As present in the body, the majority of cobalt is found in the heart, kidney, and liver, with smaller amounts in the brain and pancreas [132]. Vitamin B12 is involved in the synthesis of certain proteins, in the formation of the myelin sheath of neurons and in the normal functioning of the nervous system, since it also plays an important role in the synthesis of neurotransmitters [132]. Cobalt salts are involved in the synthesis of a glycoprotein hormone, erythropoietin, which is produced in the kidney and stimulates the production of erythrocytes in the bone marrow [133]. Accordingly, cobalt salts have been used extensively in sport medicine as a substitute for blood doping as a means to boost the number of erythrocytes and improve aerobic performance.

Vitamin B12 has the most complex molecular structure of the group of eight B vitamins, all of which are involved in the conversion of food carbohydrates into energy sources, such as glucose. Vitamin B12 plays an important role in the methylation of homocysteine to methionine within the methionine cycle, with the participation of vitamin B9 (folic acid), and conversion of the methionine back to homocysteine. Homocysteine is a sulphur-containing amino acid, whose metabolism requires the presence of vitamin B12 along with vitamin B9 (folate), vitamin B6 and riboflavin. In addition, cobalamin is necessary for the transformation of methylmalonyl-CoA, (the thioester of CoA connected to methylmalonic acid) to succinyl-CoA (which plays an important role in the tricarboxylic acid cycle). The coenzyme-forms of cobalamin participate in the degradation of leucine and of propionic acid.

3.5.2. Cobalt and diseases

Cobalt deficiency is directly related to a disturbed synthesis of B12, while cobalt overload is less frequent and its related toxicity is relatively

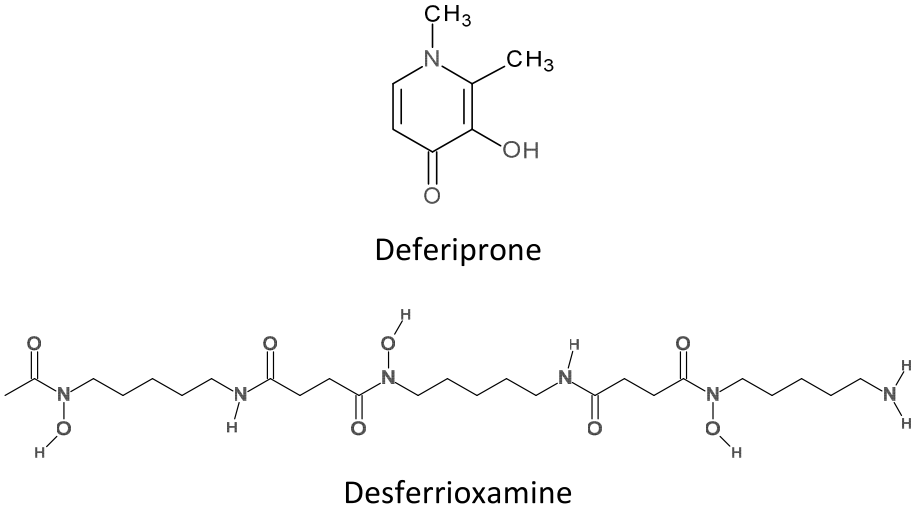

Fig. 4. Structures of deferiprone and desferrioxamine.

mild compared to those of other environmentally occurring redox active metals.

3.5.2.1. Cobalt deficiency. Cobalt deficiency results in the development of anemia, diminished functioning of the thyroid gland, and birth defects [131]. In contrast, an atypically high uptake of cobalt is manifested by an abnormal functioning of the thyroid gland and activation of erythropoiesis, which in turn causes an increased production of erythrocytes as a result of stimulated bone marrow functioning [134]. Consequently, cobalt supplementation has been used in treatment of anemia [135].

Folic acid (vitamin B9) is important in erythrocyte formation, cell growth, and in the early stages of pregnancy, and its deficiency causes impaired DNA synthesis. Folic acid and vitamin B12 deficiencies are both associated with a risk of megaloblastic anemia, a condition that is characterized by a suppressed formation of abnormal size erythrocytes. Administration of folic acid to megaloblastic anemia patients with vitamin B12 deficiency results in an increase of folic acid pool and appears to provide an effective treatment.

A deficiency in vitamin B12 or folic acid may result in hyperhomocysteinemia, a condition characterized by high levels of the sulphur containing amino acid, homocysteine, a product of methionine metabolism [136]. This condition is associated with heart disease, stroke, mental disturbances and osteoporosis, but these diseases can be treated by the administration of vitamins B12 and B6 and folic acid.

It was found that vitamin B12 supplementation was able to restore to normal, impaired sperm maturation that was linked to a low serum concentration of this vitamin [137]. Rats fed with a vitamin B12 deficient diet showed severe testicular damage which could be prevented by methionine supplementation, thus confirming the dysfunction of methionine synthase, which requires vitamin B12 as a cofactor for the regeneration of methionine from homocysteine [138,139].

Patients deficient in B12 suffer from various neurological complications, for example neuropathy which is manifested by an abnormal peripheral nervous system, myelopathy caused by spinal compression, optic neuropathy manifested by damage of the optic nerve, and dementia [140]. About one third of patients suffering from vitamin B12 deficiency have normal levels of cobalamin and therefore their levels of methylmalonic acid and homocysteine should be checked. Vitamin B12 deficiency is a serious threat mainly for the elderly, which could be due primarily to malnutrition or autoimmunity problems that affect the absorption of cobalamin. It has been shown that patients with circadian circle disorders can be cured by the administration of methylated vitamin B12, which is more effective than cobalamin in this regard [140].

3.5.2.2. Cobalt overload, toxicity and antagonism. Generally three main routes are known by which cobalt enters the human body [3]. These involve oral intake, inhalation and dermal toxicity. Cobalt and its salts are used in a variety of industries for example as an ingredient of coloured pigments. Some radioactive isotopes of cobalt, such as Cobalt 60, are used in treatment of patients in nuclear medicine [3]. A main industrial source of cobalt intoxication involves electrochemical plants where workers are exposed to cobalt-containing compounds. These workers have a significantly higher risk of developing lung cancer [141].

Cobalt has been shown to interfere with the repair of DNA and to cause DNA damage. The detailed mechanism involves crosslinking of DNA proteins, and cobalt-catalyzed formation of free radicals that can attack DNA directly [141].

The formation of free radicals in a Co(II)/Co(I)-catalyzed Fenton reaction system revealed that free Co(II)/Co(I) is able to catalyze the formation of damaging hydroxyl radicals; however, saturation of cobalt binding sites by ligand chelation (e.g. by citrate) prevented free radical formation [141].

$$Co(I) + H_2O_2 \rightarrow Co(II) + {}^{\bullet}OH + OH^{-} \text{ (Fenton reaction)}$$

The Fenton reaction also proceeds effectively with free or chelated divalent cobalt(II) according to the following equation

$$[Co(II)\text{-chelate}] + H_2O_2 \rightarrow [Co(III)\text{-chelate}] + {}^{\bullet}OH + OH^{-}$$

Which can be effectively tuned by the appropriately selection of chelators. Interestingly, when the biologically occurring antioxidants, glutathione and a dipeptide - anserine, were coordinated to Co(II), an increased formation of hydroxyl radical in the Fenton reaction occurred, as demonstrated using EPR spin trapping spectroscopy. This confirms that, under certain conditions in the presence of redox active cobalt(II), glutathione and anserine can behave as prooxidants, and thus increase the concentration of free radicals in the system. In the presence of DNA, this Co-Fenton system has been shown to cause DNA cleavage at all bases in the order G > T and C ≫ A, and this has been attributed not only to hydroxyl radicals but also to the formation of damaging singlet oxygen ($^1O_2$), as detected by EPR spectroscopy using TEMP/TEMPO spin trapping method [141].

Cobalt metal particles are a frequent source of toxicity to different organisms. A detailed EPR spin trapping study using DMPO as a spin trap revealed that, in suspension, cobalt particles react with dioxygen in the presence of SOD to generate hydroxyl radicals through the intermediate cobalt peroxo radicals Co(I)-OO$^{\bullet}$ [3]

$$\mathrm{Co(elemental)} + O_2 \rightarrow \mathrm{Co(I)} + O_2^{\cdot-} \rightarrow \mathrm{Co(I)} - OO^{\cdot} \xrightarrow{SOD} H_2O_2 + \mathrm{Co(I)} \rightarrow \text{Fenton reaction } (^{\cdot}OH)$$

These results show, that the cobalt- or generally metal-induced oxidative stress appears to be a common denominator in many oxidative stress-related diseases.

The correct biological functioning of cobalamin requires the participation of various auxiliary proteins, and further research into the origin, biological role and nature of protein-cobalamin interactions is currently active in many different laboratories around the world.

It has been reported that cobalt and manganese can bind to the same nonheme site and confer Heme-Copper Oxidase activity in a heme-nonheme biosynthetic model of myoglobin [142]. Binding of Mn^{2+} and Co^{2+} at the non-heme iron site was confirmed by the spectroscopic and X-ray measurements.

It has been shown that cobalt can replace zinc in a number of zinc-centered enzymes such as alcohol dehydrogenase. Cobalt can also increase the activity of some enzymes, such as heme oxidase in the liver. In addition, interestingly, cobalt interferes with and depresses iodine metabolism, resulting in suppressed thyroid activity, which may lead to irregular growth of the thyroid gland, known as goiter [5].

In one recent study, it was shown that cobalt does not exhibit a toxic effect on *Salmonella* Typhymurium, but it can effectively offset zinc deficiency. The data confirmed that cobalt is able to incorporate at the zinc site in bacterial proteins and thus maintain their full functionality [143].

Methionine sulfoxide reductases are a family of redox enzymes responsible for reduction of both free and protein-bound methionine sulfoxide back to methionine [138]. NMR experiments confirmed that the Zn^{2+}-containing methionine sulfoxide reductase B1 (MsrB1), in *E. coli* growing in cobalt-enriched media led to the formation of a stable Co-containing protein methionine sulfoxide reductase. Both enzymes, Zn-MsrB1 and Co-MsrB1 exhibit comparable levels of activities, in agreement with their structural similarities. NMR spectroscopy also revealed that the N-terminal site of the Co-MsrB1 is not fully disordered and that the N-terminal site of Co-MsrB1 is in contact with the metal binding site for a greater length of time than the Zn-analogue.

3.6. Copper

Copper is an integral part, or an essential cofactor, of many enzymes that are involved in electron transfer reactions. As a result of the relatively high value of the Cu^{2+}/Cu^{+} redox potential, copper-enzymes are

able to participate in many oxidation/reduction reactions [3,7,8]. By way of illustration, selected copper-containing enzymes and their biological functions are outlined in Table 2.

3.6.1. Copper in health

Copper homeostasis must be tightly controlled, since an excess of copper mediates the formation of ROS and ROS-induced damage to an organism at all levels. The correct balance between copper intake and its elimination must be achieved at the cellular level as well as at the scale of tissues and organs [3].

Such precise regulation of copper is achieved by proteins, aptly called metallochaperones, which are responsible for preserving copper (and other metals) and delivering it to protein receptors. It has been reported that such copper chaperoning is necessary for the effective expression of SOD.

For the dietary absorption of copper to take place requires the divalent metal transporter 1 (DMT1) and high-affinity copper transporter 1 (Ctr1) proteins, see Fig. 5 [144]. As taken from the diet, copper is present in the gut in its divalent form. This can be translocated into the intestine epithelial cells (enterocytes), either by the nonspecific Divalent Metal Transporter 1 (DMT1) (which also transports iron) in the form of Cu^{2+} or by a specific copper transporter (Ctr1) in the form of Cu^{+}. Copper is delivered to Ctr1 by the proteins ceruloplasmin, transcuprein, or albumin. Prior to copper uptake by Ctr1, Cu^{2+}reduced to Cu^{+} by biological reductants such as vitamin C. The absorbed copper is bound in the cytosol by the intracellular antioxidant 1 Cu chaperone (ATOX1) and the low molecular weight Cu ligand (Cu-L). The complex Cu-L transports copper into mitochondria, specifically to the copper chaperone of cytochrome C oxidase (COX17). ATOX 1 can translocate into the cell nucleus and transport copper to copper-transporting ATPase 1 (ATP7A) which is then delivered to the Trans-Golgi Network (TGN) or into vesicles to be removed from the cell [144].

Copper binds to metallothionein in the Golgi body and is deposited in the lysosomes, thus protecting the cell from the damaging effect of "free" copper. Copper that is exported from the enterocytes via the Menkes ATP-ase (MNK) protein, is then bound to the plasmatic transporter, transcuprein, and to albumin and is transported from the gut to the liver via the circulation of the blood. Hepatic copper homeostasis is a complex process that regulates the metal's secretion into the bile. Within hepatocytes, copper is transported to cytosol and mitochondria. The majority of copper is taken into the ceruloplasmin protein, then released into the circulatory system for distribution to the various tissues.

Since copper in the cytosol presents a risk for copper-induced oxidative damage, the upper limit of "free" copper, or loosely bound copper, is restricted to 10^{-18} M, which corresponds to almost vanishingly low levels of less than a single atom per cell [145]. Such a very low concentration of copper inside the cell is achieved through a combination of binding to metallothionein, and by its interaction with glutathione (GSH). Indeed, the Cu(I)-GSH complex is a highly abundant member of the copper exchangeable molecular pool in the cytosol [146], while matallothionein and GSH are principal molecules responsible for the sequestration of excess copper in living cells [147]. Furthermore, GSH is very effective in the detoxification of ROS, in copper binding and the overall maintenance of the cellular redox state.

Table 2
Selected copper-containing enzymes and their biological functions.

Enzyme	Biological function
Cu,Zn-superoxide dismutase (SOD1)	Dismutation of superoxide radical anion, signalling
Tyrosinase	Synthesis of melanin and other pigments from tyrosine
Cytochrome *c* oxidase	Oxidative phosphorylation, electron transfer from Cytochrome *c* oxidase to oxygen
Ceruloplasmin	Ferroxidase enzyme, iron metabolism
Lysyl oxidase	Biogenesis of connective tissue matrices by crosslinking the extracellular matrix proteins, collagen and elastin
Copper amine oxidase	Conversion of a primary amine functional group to an aldehyde

Enzymes containing copper ions play a key role in many physiological processes. Based on the unusual spin Hamiltonian parameters for their EPR spectra and features from their optical spectra, copper-containing enzymes are classified as: type 1 - Blue copper proteins, type 2 and type 3 copper enzymes. In Blue copper proteins, the copper ion is coordinated by two nitrogen atoms from histidine residues and one sulphur atom from a cysteine residue, to form a trigonal planar arrangement, with an additional axial site being coordinated by various donor residues. EPR spectra of proteins of this type exhibit an unusually small parallel hyperfine splitting component of the **A** tensor [8]. Type 2 enzymes possess a square planar arrangement around the copper ion, originating from the coordination of nitrogen or mixed nitrogen/oxygen donors. Type 3 enzymes comprise a dimeric structure with each copper atom coordinated by three nitrogen atoms from histidine donors. A strong antiferromagnetic coupling (antiparallel orientation of the unpaired electrons) between both copper atoms renders the protein EPR silent. The alternative oxygen carrier, hemocyanin, is a well known example of this type of enzyme.

Cu,Zn superoxide dismutase (Cu,Zn-SOD or SOD1) is an enzyme that catalyses the dismutation of superoxide radical anions to hydrogen peroxide according to the reaction [3,8].

$$2\,O_2^{-} + 2H^{+} \xrightarrow{SOD} H_2O_2 + O_2$$

Such conversion of superoxide radical anions is very important in living cells, because even though this radical species is of itself not very reactive, radicals subsequently derived from it such as the hydroxyl radical ($^{\bullet}OH$) (by Fenton reaction) are potentially damaging to all important biomolecules, including DNA, lipids and proteins. The hydrogen peroxide formed in the process is further converted to molecular oxygen and water by catalase and glutathione peroxidase.

Cu,Zn-SOD occurs in cytosol, and its protective role against ROS damage is of particular importance in cardioprotection, mainly against ischaemia-reperfusion injury. It has been documented that, in the process of mild formation of ROS during ischemic preconditioning, Cu,Zn-SOD plays an important role in regulating apoptosis and cell death [148].

In addition to Cu,Zn-SOD, three other SODs are known: Manganese superoxide dismutase (Mn-SOD, or SOD2), extracellular superoxide dismutase (EC-SOD or SOD3) and Nickel superoxide dismutase (Ni-SOD), first isolated in 1996 from *Streptomyces* bacteria. Mutations in Cu, Zn-SOD are linked with several neurological disorders (see below) [149].

Another large transmembrane protein, which contains two copper centers, is cytochrome C oxidase, an important proton pump in the mitochondrial electron transport chain that is located within the inner membrane. This enzyme couples the proton pumping process with reduction of a dioxygen molecule to form the superoxide radical anion. Cytochrome *c* oxidase increases the proton motive force used for synthesising ATP and uses an oxygen molecule as the terminal electron acceptor. This enzyme is one of the most frequent causes of respiratory chain defects in humans.

Another important copper-based enzyme is Tyrosinase, a multifunctional dimeric copper enzyme located in melanocytes. This enzyme converts tyrosine to dihydroxyphenylalanine (DOPA) and polymerization of L-DOPA results in the formation of the natural skin pigment melanin (Fig. 6). An enzyme with a similar activity to Tyrosinase is Catechol oxidase, a metalloenzyme containing three Cu centers.

Ceruloplasmin is a highly abundant protein, and a major copper carrier, in the blood: it is synthesized by hepatocytes and released into the circulatory system. The ceruloplasmin molecule contains six copper atoms. In association with transferrin, ceruloplasmin is also involved in the transport of iron in plasma, and an increased synthesis of ceruloplasmin has been reported under various pathological states of an

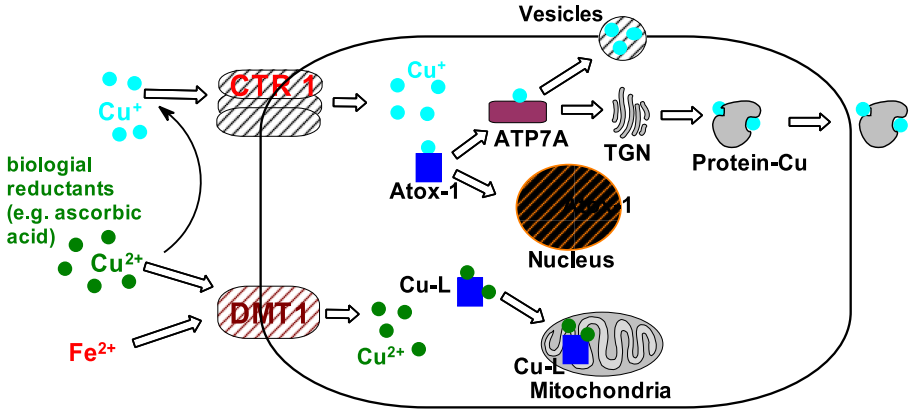

Fig. 5. A simple model of copper absorption by enterocytes. Adapted from Ref. [107].

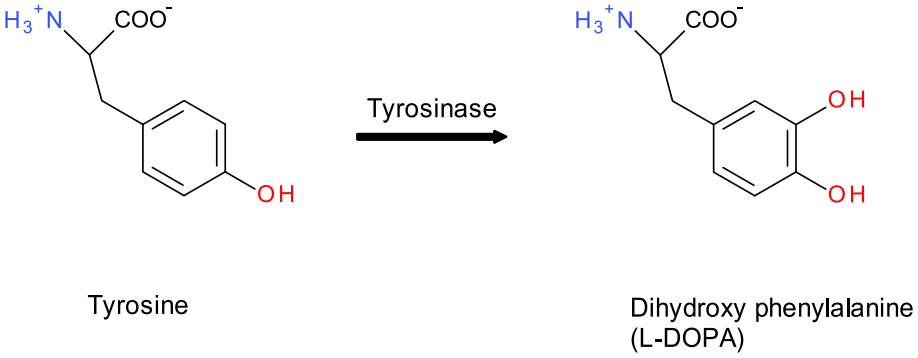

Fig. 6. Conversion of tyrosine to L-DOPA by tyrosinase.

organism [150,151].

3.6.2. *Copper and human diseases*

An imbalance of copper, either a deficiency or an overload, results in various kinds of disorders, of which a large section are neurological diseases.

3.6.2.1. Menkes and Wilson disease. Diseases caused by copper deficiency are less frequent than those resulting from overload, and include Menkes disease, neuropathy, and neutropenia.

Menkes disease, also known as Menkes syndrome or X-linked copper deficiency, is a neurological condition caused by a genetic defect in the *ATP7A* gene. *ATP7A* is an ATP-ase enzyme responsible for regulating copper levels in the body [152]. Menkes disease affects many systems in the body, clinical manifestations of which include neurological degeneration, "steely" hair, degeneration of connective tissues, and hypopigmentation of skin and hair [153,154].

Peripheral neuropathy (acute or chronic) is a disease affecting peripheral nerves, and is characterized by the gradual dysfunction of one or more nerves, resulting in muscle pain, muscle weakness, numbness, tingling and other symptoms. Those nerves affected are from sensory, motor and autonomic systems. Although the exact origin of the disease is not known, it is assumed that the altered axonal excitability that causes motor neuron death is a result of an insufficiently protective effect of copper against the abnormal release of glutamate from neurons (glutamate excitotoxicity). It is assumed that peripheral neuropathy and Menkes disease share genetic defects in the *ATP7A* gene.

More commonly, diseases are caused by copper overload. Thus, Wilson disease is a genetic disorder characterized by copper accumulation in various body tissues, such as those of the brain, liver, and eyes. The disease originates from a mutation in the ATP7B protein, a Cu-transporting P-type ATP-ase. The disturbed function of ATP7B leads to a decreased biliary excretion of copper, accompanied by the accumulation of copper in the liver and in other tissues. On their death, hepatocytes release copper to CNS which results in typical neurological abnormalities [153]. Appropriate therapy is based on the application of nitrogen-based copper chelators or zinc, which block intestinal copper absorption [155].

3.6.2.2. Alzheimer's disease. Several neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) are characterized by disturbed copper homeostasis, although the exact role of copper in the pathologies of these conditions is not fully understood [156]. Alzheimer's disease is characterized by the aberrant activity of an enzyme Acetylcholinesterase, resulting in a deficiency of acetylcholine in the brain. In addition, AD is characterized by the accumulation of the neurotoxic peptide Amyloid-β and increased levels of free redox active copper and iron, and redox inactive zinc. Several different experimental techniques revealed that the level of copper in Alzheimers related tissues is three times that in the surrounding tissues. It has also been demonstrated that Amyloid-β can reduce Cu^{2+} to Cu^{+}, and increased amounts of free copper (or iron) can catalyze the decomposition of hydrogen peroxide via the Fenton reaction [156].

$$Cu^{+}\ (Fe^{2+}) + H_2O_2 \rightarrow Cu^{2+}\ (Fe^{3+}) + {}^{\bullet}OH + OH^{-}$$

Which generates reactive hydroxyl radicals ($^{\bullet}OH$), capable of damaging DNA, lipids and proteins. It is highly significant that oxidative stress and increased markers of oxidative stress have been detected in the tissues of Alzheimers patients. To date, more than 100 various adducts of the hydroxyl radical with DNA have been detected, one of the most studied being 8-OH-Guanine, which was first isolated from human urine and is a sensitive marker of oxidative stress and a potential biomarker for

carcinogenesis [157] (see Fig. 7).

N-terminally complexed Cu(II) is reduced by electrons from the Met35 residues according to the reaction [3].

$$\text{Met-S} + \text{Amyloid-}\beta\text{-Cu(II)} \rightarrow \text{Met-S}^{\bullet+} + \text{Amyloid-}\beta\text{-Cu(I)}$$

which results in the formation of the Methionine-35 sulphide radical (Met-$S^{\bullet+}$) and cuprous species, Cu(I). The radical Met-$S^{\bullet+}$ may interact with the superoxide radical anion ($O_2^{\bullet-}$) to form Met-Sulphoxide (Met-SO)

$$\text{Met-S}^{\bullet+} + O_2^{\bullet-} \rightarrow 2\text{Met-SO}$$

Indeed, Met-SO has been found in Alzheimer's senile plaques which confirmed that these radical reactions had taken place in the AD tissues [158].

While the current treatment for AD is based on the application of cholinesterase inhibitors (galantine, rivastigmine, donepezil), approaches are being investigated which involve the development and testing of multifunctional hybrid molecules containing several functionalities, including antioxidant moieties aimed to ameliorate the oxidative stress component in AD [159].

3.6.2.3. Parkinson disease. Parkinson disease (PD) is a neurodegenerative disorder characterized by the selective and severe loss of dopamine-producing (dopaminergic) neurons in substantia nigra [127]. The clinical symptoms involve motor impairments manifested as a slowing of physical activity - bradikynesia, tremor, limb stiffness, and problems with gait and balance.

The main pathological feature of PD is the occurrence of Lewy bodies in the neurons that are responsible for the symptoms. Lewy bodies are composed mainly of the aggregated protein, α synuclein. α synuclein is a relatively small (cca 15 kDa), highly soluble unfolded protein that has "many faces". Although the normal function of α synuclein and causes of its aggregation are unknown, its localization at presynaptic terminals and the suppressed synaptic transmissions, as a response to knock down or overexpression of α synuclein, suggest that this protein may be involved in the regulated release of neurotransmitters [160].

A common feature of PD and WD is copper dyshomeostasis, and indeed, Parkinson disease is frequently also present in patients suffering from WD. It may be significant, therefore, that copper has been documented to accelerate α-synuclein aggregation [161,162]. Although the total copper level in the brains of patients with PD is not very different from healthy subjects, it has been noted that a significantly decreased content (less than half) occurs in the substantia nigra in patients with PD. A preliminary animal model study revealed that supplementation of mice with copper salts prevented striatal dopamine depletion [163]. One of the hypoxia imaging agents, t-diacetyl-bis(4- methylthiosemicarbazonato) copper(II), CuII(atsm) was found to show neuroprotective properties in several PD models, and it has been proposed that the probable mechanism of action is based on the nitration of α-synuclein [164].

3.6.2.4. Huntington disease. Huntington's disease (HD) is an inhered disease caused by the progressive degeneration of nerve cells in the brain. Clinical manifestations involve cognitive impairment, progressive motor dysfunction and psychiatric disorders. A pathological hallmark of HD involves degeneration and atrophy of the striatum, but as the disease progresses into its later stages, the cerebral cortex and other brain structures are also affected. While it is known that mutations may cause HD, the mechanism of its onset is not clear. It has been speculated that the disease progression is connected with the accumulation of copper in the brain [165], as is supported by animal studies which revealed that the advancement of the disease can be slowed by a specific copper chelator, tetrathiomolybdate [166].

3.6.2.5. Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) belongs to a group of Motor neurone disorders (MND), affecting motor neurons in the spine and brain. The disease is genetic in origin, with its most common form being due to mutations in the Cu,Zn-Superoxide dismutase (SOD1) enzyme, for which copper has an increased affinity [167]. Pharmacotherapy aimed to alleviate the toxic effects of copper, caused by copper dyshomeostasis, should be targeted toward addressing the transfer of abnormal amounts of copper to metal-deficient forms of Cu,Zn-SOD.

3.7. Zinc

Zinc is the second most abundant essential metal that is necessary for structural, catalytic and signalling processes in biological systems.

3.7.1. Zinc in health

The total quantity of zinc in the human body is about 2.5 g (Table 1), of which the major proportion is present in the musculoskeletal system. According to the WHO, zinc deficiency is the 5th most important underlying factor for deaths in developing countries [168]. Plasma and serum each contain approximately the same amount of zinc, (ca 14 μmol/L (0.1% of total zinc)) which is predominantly bound to albumin, while the vast majority of zinc in the body is bound to zinc-binding proteins in the cytosol. "Free" or loosely bound zinc is present in picomolar concentrations and is very strictly controlled [169].

Zinc can interact with various ligands and acts as a cofactor for more than 300 proteins, enabling it to take part in many biochemical processes and mechanisms. The most important of these include its participation in signalling mechanisms, thus modulating various cellular and physiological processes such as neuronal, immune, reproductive and other functions. Zinc is also essential for various enzyme activities, gene expression, and important cellular functions including their proliferation. Experimental studies have confirmed that elevated extracellular zinc levels regulate protein kinase C (PKC) and stimulate protein tyrosine phosphorylation and MAPK activities [170]. A typical transcription factor activated by zinc is the metal response element-binding transcription factor-1 (MTF-1), which is a protein composed of six zinc fingers and multiple domains that acts as a zinc sensor controlling the expression of genes important for zinc homeostasis and protection against metal toxicity and oxidative stress. These include the metallothionein, ϒ-glutamylcysteine synthetase heavy chain genes [171,172].

Since zinc acts both as an intracellular and intercellular messenger, its homeostasis has to be tightly controlled [173], and the transport of zinc across biological membranes is regulated by Zinc transporter (ZnT) and Zrt, Irt-related proteins (ZIP). While ZnT controls the transport of

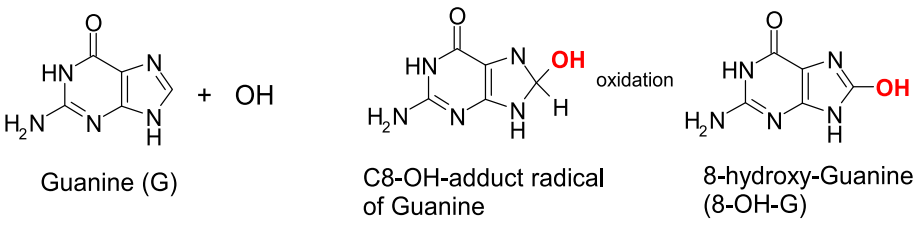

Fig. 7. Reaction of hydroxyl radical with guanine.

zinc from the cytoplasm to the extracellular environment and into the vesicles, the ZIP family of proteins is responsible for the transport of zinc from the extracellular space or the vesicles into the cytoplasm.

Despite the low abundance of zinc in the environment, cells are able to actively take up the metal to confer catalytic and structural functions for zinc proteins. Since high cellular concentrations of zinc can be toxic, cells employ a particular mechanism to store and retrieve zinc from vesicles, called zincosomes, or by the interaction of zinc with metallothionein [174].

As has already been outlined, zinc has many important functions in the human body, for example in regulating the action of insulin and hence the concentration of glucose in the blood, along with maintaining the immune system. Zinc is also required for wound healing, healthy teeth, a normal taste response and antioxidant and anti-inflammatory activities. Zinc is a diamagnetic redox-inert metal, which contains no unpaired electrons and does not participate in electron transfer reactions. The antioxidant action of zinc can be ascribed to three different mechanisms: (i) the binding to and protection of –SH groups of proteins and enzymes against attack by ROS (oxidation); (ii) by competing with redox metals such as iron and copper. This mechanism can be explained in terms of the removal of redox metals from their binding sites which are replaced by redox inactive isostructural zinc. The displaced redox-active copper or iron ions are subsequently eliminated from the cell, thus reducing their bioavailability for catalytic reactions, for example the Fenton reaction, which forms damaging hydroxyl radicals; (iii) triggering synthesis of antioxidant enzymes through expression of transcription factors such as the metal response element-binding transcription factor-1 (MTF-1) [175].

A regular intake of zinc may activate the formation of powerful antioxidants, for example metallothionein, which is one of the most efficient antioxidants known. Metallothionein consists of a group of cysteine-rich divalent metal-binding proteins, in which the metal (including zinc) is tetrahedrally coordinated by –SH(Cys) residues. A single molecule of metallothionein can bind as many as seven zinc atoms [176]. It has been proposed that metallothionein furnishes a link between cellular zinc and the redox state of the cell, which when disturbed by sustained oxidative stress, in turn triggers the release of zinc from metallothionein through zinc-thiol/disulfide exchange [177].

A connection exists between zinc and the regulation of calcium, since voltage dependent calcium channels are used to transport zinc, which are activated by electrical signals from heart cells [178]. Elevated extracellular zinc levels have been reported to increase the concentration of intracellular calcium by activation of intracellular calcium stores. Zinc and calcium are also known to exhibit a synergistic effect on DNA synthesis, MAP-kinase and mitogenic signalling in embryonic mouse fibroblast [179].

3.7.2. Zinc and diseases prevention

Tight control of the cellular zinc concentration (zinc homeostasis) is very important for the healthy functioning of organisms, a process in which a large number of proteins is involved. Thus, there are ten proteins from the Zn-T family which transport zinc from cytosol either to the extracellular space or into vesicles, fourteen proteins from the ZIP family which transport zinc in the opposite direction, and more than ten metallothioneins which serve to buffer zinc ions [180]. As already noted, “free” or loosely bound zinc is present in only picomolar concentrations and this is tightly controlled since even a small, transient increase in cytosolic free zinc may have serious health consequences.

Zinc deficiency has been associated with retarded growth and development, loss of appetite, skin inflammation, impaired function of reproductive organs, an autoimmune disease (alopecia), loss of taste acuity, problematic wound healing, and disorders of the immune system. Severe zinc deficiency is rare, and its origin may be either genetic or acquired, for example acrodermatitis enteropathica, which is an intestinal inability to absorb zinc, and is manifested clinically by skin inflammation, diarrhea and soft nails [181].

A biologically sufficient concentration of zinc is necessary for the correct functioning of the heart, healthy vessels, prevention against oxidative stress-induced diabetes, and protection of the liver against damage by alcohol. Zinc deficiency has been attributed to increased ROS formation and is associated with marked levels of oxidative damage to all important biomolecules, including DNA, proteins and membrane lipids [182]. Such ROS-induced damage, associated with low zinc levels, has been documented by increased levels of carbon centered radicals in lung microsomes [183], increased lipid peroxidation and the formation of malondialdehyde (a carcinogen) in liver microsomes [184], and a greater proneness to copper-induced oxidation of low molecular mass lipoproteins [185].

Testes are especially sensitive to low zinc levels. For example, those of rats supplied with a low zinc diet exhibited a decreased glutamine synthetase activity, a suppressed level of iron-induced 2-ThioBarbituric Acid Reactive Substances (TBARS) formation, increased markers of oxidative stress, 8-oxo-2′-deoxyguanosine levels (8-OH-dG) and an increased concentration of protein carbonyls. The observation of increased oxidative stress has been attributed both to a reduction in the activities of antioxidant enzymes and the increased formation of damaging ROS [186].

That disturbed zinc homeostasis impacts upon cardiac health is supported by an observed inhibitory effect on isoproteronol-induced cardiac oxidative injury. Several in vivo and in vitro studies have reported that ischemic injury, often caused by the action of ROS, can be prevented by the introduction of the zinc-histidine complex [187].

Zinc is a key element for the proper functioning of the immune system, and hence an insufficient zinc intake results in very serious infections, such as human acrodermatitis enteropathica (mentioned above). Indeed, zinc depletion has been shown to impair the normal functioning of all kinds of immunologically active cells. Investigations of the role of zinc in immunology have so far been mainly focused on human leukocytes, as viewed both from a molecular and a cellular perspective. In addition, the effects of immunostimulants and zinc therapy have been studied, and it was demonstrated that supplementation with zinc could restitute impaired functions of the immune system [188].

Rheumatoid arthritis is an autoimmune disease, characterized by chronic inflammation and which primarily affects the joints. Although the pathogenesis of rheumatoid arthritis is not fully understood, it appears that an immune response mediated by T-cells is a key factor for the initiation and progression of the disease [189].

It has been shown that high concentrations of zinc have a negative effect on the functioning of leukocytes [190], and that levels of zinc greater than 30 mM have more inhibitory than positive effects on the components of the immune system, according to in vitro studies. However, such inhibitory activity, conferred by high zinc concentrations can be used in appropriately targeted pharmacotherapy.

Physiological levels of zinc are also important for the proper maintenance of neurological functions. Thus, the human brain contains large amounts of zinc, as compared with other tissues, and its deficiency has been linked with neurological problems, mental disturbances, and cognitive disorders. Markers of oxidative stress in the brain, including a deficiency of zinc, represent a common denominator in several neurological conditions, including Alzheimer’s and Parkinson’s diseases [191].

Among other pathologies, Alzheimer’s disease is characterized by the marked accumulation of the amyloid-β (Aβ) peptide, which is the main component of senile plaques in brain [192]. Several studies have reported that the toxicity of Aβ can be inhibited by implementing micromolar levels of zinc, and it has been proposed that this cytoprotective mechanism operates by blocking a calcium channel pore formed by Aβ [193]. In the brains of patients suffering from Alzheimer’s disease all three metals, redox active copper and iron and redox inactive zinc are significantly elevated, in comparison with surrounding healthy tissues [194]. A protective role for zinc can be explained by its competition with

copper (or iron) to interact with Aβ, since the coordination of zinc to Aβ changes its conformation and prevents it from interacting with copper, thus circumventing the formation of hydroxyl radicals via the Fenton reaction that is otherwise catalyzed by the Cu- Aβ complex [190].

3.8. Molybdenum

In the 1930s, molybdenum, as present in bacteria, was found to be essential for nitrogen fixation and plant growth [195], but it was not until the 1950s that the metal was shown to play an important role in human health [196]. Both a deficiency and an excess of Mo may result in a variety of human diseases.

3.8.1. Molybdenum in health

Molybdenum is found in living systems at low concentrations, but most abundantly in the liver, kidney and small intestine. The only known form that can be uptaken by organisms is $[Mo(VI)O_4]^{2-}$, the oxyanion molybdate [197]. In prokaryotes, Mo enters the cell using a superfamily of integral membrane proteins, ATP-binding cassette (ABC) transporters [198]. In contrast with prokaryotes, the detailed mechanism of molybdate transport in eukaryotes is relatively poorly understood. MOlybdate Transporter1 (MOT1) is the first transporter of molybdate discovered in plant-type eukaryotes, absent in animals. MOlybdate Transporter 2 (MOT2) is encoded by *Chlamydomonas reinhardtii* gene and occurs in animals and humans [199].

About 50 molybdenum-containing enzymes are found in bacteria [200]. Mo enzymes are present in both prokaryotic and eukaryotic organisms and provide important detoxification mechanism in humans and in bacteria. Following the import of molybdate into the cell, it is next enzymatically inserted into a metal cofactor.

Due to the oscillations of Mo between several different oxidation states (+4, +5, +6), the molybdenum-coordinated cofactor can catalyze one- or two-electron transfer reactions. The existence of the Mo cofactor (Moco) was discovered in the early 1960s and its characterization was reported in the early 1980s [201]. Moco requires a defined environment, and in its unbound form is relatively labile and undergoes various transformations. Three discrete families of molybdoenzymes, the xanthine oxidase, sulfite oxidase and DMSO reductase share Moco. Molybdenum uses pyranopterin (or molybdopterin) to form the Mo cofactor (Fig. 8). The tricyclic pyranopterin-form of Moco is the simplest version of the molybdenum cofactor [202].

The Moco-dependent xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and in the next step to uric acid. During the course of the substrate oxidation, Mo is reduced and the electrons are transferred through the [2Fe–2S] core and flavin adenine dinucleotide to molecular oxygen, to form the superoxide radical anion.

The relatively weakly abundant sulfite oxidase family of molybdoenzymes is essential in higher organisms, predominantly for neuronal functioning. The molybdopterin-$Mo(VI)O_2$ core of this enzyme family contains the amino acid cysteine, bound between Mo and the molybdoprotein [203], and the relevant catalytic action includes oxidation of sulfite with the concominant two-electron reduction of Mo.

The most abundant group of molybdoenzymes are members of the DMSO reductase family, which contain a Moco derivative with two pterins coordinated to Mo. This group of enzymes also possess [3Fe–4S] or [4Fe–4S] clusters and cytochrome-b5- hemes [204], and constitute target materials for drug discovery and pharmacotherapy.

3.8.2. Molybdenum and diseases

The number of clinical diagnoses originating from a deficiency of Mo-enzymes is relatively low. This is due to the large heterogeneity of symptoms and non-specific disease progression which often leads to an erroneous attribution of a disease. Deficiencies in Mo-enzymes are manifested by a number of pathologies, ranging from the neonatal period on through to maturity [205]. In the past decade, more systematic approaches have been conducted in cohort studies with the aim to explore pathways involved in the dysfunction of Molybdenum enzymes.

An imbalance between the production and excretion of urinary acid that results in its accumulation is hyperuricemia. The primary cause of this disease is a suppressed renal excretion, while the secondary cause is more complex and involves an aberrant activity of homodimeric enzymes, xanthine oxidoreductase, which normally serve to catalyze the oxidation of hypoxanthine to xanthine or xanthine to uric acid [22]. Mammalian xanthine oxidoreductase has many important physiological roles, especially the xanthine oxidase form. Hyperuricemia is further characterized by chronic inflammation due to the abnormal release, synthesis and uptake of purines, and leads to the development of gout, as manifested by deposition of crystals in the joints, and an increased risk for the development of cardiovascular disease. The risk elements for the development of gout include genetic factors, dietary factors, cardiovascular diseases including hypertension, renal dysfunction, and obesity [206]. Dietary risk factors involve the consumption of alcohol, red meat

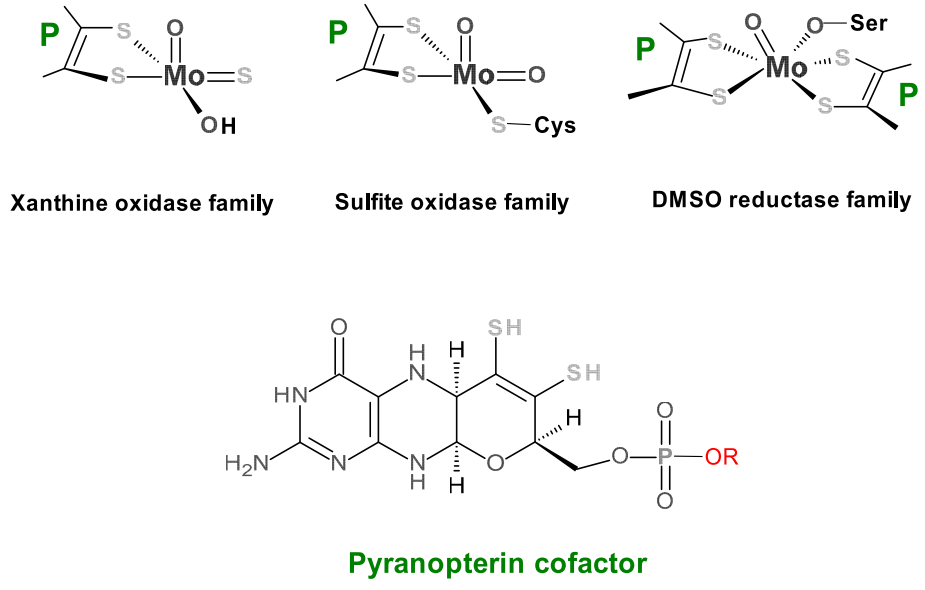

Fig. 8. Structures of the active sites in three families of Mo enzymes.

and fructose. Treatment for gout is based on the reduction of serum urate levels using xanthine oxidase inhibitors, for example allopurinol, although new drugs have recently been released [207]. Further research is focused on the mechanism of the etiology of the disease, including the identification of urate transporters.

A molybdenum cofactor (Moco) deficiency was first described more than four decades ago [208]. In fact, there are three types of Moco deficiency, termed Type A, B and C. While Type A is caused by mutations in the *MOCS1* gene, the Types B and C are caused by mutations in the *MOCS2* and *MOCS3* genes, respectively. Moco biosynthesis in eukaryotes consists of three steps and involves two major intermediates, namely cyclic pyranopterin monophosphate (cPMP) and the metal binding protein [209]. Mutations in the *MOCS1* gene disrupts the production of cyclic pyranopterin monophosphate (cPMP), which is necessary for the production of Moco. Moco plays an important role in the normal functioning of several key enzymes including sulfite oxidase [210,211]. In the absence of Moco, the sulfite oxidase process is significantly impaired and so cells cannot break down sulphite, which is highly toxic, nor a secondary product, S-sulfocysteine, that is responsible for neurological deterioration (Fig. 9) [212]. Symptoms of Mo cofactor deficiency include neonatal feeding difficulties, intense crying, neurological issues, and mental problems – for example, dysmorphic disorder, which may result in social anxiety. Neurological problems are those most serious, with respect to Mo-cofactor deficiency. As the disease progresses, patients suffer of cerebral atrophy, as is manifested by psychomotor impairment according to MRI measurements. Those patients, who survive their neonatal period, show minimal neurological progress and most probably die within the next few years of their life [213].

Treatment of Type A is based on the intravenous administration of cPMP, which appears to be helpful in preventing neurological damage. Since Type B deficiency has a different origin, the administration of cPMP has no effect [214]. However, pyridoxine supplementation can alleviate some neurological symptoms in Type B patients.

Molybdenum is an antagonist to copper [215], and hence Mo overload conditions also result in copper homeostasis disorders, as manifested as Menkes and Wilson diseases, which are characterized by copper deficiency and copper overload, respectively. The antagonism between copper and molybdenum has been proposed to be a unifying factor in the metabolism of these two metals. It has been suggested that the mechanism of copper-induced Moco inhibition is due to the inhibition of the magnesium-dependent Mo insertion reaction. Accordingly, increased copper levels, as is typical for Wilson's disease, negatively affects the biosynthesis of Moco [216].

3.9. Metals as cofactors

Cofactors and coenzymes are species that supporting the correct function of enzymes or other proteins, and hence assist in biochemical transformation processes. Some textbooks term a coenzyme as an associative non-protein factor necessary for enzymatic functioning. The coenzyme is either a tightly (permanently) bound prosthetic group or a cofactor – i.e. the loosely bound inorganic or organic molecule. Some authors use the term cofactor strictly for inorganic species. Some enzymes require several cofactors in order to function properly [217], for example the complex enzyme, pyruvate dehydrogenase requires no less than one metal ion and five organic cofactors [218].

While organic cofactors involve heme or flavin, for example, inorganic cofactors are represented by metal ions, such as mangnesium, d-transition metals and iron-sulphur clusters. Some common metal ions that are present as cofactors include Fe, Mg, Mn, Cu, Co, Mo and Zn [219]. However, cofactor often includes both inorganic and organic components. Ca is an important element in the human diet and is necessary for functioning of many enzymes, which it typically activates by allosteric regulation [50]. Some organisms require vanadium as an enzyme cofactor, for instance in the nitrogenase of the genus Azotobacter [220]. Tungsten is employed in the aldehyde ferredoxin oxidoreductase enzyme and cadmium in carbonic anhydrase [221]. The cytochrome oxidase enzymes contain the Cu^{2+} ion, catalase, nitrogenase, and hydrogenase contain either Fe^{2+} or Fe^{3+} ions, while DNA polymerase and glucose 6-phosphatase contain the Mg^{2+} ion. Arginase contains Mn, urease is known to contain Ni, and DNA polymerase, carbonic anhydrase, and alcohol dehydrogenase all contain Zn in their structures. Calcium is a cellular signalling element but is not considered as a cofactor in enzyme regulation [222].

3.10. Halogens in health and disease

Halogens are non-metallic elements which belong to Group VIIa of the Periodic table of elements. Specifically, these are fluorine (F), chlorine (Cl), bromine (Br) and iodine (I). All these elements form metallic salts and therefore the name "halogen" originates from Greek words "hal" meaning salt and "gen" meaning "to form".

Fluoride (F^-) and chloride (Cl^-) anions have small atomic radii, and are hard bases. In contrast, iodide (I^-) and bromide (Br^-) anions have large atomic radii, and are soft/border line bases.

Halogenated metabolites are often found in marine seaweeds and which participate in oxidation reactions, in the presence of light, resulting in the formation of halogenated substrates. These reactions are catalyzed by Vanadium-dependent haloperoxidases (VHPO), with bromine being incorporated most abundantly. VHPO contain vanadate as the prosthetic group and in the presence of H_2O_2 catalyze oxidation of halide ions [223].

Chlorine has an atomic mass of 35.5 and two stable isotopes ^{35}Cl and ^{37}Cl. Chloride, in its association with sodium, potassium, calcium and magnesium cations, is essential for many physiological processes. Chloride anions, together with sodium cations, are considered as the principal ionic species in the human body, being responsible for the preservation of osmotic pressure, nervous and muscular activity and transport of water and solutes between cellular compartments. The average daily chloride intake of an adult is in the range of 1800 mg–5000 mg, the most common source being table salt. Chloride anions are the most abundant extracellular anions absorbed from gastrointestinal tract. In addition to its role in electrolyte equilibrium, chloride is needed for the formation of gastric hydrochloric acid, which is secreted from the parietal (oxyntic) cells of the gastric mucosa present in

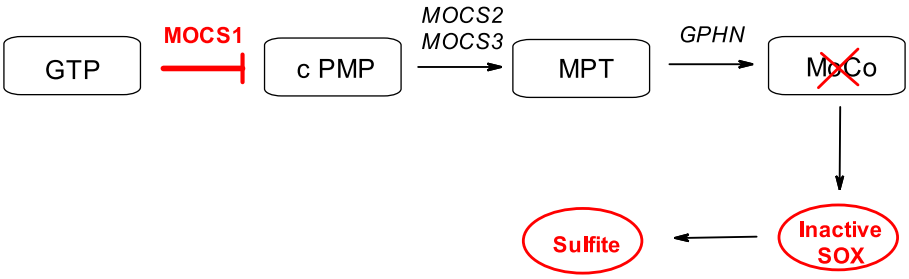

Fig. 9. Type A Moco deficiency. Moco is synthesized in several steps, from guanosine triphosphate (GTP), cyclic pyranopterin monophosphate (cPMP) and molybdopterin (MPT) via molybdenum cofactor synthetase. Disruption in the Moco synthesis, due to the mutation in the MOCS1 gene, results in the impaired function of sulfite oxidase (SOX) and accumulation of toxic sulfite and other products that are responsible for neurological damage. The GPHN gene encodes an organizational protein, gephyrin.

the glands in the fundus and body of the stomach [224]. The concentration of Cl^- follows that of Na^+ and is not controlled by homeostasis. In cases of respiratory acidosis (depletion of Cl^- due to the retention of bicarbonate anions) and respiratory alkalosis (elevated concentration of Cl^- due to the excretion of bicarbonate anions), changes in the concentration of Cl^- result in disturbances of acid-base equilibria [225]. However, disorders in acid-base equilibria are mainly a result of chloride anion losses due to vomiting and diarrhea [226].

Regulation of chloride anions is maintained by the concerted action of several mechanisms. Thus, it has been shown that chloride channels such as CIC-2 (coded by *clcn2* gene) are more permeable toward the release of chloride anions, than for these species to enter the cells. Despite the differences in essential permeability for these opposing directions, thermodynamic principles (Gibbs Free Energy) must also apply, to determine the driving forces for transport of chloride anions in and out of cells [227]. From this, it follows that an alternative transport mechanism must operate against the gradient of chemical potentials [228]. Indeed, this mechanism uses symporters, which are proteins capable of transporting two species across the membrane in the same direction, thus chloride can be transported against its gradient of chemical potential by piggybacking onto another ion transported down the gradient. The main neuronal chloride extruder is the potassium-chloride cotransporter 2 (KCC2), coded by the *slc12a5* which allows chloride to piggyback onto potassium ions that are transported along a gradient that takes them out of the cell. Since this transport involves oppositely charged ions in a stoichiometric ratio 1:1, the overall process is electroneutral.

Following efficient absorption of chloride from the gut in healthy subjects, chloride anions are maintained in the blood within a narrow concentration range, and chloride excretion is coupled to sodium and potassium loss [229]. A dietary increase of sodium chloride results in an elevation of blood pressure, and this effect depends on both sodium cations and chloride anions. The effect of serum/plasma chloride on the cardiovascular system has been studied; however, it was found that the level of chloride in plasma or serum did not correlate with the dietary intake of chloride anions [229]. It was concluded that that this is mainly due to a large time variability for chloride excretion in the urine.

Deficiency of chloride (hypochloraemia), most frequently caused by massive gastrointestinal and renal losses, is a rare condition; however, in infants, this leads to retarded growth, anorexia, agitation, lack of physical and mental energy, and various gastrointestinal symptoms [230].

Although an excess of chloride (hyperchloraemia) taken from the diet is not a common condition, a very high intake of salts or some drugs (cortisone) may lead to increased chloride levels. Hyperchloraemia may be caused by severe diarrhea resulting in the loss of bicarbonate. It may also occur with other conditions linked with massive water loss from an organism and depletion of extracellular fluids, or increased reabsorption of chloride [229].

Fluorine is the most electronegative element known, and is a highly reactive gas, which occurs in the anionic form of fluoride (F^-). Fluorides are present in air, water and in the lithosphere, and bind to metal ions. Fluoride has no biological functions with respect human growth and development [231]. Major dietary sources of fluoride are tea, fish and fluorinated salt and water. The most important fluoride salts for human use are sodium and potassium fluoride, which are very water soluble, and are permitted for addition to foods (e.g. salt) and for fluoridation of water. Calcium fluoride and sodium monofluorophosphate are also permitted as food supplements. The recommended daily fluoride intake is 3.0–3.4 mg.

Fluoride is absorbed in the stomach and small intestine by passive diffusion. Majority of ingested fluoride is absorbed, while the residual portion is excreted via the kidney and in faeces. Fluoride absorption, distribution and excretion are all dependent on pH. At low pH, the majority of fluoride is in the form of undissociated HF, which in contrast to F^-, is readily permeable across biological membranes [232].

The concentration of fluoride intake from both dietary and other sources is assessed in plasma, nails, hair, bone, saliva, sweat, urine and tooth enamel. The current intake of fluoride is estimated in blood, bone, sweat, saliva, milk and urine. The long term intake of fluoride is determined in hair, teeth and nails [233].

The incorporation of fluoride into hydroxyapatite results in the formation of fluorohydroxyapatite which protects teeth from dental caries. Fluoride taken from the diet protects teeth from caries through direct contact with enamel. Fluoride may also interfere with the metabolism of microbes present in the oral cavity, which among other effects, inhibits glycolytic enzymes.

Although the substitution of fluoride by hydroxyl anions in bone apatite changes its mineral structure, there is no direct evidence for a link between the intake of fluoride and bone health.

Supplementation of fluoride in infants (0.25 mg per day) was found to result in a higher body weight than without such supplementation [234]. It has been shown that while fluoride deficiency during development does not affect the development of teeth and caries, the susceptibility of enamel to damage by acids is higher.

Intake of large doses of fluoride ($\geq$5 mg/kg), most frequently from highly fluorinated water and/or fluoride supplements, can cause vomiting, nausea, diarrhea, kidney problems and in rare cases also death [235]. Furthermore, the long term intake of fluoride in children can lead to dental fluorosis, which is characterized by white lines and sometimes brown stains on teeth [236].

An excess fluoride intake is linked with the metabolism of calcium and the activities of many enzymes. Thus, altered enzyme activities involve glycolytic functions and cellular respiration caused by the inhibition of Na^+,K^+-ATPase [237].

During the past 20 years, organo-fluorine compounds possessing biological activities have provided a highly attractive area for the development of pharmaceutical drugs [238]. The incorporation of fluorine atoms into molecular structures, serves to protect them from degradation. The Carbon–Fluorine bond is highly polar and unreactive, and is extraneous in biological systems, meaning that enzymes do not easily degrade compounds containing them. Hence, the incorporation of fluorine atoms into the molecular structure of drugs, significantly increases their stability under physiological conditions [239].

Bromide is the negatively charged (anionic) form of bromine. In the 19th century, bromides were used as sedatives and anticonvulsants. Acute intoxication by bromide has not been reported. Long term intake of bromide (0.5–1 g per day) results in bromide intoxication [240]. This condition results from overmedication by drugs containing bromides and is known as Bromism. In addition to psychiatric disturbances and gastrointestinal problems, clinical manifestations of bromism involve restlessness, ataxia, anorexia, confusion, hallucinations, stiffness, weight loss and dermatological problems such as skin lesions [240]. The level of bromide is highest in the lungs and liver, while a major portion is also present in the skin. Depleted iodine can be replaced by bromine [241].

The mechanism of bromide toxicity involves the substitution of bromide for chloride in neural membranes of transport systems. Overall, one third of the chloride present can be replaced by bromide, and such high bromide levels results in the impairment of neural transmission [242].

Bromides are found in trace amounts in soils and water and may also be present in vegetables due to the fumigation of soil with dibromides. Bromide salts are present in some drugs, fire extinguishers, and refrigerants. Bromides used in pharmaceutical praxis are of both organic and inorganic origin. Serious bromide intoxication can be caused by drugs used as cough suppressants [243].

The oral bioavailability of bromides is about 95%, with the maximum level of bromides in serum being equilibrated within 2 h, becoming concentrated in erythrocytes and neurons [244].

It has been shown that bromides are necessary cofactors for the peroxidasin-catalyzed formation of sulfilimine crosslinks, as are

required for the development of tissues and the architecture of membranes [245]. In addition, the Bromo peroxidase enzymes of red and brown marine algae use bromide (in seawater) as a cofactor to form electrophilic brominating agents. These enzymes catalyze the oxidation of halides to hypohalous acids in the presence of hydrogen peroxide to form halogenated organic substrates [246]. In this process, a variety of compounds, containing carbon-bromine bonds are formed.

One recent study determined that the level of bromides in specific plants was above 50 mg/kg. This confirms that plants are able to take up high amounts of bromide from the environment. However, based on the relevant WHO recommendations, there is no toxicological concern over drinking herbal teas, extracts or crushed tea leaves at recommended therapeutical doses [247].

Iodine may exist in oxidation states ranging from −1 (I^- or iodide) to +7 (IO_4^- or monovalent anion, iodine oxoanion). Iodate (oxidation state +5, IO_3^-) is present in soils and rocks and is transferred to water and finally to the sea where it can accumulate in marine organisms. In land, small amounts of iodine are accumulated in plants [248].

The deficiency of iodine is a serious health problem worldwide. Iodine is necessary for the synthesis of thyroid gland hormones, which play an important role in energetic metabolism and gene expression, and hence influence many physiological functions including neurological and cognitive functions. In particular, iodine is an important component of the thyroid hormones T4 (thyroxine) and T3 (3,5,3 -triiodothyronine). Specifically, thyroid hormones are known to control such processes as mitochondrial energy metabolism, thermoregulation, metabolism of proteins and lipids and cellular oxidation. Thyroid hormones play important roles, particularly during the early growth and development and maturation of many organs, the most important being the brain [249].

Depending on their thyroid function, subjects are classified as euthyroid (normal function), hyperthyroid or hypothyroid. A number of various mechanisms can result in either a hyperthyroid or hypotheroid function of the thyroid gland, both having iodine intake as a common denominator, either excessive or insufficient.

Clinically, the consequences of iodine deficiency are referred to as Iodine deficiency disorders (IDD), and which result in hypothyroidism. IDD has been detected during the pregnancy and in infancy, and all other stages of development with the risk of developmental brain damage [250]. In 2011 it was estimated that nearly half of the European population had a too low intake of iodine as documented by a urinary iodine concentration of less than 100 μg/L. In addition to urine, iodine is eliminated from the body via faeces and sweat. A long-term iodine deficiency can lead to an enlargement of the thyroid gland (goitre) which increases the risk of thyroid cancer. A mild deficiency of iodine results in goitre in up to 20% of school children (predominantly in girls), worldwide. In severe iodine-deficient cases, a condition termed cretinism occurs, which is characterized by cognitive decline, deaf mutism, retarded growth, sexual immaturation and dwarfism [251].

Excessive intake of iodine, for example through water, as has been observed in China, may also lead to the development of goitre [252]. The long-term excessive intake of iodine may lead to either hyperthyroidism or hypothyroidism, with an increased incidence of auto thyroiditis and thyroid cancer.

The metabolism of iodine can be negatively affected by a deficiency of vitamin A and of redox metals such as Fe, Cu and Zn. Se-containing enzymes are known to play an important role in the metabolism of thyroid hormones [253].

4. Conclusion and outlook

The present paper summarizes the key biological functions of essential metals, along with various pathological states that result from their dyshomeostasis. Of the ten essential metals, four are s-block and six are d-block elements. The four s-block elements consist of two alkali metals (Na and K) and two alkaline earth metals (Mg and Ca), while the d-block essential elements include Mn, Fe, Co, Cu, Zn and Mo.

The coordination strength of s-block elements with organic ligands ranges from weak (Na, K) to intermediate (Mg, Ca), while in contrast, the d-block essential metals coordinate organic ligands more tightly. The ability of metals to coordinate ligands, is in reverse order to their mobility in biological systems. While the s-block elements, Na, K, Mg and Ca exhibit relatively high or intermediate mobilities in biological systems, the four d-block elements typically show a suppression in their mobility; the only exception being zinc, which shows a somewhat higher mobility than the other d-block elements.

Essential metals crosslink the organic “building blocks”, and so maintain the integrity of cell membranes. The preservation of the double helix structure of DNA by the presence of metal cations is also very important: thus, the charge-charge repulsion interactions between phosphate groups are compensated by their interaction with metal cations, for example Mg^{2+}. Many structural functions of solid biomolecules are a result of the presence of Zn^{2+}, Ca^{2+} and Mg^{2+} which are charge-compensated by the organic anions.

A variety of metal cations with different ionic radii and charges, including Na^+, K^+, Ca^{2+}, are employed in the transfer of information, for example across biological membranes. Transmembrane concentration gradients of these metal cations, as maintained by the membrane ion pumps, play crucial roles in the creation of electric Nernst potentials, which, for example, is important in the control of muscle contraction.

The degradation of a variety of organic compounds, including toxic metabolites such as free radicals, requires acid/base catalysis, the efficiency of which is limited to certain compartments where the pH is low, for example the stomach acid present in the GI tract. In other compartments with a physiological pH, Lewis acid or base catalysis is required, as is provided by metalloenzymes containing positively charged ions such as Mg^{2+} and Zn^{2+}.

Electron transfer reactions, including energetically demanding multielectron transfer reactions, abound in biological systems, and are mediated by the involvement of metalloenzymes which containing redox active metal centers whose activity is finely tuned by their particular ligand environment. Such metal centers involve a variety of d-block elements in various oxidation states, for example Fe^{2+}, Fe^{3+}, Cu^+, Cu^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Co^{2+}, Co^{3+}, Mo^{4+}, Mo^{5+}, Mo^{6+}.

As has been briefly outlined, essential metals play many important roles in maintaining the health of organisms, including humans. Disturbances in metal homeostasis are frequently caused by genetic defects, environmental aspects, improper diet, and other factors, and this may lead to serious health problems. Understanding the metal homeostasis more fully may lead to the development of new therapeutic agents for the clinical praxis, for example in the form of metallodrugs aimed toward the treatment of cancer, neurological disorders and other serious diseases. Since metal dyshomeostasis is very often caused by unrelated conditions or by activated compensatory mechanisms, medical intervention aimed specifically to restore metal balance may not be successful and must be undertaken with caution.

While a high intake of **sodium** has been linked with cardiovascular and renal diseases, whether Na is harmful or beneficial, actually depends on the prevailing immunological circumstances. Sodium is stored in tissues in order to activate the immune system and fight against external pathogens. However, Na-induced activation of the immune system might not only promote wound healing, but also hypertension and cardiovascular diseases. Thus the precise quantification of sodium intake should be the subject of further intervention studies [254].

Similarly to sodium, **potassium** levels are also linked to hypertension. Accordingly, in depth studies related to potassium supplementation and the treatment of hypertension and the prevention of stroke are currently being undertaken. In addition, the connection between low potassium levels and the risk of diabetes, including a potential positive effect of potassium supplementation in this respect should be the focus of detailed studies. Furthermore, a set of prevention guidelines, relevant dosage, length of therapy and other related aspects should be established

in regard to hypokalemia.

Calcium intake is inversely correlated (preventative) with the risk of lung, colon and breast cancers, although in the case of prostate cancer, the correlation is positive. The underlying mechanism for the anticancer properties of calcium should be investigated at the molecular level. The effectiveness of combined supplementation by calcium and vitamin D is currently of particular interest, especially for the prevention of cancer, and efforts to elucidate the underlying molecular mechanism are highly necessary.

Recent research regarding the physiological effects of **magnesium** has been aimed toward the kidney and lower gastrointestinal tract. However, there is an urgent need for future research to focus on the role of this metal in mental and neurological disorders, for example depression, schizophrenia, migraine and epilepsy. Disturbances in magnesium levels can also cause arrythmias, muscle cramps and other health problems, and hence levels of magnesium should be precisely determined, together with those of sodium, potassium and calcium.

All redox active **d-block metal elements** are integral parts of enzymes which catalyze various chemical reactions at physiologically compatible rates. These include, for example, oxygen transport (hemoglobin, myoglobin), the dismutation of superoxide radical anions (Cu, Zn-SOD, Mn-SOD), and the decomposition of hydrogen peroxide (Catalase).

Regarding **manganese,** the framework of its metabolism and homeostasis is, as yet, incomplete. Future progress in the development of advanced markers to monitor levels of manganese and its chronic accumulation is awaited. While levels of Mn in blood undergo rapid changes, as stored in bones, manganese has a long life of several years and provides a good biomarker for levels of this metal in the body. While the largest quantities of Mn are present in the cell nucleus, only a very limited number of intracellular transporters have been identified. Future progress is expected in the discovery of new Mn transporters in cells.

Due to the ability of **iron** to participate in redox reactions, iron levels are carefully controlled in living organisms, especially the labile iron pool. Low levels of iron are related to various anemias, whereas iron overload, which may result, for example, from hyperabsorption, can be treated by means of selective iron-chelation therapy. In addition, iron chelators have been studied, which have proved effective in the treatment of cancer, malaria and tuberculosis.

Vitamin B12 (cobalamin) is the only known vitamin containing a metal ion bound to a heme moiety, and in order to function correctly, it requires the participation of various auxiliary proteins which are currently being investigated. Indeed, it is thought that some of these proteins have yet to be discovered. So far, only a limited number of cobalt-based complexes have been studied as pharmaceutical agents, and the only known Co-based drug with antiviral properties is Doxovir, $[CoIII(bisacetylacetatonato\text{-}ethylenediimine)(2\text{-}methylimidazole)_2]^+$ which has been used to treat *Herpes simplex labialis* and viral eye-infections. Many new Co complexes, containing Schiff bases, and with different biological activities are currently under investigation, for example Co-based anticancer drugs. In addition, cobalt has been shown to interfere with hypoxia-responsive genes, although any related potential clinical applications will require further research.

Disturbances in **copper** homeostasis play important roles in the etiology of neurological disorders such as Alzheimer's, Parkinson's and motor neuron diseases. It can be expected that the role of copper, including a Cu-induced oxidative stress component, in neurological disorders will provide a solid basis for the development of new and effective therapies for these conditions.

Copper and **zinc** may mutually affect one other. Thus, a disturbed zinc homeostasis cannot be restored by zinc supplementation [255], but an excess of zinc may displace copper from its protein binding sites, which then participates catalytically in the Fenton reaction

$$\text{Protein-Cu}^{+/2+} + Zn^{2+} \rightarrow \text{Protein-Zn}^{2+} + Cu^{+/2+}$$

$$Cu^{2+} + \text{Biological reductant} \rightarrow Cu^{+} + \text{Oxidized Biological reductant}$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + {}^{\bullet}OH \rightarrow \text{Damage to biomolecules (Fenton reaction)}$$

Thus, an excess of zinc may be considered as an indirectly prooxidant agent. A focus for future research will be made on proteins that are responsible for the allocation and redistribution of zinc, and on the role of this metal in both intracellular and intercellular signalling mechanisms. Other current investigations concern the role of proteins in maintaining zinc homeostasis, which in turn affect the redox state of cells, genetic stability and inflammation.

Molybdenum is a key element, and its deficiency in Moco-dependent enzymes results in very serious and rapidly advancing neurological defects in newborn babies. Although the underpinning mechanism for this critical neurological diagnosis is gradually being explored, further progress in the identification of key molecules responsible for neuronal damage is awaited. Along with copper and iron, molybdenum may also play a role in the etiology of Huntington's disease, through its function in Mo-dependent enzymes which may cause metabolic changes. Neurological disorders are connected with the homeostasis of the amino acid cysteine, and so the relevant sulphur-containing intermediates are being investigated in the context of the overall underlying mechanism. By understanding the detailed mechanism of cysteine oxidative metabolism, some light may be cast on possible pathways for the detoxification of sulfite, a key species that is responsible for neurological damage in Moco-cofactor deficient disorders.

In this paper are surveyed selected biological roles for essential metals and the variety of diseases that may result from their disturbed homeostasis. Since each essential metal acts in a concerted action with other essential and/or non-essential metals, future research will need to examine more thoroughly the combined effects of metals from both these groups. For example, it appears important to consider essential copper, iron and zinc in combination with cadmium. Particular attention should be devoted to exploring chromium and its interactions with transition d-metal ions, which are currently attended with some controversy. Detailed studies of essential and non-essential metals will be made to explore their role in various important biological pathways, and hence with variously beneficial or detrimental effects in cells.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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