Healthy Ageing and Longevity 8
Series Editor: Suresh I. S. Rattan

Marco Malavolta
Eugenio Mocchegiani *Editors*

Trace Elements and Minerals in Health and Longevity

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Healthy Ageing and Longevity

Volume 8

Series editor

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Rapidly changing demographics worldwide towards increased proportion of the elderly in the population and increased life-expectancy have brought the issues, such as "why we grow old", "how we grow old", "how long can we live", "how to maintain health", "how to prevent and treat diseases in old age", "what are the future perspectives for healthy ageing and longevity" and so on, in the centre stage of scientific, social, political, and economic arena. Although the descriptive aspects of ageing are now well established at the level of species, populations, individuals, and within an individual at the tissue, cell and molecular levels, the implications of such detailed understanding with respect to the aim of achieving healthy ageing and longevity are ever-changing and challenging issues. This continuing success of gerontology, and especially of biogerontology, is attracting the attention of both the well established academicians and the younger generation of students and researchers in biology, medicine, bioinformatics, bioeconomy, sports science, and nutritional sciences, along with sociologists, psychologists, politicians, public health experts, and health-care industry including cosmeceutical-, food-, and lifestyle-industry. Books in this series will cover the topics related to the issues of healthy ageing and longevity. This series will provide not only the exhaustive reviews of the established body of knowledge, but also will give a critical evaluation of the ongoing research and development with respect to theoretical and evidence-based practical and ethical aspects of interventions towards maintaining, recovering and enhancing health and longevity.

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Trace Elements and Minerals in Health and Longevity

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Preface

Biological aging is a complex phenomenon consisting of loss of vitality and increased vulnerability that still lacks a shared definition and deep understanding. It is hypothesized that it can be originated by the decline of the force of natural selection with chronological age or that it is the consequence of a selection of traits that benefits a population or an ecosystem even if they are adverse to individual members. In a biological perspective, this mean that aging can be driven by the gradual accumulation of random molecular and cellular damage or that it is determined by a programmed process involving hundreds or thousands of genes acting with the purpose to eliminate older individuals from the population. Aging is also considered as a process that occurs independently by the disease of aging, by some scientists, or as the root cause of age-related diseases by others. In any case, aging remains the major cause of suffering and death worldwide and the proportion of the population affected by disabling chronic illness of aging is constantly increasing.

In this context, the development of treatments able to improve health and extend life span is highly desirable. A complete understanding of the underlying process of aging may be not necessary to start planning interventions able to reverse part of the damage that accumulates with aging, but the impact of these treatments may result to be minimal in terms of life- and health-span extension if we are not able to clearly disentangle beneficial compensatory phenomena that arise to counteract the damage associated with aging from the damage itself. A representative example of what this mean may be offered by the case of amyloid beta in Alzheimer's disease. By one side, amyloid plaques have been considered as the cause of damage, and by another side, they are considered as a protective response to other underlying causes of the disease, so that the removal of amyloid may even worsen the disease. Unfortunately, the way most intracellular signaling pathways influence aging at a biochemical and metabolic level is still largely unknown. Under a certain point of view, it may appear a paradox that in the twenty-first century, we are still not able to definitively answer the question what is aging and why we age.

With this book, we would like to contribute to the efforts made in the attempt to clarify the mysteries that control aging by focusing on a specific aspect of this process. A common feature of aging, which is frequently used to define the process of aging itself, is the loss of homeostasis (the ability to maintain internal stability in the presence of challenges) with time. In this context, it should be noted that there are various homeostatic systems which, in turn, are differently affected by aging. One of these systems is metal homeostasis (metallostasis), which is kept by a multitude of binding, buffering, and transport proteins that are highly affected by aging. The age-related loss of metallostasis appears to be a conserved phenomenon across multiple species, and there is substantial evidence that alterations in metal abundance modulate life span in humans as well as in most common animal models used in aging studies, such as rats, mice, worms, and flies.

This multi-chapter review book presents the present state of knowledge on the role of minerals and trace elements in health, aging, and longevity. The book is divided into 11 chapters, each dedicated to a specific mineral or trace element and a final chapter dedicated to the optimal and the nadir ranges of the micronutrients. All chapters have a title dedicated to a mineral or element: iron, copper, selenium, zinc, chromium, molybdenum, sodium, magnesium, and iodine. Each chapter consists of an in-depth review on the impact of each mineral or element on molecular and physiological processes of aging, with a focus on clinical, animal, and other laboratory models of interest in aging.

Engrained with the up-to-date information about role of trace elements and minerals in health, aging, and longevity, this collection is a valuable addition to the book series "Healthy Ageing and Longevity" and provides a reliable source of information and knowledge useful for understanding and developing potential interventions for modulating aging and longevity.

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Acknowledgements

The Editors, Marco Malavolta and Eugenio Mocchegiani, would like first of all to acknowledge the passing of an author of this book, a qualified diabetology specialist, an esteemed doctor, and a colleague, Dr. Massimo Boemi. He has been always highly active in research and is appreciated for his methodological and didactical approach. His chapter entitled "Water and Sodium Balance Disorders in Aging" provides an overview on the age-related changes in the peripheral and central mechanisms that regulate water and sodium homeostasis in humans and animal models. We are confident that this chapter will contribute to honoring his memory.

The co-author of the book *Trace Elements and Minerals in Health and Longevity*, Dr. Ruslana Iskra, devotes his work to Maria Iskra, a young intelligent and talented girl who loved life, and was interested in science and dreamed of a future study at the Italian Università degli Studi di Macerata. May this dedication honor its memory.

We thank all the authors and recognize the excellent quality of their contributions. While working on the progresses of the book, we have immediately raised the awareness that the book would become a key reference for aging research dedicated to minerals and trace element biology. All co-authors, young scientists, and contract researchers, who directly or indirectly have contributed with their enthusiasm and creativity, also deserve particular thanks. Marco Malavolta and Eugenio Mocchegiani also thank our series editor, Prof. Suresh Rattan, for his encouragement to begin and continue this ongoing journey of discovery and learning in the field of biogerontology. Without the dedication and creative contributions of these scientists and authors, this book would not have been possible.

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Chapter 1
Iron

Tanja Grubić Kezele

Abstract Iron is an essential component of every living organism. Iron excess and deficiency are equally detrimental for the cell and thus species longevity. They cause very large number of health disorders and organ system dysfunctions, especially development of age related diseases in elderly people. Iron deficiency increases morbidity and mortality because it causes growth arrest and cell death. Age-related accumulation of iron increases the potential for free redox-active iron, which can promote oxidative stress and mitochondrial damage and thus functional alterations of antioxidant enzymes and further increased oxidative damage to DNA, RNA, proteins, and lipids in tissues in elderly people. This further increases the risk for cancer, liver diseases, cardiovascular disorders associated with atherosclerosis, diabetes mellitus, osteoarthritis, osteoporosis, metabolic syndrome, hypothyroidism, hypogonadism, numerous symptoms and neurodegenerative disorders which accompany ageing (Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy, multiple sclerosis, etc.). Experimental animal studies provide new insights in iron manipulation mechanisms (ferritin, frataxin, mitochondrial autophagy) involved in species longevity and suggest further investigation to elucidate possible application for treatment in human degenerative and age-related diseases.

Keywords Age-related diseases · Longevity · Iron · Iron deficiency · Iron excess Oxidative stress

1.1 Introduction

Iron is an essential component of every living organism. In the human body it exists in a complex with protein (hemo-protein), as heme compounds (hemoglobin or myoglobin), heme enzymes, or non-heme compounds (flavin-iron enzymes, transferrin

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Fig. 1.1 Fenton reaction

and ferritin), which are important for cellular processes like oxygen transport, electron transfer and oxidation-reductions, mitochondrial respiration and DNA synthesis or cell growth (Abbaspour et al. 2014).

Iron can serve as an electron donor and acceptor. Within living organisms, iron can be found in reduced, ferrous (Fe^{2+}) and oxidized, ferric (Fe^{3+}) state (Waldvogel-Abramowski et al. 2014). The same chemical properties that make iron an essential component in numerous physiological processes can cause cell toxicity if iron is unshielded. A number of transporters, binding proteins, reductases and ferroxidases help maintain iron homeostasis to prevent this damage. Excess of free iron catalyzes the conversion of hydrogen peroxide to the highly reactive hydroxyl radical and hydroxyl anion, in so cold, Fenton reaction (Fig. 1.1). Hydroxyl radicals are very dangerous oxygen reactive species (ROS) that cause lipid peroxidation, oxidation of amino acids with consequent protein–protein cross-links, protein fragmentation and DNA damage (Galaris and Pantopoulos 2008).

With age, our body cells become more vulnerable to iron. Namely, in the ageing process, mitochondrial function gradually declines and increases the mutations of mitochondrial DNA (mtDNA) in all tissue cells (Fig. 1.2) (Druzhyna et al. 2008). Many studies have documented scavenging of mitochondrial ROS is the most powerful protective treatment against iron overload or homeostasis disruption (Seo et al. 2008; Altamura and Muckenthaler 2009). It is very important being able to preserve the mitochondrial membrane potential and to safeguard the morphologic integrity with associated mitochondrial enzymes such as aconitase, adenine nucleotide translocase and cytochrome coxidase and prevent degenerative processes which accompany ageing. It has been shown these enzymes mostly suffer oxidative modification under oxidative stress during ageing (Ma et al. 2009). Besides ageing process itself, iron homeostasis disruption also causes decline in mitochondrial function and plays a significant role in various degenerative diseases, possibly including age-related tissue dysfunction (Killilea et al. 2004).

Mitochondria play an important role in the biosynthesis of iron-containing proteins and in iron homeostasis because the majority of iron-sulphur-clusters (ISCs) are synthesized within mitochondria and dependent on mitochondrial function (Rouault and Tong 2005).

Since a number of ISC-containing enzymes have been quite labile when exposed to oxidative stress (e.g., nicotinamide adenine dinucleotide (NADH) dehydrogenase), release of Fe^{2+} ions from the damaged enzymes will cause further oxidative damage to mitochondria through the Fe^{2+}-catalyzed Fenton reaction (Chance et al. 1979). This reaction may be responsible for the decline of many molecular and physiological functions in ageing tissue cells (Ma et al. 2009). Age-related accumulation of iron increases the potential for free redox-active iron, which can promote oxidative stress and mitochondrial damage and thus functional alterations of antioxidant enzymes

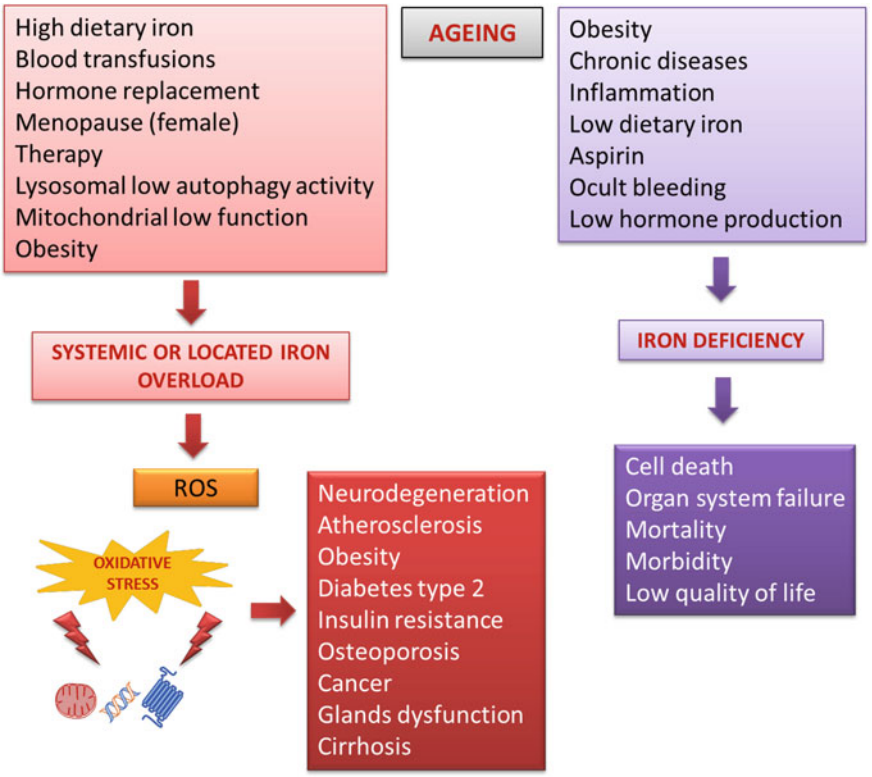

Fig. 1.2 Iron and ageing related disorders

and further increased oxidative damage to DNA, RNA, proteins and lipids in tissues in elderly people.

Lysosomes also play key roles, not only in controlled cell death and necrosis, then also in age related diseases (e.g. neurodegenerative diseases, atherosclerosis) (Fig. 1.2). Their main role is recycling damaged proteins and other macromolecules. With age, the autophagy activity becomes slower, resulting large molecules to accumulate within the cell and damaging it.

Lower autophagy activity has been associated with several age-related diseases (described later in this chapter). After lysosomal degradation of iron-containing endocytosed molecules, lysosomes can accumulate large amounts of redox-reactive ferrous iron, which may jeopardize lysosomal membranes integrity resulting in their permeabilization, cellular deficits or apoptosis induction. Beside lysosomal large influence on lifespan upon lysosome impairment in aged organisms, its dysfunction has a large influence on mitochondrial integrity too (mitochondria-lysosome relationship) (Carmona-Gutierrez et al. 2016).

Regarding ageing and age related diseases iron could be involved as a central mechanism in the pathology and toxicology of many disorders. Iron accumulation

Table 1.1 Symptoms and disorders related to iron overload and deficiency

Symptoms and disorders related to iron overload	Symptoms and disorders related to iron deficiency
Joint pain	Anemia
Chronic fatigue	Chronic fatigue
Malignant transformations	Frailty
Liver disease (cirrhosis, liver cancer)	Restless legs syndrome
Diabetes mellitus	Alopecia
Pronounced atherosclerosis	Tachycardia
Myocardial infarction	Myocardial infarction
Heart failure	Heart failure
Ischemic disorders	Acute coronary syndrome
Acute coronary syndrome	Coronary artery disease
Low libido	Pale skin
Osteoarthritis	Ischemic diseases
Osteoporosis	Osteoporosis
Obesity	Reduced physical fitness
Appetite increase	Brittle nails
Insulin resistance	Dyspnea
Metabolic syndrome	Loss of libido
Infertility	Infertility
Hypogonadism	Insomnia
Hypothyroidism	Headache
Hypopituitarism	Decreased chemotherapy outcome
Depression	Depression
Hypoadrenalism	Increased morbidity and mortality
Neurodegenerative disease	Cognitive impairment
Hypercligemia	Low concentration
Elevated liver enzymes	Central nervous system dysfunction
Elevated serum iron and ferritin	Decreased serum iron or/and ferritin

in vital organs, even in mild cases, increases the risk for liver diseases, cancer, cardiovascular disorders associated with atherosclerosis, diabetes mellitus, osteoarthritis, osteoporosis, metabolic syndrome, hypothyroidism, hypogonadism, numerous symptoms and in some cases for premature death (Fig. 1.2).

The signs and symptoms of iron overload are mainly non-specific. Thus, early diagnosis of iron overload requires consideration of this possibility when patient is having chronic fatigue, joint pain, impotence, osteoporosis and diabetes (Fleming and Ponka 2012) (Table 1.1).

Iron dyshomeostasis resulting in overload can accelerate neurodegenerative disorders and diseases, which accompany ageing (Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy, multiple sclerosis, etc.) (Fig. 1.2). It is unresolved yet whether iron accumulation causes these disorders or whether it is a consequence. However, the common feature of all these diseases is excess of oxidative stress with a ROS production like main pathogenetic item.

It is recently discussed that health can be improved with antioxidants and iron chelators that reduce oxidative stress or iron levels and slow down ageing. It is also known that blood donors exhibit lower rates of many diseases and experience better than average health, probably through controlling of circulating iron levels (Mehrabani et al. 2008).

Certain researches documented reduced lifespan in conjunction with increased levels of aggregated and insoluble proteins after high iron dietary intake, and opposite, extend lifespan due to certain iron chelator and decreased iron levels (Klang et al. 2014).

Obviously, iron accumulation is an inevitable part of the ageing process and likely threatens protein homeostasis and longevity. That is important knowledge for understanding causes of age related diseases.

Like iron overload, an iron deficiency is also detrimental for species longevity. It increases morbidity and mortality because it causes growth arrest and cell death (Table 1.1). Proper iron supplementation can keep blood iron levels normal. Because iron excess and deficiency are equally detrimental for the cell, certain regulatory mechanisms have evolved to maintain iron homeostasis at both, the cellular and the systemic level (Hentze et al. 2004).

1.2 Iron Homeostasis Regulation

There are in general four different types of cell, which play an essential role in the iron homeostasis, by controlling its content and distribution. Duodenal enterocytes serve for iron absorption, erythroid precursors for iron utilization, reticuloendothelial macrophages for iron storage and recycling, and hepatocytes for iron storage and endocrine regulation (Fleming and Ponka 2012). There is no proper physiological regulation for iron through excretion in the body, so iron absorption must be highly regulated. To maintain homeostatic balance it is necessary to absorb only 1–3 mg of iron per day via duodenal enterocytes regarding equal amount lost from desquamated cells. Dietary iron can be absorbed as part of a protein complex such as heme-protein via a specific heme transporters or as its Fe^{2+} form. A ferric reductase enzyme on the enterocytes brush border reduces Fe^{3+} to Fe^{2+} (McKie et al. 2001; West and Oates 2008). After the iron is reduced at the apical membrane, it is taken into the cell through the divalent metal transporter 1 (DMT1) (Simpson and McKie 2009).

Once iron is inside the enterocyte, it may leave the enterocyte and enter the body circulation via the basolateral transporter known as ferroportin or can be bound to ferritin, an intracellular iron bound protein. This iron will be lost from the body

when the enterocyte desquamate. Iron that enters the circulation from enterocytes is rapidly bound to transferrin, an iron-binding protein of the blood (Wessling-Resnick 2006). Transferrin delivers iron to most of cells that take up transferrin bound iron via receptor-mediated endocytosis. Erythroid precursors are the major sites of iron utilization (Frazer and Anderson 2005). These cells express high levels of transferrin receptor protein 1 (TfR1), which mediates the entry of transferrin bound iron into recycling endosomes (Skikne 2008). Reticuloendothelial cells serve as the major iron repository, regulated by hormone protein hepcidin, and represent the most dynamic iron compartment.

Reticuloendothelial cells obtain most of their iron from the phagocytosis of senescent erythrocytes (Knutson and Wessling-Resnick 2003). Similar to reticuloendothelial cells, hepatocytes are an important site of iron storage in the form of ferritin (Takami and Sakaida 2011; Pantopoulos et al. 2012; Nemeth and Ganz 2006).

There are several mayor signals that affect iron absorption but they are not the signals that regulate iron absorption directly. One signal reflects the need for iron regarding erythropoietic activity. The hormone erythropoietin stimulates red blood cell production and stimulates iron absorption indirectly, although evidence exist that is also involved in direct intestinal absorption (Skikne and Cook 1992; Latunde-Dada et al. 2006; Srai et al. 2010). Second signal depends upon the amount of iron in body stores and circulation, which stimulates the iron absorption in the proximal duodenum if its level is low. Third signal depends on oxygen saturation in the circulation.

Hepcidin is an important factor stimulated through this signals (Nicolas et al. 2002; Ganz and Nemeth 2012). Although the main producing cells are hepatocytes, it is also produced in the kidney, central nervous system, lungs, heart and other organs (adipose tissue), but with negligible effect on systemic iron homeostasis (Bartnikas 2014). When iron stores are full, in order to stop its further absorption, hepcidin down-regulates the ferroportin-mediated release of iron into the circulation (Nemeth et al. 2004a, b). The consequent iron retention in enterocytes decreases iron absorption and the iron retention in reticuloendothelial macrophages decreases iron export.

Hepcidin is an acute-phase response protein and its synthesis can be stimulated in inflammatory status (Cabantchik et al. 2005). This reaction represents an evolutionary adaptation as kind of protection from microorganisms, because hepcidin decreases the availability of circulating iron to invading microbes. However, constant inflammation can affect the iron homeostasis and lead to low quality of life regarding constant relative iron deficiency.

Conversely, inadequate hepcidin expression, due to dysregulation of either the iron-status signal or the erythroid signal, can lead to disruption of iron homeostasis in the body in the way of iron overload, and thereby shorten the life duration (Ganz and Nemeth 2012).

Namely, increased dietary iron absorption and iron released from reticuloendothelial macrophages into the blood, due to decreased hepcidin synthesis, exceeds the binding capacity of circulating transferrin creating redox-active NTBI (non-transferin bound iron; labile plasma iron) in the circulation (Brissot et al. 2012). Circulating NTBI cannot be used for heme production or up-regulating hepcidin synthesis because they require transferrin bound iron.

Although cells regulate the intake of transferrin bound iron by the expression of TfR1, the NTBI can be easily taken up by hepatocytes, cardiomyocytes, pancreatic islet cells, neurons, etc. The excess uptake of iron as NTBI contributes to increased oxidant-mediated cellular injury (Sripetchwandee et al. 2014).

1.3 Iron and Age Related Conditions and Diseases

The degenerative processes in many diseases and toxicological insults converge on iron dysregulation during ageing. This points the role of iron metabolism and implications for the use of iron chelating substances and/or appropriate antioxidants as nutritional or therapeutic agents in inhibiting the progression of these mainly degenerative diseases that accompany ageing (Mobarra et al. 2016; Fonseca-Nunes et al. 2014).

1.3.1 Iron and Cancer

It has been documented that iron is a risk factor for different types of cancers. Like said before, excess of body iron associated with tissue iron accumulation can provoke malignant transformation of cells due to higher radical production and oxidative stress induced damage (Toyokuni 2008). It is possible that hydroxyl radicals arising from Fenton reaction damage the genome. It was showed that the CDKN2A/2B (p16/p15) tumor suppressor genes were one of the major target genes in oxidative stress-induced carcinogenesis (Akatsuka et al. 2006). Indeed, iron-mediated oxidative damage appears to attack one of the most fragile sites in the genome that are susceptible to oxidative stress (Brookes et al. 2008) and interferes with cell signaling pathways involved in the cancer control (Toyokuni 2011).

Thus, iron-mediated persistent oxidative stress not only creates an environment for gene deletion but also for gene amplification (Drakesmith and Prentice 2008).

For example, persistent damage to hepatocytes in chronic viral hepatitis ultimately reduces the production of hepcidin, which promotes the absorption and deposition of iron, regardless of the iron stores (Fargion et al. 2014). Thus, hepatic iron is increased significantly in patients with chronic viral hepatitis, which causes higher risk for developing hepatocellular carcinoma (Iwabuchi et al. 2015).

In addition, it is has been suggested the association between endometriosis and ovarian cancer (Rezazadeh et al. 2005). Namely, ovarian endometriotic, so called „chocolate" cysts, are rich in blood or catalytic iron, leading to increased oxidative DNA damage of the epithelial cells which are surrounding those cysts (Rezazadeh et al. 2005).

Iron's carcinogenicity has been documented in many animal experiments. There are examples of carcinogenicity caused by iron compounds application. These include skin and soft tissue cancer (sarcoma) induced by injecting iron dextran

(Haddow et al. 1964; Chen et al. 1999), induced esophageal adenocarcinoma in rats receiving only moderately excessive doses of intraperitoneal iron dextran (Wyllie and Liehr 1998), increased tumor incidence in animals on high iron diet (Jiang et al. 2008), asbestos-induced mesothelial carcinogenesis (Bergeron et al. 1985), leukemia cell proliferation greater in iron treated animals than in controls without iron treatment (Hung et al. 2015), etc.

However, non-clinical studies, which investigated whether iron enhances carcinogenesis, provided only limited evidence relevant for cancer patients, since they were typically based on high iron doses as well as injection routes and iron formulations, which are not used in the clinical settings. Furthermore, a recent study reported that iron reduction by phlebotomy was associated with decreased cancer risks in a general population. Thus, it is important regularly to monitor serum iron level and to be very careful with the iron substitution therapy during ageing (Drakesmith and Prentice 2008).

Numerous studies have shown that iron excess plays a significant role in cancer development but still remains controversial whether iron deficiency does it.

The population-based retrospective study has presented an increased cancer risk in patients with iron deficiency anemia. Specifically the risks for pancreatic, kidney, liver and bladder cancers are significantly increased. The increased cancer risk in patients with iron deficiency anemia is probably caused by altered immune system because of iron deficiency anemia itself. This includes decreased activity of both cellular and humoral immunity thereby creating a microenvironment permissive for carcinogenesis (Park et al. 2015).

Anemia is a frequent in cancer patients as a complication too (Ludwig et al. 2015; Crawford et al. 2002) and functional iron deficiency is the predominant mechanism followed by increased hepcidin which suppresses the release of adequate quantities of iron into the circulation and inhibits intestinal iron absorption.

The most important consequence of iron deficiency is the risk for developing anemia or the aggravation of already existing anemia and thus impairment of quality of life (Fig. 1.2) (Ludwig et al. 2013). This usually is accompanied by deterioration of the performance status and impaired outcome of cancer patients (Gilreath et al. 2012). Iron is an important growth factor for rapidly proliferating cells including tumor cells (Beguin et al. 2013). Hence, it is important to review the safety for intravenous high dose iron supplementation. Likewise, no increased risk for tumor incidence and tumor progression was reported (Aapro et al. 2012).

Currently, active infections and iron intolerance are the main contraindications and limitations of intravenous iron supplementation (Sullivan 1989).

1.3.2 Iron and Atherosclerosis

It is possible that higher iron levels will promote atherosclerosis by generating more oxidative stress and promoting inflammation. There are several atherogenic steps that iron is involved. Active ferrous iron is a highly effective promoter of lipid

peroxidation and amplifies the pro-oxidant state of vascular cells. Iron increases free radical production that contributes to the oxidative modification of Low-density lipoprotein (LDL) either in the plasma or in the subintimal space where atherosclerotic lesion begins (Lynch and Frei 1993). Increased iron levels and lipid metabolism are directly linked over activity modulation of several key enzymes responsible for cholesterol and triglyceride homeostasis (Brunet et al. 1999).

In addition, the important link between iron and cholesterol metabolism is sharing the same receptor expressed on macrophages (LDL receptor-related protein, (LPR)), which play a key role in the regulation of iron homeostasis (part of reticuloendothelial cell system) (Hvidberg et al. 2005). Therefore, elevated cholesterol absorption by subintimal macrophages may also increase the iron intake. This leads to higher oxidative stress in macrophages and increased foam cells formation. Namely, during atherogenesis, blood monocytes are attracted to the subendothelial space where occurs the deposition of LDL. Later they differentiate into macrophages, and after LDL ingestion into foam cells (Vinchi et al. 2014). In animal study, the pharmacological suppression of hepcidin has decreased macrophage iron content, and increased cholesterol efflux from macrophages. Namely, decreased iron levels lowered the formation of ROS and increased the expression of cholesterol transporters, correlating with reduced foam cell formation and decreased atherosclerosis (Saeed et al. 2012).

There is evidence that atherosclerosis, coronary heart disease, stroke and peripheral arterial disease are neither prominent clinical features nor frequent causes of death in genetic hemochromatosis. This is explained by inappropriately low hepcidin (key iron influx-efflux control hormone of reticuloendothelial cells—macrophages) protein levels resulting in increase of expression of ferroportin that exports iron from macrophage. So, macrophages from hereditary hemochromatosis patients contain significantly less iron compared to macrophages from healthy people (Niederau 2000; Pietrangelo 2006), thus attenuating the inflammatory response by reducing macrophage cytokine secretion (Wang et al. 2008). It is also important to remember that hepcidin levels, a hormone that plays a central role in iron metabolism, increase by the inflammation associated with atherosclerosis.

Another critical role of redox active iron is in mediating the pro-inflammatory response in endothelial cells making them activated and dysfunctional (Libby 2002). Apart from its pro-oxidant properties in the vasculature, excess iron may enhance the atherogenicity of LDL by stimulating the synthesis of lipoprotein with low antioxidative reserve in the liver. Such lipoproteins may be primed for further oxidation in the subintima and unrecognized by the otherwise effective liver clearance system (Murray et al. 1991).

If iron is indeed involved in the pathogenesis of atherosclerosis, this may have implications in those pathologies that require long-term iron treatment (like dialysis and end-stage renal disease patients). Thus, intravenous iron treatment should be tightly controlled, iron overload markers monitored (transferrin saturation together with serum ferritin), and preferred a low-dose therapy rather than a high-dose treatment.

Iron depletion therapies may offer, either through phlebotomy or iron chelation in combination with antioxidants, a protective effect in people with increased risk

for developing age related disease (Parkinson's, Alzheimer's, atherosclerosis, etc.) (Ritchie et al. 2003; Cario et al. 2007).

1.3.3 Iron and Cardiovascular Diseases

The majority of cardiovascular disease diagnosis and deaths occur in the elderly over age of 65 years (Roger et al. 2012), perhaps due to increased exposure to cardiovascular risk factors, including iron, over longer time. Epidemiological studies documented that high ferritin levels, a marker used for stored iron, correlated with risk from coronary heart disease development or myocardial infarction (MI) (Pourmoghaddas et al. 2014).

It is documented that men and postmenopausal women have both, higher body iron levels and higher rates of atherosclerosis than premenopausal women do. It is possible, that depletion of iron stores by a regular menstrual blood loss may be kind of protection (Sullivan 1989).

It is also suggested that iron depletion protects against ischemic disease and argued that the difference in the incidence of heart disease in men and women may be caused by differences in their iron stores. Evidence for that are studies which claim that incidence for the MI is reduced in people who donor their blood (Tuomainen et al. 1997; Meyers et al. 1997).

As it was said before in the previous chapter part, high iron levels are capable of stimulating the progression of atherosclerotic lesions through producing ROS and causing lipid peroxidation of atherogenic plaque. The progression of atherosclerosis, therefore, increases the risk for ischemic cardiovascular events. Iron overload is associated with the production of free radicals that can damage tissues directly, resulting in cardiac toxicity (Gammella et al. 2015). The uptake of iron into the myocardial cells is controlled by the regulated expression of TfR1 and therefore, iron overload is observed only when the binding capacity of tranferrin is saturated. Then is formed non-transferrin bound iron or labile plasma iron (Cabantchik 2014). Iron's toxicity within cells arises from its ROS production that cause lipid peroxidation and organelle damage, which leads to cell death and fibrosis and ultimately impaired systolic and diastolic function (Gammella et al. 2015).

Iron can play also a relevant role in cardiotoxicity induced by some medications. That includes doxorubicin (DOX)-dependent cardiotoxicity, which is among the most effective antitumor medications used in a number of neoplastic diseases. Many observations indicate that susceptibility to DOX-dependent cardiac damage is bigger with high blood iron levels. During DOX degradation eventually H_2O_2 is produced, a mechanism that potentiates iron mediated ROS production and cardiotoxicity (Gammella et al. 2015).

Oxidative stress, which has a main role in this process results from the imbalance between oxidative metabolism and antioxidant activity. ROS can be tightly controlled by antioxidants administration, including nutrient antioxidants, which together with enzyme antioxidants control the ROS within physiological limits.

The effectiveness of antioxidant supplementation dependents on basal endogenous antioxidants levels like vitamin C, vitamin E and β-carotene (Roger and Go 2013). It might be advisable before starting supplementation with these antioxidants in elderly cardiovascular patients, to measure basal plasma antioxidants levels because of special precautions, which is required in this population. Namely, when taken in excess may lead to deleterious consequences (Dotan et al. 2009). Besides antioxidative strategy, iron chelation therapy has been commonly used to prevent iron-overload induced organ dysfunction (Cassinerio et al. 2012) but the evidences for efficacy and potential adverse effects are insufficient and inadequate to recommend it in the routine use for cardiovascular diseases patients (Sultan et al. 2017). However, contrary to Sullivan's hypothesis (Sullivan 1989), which says that iron depletion protects against ischemic heart disease, a significant negative correlation was identified later in studies, between transferrin saturation and coronary artery disease or MI, leading to the conclusion that high body transferrin could confer protection against development of coronary heart disease (De Das et al. 2015). Furthermore, in a study that involved 38,244 men without diagnosed cardiovascular disease, blood donations were not associated with the lower risks for MI nor fatal coronary heart disease (Ascherio et al. 2001). However, whether replenishing iron stores in patients with coronary artery disease is beneficial or not, is not sure with certainty (von Haehling et al. 2015). Epidemiological study suggest that iron deficiency is frequent in patients with coronary artery disease (Kang et al. 2012), and furthermore, recently studies indicate that iron deficiency has detrimental effects in patients with coronary artery and heart failure (von Haehling et al. 2015).

However, iron deficiency is prevalent and persistent in acute coronary syndrome associated with chronic antiplatelet therapy, anemia and increased inflammatory state with unknown prognosis (Meroño et al. 2016). Furthermore, the most common detected cause of anemia were nutritional deficiency, chronic kidney disease and blood loss due to antiplatelet agents. Hence, further research is needed to examine the role of correction of anemia in reducing long-term morbidity and mortality in these patients (Bhavanadhar et al. 2016).

Iron deficiency anemia may serve as an efficient biomarker of long-term risk and prognosis for coronary artery disease and acute coronary syndrome, heart failure and arterial hypertension prognosis.

During iron deficiency, maintenance of adequate tissue oxygenation is achieved through increase of erythropoietin production, erythrocyte concentrations of 2,3-diphosphoglycerate, and systemic arterial dilatation, due to peripheral local tissue auto-regulation. The last one leads to reduced afterload and increased stroke volume. Beside arterial dilatation, anemia also results in decreased blood viscosity, which increases venous return to the heart and thus, increases preload. Because of decreased systemic vascular resistance, a sympathetic nervous system is activated, which increases myocardial contractility and the heart rate. All this events together raise cardiac heart output. Over the long time, heart adaptations that initially increase cardiac output lead to the left ventricular hypertrophy with internal cavity enlargement and with time to irreversible pathology or heart decompensation state (heart failure or aggravate ischemic heart diseases. The effect of chronic iron deficiency on

large blood vessels is resulting in arterial hypertrophy because of sustained mechanic pressure from increased cardiac output (Farhan et al. 2016).

Iron deficiency in coronary artery disease potentiates an imbalance between myocardial oxygen supply and demand, which already exists, by reducing oxygen capacity and at the same time increased myocardial oxygen consumption through increased cardiac output. With low oxygen-saturation, due to the iron deficiency comes to impaired vascular healing among patients with coronary artery disease or acute coronary syndrome (Solomon et al. 2012).

Taking this all events together, if iron deficiency does not solve, circulus vitiosus is created that causes the deterioration of coronary artery disease and heart failure development.

1.3.4 Iron and Brain Diseases

Iron has to be maintained in a delicate balance because both, iron overload and iron deficiency are detrimental to the brain and cause neurodegeneration (Belaidi and Bush 2016).

In physiological conditions, iron-mediated ROS can positively influence the calcium levels and thus the synaptic plasticity (Muñoz et al. 2011). On the other hand, an excess of iron, with uncontrolled production of ROS, is detrimental for neuronal survival. A protective mechanism can be played by astrocytes that can uptake iron, thereby buffering its concentration in the synaptic environment. This mechanism is potentiated under astrocytes activation during neurodegenerative processes (Codazzi et al. 2015).

Iron is entering the brain over crossing the blood-brain barrier (BBB). Iron bound to transferrin is being absorbed in capillary endothelial cells via transferrin receptor (Bradbury 1997).

The main cells that supply neurons, astrocytes and microglia with iron are oligodendrocytes and choroids plexus epithelial cells (Espinosa de los Monteros et al. 1990).

All this cells take iron in a form of transferrin bound or non-transferrin bound (Ke and Qian 2007). Important mediator for iron export from astrocytes, like in other cells, is multi-copper ferroxidase ceruloplasmin. Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe^{2+} (ferrous iron) into Fe^{3+}. In that way it assists, not only in converting toxic ferrous iron into non-toxic ferric state, then also in transporting iron in association with transferrin, which can carry only ferric form (Song and Dunaief 2013). Absence or mutations of this enzyme lead to iron accumulation and neurodegeneration in the CNS. Accumulation in astrocytes may cause astrocyte cell death and further, neuron death regarding iron deprivation because of impaired iron export. These changes are causing characteristic neurologic signs and symptoms, such as cerebellar ataxia, progressive dementia and extrapyramidal signs that can occur in normal ageing process

or in neurodegenerative diseases and aceruloplasminemia (Harris 2003; Jeong and David 2006).

In iron deficiency, the brain iron content is almost unaffected and the brain is relatively protected (Beard et al. 2006), but during fetal life when the needs for embryonic and fetal development are high, the cognitive defects that arise from iron deficiency could not be corrected postnatally (Kwik-Uribe et al. 2000).

Namely, the overall role of iron in the brain is to participate in processes like neurotransmission and myelination. Iron is a cofactor for a variety of enzymes involved in maintaining healthy oligodendrocytes and myelin, regarding high rate of oxidative metabolic activity present in oligodendrocytes, and may be a crucial component of remyelination in demyelinating diseases such as multiple sclerosis (MS) (Radlowski and Johnson 2013).

Furthermore, it is an enzyme cofactor involved in synthesis of dihydroxyphenylalanine (DOPA), the precursor of catecholamine neurotransmitters such as dopamine, adrenaline and noradrenaline (Franco et al. 2015) or in synthesis of serotonin (Pino et al. 2017). Iron is also important in the process of degradation of monoamine oxidase (MAO), the enzyme responsible of oxidative deamination of dopamine, adrenaline and noradrenaline (Kumar and Aandersen 2004).

Iron levels increase in brain with ageing but the brain iron dyshomeostasis must not affect brain functioning (Bartzokis et al. 1997). This increase involves the accumulation in specific brain regions that are preferentially targeted in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). The increased iron concentrations in certain brain regions could result from the altered vascularization that is observed during ageing and in neurodegenerative diseases. MS is also a neurodegenerative disease that can be provoked with increased brain iron levels (Williams et al. 2012). It is possible that mechanisms in the brain that control iron are damaged causing these disorders via ROS and oxidative stress (Altamura and Muckenthaler 2009).

The study on autopsied aged brains documented increase of heme oxygenase-1 (HO-1), the heat shock protein involved in regulation of iron metabolism. The increase of HO-1 might be the result of an increased oxidative stress in older people (Hirose et al. 2003), but the increase of ferritin in aged brain must be elucidated.
Older individuals (60–90 years) comparing with younger subjects (28–49 years) have more iron in the microglia and astrocytes in different brain regions (cortex, cerebellum, hippocampus, basal ganglia and amygdala). Oligodendrocytes contain the largest amount of iron, ferritin and transferrin, but this content remains constant during ageing (Connor et al. 1990). Iron accumulation in microglia might stimulate the activation of these cells in the neuroinflammatory processes that contribute to AD, PD and MS.

1.3.4.1 Iron and Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia characterized by short-term memory loss, and a progressive decline in cognitive and motor functions

(Altamura and Muckenthaler 2009). In the brains of patients with AD, iron accumulation occurs without the normal increase in ferritin (Connor et al. 1992) thereby increasing the risk for oxidative stress (Thompson et al. 2003). Link between iron and AD was the observation that iron accumulates in the same brain regions where exist plaques of abnormally folded amyloid-β protein deposition (Connor et al. 1992).
Iron seems to promote both, deposition of amyloid-β protein and oxidative stress, which is associated with the plaques (Huang et al. 2000; Rottkamp et al. 2001), although some have argued that, by binding iron, Aβ-protein might protect the surrounding neurons from oxidative stress (Perry et al. 2002).
HFE (high Fe or human hemochromatosis) gene mutations are associated with increased oxidative stress and severity of AD (Braak et al. 1993; Pulliam et al. 2003).

This fact is supporting the idea that iron underlies the increase in oxidative stress that promotes neurodegeneration in AD (Moalem et al. 2000). HFE protein encoded by this gene is a membrane protein that regulates circulating iron uptake by TfR1 and the iron storage via hormone hepcidin. Its mutation is causing the iron overload and storage disorder hereditary hemochromatosis.

Iron chelators are being considered for the treatment of AD because chelation has the potential to prevent iron-induced ROS, oxidative stress and aggregation of α-synuclein and Aβ-protein (Shachar et al. 2004; Dusek et al. 2016)

1.3.4.2 Iron and Multiple Sclerosis

Excess iron can promote inflammatory states of macrophages and microglial cells, which could be beneficial in combating an infection, but can have also the negative effect in multiple sclerosis where inflammation is an important pathological component of the disease. In conditions where iron concentrations reach high levels, there can be enhanced generation of ROS leading to myelin and neuron loss followed by demyelination and neurodegeneration (Williams et al. 2012). Investigations in animal models of MS, experimental autoimmune encephalomyelitis, showed worsening of clinical course in those animals which had an iron accumulation in central nervous system (brain and spinal cord) (Ćurko-Cofek et al. 2016), especially in male rats indicating sex dependent mechanisms that must be elucidated (Ćurko-Cofek et al. 2017).

It is interesting to note that in patients with MS, iron content is elevated in deep grey matter structures and in the vicinity of lesions, and reduced in the white matter (Hametner et al. 2013). Furthermore, iron content is low in remyelinated plaques (Hametner et al. 2013), suggesting that dynamic shuttling of iron continues through the MS disease process. This reveals that the iron dysregulation associated with MS is in fact a redistribution of iron between different areas of the brain.
During remyelination, oligodendrocyte progenitor cells (OPCs) are recruited to the MS lesions and differentiated into mature oligodendrocytes which can further remyelinate a damaged axons. Considering the requirement for iron-containing enzymes in all these processes, iron levels in oligodendrocytes have an important

influence on remyelination and neuronal repair. In the situation of reduced iron availability and iron-deficient oligodendrocytes, whether through global iron deficiency (Schonberg and McTigue 2009) or impaired iron export from astrocytes (Schulz et al. 2012), it leads to reduced OPCs proliferation, oligodendrocyte differentiation and following remyelination.

Studies examining therapeutic approaches indicate that iron chelation therapy could benefit MS by regulating the immune response and limiting oxidative damage. However, experiences from other conditions indicate that iron chelation therapy in MS can cause adverse side effects that indicate close monitoring of patients during administration (Weigel et al. 2014).

1.3.4.3 Iron and Parkinson's Disease

English physician James Parkinson is the first who described Parkinson's disease. It is caused by a selective loss of the dopaminergic neurons in the pars compacta of the substantia nigra (Lozano et al. 1998).

Several researches have documented an increase in total iron concentration in the substantia nigra in the most severe cases of PD, but no changes were found in milder cases (Götz et al. 2004). Elevated iron concentrations in the substantia nigra might result from mutations in genes that are relevant for iron transport and binding. Another potential source of increased brain iron concentrations is from peripheral iron influx through a discontinued BBB, precisely via Permeability-glycoprotein (P-gp). It is an efflux pump system in the cell membrane, which might be not working well in certain brain regions in PD, thereby making the brain accessible to many foreign substances out of the brain, including serum iron. Iron deposits were found in microglia, oligodendrocytes, astrocytes located close to neurons, pigmented neurons in the substantia nigra pars compacta, but also in the putamen and pallidum of patients with PD. Numbers of ferritin-loaded reactive microglia are often associated with degenerating and neuromelanin-loaded dopaminergic cells (Jellinger et al. 1990).

Several studies have shown that iron interacts with α-synuclein, a critical target protein that regulates the supply and adequate release of synaptic vesicles with neurotransmitter dopamine in presynaptic terminals (Uversky et al. 2001). This pathological process induces the formation of neuron intracellular aggregates that disturb the cytosolic environment and interact with the vesicles, dopamine transporters and mitochondria. These disturbances might result in activation of cell death cascades. Neuromelanin, a strong chelator of cytoplasmic iron (Zucca et al. 2017), in a complex with iron activates microglia in vitro, leading to the release of neurotoxic compounds such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nitric oxide (NO) and neuromelanin itself released by dying neurons. Those factors can further induce release of neurotoxic microglial factors that can potentially lead to neurodegeneration or PD (Zecca et al. 2004).

Like said before, the ability of the lysosome to participate in autophagy becomes slower with age, resulting in the increase of non-protein "garbage" within the cells.

Less optimal autophagy has been also associated with several age related diseases, including PD (Carmona-Gutierrez et al. 2016).

Further, accumulated iron increases protein aggregation via enhanced generation of ROS and oxidative stress. Chelators of iron could help slow down the development of the PD. However, certain authors argued about implication of iron deficiency in pathogenesis of PD (Yien and Paw 2016) because it is yet not established whether iron chelators are able to remove excess iron without interfering with normal systemic iron metabolism.

1.3.5 Iron and Obesity Related Disorders

Obesity and increased adiposity are also prevalent in the elderly. The bad influence of iron on glucose metabolism causing risk for diabetes was first recognized in hereditary hemochromatosis, but high levels of dietary iron is also included in causing that risk. One of the known mechanisms is generating ROS that damage the tissue in which iron is accumulated (Simcox and McClain 2013). Nevertheless, the observed oxidative stress in islets of pancreas, and other tissues as well, is not caused only directly by the ROS generation, then also by affecting trafficking of other metals important for mitochondrial activity. For example, iron overload inhibits mitochondrial uptake of manganese, resulting in decreased activity of superoxide dismutase 2 (SOD2), enzyme that clears mitochondrial ROS and, as a result, confers protection against cell death. Experimental data on animals confirm detrimental influence of iron excess through decreased insulin secretory capacity, decreased glucose-stimulated insulin secretion, and increased β-cell death (Cooksey et al. 2004). Altogether, pancreas iron accumulation causes β-cell failure and therefore is directly involved in diabetes pathogenesis.

Iron and increased iron stores are closely associated with increased risk of type 2 diabetes, metabolic syndrome and insulin resistance (Gabrielsen et al. 2012). It was shown that ferritin blood concentration, the higher is in the individual the lower is his insulin sensitivity (Jehn et al. 2004; Gabrielsen et al. 2012).

Besides, insulin sensitivity increases as iron stores become reduced in individuals with high ferritin (Facchini and Saylor 2002; Valenti et al. 2007).

Among many epidemiological studies, that included Europeans, Africans-Americans, gestational diabetes and prediabetes across sexes, it was found strong relationship between high serum ferritin, insulin resistance and diabetes (Simcox and McClain 2013).

It is important to consider that type 2 diabetes is marked by chronic inflammation, and that ferritin is acute-phase response protein that increases with inflammation. Whether high iron is caused by diabetes in the first place is confuted in several fields. C-reactive protein (CRP) nor inflammation did not account the association between iron and diabetes risk (Padwal et al. 2015), but rather dietary iron overload (Bowers et al. 2011), particularly dietary heme iron (from meat, fish) (White and Collinson 2013) which is more efficiently absorbed than non-heme iron. Furthermore, the best

evidence that proves those statements offer studies in which iron reduction (like with phlebotomy and blood donating) increases adiponectin and improves glucose tolerance in patients with impaired glucose tolerance and very high ferritin levels (Simcox and McClain 2013; Gabrielsen et al. 2012).

Studies in vitro, on animal models and humans too, provided evidences that iron plays a direct and causal role in determining insulin-sensitizing adipokine (adiponectin) levels and diabetes risk (Gabrielsen et al. 2012) with negative correlation between adiponectin and ferritin.

Adiponectin is protein hormone that regulates glucose metabolism and fatty acid oxidation with concentrations normally increased during caloric restriction. Adipose tissue, as an endocrine organ, is a major producer of adiponectin among other adipocytokines involved in the regulation of insulin sensitivity, inflammation, atherosclerosis, etc. Adiponectin normally shows protective anti-inflammatory, anti-hyperglycemic, anti-atherogenic activities, considering the fact that obesity related-diseases are inflammatory states (Padwal et al. 2015). In the experiment with cultured cells, iron down-regulated adiponectin transcription through FOXO1 (forkhead box protein O1) transcription factor inhibition (Gabrielsen et al. 2012). Further, experiments on animals showed the loss of iron-export channel ferroportin, resulting in adipocyte iron loading, and insulin resistance (Gabrielsen et al. 2012). It is also considering that adipocyte insulin resistance is an early event in the pathogenesis of type 2 diabetes (Wlazlo et al. 2013). Supporting these findings of adipocyte overload, the contrary evidence showed that increased adipocyte ferroportin expression in hemochromatosis was associated with decreased adipocyte iron, increased adiponectin, improved glucose tolerance and increased insulin sensitivity (Gabrielsen et al. 2012).

It is obvious that dietary iron and body iron stores are certainly involved in obesity and related disorders (type 2 diabetes, metabolic syndrome, insulin resistance), through modulating the metabolism with adiponectin in response to iron stores. That was confirmed with phlebotomies in humans with reversal diabetes effect over increased adiponectin release and improved glucose tolerance (Gabrielsen et al. 2012; Nigro et al. 2014).

Iron is consider to contribute obesity in elderly people. Dietary iron intake is associated with increased appetite via increased serum leptin levels. Dietary iron overload appears to increase adipocyte iron and decreases leptin mRNA and serum protein levels in animal experiment (Gao et al. 2015).

Leptin is satiety hormone, so its lower levels with a high-iron diet provide a mechanism for increased hunger. Human ferritin levels are inversely associated with serum leptin independently of inflammation and weight, which indicates that levels of dietary iron play an important role in regulation of appetite and metabolism through leptin expression (Gao et al. 2015).

The important factors that drive iron accumulation, beside dietary iron intake, is ageing itself with no proper body losses.

Since elderly people often weight gain, due to the body's metabolism slowering with age (Alfonzo-González et al. 2006), adipose tissue expansion and fat accumulation causes dysregulation of adipokine production that strongly contributes to the onset of

obesity-related diseases (Nigro et al. 2014). In obesity, iron distribution is altered at the cellular levels, where an increased availability of fatty acids during obesity may contribute to the observed changes in macrophages polarization and their reduced capacity to handle iron, which results in adipocyte iron overload (Orr et al. 2014). In addition, the direct link between iron and obesity are findings in animal research, which have shown that iron reduction ameliorates adiposity (Tajima et al. 2012).

Furthermore, it is possible that ageing associated obesity is one of possible reasons of anemia of ageing. Namely, old age is characterized by a chronic inflammation and decline of the immune response. There are evidences with increased circulating levels of IL-6 and CRP, among other inflammatory markers (Harris et al. 1999; Krabbe et al. 2004).

It is documented that obesity negatively affects ageing by changing immune system, with reduction of T-cell precursors (Yang et al. 2009), narrowing the immune repertoire against antigens encountered later in life. In addition, chronic inflammation present in elderly, due to chronic conditions prevalent in older populations (Fairweather-Tait et al. 2014) is one of the reasons of iron deficiency anemia.

Serum iron is frequently lower, and ferritin levels higher in obese compared with normal-weight people (Cheng et al. 2012; Tussing-Humphreys et al. 2012), but with depleted iron stores, despite a higher ferritin levels (see Sect. 1.4). Iron content showed increased accumulation in adipose tissue from obese patients and negatively correlated with adiponectin expression, which could contribute to insulin resistance and development of the metabolic complications in obesity (described above) (Pihan-Le Bars et al. 2016).

Hepcidin is a pro-inflammatory adipokine released from adipocytes too, and may play an important role in hypoferremia of inflammation in obese individuals, independent from diabetes type II (Bekri et al. 2006; Gotardo et al. 2013).
As a whole, the available evidence suggests that chronic inflammation of obesity and ageing may impair iron status through hepcidin and that hepcidin is not only crucial for iron homeostasis then also for immune response (Dao and Meydani 2013).

1.4 Iron Deficiency

In iron deficiency, there are low levels of mobilizable iron with compromised supply to tissues, including bone marrow and, therefore, erythropoiesis. Iron deficiency can exist with or without anemia but the most functional deficits appear to occur with the anemia. Even mild to moderate iron deficiency anemia can affect cognitive development, immunity mechanisms, work capacity, learning ability and increase risk for sepsis during pregnancy, maternal mortality, perinatal mortality and low birth weight (Abbaspour et al. 2014). Complications associated with anemia in erderly are cardiovascular disease, increased falls and fractures, cognitive impairment, increased frailty, decreased quality of life and higher risk for morbidity and mortality (Eisenstaedt et al. 2006) (Table 1.1).

Iron deficiency anemia is very often present in the elderly people, particularly in those aged over 80 years. With ageing iron status is impaired with increased moderate anemia after age of 50 (Eisenstaedt et al. 2006; Lopez-Contreras et al. 2010) but it is not clearly understood why.

On the other hand, a number of individual or combined factors may cause iron deficiency. That includes poor diet and secondary nutritional deficiency, reduced efficiency of iron absorption as a result of reduced stomach acid production, occult blood loss (most frequently gastrointestinal bleeding), medications, chronic inflammation, chronic kidney disease and other chronic diseases (e.g. chronic infection, chronic immune activation, malignancy, atrophic gastritis, celiac disease or infection with Helicobacter pylori) and unexplained anemia (Patel 2008).

Nutritional anemia with deficiencies of iron, folate or vitamin B12 is effectively treated with certain replacement (Eisenstaedt et al. 2006; Lopez-Contreras et al. 2010). However, some studies have documented that elderly people who consumed Mediterranean diet (rich in fish, vegetables, fruit, olive oil and dairy products) had low prevalence of anemia and no measurements of infection/inflammation (Vaquero et al. 2004).

Iron deficiency anemia caused by occult bleeding requires further investigation before final treatment, anemia of chronic inflammation or chronic kidney disease may respond to treatment of the underlying disease and selective treatment with erythropoiesis-stimulating agents (ESA). However, the treatment of unexplained anemia is difficult, and there is little evidence that is effective at all (Bross et al. 2010).

Anemia in the malignancy that belongs to anemia of chronic diseases is often functional type, like it was mentioned above in the chapter about iron in cancer patients, and its treatment increases survival efficiency (Calabrich and Katz 2011).

Diseases associated with bleeding usually include colon cancer, gastric and/or peptic ulcer, hiatus hernia, colonic vascular ectasia, colonic polyps and Crohn's disease (Fairweather-Tait et al. 2014).

Furthermore, elderly people are often taking aspirin for other conditions that include atherosclerotic cardiovascular changes. Atherosclerosis, a primary cause of MI is an inflammatory disease. Aspirin use lowers risk for MI, probably through antithrombotic and anti-inflammatory effects. It has long been known that people who are taking aspirin have lower serum iron and serum ferritin levels. This effect results from possible occult blood loss and a cytokine-mediated effect on serum ferritin in individuals with inflammation, liver diseases, and infection. Aspirin lowering serum ferritin levels is more marked in those diseased subjects than in healthy ones (Fleming et al. 2001). Namely, ferritin is an acute-phase response protein, and usually is increased in inflammatory states or in conditions with damaged hepatocytes. This is why sometimes in these situations ferritin levels do not correlate with the storage iron. Therefore, a normal CRP should be used to exclude elevated ferritin caused by acute-phase reaction (Park et al. 2015).

Hemoglobin (Hb) concentration has been reported to decline with ageing. In one report, this was calculated to be 0.53–0.1 g/L/year in men and 0.05–0.09 g/L/year in women between the ages of 70 and 88 years (Nilsson-Ehle et al. 2000; Milman

et al. 2008). The decline appears to increase after the age of 80, particularly in men. It is probably a result of age related decrease in hormones production, including growth hormone, testosterone and/or insulin-like growth factor-1 (IGF-1), whose levels positively correlate with Hb in elderly people (De Vita et al. 2015). Erythrocytes released from the bone marrow are also less functional and resistant, and partially damaged with age.

All this together is contributing anemia of ageing (Fairweather-Tait et al. 2014). Nevertheless, it is possible to differentiate between pure iron deficiency anemia, anemia of chronic disease, and anemia of chronic disease with co-existing iron deficiency.

1.5 Recommended Iron Intake for Longevity in the Elderly

About 1 mg of iron is lost each day together with surface cells from skin, mucosa and gastrointestinal tract. A dietary intake of iron is needed to replace that loss including stool and urine iron. These physiological losses represent approximately 0.9 mg of iron for an adult male and 0.8 mg for an adult postmenopausal female (Abbaspour et al. 2014). Iron requirements for postmenopausal women is almost 2.5 time lower than in women of reproductive age unlike in men who require the same amount from 20 years till old age (Abbaspour et al. 2014). Most of the countries formulate their own daily nutrient recommendation or RDA (Recommended Daily Allowance) for their populations. National Academy of Sciences recommended dietary iron intake allowance for men (ages 19 and older) and postmenopausal women (ages 51 and older) at value of about 8 mg/day (Trumbo et al. 2001). Tolerable Upper Intake Levels (UL), at which no unwanted side effects are present, is 45 mg/day (Institute of Medicine (US) Panel on Micronutrients 2001) Iron deficiency is treated with about 50–100 mg elemental iron three times a day and is continued another 3–6 months to build up the body's iron reserves (Pennisi 2017).

Food sources of iron include meat and meat products, which contain heme iron, especially red meat, offal and dark poultry meat, oily fish such as tuna and sardines, cereal products, eggs, dark green vegetables and for vegetarians soybeans, tofu, lentils, kidney beans, chickpeas and baked beans. Dietary factors that could influence iron absorption as inhibitors are phytates (in wheat and other cereals, seeds, nuts, beans), polyphenols (in cocoa, berries, beans, nuts, red wine, black and green tea, coffee), calcium (in milk and milk products), some proteins (phosvitin in eggs, casein in milk) and tannins (in non-herbal teas) which chelate iron (Zijp et al. 2000). Reduced gastric acid production (gastrectomy, atrophic gastritis, drugs like antacids, proton pump inhibitors, H_2 blockers) contributes to low iron transformation from ferric to ferrous iron which is more easily absorbed. Factors that act as competitors with iron in intestinal absorption are cobalt, strontium, manganese and zinc which are sharing the same intestinal absorption pathway as iron (Rossander-Hultén et al. 1991). Facilitators of iron absorption are preventing the formation of insoluble iron compounds, and reducing the ferric to ferrous iron. They include scorbate, citrate,

some proteins, meat and fish. Iron from meat and fish as heme iron is more easily absorbed than non-heme iron from vegetables (Teucher et al. 2004).

Health disorders caused by iron deficiency must be corrected through diet or therapy, but carefully, because of the risk from increased body iron stores, as this may have detrimental effects for the cells and organs, and therefore, longevity. In elderly people, finding an adequate supply of iron may be a challenge due to impaired absorption, often a reduced food intake, and health dietary patterns with more limited diet.

1.6 Interaction of Iron with Drugs Prescribed in the Elderly

Iron can interact with certain medications. Some of them can decrease, or less often, increase iron levels. Iron itself can decrease intestinal absorption and effectiveness of certain medications when taken together at the same time. For that reason, iron supplements must be taken for couple of hours before or after taking medications.

1.6.1 Medications Which Absorption Can Be Decreased by Iron

Levodopa and Methyldopa
Iron supplements reduce the absorption of levodopa, which is used for PD and restless leg syndrome treatment, and methyldopa, which is used for high blood pressure. This is possible through intestinal chelation (Campbell and Hasinoff 1989; Greene et al. 1990) that reduces the therapy effectiveness of levodopa and methyldopa. The medications must be taken 2 h before or after taking iron supplements.
Levothyroxine is used to treat hypothyroidism, goiter, and thyroid cancer. The simultaneous ingestion of iron and levothyroxine can result in reducing levothyroxine efficacy in some patients (Campbell et al. 1992). Medication must be taken 2 h before or after taking iron supplements.

Antibiotics
Iron might decrease of antibiotic absorption. Quinolones (ciprofloxacin, enoxacin, norfloxacin, sparfloxacin, trovafloxacin and grepafloxacin) and tetracyclines (tetracycline, doxycycline and minocycline) possess iron-chelating activity. To avoid this interaction, the iron must be taken 2 h before or after taking quinolone antibiotics (Brouwers 1992) and 4 h after taking tetracyclines (Gu and Karthikeyan 2005).
Bisphosphonates are using in treatment of osteoporosis (alendronate, etidronate, risedronate, tiludronate and others). They inhibit the bone resorption by affecting osteoblasts. Iron can decrease absorption of these medications creating complexes, so bisphosphonates are never giving with food or together with iron supplements.

It is necessary to take bisphosphonates at least 2 h before iron or later in the day (Martin and Grill 2000).

Penicillamines are using for treating Wilson's disease, severe rheumatoid arthritis and cystinuria. Penicillamine is a chelating agent that reduces copper and cystine excess and rheumatoid arthritis activity. When taken together with iron supplements, iron binds penicillamine, decreases its absorption and thus lowers drug efficiency. To avoid this interaction iron must be taken 2 h before or 2 h after taking penicillamine (Lyle 1976).

Captopril (angiotensin-converting-enzyme (ACE) inhibitor) is a medication for treating high blood pressure. It can bind iron (maybe other ACE inhibitors too) and form compound that cannot be absorbed, thereby decreasing both medication and iron supplement in the blood. To avoid this interaction, the iron supplements and ACE inhibitors must be taken for 2 h apart (Campbell and Hasinoff 1991).

Some studies are suggesting taking iron to soothe and prevent cough, as a side effect symptom from ACE inhbitors (enalapril, captopril and lisinopril) (Weiss et al. 1994; Lee et al. 2001).

1.6.2 Medications that Decrease Iron Absorption

Bile Acid Sequestrants are blood cholesterol lowering medications **(Cholestyramine and Colestipol)**. They are also used to relieve itching caused by biliary obstruction. Taken together with iron supplements, bind inorganic and hemoglobin iron and inhibit its absorption (Leonard et al. 1979).

Anti-Ulcer Medications

For adequate absorption of nutritional non-heme iron, it is important to have normally produced gastric acid. Taking proton-pump inhibitors (lansoprazole and omeprazole), H_2 blockers (cimetidine, ranitidine, famotidine and nizatidine) or antacids results in a reduced secretion of gastric acid, which further reduces iron absorption. This fact is more important for patients who are already having iron deficiency and therefore suboptimal responses to iron supplementation (Ajmera et al. 2012).

1.6.3 Medication that Increase Blood Iron Levels

Oral Contraceptives may increase blood iron levels, thereby decreasing the need for extra iron (Gellert and Hahn 2017).

1.7 Iron and Species Lifespan Studies

Drosophila

Runko and co-workers showed in their study that overexpression of *Drosophila* protein frataxin in the mitochondria enhances the lifespan by increase of antioxidant capability and resistance to oxidative stress damage. Frataxin is a chaperon-like mitochondrial protein involved in assembly of ISC proteins and counteracts tissue iron accumulation. Its reduction leads to deficit of ISC enzymes and energy, and increase in mitochondrial iron level. Respiratory chain dysfunction induced by iron and ROS further leads to an oxidative stress damage, neurodegeneration (Friedreich's ataxia in humans) and diminished lifespan.

Results from Runko and co-workers suggest that *Drosophila* frataxin may function to protect the mitochondria from oxidative stresses and cellular damage likely acting on the control of iron metabolism (Runko et al. 2008).

Rich in polyphenolic compounds, green tea has been shown to increase the lifespan in various animal models, including *Drosophila.* Lopez and co-workers found that green tea increased lifespan of male flies but with negative impact on fertility. Interesting thing is that green tea protected male flies against iron toxicity. Findings suggest that green tea extends the lifespan of male flies by inhibiting reproductive potential, possibly by limiting iron uptake (Lopez et al. 2014).

High in polyphenolic content, green tea has been shown to reduce oxidative stress in part by its ability to bind free iron and in part by modulating iron regulators, specifically, mitoferrin (mitochondrial iron transporter) and reduction of mitochondrial iron (Lopez et al. 2016).

Nematodes

Caenorhabditis elegans (*C. elegans*) is widely used in studies of metal toxicity and metal homeostasis. Klang and co-workers have investigated the role of metallostasis in longevity of *C. elegans.* Their investigation included iron and other metals as well. The data showed increased accumulated levels of iron with age and that dietary supplementation with 15 mM ferric ammonium citrate reduces *C. elegans* lifespan in conjunction with increased levels of insoluble proteins. Proteomic analysis revealed widespread effects of supplemental dietary iron in multiple organelles and tissues. Furthermore, pharmacological interventions (iron chelator) to block iron accumulation attenuated proteotoxicity and extended lifespan of *C. elegans*. Taken together, these results suggest that iron accumulation with ageing contributes to age-related protein aggregation and negatively affects longevity (Klang et al. 2014).

Valentini and collaborators investigated impact of different free iron levels on oxidative stress resistance, ageing and lifespan duration in C. *elegans*. They found that 15 mM and greater of supplemental iron increased protein oxidation and shortened the lifespan of *C. elegans* and that moderate supplemental iron level (9 mM) increased oxidative stress without reducing the lifespan. Further, to test if the lowering free iron levels increases the lifespan duration in *C. elegans*, the authors used iron chelator and forced over-expression of ftn-1 (ferritin). However, they found reduced protein oxidation but without increased lifespan.

Moreover, the data showed increased expression of ftn-1 in long-lived daf-2 insulin/IGF-1 receptor mutants. Further, deletion of ftn-1 decreased resistance to oxidative stress, but enhanced daf-2 mutant longevity suggesting an effect of ferritin on signaling pathways that control ageing. This effect of ftn-1 may affect either insulin/IGF-1 signaling or some other pathway upstream or in parallel to insulin/IGF-1.

Collectively, investigators results showed that high levels of iron could increase oxidative stress damage and reduce lifespan, but overall suggest that iron levels within the normal physiological range do not promote ageing in *C. elegans* (Valentini et al. 2012).

In a Schiavi work group was investigated beneficial adaptive response to mild mitochondrial stress upon partial frataxin depletion in *C. elegans*. They showed for the first time that this conserved response of mitochondrial autophagy (mitophagy) to frataxin silencing is activated in response to mitochondrial stress in a pdr-1/Parkin-, pink-1/Pink-, and dct-1/Bnip3-dependent manner. The important finding is that this mitophagy induction extended the lifespan of C. *elegans*.

Mitochondrial autophagy is a form of autophagy, which is activated to recycle unhealthy mitochondria and the question of this investigating group was, whether mitophagy could play a specific role in mitochondrial stress control of longevity. Like said before in a part of *Drodophila* studies, the frataxin is a mitochondrial protein, which regulates the biogenesis of ISC proteins and, similar to other proteins that regulate mitochondrial respiration, frataxin deficiency leads to development of Friedreich's ataxia in humans and to developmental arrest in the nematode *C. elegans*. Frataxin suppression affects intracellular levels of adenosine triphosphate (ATP), ROS and cytosolic iron, which can initiate a hypoxia-like signaling and an iron starvation response, which further induce mitophagy and causally involve lifespan extension. The authors also identified non-overlapping hif-1 upstream (HIF-1-prolyl-hydroxylase) and downstream (globins) regulatory genes mediating lifespan extension upon frataxin and iron depletion. This data suggest that mitophagy could represent the potential target for the treatment of human mitochondria-associated and age-related disorders (Schiavi et al. 2015).

Rodents

Andziak and Buffenstein in their study refuted theory that levels of accrued oxidative damage increase with age. They compared lipid peroxidation profile and non-heme iron liver levels in long living naked mole-rats and shorter-living CB6F1 hybrid mice. The data revealed that concentrations of markers of lipid peroxidation, as well as iron, were at least twofold greater in naked mole rats than in mice. This refutes the hypothesis that prolonged naked mole-rat longevity is due to superior protection against oxidative stress or reduced level of tissue iron. In naked mole-rats, urinary isoprostane excretion declined by half with age, despite increases in tissue iron. Moreover, contrary to the predictions of the oxidative stress theory, lipid damage levels did not change with age in mole-rats. These findings suggest that age-related changes are species specific, independent of oxidative stress parameters (Andziak and Buffenstein 2006).

Pabis and collaborators examined involvement of iron metabolism in extended lifespan of long-lived rodent model. They quantified heme, tissue non-heme iron and several proteins involved in iron utilization in three different mouse models (Ames dwarfs, calorie restricted and rapamycin fed mice that extends lifespan in rodents). In addition, they studied the effects of rapamycin on 3T3 fibroblasts and measured the labile iron pool (free iron), sensitivity to iron induced cytotoxicity and expression of antioxidant genes. In long-lived mouse models, they found that NHI content tended to decrease but the changes were tissue specific and not consistent across models. Comparing changes in iron with those in cadmium, they found clear differences ruling out a generalized decrease in metal content. Preliminary data reveal that heme oxygenase was elevated in two mouse models. These findings are consistent with the idea that reduced total or labile iron plays a role in health and ageing (Pabis et al. 2017).

1.8 Conclusion

Iron is an important component of living organism, which allows normal cell and body functioning, and provides health. Important fact is that, its level must be carefully regulated, both at the systemic and at the cellular level, because excess might be detrimental for health and thus longevity. In that situation iron is a major catalyst of reactive hydroxyl radicals, that compromise cell integrity and thereby integrity of all body organs. That further accelerates ageing processes and provokes development of many health disorders and organ system dysfunctions, which together shorten species lifespan.

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Chapter 2
Copper

Miguel Arredondo, Mauricio González and Mauricio Latorre

Abstract Copper is an essential micronutrient for life. It is require by a wide range of species, from bacteria to yeast, plants and mammals including humans. To prevent the consequences of the deficit or excess of copper, living organisms have developed cellular mechanisms that regulate the uptake, efflux, storage and use of the metal. Several diseases are consequences of defects in such biological systems. Copper intake is reduce in elderly people, in some cases leading to two mayor problems for the human health. As co-factor of several antioxidant enzymes, reduction in the concentration of this metal directly affect the protective activity of these proteins, decreasing the capacity of the organism to counteract the oxidative stress damage, affecting the inflammatory/immune response and affecting the functioning of the central nervous system functioning through its participation as neurotransmitter and the ubiquitin proteasome system. Chronic copper toxicity is rare and primarily affects the liver. Wilson's disease and Indian childhood cirrhosis are examples of severe chronic liver disease that results from the genetic predisposition to the hepatic accumulation of copper. By the other hand, Alzheimer and Parkinson disease are examples of neurodegenerative disorder that may course with an alteration in copper metabolism.

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Finally, it had been developed new technology in order to study the role of copper on the ageing, highlighting advances in the field of system biology and transcriptomic.

Keywords Copper intake deficiency · Copper overload
Oxidative stress protection · Immune response · Neurotransmitters
Ubiquitin proteasome system · Omic technology · Neurological disease

2.1 Introduction

Transition metals such as copper are essential to life because of their catalytic and structural roles they play in proteins, and other biomolecules (Uauy et al. 1998). Although essential metals are normally present in trace amounts in the cell, their levels can change following environmental or nutritional variations, variables that also effected during the cycle of life. Metal overload can be toxic to the cell, causing a range of effects and leading to cell death when concentrations are extremely high (Dameron and Harrison 1998). In contrast, copper deficiency is usually the consequence of decreased copper stores at birth, inadequate dietary copper intake, poor absorption; increased requirements induced by rapid growth, or increased copper losses. To avoid copper deficiency and metal-induced toxicity, most organisms have developed several molecular and cellular mechanisms of protection. Three general mechanisms, which typically work in combination for effective homeostasis at organism levels, include reduction of metal uptake, enhanced metal export, and metal sequestration mechanisms.

2.2 Intestinal and Hepatic Tissues Determine Whole-Body Copper Distribution

Several reviews on copper metabolism have described whole-body copper metabolism (Cartwright and Wintrobe 1964; Sass-Kortsak 1965; Linder 1991; Turnlund 1998; Uauy et al. 1998). Figure 2.1 shows the hypothetical distribution of copper in a healthy adult including the molecular components associated with intestinal and hepatic copper metabolism (Gulec and Collins 2014; Lutsenko et al. 2007; González et al. 2008; Lutsenko 2016). Because extracellular copper is found predominantly as Cu^2, Steap proteins may be responsible for reduction of Cu^2 to Cu^1 before it is transported across the plasma membrane via the high-affinity copper transporter hCTR1, which is specific for Cu^1 (González et al. 2008). In addition, Cu^1 is transport directly into the cell by the divalent metal transporter DMT1. Once inside the enterocyte, mainly in the small intestine (Fig. 2.1), copper is deliver to various cell targets with the help of copper chaperones: Atx1p transfers copper to ATP7A located in the trans-Golgi network; CCS to Cu/Zn super oxidase dismutase (SOD1); and COX17

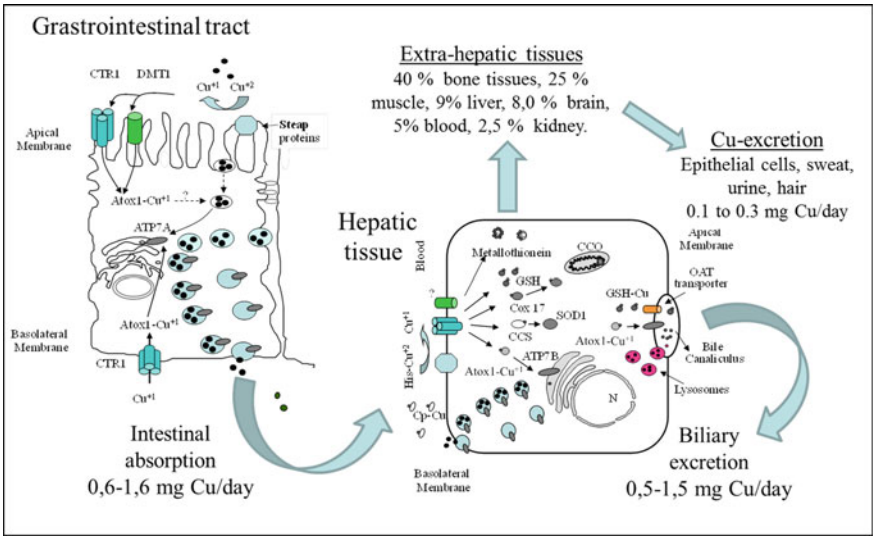

Fig. 2.1 Schematic representation of Cu distribution in the organism: from the absorption of the metal to its excretion. The processes of transport and absorption of copper have to supply an adequate amount of systemic copper to fulfill the requirement of different cupro-enzymes for this trace metal, at the same time, intestinal mechanisms must exist to prevent an excess of copper in the organism. Hepatic tissue removes copper from the circulation by rapidly trapping this metal in chelating copper proteins that transfer copper to cupro-enzymes or ceruloplasmin. Copper returns to the extra-hepatic circulation mainly bound to ceruloplasmin, while copper excess is excreted into bile. Abbreviations: Cu: copper; hCTR1: human copper transport protein 1; DMT1: divalent metal transporter 1; ATP7A: copper transporting alpha polypeptide; Cp: ceruloplasmin; His: histidine; CCS: copper chaperone for Cu/Zn superoxide dismutase; SOD: superoxide dismutase; ATP7B: copper transporting beta polypeptide; GSH: reduced glutathione; CCO: cytochrome c oxidase; OAT: organic anion transporter

through SCO1/2 to cytochrome c oxidase (COX). After that, Cu is transferred from the intestinal mucosa to the interstitial fluid and blood plasma where it is mainly complexes with albumin, amino acids (histidine, threonine, cysteine) or peptides containing these amino acids, passing into the portal circulation (Weiss and Linder 1985; Neumann and Sass-Kortsak 1967; Linder 1991).

In a second phase, the liver tissue removes the Cu from the circulation, via specific transporters such as hCTR1 and DMT1. In the cytoplasm, the Cu is transfer to the Golgi via ATP7B, where is incorporated to the Ceruloplasmin (the mayor reservoir of copper in blood), for export to extra-hepatic tissues via the systemic circulation or it can be used by cell machinery to be incorporated into newly synthesized cupro-proteins. The requirement of copper in enzyme activity, both as a cofactor and allosteric component of several cupro-enzymes, reveals that this ion is important for appropriate functional structure and catalytic activity of cupro-enzymes such as lysyl oxidase, tyrosinase, SOD1 and COX (Uauy et al. 1998). The excess of copper is bound to glutathione (GSH) and metallothionein (MT) or translocated from the

Golgi to a vesicular transport system and secreted into the biliary canaliculi (Dijkstra et al. 1996; Linder and Hazegh-Azam 1996). The average daily intake of copper in the US is about 1 mg Cu with the primary source being the diet. The bioavailability of copper from the diet is about 65–70% depending on a variety of factors including chemical form, interaction with other metals, and dietary components. The biological half-life of copper from the diet is 13–33 days with biliary excretion being the major route of elimination. Therefore, copper homeostasis is carry out by modifications in copper absorption and biliary copper excretion. The urinary, excretion of copper, is negligible in normal healthy man, being 0.1–0.3 mg/day in adults on normal dietary intakes (Buckley 1996) (Fig. 2.1).

Two genetic diseases involved in Cu transport have been recognized, Menkes Syndrome and Wilson's disease, by mutations in ATP7A and ATP7B transporter, respectively. ATP7A transporter is expressed in all tissue examined (except in liver), mutations in ATP7A in both humans (Menkes's disease) and mice (Mottled Mouse) lead to reduced intestinal basolateral export of copper and subsequent systemic copper deficiency (Mercer et al. 1999). Menkes syndrome is a disease linked to the X chromosome, which produces Cu deficiency generating progressive mental deterioration, hypothermia, connective tissue abnormalities, which eventually lead to death at approximately three years of age (Mercer 1998; La Fontaine and Mercer 2007). Interestingly, immuno-fluorescence studies using an anti-Menkes antibody suggest that ATP7A protein may concentrate copper in vesicles, which then proceed to the lysosomal pathway or to the plasma membrane for copper export, while the ATP7A itself is recycles to the trans-Golgi network (Yamaguchi et al. 1996). At the same time, Petris et al. (1996), showed that elevated copper culture conditions induced a rapid trafficking of ATP7A protein from the Golgi to the plasma membrane. Considering these data, the authors suggested the existence of a system of regulated protein trafficking which ultimately leads to the efflux of an essential yet potentially toxic ligand. In this model, the ligand (copper) itself appears to stimulate directly and specifically the trafficking of its own transporter. The implications derived from these reports, strongly support the transporter-mediated copper model shown in Fig. 2.1. Moreover, the relationship between changes in the distribution pattern of ATP7A protein and extracellular copper fluctuation are in agreement with the hypothetical alternative described in Fig. 2.1.

The major phenotypic manifestations of Wilson disease, such as liver disease and neurological abnormalities, indicate that the ATP7B protein is mainly express in the liver and the brain. Similarly to Menkes disease, mutations in the gene encoding the ATP7B also result in a severe metabolic disorder, affecting two aspects of hepatic copper metabolism i.e. ceruloplasmin formation and biliary copper excretion. Biochemical and immuno-histochemical studies have mainly localized the Wilson protein to the trans-Golgi reticulum and late endosomes (Harris and Gitlin 1996). These results are consistent with the role of Wilson ATPase in transferring copper from the cytoplasm into the secretory pathway, where it becomes available for incorporation into ceruloplasmin. In addition, ATP7B localized at the canalicular domain of the hepatocytes plasma membrane (Dijkstra et al. 1996) suggesting that it may be involved in the direct transfer of copper across this membrane. Thus, the ATPase

may represent an alternative pathway of biliary copper excretion, that works in concert with lysosomal exocytosis and GSH mediated excretion (Fig. 2.1). Therefore, a defect in ATP7B function induces a decrease of biliary copper excretion along with an increase in the hepatic content of the metal (Bartee and Lutsenko 2007) which produces neurological, psychiatric, renal, hematological and endocrine disorders (Bingham et al. 1998).

2.3 Recommended Dietary Intake of Copper

Studies using stable isotope such as ^{65}Cu, allowed estimate that the efficiency of copper absorption in humans ranges between 12 and 60% depending on copper intake, presence of dietary factors that may promote or inhibit its absorption and the copper status of the individual (Turnlund et al. 1989; revised in De Romaña et al. 2011). The total amount of copper that is retained increases with increasing intakes, reaching a plateau of approximately 1 mg/d with copper intakes of approximately 7–8 mg/d (Turnlund et al. 1989). The range of acceptable intake to prevent copper deficiency and toxicity should be based on the protection of healthy populations and should not be expected to meet requirements or prevent excess of individuals with special susceptibility. The National Academy of Sciences of USA/National Research Council (NAS/NRC) established the category of Estimated Safe and Adequate Intakes for essential nutrients when there is insufficient information to determine recommended dietary allowances (NAS 1989). Since copper requirements are not well established on a quantitative basis, this mineral is included in this category. The USA NAS/NRC has recommended that infants from 0 to 6 months of age should receive a daily intake of 0.4–0.6 mg of copper, increasing progressively up to 2 mg in children up to 10 years of age. For adolescents and adults this range is 1.5–2.5 and 1.5–3 mg respectively. World health organization has suggested a recommended daily intake of copper of 80 μg/kg for infant and young children and of 40 μg/kg and 30 μg/kg for older children and adult males, respectively (WHO 1973; WHO/FAO/IAEA 1996). Humans can adapt to excessive copper exposure from food, water, or supplements by decreasing the absorbed fraction as exposure increases (Uauy et al. 1998; Turlund et al. 1989). Risk of copper deficit or excess in apparently healthy human populations can be hypothesized under relatively rare conditions of exposure (Uauy et al. 2008; De Romaña et al. 2011). For this reason, identifying biomarkers to detect early effects of nutrient deficiency and excess has become a pressing challenge in modern nutrition. Until now, numerous efforts have been done to define new molecular markers by measuring activities in blood of copper-related enzymes, but they have failed (Suazo et al. 2008). Of the many proteins assessed as potential markers of copper status the chaperone of SOD1 (CCS) has yielded promising results, however data on its performance under different conditions are needed to confirm its use as an indicator of copper status (Olivares et al. 2008; Araya et al. 2014; Arredondo et al. 2014).

2.4 Essentiality, Deficiency, and Intake Related to Aging

The USDA recommends a consumption of 900 μg/day in adults. In general, copper deficiency can produce severe health problems, which include arthritis, osteoporosis, anemia, low body temperature, joint pain, skin inflammation and sores, among others.

While, several reports indicate that ageing does not modify significantly copper homeostasis, where the regular dietary copper intake of elderly people do not required an extra supplementation of copper to maintain health. In some cases, the inadequate intake of this metal can produce several damage, leading principally in three mayor problems related to oxidative stress protection, inflammatory/immune response and the Central Nervous System functioning (Fig. 2.2).

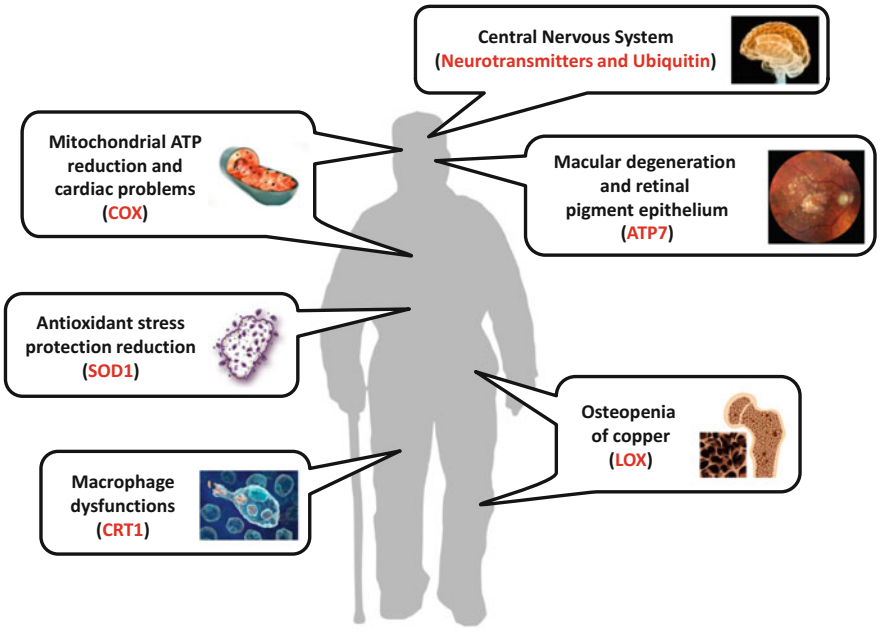

Fig. 2.2 Copper deficiency effects in elderly population. Deficiency in the intake process of copper during ageing contribute to the damage and development of age-related listed diseases. In red, molecules and cupro-enzymes affected by the decrease in the copper concentration. (All figures were obtained from free Google/images)

2.4.1 *Copper Deficiency and Oxidative Stress Protection During Ageing*

Trace elements intake (including copper) is reduce in elderly people, this lead to a decrement in the capacity of the organism to counteract oxidative stress damage. As mentioned, copper participates as co-factor of several antioxidant enzymes such as superoxide dismutase (SOD1) and catalase. In contrast to the toxic effect induced by the copper overload, reduction in the concentration of this metal directly affects over the activity of these enzymes, decreasing the capacity of the organism to reduce oxidative damage or enhance repair capacity (Mocchegiani et al. 2014).

In order to study if an increase in copper intake can generate a protective effect against oxidative stress damage, Kirchman and Botta (2007), generates a forced respiration model in yeast emulating the cellular aging process. The results indicated that supplementation of copper significantly extends the life span of the yeast. In addition, mutants for the genes *sod1* and *sod2* supplemented with copper also increase its viability, strongly suggesting that the addition of this metal may reduce the oxidative damage under the aging process.

Copper deficiency also has been linked to the appearance of osteoporosis. The lysyl oxidase (LOX) protein, also known as protein-lysine 6-oxidase, is a copper-dependent enzyme, which catalyzes the formation of aldehydes from lysine residues in collagen and elastin precursors. The reduction of its activity generated by copper intake problems leads to bone mass loss, called osteopenia of copper deficiency. The supplementation of copper improve the outcome of femoral neck fracture in old patients and increase the mineral density in bones in postmenopausal women (Saltman and Strause 1993; Delmi et al. 1990), strongly suggesting the importance of a correct dietary intake of this metal in elderly people. Moreover, patients with severe rheumatoid arthritis showed and important condition of copper deficiency, accomplished by a typically ingest too much fat and not enough fiber, zinc and magnesium (Kremer and Bigaouette 1996).

One of the mayor health problems in older population is the aging macular degeneration, leading cause of blindness in several countries. By the year 2020, it is predicted in United States that nearly three million people will suffer this disease (Friedman et al. 2004). The supplementation of several micronutrients (including 2 mg of copper) in 3640 participants between 55 and 80 years carrying a varying severity of aging macular degeneration, significantly reduce the progression of the disease in a 25% (Chew 2013). Another common eye pathology in elderly people is the retinal pigment epithelium (RPE), an ocular degeneration produced by the pigmentation of the cell layer just outside the neuro-sensory retina that nourishes retinal visual cells. The ATP7 copper protein migrates toward the apical or basal retinal epithelium, facilitating the flux of copper between the blood-brain barrier and the retina. Loss of local retinal activity of this protein in Wilson and Menkes condition contribute to the retinal degeneration (Krajacic et al. 2006).

2.4.2 *Copper Deficiency and Inflammatory/Immune Response During Ageing*

The second important alteration is the incorrect inflammatory/immune response occurred by problems in the absorption or dietary intake of copper. Several studies have been showed the role of copper in the development and maintenance of the immune system (Percival 1998). Conventionally, the immune system is divided in acquired and innate systems. In general, copper deficiency reduced the effectiveness of the acquired response.

Lukasewycz and Prohaska (1990), showed that the production of antibodies by spleen cells is reduced in mice fed with a low copper diet, presenting a reduction in the proliferation in splenocytes cells. In this study also, the authors observed that interleukin 1 and 2 are significantly greater and reduced respectively in the copper deficient mice, strongly supporting the implication of a correct copper supplementation on the immune response. In terms of the innate system, copper deficiency affects the functioning of neutrophils and macrophages. Neutropenia is a blood abnormality characterized by a low concentration of neutrophils, a clinical sign of copper deficiency. It has been postulated, that during copper deficiency scenario, exist a maturation arrest of granulocytes, increasing the number of promyelocytes and a reduction in the number of metamyelocytes. A second mechanism includes an early death of progenitor cells in the marrow, reducing the life span of neutrophils. During the phagocytosis process, macrophages produce reduced superoxide anions in order to kill of microbes. The candidacidal activity of macrophages in rats fed with a low levels of copper, are significantly reduced in relation to the adequate diet (Babu and Failla 1990).

In humans, copper-deficient marasmic children supplemented with copper significantly improve theirs phagocytic index (Heresi et al. 1985). Low copper diet in young men induces a reduction in the proliferation of mononuclear cells, including a decrease in the neutrophils amount, directly reflecting the amount of copper supplemented. Different mechanism has been proposed about the relation between copper and macrophages activation. In the site of inflammation, there is a local accumulation of copper and macrophages. The cytokines IFN-γ (interferon gamma) and TNF-α (tumor necrosis factor alpha) promotes the accumulation of the metal in phagosomes, following by the activation of CTR1 transporter, which contribute to the killing capacity of the macrophages related to the reduced superoxide anions (White et al. 2009). In addition, this rise in copper concentration is due to an elevation of the ceruloplasmin in serum, protein able to promote an anti-inflammatory activity during the immune response. On contrary, classic studies indicates that copper deficiency induce an inflammation in humans, probably linked to the reduction of ceruloplasmin and cupro-enzyme activities (Giampaolo et al. 1982; Sorenson 1988). In elder population, aged monocytes and macrophages have a reduced phagocyte capacity, this functional senescence diminished the immune response against pathogens, where the reduced intake of copper in elderly people may contributes to this phenomenon.

One of the main immune barrier is the skin. This organ contains elements of the innate and adaptive immune systems. As mentioned, the lysyl oxidase is a cuproenzyme crucial in the metabolism of collagen. Alterations in this protein leads to faulty of collagen in the body, producing wrinkles, sagging, connective tissue abnormalities and loose skin. At the same way, a decrease in the activity of the superoxide dismutase enzyme by copper deficiency conduce to skin premature aging by an accumulation of oxidative stress molecules. A second risk factor induced by copper deficiency in skin aging is the glycation of skin proteins. The hyperglycemia produced by copper deficiency leads to the glycosylation of several proteins and peroxidation. Saari et al. (1995), demonstrate that food restriction in rats reduced not only reduces the peroxidation and glycation, also reduces the symptoms of copper deficiency.

A loss in the pigmentation of hair (grey color) is classical phenotypes in old people. Several studies connect the deficiency of copper with achromotrichia (absence or loss of pigmentation in the hair). Calf feed with low concentration of copper showed after the treatment several skin problems, including loss in hair pigmentation and cytochrome oxidase activity reduction (Suttle and Angus 1976). A second study showed that low amount of copper led to alternate black and white bands in black sheep, affecting also the shape of the wool (less curly). In addition, grey hair also presents a lower concentration of copper, demonstrating a link between the metal deficiency and hair properties (Adams and Murray 1974).

Finally, there are several evidences declaring the effect of hormones over the copper levels (Malavolta et al. 2015). The expression of ceruloplasmin is significantly reduced after insulin treatment in rat hepatoma cells, a similar effects when copper (Cu^{+2}) is added into the media (Leyendecker et al. 2011). In addition, copper increase in plasma may lead to oxidative stress and tissue damage. These elevated copper levels could stimulate the formation of advanced glycosylated end products, linked to secondary complications. These results suggest that ceruloplasmin probably play a role in the pathogenesis of chronic disease complications such as diabetes mellitus, one of the highest prevalent metabolic conditions in ageing societies associated with high levels of obesity.

2.4.3 Copper and Central Nervous System

Two mayor role have been assigned to copper on central nervous system functioning. Copper concentration varies depending of the brain region. Bioavailable copper can be found in cerebrospinal (70 μM), cerebrospinal (1 μM) fluids, and principally concentrated in synaptosomes and synaptic vesicles (Stuerenburg 2000). The capacity of copper to form different complex with neurotransmitters directly affects the synaptic function of the neurons. Koefoed-Johnsen and Ussing (1958) converts the structure of frog skin membrane by the addition of copper, suggesting that this metal can modulates the permeability if plasma membrane at the presynaptic or postsynaptic levels. In this line, the stimulation of rat brain cortical synaptosomes by 50 mM

of KCl induce the release of copper from the cells, same effect occurred during the activation of NMDA receptors in primary hippocampal cultures (Kardos et al. 1989; Schlief et al. 2005).

Copper also modulates different neurons of the central nervous system in a similar way, principally blocking the neurotransmission of GABAergic and AMPAergic in olfactory bulb neurons, cortical neurons and cerebellar Purkinje cells (Opazo et al. 2014). Recently, has been demonstrated that copper also acts as an activator of the synaptic function. At the beginning, primary cultures of rat hippocampal neurons treated with 10 μM of copper block its AMPAergic activity; however, after three hours of exposure copper promotes and increase the neurotransmission in these cells (Peters et al. 2011). This biphasic effect also is correlated to neurophysiology and neuropathology implications. Primary hippocampal neurons treated by short terms with copper show a significant increase in amplitude, frequency and time constant synaptic events, supporting the role of copper as a neurotransmission in the central nervous system.

A second role for copper is its participation in the Ubiquitin proteasome system. The main function of this process is to degrade damage or unfolding proteins into small peptides. The ubiquitin protein is the small substrate added to the proteins as a signal for their degradation via proteasome. Has been demonstrated by different in vitro approaches, that ubiquitin binds copper atoms (Cu^2) through the histidine-68 residue locate in the surface of the protein and forming a metal-protein complex. Moreover, the addition of copper chelators inhibits the proteasome functioning by unknown mechanism (Ding and Lind 2009).

Even though, there is not concluded data correlating the copper deficiency with neurological disorders occurred during the aging process, it is plausible to hypostatize that copper depletion in cupro-proteins affect the correct functioning of the central nervous system. Beside its participation in neurotransmission and protein degradation, copper also play a crucial role as a co-factor in mitochondrial ATP production. The cytochrome c oxidase (COX), a mitochondrial cupro-enzyme, is essential for oxidative phosphorylation and aerobic respiration. Thus, malfunction of this protein affects the activity, signaling and survival of neuron cells, being a crucial protein in etiology, progression and prevalence of numerous human neurodegenerative diseases (Arnold 2012).

In addition, copper also has been linked to a variety of cardiovascular deficits, including anemia, high blood pressure, enhancement of inflammation, reduced blood clotting, and possibly arteriosclerosis, among others. Mutations in cytochrome c oxidase can be found in patients with hypertrophic cardio-myopathy. Copper supplementation in mice model of ascending aortic constriction can normalize gene expression related to contractility and inflammation (Zheng et al. 2015). The activity of the cytochrome c oxidase and the oxidative stress generated by mitochondria are similar in copper deficiency and aging, opening the possibility to use copper deficiency as a short-term model for studying the potential roles of mitochondria in cardiac aging (Johnson and Newman 2003). All these data strongly support the idea that copper supplementation appears to be a simple and manageable approach to

develop new clinical implantation in order to counteract the deficiency of this metal in elderly people (Table 2.1).

2.5 Copper and Species Longevity

2.5.1 Yeasts

The budding yeast Saccharomyces cerevisiae has been extensively used as a model for cellular aging. Using glycerol as carbon source, yeast generates energy by the same respiratory metabolic pathways as mammalian cells. Under this condition Kirchman and Botta (2007) showed that copper supplementation was associated with life span extension, possibly through the upregulation of oxygen-radical defense mechanisms.

In this context, the authors show that copper supplementation increased the life span of the SOD1 (found mainly in the cytosol) and SOD2 (localized in the mitochondrial matrix) mutants, suggesting that copper supplementation increases longevity by reducing or removing the superoxide production (revised in Mocchegiani et al. 2012), a process that involved iron import by Fet3p/Ftr1p (Botta et al. 2011).

Table 2.1 Effects of supplementation in copper deficiency studies

Condition	Copper supplementation protocol	Effect	References
Neutropenia	6 μg of copper (5 weeks)	Increase in activity of macrophages, NK cytotoxin and T-cell proliferation	Bala and Failla (1992)
Macular degeneration	2 mg of cupric oxide/day for 4–11 years	Reduction in apoptosis of retinal pigment epithelium	AREDS (2001)
Osteogenesis in postmenopausal women	3 mg $CuSO_4$/day for 2 months	Increase in bone mineral density	Saltman and Strause (1993)
Copper-deficient marasmic	40-50 μg/kg day	Increase in polymorph-nuclear phagocytic function	Heresi et al. (1985)
Cardiac hypertrophy	20 mg Cu/kg diet for 4 weeks	Regression of cardiac hypertrophy and activation of cytochrome c oxidase	Zheng et al. (2015)

Adapted from Mocchegiani et al. (2014)

2.5.2 Flies, Worms and Rotifers

Research involving mammals as a first approach is expensive, complex and fraught with ethical concerns. As an alternative model, invertebrate model systems such as nematodes and fruit flies, in the last decades have contribute to translational research investigating metal genetic diseases and aging process, leading to biological findings that are broadly applicable to human health and disease (Murthy and Ram 2015). In particular, the Cu homeostasis response in *Drosophila melanogaster* is highly conserved with humans, including molecular determinants, signaling pathways and regulation mechanism. Several articles have been published with studies about Cu homeostasis and flies, including dietary absorption, transport and storage, as well as regulation, together with the ever-improving arsenal of available genetic tools. In terms of aging and copper, a classic work by Massie et al. in 1984, showed that flies fed with copper (1 mM or higher) significantly reduce its life span. On contrary, a combination of copper gluconate and gluconic acid extend the life span of *D. melanogaster* by 21.5%, retarding the normal age-related accumulation of the metal.

As well, worms and rotifers organisms also have appeared as excellent models to study the influence of copper over the aging process. Studies made in *Caenorhabditis elegans*, showed that worms fed with a normal diet increase its copper levels as a function of age. Moreover, this copper accumulation contributes to age-related protein aggregation, similar phenotype occurred in humans during the formation of toxic oligomers of peptides such as β-amyloid and tau in Alzheimer's disease (Klang et al. 2014). Additionally, high concentrations of copper significantly reduce the life-span of *C. elegans*, accompanied by detrimental effects on brood size and development problems (Harada et al. 2007).

Rotifers are useful model organisms for aging research, owing to their small body size (0.1–1 mm), short lifespan (6–14 days) and the relative easy in which aging and senescence phenotypes can be measured. The life-span of the rotifer *Asplanchna brightwelli* is reduced in a 9% when is exposed to high copper concentrations. In addition, lipid peroxidation measure was significantly higher at the end of this treatment in relation to the control scenario (no Cu added), damage probably produced by the generation of reactive oxygen species (ROS) as a consequence of the high Cu diet (Enesco et al. 1989).

2.5.3 Mice

Potential risks associated with high chronic copper intake from foods and water have been a concern to health researchers, but ethical constraints limit maximum doses and exposure periods, the levels tested have not exceeded the upper limit defined as safe for humans (Araya et al. 2012).

Using mice as a model, Massie and Aiello (1984) found that increased ingestion of copper in the form of gluconate (CuG) reduced the survival time as much as 14.4% in male rats. Copper, thus may play an integral role in the degenerative processes of aging. However, earlier results on CuG human supplementation (Pratt et al. 1985) showed that 10 mg of CuG per day for 12 weeks had no significant differences in serum, hair, and urine levels of copper. In other hand, capuchin monkeys exposed to copper at doses up to 50 times the current upper level enhanced expression of genes related to inflammation and injury without clinical, blood biochemical, or histological evidence of liver damage (Araya et al. 2012).

2.5.4 Cellular Senescence: In Vitro Models

Cellular senescence had been studied for at least for four decades. It had been demonstrated that normal cells in culture had a limited ability to proliferate. Normally cells in cultures conditions initially exhibit active cell division, but, after a number of population doublings, dividing cell number decreases and at the end they cease dividing. Cellular senescence was firstly described by Hayflick and Moorhead, who demonstrated that serially cultivated human diploid fibroblasts ceased dividing after a number of population doublings and became unresponsive to mitogenic stimuli, thus entering in a condition termed replicative senescence (RS) (Matos et al. 2014).

Matos et al. (2012), showed that sub-cytotoxic copper sulfate concentration was able to induce senescence features on WI-38 human fibroblasts, similar to the effects of other well-known stress inducer premature senescence (SIPS) agents (such as: hydrogen peroxide, tertbutylhydroperoxide, ultraviolet B radiation or ethanol). They observed changes in: cell morphology (cell enlargement), changes in proliferation, in senescence-associated β-galactosidase activity, and increased senescence-associated gene expression (such as p21, apo-lipoprotein J, fibronectin, transforming growth factor β-1 (TGF β1), insulin growth factor binding protein 3, and heme oxygenase 1).

It had been demonstrated that a cellular level, resveratrol (a natural polyphenolic compound) showed to attenuate senescence features in cellular models of replicative senescence, H2O2-SIPS or cells exposed to copper, thru the resveratrol ability to activate sirtuin 1 deacetylase (Sirt1) (Matos et al. 2017). Sirt1 overexpression attenuates senescence and extends replicative lifespan in different cultured cells types, such us human endothelial cells (Ota et al. 2007); human diploid fibroblasts (Huang et al. 2008); human umbilical cord fibroblast (Yamashita et al. 2012). Then, the resveratrol effect result from its ability to promote cellular adaptive mechanisms, as autophagy up-regulation, which sustain cellular proteostasis and confer cellular resistance to stress (Matos et al. 2017).

2.6 New Technology to Study the Role of Copper on the Ageing

New technologies have emerging in order to study the role of copper on the ageing. Today, we understand that ageing is a complex process which involved several adaptation mechanism, variables and stimulus. Currently, "omics technologies" appears as an excellent alternative to integrate all this information into global mathematical models in order to contribute to a better understanding of the role of copper during the ageing (Méplan 2011).

A meta-analysis collected 27 global gene expression profiles from different age-related organism, including mice, rats and humans (de Magalhães et al. 2009). These results declared that genes encode for proteins involved in detoxification of copper where significantly overrepresented inside the group of overexpressed genes, highlighting the importance of this process during aging.

In order to obtain insight about the genetic control of copper affected pathways during ageing, a genome-wide transcriptomic assay was performed in the long-lived model of *Podospora anserine* growing in a copper deplete scenario (Servos et al. 2012). The data showed that more than 4700 genes where differentially activated. Beside components involved in oxidative stress protection and copper resistance, genes encoding for proteins related to energy metabolism, respiration, iron homeostasis and protein quality control also changes its transcriptional abundances. These results strongly support the idea that copper depletion in ageing involved the transcriptional regulation of a huge number of genes, suggesting a complex regulatory network coordinating the expression of these elements. In addition, was possible to identify new targets related with copper homeostasis and ageing, demonstrating the importance to use system biology approaches to identify new biomarkers to monitor these processes.

2.7 Copper Toxicity

Today, copper has many uses and utilities, is used as a metal or alloy in machinery, construction (mainly in domestic water pipes), transportation, and military weapons and is an important component of white gold and other alloys used for imitation jewelry, dental products, intrauterine devices, in cosmetics and as bactericide in handrails, butchery plates and clothing. While, the exposure to Cu through these items is not harmful, the exposure to elevated concentrations of Cu is damaging, because its redox activity. Similarly to iron, copper through the Fenton reaction, is capable of produce reactive oxygen species (ROS) (Gaetke and Chow 2003).

Cu is a transition metal that, like the rest of this type of metals (except Zn), has electrons unpaired in its outer orbitals. Cu as well as iron can participate in Fenton (2.1) and Häber-Weiss (2.2) reactions that produce ROS:

$$Cu^1 + H_2O_2 \rightarrow Cu^2 + OH\cdot + OH^- \quad (2.1)$$

$$Cu^2 + O_2^{\cdot}- \rightarrow Cu^1 + O_2 \quad (2.2)$$

Cu^1 salts react with H_2O_2 more efficiently than Fe^2. Therefore, the main mechanism of copper-mediated toxicosis may rest on its ability to cause ROS overproduction and subsequent pro-oxidative damage to lipids, nucleic acids, and proteins. Cu^+ ion can rapidly oxidize the S-nitrosothiol groups of proteins, as does the cupric ion in the presence of GSH, cysteines or ascorbate. This fact is of the utmost importance since in this way the ions derived from Cu can interfere with signaling pathways from which the oxidized proteins are part. There is increasing evidence that an increase in ROS (because of high ions Cu concentrations) may induce auto-phosphorylation of tyrosine kinases (TK) receptors of the plasma membrane. This activation may in turn lead to the activation of the PI3 K/Akt pathway (phosphoinositide-3-kinase/Akt), where PI3 K is recruited to the plasma membrane through its SH2 domain. Once on the membrane, PI3 K phosphorylates phospholipid PIP2 (inositol-3,4-bisphosphate) to produce PIP3 (inositol-3,4,5-triphosphate) which is required to capture proteins with PH domain (pleckstrin homology), such as Akt (also called protein kinase B, PKB) and PKD1 (protein kinase D1). Finally, PKD phosphorylates and activates Akt, which will also trigger activation and inhibition of proteins by phosphorylation. In turn, it is very interesting to note that Cu^{++} can directly activate (ligand independent) the PI3 K/Akt pathway.

Activation of TK receptors by ROS is also directly relate to the MAPK (mitogen activated protein kinase) pathway. Cu ions can induce the activation of the MAPK pathway, especially by increasing the ERK (extra-cellular regulated kinase) activity, because of an increase in ROS. Beside, an increase in ERK activity would also lead to an increase in the activity of effector caspases 3 and 7, phosphorylating and inactivating, the anti-apoptotic proteins of the Bcl-2 family, which leading to programmed cell death.

Peroxidation of low-density lipoprotein (LDL) increases atherogenic risk. Exposure of LDL to Cu^2 is one of the most common ways to initiate this oxidation in vitro. Although the relevance of these ions in the initiation of LDL oxidation in vivo is not entirely clear, it is possible that in the absence of chelating agents for Cu^2 is reduce to Cu^1 converting the hydroperoxides into alkoxyl radicals. In the absence of hydroperoxides, the oxidation would be initiated by the OH· radicals generated from the reduction of O_2 by Cu^1. Cu^2 ions can bind to a finite number of sites in the LDL molecule corresponding to histidine residues of apo-lipoproteins B-100. There is a progressive increase of TBARS in isolated human LDL, product of the oxidation of the PUFAs by reduction of Cu^2 to Cu^1. Increased ROS and LDL peroxidation may trigger pro-inflammatory stimuli inducing changes in the endothelial cell phenotype. These changes allow the passage of modified LDL particles and certain leukocyte strains through the endothelial barrier, trapping them in the sub-endothelial space. The sub-endothelial deposits thus formed stimulate the entry of macrophages and CDn lymphocytes among others, and the release of enzymes such as myeloperoxidase and iNOS inducing the formation of ROS/RNS with subsequent generation of

peroxynitrite. Thus, larger amounts of oxidized LDL (LDLox) are formed which lose their ability to bind to LDL receptors on the cell surface (Park 2009). In turn, LDLox have affinity for a family of receptors expressed in macrophages, called scavenger receptors that recognize and internalize LDLox. The changes caused by this extracellular signal cause macrophages to accumulate large amounts of cholesterol and other lipids. After a prolonged period, the macrophages will have transformed into foam cells that are part of a complex structure called "atherogenic plaque" (Brewer 2010).

In addition, Cu also cause marked disruption of the endocrine system. One of the mechanisms of Cu ions to interfere with the normal functioning of the endocrine system is shifting from its natural positions to other metals that act as cofactors, thus modifying the functionality of many transporter, receptor and enzymatic proteins. This occurs for example with the structure of the so-called Zn fingers that has the estrogen receptor protein and other steroids to bind to the acceptor site on the DNA. When there is an increase of Cu in relation to Zn, the latter is replaced by Cu causing the receptor to lose its specific function or modify it (Ziyatdinova et al. 2006). It is also widely demonstrated that copper has important effects as a cytotoxic and genotoxic agent developing undoubtedly a role in the etiopathogenesis of neoplasias. This latter mechanism relies on the production of damage in the molecular structure of the DNA by indirect route (ROS) or directly by complex formation with functional groups of the nitrogen bases that modify them by introducing mutations, or making difficult the process of repair of errors.

2.7.1 Excess/Toxicity and Age-Related Disease: Childhood and Ageing

Copper toxicosis covers the entire range of ages, so for example, hepatic copper accumulation has been observe in a variety of pediatric liver diseases including Wilson disease (WD), Indian childhood cirrhosis (ICC), the non-Indian disease termed idiopathic copper toxicosis (ICT), and disorders associated with chronic cholestasis. WD, ICC, and ICT are believed to be primary copper-associated liver diseases with distinguished epidemiologic, clinical, and biochemical characteristics as well as distinct histologic features (Müller et al. 1998).

In late 70′, Popper et al. (1979), described three cases of Indian childhood cirrhosis, a copper toxicosis different from that in Wilson's disease. They concluded that there was a distinctive pattern in the distribution of the excess copper in the last stages of the illness and they suggests that there was a genetically determined abnormality of copper metabolism. However, if ICC is due to hepatic copper toxicosis, why only Indian children should be involved, in a particular age group, from certain castes, and predominantly boys? Accumulation of copper in hepatocytes may be a secondary phenomenon and this copper in bound form is non-toxic to the hepatocytes. It is however true, that milk boiling and storing in brass utensils, which are either without

or inadequately tin coated, may be the source of high copper intake in ICC patients as 40–60~ of the dietary copper is absorbed in the gut. Thus, the accumulation of copper in ICC is well documented, but it is not likely to be the only etiology of this unique disease. In general, Cu ingested in food and water does not result in clinical toxicity because the amount is relatively low and importantly, because Cu homeostasis is well maintained by a combination of decreased absorption and increased excretion. Amongst the unusual circumstances that have led to clinical toxicity in children, is accidental ingestion of Cu sulfate, which is used as a pesticide crops.

As was commented above, the basis of Cu toxicity is ascribed to its propensity for producing free oxygen radicals within the hepatocytes resulting in severe intra-nuclear and cytoplasmic damage. This initiates infiltration of immune and connective tissue cells to repair the damage. Long-term toxicity, therefore, is typically manifested in the development of liver cirrhosis along with hemolysis and damage to renal tubules, brain and other organs. Of the clinical situations, Wilson's Disease occurs because of a genetic disorder in the excretory arm of Cu metabolism leading to large amounts of intra-hepatic Cu. However, the Indian childhood cirrhosis and the similar idiopathic copper toxicosis is thought to occur mainly due to high dietary Cu. Then, the mechanism of toxicity of Cu involved, therefore, may entail another insult (environmental or genetic) in addition to increased Cu intake. Hepatic Cu is also raised (to modest degrees) in other chronic liver disorders such as biliary atresia and chronic active hepatitis; but this is likely to be related to the effects of the disease process rather than its cause (Pandit and Bhave 2002).

Consuming Cu-contaminated water or foods is associated with development of acute gastrointestinal symptoms but not with increased mortality from liver disease. Cu poisoning may result in weakness, lethargy, and anorexia in the early stages as well as erosion of the epithelial lining of the gastrointestinal tract, hepato-cellular necrosis in the liver, and acute tubular necrosis in the kidney. The estimated lethal dose of Cu in an untreated adult is about 10/20 g (Barceloux 1999).

Chronic Cu toxicity primarily affects the liver, because it is the first site of Cu deposition after it enters the blood. Cu toxicity is typically manifested by the development of liver cirrhosis with episodes of hemolysis and damage to renal tubules, the brain, and other organs. Symptoms can progress to coma, hepatic necrosis, vascular collapse, and death. In addition, chronic Cu toxicity in dialysis patients receiving dialysis via Cu tubing, workers using pesticides containing Cu, and in infants maintained for long periods on intravenous total parenteral nutrition.

Certain medical conditions, particularly those involving obstructive bile excretions such as primary biliary cirrhosis, obstructive hepatobiliary disease, extrahepatic biliary atresia, neonatal hepatitis, choledochal cysts and a-1-antitrypsin deficiency, predispose the patient to increased Cu concentrations (Beshgetoor and Hambidge 1998).

Abnormalities of Cu binding proteins play an important role in Menkes disorder and Wilson's disease. Menkes syndrome is an X-linked Cu deficiency disorder that is usually fatal in early childhood. Patients with Menkes disorder present with mental retardation and neuro-degeneration mostly because of a deficiency of Cu-dependent enzymes necessary for brain development (Mercer 1998). Wilson's disease is an

autosomal recessive metabolic disorder characterized by a marked increase of Cu in the liver and brain (Scheinberg and Sternlieb 1996) because of a reduced capacity for biliary and other means of Cu excretion. An alteration in the ATP7B gene on chromosome 13 (Brewer et al. 1999) affects the Cu transporting enzyme, adenosine triphosphatase, which is key to excretion of Cu into the bile. Cu accumulates in a patient's hepatocytes, because it is not used in the synthesis of ceruloplasmin or excreted normally in the bile. The excess Cu builds up in the liver, leading to cirrhosis, and is deposited in other extra-hepatic tissues.

2.7.2 Neurodegenerative Diseases

Cu is an important component of proteins essential for neural functioning. However, Cu has been implicated in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS); Dementias with vascular origin (RV), spongiform encephalitis (Creutzfeld-Jakob Disease), and with Huntington's disease, among others. These diseases are characterized by the development of neuronal degeneration and progressive loss of synapses with the consequent deterioration of the superior functions, as a consequence of an increase in the concentration of Cu, Fe and Zn, especially in the tissues of the central nervous system (Cerpa et al. 2005).

All neurodegenerative diseases are characterized by the presence of intra- or extra-cellular deposits of proteins that produce neuronal damage and contribute to the development of the pathophysiological of the disease. However, these deposits cannot be consider as etiopathogenic factors responsible for the diseases, since the soluble intermediates that will later form the aggregates are more toxic than the deposits themselves (Kozlowski et al. 2009).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons, which leads to progressive muscle weakness, atrophy, and death often within 3 years of the onset of symptoms (Waggoner et al. 1999). Rosen et al. (1993), showed that 10% of cases of ALS are familial, and approximately 20% of them are due to mutations in the gene encoding cytosolic copper/zinc superoxide dismutase (SOD1). The evidence indicate that the neurological damage in the ALS arise from gain-of-function associated with these abnormal SOD1 alleles, which inducing an increased free-radical generating activity, associated with the copper bound by the mutant SOD1 enzymes (Yim et al. 1996).

Alzheimer's disease, is an incurable and progressive neurodegenerative disorder, characterized by neuronal degeneration, the gradual development of severe cognitive impairments, loss of different types of memory, personality changes, anomia, apraxia and a great variety of other characteristic signs and symptoms. One of the most evident (although not pathognomonic) histological features of this disease is the aggregates of extracellular amyloid plaques in the neocortex, containing β-amyloid peptide (Aβ) (derived from the amyloid precursor protein, APP) and a neurofibrillary intracellular framework rich in hyperphosphorylated tau protein (Hooijmans and

Kiliaan 2008). The aggregation of Aβ peptide is associated with a conformational change from α-helix to β-sheet, as a consequence of age or some pre-existing pathological condition. The amyloid precursor protein (APP) is a transmembrane protein with an extracellular region that can be divided into several domains including the copper binding domain (Cu-BD) (Kong et al. 2008), which act as reductases (Cerpa et al. 2005). Metals are significantly increased specifically within Aβ plaques, copper (390 μM), zinc (1055 μM) and iron (940 μM) are all elevated within neurons as compared with the normal age-matched cells (79; 350 and 340 μM, respectively) (Hung and Barnham 2012). Thus, Cu^2 would increase Aβ toxicity through the formation of ROS (Kozlowski et al. 2009). Although, it is unclear whether this occurs during the formation of β-amyloid aggregate or once it has matured (Andersson et al. 2002). On the other hand, it is believed that there would be anomalies in the cellular transport system of the metals involved in Alzheimer's disease (especially Fe and Cu) (Liu et al. 2006). Crouch et al. (2009) reported that an increase in the availability of intracellular Cu would increase the phosphorylation of the glycogen-synthase-kinase-3β (GSK3β) enzyme, thereby decreasing its activity, and hence tau phosphorylation.

There is an association between increased concentrations of Cu, Fe and Zn with increased deposits of β-amyloid protein in the brain. In AD, the amyloid precursor protein, which has been directly linked to early-onset forms of the disease, contains a Cu-binding site. The binding of Cu^2 to amyloid precursor proteins in vitro results in the oxidation of two cysteine molecules to cystine and produced two electrons. As only one electron is needed to reduce Cu^2, the remaining electron may be involved in the production of hydroxyl radicals. The binding of β-amyloid protein to Cu and Zn could promote ROS generation. Although, the etiology of AD is unclear, there is much evidence suggesting a state of oxidative stress as a prodrome to the symptoms that characterize this disease (Smith et al. 2007; Moreira et al. 2008). In addition, there are studies that show that lipoperoxidation and oxidation of proteins and nucleic acids occur—and even precede—the slight cognitive impairments (sometimes undetectable) that represent the early stages of AD.

Parkinson's disease (PD), like AD, is a disease with multiple etiological causes, among them genetics and environmental factors. Many researchers have demonstrated that occupational (and other environmental-related) factors are significantly associated with the incidence, prevalence and clinical progression of this disease (Gorell et al. 2004). Individuals suffering from this disease shown the characteristic tremors at rest, slowness in movements (bradykinesia), rigidity and general postural instability with changes in gait, perception, psychism and cognitive abilities. This is due to the progressive loss of dopaminergic neurons from the compact pars of the substantia nigra (SNpc). In the affected neurons can be observed round hyaline cytoplasmic inclusions, called Lewy bodies (LB). These bodies and neurite involved are composed of normal and truncated, ubiquitinated proteins, stored in the cytoplasm as undegraded products of the neurodegenerative process (Ferrer 2009). The major component of LB and aberrant neurites is the α-synuclein protein, which is found to be abnormally phosphorylated, nitrated and oxidized, and therefore much more prone to the formation of insoluble aggregates and fibers. The characterization of the interaction of Cu^2 with α-synuclein shows that this metal accelerates

the protein aggregation at physiological concentrations without altering the resulting fibrillary structures (Kozlowski et al. 2009). Oxidative stress is also a factor of undoubted importance in the pathogenesis of PD. There is a decrease in the activity of the enzymes of the antioxidant defense system and an increase in the oxidation of biomolecules (Ferrer 2009). Uversky (2007), had reported that high concentrations of metals, exposure to pesticides and herbicides, among other factors, facilitate the formation of fibers. Snyder and Friedman (1998) demonstrated that an increase in intracellular concentration of Cu in cells treated with L-DOPA and dopamine would decrease cell proliferation and increase the formation of micronuclei.

On the other hand, Atox1, a Cu transport protein, is a Cu-dependent suppressor of oxidative damage in yeast lacking SOD. Neuronal cell lines transfected with the Atox1 gene to increase the endogenous level of Atox1 expression are protected against serum starvation and oxidative stress. Thus, Atox1 may play a role in preventing neuronal cells against oxidative damage induced by Cu.

2.7.3 Treatments to Combat Metal Overload

In all this patients, the incorporation of Cu-chelating agents has been shown to prevent neuronal death (Armstrong et al. 2001; Velez et al. 2008). An approach to developing potential disease-modifying drugs for AD is to inhibit the interactions between Aβ and metals that drive the pathological features of AD, such as amyloid deposition and oxidative stress. For instance: (1) inhibiting Ab:metal interactions by selectively occupying the metal binding site on Aβ, thus preventing metal coordination; (2) to identify a class of molecules that will effectively compete with the interaction Aβ-metal ions. A logical approach is the use of metal chelators (desferrioxime, penicillamine and trientine), which have been used clinically for decades to target metal in diseases such as hemochromatosis (iron) and Wilson's disease (copper). However, these types of chelators are inappropriate as potential therapeutics for AD due to the requirement of being able to cross the blood–brain barrier (BBB); this generally requires small hydrophobic molecules (Hung and Barnham 2012).

The development of metal regulators as drugs to restore metal homeostasis in AD brains is still at its very early stage compared with other pharmacological approaches. The copper chelation is one of the alternatives as a treatment for this disease. There are several chelator specific for copper (for more details see Robert et al. 2015), among them:

(1) Pyrrolidine Dithiocarbamate (PDTC). PDTC is capable of transferring external Cu^2 into a cell to rescue in vitro hippocampal neurons from the toxicity of Aβ oligomers and to reduce tau phosphorylation in the hippocampus of transgenic APP/PS1 mice.
(2) Pyridine derivatives such as: (a) 2-methylaminopyridine derivative, that was shown to chelate Cu^2 and Zn^2. In vitro, was able to control Cu- or Zn-induced Aβ aggregation and to modify the structure of metal-induced Aβ aggregates,

possibly via a ternary 2-methylaminopyridine–Zn–Aβ complex; (b) Dipyridine derivative: ENDIP forms a highly stable tetradentate 1/1 complex at physiological pH with Cu^2 and Zn^2. This compound is able to displace Cu^2 from Aβ and thus to prevent the metal-induced amyloid aggregation and resolubilize amyloid precipitates.

(3) Reduced Schiff Base Derivatives. These compounds containing a reduced salen structure, were proposed to retrieve Cu^2 from Cu–Aβ, to prevent Aβ aggregation, and to act as antioxidants.

(4) Bis(hydrazide). In an Aβ overexpressing Drosophila transgenic model of AD, treatment with Bis(hydrazide) compound reduces the Cu-induced retinal neurotoxicity.

(5) Bis(thiosemicarbazones) (BTSC). Cu^2-(BTSC) complexes are stable with a square planar N_2S_2 geometry and are capable of crossing cell membranes.

(6) Peptides. A dodeca-peptide prochelator was designed to be enzymatically activated by β-secretase to yield a chelator able to extract copper from Aβ and to protect against copper-induced ROS formation.

(7) Multifunctional Ligands. In order to improve efficiency and selectivity, ligands able to chelate metal ions and to interact with other Alzheimer's disease targets have been proposed. For example, several ligands able to interact with Aβ, or to inhibit monoamine oxidase, AChE or BuChE.

(8) Clioquinol, PBT2, and Other 8-Hydroxyquinolines. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, is a nonspecific copper–zinc chelator, able to decrease Aβ deposits and to improve learning and memory capacities of APP transgenic mice. Unfortunately, clioquinol, induced subacute myelo-optic neuropathy and was withdrawn from the market.

The association of these diseases with transition metals is characterized by oxidative damage to lipids, proteins and nucleic acids. Oxidative damage in the brain also, could result from interactions between Cu and homocysteine, a thiol-containing amino acid. The toxicity of homocysteine/Cu co-incubation is dependent on the ability of homocysteine to reduce Cu^2, as indicated by the inhibition of toxicity with the Cu specific chelator bathocuproine disulphonate. Homocysteine, also generates high levels of H_2O_2 in the presence of Cu^2 and promotes amyloid b protein/Cu mediated H_2O_2 production and neurotoxicity. These findings suggest that increased Cu and/or homocysteine levels in the elderly could promote significant oxidative damage to neurons and may produce AD or related neurodegenerative conditions. Metal chelating agents such as ammonium tetrathiomolybdate have been shown to prevent neuronal death of rats caused by intra-hippocampal injections of cupric sulfate, ferric citrate, and zinc chloride. The toxic effects of Cu^2, Fe^3 and Zn^2 are prevented by ethylene-diaminetetraacetic acid ($CaNa_2EDTA$). Also, disodium bathocuproine disulphonate prevents neuronal death caused by Cu^2 but not Fe^3 or Zn^2, while desferrioxamine increases neuronal death caused by Cu^2. Chelating agents are recommended in severe poisoning, but little pharmacokinetic data on humans exist to guide their use. Either intravenous $CaNa_2EDTA$ or intramuscular trientine is the agent of choice in a severe ingestion. D-Penicillamine may be administered orally, if toler-

ated, to non-penicillin-allergic patients; however, case reports have not documented the effectiveness of these chelating agents following the ingestion of large doses of copper (Barceloux 1999).

Neurodegenerative conditions may also be influenced by the interaction between Cu and dopamine. In a rat substantia nigra neuronal cell line, Cu neurotoxicity was dependent on dopamine-mediated Cu uptake and one-electron reduction of amino-chrome. This result suggest that Cu neurotoxicity depends upon the formation of Cu dopamine complexes with concomitant dopamine oxidation to amino-chrome. Salsolinol (SAL), a novel dopaminergic catechol tetrahydroisoquinoline neurotoxin, may contribute to the etiology of Parkinson's disease and neuropathology of chronic alcoholism. The viability of rat pheochromocytoma (PC12) cells reduced by SAL treatment is exacerbated by Cu^2, while Cu chelator bathocupro-inedisulfonic acid ameliorates the cytotoxicity. SAL in combination with Cu^2 also induces strand scission in pBR322 and phiX174 supercoiled DNA, and causes hydroxylation of salicylic acid to produce 2,3- and 2,5-dihydroxybenzoic acids. Reaction of calf thymus DNA with SAL and Cu^2 results in substantial oxidative DNA damage as determined by 8-OH-dG formation. As the neurotoxic properties of SAL are inhibited by GSH and catalase, it is suggestive that SAL undergoes redox cycling in the presence of Cu^2 with concomitant production of ROS, particularly hydroxyl radicals, which contribute to DNA damage and cytotoxicity (Jung and Surh 2001).

A number of nutrients may interact with Cu and alter its toxicity. Information available shows that vitamin E is largely protective against Cu induced oxidative damage. Results obtained from in vitro and cell culture studies generally support the view that ascorbic acid is capable of catalyzing the initiation of oxidative damage by Cu. However, results obtained from available in vivo studies suggest that the compound is protective. High dietary ascorbic acid and Zn may protect Cu toxicity by reducing its intake. Se, beta-carotene, alpha-lipoic acid and polyphenols may also afford some protection against Cu induced oxidative damage. Further research is needed to better understand the cellular effects of Cu and its functional interaction with other nutrients.

Cu would also cause marked disruption of the endocrine system (Astiz et al. 2009). One of the mechanisms of Cu ions to interfere with the normal functioning of the endocrine system is shifting from its natural positions to other metals that act as cofactors, thus modifying the functionality of many transporter, receptor and enzymatic proteins. This occurs for example with the structure of the so-called Zn fingers that has the estrogen receptor protein and other steroids to bind to the acceptor site on the DNA. When there is an increase of Cu in relation to Zn, the latter is replaced by Cu causing the receptor to lose its specific function or modify it. In addition to the mechanisms mentioned above, it has been known for many years that copper has effects as an immuno-suppressant (Wataha et al. 2002).

2.8 New Perspectives in Copper and Ageing

Aging is a complex process influenced by several factors, including Cu. As mentioned in this chapter, the imbalance of this metal conduces to healthy problems in elder population, from nutritional problems and neurodegenerative diseases to cellular damage and molecular malfunctioning. All the showed evidences support the existence of a complex relationship at several levels between Cu and aging, which has led to the use of diverse biological models and the development of new technology to address studies in this field from an integrative and systemic point of view.

2.9 Conclusive Remarks

- Copper is an essential metal for the life of all organisms from fungi and bacteria to the human.
- Because of its redox activity, copper at high intracellular concentrations is potentially toxic to the cell.
- Wilson's disease and Menkes's disease are associated with an alteration in the transport of the metal in the organism.
- There is evidence that strongly associates copper with aging, mainly through the reduction of the ability to combat oxidative stress damage.
- Cu has been implicated in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Dementias with vascular origin, spongiform encephalitis (Creutzfeld-Jakob Disease), and with Huntington's disease, among others.

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Chapter 3
Selenium

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Abstract Selenium, discovered in 1817 by Jöns Jacob Berzelius, is known as an element with two faces in relation to human health: as a micronutrient essential for life and a potentially toxic trace element. Important biological functions of selenium are associated with selenoproteins that contain it in the form of selenocysteine (Sec), known as the 21st amino acid in the genetic code. Human selenoproteome contains 25 selenoproteins, including glutathione peroxidases, thioredoxin reductases and iodothyronine deiodinases. As a component of selenoproteins, selenium participates in defence against oxidative stress, maintenance of cellular redox status, redox signaling and thyroid hormone metabolism. Human selenoproteins are involved in a host of processes and cellular functions such as immune and anti-inflammatory reactions, cell proliferation and apoptosis, thyroid hormone activation and inactivation, fertility mechanisms, and detoxification of harmful substances. Several selenium compounds exhibit protective effects against cancer. Thus, adequate dietary selenium intake is essential for health and protects organism against diseases and age-related disorders. However, selenium can have an adverse effect on health at high exposure due to the narrow margin between the amount that is essential and the levels associated with toxicity.

Keywords Selenium · Selenocysteine · Selenoproteins · Glutathione peroxidase
Iodothyronine deiodinases · Thioredoxin reductases · Antioxidant defence
Immune function · Reproductive health · Cancer prevention · Aging · Longevity

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3.1 Introduction

Selenium (Se), a chemical element of the 16th group of the periodic table, was discovered in 1817 by Jöns Jacob Berzelius who named it after Selene, the Hellenic moon goddess (Berzelius 1818). For a long time, selenium was known only because of its toxicity and was identified as an essential element for mammals in the 1950s (Schwarz and Foltz 1957). Studies conducted in the 1970s have revealed selenium to be an integral part of the enzyme glutathione peroxidase (Rotruck et al. 1973), and subsequently other important selenoproteins such as iodothyronine deiodinases and thioredoxin reductase were discovered (Köhrle 1996; Gladyshev et al. 1996).

Selenium is included in the selenoproteins in the form of selenocysteine (Sec) recognized as the 21st amino acid, whose incorporation into the protein molecule is determined by the UGA codon (Stadtman 1996).

Human selenoproteome contains 25 human selenoproteins, involved in basic cellular processes, from maintaining selenium homeostasis to regulating the overall metabolic rate (Kryukov et al. 2003; Reeves and Hoffmann 2009). A variety of selenoproteins have been also detected in microorganisms, with at least 15 different types of selenoproteins encoded by the bacterial and archeal genomes (Kryukov and Gladyshev 2004). However, selenoprotein genes have not been found in higher plants and yeast (Lobanov et al. 2009).

Numerous animal experiments and clinical observations have shown selenium as an important micronutrient, essential in trace amounts for cellular functions in humans and animals, but toxic at higher concentrations. Endemic diseases associated with both selenium deficiency (Keshan disease and Kashin–Beck disease) and with its excess (selenosis) in some geographic regions of the world have been extensively described (Huang et al. 2013; Kraus 2015). Selenium deficiency has been implicated in a variety of conditions, including suppression of immune function, inflammatory diseases, endocrine system disorders, an impaired reproductive function, cardiac, muscular and skeletal diseases, brain disorders and cancer. Conversely, adequate selenium status is needed to the proper involvement of selenoproteins in vital processes such as defence against oxidative damage, redox signaling, control of cell proliferation and apoptosis, protection against bacterial and viral infection, thyroid hormone activation, fertility and reproduction (Spallholz et al. 1990; Snityns'kyĭ and Antoniak 1994; Arthur 2000; Arnér and Holmgren 2000; Antoniak et al. 2002a, b; Snitynsky et al. 2006; Reeves and Hoffmann 2009). Furthermore, some Se-containing compounds were shown to exhibit a chemopreventive effect against cancer (Rayman 2005; Yamanoshita et al. 2007). In addition, selenium can counteract the destructive effects of many harmful substances, including xenobiotics, natural toxins and heavy metals (Ip and Lisk 1997; Antonyak et al. 2008, 2015; Brigelius-Flohé and Kipp 2013; Antonyak and Skab 2013; Amara et al. 2013; Long et al. 2016; Hoivanovych and Antonyak 2015, 2017; Cao et al. 2017).

Thus, selenium is a trace element with a wide-ranging role in maintaining human health and can be involved in mechanisms related to human longevity. In this aspect, an adequate dietary selenium intake is of great importance. However, many people

in a number of countries may be deficient in this mineral because of the low selenium concentration in the soils or because of poor availability of soil selenium for agricultural plants (Combs 2001; Ivory and Nicoletti 2017). Therefore, selenium biofortification of plant products attracts attention as a means of preventing Se deficiency in humans and farm animals (Schiavon et al. 2016).

On the other hand, in recent decades, selenium has received much attention as a potential contaminant in the human food chain because of its biotransference and biomagnification in the components of aquatic ecosystems (Barwick and Maher 2003; Schneider et al. 2015). Therefore, monitoring the selenium content of foods is necessary to prevent the harmful effect of this element, taking into account environmental contamination by selenium from anthropogenic sources.

3.2 Selenium in the Environment

Selenium is a chalcogen with an atomic number of 34 and an atomic mass of 78.96, which occupies a position between sulfur and tellurium in the periodic table of chemical elements. Selenium exists in several oxidation states (including −2, 0, +4, and +6) that affect its solubility, mobility in the environment and bioavailability. Selenium is found in the environment as inorganic species such as selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), and a range of Se-containing organic compounds. Soluble inorganic selenium forms, the selenite and selenate, predominate in waters and are widely distributed in soils. Biologically and environmentally important organic selenium compounds include selenoamino acids selenocysteine (Sec), selenomethionine (SeMet), selenomethylselenocysteine (SeMeSeCys), the dipeptide γ-glutamyl-selenomethylselenocysteine (γ-glutamyl-SeMeSeCys), and volatile dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) (Martens 2003). Selenocysteine is the principle chemical form of Se in animal and human tissues, where Sec is used in the synthesis of selenoproteins.

The mean concentration of selenium in the earth's crust is 0.083 mg/kg. The highest levels of selenium are found in some types of sedimentary rocks such as shale (up to 675 mg/kg in black shale) and phosphatic rocks (up to 300 mg/kg). Selenium can be detected in coals and other organic-rich deposits at concentration 1–20 mg/kg (Plant et al. 2005). Selenium content of soils varies in wide range, but most soils contain it at concentrations from 0.1 to 2.0 mg/kg (Oldfield 2002). Soils with elevated levels of selenium (>0.5 mg/kg) are considered to be seleniferous, since cultivated crops and fodder plants grown on those soils absorb Se more than the maximum permissible level for human and animal consumption. In some parts of the USA, India and China, these soils contain selenium at concentrations up to 10, 20 and 20–60 mg/kg, respectively (with some exceptionally high levels of more than 100 mg/kg in the soil of Enshi County, China) (Plant et al. 2005; Yuan et al. 2012). The extremely high selenium concentration (up to 1200 mg/kg) have been reported in organic-rich soils derived from black shale in Ireland (Plant et al. 2005). In contrast to relatively limited distribution of soils with high selenium levels,

large areas of agricultural soils on a global scale have low selenium concentrations. Selenium-deficient soils have been found in Western Australia, New Zealand, China, eastern part of the Russian Federation, several parts of the USA, Canada, the United Kingdom, Finland, Denmark, and other countries (Oldfield 2002; Fordyce 2013).

Selenium is required as a trace element for vertebrates, algae and bacteria, but it has not been proven to be an essential micronutrient for higher plants. However, all plants are able to absorb selenium from the soil and convert inorganic Se compounds into Se-containing amino acids, which are then nonspecifically inserted into plant protein molecules (Abrahams 2008). Selenomethionine predominates among organic selenium compounds in plant tissues, while several plant species such as garlic, onion, leek, broccoli, and radish can accumulate selenium in the form of selenomethylselenocysteine (Wu et al 2015; Schiavon et al. 2016).

Selenium concentration in vegetation usually reflects the content of the element in soils and can range from 0.005 mg/kg in plants growing in Se-deficient soils to 5500 mg/kg in Se-hyperaccumulator plants growing in seleniferous soils (Plant et al. 2005). Selenium hyperaccumulators, which concentrate the element to toxic levels (i.e., more than 1000 mg/kg) are among the species of the genera *Astragalus*, *Haplopappus*, *Machaeranthera*, *Neptunia*, and *Stanleya* (Plant et al. 2005; Fordyce 2013). Phytoavailability of selenium depends on its chemical form and soil characteristics. Generally, the bioavailability of various forms of selenium in soils can be placed in the following order: selenate > SeMet > Sec > selenite > elemental Se > selenide (Abrahams 2008). Plants and microorganisms can volatilize assimilated selenium into the atmosphere. DMSe and DMDSe are the major gaseous compounds emitted during plant growth and from soil microorganisms in the course of selenate and selenite metabolism (Martens 2003).

In the hydrosphere, selenium levels are low and reach an average of 0.17 μg/L in seawater (Thomson et al. 2001), and 0.2 μg/L in most uncontaminated freshwaters. However, selenium concentrations can be significantly higher (up to the level 650 μg/L) in water bodies receiving irrigation drain water from seleniferous soils or agricultural lands. Selenium accumulates both in aquatic plants and in aquatic animals, and there is evidence of biotransference and biomagnification of this element in aquatic food webs with high final levels in carnivorous fish tissues (Barwick and Maher 2003; Schneider et al. 2015). In freshwater reservoirs contaminated with industrial selenium, a high level of Se accumulation in fish used in human nutrition can pose a risk to human health.

3.3 Selenium in Human and Animal Nutrition

3.3.1 Need for Selenium in Humans

The evidence of the need for selenium as a micronutrient in mammals was obtained in 1957, when it was demonstrated that sodium selenite administration prevented the

development of liver necrosis in rats receiving vitamin E-deficient diets (Schwarz and Foltz 1957). Importance of selenium to human health was clearly demonstrated in the 1970s, when it has been shown that sodium selenite supplementation is an effective means of preventing the Keshan disease, endemic cardiomyopathy in some regions of China (Keshan Disease Research Group 1979).

Another proof of the importance of selenium for health was obtained in 1979 with clinical observation of a patient supported by complete parenteral nutrition who developed a selenium deficiency accompanied by myopathy symptoms that were corrected after selenium supplementation (van Rij et al. 1979).

According to the recommended dietary allowance (RDA), established by the Food and Nutrition Board (FNB) of the US Institute of Medicine (IOM), the consumption of selenium should be as follows: 55 μg/day in persons aged 14–70 years and older; 60 and 70 μg/day in pregnant and lactating women, respectively (IOM 2000). Recommended levels of daily intake of selenium in children depend on age and are of 20, 30 and 40 μg for children 1–3, 4–8 and 9–13 years old respectively. For infants from 0 to 6 months and 7–12 months, adequate intake (AI) of selenium is of 15 and 20 μg per day, respectively (IOM 2000).

In the European Union, the European Food Safety Authority (EFSA) has established a level of adequate selenium intake for adults at 70 μg per day (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2014). Selenium deficiency in humans occurs when daily intake falls below 20 μg (Calello 2010), and 40 μg Se per day is considered the minimum intake in adult persons.

3.3.2 Source of Selenium in Human Diet

The source of selenium for humans is both the foods of animal origin (meat, marine and freshwater fish) and plant foods, such as cereals and other grain products. However, most plant foods consumed by humans are associated with the non-accumulating group of vegetation, which, if grown on non-seleniferous soils, usually contain low selenium levels (<1 mg/kg dry mass (DM)) (Abrahams 2008). Unlike agricultural crops, the seeds of Se-accumulating Brazil nut tree (*Bertholletia excelsa*) contain 8–83 mg Se per kg (Thomson et al. 2008).

Selenium content of beef meat is about 0.2–0.3 mg/kg, whereas its concentrations in heart, liver, and kidney tissues of beef are 0.55, 0.93, and 4.5 mg/kg, respectively; the average content of selenium in chicken meat is ~0.2 mg/kg; Se content of fish ranges from 0.1 to 5.0 mg/kg (Garousi 2017).

Some species of wild-grown edible mushrooms such as representatives of the genera *Albatrellus*, *Boletus*, *Macrolepiota*, *Agaricus* are naturally rich in selenium. Extremely high Se concentrations (up to 370 mg/kg DM) were detected in the rarely consumed *Albatrellus pes-caprae*, while the fruit bodies of *Boletus eduilis* contained up to 70 mg Se per kg DM (Falandysz 2008).

Selenium yeast produced by growing selected strains of *Saccharomyces cerevisiae* in Se-rich media contains 90% of its Se as selenomethionine and is a source of organic form of selenium for farm animals and humans.

3.3.3 Endemic Diseases Related to Selenium Deficiency

Although only a trace amount of selenium is required to maintain important physiological functions in the human body, it has been postulated that the vast majority of the world's population has suboptimal Se intakes (Haug et al. 2007). Between 0.5 and 1 billion people suffer from selenium deficiency, because their Se consumption is lower than the recommended intake levels for this microelement (Combs 2001). Inadequate or only marginally adequate dietary selenium intakes have been reported in many regions of the world, especially in countries where selenium content of soils is low or where soil selenium is poorly available for food crops due to soil features (Oldfield 2002). The lowest selenium consumption (7–11 μg/day) has been registered in parts of China characterized with extremely low selenium content of soils (Yuan et al. 2012). Two major endemic pathologies resulting from severe selenium deficiency in humans have been documented: Keshan disease (endemic cardiomyopathy, which got its name from a serious outbreak in Keshan County, China in 1935) and Kaschin Beck disease (osteoarthropathy), endemic in some regions of China and adjacent territories.

Keshan disease occurs in a wide geographic belt stretching from Heilongjiang Province in the northeast China to Yunnan Province in the southwest and primarily affects children under 15 years of age and young women. The disease manifests itself as an acute insufficiency of the heart function or as a chronic heart enlargement and is potentially fatal. Histopathologically, Keshan disease is characterized by multifocal necrosis and replacement fibrosis of the myocardium, which leads to acute or chronic heart failure. During 1974–1977, successful trials with the giving of sodium selenite (Na_2SeO_3) tablets to over 8000 children in the endemic regions of China have provided evidence of the efficacy of selenium in preventing the disease (Keshan Disease Research Group 1979). Although Keshan disease is closely related to poor selenium nutritional status, it has been shown that Coxsackie virus is a cofactor in the disease (Beck 1999).

Kashin-Beck disease (KBD) is a severe, progressive osteoarthropathy, endemic in a region extending from north-eastern China to Tibet in the southwest, with additional endemic regions in neighbouring areas of Russia and some regions of Vietnam and Korea (Kraus 2015). The disease starts in childhood and is characterized by chronic disabling degenerative osteoarthrosis affecting the peripheral joints and the spine with apoptosis of the cells of hyaline cartilage tissue (Yang et al. 2017). Impairment of movement in the extremities is followed by bone development disturbances such as shortened fingers and toes and in more extreme cases, dwarfism. In addition to selenium deficiency, other factors, such as iodine deficiency, contamination of grain with mycotoxin-producing fungi, and pollution of drinking water with humic

substances, have been convincingly associated with Kashin-Beck disease (Kraus 2015).

In farm animals, selenium deficiency induces poor growth, decreases reproductive fertility and causes pathological states such as nutritional muscular dystrophy (white muscle disease) in calves and lambs; stillbirths and retained placenta in ruminants; nutritional myopathy and mulberry heart disease in pigs (Koller and Exon 1986; Vlizlo et al. 2006).

3.4 Metabolism of Selenium in the Organism

3.4.1 Selenium Absorption, Distribution in the Tissues and Excretion

Selenium is present in foods in the form of seleno-amino acids and as inorganic compounds (selenate and selenite), with selenomethionine being the dominant form of dietary selenium. Selenomethionine is synthesized by plants and can be consumed by humans in plant and animal proteins that contain non-specifically incorporated SeMet. Another Se-amino acid, selenocysteine is consumed mainly in animal-derived selenoproteins. Dietary Se is readily absorbed primarily in the duodenum, but also in the caecum and colon (Bates 2009). In healthy humans, SeMet, selenate and, presumably, Sec are absorbed almost completely, while the level of selenite absorption generally exceeds 50%. Selenomethionine is absorbed via the gut methionine (Met) transporter, and Sec is taken up probably via the cysteine (Cys) transporter (Bates 2009). Selenate is considered to be transported by a sodium-mediated carrier mechanism shared with sulfur, whereas selenite is passively absorbed through the intestinal wall (Wastney et al. 2011).

After absorption, SeMet can be incorporated non-specifically into a variety of proteins in place of methionine and is present in Met-containing proteins in blood and tissues throughout the body (Burk and Levander 2005). This process is not regulated by selenium status, but depends on the availability of SeMet. However, SeMet is not available for the synthesis of functional selenoproteins until it is catabolised and converted to selenocysteine by transsulfuration pathway in the liver or kidney. Selenium then enters the regulated selenium metabolic pool and can be incorporated into selenoproteins, transported to other organs, or excreted.

Selenocysteine, taken up by the cell or derived from the degradation of intracellular selenoproteins, is catabolised by selenocysteine β-lyase with liberation of the reduced form of selenium, selenide. Ingested selenate and selenite are also metabolized to selenide. Selenide can enter the anabolic pathway by enzymatic conversion to selenophosphate (a precursor of selenocysteine in the synthesis of selenoproteins), can be transformed to an excretion form, or can be modified for transport out of the cell. Metabolism of selenide is considered to be the likely point of homeostatic regulation of selenium in the cell (Sunde 2012).

The distribution of selenium between organs and tissues is adapted to ensure the maintenance of important Se-dependent functions. At normal dietary levels, the highest selenium concentrations are found in the liver and kidney, followed by spleen, pancreas, heart, brain, lung, bone and skeletal muscle. When considering total body mass, 25–50% of body selenium was found in skeletal muscle, 16% in bone, 7–10% in blood and 4% in kidney (Zachara et al. 2001).

Homeostasis of selenium in the body is achieved through regulation of its excretion (Burk and Levander 2005). Most of the selenium is excreted by the kidneys (60%); intestinal excretion of selenium is about 35 and 5% is excreted in sweat or saliva. Under physiological conditions, Se excretion in urine is the main means of regulating the selenium content. At very high selenium intakes, the volatile Se compounds are exhaled, and the breath becomes a significant route of selenium excretion (Sunde 2012).

Most excretory metabolites of selenium have been characterized as methylated forms produced in the liver or kidneys. The major methylated Se metabolites are dimethylselenide, which is excreted through the respiratory system and sweat, especially at high levels of selenium intake (this compound is responsible for the garlic odour of persons exposed to excess selenium), and the trimethylselenonium ion (Me_3Se^+, TMSe), which is excreted in the urine. Under conditions of deficient to adequate selenium intakes, a large portion of urinary Se is present as methylated selenosugars (Francesconi and Pannier 2004).

3.4.2 Synthesis of Selenoproteins

The main biological functions of selenium in vertebrates are attributed to its biochemical effects mediated by the spectrum of selenoproteins, all of which contain selenium in the form of selenocysteine (Stadtman 1996). Synthesis of selenoproteins depends on the translational recoding of the UGA codon in mRNA (which usually acts as a stop codon during translation) to allow Sec incorporation into protein primary structure. Only proteins that are genetically programmed and perform essential biological functions are classified as selenoproteins (Hatfield and Gladyshev 2002). Although human and animal proteins also contain the non-specifically inserted SeMet, they are not considered selenoproteins. The nonspecific incorporation of amino acids SeMet or Met is directed by AUG codon and no significant distinctions in the biochemical functions have been observed.

Selenocysteine is an analogue of cysteine with a Se-containing selenol group in place of the sulfur-containing thiol group in Cys, but is more active than Cys because of a lower *pKa* value and a stronger nucleophilicity. Selenocysteine was identified as the 21st amino acid in the 1980s, when it has been demonstrated that Sec incorporation into proteins is genetically encoded (Lee et al. 1989).

The biosynthesis of selenocysteine and its cotranslational incorporation into selenoproteins are highly regulated. Unlike other amino acids, Sec is synthesized in a specific way on its transfer RNA, $tRNA^{[Ser]Sec}$, which possesses the anticodon for

UGA (Lee et al. 1989; Stadtman 1996). During this process, $tRNA^{[Ser]Sec}$ is initially aminoacylated with serine, then phosphorylated to form phosphoseryl-$tRNA^{[Ser]Sec}$, and converted to selenocysteyl-$tRNA^{[Ser]Sec}$. The carbon skeleton for selenocysteine is provided by serine, whereas selenophosphate ($SePO_3$) produced during selenide metabolism is a selenium donor compound. Process of sec-$tRNA^{[Ser]Sec}$ synthesis in mammals involves several enzymes, including phosphoseryl-$tRNA^{[Ser]Sec}$ kinase, pyridoxal-phosphate-dependent Sec synthase, and selenophosphate synthetase 2 (SPS2), which itself is a selenoenzyme (Squires and Berry 2008).

The insertion of Sec into selenoprotein primary structure is determined by the presence of UGA in the open reading frame of selenoprotein mRNA and a stem-loop structure, known as the SECIS (selenocysteine insertion sequence) element, in the 3′-untranslated region (3′-UTR). Absence of the SECIS element or its modification causes the UGA to function instead as a termination codon. In eukaryotes, two specific protein factors facilitate the Sec insertion at the UGA: the selenocysteine binding protein 2 (SBP2), which binds to the SECIS element, and elongation factor for selenocysteine (eEFsec), which binds to the sec-$tRNA^{[ser]sec}$ (Driscoll and Copeland, 2003). These proteins associate to each other and form a complex that delivers the sec-$tRNA^{[ser]sec}$ to the ribosome to incorporate Sec into the growing polypeptide chain. Several additional factors with roles in the ribosomal complex are also important for Sec incorporation into the peptide backbone of selenoproteins (Squires and Berry 2008).

The process of selenocysteine synthesis in mammals is essential for life, since the deletion of the gene encoding the selenocysteine tRNA in mice results in embryonic lethality (Bösl et al. 1997). In contrast to other amino acids, Sec is not recycled for reincorporation into new proteins but is, instead, degraded to release inorganic selenium which can be utilized for resynthesis of selenocysteine.

3.5 The Major Selenoproteins and Their Functions

The human genome contains 25 genes that encode selenoproteins, and the genome of rodents contains 24 selenoprotein-encoding genes (Kryukov et al. 2003). Among human and animal selenoproteins, glutathione peroxidases (GPXs), thioredoxin reductases (TrxRs) and iodothyronine deiodinases (DIOs) are the three most characterized families, while nearly half of the identified mammalian selenoproteins have not yet been sufficiently studied. Selenoproteins exhibit diverse patterns of tissue distribution and have the broad spectrum of biological functions such as antioxidant defence (GPX1–GPX4 and GPX6; selenoproteins K, R, W), redox signaling (TrxR1–TrxR3), thyroid hormone deiodination (DIO1–DIO3), selenocysteine synthesis (SPS2), transport and storage of selenium (selenoprotein P), and (potentially) protein folding (15 kDa selenoprotein (Sep15); selenoproteins N, M, and S) (Papp et al. 2007). Mammalian selenoproteins can be classified mainly into two groups according to the location of Sec. One group of selenoproteins possesses Sec in a site close to the C terminus of protein (TrxRs and selenoproteins S, R, O, I, and K),

while the other group (including GPXs, DIOs, selenoproteins H, M, N, T, V, and W, Sep15, and SPS2) has Sec in the N-terminal part and in most cases possesses thioredoxin fold structure (Lu and Holmgren 2009). Transcription of several selenoproteins (such as TrxR1 and GPX2) is regulated by the redox-sensitive transcription factor Nrf2/Keap1 system (Banning et al. 2005). The features of most important selenoproteins are described below.

3.5.1 Glutathione Peroxidases

Glutathione peroxidases represent a family of enzymes that catalyze the reduction of hydroperoxides (hydrogen peroxide (H_2O_2) and/or organic hydroperoxides) using reducing equivalents from glutathione (GSH) and thus play a key role in defence against oxidative stress (Arthur 2000). In humans, five Se-containing GPXs, all separate gene products, have been identified, including cellular (cytosolic) GPX1, gastrointestinal GPX2, plasma GPX3, its close homolog GPX6, and phospholipid hydroperoxide GPX4 (Kryukov et al. 2003; Lobanov et al. 2009).

GPX1

GPX1, one of the most abundant members of the GPX family, was the first selenoprotein identified in mammals (Rotruck et al. 1973). GPX1 is a homotetramer of ~23 kDa subunits, each containing a single Sec residue. The enzyme is expressed in all cell types and is localized in cytosolic, mitochondrial, and, in some cells, in peroxisomal compartments. GPX1 has substrate specificity to hydrogen peroxide and has been considered to be the major H_2O_2-reducing enzyme. However, GPX1 can also reduce lipid hydroperoxides and other soluble hydroperoxides after their release from membrane lipids (Arthur 2000; Lubos et al. 2011). GPX1 uses the reduced glutathione as an obligate co-substrate in the reduction of H_2O_2, so its activity is often discussed in parallel with the activity of the NADPH-dependent glutathione reductase, which supplies GSH for GPX1 function (Snityns'kyĭ et al. 1996; Antoniak 1998, 2000; Antoniak et al. 1999; Babych et al. 2000c; Yang et al. 2015). GPX1 accounts for 58% of total selenium in liver (Cheng et al. 1997) and is highly sensitive to dietary selenium intake. In the brain, GPX1 can play a role in protecting against neurodegenerative diseases (such as Parkinson's disease and dementia) (Power and Blumbergs 2009).

GPX2

GPX2, originally designated as gastrointestinal GPX (GPX-GI), occurs in the cell cytosol and has tetrameric structure with one Sec residue per unit, but is mainly expressed in the epithelium of gastrointestinal tract (Chu et al. 1997). The enzyme is highly expressed in rodent small intestine, whereas in humans it is also found in the liver, large intestine and several other cells. Similarly to GPX1, GPX2 reduces H_2O_2 and fatty acid hydroperoxides, but not phospholipid hydroperoxides (Arthur 2000). GPX2 was found to affect apoptosis and regulate self-renewal of the intestinal epithelium (Banning et al. 2012); this selenoprotein has also been implicated in the

control of inflammation and malignant growth. GPX2 is upregulated in colon and skin cancers and in certain cultured cancer cells. GPX2 is a target for Nrf2, and thus is part of the adaptive response (Brigelius-Flohé 2006).

GPX3

A secreted form of GPX, GPX3 is present in plasma and milk and can act as extracellular antioxidant enzyme. GPX3 activity, being very sensitive to the dietary supply of selenium, is often used as biomarker of nutritional status of this micronutrient (Combs 2015). GPX3 mRNA is found mainly in the kidney (in particular, proximal tubule epithelial cells), which is considered to be the source of GPX3 (Avissar et al. 1994). Several other cell types, including heart, lung, placenta, gastrointestinal cells, thyroid, liver, mammary gland, and white adipose tissue were shown to express GPX3 mRNA and protein (Arthur 2000; Schmutzler et al. 2007). GPX3 in this context can serve as a local source of extracellular antioxidant capacity. In the heart, GPX3 mRNA is the third most abundant selenoprotein mRNA detected; levels of GPX3 in this tissue may have a role in protecting against oxidative damage to extracellular matrix (Reeves and Hoffmann 2009). In the thyroid gland GPX3 likely serves to reduce oxidative stress (Schmutzler et al. 2007). GPx3 has been shown to be upregulated by peroxisome proliferator-activated receptor (PPAR)-induced antioxidant responses in human skeletal muscles, suggesting a role for this selenoenzyme in regulating extracellular oxidative stress that affects insulin resistance (Chung et al. 2009).

GPX4

Phospholipid hydroperoxide GPX4 is a multifunctional selenoprotein expressed in a variety of tissues (Conrad et al. 2007). GPX4 is expressed as mitochondrial, cytosolic, and nuclear forms that originate from a single gene (Ufer et al. 2008). Expression of GPX4 forms is regulated at the transcriptional level. The cytosolic form (c-GPX4) is expressed at moderate levels in most mammalian cells, whereas the mitochondrial (m-GPX4) and nuclear (n-GPX4) forms are found in large quantities in spermatoid cells (Tramer et al. 2002; Ufer et al. 2008). Unlike other GPXs, GPX4 is a monomer that can reduce phospholipid hydroperoxides, but it also catalyzes the reduction of other lipid hydroperoxides and hydrogen peroxide. GPX4 reduces complex lipid hydroperoxides even if they are incorporated in biomembranes or lipoproteins and can use a wide range of reducing substrates as well as glutathione. Depletion of GPX4 induces lipid peroxidation-dependent cell death (Imai et al. 2017). GPX4 specifically interferes with NF-κB activation by interleukin-1, reduces leukotriene and prostanoid biosynthesis, prevents COX-2 expression, and is indispensable for sperm maturation and embryogenesis (Ursini et al. 1999; Brigelius-Flohé 2006). GPX4 is also involved in the brain function, and its expression is down-regulated in Alzheimer's disease (Yoo et al. 2010). In mice, deletion of the GPX4 gene results in embryonal lethality (Ufer et al. 2008). GPX4 is highly expressed in glioma cells, and its knockdown inhibits the proliferation and migration of tumour cells (Zhao et al. 2017).

GPX6

The GPX6 enzyme is restricted in expression to the developing embryo and olfactory

epithelium in adults (Kryukov et al. 2003). GPX6 is a selenoprotein in humans, but selenocysteine is replaced by cysteine in the mouse enzyme.

3.5.2 Thioredoxin Reductases

Mammalian thioredoxin reductases (TrxRs) are selenocysteine-containing NADPH-dependent flavoenzymes that catalyze the reduction of oxidized thioredoxin (Trx) to a dithiol-containing form Trx–$(SH)_2$, which is a powerful protein disulfide reductase. Besides controlling the function of the central redox molecule thioredoxin, TrxRs can also directly reduce numerous substrates (Arnér and Holmgren 2000; Papp et al. 2007). Three isoforms of mammalian TrxR have been identified: cytosolic/nuclear TrxR1, mitochondrial TrxR2, and testis-specific thioredoxin-glutathione reductase (TGR/TrxR3). TrxR1 and TrxR2 catalyze the reduction of cytosolic and mitochondrial thioredoxins (Trx1 and Trx2, respectively), whereas TrxR3 also possesses glutathione and glutaredoxin reductase activity.

TrxRs together with thioredoxins and NADPH constitute the thioredoxin system, a major redox system involved in vital cellular processes via controlling a redox state of a variety of proteins (including enzymes and transcription factors) and other molecules (Papp et al. 2007; Lee et al. 2013; Lu and Holmgren 2014). Thioredoxin system is involved in the reduction of ribonucleotide reductase (an enzyme essential for DNA synthesis); it can also reduce methionine-sulfoxide reductase and thioredoxin peroxidase (peroxiredoxin) and is thus involved in the repair of methionine sulfoxide-oxidized proteins or redox signaling via hydrogen peroxide (Lu and Holmgren 2009). Thioredoxin system catalyzes the reduction of protein disulfide-isomerase (PDI), the major enzyme that catalyzes protein disulfide formation within the endoplasmic reticulum (ER) (Papp et al. 2007). Additional substrates include two ER proteins, calcium-binding protein 1 and 2 (CaBP1 and CaBP2) involved in calcium metabolism.

The thioredoxin system plays an important role in the regulation of gene expression through redox control of transcription factors (NF-κB, Ref-1, AP-1, and P53), glucocorticoid receptor, and apoptosis signal-regulating kinase 1 (ASK1) (Papp et al. 2007). Consequently, this system is involved in regulation of cellular activities such as cell proliferation, apoptosis, and activation of the immune response. Conversely, TrxR is transcriptionally regulated via an antioxidant-response element (ARE) (Hintze et al. 2003). The redox sensitive Sec residue within TrxR has been suggested to act as a cellular redox sensor and regulator of cell signaling in response to elevated levels of reactive oxygen species (ROS) (Papp et al. 2007).

TrxR catalyzes the reduction of NK-lysin (an antibacterial polypeptide produced by T-lymphocytes) that abolishes its cytolytic activity; thus the enzyme is involved in protecting the cell against NK-lysin cytotoxicity (Andersson et al. 1996). TrxR substrates also include a wide range of small molecules such as lipoic acid, vitamins K and C, alloxan, hydroperoxides, as well as selenium compounds (selenite, selenodiglutathione, methyseleninate, selenocysteine, and ebselen) (Arnér and Holmgren

2000). Some of these selenium compounds (selenodiglutathione and selenite) are metabolized to hydrogen selenide, the precursor of selenophosphate, which is used for the biosynthesis of selenocysteine; thus TrxR participates in selenium metabolism and have a role in controlling selenoprotein synthesis (Papp et al. 2007).

The thioredoxin system is essential for mammalian development, as evidenced by the early embryonic lethality of mice lacking Trx, TrxR1, or TrxR2 (Conrad et al. 2004). It has been shown that both TrxR1 and TrxR2 are involved in embryogenesis; however, TrxR2 is essential for hematopoiesis, heart development, and heart function, whereas TrxR1 controls developmental aspects of embryogenesis (Conrad et al. 2004). Components of thioredoxin system (Trx and TrxR1) are overexpressed in many malignant cells, which is associated with increased cell proliferation (Papp et al. 2007).

3.5.3 Iodothyronine Deiodinases

Iodothyronine deiodinases catalyze the oxidation/reduction reactions of deiodination of iodothyronines, thereby playing an important role in maintaining systemic and local homeostasis of thyroid hormones (TH): thyroxine (T_4) and triiodothyronine (T_3). The iodothyronine deiodinase family includes three enzymes: iodothyronine 5′-deiodinases type 1 (D1) and type 2 (D2) and iodothyronine 5-deiodinase type 3 (D3), encoded by three distinct genes *DIO1*, *DIO2*, and *DIO3*, respectively (Leonard 1990; Larsen and Berry (1995); Köhrle 1996; Gereben et al. 2008; Bianco 2011). The D2 and D3 enzymes are selective, that is, D2 only catalyzes outer ring deiodinataion (ORD), and D3 only catalyzes inner ring deiodinataion (IRD), while D1 is a non-selective enzyme and catalyzes both outer and inner ring deiodinataion of iodothyronines.

The deiodination process, depending on whether it occurs at the 5′- or 5-position on the iodothyronine molecule, is involved in thyroid hormone activation or inactivation. The 5′-deiodination (ORD) catalyzed by D1 and D2 leads to conversion of the prohormone thyroxine to the biologically active T_3, while 5-deiodination (IRD) catalyzed by D3 (and by D1 under certain conditions) converts T_4 to reverse T_3 (rT_3), an inactive metabolite. D3 also inactivates T_3 to 3,3′-diiodothyronine (3,3′-T_2), terminating thyroid hormone action (Köhrle 1996; Bianco 2011).

The first mammalian iodothyronine deiodinase, rat D1, was cloned and identified as a Sec-containing protein in 1991 (Berry et al. 1991). Subsequently, the D2 and D3 were also shown to belong to selenoproteins. The Sec residue in the catalytic centre plays an essential role in deiodinase activity. The three types of deiodinases show considerable similarity in their structure (~50% sequence identity) and belong to the thioredoxin fold superfamily (Callebaut et al. 2003). All three deiodinases form homodimers through disulfide bridges, but only one monomer partner is required for catalytic activity. The enzymes differ in their preference for different iodothyronines as substrates, sensitivity to inhibitors (such as 6-*n*-propyl-2-thiouracil) and expression level in tissues (Leonard 1990). While D1 and D3 are long-lived plasma

membrane proteins ($t_{1/2}$ 10–12 h), D2 is an endoplasmic reticulum resident protein with a half-life of ~40 min, which can be further decreased by exposure to physiological concentrations of its substrate, T_4, and in experimental conditions, rT_3 or high concentrations of T_3 (Gereben et al. 2008). This D2 inactivating mechanism is mediated by selective conjugation to ubiquitin; however, enzyme activity is restored after deubiquitination (Bianco 2011).

Iodothyronine deiodinases are critical for a number of cell systems, both during development and in adult vertebrates, by controlling processes of activation and inactivation of thyroid hormones; expression and activities of DIOs in cells is regulated by several hormones, including thyroid hormone, selenium, cytokines and several other factors (Larsen and Berry (1995); Köhrle 1996; Babych et al. 1998, 1999, 2000a, b; Antonyak et al. 2002a, b; Köhrle et al. 2005; Bianco 2011; Forrest and Visser 2013). These mechanisms provide an optimized control of thyroid hormone action on a cell-specific basis. In addition, deiodinase pathways also modulate thyroid hormone signaling in disease states, generally affecting the occupancy of thyroid hormone receptors and the transcription of T_3-responsive genes.

Altered expression and activity levels of DIOs have been reported in a number of tumors and cancer cell lines, suggesting a potential involvement of DIOs in cancer development, in particular in the thyroid (de Souza Meyer et al. 2005).

3.5.4 Selenophosphate Synthethase 2

Selenophosphate synthethase 2 (SPS2) in mammals is a selenoenzyme involved in the synthesis of selenoproteins by catalyzing the formation of selenophosphate from selenide and ATP. The reaction product, selenophosphate, is used as selenium donor in the synthesis of selenocysteine on sec-$tRNA^{[ser]sec}$ (Low et al. 1995).

3.5.5 Selenoprotein P

Selenoprotein P (SEPP1) is a secreted glycoprotein that contains about 50% of the plasma selenium (Burk and Hill 2009). SEPP1 differs from other selenoproteins in that it possesses 7–15 selenocysteine residues depending on species (e.g., 10 Sec residues in the human SEPP1), and the SEPP1 mRNA has two functional SECIS elements in its 3′-UTR (Turanov et al. 2015). The protein is composed of an N-terminal thioredoxin domain containing one Sec residue and a C-terminal Sec-rich region containing from 6 to 14 Sec residues (Lobanov et al. 2008). SEPP1 is synthesized primarily in hepatocytes and is secreted to the plasma in a glycosylated form; this selenoprotein is also expressed in other tissues and is presumably secreted by them (Burk and Hill 2009). Concentration of SEPP1 in plasma declines in conditions of Se deficiency and can be used as a biomarker of selenium nutritional status. Changes in the selenium intake can be reflected not only in the plasma SEPP1 level, but also in the content of Sec residues in its molecule (Turanov et al. 2015).

SEPP1 delivers selenium in the form of Sec to other organs and thus serves a key role in homeostasis and distribution of selenium in the body. Receptors such as ApoER2 and megalin, members of the lipoprotein receptor family, support the uptake of SEPP1 into tissues. ApoER2 facilitates SEPP1 uptake into the testes and brain, while megalin facilitates uptake of filtered SEPP1 into proximal tubule cells of the kidney (Burk and Hill 2009). SEPP1 may also have antioxidant functions and protects astrocytes and endothelial cells from oxidative damage. In animal model, deletion of the SEPP1-encoding gene causes an increase in Se excretion in the urine and, as a consequence, a decrease in the whole-body selenium content.

*3.5.6 Methionine-*R*-Sulfoxide Reductase (Selenoprotein R)*

Methionine-*R*-sulfoxide reductase (Msr) B1 (MsrB1), also known as selenoprotein R, is a member of the Msr family of proteins, which catalyze the reduction of oxidized methionine residues (methionine sulfoxides) (Papp et al. 2007). Oxidation of Met occurs in response to an increase in ROS and can lead to protein damage. MsrB1 also binds zinc and is localized in the cell nucleus and cytoplasm (Kim and Gladyshev 2004). It is considered that MsrB1 can potentially mediate the anti-aging properties of selenium (Papp et al. 2007). MsrB1 may also play an important role in neurologic conditions.

3.5.7 Effects of Dietary Selenium on Selenoprotein Levels

Expression of selenoproteins in tissues is controlled by the level of dietary selenium and exhibits a hierarchical style during selenium deprivation and repletion. In conditions of selenium deficiency, the significance of specific selenoproteins in specific tissues may determine the priority of their mRNA and protein expression. When selenium is limited, expression of certain selenoproteins is terminated by nonsense-mediated decay of selenoprotein mRNA and a CRL2 ubiquitin ligase-mediated degradation (Seyedali and Berry 2014; Lin et al. 2015).

The brain and endocrine tissues are preferentially supplied with selenium, thus, selenoprotein levels in the brain, testes and thyroid gland are refractory to dietary selenium deficiency. Selenoproteins expressed in the liver and kidneys are generally more sensitive to dietary selenium fluctuation and significantly decrease under selenium-deficient conditions (Allan et al. 1999; Lu and Holmgren 2009).

3.6 Biological Functions of Selenium in Humans and Animals

Given the wide range of specific functions performed by selenoproteins, it is obvious that selenium deficiency can lead to various physiological disorders and pathological conditions. Severe prolonged selenium deficiency can lead to fatal cardiomyopathy (Keshan disease), as observed in endemic regions with very low selenium content of soils. Selenium deficiency can also lead to skeletal myopathy, observed in both animals (white muscular disease) and in people who are nourished by total parenteral nutrition (Ishihara et al. 1999). Severe selenium deficiency is important contributing factor in Kashin-Beck disease (Kraus 2015).

Less-overt forms of selenium deficiency are more frequently observed, and various clinical disorders such as cystic fibrosis, rheumatoid arthritis, chronic liver disease, viral infection, cancer, systemic inflammatory response syndrome and sepsis exhibit low plasma levels of selenium (Lockitch 1989; Rayman 2012). This indicates that low selenium status can predispose to a number of diseases. Conversely, adequate selenium intake is required for normal functioning of the immune, endocrine, reproductive, cardiovascular, digestive, musculoskeletal and central nervous systems (Alissa et al. 2008; Boitani and Puglisi 2008; Mistry et al. 2012; Huang et al. 2012). In addition, selenium has protective role in several types of cancer and some selenium compounds exhibit anticarcinogenic effect (Rayman 2005, 2012). Selenium also synergizes the action of vitamin E and facilitates metabolism of many xenobiotics and natural toxins; it also has a protective role against heavy metal toxicities (Fig. 3.1). However, it has been shown that there is a U-shaped relationship between selenium concentration in the blood and the risk of disease, with possible harm occurring both below and above the physiological range for optimal activity of some or all selenoproteins (Rayman 2012). Therefore, a high intake of selenium in persons without a proven deficiency may have adverse effects (such as hyperglycaemia and atherosclerosis) (Stranges et al. 2010).

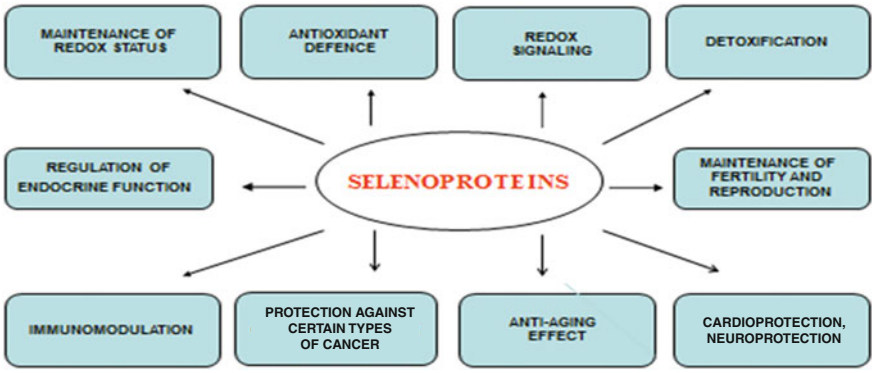

Fig. 3.1 Functions of selenoproteins in human organism

3.6.1 Selenium and the Immune Function

The immune system represents one of most selenium-sensitive target systems. Numerous studies suggest the association of selenium status with humoral and cell-mediated immune responses, survival odds in sepsis, risk of autoimmune thyroid diseases, selenium-dependent infection rates and vaccination responses (Hoffmann and Berry 2008; Rayman 2012; Ivory et al. 2017). The organs of the immune system such as spleen and lymph nodes accumulate significant amounts of selenium, and selenoproteins expressed in immune cells determine the role of this trace element in immunity (Kiremidjian-Schumacher et al. 1994; Babych et al. 1998, 1999, 2000a, b; Antonyak et al. 2002a; Snitynsky et al. 2006; Shrimali et al. 2008; Sun et al. 2017). For example, inflammatory response in the pig involves changes in the mRNA expression of 14, 16, 10, and 6 selenoprotein genes in the liver, spleen, thymus, and lymph node, respectively (Sun et al. 2017).

Selenium deficiency significantly affects the immune system and leads to impairment of T- and B-cell functions and less robust immune responses to viruses, tumours, and allergens, compared to Se-adequate controls (Spallholz et al. 1990; Hoffmann and Berry 2008). It has been shown that selenium supplementation, even in individuals without deficit of this micronutrient, has significant immune stimulatory effects, including an enhancement of activated T-cell proliferation, increased lymphocyte-mediated tumour cytotoxicity, and NK cell activity (Rayman 2000, 2012). In particular, supplementation with 100 μg Se per day (as selenium yeast) for 6 months in older Belgian residents significantly increased the proliferative response of lymphocytes to antigen challenge (Rayman 2000). Selenium supplementation increases the expression of the high affinity receptor for interleukin-2 (IL-2) in human lymphocytes, which is important for T-cell proliferation. In animal studies, the high selenium diet increased the expression of IL-2 and the high affinity chain of the IL-2 receptor accompanied by enhanced T-cell signalling and in vivo $CD4^+$ T-cell responses. Increasing dietary Se leads to a shift in the T helper (Th)1/Th2 balance toward Th1 effector cells. This effect benefits antiviral immune or antitumor responses that depend on robust Th1 immunity (Hoffmann et al. 2010).

3.6.2 Selenium and Viral Infection

Convincing evidence regarding to the involvement of selenium deficiency in viral infection were obtained from studies of Keshan disease, which has been attributed to an endemic Coxsackie virus B3 (CVB3). In animal studies, a normally-benign strain of Coxsackie virus B3 becomes virulent in Se-deficient mice and causes the development of myocarditis in the host. Mechanism of virulence is thought to be due to the accumulation of oxidative damage related mutations in the viral genome (Beck 1999). Thus, selenium deficiency can affect both the immune response of the host organism and the virus itself.

Selenium deficiency has been linked to the incidence, virulence, or disease progression of other viral infections, including human immunodeficiency virus-1 (HIV-1) and influenza virus infections (Stone et al. 2010; Hoffmann and Berry 2008). A number of studies have reported low selenium status in HIV-infected individuals, and some cohort studies have shown an association between selenium deficiency (serum or plasma selenium $\leq$85 μg/L) and progression to AIDS or mortality (Rayman 2000; Stone et al. 2010). Several randomised controlled trials have shown positive effects of selenium supplementation in HIV-infected patients (Rayman 2012).

3.6.3 Selenium and Thyroid Function

Selenium together with iodine plays a key role in the physiology of the thyroid gland and is necessary for thyroid hormone metabolism. The thyroid gland is characterized by high concentration of selenium incorporated into selenoproteins. At least 11 selenoproteins are expressed in thyrocytes (Schmutzler et al. 2007), including GPXs that contribute to the antioxidant defence of the thyroid by removing H_2O_2 generated during hormonogenesis, TrxRs that maintain the redox balance in the thyroid and perform redox control of thyrocyte functions, and DIOs involved in metabolism of thyroid hormones.

Selenium deficiency can be associated with an increased risk of thyroid disease, especially in persons with inadequate iodine intake. Combined selenium and iodine deficiency leads to severe pathologic conditions, including myxedematous cretinism prevalent in Central Africa (Köhrle et al. 2005). Decreased activity of DIOs and GPX are both considered to be responsible for the development of this disease, acting through increased oxidative damage in the thyroid tissue due to insufficient GPX and reduced thyroid hormone metabolism due to insufficient DIOs. Selenium deficiency may also be responsible for the initiation of autoimmune thyroid disorders (Köhrle et al. 2005). Selenium supplementation can be beneficial in thyroid diseases, including autoimmune thyroid disease and thyroid associated ophthalmopathy (Marinò et al. 2017; Ventura et al. 2017). Supplemental selenium also alleviates the harmful effects of excess iodine on thyroid gland.

3.6.4 Selenium and Brain Function

Selenium is a trace element crucial to brain functions. Mammalian brain cells contain genes encoding a complete set of selenoproteins, and cerebral cortex, hippocampus, cerebellum, and olfactory bulb are exceptionally rich in selenoprotein gene expression (Zhang et al. 2008). During selenium depletion the brain selenium level is maintained at the expense of other tissues whereas severe deficiency of this element causes irreversible brain injury (Burk and Hill 2009).

Three major families of selenoproteins (TrxRs, GPXs and DIOs) have the most critical role in the brain by controlling redox status, preventing and reversing the oxidative damage in neuroendocrine tissues, and providing the involvement of active thyroid hormone (T_3) in the regulation of brain functions. More than 80% of T_3 in the brain is derived from intracellular deiodination of T_4 by DIO2 (Courtin et al. 1986). Since circulating T_3 does not readily gain access to intracellular nuclear receptors, DIO2 provides an important regulatory function in the brain. As is known, thyroid hormone regulates processes associated with brain differentiation, including dendrite and axon growth, synaptogenesis, neuronal migration, and myelination (Bernal 2007). Impaired thyroid hormone production during the early development of the child leads to a deficiency in intelligence and sensorimotor functions.

Selenoproteins also contribute to optimal brain functions via redox regulation and antioxidant protection of cells (Zhang et al. 2010).

Brain is supplied by selenium through selenoprotein P, which is important not only for selenium transport, but also has a vital role in neurogenesis (Scharpf et al. 2007). SEPP1 may act directly as an antioxidant to prevent oxidative stress, or may provide selenium for biosynthesis of other antioxidant selenoproteins. Genetic deletion of SEPP1 results in decreased levels of mRNAs encoding selenoproteins in brain, most notably GPX4, and selenoproteins H, M and W (Hoffmann et al. 2007).

Selenium deficiency is associated with cognitive decline; impairment of selenoprotein functions has been implicated in Alzheimer's disease, Parkinson's disease, Huntington's disease, and epilepsy (Pillai et al. 2014). Insufficient selenium intake can be also associated with an increased risk of depression and a lowered mood status (Banikazemi et al. 2016). Selenium produces cognitive improvement and can counteract age-related neurodegeneration, which is associated with increased oxidative stress and instability of the genome, protecting the brain during the aging process (Zhang et al. 2010; Aaseth et al. 2016). Selenium has protective role in Alzheimer's disease, since it is a potent inhibitor of tau hyperphosphorylation, a critical step in the formation of neurofibrillary tangles. In addition, some Se compounds (e.g. SEPP1) protect amyloid precursor protein (APP) against excessive copper and iron deposition (Aaseth et al. 2016). Expression of SEPP1 in human brain increases during aging (Lu et al. 2004), and this effect may have a protective role against age-related neurodegeneration.

3.6.5 Selenium and Fertility

Selenium plays an important role in the male reproductive system, and the testis is one of the main target organs for this element (Boitani and Puglisi 2008). Selenium has been recognized as essential micronutrient for male fertility due to its role in the testosterone biosynthesis and formation and normal development of spermatozoa (Flohé 2007). It is considered that selenium-regulated spermatogenesis is mediated mainly by two selenoproteins: GPX4, the most abundant selenoprotein in spermatoid cells, and SEPP1, which serves as a source of selenium in the testes (Burk and Hill

2009). GPX4 has a dual role in the testes, since it functions as a GPX and also as a structural protein involved in the formation of the sperm specific mitochondrial capsule (Ursini et al. 1999). The genetic variants of the human GPX4 gene have been related to male infertility.

3.6.6 Selenium and Cardiovascular Disease

Several epidemiological studies suggest that selenium deficiency may contribute to cardiovascular disease (Shamberger 1980; Flores-Mateo et al. 2006; Kardinaal et al. 1997). Early studies have shown the significantly lower incidence of hypertensive heart disease in Se-adequate areas of the USA compared to Se-deficient areas (Shamberger 1980). A meta-analysis on the association of selenium biomarkers with coronary heart disease, which included 25 observational studies and 6 randomized trials have shown a moderate but statistically significant inverse association between selenium concentrations in several tissues and coronary heart disease outcomes. In observational studies, a 50% increase in selenium concentrations was associated with a 24% reduction in coronary heart disease risk (Flores-Mateo et al. 2006). Potential cardiovascular benefits of selenium are supported by evidence that selenoproteins prevent oxidative modification of lipids, inhibit platelet aggregation, and reduce inflammation (Rayman 2012). Selenium can exhibit protective effects against cardiotoxicity induced by several environmental pollutants such as dimethoate (Amara et al. 2013). However, randomised trials of selenium-containing supplements have not shown a significant effect on cardiovascular disease or mortality endpoints (Flores-Mateo et al. 2006; Stranges et al. 2006).

3.6.7 Role of Selenium in Detoxification of Harmful Substances

Dietary selenium was shown to be a critical modulator of intestinal cytochrome P-450-dependent metabolism of ingested drugs, carcinogens, and toxins that are absorbed by the intestinal mucosa (Pascoe et al. 1983); selenium also influences the xenobiotic metabolizing enzymes in the liver and brain (Erkekoglu et al. 2012; Caglayan et al. 2016). Selenium exerts a potent protective effect against aflatoxin B_1 (AFB_1)-induced damage to hepatocytes and other cells (Hoivanovych and Antonyak 2015, 2017; Cao et al. 2017) and protects against zearalenone-induced reproductive system damage (Long et al. 2016). In addition, selenium is known to counteract the effects of toxic heavy metals and diminish the harmful influence of Hg, Cd, Pb, Cr(VI) in the organism (Panas et al. 2003; Solohub et al. 2007; Antonyak et al. 2008; Antonyak and Skab 2013; Xu et al. 2016).

3.6.8 Effect of Selenium on Diabetes Risk

Data from several epidemiologic investigations showed correlations between abnormal glucose or lipid metabolism and decreased plasma selenium concentrations or GPX activity in diabetic subjects (Mueller et al. 2009). In addition, selenium has been shown to mediate a number of insulin-mimetic effects both in vivo and in vitro and activate key proteins involved in the insulin-signal cascade (Stapleton 2000). These data suggest that selenium can be involved in controlling the blood glucose level. However, recent animal experiments and human trials have shown a risk of prolonged high selenium intake in potentiating insulin resistance and type 2 diabetes (Zhou et al. 2013; Thompson et al. 2016).

3.6.9 Selenium and Cancer Risk

There is evidence from basic and clinical studies suggesting higher risk for cancer incidence in persons with selenium deficiency and a protective role for dietary selenium in various types of cancer (Rayman 2005, 2012; Cai et al. 2016). The results of recent meta-analysis, which included 69 studies, indicated that high selenium exposure had a protective effect on cancer risk and high serum/plasma selenium had the efficacy on cancer prevention (Cai et al. 2016). Prospective studies have provided evidence for a beneficial effect of selenium on the risk of lung, bladder, colorectal, liver, oesophageal, gastric-cardia, thyroid, and prostate cancers (Rayman 2005, 2012; Cui et al. 2017).

A number of mechanisms have been suggested to explain the anticancer effects of selenium, including: (1) reduction of oxidative stress and protection of DNA from damage; (2) reduction of inflammation; (3) induction of phase II conjugating enzymes that detoxify carcinogens and reduce DNA adduct formation; (4) enhancement of immune response; (5) increase in tumour-suppressor protein p53; (6) inactivation of protein kinase C (PKC) that plays a crucial role in tumour promotion by oxidants; (7) alteration in DNA methylation; (8) blockage of the cell cycle; (9) induction of apoptosis of cancer cells; (10) inhibition of angiogenesis (Rayman 2005; Riaz and Mehmood 2012). It has been shown that plasma selenium level should reach approximately 120 μg/L to optimise the anticancer effect of selenium, and the level of Se supplementation that reduces cancer risk is of 200 μg per day (Rayman 2005).

However, there are contradictory data regarding the effectiveness of selenium supplementation, and several recent studies have not confirmed the protective role of dietary selenium in reducing the cancer risk. (Dennert et al. 2011; Vinceti et al. 2014). These include, in particular, the increasing risk of some types of cancer such as melanoma and lymphoid cancers following selenium supplementation (Vinceti et al. 2016).

On the other hand, there is evidence of anticarcinogenic activities for several intermediary metabolites of naturally occurring organic and inorganic forms of sele-

nium. Methylselenol (CH_3SeH) that has potent anticancer properties can be produced in vivo during selenium metabolism. In particular, CH_3SeH can be formed from γ-glutamyl-selenomethylselenocysteine that is present in plants of the genera *Allium* and *Brassica* (Rayman 2005).

A range of selenoproteins have been suggested to be involved in protection against cancer, including TrxR1, Sep15 and GPX2 (Hatfield et al. 2014). However, there is evidence that these and several other selenoproteins may also be involved in the promotion of tumour growth and are over-expressed in tumour cells (Hatfield et al. 2014; Schmidt and Arnér 2016). In particular, TrxR1 is over-expressed in many cancers and malignant cell lines, which is accompanied by increased cell proliferation (Papp et al. 2007). Currently, TrxR1 is considered a promising target for anticancer drugs in view of its functional links with transcription factors (such as Nrf2, NF-κB and p53) and interaction with growth-promoting signaling pathways (Hatfield et al. 2014; Schmidt and Arnér 2016).

3.7 Health Effects of Excess Selenium

3.7.1 Selenium Toxicity and Tolerable Upper Level of Selenium Intake

Selenium toxicity in animals was first observed in the seleniferous regions in the middle of the 19th century and confirmed in the 1930s in livestock that consumed Se-accumulating plants in the western part of the USA (Spallholz 1997). In affected animals, selenium intoxication caused the acute syndrome of "blind staggers" and the chronic "alkali disease", accompanied by blindness, walking in circles, anorexia, weight loss, ataxia, and dystrophic hooves (Calello 2010).

In humans, typical symptoms of chronic selenium intoxication (selenosis) are dermatitis, hair loss, nail damage and the characteristic garlic odour of the breath (Calello 2010). These symptoms are accompanied by the toxic effects of selenium at the cellular and tissue levels, including liver and kidney damage, blood clotting, necrosis of the heart and liver, nervous system disorders and gastrointestinal disturbances. From the early 1930s to the 1960s, cases of chronic poisoning of people with selenium were often observed in Enshi County (Hubei Province of China) with a high prevalence of the disease in 1961–1964 (Yuan et al. 2012). The blood and urinary selenium levels in the affected persons were 30–100-fold higher compared with Se adequate levels, and individual daily selenium intake in this region ranged from 3.20 to 6.69 mg. In addition to selenosis in seleniferous regions, an excess of selenium in the body with toxic effects can also occur in other circumstances, including increased consumption of selenium supplements. Environmental contamination by selenium from anthropogenic sources also creates the risk of excessive selenium entry into the human body.

Based on the measurement of the indices of selenium status, it has been reported that selenium homeostasis is disturbed at the daily intake of 750 μg Se or above, whereas symptoms of selenosis (such as persistent overt fingernail changes) in susceptible persons occur at Se intake of 910 μg/day, corresponding to a blood selenium level of 1.05 mg/L (Yang et al. 1989). Thus, daily intake of selenium of 750–850 μg was suggested as the marginal level of safe consumption, and for safety, 400 μg Se per day was proposed as the daily tolerable upper level (UL) of selenium intake (Yang et al. 1989). UL is defined as the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects of almost all individuals. The FNB at the IOM of the US National Academies has set the ULs of dietary selenium intake as follows: 400 μg for persons aged 14 years and older, and for pregnant and lactating women; 45 and 60 μg for infants from birth to 6 months and 7–12 months, respectively; 90, 150 and 280 μg for children 1–3, 4–8 and 9–13 years old, respectively (IOM 2000). These levels indicate a narrow margin between amount of selenium, which is needed by the organism and selenium doses that can cause adverse effects on health. Exceeding the upper tolerable levels of selenium can have an unfavourable effect on the endocrine system, especially on the thyroid status (Winther et al. 2015), and increase the risk of type 2 diabetes (Thompson et al. 2016), some specific types of cancer such as melanoma and lymphoid cancers, and disorders of the nervous system including increased risk of amyotrophic lateral sclerosis (Vinceti et al. 2016).

3.7.2 Mechanisms of Selenium Toxicity

As regards to the mechanisms of selenium toxicity, most reports generally attribute it to the induction of oxidative stress. A number of studies have shown that both the inorganic selenium compounds and Se-amino acids, if taken in excess, provoke the formation of reactive oxygen species (Spallholz 1997; Mézes and Balogh 2009). These effects can impair the amount and/or activity of antioxidant defence mechanisms. In particular, selenite can be non-enzymatically reduced in presence of glutathione, generating selenodiglutathione and superoxide anion radical ($O_2^{-\cdot}$). Superoxide has the harmful effects on iron-sulfur clusters in mitochondrial proteins and triggers mitochondrial damage. Although the Se-amino acids (SeMet and Sec) are less toxic than selenite (Mézes and Balogh 2009), recent studies have provided evidences indicating their cytotoxicity mediated by both oxidative stress and ROS-independent mechanisms (Lazard et al. 2017). These latter mechanisms include disruption of protein homeostasis by Sec misincorporation in proteins and/or reaction of selenols with protein thiols.

In addition, the replacement of sulfur by selenium in cellular respiration enzymes can cause mitochondrial disruption, and the substitution of SeMet in place of methionine may interfere with protein synthesis (Calello 2010). The dermal/integumentary effects of excess selenium such as skin, hair, and nail damage can also be a result of selenium interpolation into disulfide bridges of keratin and other structural proteins.

3.8 Selenium and Longevity

3.8.1 Selenium Intake and Mortality Risk

Several epidemiological studies suggest an inverse association between serum selenium concentration and mortality risk (Akbaraly et al. 2005; Ray et al. 2006; Rayman 2012). However, a study with 13,887 participants with a follow-up of 12 years has shown a nonlinear association between the levels of selenium in serum and all-cause and cancer mortality. Increasing serum selenium levels were associated with decreased mortality up to 130 μg/L, while for serum selenium concentrations of over 150 μg/L a slight increase in mortality risk was observed (Bleys et al. 2008).

3.8.2 Selenium and Aging

Increased cellular production of ROS and continuous oxidative damage to cellular components contribute to the process of aging (Petropoulos and Friguet 2006). Selenium in the form of selenoproteins can potentially delay the aging process and prevent age-related diseases associated with oxidative stress in various ways: by removing free radicals and modulating oxidative damage; maintaining the redox status of cells and preventing protein oxidation; reducing methionine sulphoxides; providing the availability of active thyroid hormone; and also through its influence on redox-sensitive cellular signaling pathways. It has been shown that selenium deficiency or insufficient expression of certain selenoproteins can be accompanied by the appearance of signs of aging, suggesting the involvement of selenoproteins in protection against aging process. In particular, mice unable to express selenoproteins in epidermal cells show age-related alopecia (Sengupta et al. 2010). Decreased expression of iodothyronine deiodinases (DIO1 and DIO2) have been implicated in selenium deficiency-associated diseases of aging in mice (McCann and Ames 2011). In humans, selenium deficiency in individuals over 65 years old is accompanied with less peripheral T_3 production from thyroxine because of decreased levels of deiodinase expression (Olivieri et al. 1996). Selenium deficiency has been reported in Down's syndrome (trisomy 21), a disorder characterized by premature aging (Neve et al. 1983). Similarly, decreased selenium levels and GPX activity in blood of children affected by Kaschin-Beck disease have been associated with the presenile tissue changes observed in this disease (Li et al. 1990).

Different types of glutathione peroxidases that scavenge H_2O_2 and prevent the initiation of free radical chain reaction have important roles in protecting against free radical-induced lesions during aging. It has been hypothesized that GPX may extend life span of the organism and prevent age-related functional disorders (Riaz and Mehmood 2012). The cellular redox state maintained by thioredoxin reductase system is also essential for the physiological responses to oxidative stress and aging. TrxRs control the availability of reduced thioredoxins, which protect proteins from

oxidative damage (Pérez et al. 2011), and mitochondrial TrxR2 has a crucial role in the aging heart (Kiermayer et al. 2015).

The methionine-*R*-sulfoxide reductase B1, which catalyze the reduction of methionine sulfoxides, can also contribute to slowing down the aging process. It has been shown that mutations leading to a decrease in Msr activities are associated with a decrease in resistance to oxidative stress and to a shortening of the life span, while high level of Msr expression leads to an increase in resistance to oxidative stress and a significant increase in life span (Stadtman 2006). Therefore, MsrB1 is considered one of the selenoproteins through which selenium exhibits its anti-aging properties (Papp et al. 2007).

Aging process is accompanied by different changes in selenoprotein expression. The expression of SEPP1 in human brain increases during aging (Lu et al. 2004), and this effect, apparently, can contribute to protection against age-related neurodegeneration. The increase of GPX activity was observed in the brain of old rats as an adaptive effect to the age-related increase of peroxides (Zhu et al. 2006).

Several authors have observed significant up-regulation in GPX protein expression and cytosolic GPX activity in the liver of aged rats (Yang et al. 2015), while other studies have shown an age-related decline in GPX activity in animal liver, myocardium and skeletal muscle (Xua et al. 2007).

TrxR2 expression decreases in the heart and skeletal muscle of rats during aging (Rohrbach et al. 2006; Yoshioka 2015). However, TrxR activity was found to be elevated in cells from long-lived species of rodents, primates, and birds. Elevated TrxR activity in long-lived species reflects an increase in the mitochondrial form, TrxR2, rather than the cytosolic forms TrxR1 and TrxR3 (Pickering et al. 2017).

3.8.3 Selenium and Longevity

The cross-sectional studies have demonstrated different changes in selenium levels in blood serum and tissues from older persons (Milman et al. 2004; Letsiou et al. 2009). Findings from the ATTICA study have shown significant decrease in selenium concentration with age (18–75 years), and this decline was independent on anthropometric, lifestyle and biochemical and nutritional indices (Letsiou et al. 2009). Similarly, a study conducted in Italy on healthy subjects showed a decline in serum selenium level and GPX activity in erythrocytes with age, particularly in people over 75 years of age (Olivieri et al. 1994). A negative correlation between selenium concentration in the liver and age was observed in Greenlandic Inuit men. In contrast, Danish men displayed a positive correlation between selenium content in the liver and age (Milman et al. 2004).

Several studies have analyzed plasma selenium levels in cohorts of healthy nonagenarians and centenarians in comparison to younger elderly persons (Savarino et al. 2001; Forte et al. 2014; Alis et al. 2016). A study conducted by Savarino et al. (2001), which included 90 nonagenarians/centenarians (91–110 years, group A) and 62 elderly persons (60–90 years, group B), has shown a significant decrease in serum

selenium levels in the persons of group A when compared with group B, in both men and women. However, 84.4% of the nonagenarians/centenarians had Se concentrations equal to or greater than the lowest values of the elderly group (Savarino et al. 2001). Similar results were obtained in a study conducted in Sardinia that included 76 nonagenarians (89.0 ± 6.3 years), 64 centenarians (101 ± 1 years) and 24 middle-aged subjects as controls (61.2 ± 1.1 years). A comparison among the three classes of age showed a significant depletion of Se in nonagenarians and centenarians with respect to controls (Forte et al. 2014). In contrast, a recent study including 81 healthy centenarians (100–104 years) and 46 healthy younger elderly controls (70–80 years) showed significantly higher levels of serum selenium in centenarians compared to younger elderly persons (Alis et al. 2016). All the centenarians participating in this study were free of major age-related diseases (severe cognitive impairment, clinically evident cancer, cardiovascular disease, renal insufficiency, or severe physical impairment). Contradictory data obtained in these studies may indicate a complex relationship between selenium levels in the organism and longevity. Selenium has multiple roles in human health and is crucial for the proper functioning of the immune system; therefore, adequate Se levels can potentially impact positively human life span and health of older persons. In contrast, a decrease in selenium content during aging may be accompanied by an increased susceptibility to diseases common in the elderly, a reduced efficiency of immune system, and a deficiency in the conversion of thyroxine to T_3, which influences general metabolism. However, a decrease in serum selenium levels in the oldest old persons indicates that the need for selenium as component of selenoproteins can be more pronounced in young life periods than in old age.

Data regarding the impact of selenium on life span have been obtained in animal studies. When studying the distribution of selenium in tissues from 26 mammalian species, it has been found that the maximum life span is negatively correlated with the level of selenium in all the analyzed species, and two species of desert-dwelling African mole rats (the naked mole rat and the Damaraland mole rat), the longest lived rodents, exhibited a decreased levels of selenium utilization (Ma et al. 2015). Wu et al. (2017) reported a paradoxical effect of dietary selenium deprivation on longevity promotion together with the health deterioration in mice. According to these findings, selenium at low levels may be considered a hormetic chemical, and a dietary condition decouples healthspan and longevity. The hormetic effect on life span extension can be mediated by a non-selenoprotein pool of selenium in the organism (Wu et al. 2017).

3.9 Conclusions

Chemical element selenium for a long time was known only because of its toxic effects. Now, selenium is recognized as an essential micronutrient that influences many functions in mammalian organism. Inadequate dietary selenium intake can lead to a general decline in health, weakened immune resistance, endocrine disor-

ders, inflammatory, cardiovascular and hepatic diseases, cancer, and impaired brain function. Biologically important effects of selenium are associated with selenoproteins, all of which contain it in the form of selenocysteine residue. Selenoproteins are involved in the processes of oxidoreduction, antioxidant defence, redox signaling, thyroid hormone metabolism, selenocysteine synthesis, transport and storage of selenium, mechanisms of fertility, and immune responses. Because of the multiple roles played by selenoproteins, dietary selenium at recommended levels of consumption is essential for human health and exhibits beneficial effects.

However, selenium is considered to be one of the controversial elements, since it can exhibit harmful effects at concentrations exceeding the tolerable upper level. The U-shaped relationship has been shown between selenium concentration in the blood and the risk of disease, with possible harm occurring both below and above the physiological range for optimal activity of some or all selenoproteins. High intake of selenium can adversely affect the endocrine system and increases the risk of diabetes type 2, some specific types of cancer and nervous system disturbances.

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Chapter 4
Zinc

John H. Beattie, Marco Malavolta and Irina Korichneva

Abstract Zinc has a critical role in biology due principally to its chemical affinity for cellular thiol, imidazole and carboxyl ligands. The regulation of zinc homeostasis is critically dependent on zinc transporters and channels since free zinc or zinc associated with biomolecules is not membrane permeable. While zinc homeostasis may be maintained at different levels of dietary zinc intake, zinc status may vary with zinc intake. This chapter gives a detailed description of the current knowledge concerning the relationship between zinc homeostasis, aging and health. How age-related changes affect zinc intake, metabolism, excretion and homeostasis is described with a particular focus on the alterations of mechanisms controlling the function of zinc transporters and other proteins involved in zinc homeostasis. Evidence for the involvement of zinc dyshomeostasis in immunosenescence, cardiovascular diseases, diabetes, cancer, neurodegenerative diseases, chronic obstructive pulmonary disease and frailty is also extensively reviewed. The impact of zinc supplementation in health and diseases is approached considering data from model organisms to humans as well as the tight relationship of zinc with the insulin and insulin growth factor (IIS) pathway, which has a well-defined antagonistic pleiotropic function in aging. This chapter aims to help promote future research into the efficacy of zinc for prevention of age-related diseases and for therapeutic supplementation.

Keywords Zinc · Aging · Age-related diseases · Lifespan · Longevity
Zinc status · Zinc metabolism · Zinc homeostasis · Zinc transporters
Exchangeable zinc pool · Zinc signaling · Zinc dyshomeostasis
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4.1 Introduction

Zinc has a critical role in biology due principally to its chemical affinity for cellular thiol, imidazole and carboxyl ligands (Maret 2012). Biology has utilised this binding affinity in many ways, predominantly for maintaining the tertiary structural orientation of proteins to fulfil their function. Zinc finger and LIM domain proteins represent about 10% of the proteome and are absolutely dependent on zinc for their DNA and protein binding functions (Laity et al. 2001). Many metalloenzymes also rely on zinc because in an entatic state produced by an enforced stereochemistry and/or electronic state around the metal, it can be optimally primed for a catalytic role (Williams 1985). The dominant influence of zinc in human biology is disproportionate to its low geological abundance and implies that maintenance of health is inextricably linked with the maintenance of zinc status and metabolism. This is indeed the case and zinc status is avidly maintained across a wide range of dietary zinc intakes (King et al. 2016). Zinc is therefore described as a type 2 nutrient because it is not thought to be specifically stored within the body and homeostatic mechanisms work to maintain cellular and plasma zinc levels, even at the expense of for example growth, tissue repair and immune function (Golden 1996). The regulation of zinc homeostasis is critically dependent on zinc transporters and channels since free zinc or zinc associated with biomolecules is not membrane permeable. Human and animal health is therefore directly related to the expression and regulation of these transporters and channels and an understanding of their physiological role in human health and ageing is the subject of this review.

4.2 The Regulation of Zinc Homeostasis

Dietary zinc is absorbed primarily in the duodenum and is mostly excreted within pancreatic secretions back into the small intestine. In this way, some recycling of excreted zinc can occur which means that at the absorption level, body homeostatic mechanisms can normally be very efficient. Proportionally, very little zinc is excreted in urine or sweat but significant losses in men can occur through seminal emissions. Apart from the cellular sites of absorption and excretion, other factors can influence the efficiency of zinc homeostasis including intestinal bioavailability. Apart from reduced quantity of ingested zinc, dietary phytic acid content is the single most important factor which decreases zinc absorption (Miller et al. 2007), but dietary protein and calcium are thought to promote absorption (Miller et al. 2013). Phytate (inositol hexaphosphate) comes from plant foods and is rich in cereals, legumes and their processed products. It avidly binds Zn^{2+}, Fe^{2+}, Ca^{2+} and Mg^{2+} but quantitatively it has the most significant impact on zinc and non-haem ferrous iron bioavailability.

Nutrition has an impact on intestinal bacterial communities (Flint et al. 2017) and the impact of the gut microbiota on zinc absorption and, conversely, the impact of luminal zinc concentration on the gut microbiota, have been investigated (Skrypnik

and Sulibursk a 2018). In mice, very high levels of dietary zinc (1 g/kg) but not zinc-deficient diets (no added zinc) were found to change the microbiota β-diversity during 5 weeks of dietary intervention, as compared to control diets containing 29 mg Zn/kg (Zackular et al. 2016). However, the effect of the diets on food intake and the influence of this variable on the data were not reported. The intestinal microbiota increases in size and complexity from the duodenum to the colon and so there may be longitudinal variations in the impact of the microbiota on zinc absorption. The highest zinc absorption efficiency is in the duodenum where there is less microbial species diversity, but even in the colon, with a quantitatively rich and diverse microbiota, there is evidence that some zinc absorption or reabsorption may occur. Since the gut mucosal cells are luminally covered in secreted mucus, there is also a cross-sectional gradient of microbial speciation from the centre of the lumen to the apical membrane of the enterocytes. There is still very little information on how longitudinal and cross-sectional changes in microbial density and species diversity can impact on metal absorption.

Using dual isotope tracer techniques, fractional dietary zinc absorption can range from around 20 to >90% in adult humans (King et al. 2000) and so the capacity to modulate body zinc homeostasis is considerable. Little is understood about regulatory mechanisms for zinc excretion but both absorption and excretion appear to be influenced by zinc transporter expression and perhaps function in key cells regulating influx and efflux of zinc. Using stable isotope methodology, a multiple-compartment model of zinc has been identified in the human body (Lowe et al. 1997) but essentially there are two major kinetic pools; an exchangeable zinc pool (EZP) which constitutes about 10% of body zinc and a slowly exchangeable zinc pool comprising the remaining body zinc (Miller et al. 1994). The EZP has a turnover rate of about 3 days and includes most of liver zinc, part of other soft tissue zinc and blood plasma zinc. The latter turns over about 150 times per day, is quantitatively only about 2% of the total EZP and is resistant to reflecting changes in zinc status, which is why it is not a good biomarker to assess an individual's zinc status (King et al. 2016).

As would be expected, the size of the EZP is directly correlated with body size (body weight being the most reliable predictor), and so the EZP is simplistically normalised using body weight. However, EZP also shows a negative correlation with age and is higher in men than women, and so a normative model incorporating weight, age and gender has been suggested (Miller et al. 2017). Normalised EZP size in adult men showed a significant decrease in response to sustained (>5w) consumption of moderate and severely zinc-deficient diets. It therefore appears that while plasma zinc levels may not markedly respond to dietary zinc deprivation, the normalised EZP size does respond. It is likely therefore that the EZP response is focussed in soft tissues, particularly the liver. There is evidence that depletion of the EZP has many signalling consequences, including perhaps the production of a low molecular weight humoral factor (Ou et al. 2013) which promotes apoptosis in vascular smooth muscle (Allen-Redpath et al. 2013) and possibly other cell types.

While zinc homeostasis may be maintained at different levels of dietary zinc intake, zinc status may vary with zinc intake and there is a clear need for better biomarkers of zinc status (King et al. 2016) to determine reliable reference nutrient

values based on empirical analysis of sensitive biological targets rather than relying on the factorial approach which is currently the only method available (Gibson et al. 2016). An analysis of zinc adequacy, which may crudely be defined as the EZP size which meets the most sensitive biological requirements, is therefore essential for correctly interpreting the impact of changing zinc status. Genetic variation is another variable which may have a profound influence on zinc requirements. In extreme cases, rare single nucleotide polymorphisms of key zinc transporters such as ZIP4 (Kury et al. 2002) and ZnT2 (Chowanadisai et al. 2006) can have severe consequences, but other less severe mutations with a higher allelic frequency may have milder consequences that disadvantage the individuals (Giacconi et al. 2015) or their offspring (Alam et al. 2015) in nutritionally challenging situations, such as during dietary zinc deficiency or when demand for zinc is elevated. Epigenetic changes throughout life, stimulated by lifestyle and genetic attributes may have consequences for the process of ageing and the capacity of body to regulate zinc homeostasis (Gabbianelli and Malavolta 2018).

4.3 Age-Associated Alterations in Dietary Zinc Intake, Metabolism, Excretion and Homeostasis

Cell senescence may be caused by multiple factors. An age-related decline in mitochondrial function has been reported, resulting in deficits of bioenergetics and the compromise of cellular capacity to adapt to physiological stress (Gonzalez-Freire et al. 2015). All cellular processes including ion transport across membranes are energy-dependent and so cell senescence through mitochondrial disfunction is likely to have an inhibitory effect on the efficiency of zinc and other metal metabolism.

Indeed, we have already noted in the previous section that the EZP size decreases with age (Miller et al. 2017). One cause may be a decrease in dietary zinc intake due to a decrease in food intake (Maret and Sandstead 2006) and/or a change in food selection. For example, the elderly tend to eat less protein and since protein generally contains zinc, they also show a decline in zinc intake (Wakimoto and Block 2001). Appetite diminishes with old age, especially when taste and smell decline and dental deterioration makes eating some foods, such as red meat, more difficult (Landi et al. 2016). Psychological factors may also impact on food intake and the age-related decline in food consumption, which is known as anorexia of ageing, can appear in individuals at around 65 years of age (de Boer et al. 2013).

At an absorption level, the gut microbiota has been shown to change in density and species diversity in relation to age (Kim and Jazwinski 2018) and there is little understanding of how this impacts on zinc absorption. Absorption mechanisms across the mucosa involve a closely regulated and coordinated transport of zinc across apical and basolateral membranes utilising the plasma membrane-bound zinc transporters ZIP4 and ZnT1, respectively (Cousins 2010). Absorbed zinc is bound to serum albumin (Handing et al. 2016) and is predominantly taken up by liver hepatocytes.

However, it is ultimately excreted through pancreatic acinar cells and this process is regulated by the zinc transporters ZIP5 (on the basolateral membrane) and ZnT1 on the apical membrane (Geiser et al. 2013). The transporter ZnT2 also plays a role in vesicular excretion of zinc into the pancreatic juice. Ageing results in a decreased flow of pancreatic juice into the pancreatic duct (Torigoe et al. 2014) which may impact quantitatively on zinc excretion. Indeed, using dual-isotope tracer techniques, endogenous zinc losses in the elderly were found to be lower in elderly men compared to young men (Turnlund et al. 1986).

An overview of the regulatory mechanisms for whole body zinc homeostasis at a cellular and molecular level is presented in Fig. 4.1. The impact of cell senescence on the zinc transport efficiency of gatekeeper cells (gut mucosal and pancreatic acinar cells) is unknown but it is clear that ageing reduces zinc uptake and excretion efficiency (Turnlund et al. 1986). The function of ZIP7, an endoplasmic reticulum-located zinc transporter, is regulated by casein kinase 2-mediated phosphorylation (Taylor et al. 2012) and it is likely that other zinc transporters are similarly regulated. Mechanisms controlling transporter activation, rather than transporter expression, may be targets of the ageing process. An assessment of senescence and the function of key transporters in cells that are gatekeepers of body zinc homeostasis is currently in progress in an EU-funded RISE grant entitled "Micronutrients in Life Expectancy and Ageing (MILEAGE)".

Cell senescence can be modulated by zinc exposure. In human coronary artery endothelial cells, supraphysiological treatment with 50 μM zinc promotes the development of senescent cells whereas in zinc deficiency, senescent cells are more resistant to zinc deprivation (Malavolta et al. 2017a, b). This senescence-related change in sensitivity to zinc is probably related to modified transporter activation and/or function. Since senescence has a broad influence on cell metabolism other factors may also be involved.

4.4 Cellular and Molecular Mechanisms of Zinc Dys-Regulation in Aging

The molecular mechanisms controlling intracellular zinc homeostasis have been investigated since the discovery and cloning of zinc transporters (Palmiter and Findley 1995). With identification of 10 ZnT family proteins (SLC30 genes products) and 14 Zip (SLC39 genes) transporters the role of non-specific cationic channels in Zn permeability across plasma membrane has been demonstrated thus summarizing influx and efflux mechanisms. Finally, exponential growth of the research area brought about recent recognition of a zinc role in intracellular signaling and regulation of major signaling pathways including phosphorylation cascades and proteolysis.

Both excess of zinc and its deficiency are prejudicial to the cells. Zinc excess can be toxic to cells (Koh et al. 1996), whereas zinc deficiency can be a cause of serious metabolic disorders (Truong Tran et al. 2003). Therefore, the narrow intracellular

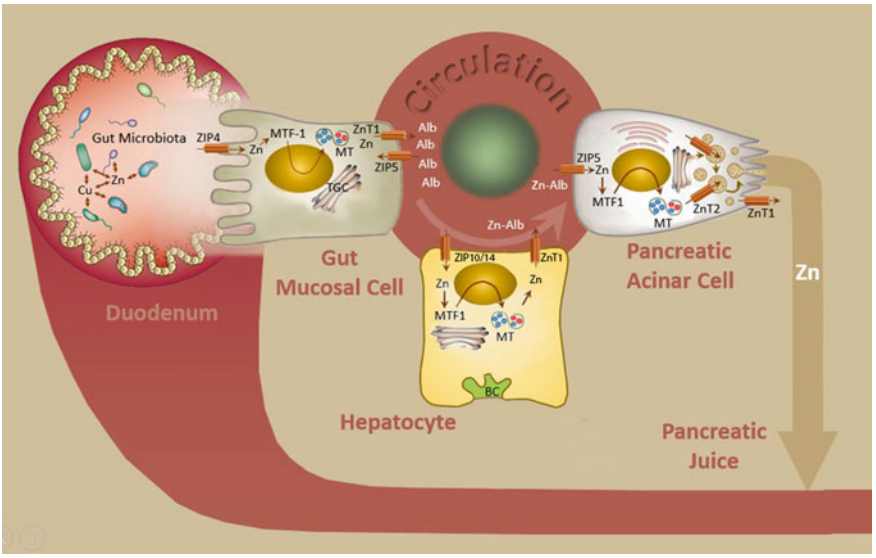

Fig. 4.1 Key cells in the maintenance of body homeostasis and metabolism of zinc. The gut microbiota and other factors such as dietary phytate may have an impact on zinc bioavailability and uptake across the gut mucosal cell apical membrane is almost entirely regulated by ZIP4. Free zinc is buffered by metallothionein (MT) through activation of metal transcription factor 1 (MTF-1) and zinc is transferred across the basolateral membrane by ZnT1. Binding to serum albumin, zinc is transported primarily to the liver where it is incorporated into hepatocytes by ZIP10/ZIP14 transportation. A large proportion of this zinc readily fluxes in and out of hepatocytes and so turnover of liver zinc is rapid. Zinc is taken up into pancreatic acinar cells through ZIP5 which can also shunt zinc from blood plasma into enterocytes. Zinc is excreted into pancreatic fluid through ZnT2-mediated vesicular secretion and also through ZnT1. Each of these key transporters may be influenced by cell senescence and which transporter is quantitatively more significant in affecting zinc homeostasis during ageing is being investigated by an EU-funded project called MILEAGE

concentration of bioavailable Zn is tightly controlled in pico to nanomolar levels. The two families of zinc transporters have opposite roles in cellular zinc homeostasis: ZnT transporters reduce intracellular zinc availability by promoting zinc efflux from cells or into intracellular vesicles (Palmiter and Huang 2004), while Zip transporters increase intracellular zinc load by allowing extracellular zinc uptake and vesicular zinc release into the cytoplasm (Eide 2004). For example, ZIP7 (SLC39A7), has been shown to be localized on the Golgi complex (Huang et al. 2005) and/or the endoplasmic reticulum (Taylor et al. 2004, 2007) and under certain conditions it releases zinc from these organelles to the cytosol (Taylor et al. 2012). The transport of zinc ions may be coupled to exchange for protons as has been shown in case of ZnT1 (Shusterman et al. 2014). More specifically, the authors demonstrated that ZnT-1 facilitates zinc efflux in a sodium-independent, pH-driven and calcium-sensitive manner. Moreover, substitution of two amino acids in the putative zinc binding domain of ZnT-1

ablated Zn^{2+} efflux and rendered the mutated protein incapable of protecting cells against Zn^{2+} toxicity.

The coordinated orchestration of a diverse family of zinc transporters also relies on non-specific cationic channels that are permeable for Zn, and in some conditions, contribute to half of the transported ion, e.g. voltage-gated calcium channels or TRP family channels (Dong et al. 2008; Gibon et al. 2011; Hu et al. 2009; Kovacs et al. 2011; Monteilh-Zoller et al. 2003) or TRP family channels (Bouron et al. 2015). For example, one of the major routes of zinc entry into most excitable cells is the L-type calcium channel (Kamalov et al. 2010) and in many other cells the TRP family channels, particularly the TRPM7 chanzyme. The further complexity of the system is determined by post-translational modifications of the transporters, notably phosphorylation as shown for Zip7 and ZnT2 (Taylor et al. 2012; McCormick et al. 2014). In addition, recently described interractions between Zn transporters and ion channels affect their function. For example, ZnT1 can interact with the beta-subunit and regulate function of LTCC (Yamasaki et al. 2012).

TRPV6 belongs to the vanilloid family of the transient receptor potential channel (TRP) superfamily. This calcium-selective channel is highly expressed in the duodenum. TRPV6 is also permeable to zinc and cadmium. Live cell imaging experiments with Fura-2 and NewPort Green DCF showed that overexpression of human TRPV6 increased the permeability for Ca^{2+}, Ba^{2+}, Sr^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+} and interestingly also for La^{3+} and Gd^{3+} suggesting that TRPV6 is not only involved in calcium transport but also in the transport of other divalent cations (Kovacs et al. 2011).

A structurally unique TRPM7 channel has been identified as having preferential permeability for Zn as compared to other cations (Monteilh-Zoller et al. 2003), and it may be itself potentially regulated by Zn while possessing an active serine/threonine kinase at the C-terminus harbouring a zinc finger structure (Clark et al. 2006; Dorovkov and Ryazanov 2004; Perraud et al. 2011; Matsushita et al. 2005). This suggests that TRPM7 may serve as a redox/zinc sensor and thus be affected by stress and aging (Krapivinsky et al. 2014). This channel has been implicated in cell de-differentiation leading to cancer development, and pancreatic cancer in particular, which has recently been shown to originate from acinar pancreatic cells (Kopp et al. 2012).

4.5 Zinc Regulation of Cellular Signaling Proteins

While several studies implicated zinc as a player in the activation of kinases (Korichneva JBC) and phosphatases, and it has even been named the «Ca of 21st century» (Frederickson et al. 2005), the term "a novel second messenger" was first used by the group of Prof. Hirano in 2008 (Hirano et al. 2008). Since then, multiple studies have demonstrated the versality of zinc functions in intracellular signaling.

It has already been mentioned that ion channels permeable for zinc, e.g. TRP family channels are at the same time inhibited by intracellular zinc. A potassium channel is another zinc target. Zn^{2+} inhibited K2P currents in one population of cells,

produced biphasic responses in another population, and increased the amplitude of the PDBu-elicited K^+ currents in a third population of myocytes, suggesting the expression of several K2P channel types, and providing insights into the interplay between PKC and PKA pathways that regulate vascular tone (Hayoz et al. 2013).

Another example of convergence of redox and zinc signaling is illustrated by the study performed on the potassium chanel KV4. The researchers investigated the redox response at the Zn^{2+} binding site in the intracellular T1-T1 inter-subunit interface of a Kv4 channel, the site that undergoes conformational changes coupled to voltage-dependent gating. The data showed that nitric oxide strongly inhibited channel activity and the effect could be reversed by reduced glutathione or suppressed by the addition of intracellular zinc ions. The authors determined two Zn^{2+}-binding cysteines that were required to observe the NO-induced inhibition (Cys-110 from one subunit and Cys-132 from the neighboring subunit). The data suggested that the interfacial T1 Zn^{2+} site of Kv4 channels acts as a Zn^{2+}-dependent redox switch (to be discussed further) that may regulate the activity of neuronal and cardiac A-type K^+ currents under physiological and pathological conditions (Wang et al. 2007).

The principles of signal transduction and a definition of a second messenger first evolved from the discovery of hormone-regulated adenylate cyclase, an enzyme that converts the signal from G-protein coupled receptors to the synthesis of second messenger cAMP. Since then, numerous consequences of cAMP-dependent signaling have been investigated in detail. The developing interest in zinc signaling prompted the analysis of its effects on membrane-associated and soluble adenylate cyclase. It has been shown that zinc inhibits hormone and forskolin stimulation of cAMP synthesis in N18TG2 cells. Crystallographic data of the truncated soluble AC suggested a possible mechanism by which zinc could inhibit AC activity, focusing on Zn^{2+} bound to a site different from Mg^{2+} binding sites involved in catalysis. Using extrinsic and intrinsic fluorescent measurements, the authors showed a conformational response of the enzyme to zinc binding (Klein et al. 2004). The in vitro results corroborate physiological data in the model of diabetic cardiomyopathy where zinc deficiency and lowered myocardial adenylate activity commonly occur, and hormonal-stimulated enzymatic activity of AC is significantly lower in diabetic rats on a low zinc diet (Mooradian et al. 1988)

The complexity of zinc effects on MAP kinases contributes to controversy between the in vitro and in vivo studies. For example, in contrast to zinc deficiency, zinc chelation inhibits activation of p38. It is not clear to what extent the production of ROS is involved in these effects and whether zinc may have direct regulatory function on the enzymes. In general, zinc deficiency is characterized by attenuation of growth factor signalling pathways and an amplification of p53 pathways, while the latter still requires zinc for normal functioning. The outcome is achieved by hypophosphorylation of the antiapoptotic kinases, AKT and ERK, under the conditions of zinc deficiency, and activation of p38 and JNK.

Perhaps a better understanding of the regulation of signaling kinases by zinc and redox changes is accomplished in the studies of PKC isoforms. PKC has always been the focus of increased interest due to the key role of this enzyme in signal transduction (Naruse and King 2000). Different isoforms translocate to different

compartments in close proximity to substrates and perform multiple cellular functions. Like other Ser/Thr kinases, PKC is sensitive to variations in zinc concentration. More than 20 years ago, Forbes and Zalewski demonstrated that micromolar concentrations of zinc chloride induced specific association of PKC with the plasma membrane or cytoskeleton (Forbes et al. 1990). The attachment of protein kinase C to the cytoskeleton was accompanied by an enhanced expression of binding sites for ^{3}H-phorbol ester, a regulatory ligand of protein kinase C. The heavy-metal chelating agent 1,10-phenanthroline completely reversed the increased [^{3}H]PDBu binding in cells pretreated with ^{65}Zn, which was associated with a decline of about 20% in cell-associated ^{65}Zn, suggesting that a relatively small pool of intracellular Zn^{2+} acts on PKC. Further numerous measurements of intracellular zinc changes under physiological stimulation of normal wild type cell lines or cultured cells reproducibly showed from 2 to 20-fold increase in zinc concentrations unlike calcium responses. The authors suggested that this 20% zinc increase could represent a membrane-associated pool. The active factor in the cytoskeleton was labile to protease, suggesting that protein kinase C binds to a cytoskeletal protein. PKC bound to a Zn^{2+} affinity column and was eluted by a metal chelator, confirming that Zn^{2+} interacts directly with PKC. The authors further proposed that putative binding sites for zinc were present in the regulatory domain of protein kinase C; however, no distinct role for zinc was obvious at that time.

Little more than a decade ago we conducted a study focusing on direct action of reactive oxygen species (ROS) on the PKC molecule. Independent of the classical lipid-mediated pathway, PKC is also controlled by a redox mechanism. PKCs embrace two independent target sequences susceptible to oxidative modification (Gopalakrishna and Jaken 2000). The two domains of PKC respond differently to redox changes. Thus, oxidation of thiols in the catalytic domains blocks enzymatic kinase function. More interestingly, oxidation within the regulatory sequence converts the protein to the catalytically competent form, while reduction reverses this process (Gopalakrishna and Anderson 1989; Knapp and Klann 2000). Physiologically, it would mean that the regulatory domain should be sensitive to lower doses of oxidants. Another manner in which such preferential oxidation might occur is through the facilitation of electron transfer by retinoids. It has been found that the redox activation of the kinase requires cofactors, retinol or its metabolites, since the high affinity retinoid binding site was mapped to the cysteine-rich regions in the regulatory domains of Ser/Thr kinases (Imam et al. 2001) Remarkably, metabolism of vitamin A (retinol) increases in heart after myocardial infarction, suggesting mobilisation of healing reserves to protect the heart from oxidative stress (Palace et al. 1999)

In the N-terminal regulatory domain, the 50 amino acid long, highly homologous stretches containing six conserved cysteine and two conserved histidine residues, tetrahedrally coordinated by two Zn^{2+} ions into a composite zinc finger are targets of redox control (Zhang et al. 1995). When oxidized, the autoinhibitory function of the regulatory domain is compromised and, consequently, cellular PKC activity is stimulated. This topological change is believed to be complemented by phosphorylation, translocation, and Ca^{2+} and phosphatidylserine binding, to lock the catalytic

domain into an active form. As a part of crosstalk, PKC becomes phosphorylated on Tyr at residues 512 and 523 under the conditions of oxidative stress (Konishi et al. 1997). Remarkably, both classical and redox activation of PKC trigger the same event, namely zinc release from the regulatory domain. Phorbol esters mimic the action of second messenger diacylglycerol by binding to the same motif within the regulatory domain and activating catalytic activity.

The interest to the mechanism of redox activation of PKC and the role of zinc in its function continues bringing more data. Recent studies show that increased concentrations of intracellular zinc affected the phosphorylation state and subcellular localization of PKCδ. More specifically, intracellular zinc inhibited the phosphorylation of PKCδ at Thr505 in a concentration-dependent manner and facilitated the translocation of PKCδ from the cytosol to the Golgi complex (Slepchenko et al. 2018).

In contrast to activation of kinases zinc ions may inhibit enzymes such as protein tyrosine phosphatases (PTPs) and the values for the K_i of these enzymes is in a low pM range. The K_i(Zn) value for inhibition of receptor PTPB is 21 pM. The binding is about as tight as the binding of zinc to zinc metalloenzymes and suggests tonic zinc inhibition. PTP1-B (PTPN1), an enzyme regulating the insulin and leptin receptors and involved in cancer and diabetes, has a K_i(Zn) value of about 5 nM (Wilson et al. 2012). The enzyme binding of zinc ions with such a high affinity confirms that such binding is physiologically significant, and the binding constants must be compatible with the cellular availability of zinc ions (Maret 2013)

To add to potential targets of cellular free (labile) zinc in signaling pathways we would like to mention the major route of calcium entry into the cell, the Orai1 channel. Using the cellular model of Esophageal Squamous Cell Carcinoma we demonstrated that elevated zinc targets specific cysteines and histidines of Orai1 to block Orai1-mediated intracellular Ca^{2+} oscillations and subsequently cell proliferation (Choi et al. 2018).

In cardiac cells, aberrant Zn^{2+} homeostasis is a hallmark of certain cardiomyopathies associated with altered contractile force. The group of SJ Pitt demonstrated that Zn^{2+} is a high affinity regulator of RyR2 displaying three modes of operation. Picomolar free Zn^{2+} concentrations potentiate RyR2 responses, at concentrations of free Zn^{2+} >1 nm, Zn^{2+} is the main activating ligand, and the dependence on Ca^{2+} is removed. Millimolar levels of free Zn^{2+} were found to inhibit channel openings (Woodier et al. 2015).

Additionally, regulation of protein function may be mediated by zinc transporters through protein-protein interaction. For example, ZnT-1, along with its ability to exchange Zn^{2+}/H^{+} inhibits the L-type calcium channel (LTCC), a major Zn^{2+} and Ca^{2+} entry pathway, the two functions are independent of each other and are mediated by different parts of the protein. The mutation analysis showed that ZnT-1 performs two structurally independent functions related to zinc homeostasis (Shusterman et al. 2017).

4.6 The Concept of Redox Zinc Switch

The tight control of free zinc ions is coordinated with their dual functions as either a pro-oxidant or a pro-antioxidant. While zinc ions are redox neutral, zinc-cysteine bonds in proteins generate redox-active coordination environments (Maret 2008). Metallothionein (MT) is an intracellular chelator of zinc and it represents the first example of a redox-active zinc protein, which changes its zinc load in response to the cellular redox state. Under certain conditions linked to cellular zinc deficiency MT may work as a donor of zinc ions to provide protection of vital enzymatic functions during stress conditions.

The concept of «Redox Zinc Switch» has been presented by us previously in detail (Korichneva 2006). The central position of zinc in the redox signaling network is built on its unique chemical nature. In proteins, zinc can bind mainly to cysteine, histidine, aspartate, and glutamateresidues. Cysteines possess the unique structural features that allow them to combine metal binding properties with catalytic activity and extensive redox chemistry. The redox inert zinc creates a redox active environment when it binds to a sulfur ligand. Within the zinc coordination center, zinc ions potentially can modulate the reactivity of cysteine thiol groups towards oxidation. Interdependency of these three aspects permits the redox regulation of proteins, metal control of redox activity, and redox control of metal-based catalysis. The most important and extraordinary property of zinc–sulfur ligand interaction is the release of zinc in an oxidative environment, converging redox signaling and zinc metabolism. Cysteine-rich zinc binding protein domains are therefore able to act as "redox zinc switches' to sense the concentrations of both zinc and oxidants. Therefore, unlike thiol oxidation/reduction, "redox zinc switches" are controlled by zinc availability. Remarkably, until recently it was assumed that the redox inert zinc protects thiolates from oxidation. However, studies suggest that a strong binding of zinc enhances the redox sensitivity of thiolates (Maret and Vallee 1998.).

The unsurpassed study illustrating the mechanism of the "redox zinc switch" operation was conducted in a relatively simple cell model of the bacterial chaperone Hsp-33. Hsp33 contains a C-terminal zinc finger domain that modulates protein activity by a redox-regulated, reversible hinge (Jakob et al. 2000.). In an oxidizing microenvironment, thiols were converted to disulfide, zinc became uncoupled, and the conformational unfolding of the protein produced an active enzyme. The reduced form in the presence of zinc was inactive. The solution structure of a recombinant 61–residue protein containing zinc-binding domain of Hsp33 suggests that loss of the bound zinc ions disrupts the well-folded structure, allowing the corresponding cysteine residues to be oxidized (Barbirz et al. 2000.). This finding implies that the redox response of zinc–cysteine clusters of Hsp33 is biphasic and emphasizes a separate role for zinc in the redox control mechanism (Won et al. 2004).

This more than a decade-old fundamental study was recently substantiated by another group that has presented corroborating data. In the theoretical model study each reaction step was characterized by its Gibbs free energy barrier (ΔG). It is predicted that the first reaction step consists in the oxidation of Cys_{263} by H_2O_2 which

is by far the most reactive cysteine ($\Delta G = 15.4$ kcal mol^{-1}). The next two reaction steps are the formation of the first S-S bridge between Cys_{263} and Cys_{266} ($\Delta G = 13.6$ kcal mol^{-1}) and the oxidation of Cys_{231} by H_2O_2 ($\Delta G = 20.4$ kcal mol^{-1}). It is then shown that the formation of the second S-S bridge (Cys_{231}-Cys_{233}) before the zinc release is most unlikely ($\Delta G = 34.8$ kcal mol^{-1}). Instead, the release of zinc just after the oxidation of the third cysteine (Cys_{231}) is shown to be thermodynamically (dissociation Gibbs free energy $\Delta G\, d = 6.0$ kcal mol^{-1}) and kinetically (reaction rate constant $K_d \approx 10^6$ s^{-1}) favored. This result is in good agreement with the experimental data on the oxidation mechanism of Hsp33 zinc center available to date (Enescu et al. 2015).

Other types of redox zinc regulations have been discovered further demonstrating the uniform nature of the concept. The universal minicircle sequence-binding protein (UMSBP), a CCHC-type zinc finger protein, has been implicated with minicircle replication initiation and kDNA segregation. Interactions of UMSBP with origin sequences in vitro have been found to be affected by the protein's redox state (Sela et al. 2008).

Redox regulation is found to be exercised by a zinc finger (ZF) in a linker that connects the catalytic domain of I-TevI to the DNA binding domain. Four cysteines coordinate Zn^{2+} in the ZF, which ensures that I-TevI cleaves its DNA substrate at a fixed distance, 23–25 nucleotides upstream of the intron insertion site. The fidelity of I-TevI cleavage is controlled by redox-responsive Zn^{2+} cycling (Robbins et al. 2011). The molecular players and targets of zinc homeostasis are summarised in Fig. 4.2.

4.7 Pathophysiological Consequences of Zinc Deregulation

Based on the chemical nature of zinc and its tight interaction with cellular molecules, the pathologies developing as a consequence of zinc dyshomeostasis (zincopathies) are closely connected to redox imbalance, oxidative stress and inflammation.

4.7.1 Age Related Zinc Deficiency and Immunosenescence

The immune system is the first candidate whose function depends on zinc. Indeed, studies revealed important roles of zinc signals in lymphocyte development and in innate and adaptive immune responses (Feske et al. 2015). The impact of zinc on the immune system is well documented in the elderly, where zinc deficiency is a common condition (Maywald and Rink 2015). It is still unclear if zinc deficiency may be considered an integrative factor in immunosenescence, the decline of the immune system with aging, but it is clearly demonstrated that both phenomena are strictly related. Considering the pivotal role of zinc signalling in the function of all cells of the immune system, it is not surprising that immunosenescence affects zinc homeostasis (Lee et al. 2008; Wong et al. 2013).

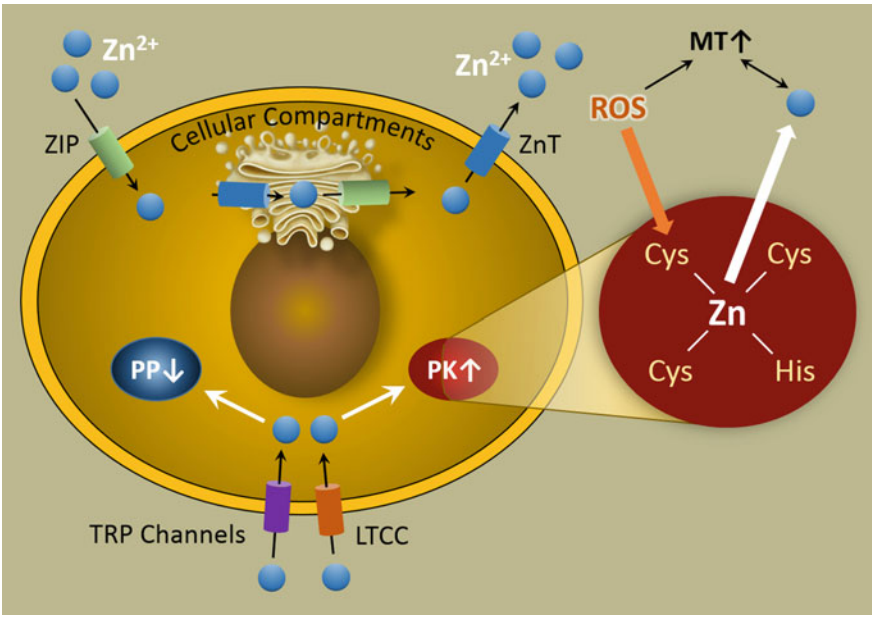

Fig. 4.2 Molecular players and targets of zinc homeostasis. Zn^{2+} entry into cells and liberation from intracellular compartments are mediated by the ZIP transporters, SLC39 family members. Cellular Zn^{2+} efflux and uptake by intracellular compartments is accomplished by ZnT transporters, members of the SLC30 gene family. Additional major routes of zinc entry from the extracellular space into most cells are L-type calcium channels (LTCC) and TRP channels. An increase in intracellular Zn^{2+} plays a signaling role by decreasing protein phosphatases (PP) and increasing protein kinases (PK). The expanded insert shows the underlying molecular mechanism of the Redox zinc switch: oxydation of one or more cysteines in the zinc finger of Ser/Thr kinases in the regulatory domain by reactive oxygen species (ROS) trigger the release of zinc ions with potential recapture upon reduction, or sequestration by metallothionein (MT), which is induced by both ROS and Zn^{2+}

There is evidence that plasma zinc levels and the capacity to maintain zinc homeostasis decline with aging as a consequence of age-related physio- and pathological changes, which may be aggravated by poor nutritional intake and genetic predisposition (Giacconi et al. 2015, 2017a, b; Mocchegiani et al. 2008a, b; Prasad et al. 1993). This condition, which may be described as "age-related zinc deficiency" (ARZD), is thought to contribute to the status called inflamm-aging (Costarelli et al. 2010), a component of immunosenescence consisting in a global reduction in the capacity to cope with a variety of stressors and a concomitant progressive increase in proinflammatory status (Franceschi et al. 2000). ARZD can also contribute to important changes in adaptive immunity including a shift of the T helper (Th) cell balance towards a Th2 response, a non-specific pre-activation of T cells and a decreased response to vaccination (Maywald and Rink 2015). Changes in innate immunity that may be aggravated by ARZD comprise a decreased Natural killer (NK) cell cytotoxicity (Mocchegiani and Malavolta 2004) as well as impaired phagocytosis and

maturation of dendritic cells (Stafford et al. 2013). Interestingly, the major clinical outcomes of ARZD consist in a higher incidence of infections, neoplasia and autoimmune diseases, all of which have been described as common consequences of immunosenescence itself. Hence, it is likely that ARZD and immunosenescence may not be simply linked but that they rather constitute different aspect of the same phenomenon.

4.7.2 Zinc Deregulation and Cardiovascular Diseases

The association between zinc deficiency and cardiovascular diseases (CVDs) has been supported by a multitude of studies (Beattie and Kwun 2004; Little et al. 2010; Beattie et al. 2012) and confirmed by various meta-analyses. A meta-analysis performed in 453 subjects from 27 case-control studies indicates that there is a significant association between low serum zinc levels and heart failure (Yu et al. 2018). Another meta-analysis performed in 2886 subjects from 41 case-control studies support that zinc deficiency is associated with myocardial infarction (Liu et al. 2015). A meta-analysis was also performed (including 14,515 subjects from 24 studies) to investigate the effects of zinc supplementation on serum lipid levels. The results suggest that zinc supplementation significantly reduced total cholesterol, LDL cholesterol and triglycerides with an overall favorable effect on plasma lipid parameters related to the risk of atherosclerosis (Ranasinghe et al. 2015).

A definitive understanding of the cellular basis for the impact of low zinc status on cardiovascular diseases has proved intractable over many years but recently, the true complexity of zinc metabolism and its consequences on cellular function has been appreciated. It is now clear that free zinc ion fluxes between extracellular, cellular and intracellular compartments initiate cell signaling pathways having consequences that are disproportionately larger than the changes in the free zinc ion concentration and that may initiate events related to the onset and progression of CVD.

One of such mechanisms involves the Nitric Oxide (NO) pathway. Indeed, zinc ions are necessary for the dimerization of endothelial NO synthase (eNOS) and the production of NO (Zalewski et al. 2018), which plays an important role in the protection against the onset and progression of cardiovascular disease. Moreover, NO is able to displace zinc from MTs and can also mobilize zinc from other internal stores, thus producing intracellular zinc signals that are responsible for a cascade of events culminating in cytoprotection and vasodilation. This could be the reason why various CVDs risk factors (such as aging, smoking and diabetes) are also known to disturb zinc homeostasis.

Another mechanism related to zinc dyshomeostasis and CVDs could be the accumulation of endothelial senescent cells. Senescent endothelial cells are not only present in human atherosclerotic lesions (Minamino et al. 2002), but they have been described as crucial factors in promoting the pathology (Childs et al. 2016; Fyhrquist et al. 2013). Both transgenic and pharmacological approaches to clear senescent cells have provided preclinical evidence that selective ablation of these cells can

contribute to ameliorate the pathological profile (Childs et al. 2016). The influence of zinc homeostasis on endothelial cell senescence appears as an important mechanism to explain the molecular basis of the association between zinc dyshomeostasis, zinc deficiency and atherosclerosis. Indeed, chronic stimulation with zinc "in vitro" (which may eventually correspond "in vivo" to a repeated mobilization of free zinc ions by chronic oxidative stress) promotes the entry of endothelial cells into senescence (Malavolta et al. 2017). Similarly, Angiotensin II induces senescence in vascular smooth muscle cells by reducing the zinc exporters ZnT3 and ZnT10 and increasing intracellular zinc (Patrushev et al. 2012;). Importantly, senescent endothelial cells show disrupted zinc homeostasis, which may be related to the susceptibility of senescent cells to undergo cell death (Malavolta et al. 2017a). Indeed, "in vitro" zinc depletion leads to a preferential apoptosis in young replicating cells rather than in senescent endothelial cells, thus suggesting a potential mechanism of accumulation of senescent cells during zinc deficiency.

The increased apoptosis of replicating cells in condition of zinc deficiency could be related to the production of a novel discovered zinc-regulated factor (Ou et al. 2013). Primary rat vascular smooth muscle cells treated with plasma from zinc-deficient rats (<1 mg Zn/kg) display increased apoptosis (Allen-Redpath et al. 2013) and changes in gene expression that are mediated by a low-molecular-weight (~2-kDa) zinc-regulated humoral factor, whose activity is reversed by repletion of zinc (Ou et al. 2013). Hence, both apoptosis of normal endothelial and vascular cells as well as increased resistance to apoptosis of senescent endothelial cells may contribute to the development of the pathology during zinc deficiency.

Moreover, zinc is a wound-healing agent, and we believe it may support survival of cardiac stem cells that are essential components of cardiac healing (Little et al. 2010). Last but not least there is convincing evidence that zinc deregulation can reduce antioxidant defense, in particular those provided by MTs, thus contributing to the development of diabetes (as described in details in the next chapter), which is a major risk factor in CVD.

Understanding of these mechanisms will lead to the development of new therapeutic modalities for the prevention and treatment of cardiac disorders that will be based on zinc manipulations.

4.7.3 Zinc Deregulation and Diabetes

Zinc is particularly important in diabetes research based on its physiological role in insulin-receptor signal transduction (Haase and Maret 2005) and insulin storage and secretion (Figlewicz et al. 1984). Deregulated zinc homeostasis is commonly observed in diabetes mellitus (DM2) and its complications (Giacconi et al. 2017a, b) and genome association studies clearly demonstrate the association of a polymorphism in the coding region of SLC30A8 (ZnT-8) gene [rs13266634; arginine (Arg)/tryptophan $(Trp)_{325}$; from herein Arg325Trp] with the susceptibility to DM2 (Saxena et al. 2007; Zeggini et al. 2007; Fan et al. 2016). The ZnT-8 was initially

described as a β-cell-specific transporter specialized in the transport of zinc into the insulin secretory granules (Chimienti et al. 2004), but further studies have shown that it is also expressed in peripheral blood lymphocytes, in subcutaneous fat tissue, and in pancreatic α-cells, where its overexpression reduces glucagon secretion (Giacconi et al. 2017a, b). The biochemical consequences of the Arg325Trp SNP are under intensive investigation. The first functional studies performed in ZnT-8 KO mice and cells transfected with low- and high- risk alleles suggested that the ZnT-8 Arg risk allele could codify for a poorly active ZnT-8 transporter, which might be responsible for a lower accumulation of zinc into β-cell granules than the Trp allele (Nicolson et al. 2009). However, subsequent studies performed in the human embryonic kidney cells 293 (HEK293 cells) demonstrated that Arg-325 variant is more active than theTrp-325 and shows accelerated zinc transport kinetics (Merriman et al. 2016). This finding is confirmed by further functional and genetic findings showing that rare loss-of-function mutations in ZnT-8 may exert beneficial effects on glucose metabolism by increasing the capacity of β-cells to secrete insulin under hyperglycemic conditions (Kleiner et al. 2018) and are associated with reduced DM2 risk in humans (Flannick et al. 2014). Other functional consequences of the Arg risk allele include an impaired glucose-stimulated insulin secretion (Kim et al. 2011) and increased hepatic insulin clearance (Tamaki et al. 2013), both of which are consistent with the involvement of this SNP in the development of D2 M.

ZnT-8 is also a major target of autoimmunity in type 1 diabetes as the SLC30A8 Arg325Trp variant is an important determinant of autoantibody specificity (Kawasaki et al. 2011).

Experimental and clinical studies also suggest that zinc deficiency might predispose diabetes mellitus and its cardiovascular complications (Mocchegiani et al. 2008a, b). A meta-analysis performed from 52 studies (n = 20,183 diabetic patients) confirm that DM2 is characterized by low zinc status accompanied by increased copper (Sanjeevi et al. 2018). Interestingly an imbalance of copper to zinc ratio is also associated with CVDs in the eldery (Malavolta et al. 2010).

It has been proposed that zinc deficiency might predispose to diabetes and its renal and cardiovascular complications by promoting oxidative stress and inflammation (Zhang et al. 2012; Mylroie et al. 2015; Shen et al. 2008).

In vitro studies performed on endothelial cells demonstrate that zinc depletion cause an increase of oxidative stress markers and an increased activity of NF-kB with the concomitant upregulation of E-selectin and monocyte adhesion (Shen et al. 2008). Conversely, zinc treatment can counteract advanced glycated end products (AGEs)-mediated endothelial dysfunction, enhances eNOS activity, and downregulate NF- kB activation (Zhuang et al. 2012). Other experiments "in vitro" have shown the capacity of zinc to regulate the synthesis of glutathione, to promote the nuclear factor-erythroid 2-related factor 2(Nrf2)-mediated heme oxygenase-1 (HO-1) induction and to enhancing the resistance to inflammation and apoptosis of endothelial cells (Mylroie et al 2015). Nrf2 is a crucial transcription factor in the antioxidant response and promotes the expression of several proteins and enzymes involved in detoxification and antioxidant response, including MTs (Fujie et al. 2016) and various zinc transporter genes (e.g., ZnT-1, ZnT-3, ZnT-6, ZnT-10, and ZIP-3) (Ishida et al.

2016). These results further confirm the existence of a tight relationship between Zn homeostasis and the antioxidant response, thus suggesting that a strict regulation of Zn homeostasis is relevant to prevent vascular damage in DM2. It is likely that MT expression induced by zinc is one of the major protective factors in diabetes. Indeed, increased pancreatic MT expression induced by zinc supplementation was shown to prevent diabetes induced by streptozotocin (Ohly et al. 2000). Moreover, several studies using transgenic technology showed that ubiquitous and tissue-specific MT overexpression can successfully ameliorate diabetic hyperglycemia and CVDs (Giacconi et al. 2018). Other mechanisms, including accumulation of senescent cells, increased apoptosis and eNOS modulation that may be involved in the pathogenesis and consequences of DM2 by zinc deficiency, have been already described in the previous chapter focused on CVDs. Other findings, already extensively reviewed (Giacconi et al. 2018), also support a role for additional zinc transporters (e.g. ZIP2) in the susceptibility to DM2 and in the response to zinc supplementation.

In conclusion, there is widespread support for a pathogenic role of deregulated zinc homeostasis in DM2 which may be useful for the development of novel therapeutic intervention in DM2 or to delay or prevent its cardiovascular complications.

4.7.4 Zinc Deregulation and Neurodegenerative Diseases

The influence of zinc homeostasis in the pathogenesis, prevention and treatment of age-related neurodegenerative diseases, such as Alzheimer's diseases (AD) and Parkinson's disease (PD) is one of the most discussed topics in zinc research. In addition to its considerable role in the stress response and in the functionality of zinc-dependent enzymes contributing to maintaining brain compensatory capacity, zinc may display neuromodulatory activity at excitatory synapses (Mocchegiani et al. 2005; Frederickson et al. 2005). In normal physiological conditions, zinc released from the synaptic vesicles modulates post-synaptic receptors. Synaptically released zinc can modulate the activity of N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors, GABAA and glycine inotropic receptors (Smart et al. 2004). However, in presence of pathological conditions, such as stroke, epilepsy and brain injury it has been documented that an excessive influx of zinc ions into the post-synaptic neurons promotes neuronal death and tissue damage (Szewczyk 2013). Indeed, when mature cortical cell cultures are exposed to several hundred micromolar concentrations of zinc (comparable to those measured "in vivo" during pathological conditions), this essential trace element can act as a potent neurotoxin (Choi et al. 1988). Conversely, treatment with EDTA, a membrane-impermeable chelator of zinc (and other cations except for calcium), reduces the influx of zinc and protect hippocampal neurons by death following transient global ischemia (Calderone et al. 2004). Hence, the regulation of zinc homeostasis in the central nervous system (CNS) is extremely critical for normal brain function. It is thus not surprising that deregulated zinc homeostasis has been implicated in a multitude of neurodegenerative diseases including AD, PD,

depression, schizophrenia and amyotrophic lateral sclerosis (ALS). The regulation of zinc homeostasis in the brain is under the control of ubiquitous and tissue specific MTs, ZnT and ZIP transporters. In this context, the brain specific MT isoform (MT3) seems to be particularly affected by aging (showing a higher increase in aging rats compared to the other isoforms) (Scudiero et al. 2017) and it is a likely candidate to play a role in some age-related neurodegenerative diseases. However, there is currently no clear association of MT3 dysfunction with a specific neurodegenerative disease and the transgenic mouse model of AD Tg2576 shows approximately a 30% decrease of MT3 (normalized to the total protein) in the extracts of whole brain homogenates compared to age-matched non-transgenic mice (Martin et al. 2006). Regarding AD, studies focused on plasma levels of zinc have reported contrasting results (Mocchegiani and Malavolta 2007), but a recent work performed in a large Australian cohort demonstrate that there is no significant difference in serum zinc among healthy controls (HC), mildly cognitively impaired (MCI) or AD subjects (Rembach et al. 2014). Although serum zinc does not seem to be involved in the pathology, there is evidence that zinc dyshomeostasis within the context of neuronal death is associated with major human neurological disorders including AD. A distinctive feature of AD is the abnormal accumulation of amyloid plaques in the neocortex. The exceptional colocalization of amyloid plaques with ZnT-3 expression and zinc in the glutamatergic synapses of the neocortex and the capacity of zinc to induce aggregation of Amyloid-β into amyloid precipitates (Bush et al. 1994) form a strong rationale for the zinc-dyshomeostasis pathological hypothesis of AD (Sensi et al. 2011). Moreover, it has been reported that the total concentration of zinc in the cortex of AD brain can rise during the pathology (Religa et al. 2006). A modern interpretation of the zinc-amyloid hypothesis suggest that amyloid plaques may "trap" zinc thus inducing neuronal depletion of this trace element with consequent cognitive and memory loss. Indeed, in experimental models (ZnT-3 KO mice) of cognitive impairment and experimental mice models of AD, treatment with zinc ionophores (compounds able to mobilize zinc and replenish zinc deficient cells), such as clioquinol or PTB2, may reverse part of the functional defects in memory loss and cognitive performances (Deshpande et al. 2009; Adlard et al. 2008). It is not surprising that these compounds have been used in clinical trials as a potential cure for AD. While the clinical trials with clioquinol have been halted due to serious side effects, those with PTB2 have produced promising results. In particular, a IIa double-blind, randomized, placebo-controlled trial found that the 250 mg dose of PBT2 was well-tolerated, significantly lowered cerebrospinal fluid levels of amyloid-β42 and improved cognition measured by a Neuro-psychological Test Battery (NTB) within 12 weeks of treatment in patients with AD (Faux et al. 2010). An important aspect of the zinc-dyshomeostasis hypothesis of AD is to understand why zinc can interacts with amyloid-β only at advanced age. One hypothesis is that this is promoted by raised extracellular zinc levels with aging (Sensi et al. 2011). This hypothesis could be of particular relevance considering that MTs, the major proteins involved in buffering of zinc, have been reported to decrease in very advanced age (despite their increase in old age) (Mocchegiani et al. 2005). This phenomenon may be related to cellular senescence (Malavolta et al. 2016) or other epigenetic mechanisms (Gabbianelli and

Malavolta 2018) that reflect human aging. The hypothesis that cellular senescence can contribute to zinc dyshomeostasis and its pathological consequences for the aging brain find further support from recent advances in the pathogenic mechanisms of PD. PD is the second most common neurodegenerative disorder after AD and is characterized by α-synuclein aggregation and death of dopaminergic neurons in the substantia nigra. Interestingly, zinc induces dopaminergic neurodegeneration resulting in PD phenotype in experimental models (Kumar et al. 2012) and postmortem studies show an increased zinc accumulation in dopaminergic neurons of PD patients (Dexter et al. 1989). This finding is particular important as excess of extracellular zinc has been shown to induce cellular senescence (Malavolta et al. 2014, 2017a) and there is substantial evidence for an implication of cellular senescence in PD (Chinta et al. 2013). The contribution of cellular senescence in PD pathology can explain the positive results obtained by treatment with piperlongumine in rotenone-induced Parkinson disease models. In these models, piperlongumine was shown to attenuate motor deficits in mice and prevent the loss of dopaminergic neurons in the substantia nigra (Liu et al. 2018a). While the authors did not explore the involvement of cellular senescence, they showed that pipelongumine can be considered among the compounds named senolytics, e.g. compunds that can selectively eliminate senescent cells (Liu et al. 2018b). Hence it can be speculated that the protective effect of piperlongumine in these models of PD could be related to a removal of senescent cells. Further studies addressing zinc homeostasis in experimental models treated with senolytics would be useful to understand the relationship between zinc dyshomeostasis and cellular senescence.

4.7.5 Zinc Deregulation and Cancer

Zinc and zinc signaling are now recognized as important targets in the treatment of cancer (Ziliotto et al. 2018). Zinc ions can act as a second messenger by inhibiting a multitude of tyrosine phosphatases involved in a cascade of signals known to promote aberrant cancer growth. Alteration of zinc transporters and MTs which can be observed in most cancer cells, in particular in pancreatic, prostate and breast cancer cells, affect the normal function of these signaling pathways (Bafaro et al. 2017). For example, in pancreatic cancer an increase in ZIP4 mRNA levels has been shown (Li et al. 2007) while most of the other zinc importers and exporters have been found to be downregulated (Yang et al. 2013). However, a high variability in zinc transporter expression has been reported in different pancreatic cancer tissues and cell lines.

In prostate cancer there is strong evidence for an involvement of zinc deregulation in the pathology. The human prostate contains 3- to 15-fold higher zinc than any other soft tissue, with a concentration raising up to 1500 μM in prostate epithelial cells (Costello and Franklin 2016). This high concentration of zinc is required to (1) inhibit the mitochondrial aconitase, which catalyze the oxidation of citrate in isocitrate, resulting in accumulation of citrate, and (2) to cause the temporary inactivity of

prostatic tissue kallikreins, a subgroup of serine proteases including prostate-specific antigen (PSA) that are responsible for regulating semen liquefaction (Verze et al. 2016). Prostate cancer is frequently characterized by an early and marked decline in the concentration of zinc (Costello and Franklin 2006) as a result of reduced levels of ZIP1 and other zinc importers (e.g. ZIP2, ZIP3 and ZIP4) (Verze et al. 2016). The absence of zinc activates an Akt-p21signaling pathway, which results in the phosphorylation of p21 and its retention in the cytoplasm with subsequent promotion of cell proliferation (Han et al. 2009).

Zinc plays an important role in normal mammary gland function; thus, it is not surprising to find that nutritional zinc deficiency is a risk factor in the development of breast cancer (McCormick et al. 2014). However, in contrast to prostate cancer there is evidence that breast cancer is characterized by higher zinc levels compared with normal breast tissue (Gumulec et al. 2014). Zinc dyshomeostasis characterizes most types of breast cancer cells, but the mechanisms involved in this dyshomeostasis are different for triple negative, HER2 positive and estrogen receptor positive tumors. Triple negative breast cancer expresses high levels of MTs, ZIP4, ZIP10 and ZIP14 and lower levels of ZIP6, ZIP9 and ZIP11 compared to normal tissues (Speers et al. 2017). Estrogen positive breast cancers express high levels of ZIP6 and ZIP8 (Chandler et al. 2016). Moreover, many patients with estrogen positive breast cancer treated with tamoxifen experience cancer relapse due to a drug resistance likely related to further alterations in zinc dyshomeostasis. Indeed, tamoxifen resistant cancer cells display an increase of intracellular zinc compared to non-resistant cells that is mediated by high levels of ZIP7 (Taylor et al. 2008). Removal of ZIP7 also blocks the activation of HER2. Moreover, HER2 positive breast cancer cells also display increased zinc levels. The raised concentration of zinc in these cells mediates the activation of the ERK and Akt kinase signaling pathways and the subsequent cascade of events culminating in cell proliferation and migration (Pisano et al. 2017). In conclusion, understanding cellular zinc homeostasis and signaling in cancer cells appears a promising strategy for the development of new drug targets to be used in the fight against cancer.

4.7.6 Zinc Deregulation and Other Age-Related Diseases

Zinc deficiency and deregulation are related to many other diseases associated with aging. Low serum micronutrient concentrations, including zinc, are an independent risk factors for frailty among disabled older women, and the risk of frailty increases with the number of micronutrient deficiencies (Semba et al. 2006). A high serum Cu to Zn ratio correlates with frailty, impairments in bone density, physical performance and overall mortality in the elderly (Gaier et al. 2012; Mocchegiani et al. 2012; Malavolta et al. 2010). Zinc seems also an important nutrient to prevent or treat sarcopenia, as pointed out in a systematic review considering the role of various mineral (van Dronkelaar et al. 2018). These data suggest that zinc supplementation may be useful to ameliorate the decline of physical performance in the elderly and to

prevent frailty. However, caution must be taken in supplementation studies performed in elderly patients, especially in those with kidney dysfunction. Indeed, a large US population-based cross-sectional study found an association with high zinc intake and increased risk of kidney stone disease (Tang et al. 2012).

Zinc deregulation has been also associated with various respiratory diseases, in particular with cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). CF is caused by genetic mutation of a chloride ion channel, the cystic fibrosis transmembrane conductance regulator (CFTR), while COPD is related to alterations of the epithelial sodium ion channel (ENaC). Both pathologies are characterized by chronic airway inflammation and obstruction, accompanied by exaggerated mucus retention and fluid imbalance. Experiments "in vitro" with patients derived cell lines, have demonstrated that the alterations of ENaC and CFTR that characterize BPCO and CF, respectively, induced a reduction of a specific zinc transporters (ZIP2), causing zinc deficiency in lung epithelial cells (Kamei et al. 2018). In turn, the zinc deficient status activates a cascade of events that culminate in the excessive production of mucus-producing genes (e.g. MUC5AC) in the lungs. These findings establish a clear mechanistic link between COPD, CF, the overproduction of mucus, and zinc deregulation in epithelial cells. While this study offers new therapeutic opportunities, it is important to remark that the defects could not be corrected by a simple zinc supplementation, as the defects arise by proteins involved in the control of zinc homeostasis rather than in a nutritional imbalance.

4.8 Zinc and Longevity from Yeasts and Invertebrates to Humans

Available data on the biology of zinc points out its strong interplay with the insulin and insulin growth factor (IIS) pathway (Malavolta et al. 2017b) Experimental manipulations that upregulate the IIS pathway promote aging, while those that downregulate these pathways extend life span and delay the onset of age-related pathologies.

Experimental models of zinc deficiency and supplementation suggest that zinc promotes the IIS pathways, thus suggesting a pro-aging role which appears to be confirmed by lifespan studies performed in animal models (Malavolta et al. 2017b).

One of the first observation was made from studies on the growth of yeasts exposed to zinc (Steenbergen et al. 1969). Yeast growth was optimal in a medium containing 1 μM Zn, while the presence of additional Zn (from 10 to 100 μM) induced a dose-dependent reduction in the lifespan. Similar findings were replicated in trematodes (cercariae and schistosomules of Schistosoma mansoni) as the longevity of the parasites appears to be inversely proportional to the zinc concentration of the medium (from 0.05 to 5 mM) (Asch and Dresden 1977). In nematodes, the addition of zinc in the medium (from 200 μM up to 500 μM) decreases dose dependently the mean and maximal lifespan, while zinc deprivation (addition of the zinc chelator TPEN from 50 μM up to 200 μM) exerts a beneficial effect (Kumar et al. 2016). The most

important finding of this study is that these results were observed only when zinc treatment was performed during early development while the same treatment performed after five days of adulthood did not affect the lifespan of the worms. The negative effects of zinc on lifespan were mainly mediated by daf16, a downstream target of the IIS pathway and ortholog of the FOXO family of transcription factors in humans. These finding indicate that the negative effects of zinc on longevity are not simply a matter of toxicity, but are likely due to their effects on signaling pathways that promote growth and development. Zinc supplementation also induces a reduction of lifespan in flies, but exerts a beneficial effect (measured as an extension of 12 days of lifespan) in the parkin mutant flies (Saini and Schaffner 2010).

However, transcript levels of the metallothionein MtnB are over-expressed in parkin mutants and MtnB is further increased (24 fold) by zinc supplementation, thus suggesting a different regulation of zinc homeostasis in these mutant flies compared to wild-type flies. It is likely that these mutant flies display a general zinc dyshomeostasis that is corrected by zinc supplementation.

These results are very similar to those obtained in a mouse model of acrodermatitis enteropathica (a human genetic disease of acute zinc deficiency) generated by a conditional knockout of ZIP4. Indeed, in this mutant mouse, high doses of zinc supplementation (250 mg/L in drinking water) exert a strong positive beneficial effect on the lifespan. (Geiser et al. 2012).

Zinc supplementation in normal mice have produced contrasting results. An antioxidant mixture including 5 mg/kg of elemental zinc in the form of gluconate induced a 10–16% increase in the lifespan of C57BL/6 mice when starting the diet at 2 and 9 months of age, but not when starting at 16 and 23 months of age (Bezlepkin et al. 1996). Zinc treatment (22 mg/L in drinking water) starting at age of 18 months in Balb/c mice housed in non-SPF conditions induced a median lifespan extension (from 27 to approximately 30 months) with an increase in 10% of maximal lifespan (from 30 to 33 months) (Mocchegiani et al. 1998). However, a detailed survival study in the long living MT1 overexpressing mice and in the respective controls (C57BL/6 J) under supplementation with a high dose of Zn (380 mg/L) demonstrated no impact on the overall survival (as observed by Kaplan-Meier analys) but a 14% in the S0 parameter of the Piantanelli mathematical model of survivorship (Malavolta et al. 2012), thus suggesting that zinc treatment increased the variability of the response in the mice.

Hence, it might be hypothesized that stochastic and microenvironmental variations regarding zinc homeostatic mechanisms, drinking behaviors of mice, subclinical diseases and infections or other microenvironmental factors related to the non-SPF conditions may affect the individual outcome of zinc supplementation.

In conclusion there is reasonable support to the idea that zinc supplementation early in life, in absence of a well-defined zinc deficiency or zinc dyshomeostasis, may promote growth and development but may negatively affect longevity. This is likely due to the impact of Zn on the IIS signaling pathway which has a well-defined antagonistic pleiotropic function in aging. Conversely, Zn treatment in adult and old organisms seems not to have mean negative effects on lifespan, but the treatment may induce an increased heterogeneity and likely a different response within the same

experimental population. Part of this heterogeneity may be attributed to differences in the machinery that regulates zinc homeostasis and thus zinc can display beneficial effects in particular conditions related to deregulated zinc homeostasis. In this case, restoring a normal zinc homeostasis through pharmacological or genetic intervention could be an interesting approach, but the complexity of the zinc homeostatic machinery makes it difficult to predict the overall outcome.

For example, the mutant drosophila with a defective MTF-1 (the sensor of free zinc in the cell that activates zinc dependent transcription) display shortened lifespan, whereas MTF-1 overexpression results in resistant flies with prolonged longevity on iron or cadmium-supplemented media but shortened life-span on zinc-supplemented medium (Bahadorani et al. 2010).

A relatively clear picture seems to arise from genetic manipulation of MTs. A longevity phenotype has been clearly shown in MT transgenic mice that overexpress the MT-1 isoform (MT1-tg) on a C57BL/6 J genetic background (Malavolta et al. 2012), as well as in cardiac-specific MT transgenic mice that overexpress the human MT2 isoform on an FVB background (Yang et al. 2006), while MT knockout mice in the 129/Sv genetic background have a shorter mean and median lifespan compared to the wild type (Kadota et al. 2015). Regarding humans, a polymorphism in the MT1a gene coding region is reported to be associated with longevity (Cipriano et al. 2006) and that MTs can be involved in the tight control of zinc homeostasis observed in the cells from centenarian offspring (Giacconi et al. 2018). Most importantly, we have recently observed that the number of MT genes in the genome, as well as MT mRNA expression (retrieved from public databases) is associated with the longevity of mammalian species even after correcting for age at sampling, body mass and phylogeny (Pabis and Malavolta, unpublished results).

4.9 Conclusions and Perspective

The relationship between Zn homeostasis, aging and health appears to be very complex and involves direct interactions (e.g. binding to target proteins or transport defects, e.g. zinc deficiency) as well as indirect interactions (e.g. mediated by oxidative stress, inflammation or cellular senescence) that can result in pathological consequences. Current evidence suggests that zinc dyshomeostasis is involved in immunosenescence, CVDs, neurodegenerative diseases, DM2, cancer, frailty and COPD and that proteins regulating zinc homeostasis can promote longevity. To better understand the molecular mechanisms and pathways that lead to imbalance in zinc both on the levels of individual cells and whole organisms, simple models like yeast or flies will help accelerate knowledge acquisition and discovery. The exponential development of molecular probes to assess ionic zinc fluctuations combined with high resolution imaging using synchrotron x-ray fluorescence techniques with help in this task. Future multidisciplinary research by biologists and chemists should yield information on genetic- and epigenetic-based changes in protein players and

their contribution to zinc dyshomeostasis. This will form the basis for prevention and supplemental therapies of age-related diseases.

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Chapter 5
Chromium in Health and Longevity

Ruslana Iskra and Halyna Antonyak

Abstract Trivalent chromium is essential to normal carbohydrate, lipid and protein metabolism. Chromium is biologically active as part of an oligopeptide—chromodulin—potentiating the effect of insulin by facilitating insulin binding to receptors at the cell surface. With chromium acting as a cofactor of insulin, Cr activity in the organism is parallel to insulin functions. Cr(III) can help enhance the role of insulin, the critical hormone that controls blood sugar and helps bring glucose into cells where it's used for bodily energy. Chromium deficiency has been suggested to lead to symptoms associated with adult-onset diabetes and cardiovascular disease, and these supplements have recently found potential as therapeutic agents in the treatment of adultonset diabetes. Cr(VI) is one of the few carcinogenic metals that directly reacts with DNA, forming adducts, and inducing mutations. The results of a wide range of studies indicate that the CpG1 methylation level of p16 could be used as a biomarker of epigenetic effect caused by Cr(VI) treatment, which can enhance cell damage by regulating its expression or affecting some transcription factors to combine with their DNA strand sites. In addition, it is difficult to distinguish between the effects caused by chromium(VI) and those caused by chromium(III) since chromium(VI) is rapidly reduced to chromium(III) after penetration of biological membranes and in the gastric environment. In addition to its role in glucose and lipid metabolism, chromium also functions as an antioxidant. Chromium(III) protects organism from oxidative stress associated with reactive oxygen species. These ROS extremely reactive chemical molecules, are considered toxic to produce oxidative damage to various cellular components which causes cellular dysfunction that accompanies aging process. The antiaging effect of chromium is undoubtedly related to the effect of chromium on insulin action. Chromium in a utilizable form, like dietary restriction, prevents hyperglycemia, hyperinsulinemia, protein glycation and extends life span. Because the body's ability to control blood glucose is critical

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to many life functions, a consequence of Cr supplementation can be improved health and reproductive outcomes as well as improved survival rate or life span.

Keywords Chromium(III) · Absorption · Biological function · Metabolism Toxicity · Diabetes mellitus · Mortality · Life span

5.1 Introduction

Chromium is a naturally occurring element found in animals, plants, rocks, and soil and in volcanic dust and gases. Chromium has oxidation states (or "valence states") ranging from chromium(-II) to chromium(VI). Chromium compounds are stable in the trivalent (III) state and occur in nature in this state in ores, such as ferrochromite. The hexavalent (VI) form is the second-most stable state. However, chromium(VI) rarely occurs naturally, but is usually produced from anthropogenic sources (EPA 1984).

Chromium levels in soil vary according to area and the degree of contamination from anthropogenic chromium sources. Chromium(VI) in soil can be rapidly reduced to chromium(III) by organic matter.

Cr(VI) is a strong oxidant—in the form of chromates and dichromates it penetrates biological membranes and reacts with cell contents, proteins or nucleic acids, while being reduced to Cr(III).

In contrast, Cr(III) is the most stable form in biological systems (Cohen et al. 1993; Losi et al. 1994) it does not penetrate biological membranes easily, and it appears that the transport of specific chromium compounds is strictly regulated by the organism. Cr(III) ion has a strong tendency to form coordination compounds with a very slow reaction rate (Mertz 1992).

That slow rate suggests that chromium would exert a structural function rather than an active site in an enzyme, which may explain that no chromium-containing enzymes have been identified (Mertz 1992).

5.2 Chromium Absorption, Blood Transport and Excretion

The total amount of Cr in the human body ranges between 0.4 and 6 mg. Daily intake strongly depends upon feed levels, and is usually approximately 15–200 μg, but may be as high as 1 mg.

Chromium is present in the diet both as the inorganic form and organic complexes. The rate of absorption of inorganic Cr is low, from 0.4–3%, and is a function of daily dose supplied. According to Anderson (1987) ingestion of daily dose of 10 μg, up to 2% is absorbed, while at the dose of 40 μg, absorption decreases to 0.5%, and at the higher doses, it remains constant at 0.4%. The absorption of Cr from CrCl3 and acetate (Mertz 1975) is approx. 0.5%, and approx. 40% for chromium

trisacetylacetonate in rats (Anderson 1987). Chromium chloride, chromium pinacolinate and chromium polynicotinate are the most common supplemental sources available. Although absorption of chromium picolinate is lower than 4%, it is still significantly greater than that of chromium chloride (Mertz 1975). There is also a report claiming that niacin-bound chromium was 672% better absorbed than chromium chloride and 311% better than chromium picolinate (Krejpcio 2001; Madhavi et al. 2013).

The absorption of Cr is facilitated by certain amino acids, such as histidine, which chelates Cr and prevents the precipitation of Cr at the basic pH in the small intestine (Mertz 1969; Mertz and Roginski 1971). Nicotnic acid and ascorbic acid are required for Cr absorption and act in synergy with this element. Ascorbic acid has been reported to enhance chromium transport or absorption in animals (Dowling et al. 1990) and humans (Mertz and Roginski 1971).

Compared with simple sugars such as glucose, fructose and sucrose, starch increased tissue chromium in mice (Dowling et al. 1990). Metals can form complexes or compete with Cr and modify its absorption. For example: Zn, V and Fe supplementation decreased the absorption of Cr (Chen et al. 1973). On the other hand, absorption of 51Cr was elevated in Zn-deficient rats and was reduced by zinc supplementation (Hahn and Evans 1975). Phytates significantly decrease the absorption of Cr in the intestines of rats, whereas oxalate act inversely (Chen et al. 1973).

Chromium is absorbed in the intestinal mucosa. In rats the middle section of the small intestine was the most active segment for Cr absorption, followed by the illeum and duodenum (Chen et al. 1973). In humans, the site of absorption also includes the jejunum (Doisy et al. 1976). The mechanism responsible for the intestinal absorption of Cr is not known.

Absorbed Cr circulates in blood bound to the β-globulin plasma fraction and is transported to tissues bound to transferrin or other complexes at the physiological concentration.

Trivalent Cr tends to accumulate in epidermal tissues (hair etc.) and in bones, liver, kidney, spleen, lungs and the large intestine. Accumulation in other tissues, especially muscles, seems to be strictly limited or non-existent (Wallach 1985). The placenta is the organ with the highest chromium amounts.

The Cr reserve relative to the body weight is higher in newborn children compared with adults (Dubois and Belleville 1991). The concentration of Cr in the lungs, aorta, heart and spleen decreases during the first months of life, whereas the liver and kidneys maintain their neonatal level up to the age of 10 years.

Absorbed Cr is excreted principally in the urine, and in small quantities in the hair, sweat and bile. The major route of elimination after absorption is faecal. Urinary excretion is the major route of elimination of Cr from the body, is a good reflection of the ingestion, but not necessarily of body status (Anderson et al. 1983).

5.3 Biological Function of Chromium

The biological function of chromium is not fully known yet. It is postulated that chromium interacts with the thyroid metabolism in humans. Binding of Cr(III) with nucleic acids has been found to stimulate the DNA-dependant RNA synthesis (Mertz 1992). The third interaction of Cr(III) is with the hormone insulin and its receptors. This suggests that Cr(III) acts with insulin on the first step in the metabolism of sugar entry into the cell, and facilitates the interaction of insulin with its receptor on the cell surface.

Older research (Schwarz and Mertz 1957, 1959) associated Cr activity in animal organisms with a substance called the glucose tolerance factor (GTF), whose active substance is Cr. Further research into the GTF however revealed that GTF activity does not correlate with Cr content (Simonoff et al. 1992; Lindemann et al. 2009). According to the latest research, GTF activity is not dependent on a unique Cr compound and the complexation of Cr by yeast is more likely simple ligand substitution by components in the growth medium. Attention has thus recently turned to chromodulin and it has been proposed that GTF is merely a decomposition product of this true biologically active form of chromium. In the 1980s, Wada et al. (1983) reported they had isolated a chromium-binding oligopeptide called the low-molecular-weight chromium binding substance—LMWCr or chromodulin. The molecular weight of the oligopeptide is ~1500 Da and it is formed by 4 types of amino-acid residues (glycine, cysteine, glutamate and aspartate). Despite its low molecular weight, it binds 4 equivalents of chromic ions in a complex of four nuclei. This oligopeptide has been isolated and purified from rabbit liver (Yamamoto et al. 1987), pig kidney (Sumrall and Vincent 1997), cattle kidney (Davis and Vincent 1997b) and colostrum (Yamamoto et al. 1983), dog liver (Wada et al. 1983) and isolated from mouse and rat kidneys (Yamamoto et al. 1983). Chromodulin is present in mammals; no paper dealing with isolation of this oligopeptide in other animal species has been published yet. The assumed mechanism for the action of chromodulin has been described by Vincent (2000; Fig. 5.1). Increased glucose concentration leads to the fast release of insulin into blood. Insulin binds to an external α subunit of the transmembrane protein insulin receptor, causing its conformation change. The receptor autophosphorylates tyrosine residues on the internal portion of its β subunit, turning the receptor into an active kinase. Chromodulin is stored in its apo-form (apochromodulin) in the cytosol (Yamamoto et al. 1989) and the nucleus of insulinsensitive cells. Increases in plasma insulin concentrations have been found to result in a movement of chromium from the blood to insulin-dependent cells (Morris et al. 1993a, b). Due to the high Cr ion binding constant of apochromodulin ($K \approx 1021$) (Sun et al. 2000), four Cr^{3+} are bound upon the entry of Cr into the cell, producing holochromodium (i.e. Cr4-chromodulin). The newly formed compound is bound to insulin-stimulated receptors, helps maintain their active conformation and enhances insulin signalling. When the level of insulin in blood decreases and receptor signalling must be interrupted chromodulin is eliminated from the cells. The high Cr-binding constant suggests that Cr might not be released from chromodulin to regenerate the apo form as formation of

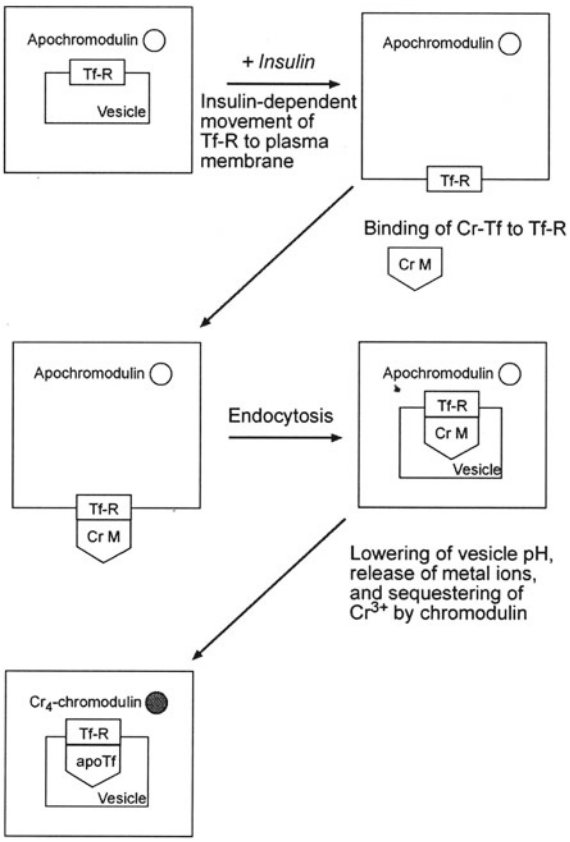

Fig. 5.1 Proposed mechanism for the movement of chromium from the blood to LMWCr (Vincent 2000, 2007). Cr—chromic ion; M—metal ion; Tf—transferrin; Tf-R—Tf receptor

the apo-oligopepetide from holochromodulin requires chelating agent activity at a low pH and increased the temperature, this is not possible if physiological conditions are to be preserved (Davis and Vincent 1997a). Loss of chromodulin from cells is consistent with increased Cr concentration in urine following the intake of carbohydrates (Anderson et al. 1982; Lindemann et al. 2009); chromodulin seems to be the main form of Cr^{3+} presence in urine.

5.4 The Role of Chromium in the Metabolism

5.4.1 Metabolism of Carbohydrates

The association between Cr and carbohydrate metabolism has been demonstrated by trials involved with results in people fed parenteral nutrition. Jeejeebhoy et al. (1977)

have published the results of a trial on women kept at parenteral nutrition for 5 years. The patients developed symptoms of diabetes together with a significant glucose intolerance and loss of weight. Insulin therapy was not efficient and it was only after supplementation of 250 μg of Cr that the state of the patients started to improve and further insulin therapy became redundant. Also, syndromes similar to diabetes mellitus, which improved significantly after Cr supplementation, have been described showing an association between reduced sensitivity of peripheral tissues to insulin and Cr deficiency (Anderson et al. 1996). The current study aimed to examine the associations of plasma chromium levels with T2DM and pre-diabetes mellitus (pre-DM) demonstrated an inverse association between plasma chromium concentrations and T2DM and pre-diabetes in a Chinese population (Chen et al. 2017). Improved glucose tolerance was however not observed in all trials. The lack of Cr deficiency or some other etiological factors may provide an explanation. A number of human studies (Anderson 2000a; Tuzcu et al. 2004), studies on pigs (Wenk et al. 1995), horses (Pagan et al. 1995; Ott and Kivipelto 1999), cattle (Subiyatno et al. 1996; Bunting et al. 1994) and rats (Kim et al. 2004) have confirmed the possibility of influencing glucose tolerance and insulin resistance by Cr supplementation. Supplementation of Cr and insulin to animal tissues in in vitro experiments has led to increased glucose oxidation, resulting in $CO_2 + H_2O$ formation, increased glycogenesis and conversion of glucose to lipids, all this was in combination with increased glucose utilisation (Anderson 1997). We have established that the action of chromium chloride and organic compounds of chromium content of glucose goes down and rises activity of hexokinase and lactate dehydrogenase (Iskra 2011; Iskra et al. 2014).

5.4.2 Metabolism of Lipids

Numerous studies show evidence that Cr is essential for lipid metabolism and reducing the risk of atherogenesis. For example, rats and rabbits fed on a Cr-deficient diet had increased total cholesterol and aortal lipid concentrations and showed increased plaque formation (Abraham et al. 1982; Pechova and Pavlata 2007). Cr supplementation has decreased the total cholesterol in their blood. An increase of HDL-cholesterol (Riales and Albrink 1981) and a decrease in total cholesterol, LDL-cholesterol and triacylglycerols (Lefavi et al. 1993) have been observed in humans after Cr supplementation.

It was established that the addition of chromium (250 mcg/kg) level in the ration of piglets leads to the decline of cholesterol content in blood plasma (Iskra 2010; Iskra et al. 2014).

Niacin, or vitamin B3, tends to reinforce chromium's beneficial effects, especially on the lipid profile, both raise levels of "good" HDL while lowering "bad" LDL and triglyceride levels (McKenney 2004).

These results are in agreement with other research (Lifschitz et al., 1980; Mossop 1983). On the other hand, Cr supplementation was not proven to have any effect in other human trials (Anderson et al. 1983; Rabinowitz et al. 1983; Offenbacher et al.

1985; Potter et al. 1985; Uusitupa et al. 1992). These ambiguous results concerning the response of blood lipids and lipoproteins to Cr supplementation may be due to differences in the Cr supplementation of different individuals. Similarly, these studies mostly ignored other main dietary factors directly impacting upon the lipid metabolism.

5.4.3 Metabolism of Proteins

It is assumed that the activity of Cr is mediated by the anabolic action of insulin, but other mechanisms cannot be ruled out (Pechova and Pavlata 2007). Evans and Bowman (1992) have demonstrated increased amino acid and glucose uptake by skeletal muscles of rats that had been incubated with Cr-picolinate. This alteration in uptake of nutrients was associated with the alteration of insulin parameters and is Cr-dependent. These observations may explain the effect of glucose tolerance as well as the increase in the percent of skeletal muscle reported by some researchers. The potential improvement of amino acid uptake by muscle cells is beneficial to the total protein deposition. Roginski and Mertz (1969) claim that Cr supplementation intensifies the incorporation of amino acids into heart proteins and amino acid uptake by tissues in rats.

It was established that the addition of chromium (250 μg/kg) level in the ration of piglets leads to increase of protein content in blood plasma (Iskra 2010; Iskra et al. 2014).

5.4.4 Chromium and Body Composition

Another effect of chromium supplementation that could be a result of its potentiation of insulin sensitivity is the redistribution of body fat, protein and water. The mechanism of this regulatory action of Cr is not known.

It has been proposed that the positive effect of chromium picolinate on body composition is through its ability to improve insulin use, thereby reducing fat deposition and improving entry of glucose and amino acids into muscle cells.

Chromium has been reported to increase lean body mass in people who exercise, such as football players, however, some follow-up studies have not supported these observations (Anderson 2000b). However, the role of chromium in the regulation of lean body mass, percentage body fat, and weight reduction is still controversial because a significant number of studies do not support the effect of chromium on body composition.

5.4.5 Metabolism of Nucleic Acids

Trivalent Cr seems to be involved in the structure and expression of genetic information in animals. The binding of Cr to nucleic acids is stronger than in other metal ions (Okada et al. 1982). Chromium protects RNA from heat denaturation. It is also clear that Cr is concentrated in cell nuclei. Cr has increased in vitro RNA synthesis in mice (Okada et al. 1983); this supports the hypothesis that Cr has an effect on gene functions. Chromium participates in gene expression by binding to chromatin, causing an increase in initiation loci and consequently, an increase in RNA synthesis. This increase is due to the induction of protein bound in the nucleus and nuclear chromatin activation (Okada et al. 1989).

5.4.6 Metabolism of Mineral Substances

There are relatively few papers on the effect of Cr supplementation on the metabolism of other mineral substances. The relation between Cr and Fe has been investigated most since both these minerals are transported as transferrin-bound. Cr is bound to transferrin that possesses two binding sites: A and B with different affinities for Fe as a function of pH. At low Fe saturation, Cr and Fe bind preferentially to different binding sites. It has been shown that Cr binds exclusively to site B. When, however, the Fe concentration is higher, the two minerals compete for the same binding sites. Thus, there is antagonism between Cr and Fe competing for this carrier (Sayato et al. 1980).

This seems to be the reason why a lower Cr retention has been identified in patients suffering from hemochromatosis than in healthy subjects or patients with a Fe deficiency (Sargeant et al. 1979). Evidence that Cr may impair Fe metabolism has been published by Ani and Moshtaghie (1992). Fe homeostasis alteration has been reported by other authors too, the most significant alteration being detected in association with Cr-picolinate supplementation (Lukaski et al. 1996). Alteration of Fe metabolism in association with Cr supplementation has also been reported by Anderson et al. (1996), decreased tissue Fe concentrations was detected in response to Cr supplementation.

Mineral metabolism in experimentally induced Cr deficiency, using goats, has been explored in detail by Frank et al. (2000a, b) on the basis of deter mining Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Se, Sr, V and Zn concentrations in the liver, kidneys, ribs and blood plasma. They detected a renal Cu concentration 43% lower compared with controls and conversely, higher Al, Co and V concentrations in the kidneys and liver. The authors attribute the increased concentrations of these minerals to a decreased Cr concentration causing subsequent freeing of binding sites on the transferrin, competed for by the individual minerals. A decreased loss of some microelements (Zn, Fe, Cu and Mn) during stress after Cr supplementation to mice has been reported by Schrauzer et al. (1986). Interaction between Cr and Cu

was studied by Stahlhut et al. (2006), Cr supplementation had no effect on the liver or plasma Cu concentrations in cows, although, supplemental Cr resulted in higher plasma Cu concentrations in calves on Day 279. Similarly Pechova et al. (2002) have detected higher plasmatic Cu concentrations in response to Cr supplementation in fattening bulls. Interactions between Cr, Ca and Mg have been reported by Moonsie-Shageer and Mowat (1993), who found Cr supplementation to be associated with Ca and Mg concentration increases on Day 7 of the trial.

5.5 Chromium and Lipid Peroxidation

In addition to its role in glucose and lipid metabolism, chromium also functions as an antioxidant. Recent studies have shown that chromium protects rats from oxidative stress associated with exposure to CCl_4. Also chromium protects against lipid peroxidation in isolated rat hepatocites (Vincent 2007; Anderson 2000a), and decreases the effects of free radicals in people with type 2 diabetes (Roussel and Zouari 1998).

We found that the action of chromium compounds decreases products of lipid peroxidation and increase of activity of the antioxidant system enzymes in blood of rats (Iskra 2011; Iskra et al. 2014). Addition of chromium citrate in a quantity of 25 μg Cr/kg body resulted to the decreased content of the MDA, activity of superoxide dismutase, but increased activity of catalase, glutathione peroxidase, glutathione reductase and the content of reduced glutathione compared to their levels in animals from group II with an experimental diabetes (Iskra and Slivinska 2015).

Chromium(III) protects organism from oxidative stress associated with reactive oxygen species.

Based on free radical theory by Harman (1956), free radicals, which are reactive oxygen species (ROS) produced in metabolic pathways, may play a critical role in aging. These ROS extremely reactive chemical molecules, are considered toxic to produce oxidative damage to various cellular components which causes cellular dysfunction that accompanies aging process. The mitochondrial respiration defined as the main source of ROS, which primarily produce cumulative damage to lipids, proteins and mitochondrial DNA and lead to cellular aging (Hoopes 2002; Piotrowska and Bartnik 2014).

However, recent workers in this and in related fields are exploring the view that superoxide radical and reactive oxygen species exert beneficial effects. Thus, such ROS are viewed as involved in cellular regulation by acting as (redox) signals, and their harmful effects are seen mostly as a result of compromised signaling, rather than due to direct damage to sensitive targets. According to some followers of this view, ROS such as hydrogen peroxide and superoxide are not just causative agents of aging but may also be agents that increase the life span by acting, for example, as prosurvival signals (Liochev 2013).

One of the controversies surrounding the use of Cr(III)-containing nutritional supplements concerns the proposed roles of such supplements as antioxidants that reduce the diabetes-related oxidative stress (Cheng et al. 2004; Vinson et al. 2002;

Peng et al. 2014), or pro-oxidants that promote the oxidative stress through the formation of ROS (Petit et al. 2005).

The dual action of Cr(III) as an antioxidant or a pro-oxidant, can be explained based on the redox reactions The reactions of Cr(III) complexes with lipid peroxides are probably responsible for the abilities of these compounds to reduce the levels of lipid peroxidation (Cheng et al. 2004; Vinson et al. 2002), but these reactions produce other strong oxidants such as Cr(V) species. These species, as well as the peroxyl (ROO·) radicals formed in the redox cycling reactions of certain Cr(III) complexes, are probably responsible for the increases in the oxidative stress markers caused by Cr(III) administration (Medeiros et al. 2003; Petit et al. 2005). Thus, Cr(III) complexes used as nutritional supplements are involved in a delicate balance between the oxidation and the reduction reactions in blood plasma, as shown most clearly by a change from a mild antioxidant effect of a prolonged treatment with Cr(III) in type II diabetic patients to a mild pro-oxidant effect of the same treatment protocol in healthy individuals (Cheng et al. 2004).

5.6 Epigenetic and Toxicological Effects of Chromium

Cr(VI) compounds induce human respiratory cancers and increase the risk of other types of human cancers (Costa and Klein 2006). Chronic poisoning by chromium compounds has been observed at the work place, by direct contact with skin and mucus membrane, or inhalation of dusts or aerosols (Ducros 1992). Because Cr is a potent sensitizer, external contacts with chromates and dichromates can induce allergic eczema in some people (Taton 1993). Reports from the chromate production industry have identified Cr(VI) as a potential carcinogen (Katz and Salem 1994).

The International Agency for Research on Cancer (IARC) has determined that chromium(VI) compounds are carcinogenic to humans. The National Toxicology Program 11th Report on Carcinogens classifies chromium(VI) compounds as known to be human carcinogens.

In laboratory animals, chromium(VI) compounds have been shown to cause tumors to the stomach, intestinal tract, and lung (Wilbur et al. 2012). Acute inhalation LC50 values in rats for several chromium(VI) compounds (sodium chromate, sodium dichromate, potassium dichromate, and ammonium dichromate) ranged from 29 to 45 mg chromium(VI)/m^3 for females and from 33 to 82 mg chromium(VI)/m^3 for males (Gad et al. 1986). Female rats were more sensitive than males to the lethal effects of most chromium(VI) compounds except sodium chromate, which was equally toxic in both sexes. Signs of toxicity included respiratory distress, irritation, and body weight depression (Gad et al. 1986).

Cr intoxication is characterised by pathologicalanatomical changes in the kidneys and liver. Acute intoxication with Cr^{6+} leads to acute renal tubular necrosis characterised by significant interstitial change and subsequent renal failure (Ellis et al. 1982; Saryan and Reedy 1988). Renal glomeruli usually remain intact. The hepatic parenchyma develops necrosis only at very high Cr^{6+} doses.

The reaction with genetic material is the basis for the carcinogenicity of some Cr(VI) salts. Cr(VI) is one of the few carcinogenic metals that directly reacts with DNA, forming adducts, and inducing mutations (Zhitkovich et al. 1996). There are a number of studies demonstrating that Cr(VI) carcinogenesis involves gene silencing and other epigenetic effects (Sun et al. 2009; Salnikow and Zhitkovich 2008). Cr(VI) has been shown to prevent the expression of inducible genes in cells by crosslinking a histone deacetylase to inducible promoters (Wei et al. 2004). The presence of this deacetylating enzyme, which removes acetyl groups from lysines in histone tails, keeps the nucleosome condensed, thereby preventing transcription factors from binding and activating gene expression (Wei et al. 2004). In order for cells to survive chronic Cr(VI) treatment, they must adapt and evade apoptosis. The loss of apoptotic activity is often accompanied by a loss of mismatch repair, since the two processes are tightly linked. Consistent with the latter, chronic exposure of cells to Cr(VI) or tumors induced by this agent in humans are often missing mismatch DNA repair capacity (Takahashi et al. 2005; Peterson-Roth et al. 2005; Reynolds and Zhitkovich 2007).

Cr(VI) exposure leads to silencing of MLH1, a component of mismatch repair, via a decreased mRNA expression resulting from enhanced H3K9 dimethylation of its promoter (Sun et al. 2009). In human lung cancers induced by Cr(VI) exposure, silencing of *MLH1* as well as tumor suppressor *p16* was correlated with DNA methylation of their promoters (Takahashi et al. 2005; Kondo et al. 2006; Ali et al. 2011).

The results of a wide range of studies indicate that the CpG1 methylation level of p16 could be used as a biomarker of epigenetic effect caused by Cr(VI) treatment, which can enhance cell damage by regulating its expression or affecting some transcription factors to combine with their DNA strand sites (Hu et al. 2016a, b).

In spite of considerable research effort, the epigenetic mechanisms of Cr(VI)-induced carcinogenesis remain largely unknown.

Nupr1 (nuclear protein 1) is a small, highly basic, and unfolded protein with molecular weight of 8800 daltons and is induced by a variety of stressors. Studies in animal models have suggested that Nupr1 is a key factor in the development of lung and pancreatic cancers, with little known about the underlying molecular mechanisms (Chen et al. 2016). Nupr1 mRNA is strongly induced by a variety of stressors such as lipopolysaccharides (Jiang et al. 1999), CCl_4 (Taieb et al. 2005), starvation (Zinke et al. 2002), cell cycle arrest and many others. Overexpression of Nupr1 has been implicated in a number of cancers. Nupr1 enhances the expression of at least two major epithelial-mesenchymal transition (EMT)-related genes, namely MMP9 and MMP13 metalloproteases (Goruppi et al. 2007). Downregulation of H4K16ac is likely a mechanism whereby Nupr1 promotes cancer development. Recent study has demonstrated that Nupr1 overexpression inhibits acetylation of lysine 16 of histone H4 (H4K16ac) (Gironella et al. 2009) and the histone acetyltransferase MOF (Kat8, Myst 1), which specifically acetylates H4K16 (Smith et al. 2005). The loss of H4K16ac and MOF correlate with increased genome instability, which is considered an important step in cancer development (Taipale et al. 2005; Li et al. 2010; Gupta et al. 2008). The loss of H4K16ac is found in a number of

tumors, including lung cancer and considered as a 'hallmark' of human cancer (Van Den Broeck et al. 2008; Song et al. 2012; Fraga et al. 2005). Cr(VI) exposure leads to increase in the level of Nupr1 in human bronchial epithelial BEAS2B cells and the loss of H4K16ac. Cr(VI)-induced reduction of H4K16ac appears to be caused by the induction of Nupr1, since overexpression of Nupr1 decreases the levels of H4K16ac, while knockdown of Nupr1 by siRNA greatly compromised the loss of H4K16ac following Cr(VI) exposure (Bollati et al. 2010). Importantly, overexpression of Nupr1 induces anchorage-independent cell growth and knockdown of Nupr1 expression prevents Cr(VI)-induced cell transformation. Together, downregulation of H4K16 acetylation through inducing Nupr1 expression might represent a new mechanism for Cr(VI) carcinogenesis.

Based upon these findings, it is conceivable that an epigenetic modification of gene expression patterns may be a key element of the developmental and carcinogenic outcomes of exposure to chromium (Schnekenburger et al. 2007)

It is thought that Cr(VI) is carcinogenic while Cr(III) has such a low toxicity that delaterious effects from excessive intake of this form do not occur readily (Barceloux 1999). It becomes toxic only at extremally high amounts. For example, cats tolerate 1000 μg Cr(III)/day and rats 100 μg Cr(III)/kg b.w. Chromium(III) compounds have a relatively low order of toxicity when ingested. Animal and human studies suggest that long-term supplemental intakes of 200 μg/day, and short-term intakes (several months) between 200 and 1000 μg/day are safe.

The safety limit for Cr^{3+} is approximately 1:10 000. Cr^{3+} toxicity is in fact lower than the toxicity of all other essential elements such as Cu, I, Zn, Mn and especially Se (Lindemann 1996). The toxicity of Cr^{6+} compounds is most probably based on an oxidative DNA impairment (Cohen et al. 1993). The details of Cr^{6+} toxic activity are however not known.

It is assumed that genotoxicity may be due to a transient form (Cr^{5+}) of intracellular origin formed by the reduction of Cr^{6+} to Cr^{3+} (Stearns et al. 1995). Extracellular reduction of Cr^{6+} to Cr^{3+} is regarded as a protective reaction (De Flora et al. 1989).

In addition, it is difficult to distinguish between the effects caused by chromium(VI) and those caused by chromium(III) since chromium(VI) is rapidly reduced to chromium(III) after penetration of biological membranes and in the gastric environment (Petrilli et al. 1986; Samitz 1970). The first defense against chromium(VI) after oral exposure is the reduction of chromium(VI) to chromium(III) in the gastric environment where gastric juice (De Flora et al. 1987) and ascorbate (Samitz 1970) play important roles by an NADPH-dependent mechanism.

However, whereas chromium(VI) can readily be transported into cells, chromium(III) is much less able to cross cell membranes (Hu et al. 2016a, b). Reduction of chromium(VI) in the red blood cell occurs by the action of glutathione. Since the red blood cell membrane is permeable to chromium(VI) but not chromium(III), the chromium(III) formed by reduction of chromium(VI) by glutathione is essentially trapped within the red blood cell (Devoy et al. 2016). Eventually the diffusion of chromium(VI), the reduction to chromium(III), and complexing to nucleic acids and proteins within the cell will cause the concentration equilibrium to change so that more chromium(VI) is diffused through the membrane. Thus, there is a physi-

ological drag so that increased diffusion results in greater chromium concentrations in the cell (Aaseth et al. 1982). It appears that the rate of uptake of chromium(VI) by red blood cells may not exceed the rate at which they reduce chromium(VI) to chromium(III) (Corbett et al. 1998).

The evaluation of toxicity of Cr^{3+} supplements has revolved around questions of genotoxicity, mutagenicity, and cancer for several reasons. First, carcinogenic Cr^{6+} is metabolized to Cr^{3+} in the body, and Cr^{3+} may be one of the ultimate species that interacts with DNA in Cr^{6+}-induced cancers. Second, the chemistry and bioavailability of Cr^{3+} is altered by its coordinating ligands (Zhitkovich 2005).

Chromium(III) nutritional supplements are widely consumed for their purported antidiabetic activities. Research results strongly support the hypothesis that the antidiabetic activity of Cr(III) and the carcinogenicity of Cr(VI) compounds arise from similar mechanisms involving highly reactive Cr(VI) and Cr(V) intermediates (Wu et al. 2016).

However, chromium is the same as any other mineral element in that the dose is the poison. The question that remains to be determined is the concentration at which the various forms of orally ingested chromium become of toxic concern because homeostatic mechanisms are unable to prevent chromium accumulation in high enough quantity in cells that will allow chemical reactions that can cause non-repairable damage to occur.

It thus seems plausible that, under an oxidative-stress situation that might compromise life before reproduction is attained, the balance between dedicating resources to prolong lifespan or to reproduction be shifted toward the lifespan side. This hypothesis has recently received some scientific support by the discovery of what is known as the hormetic effect, according to which low doses of either toxic substances may provoke a protective effect, sometimes resulting in a lifespan prolongation rather than in the expected lifespan shortening. The underlying principle might be an action–reaction mechanism by which the biochemical defences unchained by the stress situation exceed the insults produced by it (overcompensation).

Chromium(VI) and other heavy metals have recently been reported to show a relatively low toxicity, as well as a hormetic effect, in long-term viability of fish (Perez-Benito 2006; Johnson and Radhakrishnan 2015).

Since redox processes play a crucial role in living organisms, and are thought to unchain a cascade of reactions leading to senescence and other degenerative diseases in aerobes, the effect of moderate oxidants on their lifespan is a topic of some interest.

5.7 Chromium Deficiency

Papers dealing with the experimental study of Cr deficiency are relatively scarce and most of the existing ones quote results of experiments on laboratory animals. Anderson (1994) has summed up the results of a number of trials on humans, rats, mice and other animal species in a review of physiological and biochemical symptoms of Cr deficiency that we present in Table 5.1. Frank et al. (2000a, b) have

Table 5.1 Symptoms of Cr deficiency (Anderson 1994)

Function	Species
• Glucose intolerance	• Humans, rats, mice, monkeys, Guinea pigs
• Increased circulating insulin	• Humans, rats, pigs
• Glycosuria	• Humans, rats
• Hunger hyperglycemia	• Humans, rats, mice
• Growth disorders	• Humans, rats, mice, turkeys
• Hypoglycaemia	• Humans
• Increased serum cholesterol and triacylglycerols	• Humans, rats, mice, cattle, pigs
• Increased incidence of aortal plaques	• Rabbits, rats, mice
• Increased surface of aortal plaques of the inner surface	• Rabbits
• Neuropathy	• Humans
• Encephalopathy	• Humans
• Corneal lesions	• Rats, monkeys
• Increased intraocular pressure	• Humans
• Reduced fertility and number of sperm cells	• Rats
• Diminished longevity	• Rats, mice
• Reduced insulin binding	• Humans
• Reduced number of insulin receptors	• Humans
• Reduced muscle proportion	• Humans, pigs, rats
• Increased proportion of body fat	• Humans, pigs
• Reduced humoral immune response	• Cattle
• Increased morbidity	• Cattle

studied experimentally induced Cr deficiency in goats. The population with a Cr deficiency showed higher weight gains (31.1 ± 11.7 vs. 20.0 ± 7.3 kg) for the period of monitoring (84 weeks) compared with the control group. The authors explain this unexpected effect by the possibility that Cr deficiency has impaired glucose tolerance and increased insulin release subsequently leading to hyperinsulinemia. Cr deficiency has also led to an increase in haematological parameters (haemoglobin, haematocrit, erythrocytes, leucocytes and mean erythrocyte volume); increased total protein concentrations and hyperinsulinemia were observed compared with the group of controls as well.

5.8 Dietary Chromium Intake and Recommendations

Trivalent chromium, the form found in foods and nutrient supplements, is considered one of the safest nutrients (NRC 1989). The Environmental Protection Agency has established a reference dose, defined as "an estimate of a daily exposure to humans, including sensitive subgroups, that is likely to be without an appreciable risk of delaterious side effects over a lifetime, that is 350 times the National Research Council's upper limit of the "safe and adequate range".

Table 5.2 Dietary Cr intakes in various countries (Kumpulainen 1992)

Mean Cr intake (μg/d)	Country	Type of diet
62	Germany	Self-selected
62–89	USA	Formulated to meet US dietary requirements
31	Finland	Self-selected
56	Canada	Self-selected
24.5	UK	Self-selected
28	USA	Self-selected
49	Turkey	Self-selected
50	Switzerland	Mixed institutional
50	Sweden	Average market basket
85	Brazil	Average market basket
75	Iran	Self-selected
60	Italy	Self-selected
60	Spain	Self-selected
105	Sudan	Self-selected

Recent studies obtained as part of the Trace Elements in Food Research Programme of the FAO European Research Network on Trace Elements (FAO 1989) demonstrated that the Cr content in animal foodstuffs such as meat, poultry and fish is low providing 2 μg Cr (Anderson et al. 1992). Most dairy products are also low in Cr and provide <0.6 μg/serving. Whole wheat and wheat flour contain 5–10 μg of Cr/kg (Anderson et al. 1992). Pulses, seeds and dark chocolate may contain more chromium than most other foods (Jorhem and Sundstrom 1993). Certain species such as black pepper contain high concentrations of Cr (Anderson et al. 1992). Some brands of beer contain significant amounts of $C\Gamma$, some of which presumably comes from the brewing containers (Anderson and Bryden 1983). According to Mertz et al. (1974) the best known chromium complex is the glucose tolerance factor, found in brewer's yeast.

Most of the average daily dietary Cr intake estimates representing various populations living in 14 different countries ranges between 30 and 60 μg. WHO (1996) estimates that the daily minimum population mean intake likely to meet normal requirements for chromium might be approximately 33 μg/person.

Kumpulainen (1992) compared the average dietary Cr in various countries (Table 5.2). It is thought that dietary chromium intake in the USA and other developed countries is sub-optimal and is 50–60% of the minimum US suggested safe and adequate daily intake of 50 μg (Anderson et al. 2003).

The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) of Cr as proved by the Food and Nutrition Board of the US National Academy of Science in 1989. For adults and adolescents that range was 50–200 mcg (NRC 1989). In 2001, DRIs for chromium were established. The research base was insufficient to establish RDAs,

Table 5.3 Adequate intakes (AIs) for chromium (RDI 2001)

Age	Infants and children (μg/day)	Males (μg/day)	Females (μg/day)	Pregnancy (μg/day)	Lactation (μg/day)
0–6 months	0.2				
7–12 months	5.5				
1–3 years	11				
4–8 years	15				
9–13 years		25	21		
14–18 years		35	24	29	44
19–50 years		35	25	30	45
>50 years		30	20		

so AIs were developed based on average intakes of chromium from food as found in several studies (DRI 2001). Chromium AIs are provided in Table 5.3.

Adult women in the United States consume about 23–29 μg of chromium per day from food, which meets their AIs unless they're pregnant or lactating. In contrast, adult men average 39–54 μg per day, which exceeds their AIs. Recently, a new daily adequate intake (AI) has been set at 35 μg/day for males and 25 μg/day for females (DRI 2001), which more accurately reflects normal dietary intakes. Because pregnancy and lactation are known to deplete chromium stores, taking 30 μg daily is recommended for pregnant women, and women who are breastfeeding should take at least 45 μg daily.

The average amount of chromium in the breast milk of healthy, well-nourished mothers is 0.24 μg per quart, so infants exclusively fed breast milk obtain about 0.2 μg (based on an estimated consumption of 0.82 quarts per day) (DRI 2001). Infant formula provides about 0.5 μg of chromium per quart (Cocho et al. 1973). No studies have compared how well infants absorb and utilize chromium from human milk and formula (DRI 2001; Stoecker 2001).

It is believed that intense exercise and traumatic injury also increase the body's demand for chromium (Anderson et al. 1997).

Other health care professionals recommend more chromium to help with blood sugar control, especially for people with existing cases of mild or serious insulin-resistance or diabetes.

Dr. Anderson postulates that doses well above these recommended minimum levels may be necessary to treat chronic diseases. Citing a study conducted in China, he notes that patients there received up to 1000 μg of chromium per day, a dose that proved "highly effective" in relieving many of the symptoms of type II diabetes (Anderson 1997, 1998).

5.9 Diabetes Mellitus and Chromium

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. In the form of this disease known as maturity-onset diabetes, the pancreas often continues to secrete normal amounts of insulin, but this insulin is ineffective in preventing the symptoms of diabetes which includes heperglycemia, impaired carbohydrate metabolism, glycosuria and decreased insulin sensitivity (Press and Geller 1990).

Diabetes in particular has been growing at the particularly high rate and is now one of the world's most common long term health conditions.

It is estimated that 415 million people are living with diabetes in the world, which is estimated to be 1 in 11 of the world's adult population. 46% of people with diabetes are undiagnosed. The figure is expected to rise to 642 million people living with diabetes worldwide by 2040.

The International Diabetes Federation (IDF) currently states that the top 5 countries with the highest amount of people with diabetes are as follows:

China: 109 million
India: 69 million
USA: 29 million
Brazil: 14 million
Russian Federation: 12 million

According to the 2013 IDF in Europe, diabetes is estimated to affect 56.3 million adults aged between 20 and 79—8.5% of the adult population. Even more worryingly, this figure is set to increase: by 2035, it is estimated that nearly 70 million people will be living with diabetes in Europe, driving regional prevalence to beyond 10%.

For the 45–74 year-old age group, the prevalence is higher among men, while for those above 75 years, the rate is higher among women.

In several European countries, diabetes and its complications have caused the greatest increase of deaths over the past 20 years. Diabetes is ranked among the leading causes of cardiovascular disease, blindness, renal failure and lower limb amputation. About 75% of people with diabetes die of cardiovascular events—the number one cause of death in Europe. People with type 2 diabetes have a 2–4 times higher risk of coronary heart disease than the overall population.

Of great concern is that children and adolescents are also now developing type 2 diabetes, largely due to the high levels of obesity in these age groups. Estimates suggest that 1 in 5 children in Europe is overweight and that each year 400,000 children become overweight.

In the late 1950s Schwarz and Mertz (1959) first demonstrated that the dietary-induced impairment of glucose tolerance in rats could be reversed by the administration of trivalent chromium compounds. Since then, the possible beneficial role of Cr(III) in carbohydrate, protein and fat metabolism has been extensively studied in

various experimental systems (Wrobel et al. 1999). Experiments carried out so far in humans can be classified into 3 groups: studies in long-term parenteral nutrition, administration of high glucose doses, supplementation with various compounds of Cr(III) (Wrobel et al. 1999).

Also in parenteral nutritional studies chromium deficiency cases were observed and glucose tolerance was reversed by daily Cr(III) supplementation (Jeejeebhoy et al. 1977). Further experiments carried out at constant or elevated glucose concentration in plasma, showed an inverse relationship between plasma insulin and plasma chromium (Morris et al. 1993a, b).

Anderson et al. (1991) studied the effect of Cr(III) supplementation in healthy subjects, in patients with type 2 diatetes and/or lipemia. The results indicated that Cr(III) is in some way required for insulin action improving blood glucose, insulin and lipid indices.

Glucose intolerance, related to insufficient dietary chromium is a widespread health problem. On the other hand, improved chromium nutrition leads to improved sugar metabolism in hypoglycemics, hyperclycemics and diabetics (Anderson 1989; Shinde Urmila et al. 2004). Absorption of chromium from the gut and its urinary excretion are significantly higher in insulin-requiring diabetes than in healthy subjects. Chromium maintains normal glucose tolerance primarily by regulating insulin action. In the presence of chromium, much lower amounts of insulin are required (Anderson 1989). It is important to keep insulin at low levels to prevent secondary signs of diabetes. There is very strong evidence that insufficient dietary chromium leads to impaired glucose tolerance that can be alleviated by supplemental chromium.

Fasting glucose, circulation insulin, insulin binding, circulatory glucagon, and β-cell sensitivity improve with increasing chromium status (Jeejeebhoy et al. 1977). Response to chromium is related to degree of glucose intolerance. Subjects with hypoglycemia, hyperglycemia and maturity-onset diabetes have shown to respond to supplemental chromium, while subjects with normal glucose tolerance with no signs of marginal chromium deficiency do not respond to supplementation with chromium.

The prevalence of type 2 diabetes and impaired glucose tolerance (IGT) increases with aging (Chang and Halter 2003).

Insulin secretion (both first and second phase) normally decreases at a rate of 0.7% per year with aging; this decrease in β-cell function is accelerated about two-fold in people with impaired glucose tolerance—first phase to a greater extent than second phase. Finally, aging per se has no effect on insulin sensitivity independent of changes in body composition (Szoke et al. 2008).

Chromium status decline with age. Recent, well-controlled, double-blind studies reported that the glucose tolerance of the elderly subjects given supplemental chromium improved. However, if subjects were eating well-balanced diets with a mean Cr intake more than 37 μg Cr/d, glucose tolerance was unchanged.

Urberg and Zemmel (1987) showed that fasting glucose and glucose tolerance of elders also improved following daily supplementation with 200 μg Cr as $CrCl_3$ together with 100 μg of nicotinic acid, but not with Cr alone (Anderson 1989). Cefalu

(1998) demonstrated that chromium picolinate appears to increase insulin sensitivity in moderately obese, nondiabetic subjects.

The number of in-dividuals with impaired glucose tolerance is alarmingly high. Metabolic syndrome, also known as Syndrome X, is a combination of medical conditions characterized by abnormal glucose metabolism, elevated insulin levels, excess weight and abdominal fat distribution, disturbances of normally healthy lipid levels, and high blood pressure—all of which are associated with the subsequent development of type II diabetes and cardiovascular disease.

While diabetes and cardiovascular disease are well-recognized threats to overall health, some researchers believe that elevated blood sugar—even absent these other conditions—contributes directly to aging. By interacting with proteins and nucleic acids, excess glucose molecules wreak havoc with tissue elasticity and normal function (Turkoski 2004). Thus, controlling blood sugar may actually put the brakes on the aging process, and should be an essential component of any life-extension strategy.

5.10 Effects of Chromium on Life Span

Some of the original studies from the 1960s (Schroeder et al. 1963; Schroeder 1968) were life-term studies with mice and rats given 5 ppm Cr from Cr acetate (an organic form of trivalent Cr) in drinking water showed increased growth over unsupplemented controls for both males and females and decreased mortality of males. In a study of controlled stress, the percentage of rats surviving was greater for the Cr-supplemented group (Mertz and Roginski 1969). Evans and Meyer (1992) fed three groups of rats 1 ppm Cr from either Cr chloride, Cr nicotinate, or CrPic. The authors followed plasma glucose and glycated hemoglobin throughout the study. After 41 months of supplementation, all rats fed the supplemental Cr from Cr chloride or Cr nicotinate had died while 80% of the rats fed CrPic remained alive; median life span for the first two groups of rats was 33 months (a fairly normal life span for laboratory rats) while median life span for the rats supplemented with CrPic was 45 months (Evans and Meyer 1994).

A series of studies with broilers provides clear evidence of an effect of Cr on mortality. The first two studies (Hossain et al. 1998) were conducted in Brazil using a high Cr-yeast and the latter three studies (Kim et al. 1996a, b, 1997) in Korea using CrPic.

Mortality rate was significantly lower for Poultry, that were supplemented with 150 PPb Cr^{+3}—yeast from 1 to 56 days of age. This reduction in mortality rate could be due to the role of Cr^{+3}—yeast in improving birds health and increase immune response which result in reducing heat stress (Hossain et al. 1998).

Several points can be made from the studies. First, when mortality is low (and presumably the cumulative stress from crowding, disease challenge, heat, etc. is low) there is less of a response to supplemental Cr but when mortality (and presumably

stress load) is high then the response to Cr can be quite large. A second point to be made is that the response is clearly dose dependent.

A question that naturally arises is whether there are potential Cr effects on mortality in reproducing sows. It would seem from our understanding of nutrient responses that the potential for a response would be greatest in those situations where there was the greatest departure from normal performance, i.e. when performance was most compromised. The same concept would exist with regard to potential mortality benefits in sows. One of the earliest studies reported does illustrate very clearly the potential benefit of Cr supplementation on some of these parameters. A large study (Campbell 1996) from Australia involving over 800 sows wherein supplementation of 200 ppb Cr from CrPic resulted in a highly significant improvement in farrowing rate (from 79.0 to 92.4%; $p < 0.001$). Numerical reductions of more than 60% in abortions, natural sow deaths, and sows that returned to estrus and were rebred were also observed with the supplementation. Every aspect of reproductive health that was recorded was benefited by supplementation. Of particular note should be the reduction in mortality—a response consistent with the broiler observations.

Numerical improvements in mortality were also observed in the study of Hagen et al. (2000). The reduction in mortality was greater in first litter sows and sows older than three parities (both of which had mortality substantially greater than that of the second and third parity sows). In this study, there were also improvements in litter size and wean to first service interval. The improvements in litter size were seen for sows of all parities and the improvements in wean to first service interval were most pronounced in first parity females (when the interval was greater than that of older sows). These effects have much statistical strength given the size of the study; this study utilized 48,000 sows that farrowed almost 100,000 litters for almost 1,000,000 pigs.

Because the body's ability to control blood glucose is critical to many life functions, a consequence of Cr supplementation can be improved health and reproductive outcomes as well as improved survival rate or life span.

Cerami (1985) first suggested that elevated blood glucose levels may decrease survival by accelerating the process known as protein glycation. Later, Masoro et al. (1992) demonstrated that food restriction in rats resulted in decreased serum glucose concentrations with a concomitant decrease in glycation of hemoglobin.

The results demonstrate that chromium in a utilizable form, like dietary restriction, prevents hyperglycemia, hyperinsulinemia, protein glycation and extends life span. An increase in life span of rodents resulting from chromium supplementation has been noted previously (Mertz and Roginski 1969; Schroeder 1968). Additions of CrO_3 to the drinking water increased the survival of rats compared to unsupplemented controls. A significant increase in the mean age of the tenth-percentile survivors was observed when male rats fed a chromium deficient diet were supplemented with chromium acetate in the drinking water (5 μg Cr^{+3}/ml). In addition, the survival of male mice fed a chromium deficient diet was significantly increased when the mice were supplemented with chromium acetate in the drinking water (5 μg Cr^{+3}/ml).

The antiaging effect of chromium is undoubtedly related to the effect of chromium on insulin action, since several investigations with cell cultures, animals and human,

provide evidence that chromium increases insulin sensitivity. Investigators discovered that the symptoms of diabetes which developed during long-term parenteral nutrition could be prevented by the addition of chromium to the intravenous solution (Jeejeebhoy et al. 1977; Freund et al. 1979). When either swine or heifer calves were fed chromium picolinate in the diet, increased plasma glucose clearance rates were accompanied by decreased plasma insulin levels (Evock-Clover et al. 1993).

Evans and Bowman (1992) and Evans and Pouchnik (1993) discovered that insulin internalization was markedly increased in rat muscle cells cultured in a medium that contained chromium picolinate and the increased internalization rate was accompanied by a marked increase in the uptake of both glucose and leucine. The effect was specific for chromium picolinate since neither zinc picolinate nor any other form of chromium tested was effective.

Schroeder et al. (1963) established the fact that chromium deficiency leads to increased mortality while other results prove that the form in which chromium is ingested also influences the aging process. Chromium picolinate is a neutral, lipophilic complex while chromium nicotinate is a charged complex (Evans and Pouchnik 1993). Chromium chloride of course dissociates into the ionic components. Absorption experiments demonstrate that the charged forms of chromium are not readily absorbed when mixed with the diet and tests of biological function indicate that chromium nicotinate and chromium chloride are either unstable in physiological media or are simply not utilized by cells (Mertz and Roginski 1969; Evans and Meyer 1992; Evans and Pouchnik 1993).

Glucose is the preferred carbon and energy source in prokaryotes, unicellular eukaryotes, and metazoans. However, excess of glucose has been associated with several diseases, including diabetes and the less understood process of aging. On the contrary, limiting glucose (i.e., calorie restriction) slows aging and age-related diseases in most species. Understanding the mechanism by which glucose limits life span is therefore important for any attempt to control aging and age-related diseases. The pro-aging effect of glucose signaling on life span correlated with an increase in reactive oxygen species and a decrease in oxidative stress resistance and respiration rate (Roux et al. 2009).

Thus, when adequate quantities of chromium are ingested in a form that can be absorbed and utilized inside the body, insulin resistance and the subsequent hyperinsulinemia which occurs can be prevented. Because hyperinsulinemia has been associated with long term deleterious effects (Reaven 1988), we suggest that chromium, like dietary restriction, increases longevity by preventing development of symptoms associated with insulin resistance.

5.11 Conclusions

Trivalent chromium is essential to normal carbohydrate, lipid and protein metabolism. Chromium is an essential micronutrient which is required for the normal functioning of insulin and regulation of blood sugar levels. It acts as a vital

antioxidant for maintaining insulin homeostasis. Cr(III) supplementation may be therapeutic or useful as an adjunct treatment for some cases of type 2 diabetes or other disorders caused by insulin insensitivity.

Cr(VI) is a strong oxidant—in the form of chromates and dichromates it penetrates biological membranes and reacts with cell contents, proteins or nucleic acids, while being reduced to Cr(III). Research results strongly support the hypothesis that the antidiabetic activity of Cr(III) and the carcinogenicity of Cr(VI) compounds arise from similar mechanisms involving highly reactive Cr(VI) and Cr(V) intermediates.

The pro-aging effect of glucose signaling on life span correlated with an increase in reactive oxygen species and a decrease in oxidative stress resistance and respiration rate. Chromium(III) protects organism from oxidative stress associated with reactive oxygen species. The results demonstrate that chromium in a utilizable form, like dietary restriction, prevents hyperglycemia, hyperinsulinemia, protein glycation and extends life span. Chromium, like dietary restriction, increases longevity by preventing development of symptoms associated with insulin resistance.

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Chapter 6
Boron in Aging and Longevity

Forrest H. Nielsen

Abstract Only limited direct evidence from Drosophila studies exists to indicate that boron promotes healthy aging and longevity. However, substantial indirect evidence supports the suggestion that boron does have such an effect. Boron has bioactivity that affects the formation and activity NAD^+ and SAM, which have been shown to affect aging and longevity. Evidence has been provided to indicate, that through affecting these two biomolecules, boron has beneficial effects on inflammatory and oxidative stress, DNA damage detection and repair, and SAM involvement in methylation and homocysteine levels. Through these effects, nutritional intakes of boron have been shown to moderate or alleviate several pathological conditions associated with aging, including cancer, cognitive decline, sarcopenia, and bone health. These findings indicate that a diet rich in boron will promote healthy aging and longevity.

Keywords Nicotinamide adenine dinucleotide · S-adenosylmethionine
Inflammatory stress · Oxidative stress · DNA damage · DNA repair
Homocysteine · Cancer · Cognitive function · Sarcopenia · Bone

6.1 Introduction

The first indication that boron could affect the aging process came from experiments with Drosophila fruit flies (Massie et al. 1990; Massie 1994). Drosophila reared on a medium containing 0.62 μg/g boron exhibited a 9.5% increase in life span when the medium was supplemented with 10.7 μg boron/g as sodium borate. On the other hand, adding 43, 216 and 430 μg boron/g medium decreased lifespan by 21.2, 51.9, and 69.2%, respectively. It was also found that adding 4.3 or 21.6 μg boron/g to a diet containing 31.1 μg boron/g had no effect on the longevity of old mice. No suggested mechanism was given for the boron effect on the longevity of Drosophila. However, it is now known that mineral element intake can affect changes associated

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with aging and many of the chronic and neurodegenerative diseases that decrease longevity. Studies with cells, animals, and humans have indicated that boron may be one of those mineral elements.

6.2 Boron Biochemistry Related to Aging and Longevity

The biochemistry of boron provides some insight as to why boron might affect aging and longevity. Boric acid forms ester complexes with hydroxyl groups of organic compounds (Hunt 1998). This preferably occurs when the hydroxyl groups are adjacent and in the *cis* formation. This property results in the formation of complexes with several biologically important sugars, including ribose (Hunt 1998).

Ribose is a component of adenosine. Some of biomolecules that have been associated with aging and longevity contain adenosine or are formed from adenosine precursors. Among these biomolecules is oxidized nicotinamide adenine dinucleotide (NAD^+), which has the ability to bind two borate molecules to the ribose moiety and this binding affinity is reduced by phosphorylation and reduction in charge (Kim et al. 2003, 2004). Boron may also bind to precursors of NAD^+ because they contain the ribose moiety; these include nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN). Products of enzymatic reactions in which NAD^+ is a co-substrate also have the ability to bind boron; these include adenosine diphosphate (ADP) ribosyl cyclase, O-acetyl-ADP-ribose, and ADP ribose.

Another molecule associated with aging and longevity is S-adenosylmethionine (SAM). Boron has a higher affinity for SAM than most other recognized boron ligands in animal tissues (Ralston and Hunt 2001). About 95% of SAM is used in methylation reactions, which influence the maintenance and activity of DNA, RNA and proteins (Loenen 2006).

6.3 NAD^+ and SAM Biochemistry in Aging and Longevity

6.3.1 NAD^+ in Aging

Cellular NAD^+ concentrations increase under conditions that increase life span and decrease during aging or conditions that decrease life span (Verdin 2015). The effect of NAD^+ on aging and life span apparently occurs through its enzymatic roles. Nicotinamide adenine dinucleotide is a coenzyme in oxidation-reduction reactions and is a co-substrate for the sirtuins (SIRTs), adenosine diphosphate (ADP)-ribose transferases (ARTs), poly(ADP-ribose) polymerases (PARPs), cyclic ADP-ribose (cADPR) synthases (CD38 and CD157) (Verdin 2015).

There are seven SIRTs in mammals; three of these, SIRT1, SIRT3, and SIRT6 have been associated with aging and longevity (Verdin 2015). Calorie restriction, which

increases life span, depends upon NAD^+ sensing by SIRT1. Located in the nucleus, SIRT6 is linked to aging by regulating telomere stability and inflammation through NF-κB signaling. Loss of SIRT6 results in progeria, and gain of SIRT6 function extends life span in mice. The major mitochondrial deacetylase, SIRT3, expression is enhanced by calorie restriction and depressed by a high-fat diet, factors that affect aging and longevity.

The DNA-dependent nuclear PARP1 is important in in DNA damage detection and repair and for the decision by a cell to repair itself or die after a genotoxic insult (Verdin 2015). In addition, PARP1 is required for the assembly of cytoplasmic stress granules that regulate the stability and translation of mRNA in response to stress, which affects aging.

One hypothesis to explain aging is the free radical theory. This theory proposes that the accumulation of reactive oxygen species or oxidative stress causes cell and organs to age. Overexpression of the cADPR synthase, CD38, which decreases cellular NAD^+ concentrations, have been associated with increased oxidative stress and decreased expression of proteins that have roles in anti-oxidant response and DNA repair (Verdin 2015).

6.3.2 SAM in Aging

Factors affecting methylation of both nuclear and mitochondrial DNA and elevating homocysteine influence aging and age-related disorders (Park et al. 2012; Iacobazzi et al. 2013; Zhang et al. 2015). Longevity and aging have been associated with SAM and its effect on DNA methylation or epigenetic alterations in animal and cell models. For example, increased liver SAM and methyltransferase activity correlated with elevated global DNA methylation in long-lived Snell Dwarf mice (Vitvitsky et al. 2013). Regulating SAM was found to extend *Drosophila* lifespan (Obata and Miura 2015). Methionine restriction, which increases lifespan, increases hepatic global DNA methylation in adult but not young mice (Mattocks et al. 2017). DNA hypomethylation of telomerase reverse transcriptase induced by homocysteine was found to increase senescence of endothelial cells (Zhang et al. 2015).

DNA methylation resulting in epigenetic modification is influenced by nutrients that affect levels of the methyl donor, SAM, and the methyl transferase inhibitor, S-adenosylhomocysteine (SAH), which can be hydrolyzed into homocysteine (Park et al. 2012). For example, cognitive impairment in aging has been associated with impaired SAM methylation reactions and homocysteine neurotoxicity caused by vitamin B deficiencies (Selhub et al. 2010). Also, methyl-deficient diets and supplementation with phytonutrients such as polyphenols and isoflavones alter epigenetic patterns, which have been associated with anti-cancer effects (Park et al. 2012).

6.4 Boron Association with Aging and Longevity Through Affecting NAD^+ and SAM Formation and Activity

Nutritional amounts of boron (amounts found in diets following dietary guidelines) have been found to affect NAD^+ and SAM formation, metabolism, and activity in animal and cell models. This is the basis for the suggestion that boron has beneficial effects in animals and humans (Nielsen and Meacham 2011; Nielsen 2014, 2017). This also can be the basis for the concept that boron can affect aging and longevity.

6.4.1 Inflammatory and Oxidative Stress

The binding of extracellular NAD^+ to the membrane receptor CD38, an ADP-ribose cyclase, converts NAD^+ to cADPR. Cell culture studies show that boron binds to and is a reversible inhibitor of cADPR, which binds the ryandodine receptor and induces the release of Ca^{2+} from the endoplasmic reticulum. (Henderson et al. 2009). Boron in concentrations that are found in blood was found to decrease Ca^{2+} release from ryandodine receptor-sensitive endoplasmic reticulum stores. Elevated Ca^{2+} release results in increased inflammatory and oxidative stress through the release on inflammatory neuropeptides, cytokines, prostaglandins, and leukotrienes (Weglicki 2012). Aging is associated with a chronic increase in proinflammatory cytokines, especially Interlukin-1 (IL-1) and interlukin-6 (IL-6) (Roubenoff et al. 1998). In the Framingham Heart Study, increased amounts or production of tumor necrosis factor-α (TNF-α) and IL-6 were associated with increased mortality in community-dwelling elderly adults (Roubenoff et al. 2003). Thus, boron may beneficially modify age-promoting oxidative and inflammatory stress.

Further support for boron affecting aging through alleviating oxidative and inflammatory stress is numerous studies showing that boron modulates this induced stress in animals and cells. Cell culture studies have shown that boric acid inhibits lipopolysaccharide (LPS)-induced TNF-α formation through a thiol-dependent mechanism in human monocytic leukemia THP-1 cells (Cao et al. 2008) and calcium fructoborate decreased IL-1β release by LPS-stimulated murine macrophage RAW 264.7 cells (Scorei et al. 2010). Animal studies have found that boric acid increases the anti-oxidant capacity of spleens in rats (Hu et al. 2014); prevents damage to membranes of the cerebral cortex through anti-oxidant action in rat pups from alcohol-treated dams (Sogut et al. 2015); and inhibits lipid peroxidation induced by arsenic trioxide in rats (Kucukkurt et al. 2015). Borax was found to increase serum total-anti-oxidant activity, and hepatic expression of both Cu-Zn superoxide dismutase and Mn-superoxide dismutase mRNA in rats injected with sheep red blood cells in the footpad (Bhasker et al. 2016).

Human studies also have found boron modulates oxidative and inflammatory stress. These studies include two that found that boron supplementation reduces C-reactive protein (CRP) in individuals with serum concentrations greater than

3.0 mg/L, which is considered an indicator of chronic inflammatory stress. Boron supplemented at 3.0 mg/day as calcium fructoborate significantly decreased elevated serum CRP concentrations in 29 patients with stable angina pectoris, while 29 patients without supplementation showed no change at both 30 and 60 days (Militaru et al. 2013). In another double-blind, placebo-controlled clinical study, groups of 26–28 healthy individuals with mean serum CRP concentrations >3.0 mg/L were given a placebo or supplemented with boron at either 1.5 or 3 mg/day as calcium fructoborate for 30 days (Rogoveanu et al. 2015). Compared to the placebo group, both boron supplementations significantly reduced the serum concentrations of CRP, IL-6, and monocyte chemoattractant protein-1.

6.4.2 DNA Damage Detection and Repair

The changing of cellular Ca^{2+} release by boron through reversible cADPR inhibition also has been found to activate eukaryotic initiation factor-α (eIF2α), which protects cells by redirecting gene expression to manage endoplasmic stress (Kobylewski et al. (2017). Gene expressions that were ultimately increased by boron were DNA damage-induced protein 34 (GADD34) and homocysteine-induced endoplasmic reticulum protein (Herp). These gene expression changes and the finding that boron binds NAD^+ and an enzymatic product of this molecule, cADPR, which affects the action of cADPR, allows for the speculation that boron may influence aging and longevity through means other than just moderating oxidative and inflammatory stress. This would include boron affecting telomere stability and DNA damage detection and repair actions of SIRTS and PARP1. Indirect evidence for boron having such an effect has been reported. Cell culture studies have found that borate inhibited micronucleus and sister chromatid exchange formations induced by aflatoxin B1 (Turkez et al. 2012); boric acid provided protection against the induction of DNA strand breaks and micronuclei by lead and cadmium toxicity in V79 cells (Üstündağ et al. 2014); and boric acid protected chromosome structure in human primary alveolar epithelial cells treated with nicotine (Turkez et al. 2016) and reduced the formation of DNA double strand breaks in human epithelial cells treated with irinotecan, etoposide, and doxorubicin (Tepedelen et al. 2016).

6.4.3 SAM and Homocysteine Metabolism

Support for the suggestion that boron might affect aging and longevity through affecting SAM formation and/or utilization are the findings that plasma homocysteine increased (Nielsen and Stoecker 2009; Nielsen 2009) and liver SAM decreased in rats fed apparently deficient (0.05–0.15 mg/kg) dietary boron and compared to rats fed supplemented with boron (3 mg/kg diet) (Nielsen 2009). In a randomized, double-blind, parallel clinical trial, boron supplemented at 3 mg/day as calcium fruc-

toborate decreased homocysteine concentrations (Rogoveanu et al. 2015). Another finding supporting the concept of boron affecting SAM utilization is that the bacterial quorum-sensing signal molecule, auto-inducer-2, is a furanosyl borate ester synthesized from SAM (Chen et al. 2002). Quorum sensing is the cell-to-cell communication between bacteria accomplished through the exchange of extracellular signaling molecules (auto-inducers).

6.5 Boron Effects on Aging-Related Changes and Pathology

Numerous studies have shown that boron in nutritional or physiological amounts ameliorates pathology associated with shortened age and longevity. Some of the beneficial effects of boron likely occurred through affecting NAD^+ and SAM as described above.

6.5.1 Cancer

Alleviating inflammatory and oxidative stress might be the reason for boron being associated with reduced risk for some cancers. Based on a study of 95 cases and 8720 controls, low dietary boron was associated with increased prostate cancer risk (Cui et al. 2004). The protective effect of boron became stronger with increasing amounts of boron consumed as a constituent of foods. Since then, boric acid in concentrations similar to that in blood has been found to inhibit the proliferation of some human prostate cancer cells in vitro (Barranco and Eckhert 2004; Kobylewski et al. 2017). Supra nutritional amounts of boron was found to decrease prostate cell proliferation in vitro through an apoptopic effect including decreasing telomerase enzyme activity (Korkmaz et al 2014), and decrease growth and mitotic figures in human prostate adenocarcinoma tumors in nude mice (Gallardo-Williams et al. 2004). Limited studies have found boron to be inversely associated with other forms of cancer. Cervical smears from 587 women with a mean boron intake of 1.26 mg/day found 15 cases with cytopathological indications of cervical cancer, but none was found in 472 women with a mean boron intake of 8.41 mg/day (Korkmaz et al. 2007). In a study of 763 women with lung cancer and 838 matched controls, boron was inversely related with the incidence of lung cancer (Mahabir et al. 2008). Boron was found to inhibit the proliferation of cultured breast cells in a dose-dependent manner (Scorei et al. 2008).

6.5.2 Cognitive Function

The beneficial effect of boron on cognitive function might involve the modulation of oxidative stress and increased homocysteine, factors that have been implicated in cognitive decline of aging (Secher et al. 2012). In one study, folate supplementation, which decreased elevated homocysteine, was found to improve memory, sensorimotor speed and information processing speed (Durga et al. 2007). Boron deprivation of older men and women altered electroencephalograms (EEG) such that there was a shift toward more activity in the low frequencies and less activity in the high dominant frequencies of the EEG spectrum (Penland 1994, 1995, 1998). A similar effect was found in rats (Penland and Eberhardt 1993; Penland 1998). The EEG changes induced by boron deprivation are similar to that found in non-specific malnutrition and heavy metal toxicity. Increased low-frequency activity is typical of states of reduced behavioral activation and mental alertness, and has been associated with impaired vigilance and psychomotor tasks. Decreased high-frequency activity has been associated with impaired memory performance. These EEG findings support the finding that boron supplementation after deprivation under well-controlled dietary conditions improved psychomotor skills of motor speed and dexterity, and cognitive processes of attention of and short-term memory (Penland 1994, 1995, 1998).

Fatty acids have been suggested to affect the risk for cognitive impairment and dementia (Secher et al. 2012). One study found that the regular consumption of n-3 polyunsaturated fatty acid (PUFA) rich oils or fish was associated with a decreased risk for dementia, and regular consumption of n-6 PUFA-rich oils was associated with increased risk for Alzheimer's disease and dementia (Barberger-Gateau et al. 2007). In addition to having an effect on neuron membrane fluidity and their vascular properties, PUFAs affect neuroinflammation; n-3 PUFAs are anti-inflammatory and n-6 PUFAs are pro-inflammatory (Secher et al. 2012). Boron might have a similar effect as n-3 PUFAs on aging brain health. An experiment with rats found that boron affected behavior differently when dietary fat was fish oil (high in n-3 PUFA) instead of safflower oil (high in n-6 PUFA). Boron-deprived (0.1 mg/kg diet) rats were less active than boron-supplemented (3.0 mg/kg diet) rats when fed safflower oil based on reduced number, distance, and time of horizontal movements, front entries, margin distance, and vertical breaks and jumps in a spontaneous activity evaluation. Feeding fish oil instead of safflower oil attenuated the activity response to boron deprivation, which suggests that boron was acting similarly to n-3 PUFA on central nervous system function (Nielsen and Penland 2006).

6.5.3 Sarcopenia

Sarcopenia is the loss of muscle mass and strength that occurs with aging (Morley et al. 2001). An age-related decrease in estrogen and testosterone apparently accelerates sarcopenia through a decrease in muscle anabolic potential (Secher et al. 2012).

Insulin, which has an anabolic effect on muscle protein, also apparently has a role in the onset of sarcopenia (Secher et al. 2012). Decreased vitamin D status has been associated with decreased muscle strength and gait speed, impaired equilibrium, and increased risk of fall and fractures (Gerdhem et al. 2005). Boron has been shown to influence the activity of these three hormones, which suggests boron intake might affect the development of sarcopenia.

Boron supplementation (3.0 mg/day) after boron deprivation 0.25 mg/day for 119 days has been reported to increase serum estrogen and testosterone in postmenopausal women (Nielsen et al. 1987). Boron supplementation also has been shown to increase plasma testosterone in rats (Naghii and Samman 1997). In addition to increasing circulating estrogen, boron has been found to increase the efficacy of estrogen supplementation in both rats and humans. In ovariectomized rats fed a diet containing 0.4 mg/kg boron, a 5 mg/kg boron supplement significantly increased the beneficial effect of 17β-estradiol supplementation on trabecular bone quality (Sheng et al. 2001a). The combination of boron and 17β-estradiol, versus either of these alone markedly improved the apparent absorption of calcium, phosphorus and magnesium and the retention of calcium and magnesium (Sheng et al. 2001b). Boron supplementation alone did not significantly improve any of these variables in the ovariectomized rats, which suggests that boron was enhancing the effect of 17β-estradiol. In postmenopausal women, the increases in serum 17β-estradiol and plasma copper induced by estrogen therapy were significantly higher with a boron intake of 3.25 mg/day instead of 0.25 mg/day (Nielsen et al. 1992). The higher boron intake also enhanced the effect of estrogen therapy on serum triglyceride and immunoreactive ceruloplasmin concentrations. The combination of estrogen therapy and higher boron intake was most effective in increasing serum 25-hydroxy vitamin D concentration in the postmenopausal women.

Limited evidence suggests that boron can facilitate insulin action. In rats fed a diet containing 0.2 mg/kg boron, a supplement of 2 mg/kg boron reduced plasma insulin but did not change plasma glucose concentration (Bakken and Hunt 2003). Peak insulin release from isolated perfused pancreas of boron-deprived chicks was almost 75% higher than from pancreas of boron-supplemented chicks (Bakken and Hunt 2003). The difference was especially noticeable when the perfusate was supplemented with glucose. These findings suggest that boron may reduce the amount of insulin needed to maintain plasma glucose. An effect on insulin utilization could be the basis for the observation that boron deprivation induced a modest but significant increase in fasting serum glucose concentration in older men and women fed a low-magnesium, marginal copper diet (Nielsen 1994).

The seminal finding indicating that boron is a bioactive beneficial element in nutritional amounts was that boron deprivation exacerbated gross bone abnormalities in chicks fed marginal amounts of vitamin D (Hunt and Nielsen 1981; Hunt 1989). Subsequently, it was found that boron deprivation exacerbated marginal vitamin D deficiency-induced decreased calcium and phosphorus absorption and balance in rats (Hegsted et al. 1991) and increased plasma triglycerides and decreased growth and femur calcium concentration in chicks (Hunt et al. 1994; Bai and Hunt 1996). Boron supplementation also has been found to increase plasma 1, 25-hydroxy-vitamin

D concentrations in rats (Naghii and Samman 1997). In older men and women, boron supplementation (3 mg/day) after 63 days of boron deprivation (0.25 mg/day) increased serum 25-hydroxy-vitamin D concentrations (Nielsen et al. 1990; Nielsen 1996).

Support for boron affecting muscle anabolism recently has been provided by a cell culture study (Apdik et al. 2015). A low dose (81.9 μm) boron treatment increased myogenic gene expression of myosin heavy chain, MyoD, myogenin, and desmin at day 4 of differentiation of human adipose-derived stem cells.

6.5.4 Mortality

Increased amounts or production of catabolic cytokines TNF-α and IL-6 have been found to be associated with increased mortality in community-dwelling elderly adults (Roubenoff et al. 2003). This finding suggests that boron might affect mortality through its effect on TNf-α and IL-6 as described above. A geographical study in Northern France found boron to be inversely with mortality (Yazbeck et al. 2005).

6.5.5 Bone Health

Osteoporosis, which increases the incidence of bone fractures, commonly occurs with aging. Significant evidence indicates that boron is beneficial for bone health, especially trabecular and alveolar bone. This beneficial effect likely occurs through boron-induced beneficial changes in factors (described above) affecting bone formation and maintenance. These factors include oxidative and inflammatory stress, vitamin D and estrogen function, and NAD^+ activity.

Microcomputed tomography of the fourth lumbar vertebra found that boron deprivation (0.1 vs. 3 mg/kg diet) decreased bone volume fraction and trabecular thickness and increased trabecular separation and structural model index (a lower value or more plate-like structure is preferable) in rats (Nielsen and Stoecker 2009). Boron deprivation (0.07 vs. 3 mg/kg diet) in rats also has been shown to decrease trabecular and alveolar bone volume/total volume in bone repair initiated immediately after tooth extraction (Gorustovich et al. 2008).

Cell culture studies have indicated that boron may be beneficial for bone formation and maintenance in humans. Boron in physiological amounts increased mineralized nodule formation and mineralized tissue-associated mRNA expression to type 1 collagen, osteopontin, bone sialoprotein, osteocalcin and runt-related transcription factor 2 by cultured osteoblasts (MC3T3-E1) (Hakki et al. 2010). In addition, boron supplementation increased bone morphogenetic protein-4, -6 and -7 levels. Physiological concentrations (amounts normally found in tissues) of boron increased calcium deposition in cultured human bone marrow stromal cells (Ying et al. 2011).

In addition boron increased mRNA expression of alkaline phosphatase, osteocalcin, collagen type 1, and bone morphogenetic protein 7 in these cells.

Animal studies indicate that boron can be beneficial for bone formation and strength. The modification of bioactive glasses (used for bone tissue engineering and regeneration) to contain boron enhances bone formation (Gorustovich et al. 2006; Wu et al. 2011; Doğan et al. 2014). Reported findings indicating that boron is beneficial to bone strength include boron deprivation decreasing strength variables determined by a three-point bending test of femurs of female rats (Nielsen 2004) and femurs of pigs (Armstrong et al. 2002).

A human boron supplementation study also has indicated that boron could be beneficial for bone maintenance. Six months of providing 226 mg/day of calcium fructoborate incorporated into margarine improved bone density in 66 of 100 patients with osteoporosis (Scorei and Rotaru 2011). Because the supplement provided only 20 mg/day calcium, the improvement was attributed to the additional 5.65 mg/day boron.

6.6 Beneficial and Safe Intakes of Boron

Both animals and humans deprived of boron exhibit positive health benefits when dietary intakes are increased in nutritional amounts (Nielsen and Meacham 2011; Nielsen 2017). In human depletion-repletion experiments, participants responded to a 3 mg/day supplement after consuming a diet supplying boron at only 0.2–0.4 mg/day (Nielsen 1994, 1996; Nielsen et al. 1987, 1992). Human and animal findings were used to arrive at a mean population boron intake of 1.0 mg/day to meet the normative needs of adults (World Health Organization 1996). Thus, achieving a boron intake between 1.0 and 3.0 mg/day apparently could be considered adequate to obtain any benefits that boron has on aging and longevity.

Boron is a relatively non-toxic food component. The safe upper intake level (UL) of boron in the United States and Canada has been set at 20 mg/day for adults (Institute of Medicine, Food and Nutrition Board 2001). The World Health Organization (1996) first suggested that 13 mg/day would be a safe intake but later increased this to 0.4 mg/kg body weight or about 28 mg/day for a 70 kg person (World Health Organization, International Programme on Chemical Safety 1998). The European Union established an UL based on body weight that translates to about 10 mg/day for adults (European Food Safety Authority 2004).

An intake of 1.0–3.0 mg/day, which is well below the UL, can be achieved by consuming foods of plant origin. Foods rich in boron include fruits, leafy vegetables, nuts, legumes, and pulses (Hunt and Meacham 2001; Choi and Jun 2008). Beverages based on fruits and grains, such as wine, beer, and cider are also good sources of boron.

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Chapter 7
Molybdenum

Yosef Dror and Felicia Stern

Abstract The transition metal molybdenum (Mo), with atomic weight 95.9, is the heaviest essential element with an established RDA, and with the lowest intake and blood concentration. Mo activates in humans 4 enzymes. In bacteria and archaea, about 100 enzymes are activated by Mo. Each of the human enzymes contains a pterin-based Mo cofactor (Moco) at its active site. These enzymes are involved in the decomposition of some metabolites, thus enabling their products to be excreted by the kidneys. As well, they decompose many drugs, and consequently affect their pharmacokinetics. This activity is especially important to the elderly people, who consume drugs at escalating rates. The Mo enzyme activities highly depend on the Mo blood concentrations. Mo might interact with other metal ions, and thus affect the enzymatic actions activated by Mo. Lower Mo concentrations (within the regular range) presumably have a physiological preference over the higher concentrations, particularly in type 2 diabetic patients. By eliminating bean consumption, diabetics might decrease dietary Mo intake. Episodes of Mo deficiency and overt toxicity in healthy people are very rare. A routine inclusion of Mo in the marketed 'multivitamins' is questionable, because it is not supported by a robust data.

Keywords Molybdenum · Moco · Sulfite oxidase · Xanthineoxireductase Aldehyde oxidase · Drug pharmacokinetics

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7.1 Introduction

Molybdenum (Mo) is a metallic element of the second transition series, with atomic number 42 and atomic weight 95.94. Mo has chemical properties similar to those of chromium. Mo is commonly used in steel alloys because it imparts hardness, strength, heat resistance, and corrosion resistance to these alloys. Mo is present in all plant and animal tissues and is considered an essential micronutrient for most life forms (Barceloux 1999). It is the heaviest catalytic metal in humans (and other mammals). Physiologically, it exists in the body as an ion. Although Mo is the most abundant transition metal in seawater (107 nM), it is present in low concentrations in most freshwaters, typically <20 nM (Glass et al. 2012), in soils 1.1 mg/kg (Tejada-Jiménez et al. 2013) with a range of a trace to 40 mg/kg (US Department of the Interior 1998) and in human serum, 1.15 ng/mL. The transition element Mo is essential for biological systems, as it is required by enzymes catalysing diverse key reactions in the global carbon, sulfur and nitrogen metabolism. The metal is biologically inactive unless it is complexed by a special cofactor. With the exception of bacterial nitrogenase, where Mo is a constituent of the FeMo-cofactor, Mo is bound to a pterin, thus forming the Mo-cofactor (Moco), which is the active compound at the catalytic site of all other Mo-enzymes (Vieira et al. 2011).

Mo is scarcely known by the nutritionists, because deficiency or toxicity overt symptoms are very rare in humans. In some textbooks dealing with trace element issues that were published during the 80s and the 90s, Mo was thoroughly ignored. In more recent studies, two most important issues concerning Mo have been almost disregarded, and therefore have been studied on a limited scale:

A. The effect of Mo concentrations on Mo enzymes that control the pharmacokinetics of the medications. In this matter, the elderly with their high medication use and their dependency on stable and accurate dynamics of the pharmacokinetics, might be the most vulnerable individuals to the fluctuations in Mo blood concentrations.
B. The disadvantage of the upper population quartiles of Mo blood concentrations in comparison with the lowest concentration quartiles. Concerning this matter, Mo concentrations are higher in type 2 diabetic patients (Menke et al. 2016; Flores et al. 2011), spermatogenesis is lower (Meeker et al. 2008) and the viability of the T-helper lymphocytes (Jurkat cells) is higher at the lower Mo concentrations (Caicedo et al. 2007).

Mo comprises one of the nine microelements with established RDA. Among these elements, it has the lowest serum concentration and the lowest established RDA (Fig. 7.1). In the human nutrition literature, there is the scarcest data for Mo body fluids, and a minimal data for deficiency and toxicity symptoms and cut-offs. The intake of the Mo in the US ranged from 120 to 240 μg/d (1.2–2.5 μmol/d) (Tsongas et al. 1980) or 76 μg/d for females and 109 μg/d, for males. Data from the National Health and Nutrition Examination Survey (NHANES III) indicated that the median intake of Mo from supplements was 23 mg/d. The RDA and the tolerable upper intake

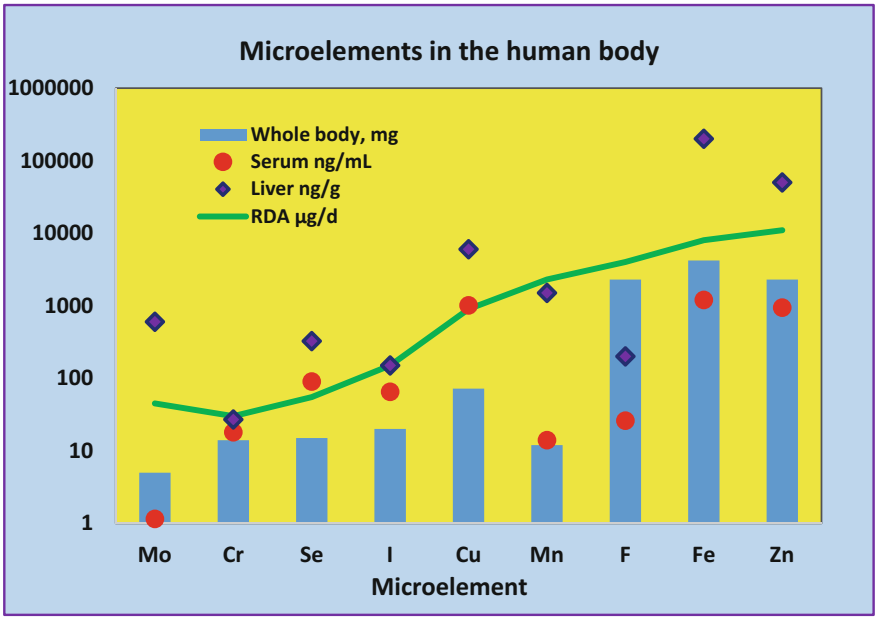

Fig. 7.1 A comparison between Mo concentrations in whole body, serum and liver as well as Mo dietary requirements with those of other microelements (ascending RDA order)

level (UL) for the adults were set by the Institute of Medicine (IOM) as 45 µg/d and 2 mg/d, respectively (Institute of Medicine 2000). All the other micronutrients with established RDAs have much higher serum concentrations, with those of zinc and copper laying at the range of ~1000 ng/mL (Fig. 7.1).

Mo comprises the tinniest amount of all the essential microelements in the human body. Its concentration is similar to those of the ultra-microelements, such as cobalt, nickel, or the toxic metals, such as cadmium, mercury, lead and silver (Otten et al. 2006). Mo is very abundant in the oceans in the form of the molybdate anion. In soils, the molybdate anion is the only form of Mo that is available for plants, fungi, and bacteria (Mendel and Kruse 2012).

7.2 Physical Characteristics

Mo atomic radius is 1.40 Å, its density is 10.28 g/mL, the melting point is 2617°, and the boiling point is 4825 °C. Mo common mineral compounds are: molybdenite (MoS_2); powellite ($CaMoO_4$); wulfenite ($PbMoO_4$); ferrimolybdite ($Fe_2(MoO_4)_3 \cdot n(H_2O)$; and ilsemannite ($Mo_3O_8$). The global production (2013) was 258,000 tonne/year (Index Mundi). The ores concentration is 0.5% (Chappell et al. 1979). The oxidation states range from -II to VI. Mo belongs to the class of inorganic

compounds known as homogeneous transition metal compounds. The pure metal is silvery white in color, fairly soft, and has one of the highest melting points of all pure elements. Mo is used in metallurgical applications, in oil pipelines, aircraft and missile parts, in filaments of some X-ray tubes, particularly in mammography applications, in some lubricants, and in the nuclear isotope industry (Barceloux 1999). Mo pigments range from red-yellow to a bright red orange and are used in paints, inks, plastics, and rubber compounds (HMDB).

7.3 Occurrence

Plants and animals generally contain Mo in amounts of a few parts per million (HMDB). The major anthropogenic sources of Mo in the environment are the following: combustion of coal, municipal sewage sludge, and industrial or mining operations. The daily intake of Mo from the air and from drinking water is negligible compared to its absorption from the diet. The concentration in fly ash ranges from 7 to 160 mg/kg. The concentration in soil averages ~1–2 mg/kg. Mo-deficient and Mo-excessive soils are defined by concentrations of 0.2 and 0.7 mg/kg, respectively. Most natural waters contain low levels of Mo in the range of <2–3 μg/L (Barceloux 1999). Concentrations of Mo in water are variable, ranging from 0.01 mg/L in the ocean up to 30 mg/L, in some American rivers. Mo concentrations in drinking water are generally low (up to 6 μg/L). In the vicinity of the areas where Mo ore is mined, drinking water contamination may occur, leading to intakes of >1000 μg/d (Ćurković et al. 2016). In the British waters data for 1735 groundwater samples from across Britain have a 10–90th percentile range for Mo of 0.035–1.80 μg/L with a median of 0.20 μg/L (Smedley et al. 2014). Tobacco contains only small concentrations (0.3–1.76 μg Mo/g) and, therefore, smoking probably is not a significant source of Mo (Barceloux 1999).

7.4 Catalytic Activity

A recent opinion, based on in silico calculations, claims that almost half of all the enzymes (47%) must associate with a particular metal to function (as metalloenzymes). A catalogue of the principal type of enzyme that uses each metal reveals that iron (81%), copper (93%) and Mo plus tungsten (81%) are most commonly used as conduits for electrons in oxidoreductases. Cobalt and Mo are found almost exclusively in association with cofactors in vitamin-B12-dependent and molybdopterin-dependent enzymes (Waldron et al. 2009). Mo occurs in a wide range of metalloenzymes in bacteria, archaea, fungi, algae, plants and animals, where it forms part of the active sites of these enzymes. However, in order to gain biological activity, Mo requires the coordination by a pyranopterin, thus forming a prosthetic group named Mo-cofactor (Moco). Mo is bound to the pterin-based Moco of those enzymes,

which in most cases harbours additional prosthetic groups for intramolecular electron transfer (Belaidi et al. 2015). Mo participates only in <1% of all the activated metalloenzymes (Waldron et al. 2009).

Mo has a versatile redox-chemistry that is used by the enzymes to catalyse diverse redox reactions. This redox-chemistry is controlled both by the different ligands at the Mo atom and the enzyme environment. Mo-containing enzymes are essential for life, since they hold key positions both in the biogeochemical redox cycles of nitrogen, carbon, and sulfur on earth and in the metabolism of the individual organism. So far, >100 enzymes are known to be Mo-dependent. The vast majority of them are found in bacteria, whereas in eukaryotes only 7 Mo-dependent enzymes have been identified. Mo is an essential trace element, i.e. the organism needs it only in minute amounts. There is a trace requirement for Mo in plants, and soils can be barren due to Mo deficiencies (Mendel and Kruse 2012; Schwarz 2016). Mo ion in mammals and presumably in all higher animals activates only 4 enzymes whereas in plants, Mo catalyses also nitrate reductase (NR) (Tejada-Jiménez et al. 2013).

In humans, deficiency symptoms are confined to rare congenital diseases or enteral and parenteral feeding, whereas toxicity is mainly confined to the industrial or the environmental contaminations (Freeland-Graves and Turnland 1996; Turnlund et al. 1995). Therefore, Mo has attracted only a limited attention for its activities, description of deficiency or toxicity, and more importantly for the monitoring of the normal serum level.

However, it might show a pivotal role in the maintenance of optimal health status, in particular, in the frail elderly. The accepted adequate intake and the UL are only a general frame for the optimal intake that is located somewhere in this frame with some unknown nadir range that warrants optimal serum concentration and optimal intake.

7.5 Mo Enzymes in Humans

The versatile redox chemistry of Mo is mirrored by the plethora and complexity of enzymes using Mo in bacteria and plants. Nature has developed two very different systems to control the redox state and catalytic power of Mo (Schwarz et al. 2009).

Four Mo-dependent enzymes are known in humans, each harboring a pterin-based cofactor (Moco) in the active site (Maia and Moura 2015):

a. Sulfite oxidase (SO)
b. Xanthineoxireductase (XOR)
c. Aldehyde oxidase (AO)
d. Mitochondrial amidoxime-reducing component (mARC).

Mo is considered an essential element, because it is required for the function of the above enzymes, which play an essential role in the catabolism of the sulfur amino acids and the compounds, such as purine and pyrimidine. Mo enzymes catalyze key redox reactions in the global carbon, sulfur, and nitrogen cycles. Their overall

reaction is characterized by the transfer of an oxygen atom to or from a substrate in a two-electron transfer reaction. Except for the well-known activities of the Mo-enzymes in the purine and sulfur metabolism, these enzymes are involved in drug metabolism and clearance. A study of the major clearance pathways of the top 200 most prescribed drugs showed that 73% of all the drugs were eliminated primarily through hepatic metabolism, 25% by renal and 2%, by biliary clearance (Di 2014).

With the increased role of the drug metabolism in our lifestyle, the activity of the Mo enzymes in drug metabolism should attract a particular interest.

Though serum Mo in humans lays at a very low concentration range in comparison to all other microelements, Mo might have a critical role in the metabolic clearance of drugs. Drug metabolism and pharmacokinetic disciplines play central roles in our understanding of the fate of most drugs in the body. Because most marketed drugs are cleared by metabolism, it is not surprising that a clear understanding of metabolism and metabolic enzymes and pathways is critical in drug discovery to optimize drug activities (Fan et al. 2016).

With the accelerated extension of longevity, drug clearance and its pharmacokinetics become one of the most important issues in our welfare, our lifestyle and probably even our longevity. Human Mo enzyme activities, besides being dependent on Mo concentration and kinetics they are also dependent on the interactions with other compounds and other metals. The stability of all of these parameters might seriously affect drug kinetics (Rowland et al. 2012). Drug metabolizing enzymes play a very important role in the drug clearance by converting lipophilic molecules to more water-soluble metabolites. Certain drug metabolizing enzymes are polymorphic, leading to a high pharmacokinetic variability, toxicity or loss of efficacy. Metabolites generated by the drug metabolizing enzymes, such as AO and XOR can be pharmacologically active, and reactive metabolites that might cause toxicity are formed during the bioactivation (Di 2014). Current estimates suggest that only 10% of potential drug targets are currently addressed, and in the future, new target classes for drug discovery will be actively pursued. In recent years, the number of compounds in clinical trials targeting kinase inhibition has risen dramatically. It is predicted that the number of compounds targeting ion-channel modulation will also significantly increase. Clearance of drugs would more than likely be mediated by a non-cytochrome 450 based mechanisms, and alternative metabolic pathways, for example, pathways mediated by AO would become more important (Pryde et al. 2010).

Mo hydroxylases, which include AO and XOR, are involved in the metabolism of some medicines in humans. They exhibit oxidase activity towards various heterocyclic compounds and aldehydes. The liver cytosol of various mammals also exhibits a significant reductase activity toward nitro, sulfoxide, N-oxide and other moieties, catalyzed by AO. There is a considerable variability in AO activity in the liver cytosol of mammals, with humans showing the highest activity. Various drugs have inducing or inhibitory effects on AO and XOR. AO linked activities are markedly inhibited by menadione, isovanillin, chlorpromazine and estradiol, and XOR is inhibited by oxypurinol (allopurinol, a drug used to treat gout) (Kitamura et al. 2006). To avoid potential drug interaction risks, such as a toxic excess of drug bioavailability or a

loss of drug efficacy, caution is suggested in the use of XOR inhibitors, as in the case of hyperuricemic patients affected by gout or tumor lysis syndrome, when it is necessary to simultaneously administer therapeutic substances that are activated or degraded by the drug-metabolizing activity of XOR (Batteli 2016).

7.5.1 Mo Cofactor (Moco)

Moco is synthesized by a conserved biosynthetic pathway. Moco consists of a Mo atom covalently bound via the dithiolate moiety of a fully reduced pterin backbone. Synthesis and incorporation of the Moco are essential for the catalytic activity of all Mo-containing enzymes with the exception of nitrogenase. Moco enzymes catalyze redox reactions using water as oxygen acceptor or donator. Besides their name-giving functions, Mo-enzymes have been recognized to catalyze novel reactions, including the reduction of nitrite to nitric oxide (Schwarz 2016). Moco biosynthesis follows an evolutionarily highly conserved pathway and genetic deficiencies in the corresponding human enzymes result in Moco deficiency, which manifests itself in severe neurological symptoms and death in childhood (Kasaragod and Schindelin 2016; Schwarz 2016). Disease-causing symptoms mainly go back to the lack of SO activity, an enzyme in cysteine catabolism. SO is the most important Mo enzyme, which is mainly found in the liver, where it catalyzes the oxidation of sulfite, which is generated during the catabolism of cysteine (Belaidi et al. 2015). Interestingly, most of the symptoms of Moco deficiencies are mirrored in isolated SO deficiency, leading to sulfite accumulation. Therefore, SO is seen as most important Moco-dependent enzyme, and sulfite accumulation presents the primary cause of neurodegeneration in both disorders of Moco and SO deficiencies. Sulfite accumulation is accompanied by changes in other S-containing metabolites, such as cysteine, S-sulfocysteine, thiosulfate, homocysteine and taurine (Schwarz 2016; Belaidi et al. 2015; Hobson et al. 2005).

7.5.2 Sulfite Oxidase (SO)

This protein is involved in the sulfur pathway metabolism, which is part of energy metabolism. Mammalian SO is a dimeric enzyme consisting of Moco and heme-containing domain. Animal SO harbors a cytochrome *b*5-type hem domain in addition to a Moco domain. The catalytic cycle of SO involves electron transfer from sulfite to Moco, followed by two electron-transfer steps via the cytochrome *b*5 domain to the terminal electron acceptor cytochrome *c* (Protein knowledgebase, sulfite oxidase; Belaidi et al. 2015). In the absence of SO activity (as seen in Moco deficient mammals and SO deficiency), sulfite accumulates within the cell and has been found to increase reactive oxygen species (Schwarz 2016; Qin et al. 2011). Endogenous sulfite is an intermediate metabolite produced in the degradation of the sulfur-containing amino

acids, cysteine and methionine. SO catalyzes the oxidation of sulfite to sulfate in most tissues, including the brain. Disruption of this pathway occurs in the inborn error of metabolism isolated sulfite oxidase deficiency and results in progressive neurodegeneration (Hobson et al. 2005; Kasaragod and Schindelin 2016; Schwarz 2016).

7.5.3 Xanthineoxireductase (XOR)

This protein is a key enzyme in purine degradation. It catalyzes the last two steps of purine catabolism in the highest uricotelic primates, i.e., the oxidation of hypoxanthine to xanthine and the oxidation of xanthine to uric acid. This activity has a rate-limiting effect on the recovery of nucleotides, because it interferes with the purine salvage pathway by producing the irreversible products, xanthine and uric acid. XOR function is not fully understood, and appears wider than merely a housekeeping role in nucleic acid metabolism. XOR has different enzymatic activities; it can act as (1) an oxidized NAD+-dependent dehydrogenase; (2) an O_2-dependent oxidase; (3) a reduced NADH oxidase; or (4) a reductase for N-oxide, nitrite and nitrate. The products of XOR activity have both oxidant and antioxidant properties and are involved in the regulation of vascular tone and blood pressure as well as in the induction of inflammation and the reparative response. XOR has a low specificity for substrates and is able to metabolize a number of endogenous metabolites and a variety of exogenous compounds, including drugs, thus playing a significant role not only as a xenobiotic detoxifier, but also as a drug-metabolizing enzyme. The role of XOR as a drug-metabolizing enzyme is due to the poor specificity of its enzyme action that allows the utilization of a variety of substrates, including a wide range of xenobiotics. Specifically, the XOR activity is directly involved in the metabolism of a number of antiblastic and antimetabolic drugs used against neoplasia, autoimmune disease and viral infection (Protein knowledgebase, xanthine dehydrogenase/oxidase; (Batteli 2016)). XOR deficiency results in the accumulation of xanthine in urine leading to a disease termed xanthinuria with a very low level of plasma uric acid and high levels of xanthine (Schwarz 2016).

7.5.4 Aldehyde Oxidase (AO)

Mammalian AO enzymes hydroxylate the rings of various aza-, oxo-, and sulfoheterocycles and oxidize iminiuim functions to cyclic lactams. AO enzymes also act as reductases, reducing N-oxides, sulfoxides, nitro compounds, and heterocycles, particularly under hypoxic conditions. AO might be a prominent source of superoxide generation via the one-electron reduction of molecular oxygen (Protein knowledgebase, aldehyde oxidase).

AO plays a key role in the metabolism of xenobiotics and drugs containing aromatic azaheterocyclic substituents. It participates in the bioactivation of prodrugs, such as famciclovir, catalyzing the oxidation step from 6-deoxypenciclovir to penciclovir, which is a potent antiviral agent. It also metabolizes the non-benzodiazepine hypnotic, zaleplon. AO in the mammals is characterized by a maximum of 4 *AOX* genes coding for a corresponding number of AOX isoenzymes. This group of enzymes has a particular ability, in the medical and toxicological fields, to metabolize a wide range of drugs and environmental toxicants (Pryde et al. 2010; Kitamura et al. 2006; Terao et al. 2016).

The number of drugs metabolized by AO is large. They include antitumor, immunosuppressive, antimalarial, and antiviral agents as well as molecules acting in the central nervous system. Among the antitumor and immunosuppressive agents, the oldest examples of drugs metabolized by AO enzymes are methotrexate and 6-mercaptopurine. In respect to the role of AO enzymes in the metabolism of drugs in humans, some issues should be discussed. Oxidation of drugs by AO enzymes does not necessarily lead to metabolic inactivation. In prospective, human AO-dependent activation of pro-drugs may be a strategy to be pursued in the oncologic field to increase the therapeutic index of antitumor agents by increasing the tumor selectivity of their pharmacological action. Moreover, the AO-dependent metabolism of drugs may be relevant in tissues and organs other than the liver. On the basis of the AO very broad (>100) substrate spectrum, a general role in biotransformation and detoxification can be assumed. AO was also suggested to participate in the metabolism of neurotransmitters and in the formation of retinoic acid (Mendel and Kruse 2012; Terao et al. 2016).

AO and XOR isoenzymes are classified as molybdo-flavoenzymes, since they require FAD besides Moco to oxidize their substrates. AO is homologous with XOR, another mammalian molybdoflavoprotein, and both AO and XOR show a remarkable degree of similarity in their amino acid sequence. Like XOR, AO is active as a homodimer composed of two identical subunits of about 150 kDa (Terao et al. 2016). The amino acid sequence of AO and XOR are remarkably similar, with ~86% homology (Li et al. 2009).

Some drugs are metabolized by both AO and XOR. Generally, AO has lower substrate specificity than XOR. AO inter-individual differences in AO activities have been observed in humans, with 50-fold in the benzaldehyde metabolism between individuals (Sanoh et al. 2015).

7.5.5 Comparison Between AO and XOR Activities

Both AO and XOR exhibit broad specificity, accepting a variety of reducing substrates, including purine, pteridine, aldehyde, and NADH. But, AO catalyzes the oxidation of aldehydes and NADH more efficiently, with lower *Km*, whereas XOR has higher affinity for xanthine, hypoxanthine, pteridine, and purine (Li et al. 2009). Age, gender, and toxic agents might have a major effect on the AO activity and this

parameter should be seriously considered in drug development methodology (Terao et al. 2016; Tayama et al. 2012).

7.5.6 *Mitochondrial Amidoxime-Reducing Component (mARC)*

The mitochondrial amidoxime reducing component (mARC) is the lately discovered Mo-containing enzyme in mammals. In the presence of NADH, mARC proteins (the two homologs, mARC1 and mARC2) exert N-reductive activity together with the two electron transport proteins cytochrome b5 type B and NADH cytochrome b5 reductase. This enzyme system is capable of reducing a great variety of N-hydroxylated substrates. It plays a decisive role in the activation of prodrugs containing an amidoxime structure, and in detoxification pathways, e.g., of N-hydroxylated purine and pyrimidine bases. mARC acts as a component of an N-hydroxylated prodrug-converting complex required to reduce N-hydroxylated prodrugs, such as benzamidoxime (Ott et al. 2015). It is also able to reduce N(omega)-hydroxy-L-arginine (NOHA) and N(omega)-hydroxy-N(delta)-methyl-L-arginine (NHAM) into L-arginine and N(delta)-methyl-L-arginine, respectively (Protein knowledgebase, MARC1). There are also hints that the mARC-containing enzyme system acts as a regulator for NO biosynthesis (Havemeyer et al. 2011). The physiological relevance of mARC is largely unknown (Ott et al. 2015).

7.5.7 *Possible Role of Moco in the NO Synthesis*

The 4 enzymes, activated by Moco, might be involved in the synthesis of nitric oxide (NO) signaling molecule, which is participating in several physiological processes, in prokaryotes as well as in eukaryotes. Nitrite is recognized as an important source of signaling NO, and as a 'storage form', that can be made available to maintain NO formation under conditions of hypoxia. Thus, it is particularly relevant to cell signaling and survival under challenging conditions. Nitrite can exert a significant protective action during ischemia and other pathological conditions. The non-respiratory nitrite reduction to NO is carried out by non-dedicated nitrite reductases, making use of metalloproteins present in cells to carry out other functions, such as several molybdoenzymes, which are a new class of nitric oxide-forming nitrite reductases (Maia and Moura 2015).

7.6 Human Exposure

The Mo RDA for adults is 45 μg/d (Institute of Medicine 2000). Mo comprises a small amount of the sum of all the RDAs for microelements, which totals 26.48 mg/d. For some of the micronutrients, the allowance, as calculated per body weight, might differ remarkably from the recommendations for the animal husbandry or experimental animals. While for the animals, the requirement is estimated according to accelerated growth and high production, for humans, the guidelines for the optimal intake should be based on the lowest risk of morbidity, the highest longevity, and the highest quality of life (Yetley et al. 2016). Much more research is needed to characterize better markers of micronutrient status in terms of metabolic effects, and large-scale trials of different doses of micronutrients are required with precise outcome markers to optimize intakes in different groups of patients as well as in the general population (Shenkin 2006). *Because Mo might have a considerable effect on the metabolism of drugs, the needs for the most appropriate Mo status might be more critical in humans than in the experimental animals.*

Mo is considered as an essential element with all the 'multivitamins' and enteral and parenteral formulas containing Mo (Iacone et al. 2016). Deficiency has been described in adult patients and preterm infants, who received very long-term enteral formulas without Mo supplementation. Clinical sequelae seen in adults with Mo deficiency include cardiac and neurologic, symptoms, such as tachycardia and coma. Today all such formulas are supplied with accurate amounts of trace elements. There is a requirement of 1 μg/kg/d of parenteral Mo and 4–6 μg/kg/d of enteral Mo for the low birth weight infant. The following amounts of Mo are added to the parenteral formulas for preterm infants: 2 μg/d until 6 months; 0.3 μg/kg/d thereafter, and for enteral feeding, 4–6 μg/kg/d (Finch 2015). Mo content in two enteral formulations for malnourished surgical patients is reported as 10 μg/100 mL (Klek et al. 2017). Daily requirement for Mo for surgical patients is 20 μg/d (Braga et al. 2017).

In a Winnipeg survey, the average Mo value in human milk was 5.1 ng/mL. A survey of infant formulas in the US and Canada showed that the average content was 37 ng/mL (7 fold higher than the average human milk content) with a range of 15–80 ng/mL. The formula with the highest Mo content contained 16 fold the concentration of the human milk (Abramovich et al. 2011). This comprehensive survey shows that the Mo content of the formulas is not based on the human milk content, and is extremely exaggerated without any logical justification.

Mo is rapidly absorbed from food and water by gut and placenta when present as the moblydate or trioxide, but not as disulfide, and it is rapidly excreted via the urine. In a study performed in three communities in Croatia, where people use drinking water from wells, the urinal concentration was higher than that in serum (Ćurković et al. 2016). Exogenous or endogenous inorganic sulfate specifically increases Mo excretion and reduces its retention in tissues. High exposure is rare, but high intakes (10–15 mg/d) were documented in India, Armenia and Turkey. No biochemical or clinical effects were observed in humans whose water supplies contained up to

50 μg/L. Increased urinary excretion was observed in humans whose water supplies contained 50–200 μg/L (Chappell et al. 1979).

7.7 Tissue Concentrations

The total body Mo ranges from 900 to 5400 μg depending on intake (Novotny and Turnlund 2007). Mo is widely distributed in the tissues of the body ranging from approximately 3 μg/g in the liver to approximately 0.15 μg/g in the lung, brain, and muscle. The skeleton contains over 50% of the total body Mo, presumably due to the large surface area of the mineral phase of the bone and the possibility of phosphate-molybdate exchange reactions in hydroxyapatite crystals. Tooth enamel has appreciable quantities of Mo of the order of 5 μg/g, and might confer some caries resistance. Rats on a normal dietary intake of Mo have a higher concentration in their livers than in most other tissues, such as kidney, spleen, brain, muscle (Chappell et al. 1979). Tissue concentrations in Australian sheep, mean and range (μg/g): kidney, 0.44 (0.028–2.28); liver, 1.05 (0.12–2.18); and muscle, 0.014 (Maclachlan et al. 2016). Molluscs and insects accumulate Mo at a range of 0.2–1.05 μg/g of dry matter. Mice and vole species incorporate 0.35–0.65 μg/g, whereas shrews store 1.5–2.5 μg/g, i.e. insectivores have significantly higher Mo contents than rodents. The amounts of Mo accumulated by wild and domestic mammals are highest in the liver and kidneys, and lowest in muscle tissue and hair.

In a Korean study in cadavers the following Mo concentrations were found (μg/g): nails, 1.9; liver, 0.73; kidney, 0.27; hair, 0.25; lung, 0.10; heart, 0.09; spleen, 0.08; and cerebrum, 0.05 (Young et al. 2002). In a survey of mineral tests performed by 6 laboratories in the US, the average Mo hair content was 0.63 μg/g with a range of 0.04–2.8 μg/g. For a comparison, zinc average content was 182 μg/g (Cleeman 2001).

The best indicators of Mo deficiency and intoxication are liver, kidneys, blood and milk concentrations. Milk delivers sufficient Mo to the newborns (Anke et al. 2007). Bone, tendon, and cartilage abnormalities, as well as osteoporosis, have been seen in animals with molybdenosis (Chappell et al. 1979). The high and the fluctuating Mo concentrations in the liver, and the high activities of the Mo enzymes in the liver have a major potential for high fluctuations in the drug pharmacokinetics. A stable Mo concentration is presumably a prerequisite for stable drug activities.

7.8 Blood Concentrations

Mo blood concentration (whole blood and serum or plasma) in humans is highly affected by Mo intake (Turnlund and Keyes 2004; Hays et al. 2016). Mo intake and blood concentration presumably control the activities of the Mo enzymes in some health disorders. We have tried to collect all the information available for Mo

concentrations in blood. We have collected data from 17 and 4 studies for serum and whole blood concentrations, respectively, determined in Japan, China, Israel, Croatia, Italy, Switzerland, Belgium, Germany, Poland, Sweden, Norway, France, Canada, Mexico, and Venezuela, (with ~5000 subjects). Since most of the studies checked Mo serum concentrations, we calculated an average of all the available data, assuming that the 4 studies would not significantly affect the resultant average value. We attained an average concentration of 1.08 ng/mL (10.9 nM), the mode, the lowest, and the highest average concentrations being 0.81, 0.4, and 3.8 ng/mL, respectively. The Mayo Clinic Laboratories might well represent the US Mo concentration values with an average of 0.43 ng/mL, a normal range for whole blood of 0.6–4 ng/mL, and a normal range for serum of 0.3–2 ng/mL (Mayo Clinic 2017).

7.9 Mo Excretion

Mo retention and turnover are regulated by urinary excretion, and excess Mo is rapidly excreted in the urine. At high levels, at the range of 100–1500 μg/d, almost all of the Mo was excreted in the urine. At this range of administration, plasma concentration increases linearly from 0.6 to 4.4 ng/mL (Hays et al. 2016). An increase in the dietary Mo results in its more rapid turnover of the body's Mo stores. This was demonstrated by the data on total Mo excretion and the excretion of both oral and intravenous tracers. When dietary intake was very low, ~60% of total Mo was excreted via urine. When the dietary intake increased, >90% of the total Mo was excreted via urine (Institute of Medicine 2000).

Urine is the predominant route of elimination, with upwards of 90% of an oral dose being eliminated via urine with a half-life of less than 12 h. In plasma, elimination has been described with a biexponential function with mean half-lives of 0.5 and 6.6 h, respectively (Hays et al. 2016).

Mo urinal excretion highly fluctuates between individuals. In 10% of 35 Japanese male adults, Mo excretion was <0.2 ng/L while in 10% of the subjects at the higher level of excretion, it was >3 ng/mL (Yoshida et al. 2006). In the US, the average concentration of urinary Mo is 69 μg/L (Institute of Medicine 2000), a value that might reflect an average daily intake of 76 μg/d for females and 109 μg/d for males (Institute of Medicine 2000).

In premature infants and young men, isotope studies confirm efficient absorption and urinary excretion increasing in parallel (Disease Month 2004). Transitioning of Mo intake from 22 to 72 μg/d resulted in tripling of the fraction of plasma Mo excreted into urine. Further transitioning from 121 to 467 μg/d resulted in an additional doubling of fractional transfer of plasma Mo into urine (Novotny and Turnlund 2007). Because urine Mo is highly related to plasma level and Mo intake, urine Mo reflects plasma/serum Mo concentration (Hays et al. 2016). The fraction of Mo excreted in the urine is very different from that of most minerals. Extremely small amounts of copper are eliminated in the urine, and under usual conditions, the amount does not

vary with dietary intake. More zinc is excreted in the urine, but this is also a small fraction of dietary intake (Turnlund et al. 1995).

7.10 Food Sources

Typical intakes of Mo are probably in the range of 1.5–2.5 μg/kg/d per body weight. An average dietary Mo content in representative total diets from 11 countries was 230 μg/kg/d of dry matter (WHO 1996). High variation is expected for Mo intake, because Mo content in food is highly affected by soil and water concentrations (Institute of Medicine 2000; O'Connor et al. 2001; Smedley et al. 2014). The following food Mo concentrations have been collected, μg/kg: pulses, 2.5; cereals, excluding wheat, 1.5; wheat, 0.3; milk, 0.26; eggs, 0.18; fruits, 0.13; fish, 0.12; vegetables, 0.08; sugar, 0.08; meat, 0.07; and oil, 0 (Rajagopalan 1988; Ali et al. 2014). The main contributors of Mo in North American diet are legumes, grain products and nuts (Hays et al. 2016). The content of Mo in foods as published in a German list varies from 10 to 6000 μg/kg dry matter: cereal products, sugar- and starch-rich food, luxury food, bread, rolls, cake, spices and most kinds of fruits, 10–400 μg/kg dry matter. Vegetable foodstuffs as part of mixed diets in Germany deliver 70% of the human intake, animal foodstuffs about 20% and beverages less than 10% (Anke et al. 2007). The Mo contributors of the daily Japanese menu containing 225 μg/d, % are: rice, 45; pulses, 29; vegetable and fruits, 11; wheat, 4; eggs, 6; and fish, 5 (Hattori et al. 2004). In Cameroon, the Mo contributors in the daily intake of 195 μg/d, % are: cereals and cereal products, 30; tubers and starches, 8; fruits, vegetables and oilseeds, 49; and beverages, 6 (Gimou et al. 2014).

The Western diet contains 200–500 μg/d of Mo (Abumrad 1984). The following daily intakes have been collected μg/d: Britain, 130; US, 330 (range of 210–460); and New Zealand, 46–96 (Chappell et al. 1979). Most natural waters contain low levels of Mo, in the range of 2–3 μg/L (Barceloux 1999). Legumes contain symbiotic nitrogen binding bacteria called rhizobia, which produce nitrogen compounds that help the plant to grow and compete with other plants (Thrall et al. 2011). Mo is essential for microbial nitrogen assimilation due to its presence in nitrogenase (Glass et al. 2012). Probably, beans contain the highest Mo concentrations of all foodstuffs (Seifert et al. 2007). However, there are very high variations in Mo concentrations between land areas, bean species, varieties, and seasons (Ojeda et al. 2015; Nobile et al. 2016; O'Connor et al. 2001).

7.11 Mo-Tungsten Interaction

Mo and tungsten (symbol W, atomic number 96, and atomic weight 184) enzymes show common structural features, with the metal being bound by a pyranopterin-dithiolene cofactor called molybdopterin and tungstopterin, respectively. They are

also similar in functional aspects. Mo is in many ways the twin element of tungsten. However, tungsten does not act as a catalytic metal in animals, but only in microorganisms (Holm et al. 2011; Pushie et al. 2014; Bevers et al. 2009). In animals, tungsten competes with Mo and occasionally induces Mo deficiency. In bacteria, there are cases in which tungsten has been successfully substituted for Mo, although sometimes with a change in pH activity profile (Holm et al. 2011). Tungsten concentration remarkably affects Mo activity in bacteria. However, humans are strictly dependent on the availability of Mo, while they are independent of tungsten (Bevers et al. 2009).

7.12 Mo Deficiency and Congenital Metabolic Defects

Mo is characterized by extremely rare episodes of deficiency. The two known phenomena are an episode of a long-term total parenteral nutrition (TPN) and very rare episodes of congenital error in Mo enzymes and Moco. The patient on TPN presented with symptoms of amino acid intolerance, irritability, coma, hypermethioninemia, increased urinary xanthine and sulfite and decreased urinary uric acid and sulfate. These abnormalities were alleviated after the patients were administered TPN containing Mo concentrations of 19, 47.5 and 190 μg/d (Stehle et al. 2016).

Moco deficiency is a rare severe autosomal recessive inborn error of metabolism that leads to the deficiency of the reducing component of all Mo enzymes (SO, XOR, AO, and mARC). The resultant accumulation of sulfite, taurine, S-sulfocysteine and thiosulfate contributes to the severe neurological impairment (Atwal and Scaglia 2016). The incidence is estimated between 1 in 100,000–200,000 live births, and over 100 patients from multiple ethnic groups have been identified worldwide. Congenital metabolic defects caused by Moco deficiency are characterized by a neonatal presentation of intractable seizures, feeding difficulties, severe developmental delay, microcephaly with brain atrophy and coarse facial features.

Biochemical symptoms of Moco deficiency are elevated levels of xanthine, hypoxanthine and sulfites, low levels or non-detectable levels of uric acid and elevated levels of *S*-sulfocysteine in plasma and urine. Furthermore, urothione, the Moco degradation product, is absent in the urine of Moco-deficient patients. Most infants die in early childhood; those who survive the neonatal period have a profound developmental delay, with abnormal tone, ocular lens dislocation, dramatic and progressive loss of white matter in the brain. Most of the clinical findings observed in patients are attributable to the loss of SO activity and the subsequent neurotoxic effect of sulfite accumulation. Moco and SO deficiencies are clinically indistinguishable and only biochemical and genetic tests can differentiate between these two disorders (Jakubiczka et al. 2016).

7.13 Mo Toxicity

7.13.1 Mo Toxicity in Humans

As the tinniest essential trace element (with established RDA) in the human body, Mo is highly affected by the concentrations of other essential and nonessential trace metals. The chemical toxicity of a given element is related to its interactions with the biochemical processes in the human body. Some of these interactions may be beneficial or even essential, whereas others may be detrimental. Even moderate concentrations of nonessential elements, such as aluminum, nickel, titanium and uranium, might be strong competitors of essential elements in the biochemical process and can influence general human health. Pollution has a large impact on the accumulation rate of toxic elements in the human body (Zeneli et al. 2015).

Symptoms of toxicity were exhibited by humans exposed to environmental contamination, such as intake of 10–15 mg/d of Mo, resulting in gout-like symptoms associated with high blood concentrations of Mo, uric acid, and xanthine. It was also reported that an intake of 0.54 mg/d of dietary Mo resulted in significant urinary copper losses (Freeland-Graves and Turnlund 1996).

7.13.2 Mo Effect on Copper Metabolism

Mo metabolism is related to copper and sulfur metabolism. Mo salts are capable of inhibiting the absorption of iron and copper and the formation of copper molybdate or thiomolybdate compounds. Copper generally has a beneficial effect on the symptoms caused by excessive Mo concentration. Mo affects copper utilization, and increased Mo intake results in copper depletion (Chappell et al. 1979). Because Mo toxicity is associated with depleted copper stores, humans with an inadequate intake of dietary copper could be at greater risk of Mo toxicity (Vyskočil and Viau 1999).

7.13.3 Mo Toxicity in Plants

Dicotyledonous species generally are less tolerant to excess Mo than are the monocotyledonous species (Gupta and Gupta 1998). Fertilizers for leguminous plants incorporate Mo salts, and standard commercial fertilizers contain approximately 2–6 mg/kg (Barceloux 1999). Mo is particularly important for microbial nitrogen assimilation due to its presence in nitrogenase, the enzyme that performs N_2 fixation, and in nitrate reductase, the enzyme that performs the first step in nitrate (NO_3^-) assimilation, reduction of NO_3^- to nitrite (NO_2^-). N_2 fixation requires more Mo than NO_3^- assimilation, while other more chemically-reduced forms of N, such as NH_4^+, do not require Mo for assimilation (Glass et al. 2012).

7.13.4 Mo Toxicity in Animals

Ruminants are more sensitive to Mo than monogastric animals, but the basis for the toxicity of Mo in ruminants is not relevant for humans (Institute of Medicine 2000). In a decreasing order, the following animals are sensitive to high Mo intake: sheep, pigs, rats, guinea pigs and poultry (Chappell et al. 1979). Mo toxicity in ruminants has been observed in many locations. Generally, where such symptoms are observed, the feed contains 6–36 mg/kg of Mo (Miller and Engel 1960). The rumen is the site of significant interactions between copper, sulfur and Mo. It also shows reactions between copper, sulfur, and iron. The interaction between Mo and sulfur results in the formation of thiomolybdates, which readily bind copper and in the absence of adequate quantities of rumen copper are absorbed into the animal and bind to copper in plasma and other tissues. This is the cause of thiomolybdate toxicity, often misleadingly called copper deficiency. Another interaction between iron, sulfur and copper, might intensify the thiomolybdate problem by making copper unavailable to bind to the thiomolybdates (Gould and Kendall 2011).

7.14 Tolerable Upper Intake Level (UL)

The UL for Mo was set by the IOM as 2 mg/d (Institute of Medicine 2000), an amount that is ~45 times the suggested intake for adults. The UL refers to the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population. Since human studies are limited in their scale and most limited in observations of excess metal consumption that is far above the suggested intake, animal experimentation was the main basis for UL evaluation. The UL for Mo is based on adverse reproductive effects in rats fed high levels of the mineral (Institute of Medicine 2000). A negative effect of Mo excessive consumption on animals cannot serve as a basis for establishing UL in humans.

7.15 Saturation of the Mo Enzymes

Metalloenzymes, such as XOR, must bind ionic metal for their activity and must be saturated by the ionic metal for their maximal activity. In the mouse fibroblastic cell line, the highest XOR activity was observed at the Mo concentration of ~20 mM (1800 μg/mL) with about a linear increase from zero concentration to 10 mM Mo. Within the data we have collected, the average Mo concentration in the human serum is 0.0014 μg/mL, and in human liver, 0.73 μg/g. The concentrations in these two tissues are far below the Mo saturation level as measured in mouse fibroblastic cell line for XOR (Falciani et al. 1994). In a rat experiment, tissue Mo concentrations dramatically responded to the dietary Mo and reached the plateaus at various levels

of dietary Mo. When dietary Mo increased, Mo concentrations in the liver increased up to 200 μg Mo/kg diet level. Beyond this level, no further increase in tissue concentration occurred. Supplementation up to 50 μg/kg diet almost doubled the hepatic SO activity relative to the activity in the group fed the basal diet. Further supplementation of dietary Mo did not have a significant additional effect on the activity. The results suggest that the activities of the Mo-enzymes reflect the concentrations of dietary Mo and may be useful in the assessment of the nutritional status of Mo (Wang et al. 1991). Since Mo enzymes have a major effect on drug metabolism, Mo intake might have a critical role in the drug pharmacokinetics.

The information presented for the Mo enzyme saturation by the Mo ion is quite limited for such a critical issue regarding the possible effect of Mo on the drug pharmacokinetics (Falciani et al. 1994). The effect of Mo enzyme saturation on drug metabolism might justify an examination of the impact of the Mo intake on the drug pharmacokinetics. Fluctuations in Mo intake, such as in the case of traveling to another country, might change the Mo tissue concentration and increase or decrease the rate of the drug degradation. In critical morbidity, such an outcome might have a harmful effect.

7.16 The Effect of Mo Enzymes on Drug Metabolism

Hepatic clearance plays a key role in determining the systemic exposure of drugs and metabolites, which in turn has a major effect on variability in the beneficial and adverse effects of medications. Aging results in a number of significant changes in the human liver, including reductions in liver blood flow, liver size, drug-metabolizing enzyme content, and pseudocapillarization. Drug metabolism is also influenced by comorbid disease, frailty, concomitant medicines, and epigenetics. These changes have the potential to alter the hepatic clearance of drugs. There is a growing evidence that the age-related changes in the liver not only result in a decrease in the hepatic clearance of unbound drugs, but also influence variability in response to medications in older people. Misinterpretation of these age-related changes in total hepatic clearance could lead to recommendations to actually increase the dose rate in older people, whereas the unbound clearance is decreased, leading to a considerable risk of toxicity (McLachlan and Pont 2012).

Mo enzymes are only a minor part of the drug degradation system, but because of their extensive activity in drug decomposition, their fluctuating activities might deteriorate the pharmacokinetics of many drugs. Metabolites generated by the drug metabolizing enzymes can be pharmacologically active, and reactive metabolites formed during bioactivation might cause toxicity (Di 2014). Therefore, a comprehensive understanding of the factors that influence hepatic metabolism in older people is critical to allow individualization of drug and dose selection for older people, and thus achieve optimal outcomes for this patient population (McLachlan and Pont 2012).

7.17 Increased Medication Intake

The striking effect of Mo enzymes on drug metabolism has a particular impact on the elderly people, because this population segment consume medications at a much higher rate than any other subgroup in the population. With the general trend of increase in longevity, the marked increase in the old adult population, the expansion of the pharma industry, and the increased role of the medications in our life quality and longevity, there is a continuous increase in the medication intake worldwide (Gransjön et al. 2017). In the US NHANES, 51% of the adults reported use of any drug, and a significant increase of about 0.68%/year in overall prescription drug use and polypharmacy were observed. These increases persisted after accounting for changes in the age distribution of the population. The prevalence of prescription drug use increased in the majority of, but not all, drug classes (Kantor et al. 2015).

In Germany, the proportion of antihypertensive drugs use among adults with hypertension significantly increased over time from 53.8% in 1998 to 71.6% in 2008–2011 (Sarganas et al. 2016). In Sweden, medication use increased from 1987 to 2007 for both genders and in all age groups; in the age group 78 year, from 2.8 drugs in 1987 to 5.8 drugs in 2007, and corresponding figures for the age group >96 year was from 3.6 to 7.7 (Gransjön et al. 2017). In the elderly population in Israel, an increased intake of medications was observed with advanced age. At least one medication a day was routinely prescribed by a physician in ~81% of subjects aged 65–74 year and in ~94% of subjects aged >75 year (Brodsky et al. 2007).

7.18 The Advantage of Lower Mo Concentrations in Some Health Disorders

We have collected some information showing the advantage of the lower Mo concentrations over its higher concentrations in type 2 diabetes and spermatogenesis. Elevated incidence of type 2 diabetes and impaired spermatogenesis disorders might become biomarkers for the major health burdens, such as high incidence of other non-communicable diseases (NCDs), mortality, and disability.

7.18.1 Type 2 Diabetes

Some limited information has suggested a relationship between type 2 diabetes and higher serum Mo. In a small study conducted in Mexico, higher serum Mo concentrations were found in diabetic patients compared to healthy controls. Mo concentrations in serum tended to be elevated in diabetic patients as referred to control subjects and significantly increased in severe complications as compared to slight-to-moderate concentration groups. Serum Mo concentrations correlated directly with several

parameters characterizing the progress of diabetes. In patients with severe complications elevated serum Mo concentrations were closely associated with nephropathy, neuropathy, serum creatinine and urine total proteins, and glycosylated hemoglobin. It remains uncertain why serum Mo presented a tendency toward higher concentrations in diabetic patients versus healthy individuals and was significantly increased in severe versus slight-to-moderate complications. It is also unclear how Mo–sulfur species required for Cu chelating are generated in diabetic patients (Flores et al. 2011). In the NHANES 1999–2010, Mo was consistently positively associated with diabetes prevalence; a much lower relative risk for diabetes was found for urinary Mo excretion at the 1st quartile than at the 2nd to the 4th quartiles;.also, higher quartiles of Mo were associated with greater homeostatic model assessment (HOMA) values of insulin resistance (Menke et al. 2016).

In a small group of obese patients with gestational diabetes visiting prenatal clinic at the Maternity Hospital in Kuwait, a slightly elevated level but still significant of blood Mo concentration was observed (Al-Saleh et al. 2007). In a study conducted in Tehran in type 2 diabetic females aged 35–55 year, hair Mo content was higher by 33% than in the control. Similarly, hair cadmium, copper, aluminum, and lead concentrations were elevated in these females (Tadayon et al. 2012). An in vitro study, suggests that Mo is capable of inducing toxicological effects in pancreatic β-cells that result in dysfunction and apoptosis (Yang et al. 2016). Because type 2 diabetes is of major health concern worldwide, the association between Mo and diabetes should attract the attention of the health authorities in each country.

The practice of Mo incorporation into ‘multivitamins’ has never been supported by robust data and presumably has never been supported by any data. Mo deficiency has never been shown, except for in one patient on a long-term TPN and in the very rare cases of congenital diseases (Abumrad et al. 1981). Therefore, it would be prudent to consider the possibility of Mo free ‘multivitamins’ for diabetic patients.

7.18.2 Impaired Human Spermatogenesis and Lower Testosterone Levels

Exposure to environmental contaminants rather than genetic defects accounts for the defects in reproductive functions. Heavy metals are found to increase the reactive oxygen species leading to oxidative stress, induce DNA damage and cause apoptosis of sperm cells with the ability to disrupt blood-testis-barrier and affect spermatogenesis events. Semen analyses are conducted worldwide in infertility infirmaries with some routinely available parameters for its quality. The major effects observed are the decrease in sperm count, the abnormal increase in sperm counts, sperm DNA damage, and impaired sperm motility. Except for lead, mercury, cadmium, arsenic, and aluminum, essential elements also affect semen quality (Jenardhanan et al. 2016). In a study conducted in two Michigan infertility clinics, the investigators found signif-

icant or suggestive associations and dose-dependent trends between blood Mo and declined sperm concentration and morphology (Meeker et al. 2008).

Compared with Mo levels at the lower range, the middle and high Mo levels (70th to 85th percentile and >85th percentile, respectively) were associated with 23 and 60 ng/dL reductions in testosterone levels, respectively. Mo concentration was also associated with a significant inverse trend in free androgen index (Meeker et al. 2017).

7.18.3 Bone Mineral Density

Bone mineral density (BMD) is a noninvasive measure of bone health that depends on peak bone mass reached in adolescence and subsequent bone loss. BMD is lower in women than men and decreases substantially with age in both genders, leading to complications such as osteoporosis, which increases the risk of subsequent fractures. In an analysis, which used publicly-available data derived from the US NHANES 2007–2010, there was a statistically significant inverse association between urine Mo and lumbar spine BMD among 50 to >80 year women. Similarly, there was a statistically significant dose-dependent decrease in lumbar spine BMD with increasing urine Mo quartiles among these women from the lowest to the highest quartile. The potential biological mechanisms through which Mo may act to influence BMD have not been identified or even largely explored, but it is plausible that this trace element may disrupt levels of steroid sex hormones, which are necessary for promoting and maintaining bone health. Notably, early animal studies have shown that Mo may influence normal bone development through its antagonistic relationship with copper. Additionally, since bone tissue serves as a reserve for minerals, such as Mo, it is possible that the inverse association between urine Mo and BMD observed in older women might be due to the release of Mo from bone demineralization associated with aging (Lewis et al. 2016).

7.18.4 Liver Malfunction

In an analysis, which used publicly-available data derived from the US NHANES 2007–2010, participants who reported liver problems had increased creatinine-adjusted levels of Mo in their urine. The hepatotoxicity of the metal has been observed in a few animal studies after chronic Mo exposure and the mechanism was thought to be an induction of fatty changes in the liver. However, no convincing epidemiologic studies in humans have confirmed the findings observed in animals (Mendy et al. 2012).

7.18.5 Susceptibility of Jurkat Cells to Metal Concentrations

Jurkat cells are human T-helper lymphocytes that enable in vitro measurement of cell viability under stress conditions induced by foreign antigens or elevated concentrations of metals. Exposure in vitro of an isolated cell to ion metals, including Mo showed adverse effects on some of the studied parameters, such as DNA damage, apoptosis, viability, and proliferation inhibition. The average concentration to cause a harmful outcome was lower for Mo than for zirconium, beryllium, chromium, aluminum, and iron (Caicedo et al. 2007). This study shows that ion metals might exert harmful effects on some sensitive cellular activities even at concentrations that are essential for normal biochemical activities, and that Mo does not behave as an inert ion even at low concentrations.

7.19 U-Shaped Curves and Nadir Values

Presently, no U-shaped curves are available for the effect of Mo concentrations on the relative risk of morbidity and mortality. The scope of blood (serum/plasma) determinations is very limited, and no consensus has been reached for the optimal cut-off points for Mo intake or blood concentration. The public health services and medical teams worldwide are not aware of the advantage of adjusting optimal serum Mo levels according to the relative risk of morbidity and mortality. However, presently, nobody knows what are the optimal Mo concentrations and the optimal Mo intake, and even no motivation arises to assay Mo levels. Thus, no research funds are invested in the determination of Mo optimal concentrations. In the absence of available data for establishing the optimal intake (Otten et al. 2006), which determines blood concentrations, no nadir value has been established for Mo blood concentrations.

7.20 Mo Supplementation

The available data for the recommended intake of 45 μg/d and of the UL of 1100–2000 μg/d (Institute of Medicine 2000) is not supported by robust data (Hays et al. 2016). For most nutrients the recommended intake is actually based on an average intake without any physiological endpoint (Yetley et al. 2016). The Mo intake highly depends on the diet type (generally higher Mo intake is found in a vegetarian diet) and on the land area (the higher the soil content the higher the intake). Mo intake in the studies widely varied between societies and land areas. The following data have been collected μg/d: in Germany: mixed diet, 95, vegetarian diet, 175; in Mexico, 185 (Holzinger et al. 1998); in the US adults, 160 (Hunt and Meachen 2001); in another US study, 120–240 μg/d (Tsongas et al. 1980); in Japan, 225 (Hattori et al. 2004); in Belgium food survey, 87 (Van Cauwenbergh et al. 1997). A median of

150 μg/d, with extreme values of 928 in China, 523 in India and 58 in Germany, was calculated from 21 observations in 11 countries (Van Cauwenbergh et al. 1997).

It might be suggested that for the majority of the people, Mo intake exceeds the optimal level. This is claimed in the light of the data found for the disadvantage of the higher percentiles of Mo intake, the higher urine excretion and the higher Mo blood concentrations found in in vitro and some human studies. Since Mo intake mainly depends on soil content, the ability to optimize Mo status is limited. However, legumes, particularly beans, comprise the main source of Mo intake (Seifert et al. 2007). Monitoring of beans intake might substantially decrease Mo intake. Presently, the information presented here does not support any suggestion of intake for the general population. However, for diabetic patients we cannot ignore the data shown here. In the absence of contradictory information, there is no need to incorporate Mo in any 'multivitamin' supplement for this group of patients. Monitoring Mo intake by decreasing beans intake or by consuming beans with a low Mo content should be considered.

Mo content at a range of 20 μg/d for TPN adult patients suggested by ESPEN sounds reasonable. Presently, the TPN formulas contain a very high content of Mo. In extensive surveys of enteral formulas and TPN for the adults, the Mo content was the second highest among all the other micronutrients and was higher far above the ESPEN recommendations (Iacone et al. 2016; Stehle et al. 2016).

7.21 The Present State of Mo Research

We have presented here some crucial nutritional issues regarding Mo.

A. What should be the optimal Mo intake?
B. What is the nutritional status of the Mo in various societies?
C. Is the present practice of Mo supplementation or Mo incorporation in nutritional formulas justified?
D. What is the relationship between serum Mo and the pharmacokinetics of drugs, and in particular the relationship between serum Mo and the pharmacokinetics of drugs for chronic diseases?

The available data on these critical issues is most limited. Moreover, the nutritional research of Mo is most limited. The number of topic items for "molybdenum intake" for the year 2015 is on decreasing trend in comparison to other microelements and to the former years. The number of "metal intake" topics for the year 2015 for zinc, iron, copper, iodine, and selenium lay at the range of 130–400 for each metal, while for molybdenum only 11 items were found (Web of Science). Thus, an extensive study of Mo role in human nutrition is required.

7.22 Conclusions

1. Molybdenum (Mo) functions in humans as a cofactor for 4 enzymes, SO, XOR, AO and mARC.
2. Though Mo deficiency and toxicity are very rare, Mo status has a critical role in human health.
3. Mo concentration, presumably, has a major effect on drug pharmacokinetics.
4. Mo intake highly varies between people according to the content of the soil wherein the foods were grown.
5. Mo intake presumably exceeds the optimal level.
6. Diabetic patients are particularly sensitive to higher Mo intake.
7. Mo intake might be controlled by excluding beans intake or by consuming beans with low Mo content.
8. The practice of Mo incorporation into commercial supplements is questionable.

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Chapter 8
Water and Sodium Balance Disorders in Aging

Massimo Boemi and Maria Paola Luconi

Abstract The elderly population is growing rapidly and people with more than 60 years is increasing faster than adults of any younger decades. The mechanisms involved in water and sodium metabolism are complex and change with age; they include vasopressin system, renin-angiotensin-aldosterone system (RAAS) and the action of the natriuretic peptides. Fluid and sodium balance is generally conserved but this homeostasis could be lost due to illness conditions (physical and/or cognitive) and some pharmacological treatments. Consequently, the serum sodium imbalance is one of the most common finding in clinical practice and a major cause of hospital admissions. Hyponatremia and hypernatremia are defined respectively as serum sodium level <135 and >145 mmol/l. Both electrolyte disorders are associated with high morbidity and mortality in humans as well as in animals. At the same time, they are potentially preventable, thus an early diagnosis with the recognition of the underlying causes and a proper management may avoid negative outcomes. This chapter attempts to provide an overview on the age-related changes in the peripheral and central mechanisms that regulate water and sodium homeostasis in humans and animal models. It will describe the clinical manifestations of dehydration and volume overload and it will address the current challenges in diagnosis and treatment of hyponatremia and hypernatremia.

Keywords Water · Sodium · Osmolality · Dehydration · Hyponatremia Hypernatremia · Aging

8.1 Introduction

The elderly population is growing rapidly, especially as a result of socio-economic progressions and improvements in clinical management (http://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2015_Report).

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Aging is associated with changes in the complex systems that regulate water and sodium homeostasis in both humans and animals: hypothalamic vasopressin secretion, thirst perception and kidney function. In particular, some structural and functional renal changes have been described with aging; they lead to a reduced ability to concentrate or dilute urine (Cowen et al. 2013; Muntner 2009; Koch and Fulop 2017). Moreover, in the elderly the functional and cognitive decline may reduce the ability to assume a proper amount of water or communicate its need (Koch and Fulop 2017; Phillips et al. 1991).

Plasma osmolality is normally maintained within an extremely narrow range of variation. Hypo-osmolar and hyper-osmolar states are conditions respectively due to an excess and a deficiency of body water relative to body solute. Because the sodium is the main constituent of extracellular (and plasma) osmolality, hypo-osmolar and hyper-osmolar states generally reflect changes in sodium concentration and correspond with hyponatremia and hypernatremia (Verbalis 2003; Adler and Verbalis 2006; Anderson et al. 1985).

A great number of potential causes of sodium imbalance is recognized, as well as a wide spectrum of clinical manifestations ranging from the absence of symptoms to severe neurological signs (Spasovski et al. 2014; Kim 2006). Hyponatremia and hypernatremia are both associated with increased morbidity, length of hospitalization and mortality; a proper assessment with the research of underlying causes represent a challenge for clinicians (Liamis et al. 2013; Wald et al. 2010; Snyder et al. 1987; Palevsky et al. 1996).

8.2 Water Homeostasis in Humans: Mechanisms and Age-Related Changes

The body of healthy adults has constituted by water for about 55–65%; this percentage decreases proportionally with age because of the typical changes of senescence, in particular the reduction of water-rich tissues (such as muscle mass). Water exerts many functions: structural, carrying (for nutrients and toxics), thermoregulatory (to maintain body temperature stable during changes of external temperature) and regulatory in chemical reactions (such as hydrolysis reaction) (Verbalis 2003).

Approximately 55–65% of total body water (TBW) is contained in the intracellular fluid (ICF), and the other 35–45% in the extracellular compartments (ECF). The latter is divided between the interstitial fluid (about three quarters of the total) and intravascular fluid or blood volume (the other one quarter). Biological membranes are semipermeable, namely freely permeable to water but not to aqueous solutes. Water shifts across cell membrane from a compartment with a lower solute concentration to another with a higher one, until the osmotic pressure (a function of the concentration of all the solutes in a compartment) has been equalized on both sides. The membrane-bound Na^+/K^+ pumps maintain Na^+ in a primarily extracellular location and K^+

in a primarily intracellular location, so ICF and ECF differ consistently in solute composition, but their osmotic pressure is equivalent (Verbalis 2003; Fanestil 1994).

The osmolality is defined as the concentration of all the solutes in a given weight water. Plasma osmolality can be measured directly or estimated with the following formula:

$$\text{Plasma Osmolality} \sim 275-295\ \text{mOsm/Kg}H_2O$$
$$= 1863 \times \text{Serum } Na^{+}(\text{mmol/l}) + \frac{\text{Glucose (mg/dl)}}{18} + \frac{\text{Glucose (mg/dl)}}{2.8}$$
$$BUN = Bread\ Urea\ Nitrogen$$

Of all solutes, those impermeable to cell membranes (such as, Na^+ and mannitol) are "effective solutes"; they create osmotic pressure gradients leading to the shift of water between ICF and ECF compartments. On the contrary, the solutes permeable to cell membranes (such as, urea and ethanol) are "ineffective solutes", not able to create an osmotic pressure gradient. Glucose has a double action: at physiological plasma concentrations, it acts as an ineffective solute and it is moved through the membranes by active transport mechanisms, on the contrary, when augmented (i.e. in case of insulin deficiency and impaired tissue uptake), it becomes an effective extracellular solute (Verbalis 2001; Vokes et al. 1987).

Under physiological condition, the human body maintains plasma osmolality and serum sodium concentration within an extremely narrow range, respectively 275–295 mOsm/Kg H_2O and 135–145 mmol/L (mEq/L), to avoid osmotically-induced cellular dysfunction. The water metabolism depends on the interaction of central and peripheral mechanisms involving three principal sites: the hypothalamus, the center of thirst and the kidney (Spasovski et al. 2014).

8.2.1 Osmoreceptive Neurons, Baroreceptors and Arginine Vasopressin (AVP)

The osmoreceptive neurons are located in the anterior hypothalamus; they are sensible to changes of cell stretch due to variation of effective plasma osmolality. A decrease in cell stretch (cell dehydrated) in response to increased plasma osmolality (or tonicity) triggers their firing activity and lead to both augmented thirst and secretion of arginine vasopressin (AVP) (Spasovski et al. 2014).

AVP, also called anti-diuretic hormone (ADH), is a nine-amino-acid peptide produced by magnocellular neurons located in the hypothalamic supraoptic and paraventricular nuclei (3rd ventricle). After synthesis, AVP is transported to the bloodstream through the axon terminals located in the posterior pituitary. It exerts an anti-diuretic action by regulating water permeability of the nephron. AVP binds vasopressin-2 (V2) receptors and determines the insertion of aquaporin-2 water channels (AQP2) into the apical surface of the collecting duct principal cells. By the action of these

channels, free water is re-absorbed and a more concentrated urine is excreted (Knepper 1997). Usually, an increase in plasma osmolality as small as 1–2% is enough to increase the plasma vasopressin concentration of 1 pg/ml. Maximum antidiuresis is obtained in presence of ADH concentrations above 5 pg/ml (Wong and Verbalis 2002).

Baroreceptors are stretch-sensitive receptors located in carotid sinus, left atrium, aortic arch, pulmonary veins and large arteries. Their discharge rate decreases concordantly with the reduction of effective circulating volume, stimulating the release of ADH and the activity of efferent symphatetic nerve (vasoconstriction and increase in heart rate). A reduction in blood pressure of at least 5% is required to elicit the increase of serum vasopressin concentration; in case of worsening circulatory hypovolaemia, vasopressin concentration dramatically increases and baroregulation overrides the osmoregulatory system (Spasovski et al. 2014; Baylis 1983).

AVP may also be released in response to pathological conditions, such as pain, nausea, medications, endogenous stimulus (acetylcholine, histamine, dopamine, prostaglandins, bradykinin, neuropeptide Y, angiotensin I) or medications. On the contrary, its release is inhibited by nitric oxide, atrial natriuretic peptide (ANP) and opioids. (Adler and Verbalis 2006; Schlanger et al. 2010). Schwartz WB and Bartter FC defined this condition as "syndrome of inappropriate antidiuretic hormone secretion" (SIADH). Now the term "syndrome of inappropriate antidiuresis" (SIAD) is preferred because it includes the increase of water permeability in the collecting duct due to genetic factors or drugs (vasopressin-independent) (Spasovski et al. 2014; Schwartz et al. 1957).

8.2.2 Thirst

Thirst is the stimulus of water ingestion in response to the perception of body fluids deficit. Thirst center is located near the hypothalamic osmoreceptors. It is elicited, in humans and animals, by an increase in the extracellular fluid osmolality (with secondary reduction in intracellular volume) or by an intravascular hypovolaemia (with secondary reduction in extracellular volume) (Verbalis 2003). Studies in humans have described that a minimum increase in plasma osmolality above basal levels may produce the sensation of thirst, conversely a greater threshold of blood volume depletion (hypovolaemia and hypotension) is needed for producing thirst (Robertson 1983; Thompson et al. 1986). This minor response to baroreceptor activity is probably a result of human adaptation to blood fluctuations, such as the orthostatic pooling of blood in the lower body during erect posture (Verbalis 2003).

Thirst and sodium appetite are also regulated by hormonal signalling systems, such as the activation of the renin-angiotensin-aldosterone system (RAAS) in presence of hypovolaemia and hypotension (Phillips et al. 1985).

Under normal physiological conditions, the threshold for releasing vasopressin (and subsequent antidiuresis) is lower than that for triggering thirst; the osmotic threshold for thirst is about 5–10 mOsm/Kg H_2O lower than for ADH secretion

(Knepper 1997). The intake of water is generally in excess of true 'need'. When it is inadequate to supply body needs, even in the presence of AVP-induced maximal antidiuresis, plasma osmolality rises to levels able to stimulate thirst and produce water intake proportional to the elevation of osmolality. Thirst is a defence behaviour essential when pituitary and renal mechanisms are insufficient to maintain plasma osmolality within a narrow range and to avoid dehydration (Verbalis 2003).

8.2.3 Kidney and Renin-Angiotensin-Aldosterone System (RAAS)

The mechanism by which the kidney produce maximum urine osmolality is complex and depends on many factors: vasopressin stimulus, renal blood flow and glomerular filtration rate (GFR), solute load, sodium and urea transporters, water channels (or aquaporins) functions. The countercurrent configuration of the loops of Henle creates a gradient of solutes from the cortex to the medulla via the re-absorption of both sodium and urea from the lumen; this system is essential in order to concentrate the urine (Schlanger et al. 2010). The maximum medullary gradient is reached in case of dehydration (~1.200 mOsm/L) (Gottschalk and Mylle 1958).

Thirteen aquaporins (AQPs) have been identified. AQP1 is a constitutive vasopressin-independent water channel located on the apical and basolateral membranes in the proximal convoluting tubules (PCT), on the thin descending limb of the loop of Henle (tDL), and on the endothelium of the descending vasa recta. The PCT is responsible for the re-absorption of 60% of the glomerular ultrafiltrate, whereas the tDL is essential in maintaining the countercurrent multiplier. AQP3 and AQP4 are constitutively active and located on the basolateral membrane. Studies have shown a greater expression of AQP3 in the presence of ADH. Finally, AQP2, as described above, is a vasopressin-dependent water channel located in the apical membrane of collecting tubules. (Agre 2006; Knepper 1994).

RAAS is a hormonal system strictly involved in water and salt homeostasis. In response to hypovolaemia and ECF depletion this system activates and elicits thirst (Abdelaal et al. 1976). An effective volume depletion induces at kidney level the secretion of renin from the juxtaglomerular apparatus, via both a reduction in renal perfusion pressure and an increase in the sympathetic nervous system activity, with secondary elevation of circulating levels of Angiotensin II (Ang II) (Fitzsimons 1998). The latter one generates the sensation of thirst sending signals to the median preoptic region of the hypothalamus (Simpson 1981).

Aldosterone is an adrenal mineralocorticoid hormone, primarily secreted in response to elevation of Ang II, but also stimulated by high serum potassium concentrations. In tubular epithelial cells of the distal nephron, aldosterone binds mineralocorticoid receptors (MR), increases sodium re-absorption and sodium-potassium exchange by enhancing the synthesis and activity of ion channels, in particular the epithelial sodium channel (ENaC) (Masilamani et al. 1999) Aldosterone is inhib-

ited by the atrial natriuretic peptide (ANP) and by hyperosmolality. Moreover, other natriuretic factors may override its effects through a phenomenon known as "escape from mineralcorticoids", in which sodium balance is re-established after a period of sodium retention and ECF expansion (Schneider et al. 1985).

8.2.4 Natriuretic Peptides

Natriuretic peptides are three endogenous hormones, atrial (ANP), brain (BNP) and C-type (CNP), widely expressed in brain tissue, particularly in regions related to thirst, sodium appetite and body fluid homeostasis (Antunes-Rodrigues et al. 2004).

ANP is a small and short-lived peptide secreted by the atrium in response to changes in volume status and blood pressure (atrial stretch) (Sagnella et al. 1986).The effects of this hormone include an augmented glomerular filtration, and renal excretion of sodium and water (natriuresis and diuresis) with a secondary reduction in blood volume and pressure. It inhibits RAAS and the hypothalamic secretion of AVP (Curry 2005).

8.2.5 Age-Related Changes of Water Balance in the Elderly

Aging is associated with significant changes in health and cognitive status and with a parallel increase in the prevalence of chronic kidney disease (CKD). In elderly people, the renal blood flow and GFR decline, the weight and size of kidney reduce with a concomitant creatinine clearance decrease, estimated in cross-sectional studies of 0.8 ml/min/1.73 m^2 per decade after the age of 40 years (Cowen et al. 2013; Muntner 2009). Changes in renal blood flow may be influenced by age-related modifications of cardiovascular hemodynamics. In addition, structural renal changes occur, including glomerulosclerosis, interstitial fibrosis, tubular atrophy and arterial hyalinosis. All these modifications lead to a reduced ability to concentrate or dilute urine (Koch and Fulop 2017). In healthy elderly individuals compared with healthy young adults, the maximum urinary concentration decreases from 1.200 to 700–800 mOsm/Kg H_2O, at the same time, the maximum urinary diluting varies from 50 to less than 100 mOsm/Kg H_2O (Rowe et al. 1976; Crowe et al. 1987).

Renal ability to excrete free water depends on the presence of an adequate concentration of solute in the diluting region, on a functional intact distal diluting site (distal tubule and ascending limb of Henle's loop), and on suppression of ADH in order to avoid water reabsorption in the collecting duct (Koch and Fulop 2017). These mechanisms are frequently altered in aging. On the other hand, age-related difficulties in renal concentrating capacity are mainly caused by the reduced number of functioning nephrons (with solute diuresis in the remaining intact ones) and by an abnormally high secretion of ADH in response to appropriate stimuli (Rowe et al. 1976; Davies et al. 1995). The impairment of the urine concentrating ability

also results from the reduction of the osmotic gradient in the medullary region. The intake of a low-protein diet may contribute to the decreased medullary tonicity; in fact urea, the greater contributor, derives from the breakdown of proteins (Schlanger et al. 2010; Dontas et al. 1972; Sands 2012).

In aging the osmoregulatory system appears to be maintained or increased, but collecting duct sensitivity to ADH is impaired and thirst stimulus is diminished, increasing the risk of developing water depletion and hypernatremia (Ledingham et al. 1987; Hypodipsia in geriatric patients 1982). In healthy elderly subjects compared to young ones, elevated basal plasma vasopressin levels have been found; these may reflect a deregulation in central control systems for AVP release. ADH secretion is no more strongly correlated with plasma osmolality and sleep-associated vasopressin peak is reduced or absent with higher urinary flow rate at night compared to daytime (Koch and Fulop 2017; Asplund and Aberg 1991). Differently, the ADH response to volume-pressure stimuli is markedly impaired in elderly subjects with an inadequate increase during orthostatic maneuvers or position changes (Rowe et al. 1982).

Older adults often assume multiple medications for a variety of clinical conditions, and some of them may alter vasopressin secretion. Moreover illnesses affecting neurohypophysis (tumors, granulomatous disease, vascular damage, and trauma) result in impaired ADH secretion and reduced thirst perception (Phillips et al. 1991). Mental and physical impairment may limit the ability to communicate the need of water intake, as well as to assume an adequate amount of fluids (Koch and Fulop 2017). Older subjects aged 67–75 years present a lower awareness of thirst in condition of water depletion with a subsequent increased plasma osmolality, but the real grade of misperception in healthy elderly is unknown (Robertson 1983; Phillips et al. 1984).

The serum activity of angiotensin-converting enzyme (ACE) has found reduced in older individuals compared to younger men; moreover there are an impaired capacity to reabsorb sodium and a decreased tubular responsiveness to aldosterone (Conti et al. 2012; Bauer 1993). Furthermore, Ang II alterations can lead to a reduced thirst perception (Phillips et al. 1991; Macías Núñez et al. 1978).

In older people, ANP levels are found increased (probably due to an enhanced atrial sensitivity to afferent system), and its renal effect may be exaggerated (Pollack et al. 1997). The increase of ANP produces a suppression of renin and consequently of Ang II and aldosterone. Moreover, ANP suppresses AVP secretion (Ohashi et al. 1987; Heim et al. 1989).

The described age-related changes in the mechanisms that regulate water homeostasis are summarized in the Fig. 8.1.

Dehydration, described as a depletion of total body water (TBW), is a common problem in older people. It is defined hypotonic (hypo-osmolar), if there is a concomitant salt loss or hypertonic (hyper-osmolar), if there is a free water loss. It is important to underline that TBW decreases in older people down to 50% (Olde Rikkert et al. 1997). Hypertonicity is associated with high morbidity (in particular neurological sequelae) and mortality (Rondon-Berrios et al. 2017). It is estimated that about 17% of older adults hospitalized for dehydration die within 30 days (Waikar et al. 2009). Marra MV et al. studied 247 older subjects resident in eight community-based long-

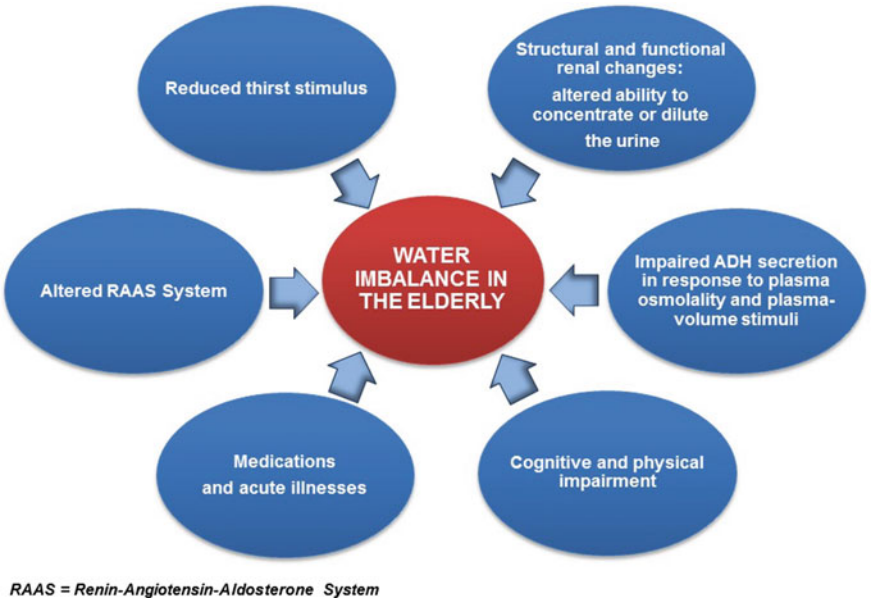

Fig. 8.1 Mechanisms involved in water imbalance in the elderly

term care facilities; approximately 38% of them were found dehydrated and 31% showed impending dehydration (serum osmolarity 295–300 mOsm/kg H_2O) (Marra et al. 2016). The general recommendations for daily total water intake vary from 1.0–2.2 L per day in women and 1.2–3.0 L per day in men (Koch and Fulop 2017; Morley 2015).

Clinical manifestations of dehydration in older adults include: thirst (even if the stimulus is reduced), dry mouth and tongue, sunken periorbital areas, confusion, fatigue, dizziness, delirium (common symptom), body weight loss, hypotension and tachycardia (especially with a concomitant use of anti-hypertensives and diuretics) (Flaherty and Morley 2013). In elderly people with cognitive impairment or overt dementia, dehydration can cause hallucinations, depression, anxiety and agitated behaviour (Wilson and Morley 2003).

An acronym screening tool to assess dehydration risk has been developed by the Dehydration Council (Fig. 8.2) (Lawhorne L 2008).

8.3 Water Balance in Aging Animal Studies

As in humans, animal models of aging have shown a reduced thirst and an impaired ingestive behaviour in response to hyperosmotic stimuli, hypovolemic stimuli and dehydration (Thunhorst et al. 2009; McKinley et al. 2006).

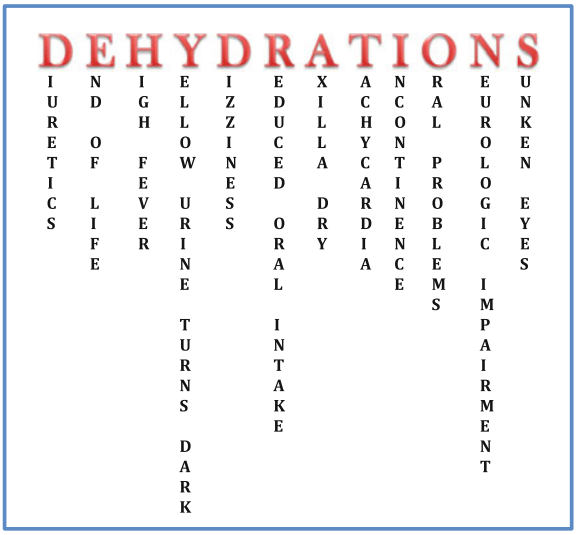

Fig. 8.2 Acronym screening tool to assess dehydration risk developed by the Dehydration Council (Lawhorne L 2008)

In animals, drinking behaviour is stimulated by 1–4% increases in plasma osmolality above basal levels, but aging alters this mechanism (Koch and Fulop 2017). McKinley et al. have demonstrated a decreased fluid intake in response to polyethylene glycol in the Munich Wistar rat aged 18–24 months, when compared with younger counterparts (6 and 15 months old). (McKinley et al. 2006). An impaired fluid intake has been found also in old mice and rats (Brown Norway and Munich Wistar rats) exposed to a condition of dehydration, produced by fluid deprivation (with or without diuretic treatment) or by exposure to a heated environment. (Thunhorst et al. 2009; Silver et al. 1991). Furthermore, aged rodents show a reduction in baroreceptor sensitivity and sodium appetite (Thunhorst and Johnson 2003; Barringer and Buñag 1991).

Numerous studies have described specific functional and structural changes of aging animals' kidney. (Begg 2017). In particular, the reduction of GFR, the decrement in number of aquaporin (AQP2 and AQP3) and urea transporter proteins, and the impaired renal tubular sensitivity to vasopressin may play a role in the development of water imbalance (Schlanger et al. 2010). On the contrary, AVP levels are found to be similar to that of younger age groups, suggesting the absence of a pituitary secretory defect. In old rats, renal V2 receptor expression (number of binding sites for AVP in the basocellular membrane) and second messenger response to AVP-V2R signalling are down-regulated, they both lead to a decreased renal responsiveness to the hormone (Catudioc-Vallero et al. 2000). Under conditions of dehydration or chronic infusion of dDAVP, a V2-selective agonist, the number of AQP2 in the apical and sub-apical membrane of collecting tubule cells increases, but urine osmolality doesn't augment in parallel, as expected (Combet et al. 2003; Tian et al. 2004). Probably the impairment of the AQP2's phosphorylation interferes with its relocation to the apical membrane of collecting tubule cells (Combet et al. 2008) The urea

transporters (UT-A1, UT-A2, UT-A3, UT-B) are responsible for the maintenance of the countercurrent mechanism of kidney in order to produce a concentrated urine; some of these have been altered with aging (Sands 2012; Sands 2004). Furthermore, in the thick ascending limb of the loop of Henle, there is a sodium chloride transporter, the NKCC2/BSC1, that actively transports NaCl under the stimulus of AVP increasing the tonicity in the medulla (Tian et al. 2004; Di Stefano et al. 1991). A study conducted in F344BN rats, showed that the ones 30 months old had a minor abundance of NKCC2/BSC1 co-transporters in the outer medulla and less β and γ subunits of ENaC (Epithelial Sodium Channel) if compared with younger cohorts. Moreover, following a moderate water restriction, the increase in the expression of NKCC2/BSC1 co-transporters was less than expected despite a normal AVP secretory response (Tian et al. 2004).

In animal models of aging, the inability of diluting urine is less evident than the concentrating impairment and it occurs later. AQP2 and UT-A1/UT-A2 downregulation leads to a minor tonicity in the renal papilla explaining the diluting inability; NKCC2/BSC1 co-transporter is involved in both the mechanisms of diluting and concentrating urine. The reduction in number of sodium chloride transporters produces a less reabsorption of NaCl, an increase in delivery of the solute load to the collecting tubule, and finally, a decrease in the solute free water excretion (Tian et al. 2004; Di Stefano et al. 1991).

In conclusion, the alterations found in the kidney of aging rodents are similar to that described in older humans. The reduced expression of V2R receptor binding, AQP2 and AQP3 may explain an excessive water excretion; while the minor number of urea and sodium chloride transporters may alter the maintenance of the countercurrent multiplier system with secondary inability in diluting and concentrating the urine. All these changes may contribute to the development of water imbalance and sodium disorders.

8.4 Sodium Balance Disorders Associated with Aging

Water homeostasis is a balance between the intake and the excretion of water. Water balance disorders can be divided into hypo-osmolar and hyper-osmolar ones, depending on existing excess or deficiency of body water relative to body solute. Normal serum sodium concentration ranges from 135 to 145 mmol/l (mEq/l). Sodium is the main constituent of plasma osmolality, therefore hypo-osmolar and hyper-osmolar states generally reflect changes in its concentration and correspond respectively with hyponatremia and hypernatremia (Verbalis 2003; Adler and Verbalis 2006; Anderson et al. 1985).

Table 8.1 Classification of hyponatremia (Spasovski et al. 2014)

Biochemical severity	**Mild:** serum Na concentration 130–135 mmol/l **Moderate:** serum Na concentration 125–129 mmol/l **Profound:** serum Na concentration <125 mmol/l
Time of development	**Acute:** hyponatremia that is documented to exist <48 h **Chronic:** hyponatremia that is documented to exist for at least 48 h *In the absence of clinical or anamnestic information useful to classify hyponatremia, this must be considered chronic*
Clinical features	**Moderately syntomatic:** any biochemical degree of hyponatremia in the presence of moderately severe symptoms • Nausea without vomiting • Confusion • Headache **Severely syntomatic:** any biochemical degree of hyponatremia in the presence of severe symptoms • Vomiting • Cardiorespiratory distress • Abnormal and deep somnolence • Seizures • Coma (Glasgow coma scale ≤8)

Na = Sodium

8.4.1 Hyponatremia

8.4.1.1 Definition and clinical aspects of hyponatremia

Hyponatremia is the most common electrolyte disorder that clinicians face in clinical practice (Upadhyay et al. 2009). It is especially found and often neglected in older adults (Soiza et al. 2014).

Hyponatremia is defined as a serum sodium concentration <135 mmol/l (mEq/l). The incidence of this disorder in elderly population varies between 0.2 and 29.8% depending on the criteria used to define both "hyponatremia" and "elderly" (Hawkins 2003). The incidence is reported to be approximately 11% among the elderly outpatients and 22% among those resident in long-term care facilities (Miller et al. 1996; Miller 1998).

Hyponatraemia is classified depending on biochemical degree (mild, moderate and profound), time of development (acute or chronic) and clinical features, a wide variety of manifestations ranging from the absence of symptoms to lethargy, convulsion and coma (Table 8.1) (Spasovski et al. 2014).

While acute onset of hyponatremia is accompanied by severe and well recognized signs, older patients with chronic disorders present a variety of manifestations: neurocognitive decline, gait disturbances, osteoporosis and falls (and consequent high risk of bone fractures), need for hospital admission and long-term care (Liamis et al. 2013; Renneboog et al. 2006; Hoorn et al. 2011; Verbalis et al. 2010). Moreover, all forms of hyponatremia are independently associated with in-hospital mortality (Wald et al. 2010).

Renneboog B et al., during a "dynamic walking test" consisting of 3 stereotype steps in tandem, found that participants improved in tasks after correction of serum sodium level (Renneboog et al. 2006).

Studies in animal models show that mild hyponatremia is a possible cause of gait abnormalities, falls and cognitive deficits due to subtle neurological changes; the rats may develope gait and memory deficits in condition of chronic hyponatremia (Negri and Ayus 2017; Fujisawa et al. 2016).

Several studies have demonstrated an increased risk of bone fracture in older adults and aging animals with hyponatremia. Verbalis JC and Colleagues have shown in a rat model that the chronic imbalance is associated with bone loss and augmented osteoclastogenesis (Verbalis et al. 2010). About 30% of total body sodium is stored in the bone, probably the bone matrix resorption is a defensive mechanism of the body to preserve sodium balance at the expense of bone structure. Follow-up studies confirmed that this process occurred in aging animals but at a lesser degree than in the younger cohorts (Verbalis et al. 2010).

Also in humans, chronic hyponatremia seems to be associated with bone demineralization and reduced bone quality, but the studies have reported conflicting results. In a cross-sectional study, Verbalis JC et al. have demonstrated a significant positive correlation between femoral BMD and plasma sodium concentration in hyponatremic patients, whereas no association was found in the normonatremic ones (Verbalis et al. 2010). Also Kinsella S and Colleagues described a decrease in BMD in a group of hyponatremic subjects compared to normonatremic counterparts, but their findings suggest that the increased risk for bone fractures caused by hyponatremia occurs independently of BMD (Kinsella et al. 2010) On the contrary, Hoorn EJ et al. found no relationship between hyponatremia and BMD, even if hyponatremic patients showed an high risk for fractures due to the increased number of falls (Hoorn et al. 2011).

Finally, subsequent animal studies have implicated hyponatremia in the development of other pathological conditions, regarding the heart, the skeletal muscle and the gonads. The reason is that hyponatremia may exacerbate the well-known aspects of senescence, as osteoporosis, cardiomyopathy, sarcopenia, hypogonadism and changes in body composition (Cowen et al. 2013).

Depending on plasma osmolality, hyponatremia can be divided in two main categories: non-hypotonic and hypotonic (Fig. 8.3).

Non-hypotonic hyponatremia may be classified into isotonic and hypertonic hyponatremia. **Isotonic hyponatremia** is also known as "pseudo-hyponatremia". Under this condition, plasma sodium level seems to be reduced due to an exaggerate dilution by non-aqueous material (such as, in diseases characterized by augmented

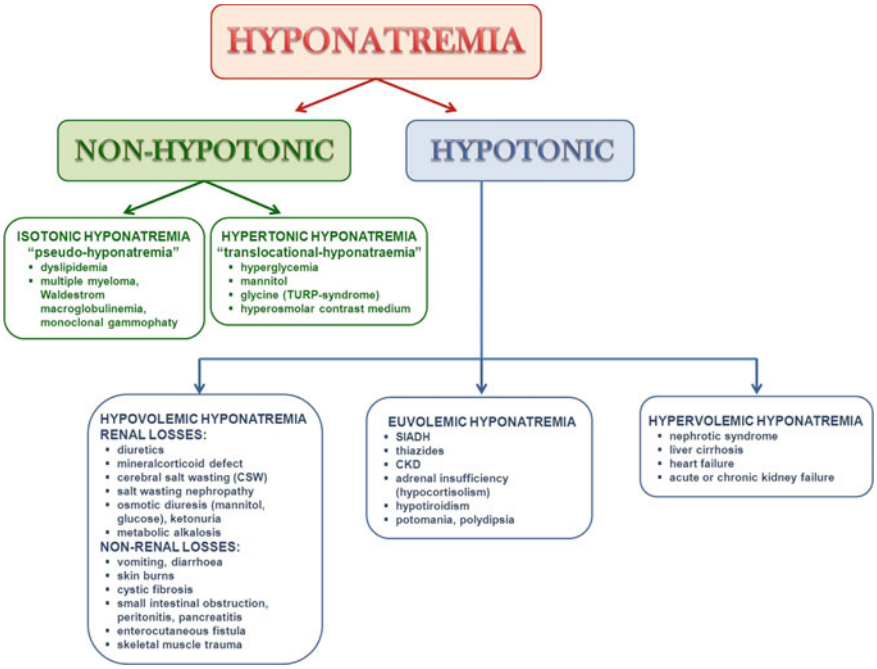

Fig. 8.3 Clinical approach to hyponatremia (Spasovski et al. 2014)

intravascular proteins or lipids), but the effective concentration in the aqueous portion of a plasma sample is normal (Kim 2006) In **hypertonic hyponatremia** an excess of "effective osmoles" in the ECF augments its tonicity and attracts water from the intracellular compartment causing a "translocational-hyponatraemia", or hyponatremia by dilution due to hyperosmolality. If water returns to the intracellular space, serum sodium concentration increases until normal values.These osmoles may be: glucose in uncontrolled hyperglycemia, mannitol, glycine, hyperosmolar contrast medium (Hillier et al. 1999; Aviram et al. 1967; Desmond 1970).

The **hypotonic hyponatremia** is further differentiated in hypovolemic, hypervolemic and, the most common form, euvolemic. ECF status may be assessed by physical exam (vital signs, response to orthostatic maneuvers, jugular venous pressure, skin and mucous turgor, peripheral edema, plasmatic levels of urea and uric acid) and by measuring urinary sodium excretion (Spasovski et al. 2014).

Hyponatremia may occur in case of extracellular volume depletion, **hypovolemic hyponatremia**, due to renal or extrarenal losses, with or without deficit of total body sodium. The ADH secretion is abnormally stimulated and it may lead to water retention despite hypotonicity (Spasovski et al. 2014). ECF depletion is common in elderly patients treated with diuretics, in particular thiazides, which interfere with urinary diluting ability (not with concentrating ability), thus leading to an excess of free water excretion (Friedman et al. 1989).

Hyponatremia may also be recognized in condition of ECF expansion, **hypervolemic hyponatremia**. In renal failure, the ability of kidney to dilute urine and excrete free water is decreased. In severe CKD, the urine osmolality is similar to serum osmolality, condition known as isosthenuria; fluid restriction is able to limit the development of hyponatremia. In nephrotic syndrome, the water retention due to activity of ADH is generally balanced by intense sodium retention; it is the administration of diuretics that, in combination with high level of ADH, may lead to moderate hyponatraemia (Spasovski et al. 2014).

Approximately 20–30% of patients with chronic heart failure NYHA classes III and IV have hyponatremia, which augment the severity of the disease and the risk of death. The renal sodium retention causes the increase of ECF but effective circulating blood volume is generally reduced due to impaired cardiac output. In elderly individuals, the congestive heart failure is associated with further reduction of renal perfusion and GFR, leading to the development of hyponatremia if the activation of RAAS (secondary hyperaldosteronism) has been overcome. The activation of RAAS and the secretion of vasopressin (baroreceptor-mediated) limit urinary sodium excretion; although the treatment with diuretics (especially thiazides) may produce hyponatremia (Albert NM 2007; Goland et al. 2011).

In liver failure, as well as in heart failure, there are sodium retention and reduction of effective circulating blood volume. Baroreceptors stimulate the vasopressin release, moreover the simultaneous use of mineralocorticoid receptor blockers (alone or in combination with loop diuretics), may contribute to the development of hyponatremia(Kim et al. 2008).

Elderly individuals with immobility syndrome (IS) are unable to perform daily tasks because of motor deterioration; they usually experience muscle rigidity, orthostatic hypotension, low cardiac output, increase in total body water and finally lower serum sodium. Musso C et al. found in the elderly with IS, compared to healthy mobile elderly, a higher total body water. They also described in this group no correlation between plasma osmolality and vasopressin level, in contrast with the mobile group, for which there was a positive correlation between plasma osmolality and plasma vasopressin (Musso et al. 2009).

Euvolemic hyponatremia is caused by normal or slightly increased extracellular volume in the absence of edema. It results from an excessive fluid intake in presence of an impaired free water excretion, due to inappropriate release of vasopressin or low sodium intake. The “syndrome of inappropriate antidiuresis” (SIAD) or “inappropriate antidiuretic hormone secretion” (SIADH) accounts for approximately half of cases. It is the most common cause of hyponatremia in elderly populations (Cowen et al. 2013; Anpalahan 2001; Hirshberg and Ben-Yehuda 1997). This syndrome was first described by Bartter FC and Schwartz WB in 1957; the diagnostic criteria were summarized 10 years later (Table 8.2) (Schwartz et al. 1957; Bartter and Schwartz 1967; Janicic and Verbalis 2003) In SIADH, the vasopressin secretion is inappropriate and independent from effective plasma osmolality or circulating blood volume (increased pituitary secretion or ectopic production). Excessive antidiuresis may also result from hyper-activity of ADH in the collecting duct (in example, gain-of-function mutation in its receptor). In elderly people, a large variety of diseases and medications

Table 8.2 Diagnostic criteria for SIADH (Schwartz et al. 1957; Bartter and Schwartz 1967; Janicic and Verbalis 2003)

Essential criteria	• Hyponatremia <135 mmol/l and effective serum osmolality <275 mOsm/kg H_2O • Urine osmolality >100 mOsm/kg H_2O • Clinical euvolemia • Urine sodium concentration > 30 mmol/l with normal salt and water intake • Absence of adrenal, thyroid, pituitary or renal insufficiency • No recent use of diuretic agents (or drugs that may cause SIADH)
Supplemental criteria	• Serum uric acid <4.0 mg/dl (<0.24 mmol/l) • Serum urea <21.6 mg/dl (<3.6 mmol/l) • Failure to correct hyponatremia by infusion of 0.9% NaCl • Fractional sodium excretion >0.5% • Fractional urea excretion >55% • Fractional uric acid excretion >12% • Successful correction of hyponatremia by fluid restriction

SIADH remains a diagnosis of exclusion

may cause SIADH (central nervous system injury, pulmonary disease, malignancies …); it has also been described an idiopathic form that accounts for about one-quarter to one-half of cases (Cowen et al. 2013; Anpalahan 2001; Hirshberg and Ben-Yehuda 1997). Possible causes of SIADH are summarized in Table 8.3.

Hypothyroidism and adrenal insufficiency are other causes of euvolemic hyponatremia (Spasovski et al. 2014). Secondary adrenal insufficiency is associated with reduced or absent secretion of adrenocorticotrophic hormone (ACTH) and thus with hypocortisolism. Low plasma cortisol levels fail to inhibit the central secretion of both ACTH and ADH leading to impaired free water excretion and hyponatremia(Faustini-Fustini and Anagni 2006) Severe hypothyroidism is a rare cause of hyponatremia; serum sodium concentration approximately decreases by 0.14 mmol/l for every 10 mcUI/ml rise in thyroid-stimulating hormone (TSH). The reduction in cardiac output and GFR may result in both sodium disorder and myxoedema (Warner et al. 2006; Kilpatrick 2006).

8.4.1.2 Treatment of hyponatremia

Recommendations for treatment of hyponatremia differ on the basis of expert panel consensus considered. In the elderly, the underlying cause should be researched and corrected with particular attention to multiple medications and volemic status. The optimal drug prescribing is one of the cornerstones of a comprehensive geriatric assessment; therapy should be reviewed with the aim to stop unnecessary drugs and

Table 8.3 Causes of SIADH (Spasovski et al. 2014; Ellison and Berl 2007)

Malignancies	• Carcinoma (lung, gastrointestinal tract, genitourinary tract...) • Lymphomas • Sarcomas • Neuroblastoma • Thymoma
Pulmonary diseases	• Asthma • Infections • Cystic fibrosis • Chronic obstructive pulmonary disease (COPD) • Lung failure; treatment with positive-pressure breeding
Nervous system diseases	• Vascular damage • Infections • Brain tumors • Head trauma • Other (hydrocephalus, delirium tremens, cavernous sinus thrombosis…)
Drugs	• Drugs that stimulate release of vasopressin or enhance its action • Vasopressin analogues • Mixed or uncertain action (ACE inhibitors, clofibrate, cyclophosphamide, colchicine, vincristine, carboplatin, etoposide, carbamazepine, oxcarbazepine, clozapine, amiodarone, proton pump inhibitors, SSRIs…)
Other causes	• Idiophatic • Hereditary (gain-in-function mutation of the vasopressin V2 receptor) • Transient (exercise-induced, general anaesthesia, nausea, pain, stress)

adjust the doses on the basis of efficacy and organ damage (in particular, CKD or liver failure) (Soiza et al. 2014). The over-prescription of thiazide diuretics, serotonin reuptake inhibitors or proton pump inhibitors is usually observed; it may cause fragility fractures and hyponatremia to a much greater extent in the elderly than in other age groups (Schlanger et al. 2010; Soiza et al. 2014; Cumming et al. 2014).

In inpatient settings the infusion of **isotonic saline (0.9% NaCl)** is indicated in all forms of hypovolemic hyponatremia. It produces expansion of extracellular fluid and suppresses the non-osmotic vasopressin secretion (Verbalis et al. 2013).

Intravenous **hypertonic saline (3% NaCl)** is reserved for any forms of severe hyponatremia, acute or chronic, because this solution overrides the ability of kidney to concentrate urinary sodium. It is recommended an infusion bolus of 100–150 ml

over the first 10–20 min, then the check of serum sodium level and eventually the repetition of another infusion bolus in the next 20 min. If 3% NaCl is given as continuous infusion, the increase of serum sodium of 1 mmol/l/hour is desirable. The infusion may be stopped when the symptoms improve or when the serum sodium concentration increases 6–10 mmol/l in 24 h (maximum 18 mmol/l in 48 h) or reaches 130 mmol/l. A too rapid correction exposes the patient to the risk for osmotic demyelination, thus it is recommended a control of serum sodium every two hours to adjust the rate of infusion. Severe chronic forms of hyponatremia, hepatic failure, alcoholism, potassium depletion and malnutrition increase the risk for cerebral edema associated with an aggressive correction (Verbalis et al. 2013; Mohmand et al. 2007; Whitmire 2008).

Whenever feasible, medical nutrition therapy should be modified to support efforts of restoring water and sodium homeostasis. For patients who are receiving enteral nutrition, fluid intake can be increased by switching to a less calorically dense enteral formula and by augmenting tube flushes, whereas sodium intake can be increased by administering a formula with higher sodium content (Whitmire 2008). The following equation can be used to estimate the amount of sodium required:

$$\text{Sodium requirement} = \text{TBW (1)} \times [\text{desidered plasma Sodium (mmol/l)} - \text{current plasma Sodium (mmol/l)}]$$

$$TBW = Total\ Body\ Water$$

Diuretics (loop diuretics) may be given orally or intravenously to treat hypervolemic hyponatremia or SIADH. These induce a copious water diuresis but also urinary sodium excretion, for this reason sometimes they may worsen hyponatremia (Soiza et al. 2014; Decaux et al. 1981).

Fluid restriction to about 0.8–1.0 L in 24 h is the first line treatment of the SIADH, but this is often ineffective and ill-tolerated, especially in young adults. Moreover, the SIADH is characterized by a reset of the "thirst osmostat", meaning that the plasma osmolality cut-off able to trigger thirst is lower with secondary augmented fluid intake (Smith et al. 2004) For patients receiving central parenteral nutrition, the formula administered should be concentrated to restrict the amount of water and the effective osmolality of the solution should be adjusted to exceed urine osmolality. It should be considered that there are maximum osmolality constraints inherent with peripheral parenteral, thus the possibility to control the effective osmolality of regimens is limited. For patients on enteral nutrition, the intake of electrolyte-free fluids should be replaced by a formula with higher electrolyte concentration (Whitmire 2008). Predictors of failure of fluid restriction include the high urine osmolality and the increase in serum sodium less than 2 mmol/l/day (Koch and Fulop 2017).

A direct specific treatment of SIADH, and potentially of hypervolemic hyponatremia or other forms of euvolemic hyponatremia, is the administration of oral vasopressin renal V2-receptor antagonists, **vaptans**. Tolvaptan, an orally available antagonist, has been demonstrated to possess high efficiency in the correction of hyponatremia with minor side effects but high cost. Tolvaptan is available as a tablet, usually taken once a day in the morning. The recommended dosage for SIADH is 15–30 mg

per day. It targets the pathological mechanism underlying the hyponatremia in this condition, an excessive ADH secretion; for this reason serum sodium levels falls again when treatment is discontinued. Patients receiving tolvaptan should discontinue any previous fluid restriction and drink fluids ad libitum (Soiza et al. 2014; Gross 2012). A major concern in the treatment of older people is the safety profile of vaptans, considering that their aquaretic action increases the risk for volemic depletion. In the SALT trials (Study of Ascending Levels of Tolvaptan in Hyponatremia) the adverse effects seen with tolvaptan were similar to that with placebo; moreover the EVEREST study demonstrated a good safety profile after a median 9.9 months of treatment with tolvaptan, but only 10% of subjects enrolled had hyponatremia at baseline (Spasovski et al. 2014; Schrier et al. 2006; Konstam et al. 2007). Tolerability and safety of tolvaptan were also assessed in subsequent trials, however caution and further evaluations are needed in the elderly (Soiza et al. 2014).

8.4.2 Hypernatremia

Definition and clinical aspects of hypernatremia

Hypernatremia is a common electrolyte disorder resulting from a deficit of body water relative to body solutes. Hypernatremia is defined as a serum sodium concentration >145 mmol/l (mEq/l); it is an hyperosmolar disorder because sodium is the main constituent of plasma osmolality. It should be remembered that, if hypernatremia is always synonymous of hyperosmolality, hyperosmolality can exist without hypernatremia, in case of excess of non-sodium solute relative to TBW. The latter condition may occur with marked elevation of plasma glucose in non-ketotic hyperglycaemic hyperosmolar syndrome (Verbalis 2003).

As well as hyponatremia, hypernatremia is associated with increased morbidity and mortality especially in the elderly and critically ill patients. Approximately 1% of patients over 60 years old admitted in hospital for acute illness are found to be hypernatremic (Liamis et al. 2013). Mortality rates are greater than 40% and related to the underlying cause of hypernatremia (Liamis et al. 2013; Palevsky et al. 1996). Liber M et al. observed that elderly with hypernatremia admitted in hospital had lower baseline functional and cognitive status and higher APACHE II scores if compared with the normonatremic counterparts (Liber et al. 2016).

Hypernatremia may result from increased water loss (the majority of cases), decreased water intake or sodium gain.

Thirst is a defence mechanism against hypernatremia, therefore older subjects with a decrease in thirst stimulus, with cognitive impairment or difficulties to obtain adequate fluids have an high risk for hypernatremia (Wong and Verbalis 2002; Palevsky 1998). Hyperosmotic state is further exacerbated by the inability to concentrate urine that occurs with aging and some medications (Danowski et al. 1946).

They have been described in a previous paragraph the impairment of thirst behaviour, the reduction of baroreceptor sensitivity and sodium appetite, and the

Table 8.4 Classification and causes of hypernatremia (Adrogué and Madias 2000)

Hypovolemic hypernatremia	Renal losses: • Osmotic diuresis (glucose, mannitol, urea) • Loop diuretics • Post-obstructive polyuria • Nephropathy Extra-renal losses: • Gastrointestinal losses: diarrhoea, vomiting, fistulas… • Dermal losses: skin burns, excessive sweating, fever…
Hypervolemic hypernatremia	Increased sodium Intake: • Hypertonic saline infusion • Sodium bicarbonate infusion • Hypertonic dialysis • NaCl-rich emetics • Enteral nutrition or parenteral nutrition Increased sodium Re-absorption by kidney: • Mineralcorticoid excess (primary aldosteronism) • Endogenous hypercortisolism
Euvolemic hypernatremia	• Central diabetes insipidus • Nephrogenic diabetes insipidus • Mechanical ventilation (tachypnea) • Impaired thirst perception • Inability (physical or mental) to access fluids • Hypodipsia or adipsia

alterations of kidney function that occur in animals with aging and lead to the development of hypernatremia.

On the basis of volemic status this disorder can be divided in three categories: hypovolemic hypernatremia (water deficit in excess of sodium deficit), hypervolemic hypernatremia (sodium gain in excess of water gain), and euvolemic hypernatremia (pure water loss) (Table 8.4) (Adrogué and Madias 2000).

The assessment of volemic status and urinary sodium helps in defining the etiology of hypernatremia. The urine osmolality evaluation is an useful but not validated tool. The following formula may be used to calculate free water fluid deficit:

$$\text{Free Water Fluid Deficit} = \frac{\text{Serum } Na^{+} \text{ (mmol/L)}}{140} \times \text{usual BW (kg)} \times 0.5 - [\text{BW (kg)} \times 0.5]$$

$$BW = Body\ Weight$$

Neurological manifestations are common, even if elderly hypernatremic patients may be asymptomatic or have few symptoms until serum sodium levels greater than

Table 8.5 Clinical manifestations of hypernatremia (Kim 2006)

Common	• Insomnia, lethargy, depression of sensorium, irritability, focal neurologic deficits • Fever • Nausea, vomiting • Labored respiration • Restlessness, muscle weakness
Rare	• Muscle spasticity • Intense thirst (absent in the elderly; dissipation trend when disorder evolves) • Seizures, coma
Other	• Manifestations of underlying cause: i.e. volume depletion, confusion, orthostatic hypotension, tachycardia…

160 mmol/l (Arieff and Guisado 1976) Clinical manifestations of hypernatremia are summarized in Table 8.5 (Kim 2006).

8.4.2.1 Treatment of hypernatremia

In condition of hypertonicity, brain cells initially uptake electrolytes and loss water. With a prolonged hyperosmolar status, the electrolytes in excess are replaced by organic solutes, historically termed "idiogenic osmoles" because considered to be produced by the brain cells themselves.

Subsequent studies in animals and cultured cells have found that the "idiogenic osmoles" are the same organic osmolytes used by all organisms for volume regulation; in the mammalian brain they include myo-inositol, taurine, glycerylphosphorylcholine and betaine, uptaked from extracellular fluids through the activation of sodium-dependent cotransporters. The accumulation of these osmolytes leads to the shift of water from the cerebrospinal fluid into the brain, with gradual restoring neuron volume. The cerebral adaptation in hypernatremia explain why many subjects are asymptomatic (Kim 2006).

Before correction of hypernatremia, it is important to define if the rise in serum sodium is acute (<48 h) or chronic (>48 h). The rapid correction of a chronic state of hypertonicity can result in increased intracranial pressure and brain stem herniation. The myelinolysis (cerebral edema) is the most fearsome complication of hyperosmolar settings (hypernatremia or hyper-hypoglicemia), as well as of rapid correction of severe hyponatremia (Lien et al. 1990; Adrogué and Madias 2000).

The treatment of hypernatremia differs on the basis of the underlying pathogenesis. The gastrointestinal tract is able to absorb a large amount of water and solutes, but in presence of critical illnesses or gastrointestinal diseases, this ability may be compromised with the subsequent risk of abdominal distension, vomiting and pulmonary aspiration. Moreover, in patients who are receiving enteral nutrition, the

additional load of fluids should be considered. For this reasons, the proper route of water replacement, oral or intravenous, must be identified. In hypovolemic disorders with loss of total body sodium along with free water, isotonic saline (0.9% NaCl) infusion is recommended. In euvolemic hypernatremia, with free water depletion and normal total body sodium, therapy consists of intake of water. In edematous and hypernatremic patients (excess of total body sodium and free water) therapy consists of sodium and fluid restriction. Diuretics may be considered in association with free water intake (Adrogué and Madias 2000).

In patients with prolonged plasma hyperosmolality, the aim of treatment is the decrease in serum sodium of 8–10 mmol/l in the first 24 h or achieving a serum sodium level of 145 mmol/l (Adrogué and Madias 2000).

Rehydration in the elderly is a challenge for the risk of fluid overload (particularly in patients with CKD or heart failure) and for improper inhibition of vasopressin secretion with subsequent lower thirst perception (Koch and Fulop 2017).

8.5 Conclusion

Hyponatremia and hypernatremia are the most common electrolyte disorders seen in the elderly; they substantially reflect changes in plasma osmolality.

The numerous causes recognized include: cognitive impairment, alteration of thirst, changes in fluid intake behaviour, kidney's inability to concentrate or dilute the urine, assumption of multiple medications and acute illnesses. Some of these alterations have been recognized also in animal models of study.

The sodium disorders may result in a wide spectrum of clinical manifestations, ranging from the subtle alterations of chronic forms to the severe neurological signs of acute conditions. They also represent a major cause of hospitalization in the elderly.

Despite the economic burden and the severity of clinical implications, not rarely these electrolyte disorders are under-estimated or neglected. The challenge of clinicians should be primarily that of preventing sodium and water imbalance in the elderly. If the disorder has already been established, the efforts should focus on the research and treatment of the underlying cause, with particular attention to multiple medications.

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Chapter 9
Magnesium Role in Health and Longevity

Mario Barbagallo and Ligia J. Dominguez

Abstract Reduced Magnesium (Mg) intake is a frequent cause of Mg deficit with age. A decreased intestinal Mg absorption and an increased Mg loss may also contribute, as well as the use of medicaments. Furthermore, Mg requirements may be higher with aging. Alterations of Mg metabolism with age, cellular Mg transport systems and problems with measurement methods are discussed. Mg contained in water is more bio-available than Mg in food and it is a possible alternative to Mg supplementation in the correction of Mg deficiencies. Mild to moderate Mg deficits are generally asymptomatic and clinical signs are usually absent or non-specific. Hyperemotionality, tremor, asthenia, sleep disorders, and amnesic and cognitive disturbances are frequent in older adults, and may be often overlooked or confused with age-related symptoms. Chronic Mg deficiency results in oxidative stress and chronic, low-grade inflammation, which may be linked to several age-related diseases, and to the aging process itself. Mg deficit-related conditions may involve different tissues and organs, including hypertension and cardiovascular diseases, diabetes mellitus and metabolic syndrome, asthma and airways constrictive syndromes, depression and psychiatric disorders, Alzheimer's disease and neuromuscular diseases (chronic fatigue, muscle pain, fibromyalgia), fragility fractures, and cancer. Keeping an optimal Mg balance throughout life might help to prevent some chronic disease associated to aging, and to extend healthy life. This needs to be proven by future studies.

Keywords Magnesium · Mg · Oxidative stress · Aging · Mg water · Mg food
Bone · Fracture · Hypertension · Health · Longevity

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9.1 Introduction

Magnesium ion (Mg) is the second most abundant cation after potassium (K) in the intracellular compartment and the fourth most common mineral in the human body after calcium (Ca), sodium (Na) and K; Mg atomic weight is 24.305 g/mol, and its atomic number is 12 (Table 9.1). Mg is a member of Group IIA in the periodic table and thus an alkaline-earth element; it forms most stable complexes with phosphate and carboxylate anions or with nitrogen base. Mg is an essential cofactor for numerous biological processes, and it is required for energy production, oxidative phosphorylation, glycolysis, protein synthesis, and nucleic acid synthesis and stability (Saris et al. 2000). Mg plays a role in the active transport of other ions across cell membranes, such as Ca and K, modulating neuron excitability, muscle contraction, and normal heart rhythm. Mg has a key role in the adenosine triphosphate (ATP) synthesis in mitochondria, to create a complex with Mg (MgATP) (Barbagallo and Dominguez 2007). Cell signaling requires MgATP for protein phosphorylation and for the synthesis and activation of cell-signaling molecule cyclic adenosine monophosphate (cAMP) involved in a myriad of biochemical processes (Reinhart 1988).

Mg has critical role in modulating a wide variety of critical cellular activities and metabolic pathways. Mg is cofactor in over three hundred enzymatic reactions, and in particular is required for the activity of all rate-limiting glycolytic enzymes, protein kinases, and in all phosphorylation processes and in all reactions that involve ATP utilization and transfer (Barbagallo and Dominguez 2007). Mg has a weak calcium antagonist action and has many structural functions (multi-enzyme complexes, i.e., G-proteins, *N*-methyl-D-aspartic acid [NMDA] receptors, mitochondria, polyribosomes, proteins and nucleic acids synthesis, etc.).

Therefore, Mg is a critical factor for normal cellular and body homeostasis (Table 9.2). Over the past decades, the clinical relevance and biological significance of Mg have been documented, as well as the impact of Mg on molecular and physiological processes of aging, and on health and age-related clinical diseases.

Table 9.1 Characteristics of ionic magnesium

• Element category: alkaline earth metal
• Atomic number: 12
• Atomic weight: 24.305 g/mol
• Valence: 2
• Normal serum: 0.75–0.95 mmol/L; 1.7–2.5 mg/dL
• Total body content: 24 g
• Distribution in serum: – free ionized 70–80% – protein-bound 20–30% – complexed 1%

Table 9.2 Physiological roles of magnesium

Enzyme function	
Enzyme substrate • Kinases • ATPases/GTPases • Cyclases	*Direct enzyme activation* • Phosphofructokinase • Creatine kinase • 5-phosphoribosyl-pyrophosphate synthase • Adenylate cyclase • Na-K-ATPase
Structural function	
• Proteins • Polyribosomes • Nucleic acids	• Multiple enzyme complexes • Mitochondria
Calcium antagonist	
• Muscle contraction/relaxation • Neurotransmitter release • Action potential conduction in nodal tissue	
Membrane function	
• Cell adhesion • Transmembrane electrolyte flux	

9.2 Magnesium Metabolism and Requirement

In the human body, approximately 24 g (1 mol) of Mg are present, of which about 65% are stored in bone and 34% in the intracellular space. Less than 1% of total Mg is contained in blood serum; normal serum Mg concentrations range between 0.75 and 0.95 mmol/L (1.7–2.5 mg/dL or 1.5–1.9 meq/L). The levels of Mg in the plasma of healthy people are extremely constant and are tightly controlled and maintained within this narrow range by the small intestine and the kidney; both increase their fractional Mg absorption under conditions of Mg deprivation. If Mg depletion continues, the bone store helps to maintain serum Mg concentration by exchanging part of its content with extracellular fluid (Fig. 9.1) (Barbagallo et al. 2003).

In the serum, Mg exists in three forms: a protein-bound fraction (25% bound to albumin and 8% bound to globulins), a chelated fraction (12%), and the metabolically active ionized fraction (Mg-ion: 55%). Hypomagnesemia is defined as a serum Mg level less than 0.75 mmol/L (Barbagallo et al. 2003). Intracellular Mg concentrations are highly regulated and Mg itself acts as an intracellular regulator of cell cycle control and apoptosis. Intracellular Mg exists mainly in a bound form. Plasma Mg levels do not always reflect intracellular or total Mg.

Mg balance is controlled by Mg intake, by its absorption through intestine, by the renal excretion, and by the Mg requirement of different tissues (e.g., skeletal and cardiac muscle uptake and usage) (Quamme 2008). Daily Mg requirement is calculated to be around 300–400 mg in healthy adults (5–6 mg/kg/day) but may be

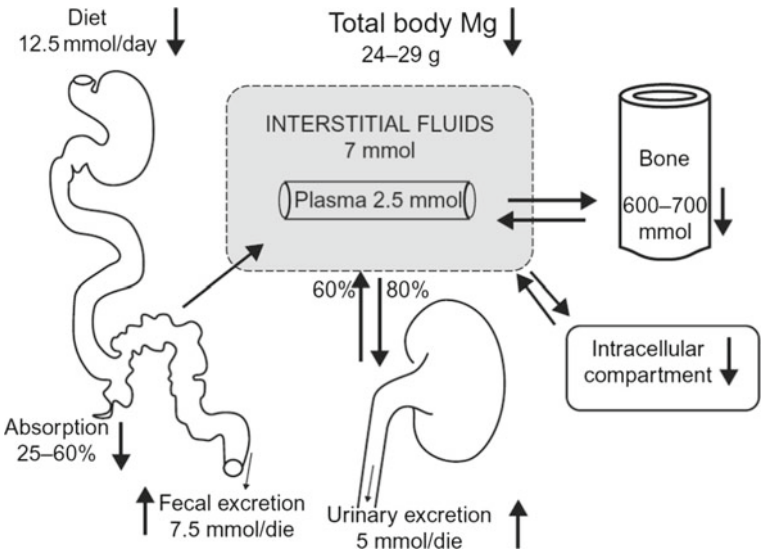

Fig. 9.1 Magnesium homeostasis and age (arrows indicate possible sites of alterations with aging)

Table 9.3 Determinants of magnesium equilibrium

• Gastrointestinal absorption and renal excretion are the main determinants of Mg equilibrium
• Healthy individuals need to consume 0.2–0.4 mmol/kg of body weight/day to stay in balance
• Extracellular Mg is in equilibrium with that in the bone, kidneys, intestine, and other soft tissues
• Bone is the main reservoir of Mg
• Primary renal disorders cause hypomagnesemia by decreased tubular reabsorption of Mg
• Osmotic diuresis results in magnesium loss
• Drugs may cause magnesium wasting

higher in several physiological conditions (i.e. pregnancy, aging, exercise, etc.) and diseases (type 2 diabetes, infections, etc.). The main site for Mg absorption is the small intestine. Healthy persons need to consume 0.2–0.4 mmol/kg/day to maintain the balance (Table 9.3). Since the Mg stored in the bone cannot quickly exchange with the Mg in extracellular fluids, the quick Mg needs are provided by the Mg stored in the intracellular compartment. Kidney also has a key role in Mg homeostasis and about 120 mg of Mg are excreted into the urine each day (Saris et al. 2000). Renal Mg handling is tightly dependent on Mg body status, since Mg deficiency increases renal Mg reabsorption across all nephron segments. In fact, urinary excretion is reduced when Mg is depleted (Shils 1969). Diuretic drugs may also modify renal Mg handling by reducing Mg reabsorption (Quamme 1997).

Although no known hormonal factor is specifically involved in the regulation of Mg metabolism, many hormones are recognized to have an effect on Mg balance and transport. Among them, parathyroid hormone (PTH), calcitonin, catecholamines, and insulin have a major role (Barbagallo et al. 2003, 2007).

9.3 Magnesium Measurements Methods

Measurements of total serum Mg concentrations (MgT) are not an accurate measure of the body Mg status; MgT measurements are useful in epidemiological studies, but do not detect subclinical Mg deficit in an individual basis (Elin 2010). Others, more precise and expensive techniques, such as ^{31}P-NMR spectroscopy, still remain mainly a research tool. The development of Mg-specific ion-selective electrodes, that measure the active ionized fraction of Mg, has been more appropriate, allowing measuring extracellular free levels of Mg with a higher sensitivity than MgT in order to detect subclinical Mg deficits in several medical conditions (Barbagallo et al. 2014; Resnick et al. 1997).

9.4 Cellular Magnesium Transport System

Transient receptor potential (TRP) is a family of protein containing both, a cation-permeable ion channel and a kinase domain (Clapham et al. 2001). TRPM7 is a divalent cation-selective ion channel that is permeable to Ca^{2+} and Mg^{2+}. The channel is downregulated by intracellular levels of Mg^{2+}, MgATP, and other Mg-nucleotides (Penner and Fleig 2007). The channel plays a key role in Mg homeostasis due to its preference for divalent ions (Romani 2011). TRPM7 represents a ubiquitous homeostatic mechanism that regulates Ca^{2+} and Mg^{2+} fluxes. Reducing the cellular levels of Mg stimulates the activation of TRPM7-mediated currents (Faouzi et al. 2017).

9.5 Magnesium Deficiency with Aging: Causes and Mechanisms

Mg deficits are common in old age (Barbagallo and Dominguez 2010). Total plasma Mg concentrations do not change with age (Yang et al. 1990). Differences in Mg levels are generally related to the presence of age-related diseases and alterations in renal function. Studies using 24-h Mg retention showed an increased Mg retention in old age, suggesting a significant subclinical Mg deficit, not detected by total serum Mg (Gullestad et al. 1994). In healthy persons, we showed that intracellular free

Table 9.4 Main mechanisms of magnesium deficit with aging

Primary Mg deficit
• Inadequate Mg dietary intake • Reduced efficiency of Mg absorption (associated with reduced vitamin D levels)? • Increased urinary excretion of Mg (Mg) (associated with age-dependent reduction of kidney function and of Mg tubular reabsorption)
Secondary Mg deficiency
• Associated with age-related diseases and comorbidities • Increased urinary Mg loss secondary to drugs (i.e. diuretics) frequently used in the older persons

Mg decreases with age; we have specifically studied the behavior of intracellular Mg content with age, using the gold standard method (31P-NMR spectroscopy) in healthy young and older persons and have shown a continuous age-dependent fall of intracellular Mg levels in red blood cells of healthy older adults (Barbagallo et al. 2000), while total serum Mg was not significantly altered with age. It has been shown that many older adults are prone to chronic latent Mg deficiency (Table 9.4), and epidemiological data from US and Europe have confirmed that low Mg intake is a common condition in older persons (Ford and Mokdad 2003; Galan et al. 1997).

However, it has been suggested that Mg requirement do not change with age (Hunt and Johnson 2006). Data from the National Health and Nutrition Examination Survey (NHANES) III showed that Mg intake tend to decrease with age (Ford and Mokdad 2003). In addition, older adults affected by chronic conditions and on chronic drug treatment have an additional risk of being Mg deficient. The recommended Mg intake in the US population is 420 and 320 mg/day for men and women, respectively, but Mg intake in the US older population is far below (225 and 166 mg/day for men and women, respectively) (Table 9.5) (Ford and Mokdad 2003). Sixty eight per cent of US adults consume less than the recommended daily allowance (RDA) of Mg, Forty-five per cent consume less than 75% of the RDA, and nineteen per cent consume less than 50% of the RDA (King et al. 2005). The "Suppléments en Vitamines et Minéraux AntioXydants" (SU.VI.MAX) study showed that seventy-seven per cent of women and seventy-two per cent of men have dietary Mg intakes lower than RDA; twenty three per cent of women and eighteen per cent of men consumed less than 2/3 of these RDA (Galan et al. 1997).

Decreased intestinal Mg absorption may further contribute to Mg deficiency in the elderly (Coudray et al. 2006). Mg absorption tends to decrease with age. Duodenum and ileum are mainly involved in Mg absorption and both passive and active transport processes are involved. The alterations of the intestinal absorption of Mg in old age may be also aggravated by the frequent age-related impairment of vitamin D homeostasis. Kidney active reabsorption of Mg takes place in the loop of Henle and in the proximal convoluted tubule. A latent primary renal disorder may also be associated to an increased Mg loss linked to a reduced tubular reabsorption.

Table 9.5 Recommended dietary allowance (RDA) for magnesium (AI: adequate intake when RDA cannot be determined)

Life stage	Age	Females (mg/day)	Males (mg/day)
Infants	0–6 months	30 (AI)	30 (AI)
Infants	7–12 months	75 (AI)	75 (AI)
Children	1–3 years	80	80
Children	4–8 years	130	130
Children	9–13 years	240	240
Adolescents	14–18 years	410	360
Adults	19–30 years	400	310
Adults	31 years and older	420	320
Pregnancy	18 years and younger	–	400
Pregnancy	19–30 years	–	350
Pregnancy	31 years and older	–	360
Breast-feeding	18 years and younger	–	360
Breast-feeding	19–30 years	–	310
Breast-feeding	31 years and older	–	320

Secondary Mg deficiencies may also be associated with drug use or with pathological conditions (i.e., type 2 diabetes mellitus, insulin resistance, alcoholism, hyperadrenoglucocorticism, HIV/AIDS, acute myocardial infarction, stroke, etc.). Mg depletion due to an excess urinary loss may be related to treatment with loop diuretics. Patients receiving long-term treatment with thiazide diuretics are also at risk, especially in old age. Hypokalemia is often associated with diuretic-induced Mg depletion. It has been reported the finding of hypomagnesemia in 38–42% of hypokalemic patients. The correction of a K deficit may be difficult to achieve unless the Mg deficit is also corrected, hence patients with hypokalemia should be evaluated for Mg deficiency. Other commonly used medications may diminish Mg absorption and/or reduce Mg levels (e.g., H_2 blockers, proton pump inhibitors, antacids, antibiotics, antihistamines, antivirals, and antiepileptic drugs, among others).

Western diets, generally very low in whole grains and green vegetables, and high in refined foods, are often severely deficient in Mg (Table 9.6). Food processing may significantly lower Mg content. Because most of the Mg present in food is lost in cooking or refining procedures, diets that provide a high proportion of daily calorie requirements from refined or processed foods are likely to be low in Mg (Durlach et al. 1985). Cooking, especially boiling of foods may cause a significant loss of Mg. Processed food accounts for a substantial portion of the diet in western countries, which makes more probable the establishment of a state of true or relative Mg deficiency (Barbagallo and Dominguez 2010). Furthermore, phytic acid found in certain foods lowers the absorption of Mg. Some pesticide agents, commonly used in the crops, such as glyphosate, may chelate minerals including Mg (Cakmak et al. 2009), further decreasing the content of Mg in soil and in some crops. Organic food,

Table 9.6 Some food sources of magnesium

Food	Serving	Magnesium (mg)
Cereal all bran	1/2 cup	112
Cereal oat bran	1/2 cup dry	96
Brown rice, medium-grain, cooked	1 cup	86
Fish, mackerel, cooked	3 oz	82
Spinach, frozen, chopped, cooked	1/2 cup	78
Almonds	1 oz (23 almonds)	77
Swiss chard, chopped, cooked	1/2 cup	75
Lima beans, large, immature seeds, cooked	1/2 cup	63
Cereal, shredded wheat	2 biscuits	61
Peanuts	1 oz	48
Molasses, blackstrap	1 tablespoon	48
Hazelnuts	1 oz (21 hazelnuts)	46
Okra, frozen, cooked	1/2 cup	37
Milk, 1% fat	8 fluid ounces	34
Banana	1 medium	32

from pesticide-free soils, was found to have significantly more Mg than non-organic control food (Griffiths et al. 2012).

Because of this substandard dietary content of Mg in developed countries, Mg intake is often significantly reduced. In this context, Mg intake derived from drinking water rich in Mg may represent a possible alternative to supplements in Mg deficits (Galan et al. 2002).

In the SU.VI.MAX cohort, drinking water contributed 6–17% of total daily Mg intake depending on the Mg concentration of the mineral water used. Drinkers of mineral water rich in Mg and water with a moderate mineral content had Mg intakes significantly higher than those of drinkers on low mineralized or tap water. Therefore, mineral-rich water may provide an important supplementary contribution to total Mg intake (Galan et al. 2002). In addition, bio-availability of Mg in water is higher when compared to Mg in food and it is easy to add Mg to water, but virtually impossible to add Mg to foods. Mg content may be important not only in drinking water, but also in water used for cooking, since the concentration of Mg in water may interfere with the leakage of Mg in food during cooking, and may reduce the loss of Mg in the cooked/boiled food.

9.6 Magnesium, Inflammation and Oxidative Stress

Hypomagnesaemia has been shown to cause increased production of oxygen free radicals. Poor Mg diets are associated with a low-grade chronic inflammatory state, both, by initiating an excessive production and release of interleukin (IL)-1β and tumor necrosis factor (TNF)-alfa, and by stimulating the synthesis of nitric oxide and some inflammatory markers (Kramer et al. 2003; Mazur et al. 2007). Mg deficiency also increases the aggregation and adhesiveness of platelets, and inhibits growth and migration of endothelial cell, potentially modulating microvascular functions (Mazur et al. 2007).

In animals, several studies have shown that Mg deprivation causes: (i) marked elevation of proinflammatory molecules TNF-alfa, IL-1-beta, IL-6, vascular cell adhesion molecule (VCAM)-1, and plasminogen activator inhibitor (PAI)-1 (Malpuech-Brugère et al. 2000; Mazur et al. 2007); (ii) increased circulating inflammatory cells (Galland 1988), and (iii) increased hepatic production and release of acute phase proteins (i.e., complement, alfa2-macroblobulin, fibrinogen) (Bussière et al. 2003; Mazur et al. 2007).

In humans, clinical data have shown that low serum Mg levels as well as inadequate dietary Mg are strongly related to low-grade systemic inflammation (King et al. 2005; Guerrero-Romero et al. 2011; Song et al. 2007). Several other studies have confirmed an inverse relationship among Mg intake, serum Mg and inflammation markers. Data from the Women's Health Study have shown that Mg intake is inversely related to systemic inflammation, measured by serum C-reactive protein (CRP) concentrations, and with the prevalence of the metabolic syndrome in adult women (Song et al. 2005). Using the 1999–2002 NHANES databases, King et al. found that dietary Mg intake was inversely related to CRP levels. Among 70% of the population not taking supplements, Mg intake below the RDA was significantly associated with a higher risk of having elevated CRP (King et al. 2005).

Mg deficiency has been associated with increased oxidative stress and decreased antioxidant defense competence. Previous studies have shown compellingly that Mg deficiency results in an increased production of oxygen-derived free radicals in various tissues, increased free radical-elicited oxidative tissue damage, increased production of superoxide anion by inflammatory cells, decreased antioxidant enzyme expression and activity, decreased cellular and tissue antioxidant levels, and increased oxygen peroxide production (Weglicki et al. 1996; Mazur et al. 2007)

In rats, Mg deficiency has been shown to increase lipid peroxidation and malondialdehyde and to decrease hepatic glutathione, superoxide dismutase, and vitamin E (Calviello et al. 1994). We have suggested a link between the action of Mg in altering the antioxidant capacity and in activating oxidative stress, inflammation, and lipid oxidation with insulin resistance, diabetes, and cardio-metabolic syndrome (Barbagallo and Dominguez 2007).

Aging is characterized by a chronic, low-grade inflammatory state that involves several tissues and organs, and that has been named "inflammaging" (Franceschi et al. 2017).

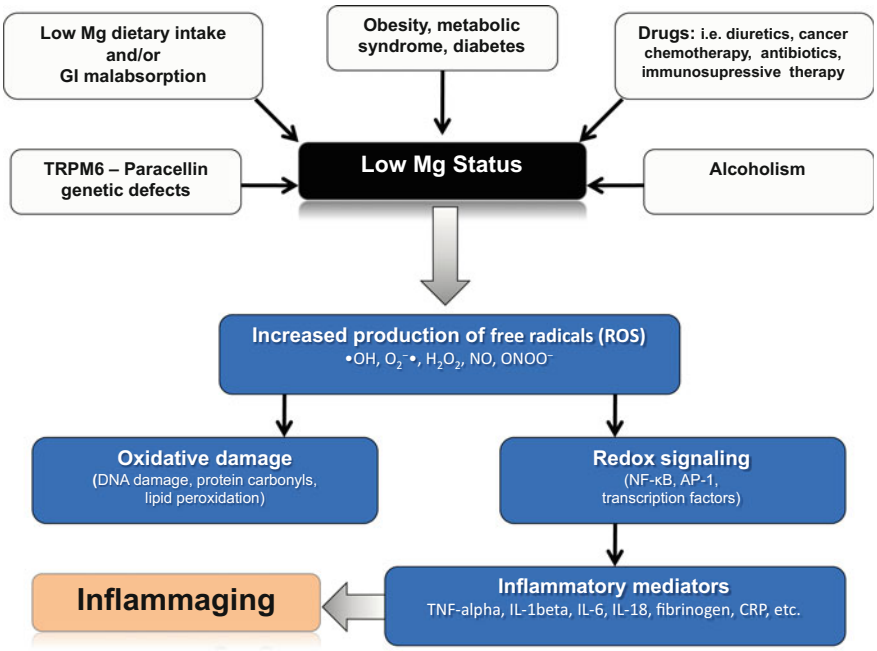

Fig. 9.2 Chronic magnesium deficit has been proposed as one of the physiopathological links that may help explain the interactions among inflammation, oxidative stress, and aging

We have suggested that the Mg deficiency through its role in facilitating an impairment of the redox status and a low-grade inflammation may be a link to several age-related diseases and/or to accelerated aging (Fig. 9.2) (Barbagallo et al. 2009; Barbagallo and Dominguez 2010). Mg itself possesses antioxidant properties scavenging oxygen radicals possibly by affecting the rate of spontaneous dismutation of the superoxide ion (Weglicki et al. 1992).

9.7 Mg and the Immune Responses

There is evidence that Mg may play a role in the immune response as a cofactor for immunoglobulin (Ig) synthesis, C3 convertase, immune cell adherence, antibody-dependent cytolysis, IgM lymphocyte binding, macrophage response to lymphokines, and T helper-β cell adherence (Tam et al. 2003; Galland 1988). In addition, Mg deficiency seems to accelerate thymus involution. One of the most remarkable results regarding effects of Mg deficiency on the organism is the higher level of apoptosis shown in thymuses from Mg-deficient rats as compared with controls (Malpuech-Brugère et al. 1999). Mg-deficient diet has been shown to alter polymorphonuclear cell number and function, together with an increased number of

neutrophils, related to an increased activity of phagocytosis (Bussière et al. 2003). Mg is also involved in human cell apoptosis. Fas-induced β-cell apoptosis is Mg-dependent. Elevation of intracellular free Mg levels is needed for Fas molecule binding expression on the β-cell surface to trigger signaling pathways that cause apoptosis and cellular death (Chien et al. 1999).

9.8 Magnesium Deficits and Aging Related Diseases

9.8.1 Clinical Signs and Symptoms

Severe Mg deficit may be associated with neuromuscular symptoms, such as weakness, tremor, muscle fasciculation, dysphagia, positive Chvostek's sign (facial twitching as a reaction to the tapping of the facial nerve), and positive Trousseau's sign (spasm of muscles of the hand and forearm following the application of a pressure cuff, transiently occluding the brachial artery). Neurologic disturbances may involve both, the sympathetic and parasympathetic nervous systems, causing orthostatic hypotension or borderline hypertension.

Mild to moderate Mg deficits are generally asymptomatic and clinical signs are usually absent and/or non-specific. Subjective symptomatology may include non-specific manifestations, such as anxiety, hyperemotionality, fatigue, depressive symptoms to major depression, headache, insomnia, light-headedness, dizziness, and nervous fits. Peripheral signs are commonly present such as myalgias, acroparesthesias, and cramps. Functional complains may be present and are non-specific and may include chest pain, *sine materia* dyspnea, precordialgia, palpitations, extrasystoles and other arrhythmias, etc. Hyperemotionality, tremor, asthenia, sleep disorders and amnesic and cognitive disturbances are particularly important in elderly patients, and may be often overlooked or confused with age-related symptoms.

Elin suggested to name this common condition of persons with mild, chronic, negative Mg balance, associated with a non-specific symptomatology, as a syndrome of "*Chronic Latent Magnesium Deficiency*" (CLMD) (Elin 2010). Persons affected of CLMD have a total serum Mg concentration still within the lower part of the reference interval (latent), and from a clinical standpoint are generally undiagnosed being considered as having normal Mg status.

However, a chronic low Mg status has been associated with numerous pathological conditions characterized by a chronic inflammatory stress component. In humans, Mg deficiency through exacerbating chronic inflammatory stress may contribute significantly to the occurrence of several chronic age-related diseases.

9.8.2 *Magnesium, Hypertension and Cardiovascular Diseases*

Chronic Mg deficits have been linked to an increased risk of numerous preclinical and clinical cardiovascular outcomes, mostly observed in older populations, including hypertension, ischemic heart disease, cardiac failure and cardiovascular mortality, stroke, cardiac arrhythmias, atherosclerosis, endothelial dysfunction, alterations in lipid metabolism, platelet aggregation/thrombosis, inflammation, oxidative stress (Paolisso and Barbagallo 1997; Barbagallo et al. 2007).

Kobayashi in 1957 first noted that the nature of drinking water might influence death rates from cardiovascular disease; the incidence of stroke was significantly lower in areas with hard water (mainly linked to Mg and calcium content) (Kobayashi 1957). Schroeder surveyed the hardness of drinking water in the US, analyzing the relationship between the death rates and the water hardness found that death rates from cardiovascular diseases (particularly from coronary heart attacks in white men 45–64 years old) was significantly higher in states with soft water than in states with hard water (Schroeder 1966).

Mg has a crucial role in cardiovascular homeostasis. Although not directly involved in the biochemical process of contraction, Mg modulates vascular smooth muscle tone and contractility by affecting calcium ion concentrations and its availability at critical sites (Altura and Altura 1981; Altura et al. 1984). Consistent with the above, not only calcium-induced contraction in vascular smooth muscle is sensitive to changes in Mg concentration, but direct reduction of extracellular Mg raises smooth muscle Ca content, while conversely, elevations in Mg concentrations reciprocally lower calcium content in smooth muscle (Turlapaty and Altura 1980). Moreover, Mg directly affect uptake, distribution, and content of calcium in vascular smooth muscle cells, and can itself function as a nature's weak physiologic calcium channel blocker (Iseri and French 1984), modulating calcium-channel activity in heart cells (Agus et al. 1989). In view of such direct and indirect actions of Mg on cardiac and vascular smooth muscle cells, it is reasonable to suggest that Mg deficiency might be relevant to disorders of blood pressure homeostasis, such as hypertension. Indeed, vascular hyperreactivity and frank hypertension can be induced by depleting of Mg both the in vitro environment, or in the organism as a whole (Altura and Altura 1991). Measurements of serum Mg levels are not useful, since no distinct alterations of circulating total Mg levels have been identified in essential hypertension. However, several abnormalities of Mg metabolism have long been recognized in hypertensive subjects. Epidemiologic studies have suggested an inverse relationship between Mg dietary intake and hypertension, lower dietary Mg intake being associated with higher blood pressure (Joffres et al. 1987). In aging populations, a gradual rise in blood pressure, a gradual fall in total serum Mg levels with age (Petersen et al. 1977), and an age-related suppression of intracellular free Mg (Barbagallo et al. 2000) have been observed, suggesting a possible role for Mg deficit in hypertensive states. Fasting levels of intracellular free Mg were found significantly suppressed in hypertensive patients as compared with normotensive controls (Resnick et al. 1984). Moreover,

in different experimental rat models of hypertension, diets that raised or lowered intracellular free Mg, consistently lowered and raised blood pressure, respectively. Abnormalities of Mg urinary excretion in hypertensive experimental models have also been described (Barbagallo et al. 1992). Similarly, the ability of a high salt diet to elevate blood pressure was shown to be related to intracellular free Mg in humans (Resnick et al. 1994).

Mg was first recommended to lower blood pressure in patients with malignant hypertension as early as 1925 (Blackfan and Hamilton 1925). Therapeutical use of Mg has consistently been found beneficial in preeclampsia and eclampsia (Chien et al. 1996) and in patients with malignant hypertension (Winkler et al. 1942), while the response to Mg in essential hypertensives is heterogeneous (Resnick and Laragh 1985). In some studies, Mg supplementation may have significant hypotensive effects, while in others blood pressure may not change or may worsen. A Cochrane review suggested that there was not yet enough information to suggest the use of Mg in hypertension despite a small statistical reduction in diastolic blood pressure (Dickinson et al. 2006). Thus, even if a role for decreased Mg levels in the pathophysiology of hypertension appears likely, a consistent, reproducible effect of Mg supplementation on blood pressure has not yet been confirmed in hypertension and further data are needed to consider Mg as a non-pharmacological tool for treating hypertension. Among the reasons for this are the virtual absence of adequately designed clinical trials of Mg therapy in hypertension, the differing treatment schedules used in a number of smaller clinical reports, and a failure to appreciate the heterogeneity of the underlying mechanisms contributing to hypertension. Thus, long-term prospective therapeutic trials of Mg in hypertension are clearly needed in the near future.

Mg deficiency may also have a role in the development of atherosclerosis. Contrasting results have been reported on the relationship between serum lipids and total and ionized serum Mg concentrations. Serum Mg has been found to be positively (Randell et al. 2008) or negatively (Corsonello et al. 2007) associated with serum lipid levels. Binding interactions between Mg and lipoproteins may, at least partially, account for these contrasting results, although it is also possible that the relationship between Mg status and lipids in healthy persons may be different from that in patients with chronic conditions, such as obesity, diabetes, and hypertension (Corsonello et al. 2007). Low Mg status has been suggested to contribute to vascular calcification, altered lipid accumulation, and reduced cholesterol transport by high density lipoprotein (HDL) (Rayssiguier 1984). Mg has been suggested to have role in preventing atherosclerotic plaque formation, and to have a positive effect on metabolic lipid profiles.

Rosanoff and Seelig proposed that Mg may act as a weak inhibitor and a modulator of 3-hydroxy-3-methylglutaryl-CoA-reductase activity. Mg is also essential for the activity of other enzymes of the lipid metabolism such as lecithin cholesterol acyl transferase (LCAT), which regulates low density lipoprotein-cholesterol, HDL-cholesterol, and triglyceride levels, and thus may modestly help to raise HDL-cholesterol and lower triglycerides (Rosanoff and Seelig 2004).

Mg may be beneficial as a support in the treatment of atrial and/or ventricular arrhythmias, in particular when there is co-existent hypokalemia (McLean 1994). Indeed, Mg plays a role in the heart's electrical conduction and Mg deficiencies have been linked to many cardiovascular conditions. Mg deprivation has been suggested to compromise cardiovascular health and favor the occurrence of heart arrhythmias. Hypomagnesemia is relatively common in patients presenting with atrial fibrillation (AF) (Singh et al. 1976) and low serum Mg was suggested to be moderately associated with the development of AF (Khan et al. 2013).

Dietary Mg restriction to about one third (33%) of the RDA induced heart rhythm changes including AF and flutter that responded quickly to Mg supplementation (Nielsen et al. 2007). A meta-analysis conducted in Canada has suggested that intravenous Mg administration is an effective and safe strategy for the acute management of AF. An overall favorable response was achieved in 86 and 56% of patients in the Mg and control groups, respectively (OR 4.61; 95% CI 2.67–7.96) (Onalan et al. 2007). Intravenous administration has been proposed as a very effective and safe treatment for torsade de pointes, because its application is rapid and simple (Tzivoni et al. 1988; Gupta et al. 2007).

Major cardiac effects of Mg are prolongation of atrial and atrioventricular nodal refractory periods, which may facilitate rate and rhythm control in AF (DiCarlo et al. 1986). These antiarrhythmic actions of Mg may, at least in part, help to explain the possibility that a high Mg dietary intake may reduce the risk of sudden death. Women in the highest quartile of Mg intake were found to have a reduced risk of sudden cardiac death (Chiuve et al. 2013).

In patients with severe congestive heart failure (New York Heart Association functional classification IV), under optimal medical cardiovascular treatment, oral Mg supplementation improved clinical symptoms and survival outcomes as compared to placebo (Stepura and Martynow 2009).

9.8.3 Magnesium and Type 2 Diabetes

The link between Mg deficiency and type 2 diabetes mellitus (DM2) is well known. DM2 is frequently associated with both extracellular and intracellular Mg depletion, in particular in those patients with poorly controlled glycemic profiles, with longer duration of the disease, and with the presence of micro- and macrovascular chronic complications (Mather and Levin 1979; Schnack et al. 1992; Barbagallo and Dominguez 2007, 2015). Depletion in intracellular and/or ionized plasma Mg can be found in individuals with normal total serum Mg (Resnick et al. 1993; Barbagallo et al. 2014). Among the mechanisms that may favor Mg depletion in diabetes, the most important are a low Mg dietary intake and an increased Mg urinary loss, while absorption and retention of dietary Mg seems to be unchanged in patients with DM2 (Wälti et al. 2003). An inverse association between dietary Mg and the incidence of DM2 has been reported. A diet deficient in Mg is associated with a significant impairment of insulin-mediated glucose uptake and with a considerable increased risk of

developing glucose intolerance and diabetes (Barbagallo and Dominguez 2007). Mg depletion in DM2 is associated with renal Mg and calcium wasting. Hyperglycemia and hyperinsulinemia may both have a role in the increased urinary Mg excretion contributing to Mg depletion (McNair et al. 1982). Hyperglycemia, which is a hallmark of lack of good metabolic control, may have a role in urinary Mg wasting. Hyperinsulinemia, present in insulin resistant states, may contribute per se to the urinary Mg depletion and the reduced insulin sensitivity, and may itself affects Mg transport (Djurhuus et al. 1995). Lower Mg levels may not only be a consequence, but may also predispose to the development of DM2.

Mg deficits have been associated with an increased risk for the development of glucose intolerance, cardiometabolic syndrome, and DM2 (He et al. 2006; Lopez-Ridaura et al. 2004; Song et al. 2004). Intracellular Mg depletion, causing a defective activity of the tyrosine kinase insulin receptor, as well as other Mg-dependent kinases of the insulin signaling, impairs insulin sensitivity and may contribute to the development of clinical conditions associated with insulin resistance, such as glucose intolerance and DM2. Inflammation and oxidative stress have been proposed as additional mechanisms by which Mg is linked to insulin resistance/metabolic syndrome. More generally, chronic hypomagnesemia and conditions commonly associated with Mg deficiency, such as DM2 and aging, are all associated with an increase in free radical formation with subsequent damage to cellular processes (Barbagallo and Dominguez 2007). The hypothesis that a dietary Mg deficit would induce and/or exacerbate insulin resistance is confirmed by data, both in experimental animals and in humans, showing that dietary-induced Mg deficiency is associated with insulin resistance (Schnack et al. 1992). A Mg-deficient diet in sheep caused a significant impairment of insulin-mediated glucose uptake (Matsunobu et al. 1990), while Mg supplementation delayed the development of diabetes in a rat model of diabetes (Balon et al. 1995). Higher Mg intake is associated with lower fasting insulin concentrations among women without diabetes (Fung et al. 2003), and a significant negative correlation is present between total dietary Mg intake and the insulin responses to an oral glucose tolerance test (Humphries et al. 1999). The increased risk for developing glucose intolerance and DM2 in persons with dietary and/or serum Mg deficits have suggested potential benefits of Mg supplementation in persons with DM2 or with risk factors for diabetes. The use of Mg supplements has been proposed as a potential tool for the prevention and the metabolic control of DM2. Benefits of Mg supplements on glycemic profile in most, but not all, studies does not explain whether according to meta-analysis a net beneficial effect is to be expected. While the body of evidence from epidemiological studies consistently shows a strong inverse relationship between dietary Mg intake and the risk of developing DM2, research concerning Mg supplementation in people with or at risk of diabetes is limited (Rodríguez-Morán et al. 2011; Von Ehrlich et al. 2014). A recent systematic review and meta-analysis including eighteen double-blind randomized controlled trials (12 in people with diabetes and 6 in people at high risk of diabetes) showed that Mg supplementation appears to have a beneficial role improving glucose parameters in people with DM2 and also improving insulin-sensitivity parameters in those at high risk of diabetes (Veronese et al. 2016).

9.8.4 Magnesium in Asthma and Airway Constriction

The first suggestion of a role for Mg in asthma was proposed in 1940 by an anecdotal report of Haury in two hospitalized patients having acute exacerbations of asthma who had a favorable clinical response after intravenous Mg sulfate administration. Haury reported that both patients were relieved immediately and remained free from symptoms for eighteen and twenty-four hours, respectively (Haury 1940).

Afterwards, the possible role of Mg in the pathogenesis of bronchial constriction as well as in its treatment regained considerable attention, particularly because of several reports confirming positive results of Mg administration in acute airway constriction (Okayama et al. 1987; Bloch et al. 1995) although some studies reported negative results (Tiffany et al. 1993; Bernstein et al. 1995). Even in the absence of an acute exacerbation, the functional pulmonary tests have been shown to improve with the administration of intravenous Mg and the action of Mg appears to be additive to the bronchodilating effect of the anti-asthmatic medicaments terbutaline (Skorodin et al. 1994) and salbutamol (Rolla et al. 1994).

Mg has modulatory effects on the contractile state of smooth muscle cells in various tissues: hypomagnesemia leads to contraction and hypermagnesemia leads to relaxation. Potential mechanisms for the direct relaxing effects of Mg on bronchial smooth muscle include calcium channel blocking properties (Iseri and French 1984), inhibition of cholinergic neuromuscular transmission with decreased sensibility to the depolarizing action of acetylcholine (McLean 1994), stabilization of mast cells and T-lymphocytes (Chyrek-Borowska et al. 1978), and stimulation of nitric oxide (Kemp et al. 1994) and prostacyclin (Nadler et al. 1987). In accordance with this hypothesis, Britton et al. showed that dietary Mg intake was independently related to lung function and to the occurrence of airway hyperreactivity, suggesting that a low Mg intake may be involved in the etiology of asthma (Britton et al. 1994). Significant positive independent associations of dietary Mg intake and lung function, airway reactivity to inhaled methacholine, and respiratory symptoms (wheezing) in the general population were reported (Britton et al. 1994).

Serum Mg measurements are not clinically useful for predicting the severity of the asthmatic attack, nor are they predictive of the response to Mg infusion, and no differences were present in serum Mg in asthmatic patients during acute exacerbation compared to a non-asthmatic population (Falkner et al. 1992). Conversely, cellular skeletal muscle Mg was found to be lower in the asthmatic patients when compared to non-asthmatic controls (Gustafson et al. 1996). We have shown a strong and direct relationship between the intracellular Mg levels and the methacholine bronchial reactivity in asthmatic patients suggesting a key role of intracellular Mg alterations and favorable effects of Mg administration in these patients (Dominguez et al. 1998).

Altogether the data are in agreement for a role of a deficit in cellular and body Mg as a relevant contributor to an increased reactivity and contractility of smooth muscle in the vascular and bronchial tissues, causing both vasoconstriction and bronchoconstriction (Seelig 1994).

9.8.5 *Magnesium, Depression, Other Psychiatric Disorders, and Neuromuscular Symptoms*

Mg deficits have been associated with numerous acute and/or chronic psychiatric disorders including depression, hypochondriasis, generalized anxiety, behavioral alterations, panic attacks, hyperexcitability, cephalalgias, as well as focal seizures, ataxia, anxiety, dizziness, tremor, irritability, insomnia, and psychotic behavior. Neuromuscular symptoms may include age-related muscular weakness, asthenia and myalgias (e.g., fibromyalgia and chronic fatigue syndrome). These conditions are generally, at least in part, reversible (Durlach et al. 2000).

As regards the central nervous system, Mg deprivation has been suggested to cause electrophysiological signs of hyperexcitability. In Mg deficient rats, changes in the electroencephalogram (EEG) were studied during auditory stimulation and correlated with behavioral alterations. The EEGs showed consistent changes with spike activity, initiating in the hippocampus and then spreading to the neocortices bilaterally, suggesting that behavioral changes induced by auditory stimulation in Mg-deficient rats are due to a Mg-related increased excitability of the central nervous system, resulting in seizures in deeper brain structures, particularly in the limbic system, later developing secondary generalization and projecting secondarily to the neocortices (Goto et al. 1993).

In humans, many characteristic signs and symptoms of Mg deficiency have been associated with neural and neuromuscular hyperexcitability (Galland 1991). Durlach have reviewed a number of possible mechanisms of Mg deficiency, which may induce depolarization and mediate central nervous hyperexcitability (Durlach et al. 2000). These include the previously described effects of Mg affecting cellular calcium homeostasis, increased susceptibility to peroxidation, increased activity of excitatory neurotransmitters, such as acetylcholine, catecholamines, *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors of excitatory aminoacids, decreased activity of inhibitory neurotransmitters, such as gamma-aminobutyric acid (GABA), taurine, glutaurine, adenosine and K receptors of opioids. Systemic effects that may also be involved include increased production of inflammatory mediators: neuropeptides, prostanoids, cytokines and decreased activity of anti-oxidant defenses.

Because of these important connections with the biological and transduction pathways implicated in the pathophysiology of depression, and in particular its role on the ion channel of the NMDA-receptor complex that is subject to voltage-dependent regulation by Mg ions (Decollogne et al. 1997), Mg supplementation has been suggested to be useful for the treatment of depression (Eby and Eby 2010; Derom et al. 2013). No significant correlation between total plasma Mg levels, severity of depression, and anxiety were observed (Barra et al. 2007; Kirov et al. 1994), although antidepressant drugs sertraline and amitriptyline have been shown to increase intracellular Mg levels (Nechifor 2009).

A systematic review including twenty-one cross-sectional studies, three intervention trials, one prospective study, one case only study, and one case series study,

concluded that a higher intake of dietary Mg was associated with lower depression symptoms (Derom et al. 2013).

Mg appears to be effective to some extent in the treatment of depression but data are scarce and incongruous. Oral Mg supplementation may help in the prevention of depression and might be used as an adjunctive therapy. However, more interventional and prospective studies are needed in order to further evaluate the benefits of Mg supplementation for the treatment of depression.

Mg has also been used as an adjuvant in the treatment of insomnia. Thus, Mg as a natural NMDA antagonist and a GABA agonist, may have a relaxant effect, may increasing melatonin levels, and may facilitate sleeping well (Abbasi et al. 2012).

9.8.6 Mg and Alzheimer's Disease

The role of Mg in dementia and other degenerative disorders has been the focus of increased attention (Glick 1990). Some epidemiological, experimental and clinical data have linked Mg depletion to dementia and Alzheimer's disease (AD) although the mechanisms of this association have not been clearly defined yet. Mg insufficiency and its altered concentrations in the brain, as well as the effects of Mg supplementation in AD, have been investigated. Total serum Mg levels, ionized plasma Mg levels, and Mg content in various tissues of patients with Alzheimer's disease in clinical, experimental and autopsy studies have consistently shown to be reduced (Vural et al. 2010; Cilliler et al. 2007; Barbagallo et al. 2011; Andrási et al. 2005). Mg concentration affects multiple biochemical mechanisms in the brain, which are involved in the cognitive process, including NMDA-receptor response to excitatory amino acids, cell membrane fluidity and stability, and toxic effects of calcium (Barbagallo and Dominguez 2010). In addition, high intake of a neurotoxic metal, such as aluminum, which inhibits activity of Mg-requiring enzymes, impairs transport of Mg and/or enhances transport of the neurotoxic metal into brain tissue, has been hypothesized to have a role to alter incorporation of Mg into brain neurons (Glick 1990).

9.8.7 Magnesium and Bone Disease

Mg deficiency has been proposed as a potential risk factor for developing osteoporosis and fragility fractures. Insufficient dietary Mg intake has been associated in humans with low bone mass and postmenopausal osteoporosis. Epidemiologic studies have demonstrated a positive significant correlation between dietary Mg intake and bone density and/or an increased rate of bone loss with low dietary Mg intake (Tucker et al. 1999; New et al. 2000). In two thousand thirty-eight older black and white men and women aged 70–79 at baseline enrolled in the Health, Aging and Body Composition Study, higher Mg intake, assessed using a semiquantitative food frequency questionnaire, was found to be associated with higher bone mineral den-

sity in healthy older white (but not in black) participants (Ryder et al. 2005). The effect of selective dietary Mg depletion has been extensively studied in experimental rat model. Preclinical studies have shown that Mg-depleted mice with frank hypomagnesemia had impaired bone growth, decreased bone formation, increased bone resorption, osteoporosis, and increased skeletal fragility (Kenney et al. 1994; Rude et al. 1999).

The pathophysiologic basis for this effect of Mg on bone, however, remains unknown, although elevated serum concentrations of inflammatory cytokines may play a role. The possible role of Mg deficiency in determining bone loss is confirmed by data from Rude and Gruber showing an increased osteoclastic bone resorption associated with an increased concentration of substance P and TNF-alfa in bone from Mg-deficient rats (Rude and Gruber 2004).

Mg is required for activation of vitamin D and Mg deprivation is associated with hypoparathyroidism, low production of 1,25-OH_2 D_3, and resistance to PTH and vitamin D actions (Medalle et al. 1976). The combined effects of Mg deficiency and low PTH and 1,25-OH_2 D_3 synthesis and secretion may also contribute to impair bone growth and mineralization and to reduce bone quality, strength, and bone mineral density. Oral Mg supplementation suppresses bone turnover and may help to prevent osteoporotic disease (Aydin et al. 2010; Dimai et al. 1998).

A recent report in a large cohort of American men and women involved in the Osteoarthritis Initiative followed over a period of 8 years showed that women meeting the recommended Mg intake were at a 27% lower risk for future fractures, suggesting a protective effect of Mg on the risk of incident fragility fractures (Veronese et al. 2017).

9.8.8 Mg and Muscular Disease (Chronic Fatigue, Muscle Pain, and Fibromyalgia)

Muscle pain may be associated with Mg deficiency. The original symptoms, which may have been due to Mg deficiency, include weakness and night cramps. Fibromyalgia is a rheumatic disease characterized by muscular pain and tenderness associated with a non-specific general symptomatology that includes fatigue, sleep disorders, bowel dysfunction, and headache, among others. The etiology is still unknown, although it has been suggested that deficiency in trace elements may contribute to the development of fibromyalgia (Sendur et al. 2008).

There are limited data about the effects of Mg treatment on fibromyalgia symptoms, although it has been suggested that Mg supplementation may have a role in reducing pain, tenderness, and symptom severity in patients with fibromyalgia (Bagis et al. 2013).

Table 9.7 Mechanisms by which low magnesium status may affect muscle, increasing oxidative stres

• Energetic metabolism (oxygen uptake and energy production)
• Transmembrane transport
• Muscle contraction and relaxation (by means of MgATP and the release of Ca)

9.8.8.1 Mg and Muscle Performance

Mg status is crucial to muscle ATP concentration, muscle energetic metabolism, transmembrane transport, and muscle contraction and relaxation, while Mg deficiency is associated with poor physical performance. In agreement with the role of Mg deficiency in increasing oxidative stress and inflammation (Weglicki et al. 1996), Mg depletion is also associated with impaired intracellular calcium levels and muscle cells structural damage, affecting energetic metabolism (oxygen uptake and energy production), transmembrane transport, and muscle contraction and relaxation (Table 9.7) (Rock et al. 1995). A significant, independent and strong relationship was found between circulating Mg and muscle performance, which was consistent across several muscle parameters for both men and women (Dominguez et al. 2006).

Mg supplementation (up to 8 mg/kg daily) enhanced muscle strength, enhanced endurance performance, and decreased oxygen use in young volunteers (Brilla and Haley 1992). In older persons, oral Mg supplementation (300 mg per day) improved physical performance, in particular in those participants with a low Mg dietary intake, suggesting a role for Mg supplementation in preventing or delaying the age-related decline in physical performance (Veronese et al. 2014).

9.8.9 Magnesium and Cancer

A complex relationship links Mg and cancer. In animal model, Mg may exert a protective effect in the early phases of chemical carcinogenesis. It has been reported that Mg inhibits nickel-induced carcinogenesis in the rat kidney (Kasprzak et al. 1994), and protects against 3-methyl-cholantrene-induced fibrosarcomas in rats (Patiroğlu et al. 1997). Mg acts as a protective agent in colorectal cancer in experimental models by inhibiting c-myc expression and ornithine decarboxylase activity in the mucosal epithelium of the intestine (Mori et al. 1997). It has been suggested that a decrease in Mg intake may increase cell proliferation by activating Ca channels (TRPM7), which can provide the milieu for the development of cancer (Hanano et al. 2004). A higher serum Ca/Mg ratio has been shown to be associated with an increased risk of postmenopausal breast cancer (Sahmoun and Singh 2010). Likewise, an increase in dietary Mg consumption has been reported to be inversely related to the risk of developing colorectal adenomas and colorectal cancer (Wark et al. 2012).

The relationship of cancer protection and Mg intake is not clear because Mg content in the diet is closely related to fiber and largely obtained from green vegetables. There is general agreement about the inverse significant correlation between the risk of cancer and the regular consumption of fruit, whole cereals and vegetables, rich sources of fiber, micronutrients, vitamins and minerals, including Mg.

9.9 Role of Magnesium in the Aging Process and Longevity

In cellular systems, Mg is highly required to maintain genomic stability. Mg has stabilizing effects on DNA and chromatin structures and is an essential cofactor in almost all enzymatic systems involved in DNA processing. Furthermore, as essential cofactor in nucleotide excision repair, base excision repair, and mismatch repair, Mg is required for the removal of DNA damage generated by environmental mutagens, endogenous processes, and DNA replication (Hartwig 2001). Thus, Mg deficiency increases the susceptibility to oxidative stress and immune dysfunction, which may decrease membrane integrity and function and contribute to several mitochondrial alterations with age (decreased number, morphology modifications, increased DNA mutations, decreased biogenesis, decreased autophagy, increased apoptosis) (Table 9.8) (Barbagallo and Dominguez 2010).

Mg has a central role in direct regulation of protein synthesis and in ancillary processes as a response to membrane perturbation and repair (Hartwig 2001; Rubin 2005). DNA is continuously damaged by environmental mutagens and by endogenous processes. Intracellular free Mg increases in cells facing apoptosis. Mg raise is an early event in apoptosis, possibly linked to a mobilization of Mg from the mitochondria, and appears to be a "second messenger" for downstream events in apoptosis (Chien et al. 1999).

There is increasing evidence from animal experiments and epidemiological studies, that Mg deficiency may decrease membrane integrity and membrane function, increasing the susceptibility to oxidative stress, cardiovascular heart diseases, as well as accelerated aging. The aging process is associated with a shortening of telomeres, repetitive DNA sequences, and associated proteins that cap and protect the ends of

Table 9.8 Effects of aging on the mitochondria by which oxidative stress may be increased

• Decreased number
• Morphology modifications
• Increased DNA mutations
• Decreased biogenesis
• Decreased autophagy
• Increased apoptosis

chromosomes. Low telomerase activity is associated with increased catecholamines while the sensitivity of telomere synthesis to Mg ions is primarily seen for the longer elongation products (Blackburn 2000). Several studies have reported alterations in cell physiology with senescence features during Mg deficiency in different cell types. Mg-related alterations may include reduced oxidative stress defense, cell cycle progression, culture growth, and cellular viability and activation of the expression of proto-oncogene and of transcription factors (Sgambato et al. 1999). Culture of primary fibroblasts in Mg-deficient media caused a loss of replicative capacity and an acceleration of the expression of biomarkers associated with senescence and in telomere attrition. In addition, a significant decrease in the replicative lifespan was seen compared to fibroblast populations cultured in normal Mg media conditions (Killilea and Ames 2008).

Because of the crucial role of Mg in stabilizing DNA, reducing the potential for oxidative stress and promoting DNA replication and transcription, a Mg deficiency might lead to an increased genomic instability, inhibited DNA repair, and altered function of mitochondria, thus contributing to cellular senescence and accelerated aging (Killilea and Maier 2008; Hartwig 2001). Mg protects against these effects and against the shortening of telomeres seen with lower Mg, associated with a reduction in life expectancy. It has been hypothesized that because of the effects of Mg supple-

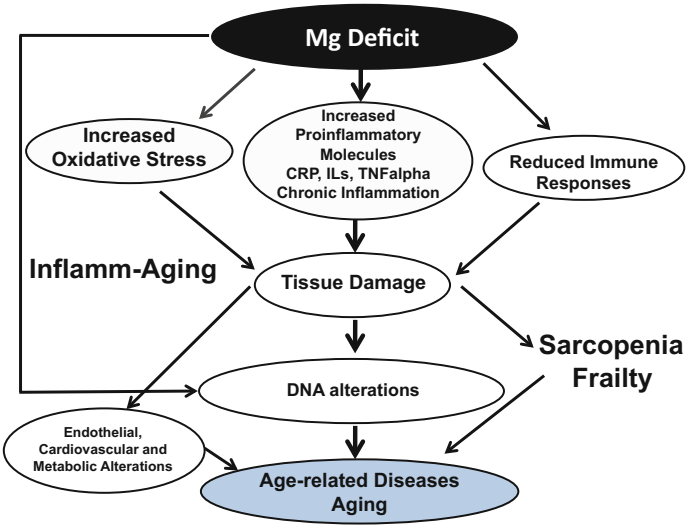

Fig. 9.3 Different factors may converge in old age and determine a low magnesium status. Because magnesium acts as an antioxidant reducing the production of free radicals by the mitochondria, its deficit may contribute to the accumulation of oxidative damage, which in turn may trigger the release of inflammatory mediators conforming a state identified as "inflammaging". This term refers to the low-grade chronic inflammation frequently seen in old age and associated with several age-related chronic conditions

mentation in maintaining telomere length, it may also have a role in extending the life span (Rowe 2012).

9.10 Conclusions

Aging is frequently associated with chronic Mg deficiency. Several age-related chronic conditions have been linked to a low-grade chronic inflammation and/or to an excessive production of oxygen-derived free radicals. Since Mg deficits trigger both these conditions, we have previously hypothesized that a chronic Mg insufficiency may help to explain the interactions between low grade chronic inflammation and oxidative stress with the aging process and/or age-related diseases (Fig. 9.3) (Barbagallo et al. 2009; Barbagallo and Dominguez 2010).

It is thus possible to postulate that preserving an optimal Mg homeostasis throughout life might help to prevent some aging-related conditions, associated with Mg inadequacy, and may lengthen healthy life. In this context, also the possible role of Mg supplementation remains unclear. Very few prospective blind studies on the effects of Mg deficiency treatment in older adults have been performed. The possibility that Mg may supplementation may become a safe and economic health strategy in the aging population is a suggestive hypothesis that needs to be proven by future prospective studies.

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Chapter 10
Iodine

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Abstract Iodine is an essential micronutrient for health and maintenance of thyroid function in humans and other vertebrates. The major effects of iodine in the organism are mediated by its action as a structural constituent of thyroid hormones, thyroxine and triiodothyronine, potent regulators of cellular metabolism and growth and development processes. As a component of thyroid hormones, iodine has a crucial role in prenatal and early postnatal ontogenesis due to its involvement in the regulation of neurodevelopment, maturation of the musculoskeletal and respiratory systems and the formation of cognitive function. The adequate iodine status is also an important factor in preventing thyroid disorders and maintaining proper mental and physical health in adulthood. Iodine exhibits some effects not mediated by its action in the composition of thyroid hormones and can be involved in the prevention and inhibition of tumour growth. Iodine deficiency in organism has multiple adverse health consequences, including goitre, hypothyroidism and an increased risk of developing several types of cancer. The activity of the thyroid gland changes with age, and alterations in its function can be associated with longevity; thus, peculiarities of iodine metabolism in older age are of particular interest.

Keywords Iodine · Thyroid gland · Thyroid hormones
Iodothyronine deiodinases · Iodine deficiency disorders · Goitre
Neurodevelopment · Cognitive function · Cancer prevention · Health · Aging
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10.1 Introduction

Iodine (I) [*Gr.* = violet], a halogen of atomic number 53 and atomic mass 126.9 amu, was discovered in 1811 by Bernard Courtois, who separated it from seaweed ash, and was independently identified as a chemical element in 1813 by Joseph Louis Gay-Lussac and Humphry Davy (Kendrick 2016). Along with the 200-year period of fundamental research and practical use of iodine, this element has a thousand-year history of application in medicine. Medications with iodine-rich brown algae *Sargassum* and the thyroid gland of animals were used in ancient China and the ashes of marine sponges were applied in medieval Europe, presumably for the treatment of goitre (Langer 1960). Progress in biochemistry and endocrinology from the late 19th to the middle of the 20th century and subsequent advances in molecular biology made it possible to clarify the biological role of iodine, its metabolism in human organism and the health risks associated with iodine deficiency.

The thyroid gland, which can effectively concentrate iodine from the blood, provides the accumulation and organification of iodine in humans and vertebrate animals. Thyroid tissue is unique among vertebrate tissues because of its ability to synthesize 3,5,3′,5′-tetraiodothyronine (thyroxine, T_4) and 3,5,3′-triiodothyronine (T_3), the only known to date iodine-containing natural compounds that exhibit hormonal activity (Felig et al. 1995; Nicola and Carrasco 2014). Thyroid hormones are involved in the regulation of a wide range of metabolic and physiological processes and mediate the main functions of iodine in human organism. These bioregulators are critical for growth, development, differentiation and maintenance of metabolic balance (Cheng et al. 2010). As an integral component of thyroid hormones, iodine is especially required for development and proper activity of the nervous system and brain maturation (Hetzel 2005; Bernal 2017), as well as for the maturation of the respiratory and musculoskeletal systems during the prenatal and early postnatal periods (Forhead and Fowden 2014). Thyroid hormones are also important for the maturation of the reproductive system and the maintenance of a healthy pregnancy (Krassas et al. 2010), for the functioning of cardiovascular (Klein and Danzi 2016), gastrointestinal (Sirakov and Plateroti 2011) and other vital system throughout life. Iodine exhibits some effects independently of its action in composition of thyroid hormones (such as antioxidant effect) and participates in autoregulation of thyroid functions (Gaertner 2009; Venturi and Venturi 2014). In addition, iodine exhibits an antiproliferative effect and can be involved in prevention of certain types of cancer (Venturi et al. 2000; Brown et al. 2013; Rappaport 2017).

Iodine deficiency in humans leads to metabolic and functional disturbances as a consequence of inadequate formation of thyroid hormones. The spectrum of these disease states is collectively referred to as iodine deficiency disorders, widespread in the world's population (Hetzel 1983; Zimmermann 2009). Endemic goitre is the most visible manifestation of iodine shortage in the organism; however, most severe consequences of iodine deficiency include neurodevelopmental abnormalities and infant mortality (Zimmermann 2016).

Most metabolic disorders observed in conditions of iodine deficiency can be prevented by normalizing iodine intake (Hetzel 2005; Zimmermann 2009). However, the problem of iodine deficiency in population still remains unresolved in many countries. Nearly 1.9 billion people worldwide have inadequate iodine consumption and are at a risk for iodine deficiency disorders, with children, pregnant and lactating women being the most vulnerable groups (Andersson et al. 2012; Zimmermann 2007, 2016). Thus, iodine deficiency is one of the global health problems and attracts considerable attention of the world's scientific community.

In addition, iodine is an element associated with global environmental processes, and its speciation in the atmosphere can contribute to the destruction of atmospheric ozone (Saiz-Lopez et al. 2012). Studies on the global cycle of iodine, its evolutionary significance and essentiality for living systems and various aspects of human health are among actual issues in many areas of scientific research. The aim of this chapter was to analyze the distribution of iodine in the environment, its metabolism in human organism and its importance for human health.

10.2 Iodine Distribution in the Environment

Iodine is an ultra-trace element of the Earth's crust (mean concentration is about 0.25 mg/kg) with an uneven and highly variable distribution in different compartments of the natural environment (Fuge and Johnson 2015). Its concentration is generally low in the lithosphere and reaches higher levels in seawater and in marine and oceanic sediments. Iodine occurs in different oxidation states (from −1 to +7), with I^- (iodide), IO_3^- (iodate) and organic iodine compounds being predominant in the environment (Küpper and Kroneck 2015).

A key part of the global biogeochemical cycle of iodine is its volatilization from the surface of the World Ocean to the atmosphere. Volatile iodine species include molecular iodine (I_2) and organic compounds (CH_3I, CH_2I_2 and CH_2ClI), produced by macrophytic algae, phytoplankton and bacteria (Leblanc et al. 2006). Iodine concentration in the seawater amounts on average 45–60 μg/L, while most freshwater systems contain it at a concentration of <20 μg/L with many values in the range from 0.5 to 5 μg/L (Fuge and Johnson 1986). The total concentration of iodine in the atmosphere is in the range of 10–20 ng/m^3 and includes both the organic and inorganic fractions. In wet and dry precipitation, iodine is transferred from the atmosphere to the land surface and infiltrates the soils, where its concentration varies in the wide range (0.5–50 mg/kg), depending on the soil type and location. The near-coastal soils are enriched in iodine, while those located far from marine influence are relatively depleted in this element (Fuge and Johnson 2015). Iodine is generally strongly adsorbed in soils due to its binding by organic matter and other soil components, while only small amount of the total soil iodine is available for uptake by plants. Heavy rainfalls, seasonal flooding, soil erosion and weathering, excessive use of fertilizers deplete the soil content of iodine. Agricultural cultivation of iodine

depleted soil results in the production of iodine deficient crops that increase risk of iodine deficiency in the residents of such regions.

Iodine has a strong tendency to accumulate in the biosphere and is found in all groups of terrestrial, soil and aquatic biota. Iodine content is usually low in natural vegetation (less than 1 mg/kg), but in some agricultural plants it can be significantly increased by using iodine-containing fertilizers (Medrano-Macías et al. 2016). Marine algae and invertebrates accumulate iodine in much larger quantities than terrestrial biota, with brown algae (Phaeophyceae) being the most potent iodine bioaccumulators among all living organisms. The highest iodine contents have been reported in the species of the genus *Laminaria* with the maximum levels in *Laminaria digitata* (up to 6.12–8.17 g/kg of dry weight) (Teas et al. 2004). To date, more than a hundred iodine-containing natural products have been identified, most of them derived from marine organisms (Küpper and Kroneck 2015). Many of recently discovered compounds of marine origin were found to possess biological activities, including anticancer and antibiotic properties (Gribble 2015).

Iodine is necessary for all classes of vertebrates for the synthesis of thyroid hormones that control embryonic development, postnatal growth and metabolic rate (Tata 2011; Mullur et al. 2014). In amphibians and teleost fishes, thyroid hormones are involved in the control of metamorphosis (Tata 2011). In mammals, thyroid hormones are closely related to the regulation of growth and development processes (Forhead and Fowden 2014). In homeothermal organisms, thyroid hormones play an important role in the regulation of basic metabolism and the processes of obligate and adaptive thermogenesis.

10.3 Iodine in the Human Organism

Iodine is an essential micronutrient for normal growth, metabolism and thyroid function in humans and is primarily used as a substrate for the processes of hormonogenesis in the thyroid gland (Kopp 2005). Total amount of iodine in the human body ranges from 15 to 20 mg with 70–80% of it being stored in the thyroid (Hetzel and Maberly 1987). The major part of iodine enters the human organism via the gastrointestinal tract, including more than 90% of iodine derived from food. Other routes of iodine entry (inhalation, dermal absorption) have only a minor role in iodine intake by humans.

10.3.1 Iodine Absorption in the Digestive Tract

The biologically available form of iodine for humans is an iodide anion that is readily absorbed into the bloodstream from the small intestine, mostly from the duodenum (Josefsson et al. 2002). Other forms of dietary iodine are converted into iodide in the gastrointestinal tract prior to absorption. Iodine-containing organic compounds

are usually decomposed during digestion of food with the release and subsequent absorption of iodide.

Iodide uptake in the intestine is mediated mainly by sodium iodide symporter (Na^+/I^- symporter, NIS) that is identical to the thyroidal NIS (Nicola et al. 2009, 2015). In the small intestinal epithelium, NIS is located in the brush border of enterocytes and can mediate iodide active transport from the intestinal lumen to the cells. Subsequently, iodide can reach the bloodstream, probably through the Cl^- channels (Nicola et al. 2009). Closely linked to NIS is Na^+-multivitamin transporter (SMVT), also capable of transferring iodide, but with a lower affinity. The SMVT may provide a complementary pathway for iodide uptake in the small intestine (de Carvalho and Quick 2011). Iodide is transported via the blood, being weakly bound by plasma proteins. A small fraction of the plasma iodide is in an ionic form.

10.3.2 Iodine Accumulation in the Thyroid Gland and Extrathyroidal Tissues

Iodide is absorbed by thyroid cells in an amount necessary to maintain an adequate thyroid function. In conditions of sufficient iodine intake, the adult human thyroid gland contains 12–15 mg of this element (Hetzel and Maberly 1987). Under physiological conditions, the thyroid absorbs about 60 μg of iodide per day; however, in cases of iodine deficiency, the level of daily iodide uptake by the thyroid gland can be reduced to 20 μg or less (DeGroot 1966). Iodide uptake by human foetal thyroid begins around 10–12 weeks, but increases significantly after 22 weeks of prenatal development until term. Thyroidal iodide uptake is higher in adolescents than in adults and decreases progressively with age (Verger et al. 2001).

Iodide enters the thyroid cells via two routes: (1) active transport involving membrane Na^+/I^- symporter and (2) diffusion through ion channels (McLanahan et al. 2008). The main stages of iodine metabolism in the thyroid are associated with the oxidation of iodide and its subsequent organification followed by the formation and release of thyroid hormones. The formation of iodolipids and deiodination of iodothyronines with the release of iodide also take place in thyroid cells (Köhrle et al. 2005; Gaertner 2009).

In addition to the thyroid gland, a certain amount of iodine can accumulate in some extrathyroidal tissues, mainly in the gastric mucosa, salivary glands and lactating mammary gland (Spitzweg et al. 1998; Dohan et al. 2003; Angelousi et al. 2016). All these cells express the Na^+/I^- symporter and form the major extrathyroidal iodide pool, which can be maintained depending on the level of iodide intake and the physiological status of the organism. In these tissues, as in the thyroid gland, NIS is located in the basolateral membrane of epithelial cells and mediates the uptake of iodide from the bloodstream (Nicola et al. 2009). However, unlike the thyroid gland, nonthyroidal tissues do not possess the ability to organify accumulated iodide (with the possible exception of the lactating mammary gland) (Carrasco 2005).

The mammary gland during lactation concentrates iodide almost as actively as the thyroid and secretes it into milk, providing a substrate for thyroid hormone synthesis in newborns. NIS is present in the healthy breast exclusively during late pregnancy and lactation, in contrast to its constitutive expression in the thyroid (Tazebay et al. 2000). Conversely, malignant cells of the non-lactating mammary gland have been shown to express Na^+/I^- symporter and accumulate iodide (Tazebay et al. 2000; Angelousi et al. 2016).

Iodide accumulation in the gastric mucosa and salivary glands may provide the formation of its endogenous reserve, which can be reabsorbed in conditions of low dietary iodine intake. From the gastric mucosa, iodide is released into the stomach lumen, possibly through the Cl^- channels (Josefsson et al. 2002). Iodide secreted in the saliva and gastric juice can be reabsorbed in the small intestine along with newly ingested iodide.

10.3.3 Excretion of Iodine from Human Organism

Iodine is removed from human body primarily through the excretory system in the form of iodide. Renal excretion accounts for about two thirds of iodide cleared from plasma, and urinary iodide concentration (UIC) is an indicator of recent dietary iodine intake. The remaining part of iodine is excreted through the digestive tract (about 1%), exocrine glands and in the exhaled air (Hetzel and Maberly 1987). Under conditions of strenuous physical activity, iodine can be intensively excreted through the sweat glands. In areas with low iodine intake, the loss of iodide in sweat can be equal to that in urine and can partially contribute to iodine deficiency in people who perform heavy workloads (Smyth and Duntas 2005).

The release of iodine into breast milk is an important route of iodine excretion during lactation. Iodine in milk is represented by iodide and thyroid hormones, mainly thyroxine. Breast milk iodine concentration (BMIC) is closely related to the level of iodine intake and, together with iodine concentration in urine, is considered a biomarker of iodine nutrition in lactating women (Dold et al. 2017). In conditions of iodine sufficiency, BMIC is in the range of 100–150 μg/L, but it has been reported to vary from 5.4 to 2170 μg/L in worldwide studies (Azizi and Smyth 2009). Iodine content in colostrum is significantly higher than in mature milk (Moon and Kim 1999).

10.4 Iodine Turnover in the Thyroid Gland

10.4.1 Thyroid Function and Its Regulation

The thyroid gland has a major role in the concentrating and storing of iodine and is the only specialized endocrine organ capable of synthesizing thyroid hormones, although the expression of genes involved in this process is also found in several nonthyroidal tissues (Sellitti et al. 2000; Kim et al. 2017). Thyroid-stimulating hormone (TSH, thyrotropin), produced by anterior pituitary, is the principal regulator of thyroid gland. TSH is required for the differentiation and proliferation of thyrocytes, the processes of iodide uptake and the synthesis and release of thyroid hormones. Thyrotropin itself is under the control of thyrotropin-releasing hormone (TRH) secreted from the hypothalamus (Felig et al. 1995). These hormones together with thyroxine and T_3 form the hypothalamus-pituitary-thyroid axis, which is regulated by the feedback mechanisms.

Thyrotropin controls thyroid functions and modulates the expression of thyroid-specific genes via interaction with the class A G-protein-coupled receptor located at the basolateral surface of thyrocytes (Kleinau et al. 2017). The regulatory effects of thyrotropin are mostly mediated by the cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent pathway and involve protein kinase A, but TSH can induce also the phospholipase C signal transduction system (Corvilain et al. 1994). A number of additional regulatory factors, including hormones (insulin, glucocorticoids, estradiol), cytokines and growth factors (such as insulin like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β)), as well as follicular thyroglobulin and iodide itself, are involved in the regulation of thyroid functions and can interfere with TSH-stimulatory effects (Deleu et al. 1999; Santin and Furlanetto 2011; Nadolnik 2012; Pesce and Kopp 2014). The transient inhibition of thyroid hormone production by excess iodide is known as the Wolff–Chaikoff effect (Wolff and Chaikoff 1948).

In addition to the synthesis of thyroid hormones, the thyroid gland is capable of iodinating polyunsaturated fatty acids. A range of iodolipid compounds belonging to iodoaldehydes and iodolactones have been identified in human and animal thyroids, with 2-iodohexadecanal (2-IHDA) being the main compound of iodolipid fraction (Gaertner 2009; Rossich et al. 2016). It has been suggested that iodoaldehydes and iodolactones can demonstrate regulatory effects and mediate the inhibitory influence of excess iodide in the thyroid gland (Gaertner 2009).

10.4.2 Membrane Transport of Iodide in Thyrocytes

The ability of the thyroid gland to accumulate iodine was first demonstrated in 1895 by Eugen Baumann, who reported high concentrations of protein-bound iodine in thyroid extracts (Baumann 1895), and two decades later the thyroidal absorption of iodide was described (Marine and Feiss 1915). Significant progress in clarifying the

mechanism of iodide transport and its regulation in the thyroid and extrathyroidal tissues was achieved after characterization of the Na^+/I^- symporter and cloning of genes encoding NIS from rat and human in 1996 (Dai et al. 1996; Smanik et al. 1996).

The Na^+/I^- symporter, also known as SLC5A5 (solute carrier family 5, member 5) is located at the basolateral plasma membrane of thyrocytes and actively cotransports two sodium cations per each iodide anion using an electrochemical sodium gradient generated by Na^+/K^+-ATPase (Dohan et al. 2003; Carrasco 2005; Portulano et al. 2014). NIS has a high affinity for iodide, which allows the thyroid gland to concentrate this anion 20–40 times compared to its concentration in blood plasma. Besides iodide, NIS is capable of transporting other anions, including nitrate (NO_3^-), thiocyanate (SCN^-), and perchlorate (ClO_4^-) that can act as competitive inhibitors of iodide uptake (Tonacchera et al. 2004).

Spontaneous mutations of *SLC5A5* (*NIS*) gene have been identified as causes of a congenital iodine transport defect, an autosomal recessive disorder, leading to thyroid dyshormogenesis and hypothyroidism (Nicola and Carrasco 2014). Conversely, NIS expression is significantly higher in hyperfunctioning thyroid gland in patients with Graves' disease than in healthy thyroid tissue.

Membrane transport of iodide in thyrocytes is controlled primarily by TSH, which up-regulates the expression of Na^+/I^- symporter by either induction of NIS mRNA or posttranslational modification of NIS molecule (Dohan et al. 2003). Regulatory effects of TSH on NIS expression are mainly mediated by activation of adenylyl cyclase, protein kinase A and transcription factor CREB (Portulano et al. 2014). Estradiol down-regulates the expression of NIS and iodide uptake in thyroid cells that possibly explains the higher incidence of goitre in women (Santin and Furlanetto 2011). Insulin and IGF-1 down-regulate the *NIS* gene expression, with phosphatidylinositol-3-kinase (PI3K) participating in their inhibitory effects (García and Santisteban 2002). Tumour necrosis factor-α (TNF-α), TGF-β and some interleukins also inhibit NIS expression in thyrocytes (Pesce and Kopp 2014). Excess iodide was shown to reduce the iodide uptake due to down-regulation of the *NIS* gene transcription through activation of PI3K/Akt pathway (Nascimento et al. 2016).

Iodide transport through an apical membrane of the thyrocyte (iodide efflux) to the intraluminal compartment of the follicle is mediated, in part, by a transporter known as pendrin (SLC26A4, solute carrier family 26, member 4) in conjunction with several other channels such as CLCN5 (chloride channel 5) and calcium-activated anion channel anoctamin 1 (TMEM16A) (Kopp et al. 2017). Biallelic mutations in the *SLC26A4* gene lead to Pendred syndrome, an autosomal recessive disorder characterized mainly by sensorineural deafness, goitre and partial defect in iodide organification (Royaux et al. 2000).

10.4.3 Synthesis of Thyroid Hormones

The central stage in the synthesis of thyroid hormones is iodide organification that takes place in the follicular lumen. This process involves a 660 kDa homodimeric glycoprotein thyroglobulin (TG) that serves as an iodine acceptor in the process of organification, as the scaffold for the synthesis of thyroid hormones, and as an intraglandular store of iodine (Di Jeso and Arvan 2016).

Iodide, being transferred to the outer apical side of the thyrocyte, is oxidized on the cell-colloidal interface and is rapidly organified by the covalent binding to the selected tyrosyl residues of TG with formation of mono- and diiodothyrosines (MIT and DIT, respectively). This process, referred to as organification, is catalyzed by membrane-bound thyroperoxidase (TPO) in the presence of hydrogen peroxide (H_2O_2), which acts as electron acceptor (Ruf and Carayon 2006). Hydrogen peroxide is generated by two members of the NOX (NADPH-oxidase) family: dual oxidases 1 and 2 (DUOX1 and DUOX2, respectively) (Sumimoto 2008).

In the subsequent coupling reaction, also catalyzed by TPO, two iodinated tyrosyl residues within the TG molecules are coupled to form iodothyronines (either T_4 or T_3). Iodinated molecules of TG containing MIT, DIT, T_4 and T_3 are accumulated in the intraluminal colloid of the follicle. To release thyroid hormones, iodinated thyroglobulin is internalized into thyrocytes by micro- and macropinocytosis and undergoes proteolysis in lysosomes (Kopp 2005). This process is accompanied by the release of T_4 and T_3 that are secreted into the bloodstream at the basolateral membrane. The levels of T_4 and T_3 secretion from human thyroid are 80–100 μg and 3–15 μg per day, respectively (Hetzel and Maberly 1987; Nicola and Carrasco 2014). Mechanism of T_4 and T_3 secretion from the thyrocyte involves specific membrane transporters of thyroid hormones, in particular, the monocarboxylate transporter MCT8 (Visser et al. 2011).

Small amount of TG molecules (about 10%) can be subjected to the process of transcytosis (i.e., the vesicular transfer of TG from the apical to the basolateral membranes bypassing the lysosomes) that results in the release of TG into the blood (Tuma and Hubbard 2003). Megalin (gp330), a member of the family of low density lipoprotein receptors, was shown to mediate the TSH-stimulated uptake of TG in thyrocytes and its transcytosis.

Process of hormonogenesis is regulated mainly by TSH, which stimulates the expression of thyroid-specific genes, including those encoding TG and TPO expression. TSH-dependent cAMP cascade is the major regulator of hormone secretion of thyrocytes, whereas TSH-stimulated phospholipase C-dependent pathway activates the formation of H_2O_2 and TG iodination (Kopp 2005). Process of iodide organification is transiently blocked by excess iodide (Koukkou et al. 2017).

10.5 Transport of Thyroid Hormones into the Cells

The entry of thyroid hormones into target cells is mediated by transport proteins, localized in the plasma membrane. These include the Na^+/taurocholate cotransporting polypeptide (NTCP), the members of the family of Na-independent transporters of organic anions (OATP, organic anion transporter polypeptide), L-type amino acid transporters LAT1 and LAT2, and monocarboxylate transporters (MCT). However, only MCT8 and OATP1C1 exhibit a high degree of specificity for iodothyronines (Visser et al. 2011).

10.6 Deiodination of Thyroid Hormones

Studies conducted in the 1970s have shown that about 80–90% of thyroxine secreted from the thyroid gland undergoes deiodination in peripheral tissues with the formation of T_3 and reverse triiodothyronine (rT_3) (Chopra et al. 1978). Subsequently, deiodination process was recognized as an important stage in the metabolism of iodothyronines and in the iodine turnover in the organism (Leonard and Visser 1986). In humans, thyroxine deiodination produces about 80% of T_3 and almost 95% of rT_3 (Leonard and Visser 1986). In addition, about 10% of T_4 initially produced, is deiodinated intrathyroidally with formation of T_3 (Nicola and Carrasco 2014).

Catalysis of oxidation/reduction reactions of iodothyronine deiodination involves three selenium-containing enzymes: iodothyronine 5′-deiodinases type 1 and type 2 (D1 and D2, respectively) and iodothyronine 5-deiodinase type 3 (D3) (Leonard 1990; Bianco et al. 2002; Köhrle et al. 2005). The first two enzymes catalyze the deiodination of the outer (phenolic) ring of thyroxine at the 5′-position that leads to the formation of T_3 (the biologically active form of thyroid hormone). The D3 enzyme is responsible for the deiodination of the inner (tyrosyl) ring of thyroxine in the 5-position, resulting in the formation of 3,3′,5′-triiodothyronine (rT_3), which is generally considered an inactive form of thyroid hormone (although rT_3 can exhibit some local non-genomic effects). Hence, by catalytic conversion of thyroxine to T_3 or rT_3, iodothyronine deiodinases control the activation or inactivation of the thyroid hormone, respectively. The ratio between these two routes of T_4 conversion is regulated by the metabolic situation in the cells and physiological status of the organism. Expression of iodothyronine deiodinases was found to be tissue-specific and regulated by a number of factors (hormones, cytokines, growth factors, neurohumoral and alimentary factors) (Bianco et al. 2002; Antonyak et al. 2002; Köhrle et al. 2005; Bianco 2011; Antonyak and Vlizlo 2013).

These three enzymes share significant homology of the primary structure and possess the amino acid selenocysteine residue Sec (SeCys) in the active site. The Sec incorporation into enzyme molecules is determined by UGA codon and by a segment SECIS (selenocysteine insertion sequence), which is located in the 3′-untranslated region of selenoprotein mRNA. Several other factors are involved in this process,

namely: selenocysteine-specific $tRNA^{Ser(Sec)}$, specific for eukaryotes elongation factor eEFsec and the SECIS-binding protein SBP2 (SECIS binding protein 2) (Bianco et al. 2002).

D1 catalyzes primarily 5′-deiodination of T_4 and other iodothyronines, and its activity leads to the release of the main portion of T_3 into the blood. D1 is widely expressed in tissues, with the highest levels in the liver, kidneys and thyroid gland (Leonard 1990; Köhrle et al. 2005). Consequently, both the liver and kidney are the most important organs of extrathyroidal T_3 formation.

D2 specifically catalyzes deiodination of the outer ring of T_4, without showing an affinity for T_3 (Leonard 1990). The D2 enzyme is primarily responsible for the local T_3 production in target cells, and its activity is regulated by ubiquitination/deubiquitination mechanism (Bianco 2011). Expression of D2 has been found in the cerebral cortex, anterior pituitary, placenta, brown adipose tissue, thyroid gland, cardiac and skeletal muscles, skeleton, skin and haemo- and lymphopoiesis systems (Croteau et al. 1996; Soutto et al. 1998; Babych et al. 1999, 2000a, b; Antonyak et al. 2002; Bianco 2011).

The D3 enzyme, which is expressed in brain, placenta, skin and some other tissues, catalyzes T_4 conversion to rT_3 and deiodination of T_3 to 3,3′-diiodotyronine (3,3′-T_2) (Leonard 1990; Huang 2005). The physiological role of D3 is considered as the inactivation of excess thyroxine and T_3 by converting them to the inert compounds. High levels of 5-deiodination activity are found in embryonic tissues.

Alterations in the thyroid hormone deiodination system are observed in the ontogenesis of humans and animals, during pregnancy and other physiological states and in diseases (Bianco 2011; Antonyak and Vlizlo 2013). A range of studies have shown a correlation between the expression of 5′-deiodinases and cell differentiation; the changes in the deiodinase expression have been also detected in malignant cells (Schreck et al. 1994; Babych et al. 1998, 1999; Gouveia et al. 2005; Huang 2009; Miro et al. 2017).

10.7 Biological Effects of Thyroid Hormones in the Organism

Most functions of iodine in mammals are mediated by the effects of thyroid hormones, known as potent bioregulators of metabolic processes. Thyroid hormones are involved in maintaining the basic metabolism and regulation of cell proliferation, differentiation and apoptosis (Felig et al. 1995; Hulbert 2000; Babych et al. 2000c; Tata 2011; Mullur et al. 2014; Deng et al. 2017). These hormones are involved in the regulation of growth and development of the organism, tissue regeneration, maturation and maintenance of proper functioning of organ systems (Forhead and Fowden 2014). Their importance is proved for the maturation of the central nervous system (Calza et al. 2015; Bernal 2017), regulation of functions of cardiovascular (Klein and Danzi 2016), musculoskeletal (Anwar and Gibofsky 2010), gastrointestinal (Sirakov and

Plateroti 2011; Brown et al. 2013), reproductive (Krassas et al. 2010) systems. Thyroid hormones also participate in the regulation of haematopoiesis, immune function, respiratory function and blood cell metabolism (Sukhomlinov et al. 1986; Snitynsky and Antonyak 1995; Antoniak 1999; Babych et al. 2000c, d, e; Jara et al. 2017).

Although thyroxine is the main thyroid hormone secreted by the thyroid gland, it is considered a prohormone of T_3, which is able to regulate gene expression. The T_3 was demonstrated to be present in human plasma by J. Gross and R. Pitt-Rivers in 1952, and its role in the regulation of transcription process was shown in the 1960s by J. R. Tata and colleagues (Tata 2011). A range of vitally important genes were shown to be directly activated by T_3 at the transcriptional level (Huang et al. 2008). The T_3-target genes include the genes of structural and regulatory proteins, those that participate in metabolic processes, detoxification, transduction of regulatory signals, adhesion and cell migration. In addition, a number of transcription factors and cell cycle regulators are activated by T_3 (Tarım 2011). These effects of the thyroid hormone largely mediate the influence of iodine on metabolic processes in human and vertebrate animals.

Thyroid hormone nuclear receptors (TRs) are non-histone proteins acting as ligand-dependent transcriptional activators by binding to the response-elements (TRE) in promoter regions of target genes (Zhang and Lazar 2000; Antoniak et al. 2000; Boelen et al. 2012). TR expression is determined by *TRα* and *TRβ* genes, located in humans on chromosomes 17 and 3, respectively (Yen 2001). Genomic effects of thyroid hormone are mediated by TR receptor isoforms α1, β1 and β2 that regulate target genes in the presence or absence of T_3 by involving the co-regulatory complexes (nuclear corepressors and coactivators) (Boelen et al. 2012). The TRs often act as homodimers, but also as heterodimers with the retinoid-X receptor. The TRα1 and TRβ1 are widely expressed in tissues, with high level of TRα1 expression in the cardiac and skeletal muscles and TRβ1 as the main TR isoform in the liver. Expression of TRβ2 is restricted to the anterior pituitary gland, hypothalamus, the developing brain and the inner ear (Yen 2001).

Iodothyronines can act also through non-genomic mechanisms that are not initiated by liganding of T_3 to nuclear receptors (Davis et al. 2016). These effects may be initiated by interaction of iodothyronines with the binding sites located in the plasma membrane or in cytoplasm. Plasma membrane-initiated actions begin at a receptor on the integrin αvβ3 that activates mitogen-activated protein kinase (MAPK) cascade. The T_3 can also activate phosphatidylinositol 3-kinase (PI3K) pathway by a mechanism that may be cytoplasmic in origin or may begin at integrin αvβ3 (Cheng et al. 2010). These mechanisms can potentially influence gene expression. In addition, iodothyronines have effects on mitochondrial energetics by modulating the basal proton leak in mitochondria that accounts for heat production and cellular oxygen consumption (Felig et al. 1995). Thyroid hormone can also act on the mitochondrial genome via imported isoforms of nuclear TRs to affect several mitochondrial transcription factors (Cheng et al. 2010).

10.8 Iodine in Human Nutrition

10.8.1 Recommended Norms for Iodine Intake

Inadequate intake of iodine is associated with a broad spectrum of thyroid disorders related to both the deficiency and excess iodine in the organism. To prevent the development of adverse effects, especially those associated with insufficient iodine intake, and to maintain proper thyroid function, optimal levels of iodine intake have been established by international health agencies for different population subgroups. According to the recommendations of the World Health Organization (WHO) based on the propositions of the United Nation Children's Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), the daily intake of iodine should be as follows: 90 μg for preschool children (0–59 months); 120 μg for schoolchildren (6–12 years); 150 μg for adolescents (above 12 years) and adults; 250 μg for pregnant and lactating women (WHO 2007). When assessing the level of iodine intake using the urinary iodine concentration as an indicator, iodine consumption can be considered adequate when the UIC is 100–199 μg/L in the general population and 150–249 μg/L in pregnant women (WHO 2007).

Several studies recommend a higher daily intake of iodine in pregnant and lactating women: 250–300 and 225–350 μg, respectively, because of significant increase in iodine requirements in women during pregnancy and lactation and the positive effects of this element on the development of the child in prenatal period and in infancy (Delange 2004, 2007). In particular, need for iodine increases by ≥ 50% during pregnancy (Zimmermann 2016) due to several factors: increased demand for T_4 to maintain normal metabolism in the mother; transfer of T_4 and iodide from mother to foetus; increased loss of iodide through the kidneys (Delange 2004). Since part of the maternal iodine is taken up by the growing foetus and placenta, a decrease in the amount of absorbed iodine, available to the mother, is more obvious in the second half of pregnancy (Verger et al. 2001).

10.8.2 Sources of Iodine and Levels of Iodine Intake

The estimated daily iodine intake in residents of different countries varies widely, from < 50 μg in the population of iodine deficiency areas to 500 μg and above in people who regularly consume marine foods (WHO 2004; Zava and Zava 2011; Abt et al. 2016). In several areas of Africa, Asia, Latin America, and parts of Europe, iodine consumption is insufficient and varies from 20 to 80 μg per day, while dietary intake of iodine by residents of Japan, the USA and Canada is higher compared to the population of other countries (WHO 2004). Recent findings based on iodine concentration in food samples suggest that the mean daily iodine intake for the total USA population is 216.4 μg per person (Abt et al. 2016). In contrast, iodine intake

by the population of Japan is 1–3 mg/day, but in some cases this index is significantly higher (up to 10 mg/day and above) (Zava and Zava 2011).

Food analysis shows that iodine levels in the main food groups range as follows (data indicate geometric mean for each group): marine fish (1294.6 μg/kg) > freshwater fish (102.8 μg/kg) > leafy vegetables (88.8 μg/kg) > milk and dairy products (83.9 μg/kg) > other vegetables (80.1 μg/kg) > meat (68.4 μg/kg) > cereals (56.0 μg/kg) > fresh fruits (30.6 μg/kg) > bread (18.3 μg/kg) > water (6.4 μg/L) (Fordyce 2003). In addition, iodine concentrations in edible marine invertebrate species vary from 308 μg/kg in crab tissues to 1300–1400 μg/kg in shrimp and mussels (Wayne et al. 1964), while commercially available seaweed products contain iodine averagely 16–1540 mg/kg dry weight (up to 8.17 g/kg in kelp granules made of dried algae) (Teas et al. 2004). Consequently, marine algae, fish and shellfish are the most potent natural source of iodine in human nutrition. These foods are traditionally consumed by the population in Asian countries, with seaweed accounting for 10–25% of people's diet in Japan (Yuan and Walsh 2006).

In addition to marine foods, milk and dairy products, being particularly important sources of iodine for children and pregnant women, also make a significant contribution to iodine intake by population. In some countries milk has been shown to be the principal source of dietary iodine. Contribution of milk and dairy products to the total level of iodine consumption comprises about 50% in the USA, Canada and Europe (Fordyce 2003). Iodine concentration in cow's milk was shown to vary from 50–130 μg/L to considerably higher levels (up to 2000 μg/L), depending on the stage of lactation, seasonality and composition of cattle feeds (Fordyce 2003; Flachowsky et al. 2014).

10.8.3 Inhibitors of Iodine Uptake

Regardless of iodine levels in foods, the absorption of this micronutrient in the gastrointestinal tract and in the thyroid depends on food composition and on the presence of various xenobiotics that can get into agricultural products as a result of environmental pollution (Miller et al. 2009). NIS inhibitors such as perchlorate and thiocyanate present in food can interfere with iodide uptake in the intestine, thyroid and lactating mammary gland and reduce its content in breast milk, creating a risk of iodine deficiency in infants (Pearce et al. 2007). Excess iodide inhibits iodide absorption in the intestine and in the thyroid gland (Nicola et al. 2015).

Plant secondary metabolites, such as glucosinolates and cyanogenic glycosides, can affect iodine uptake, since degradation and detoxification of these compounds in the digestive tract lead to the formation of thiocyanate (Gaitan 1990). These compounds are referred to as goitrogens, as if consumed in high amounts, can contribute to development of goitre and hypothyroidism. Several flavonoids present in plants (including soy isoflavones) also have a goitrogenic potential due to the inhibition of the iodide uptake and thyroperoxidase activity (Gonçalves et al. 2017).

10.9 Consequences of Inadequate Iodine Intake

10.9.1 Iodine Deficiency Disorders

In conditions of unbalanced nutrition of people living in areas with low content of iodine in soils (areas remote from the marine environment, mountain areas, etc.), the amount of iodine entering the body can be less than optimal demand. Long-term deficiency of iodine in the organism leads to a decrease in the synthesis of thyroid hormones and is associated with a range of metabolic and functional disorders that result from thyroid dysfunction. These include goitre, hypothyroidism, and impaired growth and development in children (Zimmermann 2009). Furthermore, the iodine deficiency increases pregnancy loss, foetal development anomalies and infant mortality and is the leading preventable cause of mental deficiency in childhood (WHO 2007; Zimmermann 2007). These disease states are generally referred to as "iodine deficiency disorders" (IDD) (Hetzel 1983; WHO 1994).

As is known, thyroid function is controlled by the dynamic interrelationships between the hypothalamus, the pituitary gland and the thyroid. Under physiological conditions, the function of the thyroid gland is tightly controlled by TSH, whose secretion typically increases when iodine intake declines below the 100 μg/day (Zimmermann 2009). TSH stimulates the thyroidal iodide uptake from the blood and its recycling within the thyroid, and increases the efficiency of thyroid hormone production by up-regulating the genes of NIS and components of the hormone synthesis system. However, this mechanism can fail in conditions when the supply of iodine is chronically too low to maintain an adequate function of the thyroid gland. A very low level of iodine intake during a certain period can reduce thyroid hormone production even in the presence of elevated TSH levels. Consequently, depending on the level of decrease in iodine intake and the duration of iodine deficiency, there may be different degrees of thyroid dysfunction. Based on the UIC measurement, the degree of iodine deficiency in the population is classified as mild (UIC 50–99 μg/L), moderate (UIC 20–49 μg/L) or severe (UIC < 20 μg/L) (WHO 2007).

In conditions of long-term iodine intake in amounts below the 50 μg/day, the stimulating effect of elevated serum TSH levels on thyrocytes leads to the enlargement of the thyroid gland, known as goitre. Goitre is usually the earliest clinical sign of iodine deficiency, and can be regarded as an adaptive disease that develops in response to an insufficient supply of dietary iodine (Stanbury et al. 1954). Goitre is 20–30 times more common in women than in men, and is most commonly observed in young girls at the age of puberty. A more severe iodine deficiency (in conditions of daily iodide intake below 10–20 μg) can lead to hypothyroidism (insufficient production of thyroid hormones). Although many people with goitre have normal thyroid hormone levels, studies have shown that more than 30% of the persons in endemic areas are hypothyroid, despite the enlargement of their thyroid gland (Kapil 2007).

Being the most common manifestation of iodine deficiency, endemic goitre has for centuries prevailed in the inhabitants of the mountainous regions, such as the

Himalayas, Alps, and Andes and in areas with frequent flooding. In addition to suffering from goitre, a certain proportion of the population in these regions was affected by endemic cretinism, manifested by mental deficiency, neurological disorders, short stature and often deafness. The relationship between iodine scarcity in environmental components and the development of endemic goitre and cretinism was established by G. A. Chatin in the 1850s (Chatin 1853).

Iodine deficiency can arise in the organism at any age, but children, pregnant women and lactating mothers are the most vulnerable groups for IDD (WHO 2007; Delange 2007; Zimmermann 2016; Nyström et al. 2016). Severe iodine deficiency in pregnant women can lead to the pregnancy loss, stillbirth and perinatal mortality, and adversely affects the development of a child (WHO 2007). This especially refers to the period before the onset of foetal thyroid function (the second trimester of pregnancy), when human foetus is entirely dependent on the maternal thyroid hormone. Subsequently, after the foetal thyroid gland begins to function, maternal iodine is necessary for the production of foetal thyroid hormones involved in the regulation of development and maturation of the brain, musculoskeletal, respiratory, auditory and other systems of the foetus (Forhead and Fowden 2014).

Daily iodide intake below about 10–20 μg in pregnant women can cause maternal and foetal hypothyroidism and result in major neurodevelopmental deficits and goitre in their offspring. The most severe consequence of iodine shortage during the prenatal period is cretinism, accompanied by an intellectual disability that arises primarily as a result of irreversible brain damage caused by a deficiency of thyroid hormones (Zimmermann 2016). The lesser degrees of iodine deficiency in pregnant and lactating women can also cause thyroid dysfunction and potentially affect neurodevelopment in infants and children with long-term adverse consequences. A study, performed in Australia has shown that even mild iodine deficiency in pregnant women was associated with lower education outcomes in their children aged 9 years (Hynes et al. 2013). The authors have concluded that the adverse impacts of maternal mild iodine deficiency on foetal neurocognitive development are not ameliorated by iodine sufficiency during childhood.

Being especially required by infants and their mothers, iodine is an important micronutrient for growth, physical and mental development of preschool and school-age children. Iodine deficiency in children is characteristically associated with goitre. Goitre rate increases with children's age and reaches a maximum in adolescence. Inadequate iodine intake during these periods of life can lead to retarded physical development, delayed puberty, weight gain, slower growth and decreased intelligence with a decline in cognitive function, including memory and thinking skills. The observational studies have reported differences in intelligence quotient (IQ) between groups of children living in iodine-sufficient areas and those from the areas of iodine deficiency (Bleichrodt and Born 1994; Qian et al. 2005). In particular, meta-analysis made in China has shown a 12–13 point lower IQ score in children of lower iodine status (Qian et al. 2005). A decrease of 6.9–10.2 IQ points is observed in iodine-deficient children of 5 years old and under in comparison with iodine replete children (Bougma et al. 2013). The lowering of intelligence due to iodine shortage affects the educational potential of children. According to Wolka et al. (2013), children with

goitre had a 1.8 times greater odds of having a below-average academic achievement than children who did not have goitre (Wolka et al. 2013). Similar negative effect of iodine deficiency on the mental performance of schoolchildren has been revealed in other studies (Pineda-Lucatero et al. 2008). The adverse health consequences of iodine deficiency in the periods of childhood and adolescence can lead to reduction in both productivity and intellectual potential in adulthood.

Adequate iodine intake is necessary for health also in adult age, and iodine status is a key determinant of thyroid disorders in adults. Severe iodine deficiency in adult persons causes goitre and its complications, hypothyroidism, endemic mental deficiency, and decreased fertility rate. In conditions of mild and moderate iodine deficiency, increased thyroid activity can compensate for low iodine intake and maintain euthyroidal state in most persons, however, long-term thyroid stimulation results in an increase in the prevalence of toxic nodular goitre and hyperthyroidism in populations (Zimmermann and Boelaert 2015).

10.9.2 Hypothyroidism

Lack of iodine, leading to inadequate synthesis of thyroid hormones, is one of the common causes of hypothyroidism, which is defined as a complex of clinical symptoms caused by impaired thyroid function or insufficient action of thyroid hormones in target tissues (Almandoz and Gharib 2012). Apart to the conditions of iodine deficiency, hypothyroidism can also be caused by a disruption in the ability of the thyroid gland to absorb and organify iodide as a result of congenital defects in the expression of NIS and components of the hormone synthesis system; the harmful effects of alimentary factors, medications and environmental contaminants; because of the destructive treatment of thyrotoxicosis or due to a chronic autoimmune disease. In addition, rare cases of hypothyroidism can result from hypothalamic or pituitary dysfunction (Persani and Bonomi 2017).

The initial stage of hypothyroidism is latent (subclinical) hypothyroidism, which is defined biochemically by an elevated serum TSH concentration (above of 4.0 mU/L) and normal serum free thyroxine (fT_4) level (Almandoz and Gharib 2012; Schübel et al. 2017). Subclinical hypothyroidism often occurs asymptomatically, but nearly 30% of patients with this condition may have symptoms that are suggestive of thyroid hormone deficiency. According to epidemiologic studies, subclinical hypothyroidism occurs in 3–10% of the general population and is more common in women than in men (Schübel et al. 2017). Latent hypothyroidism can progress to overt hypothyroidism from 2–5% (Khandelwal and Tandon 2012) to about 18% of affected patients per year (Parle et al. 1991). Overt hypothyroidism is defined as a combination of low fT_4 and high TSH concentrations (Bensenor et al. 2012). In general, hypothyroidism affects 3–8% of men and 5–20% of women, and occurs most frequently in older women (Laurberg et al. 2005; Bensenor et al. 2012).

Hypothyroidism, in particular its subclinical form, is the most common pregnancy-related thyroid disorder, affecting 3–5% of all pregnant women, and is associated with

a higher risk of pregnancy loss, placental abruption, premature rupture of membranes, and neonatal mortality (Maraka et al. 2016). High TSH levels in pregnant women have been also associated with an increased risk of developing neurocognitive deficits in offspring.

10.9.3 Tumour-Promoting Effects of Iodine Deficiency

The prevalence of goitre and thyroid nodules is known to be higher in populations living in areas with iodine deficiency (Carlé et al. 2014), and in many cases goitre and nodularity precede the development of thyroid cancer. The relationship between iodine deficiency and thyroid cancer incidence was demonstrated in the 1920s by Carl Wegelin, who argued that thyroid cancer was more common in endemic goitre areas, with a frequency ranging from 1.04% in central Switzerland (the area of endemic goitre) to 0.09% in Berlin (non-endemic region) (Wegelin 1928). In animal studies, iodine deficiency induces thyroid tumours and promotes thyroid carcinogenesis under an influence of carcinogens, such as N-bis(2-hydroxypropyI)-nitrosamine (DHPN) or N-nitrosomethylurea (NMU) (Ohshima and Ward 1986; Zimmermann and Galetti 2015).

A number of studies suggest that iodine deficiency can have a causative role in the incidence of breast cancer (Stadel 1976; Aceves et al. 2013; Rappaport 2017) and increases the risk of endometrial, ovarian and stomach cancers (Stadel 1976; Gołkowski et al. 2007).

10.9.4 Prevention of Iodine Deficiency

It has been proven that most of metabolic abnormalities observed in the circumstances of iodine deficiency, are preventable and can be avoided by normalizing the iodine intake. The use of iodine to prevent iodine deficiency disorders began with the research of David Marine, who first used low doses of iodide to reduce the manifestations of goitre in schoolchildren in 1917 (Marine and Kimball 1917). Then the program of iodization of table salt was developed in the USA and Switzerland in the 1920s, and subsequently this practice was introduced in a number of countries. By the 1970s, it became apparent that iodine supplementation reduced the level of intellectual impairment and infant mortality rates (Hetzel 1983). In 1993, WHO and UNICEF recommended universal salt iodisation as the main strategy to achieve elimination of IDD in the world population (WHO 1994), with iodised salt containing iodine in an amount of 15–80 mg/kg. Since then, there has been significant progress in increasing the use of adequately iodised dietary salt worldwide, and as a result, many countries have achieved, or are now on the threshold of achieving IDD elimination. In 2015, around 100 countries had national iodized salt programs (Zimmermann and Galetti 2015).

However, despite the fact that IDD have been eliminated in most areas with severe iodine deficiency, many countries, including industrialized ones, still show a mild to moderate degree of iodine deficiency. The IDD remain a global public health problem, as approximately 1.9 billion people are at risk worldwide (Andersson et al. 2012). On the basis of current surveys performed in 152 countries, 29 are affected by iodine deficiency (Zimmermann and Boelaert 2015). Globally, 29.8% of school-age children (241 million) are estimated to have insufficient iodine intakes (Andersson et al. 2012), while in Europe this index comprises about 44% (Taylor et al. 2014). Many pregnant women in European and other countries are also at risk for IDD due to inadequate iodine intakes (Zimmermann 2007). Dietary deficiencies of the micronutrients (selenium, iron, and vitamin A) may interact with iodine deficiency and affect the response of iodine-deficient persons to iodine supplementation (Zimmermann 2009).

To date, various strategies have been developed in order to increase iodine content in people's diet and in the foodstuffs. Biofortification of food plants with iodine is considered as an effective method of iodine supply to the population and a novel strategy for the prevention of iodine deficiency in humans (Gonzali et al. 2017). The use of iodine supplements for feeding livestock and poultry remains an important way of increasing iodine concentration in dairy products and in eggs (Flachowsky et al. 2014). One way to increase iodine intake in a population is the producing of iodine-enriched food products such as bread, sweets, etc. Consumption of foods of marine origin is also important for increasing the level of iodine intake; however, a high level of consumption of iodine-rich foods, mainly seaweed, can adversely affect people with thyroid disorders.

10.9.5 Excessive Iodine Intake

Excessive iodine intake in humans occurs less often than iodine deficiency, since common diets consisting of natural foods (with the exception of marine products) usually contain less than 1 mg of iodine per day. However, such a situation can arise when the diet contains a large amount of marine fish or seaweed or when a person takes medications containing iodine such as amiodarone and diagnostic contrast agents. In particular, taking an antiarrhythmic medication amiodarone leads to an additional intake of 3–21 mg of iodine daily (Aceves et al. 2013). Intake of excess iodine can also occur when, in the context of iodine prophylaxis, salt iodisation is excessive and poorly controlled (WHO 2007).

There are a variety of data relating to people's tolerance to different doses of iodine. While some studies show that consumption of iodine at a dose of 2 mg per day should be considered harmful to humans (Wolff 1969), there are many studies suggesting that low and intermediate doses of iodine (1.5–8 and 10–32 mg/day, respectively) are well tolerated in euthyroid persons (Backer and Hollowell 2000; Bürgi 2010). High tolerance to iodine is observed in all animal species studied, pointing to a wide margin of safety for this element (WHO 1996).

On the other hand, long-term consumption of excess iodine from medical sources, foods or food additives with exceptionally high iodine content has been associated with the development of thyroid autoantibodies and may lead to alterations in thyroid function, including autoimmune thyroiditis, goitre, hyperthyroidism and hypothyroidism (Luo et al. 2014; Foppiani et al. 2016). Therefore, it is important to take into account the maximum safe level of iodine intake, especially for population subgroups at risk of developing thyroid dysfunction.

The ICCIDD has proposed that 150–299 μg iodine per day is adequate to cover the thyroid requirement, and the RDA (Recommended Dietary Allowances) suggested the maximal allowable dietary dose of iodine 1.0 mg/day for children and 2.0 mg/day for adults (Aceves et al. 2013). According to recommendation of health agencies, a tolerable upper level (the approximate threshold below which notable adverse effects are unlikely to occur in the healthy population) of iodine intake is of 1100 μg per day in adults (Leung and Braverman 2014). In Japan, a safe upper limit of iodine intake was set by the Ministry of Health, Labor and Welfare at a level of 3 mg/day (Zava and Zava 2011). When estimating iodine intake in the population, WHO recommends that UIC values ≥ 300 μg/L and ≥ 500 μg/L be considered as indicative of excessive iodine intake in children and adults, and in pregnant women, respectively (WHO 2007).

10.9.6 Effects of Excessive Iodine Intake

The organism of healthy persons possesses mechanisms of adaptation to the conditions of excess iodine, and the majority of people tolerate a wide range of dietary iodine levels. When iodide is consumed in large amounts, its absorption in the gastrointestinal tract can be reduced due to inhibition of NIS in the cells of the small intestinal mucosa (Nicola et al. 2015). At the level of the thyroid gland, the excess iodide can inhibit the production of thyroid hormones (the Wolff–Chaikoff effect) by affecting each step leading to their secretion, namely: iodide uptake, iodide organification and secretory process itself (Ferreira et al. 2005), but the rate-limiting step for these effects depends on thyroperoxidase activity. The inhibition is usually transient (lasting 24–48 h) and is followed by normalization of thyroid hormone production (the "escape" from the Wolff–Chaikoff effect). The transient inhibition of thyroid hormone production is an autoregulatory mechanism that shields organism against hyperthyroidism in conditions of abundant iodine supply, while the resumption of thyroid function provides the maintenance of euthyroid state. Mechanism for the acute Wolff–Chaikoff effect is considered to be mediated by generation of intrathyroidal iodolipid compounds (iodolactones, iodoaldehydes) and their inhibitory effects on iodide organification catalyzed by thyroperoxidase (Pramyothin et al. 2011).

Unlike to healthy individuals, the persons with thyroid disorders might fail to "escape" from the Wolff-Chaikoff effect that can lead to iodine-induced hypothyroidism. Susceptible persons include patients with the autoimmune thyroid disease, thyroiditis, amiodarone-induced thyrotoxicosis of type 2; those with a previous

surgery, treatment with radioiodine or antithyroid drugs for Graves' disease; patients treated with interferon-α, and persons concomitantly using the potential goitrogens (Leung and Braverman 2014). Exposure to high concentrations of iodide in healthy persons may also lead to a decrease in production of thyroid hormones that is accompanied by a mild increase in the serum TSH level (often to the upper limit of the normal range) (Pramyothin et al. 2011). Hypothyroid state has been also observed in infants born to mothers who consumed excessive amounts of seaweed during pregnancy and lactation (Shumer et al. 2013). There have been reports of several cases of congenital hypothyroidism in newborns caused by maternal intake of excess iodine tablets during pregnancy (Connelly et al. 2012).

In some susceptible persons, excess iodine leads to hyperthyroidism, that is, excess thyroid hormones in the circulation because of their increased production by a hyperactive thyroid gland. The elevated levels of thyroid hormones suppress TSH secretion from the pituitary in a negative feedback loop (Sharma et al. 2011). Iodine-induced hyperthyroidism, also known as the Jod–Basedow phenomenon, was first described in the early 1800s in the context of the treatment the endemic goitre. It has been observed that in patients with goitre treated with iodine, thyrotoxicosis develops more often than in persons without goitre (Coindet 1821). This type of hyperthyroidism is much more common in iodine-deficient areas than in areas where the diet contains sufficient amount of iodine. The risk factors for iodine-induced hyperthyroidism include long standing iodine deficiency, latent Graves' disease and nontoxic or diffuse nodular goitre (Leung and Braverman 2014). Iodine-induced hyperthyroidism has also been observed in euthyroid patients with nodular goitre in iodine-sufficient areas in conditions of excessive iodine supplementation. In general, hyperthyroidism is found in about 2% of women and 0.2% of men (Pearce 2006). Subclinical hyperthyroidism, defined as low serum TSH in the presence of normal thyroid hormone levels, affects about 3% of the population.

Besides iodine-induced alteration in thyroid function, excessive iodine intake also affects other aspects of thyroid health. High levels of iodine intake can increase the prevalence of autoimmune thyroiditis, as shown in animal model and in humans (Kahaly et al. 1998; Harach and Ceballos 2008; Leung and Braverman 2014). Autoimmune thyroiditis is more common in iodine-replete areas compared with areas of iodine deficiency, and its incidence increase after iodine prophylaxis both in non-goitrous and iodine-deficient areas (Harach and Ceballos 2008). In particular, a study conducted by Kahaly et al. (1998) has shown that high microsomal and thyroglobulin autoantibodies titres with marked lymphocyte infiltration in the thyroid gland were present in six of 31 goitrous patients (19%) who received iodine at a dose of 0.5 mg/day for 6 months; while iodine-induced hypo- and hyperthyroidism developed in four and two of them, respectively.

10.10 The Extrathyronine Effects of Iodine in the Organism

Apart from the participation in the structure of thyroid hormones, iodine can have some additional functions in human organism that are not mediated by iodothyronines. A number of studies have shown the antioxidant effects of iodine, its effects on cell proliferation and promotion of apoptosis, induction of cell differentiation, protective effects against some types of cancer, anti-inflammatory effects, and possible participation in mechanism of cell mediated immunity (Venturi 2011; Swietaszczyk and Pilecki 2012; Aceves et al. 2013; Venturi and Venturi 2014).

In many patho-physiological conditions, effects of iodine are mediated by iodolipid compounds, formed in the thyroid gland. In addition to the involvement of iodolipids in mediating the Wolff–Chaikoff effect, these compounds can mediate the inhibitory effects of excess iodide on thyroid cell proliferation and modulate the signals of TSH and cytokines (Gaertner 2009; Soriguer et al. 2011; Swietaszczyk and Pilecki 2012; Rossich et al. 2016). In particular, 2-IHDA has a suppressive effect on adenylyl cyclase, which participates in cAMP formation, whereas 6-iodo-5-hydroxyeicosatrienoic acid δ-iodolactone (6-IL) can inhibit calcium-dependent protein kinase C and signaling pathways induced by local growth factors. Iodolipid compounds also show antiproliferative effects and trigger apoptosis in thyrocytes and some other types of cells (Swietaszczyk and Pilecki 2012; Rossich et al. 2016).

10.10.1 Antioxidant Function of Iodide

Iodine can act both as the oxidizing and reducing agent, depending on its chemical form and surrounding milieu. In the thyroid, iodide oxidation underlies the processes of tyrosine iodination and the formation of thyroid hormones. At the same time, iodide, by releasing electrons, is a reducing agent and, hence, can perform an antioxidant function. As electron donor, iodide can quench $^{\cdot}OH$–radical and H_2O_2; however, iodide can also act as a free radical capable of iodinating tyrosine, histidine and some polyunsaturated fatty acids in cell membranes making them less reactive with oxygen radicals (Smyth 2003; Aceves et al. 2013).

Together with peroxidases that transfer electrons to hydrogen peroxide, iodide can contribute to H_2O_2 detoxification and reduce cell damage (Venturi 2011). Iodide has been suggested to have an antioxidant function in the thyroid, mammary gland, stomach and salivary glands, where it is accumulated (Venturi et al. 2000; Smyth 2003; Venturi and Venturi 2014), while insufficient iodide intake leads to accumulation of malondialdehyde and other products of lipid peroxidation. In addition, iodide increase the total antioxidant status in human serum (Soriguer et al. 2011), and protect brain cells from lipid peroxidation (Venturi and Venturi 2014).

Some authors hypothesize that iodide together with peroxidases plays an antioxidant role in all organisms containing cells capable of concentrating iodine—from

algae to vertebrates and can be considered the most ancient antioxidant in the evolutionary sense (Venturi et al. 2000; Venturi and Venturi 2014).

10.10.2 Antitumor Effects of Iodine

It has been demonstrated that iodine exhibits an antiproliferative/cytotoxic effect on a number of malignant cells and has a protective role in several types of cancer, including thyroid, breast and stomach cancer (Venturi et al. 2000; Brown et al. 2013; Rösner et al. 2016; Rappaport 2017). Since the 1920s, there are a number of studies suggesting a lower risk of developing thyroid cancer with a higher level of iodine intake (Zimmermann and Galetti 2015). At the same time, a moderately elevated level of iodine consumption can contribute to reducing risk of several extrathyroidal cancers. In animal and human studies, iodine administration has been shown to cause regression of both iodine-deficient goitre and benign pathological breast tissue (Cann et al. 2000). The evidence of the antineoplastic effect of iodine in extrathyroidal tissues was obtained in the 1990s (Kato et al. 1994). Subsequently, it has been found that molecular iodine, which can be taken by cells through a pathway not mediated by NIS, reduces tissue neoplasia much more effectively than iodide. Clinical data and animal studies suggest that I_2 has a suppressive effect on the development of both benign and cancerous pathologies in mammary and prostate glands and is less thyrotoxic than iodide (Anguiano et al. 2007; Aceves et al. 2013). Dose-response studies in humans have shown that iodine at concentrations of 3, 5, and 6 mg/day, mainly in the form of I_2, has significant beneficial actions in benign pathologies (mastalgia or prostatic hyperplasia) and exhibits antineoplastic effects in early and advanced breast cancer, without the side effects of these doses (Ghent et al. 1993; Kessler 2009; Anguiano et al. 2010; Aceves et al. 2013). Higher concentrations of iodine (9 and 12 mg/day) resulted in the same benefits, but caused transient hypothyroidism in 20% of the studied patients with minor side effects that disappeared when the high iodine supplementation was stopped (Kessler 2009).

The antitumor effects of iodine can be mediated by different mechanisms, including direct actions in which oxidized iodine dissipates the potential of the mitochondrial membrane, thus inducing mitochondria-mediated apoptosis, and indirect effects through the formation of iodolipid compounds (Aceves et al. 2013). Iodolipids can alter both expression and action of peroxisome proliferator-activated receptors (PPARs) type gamma that, in turn, trigger apoptotic or differentiation pathways (Nuñez-Anita et al. 2009; Aceves et al. 2013).

10.10.3 Anti-Inflammatory Effects of Iodine

The available data suggest that iodine has an anti-inflammatory effect (Soriguer et al. 2011), which can be mediated, in part, by its ability to neutralize the reactive oxygen

species. Molecular iodine has been also shown to inhibit the NO generation and expression of TNF-α in monocytes/macrophages (Moore et al. 1997).

10.11 Beneficial Effects of Iodine in Human Health and Longevity

Iodine is essential for health at every stage of human life mainly because of its indispensable role in the production of thyroid hormones that control the key metabolic processes in the organism. In addition, iodine is involved in the processes of thyroid autoregulation and can have some functions in human body that are not mediated by iodothyronines (Fig. 10.1). As above mentioned, iodine in the thyroid gland can participate in formation of iodolipids that exhibit regulatory and antiproliferative effects; such compounds may also play a role in the proliferative control of extrathyroidal tissues. However, with the exception of the effects of iodine as a component of thyroid hormones, other mechanisms of iodine regulatory action have not been extensively studied. The available data on the long lifespan of people who traditionally consume iodine in moderately high amounts (for example, the population of Japan) indicate the important role of this micronutrient in human health, but it is difficult to analyze the role of iodine in longevity, because of physiological changes in thyroid function with age.

It can be considered that the most significant effects of iodine as a component of iodothyronines are manifested in the early period of development of human organism (prenatal period, infancy and childhood). The essentiality of iodine at these ontogenetic stages is proved by data on the improvement of foetal and neonatal neurodevelopment and the reduction of infant mortality after iodine prophylaxis in pregnant women. In particular, iodine supplementation before or during early pregnancy increases birth weight, reduces perinatal and infant mortality rates, eliminates the cases of cretinism and generally increases developmental scores in young children by 10–20% (Zimmermann 2016). Improvement of neurocognitive function in infants and children after iodine supplementation in pregnant and lactating mothers is associated with the indispensability of thyroid hormones for normal neuronal migration and myelination of the brain during foetal and early postnatal life. Iodine supplementation in moderately iodine-deficient children improves their mental performance due to the effect of iodine on cognition and increases their motor function and somatic growth (Zimmermann 2007).

Iodine also has an important role in the mental and physical health of adult and older persons. Adequate iodine intake is necessary for the proper functioning of the central nervous system, cardiovascular, gastrointestinal, immune, hematopoietic and endocrine systems throughout life. The available data show that adult population living in areas with iodine deficiency are characterized by a high degree of apathy, low educability, poor performance, lack of physical energy and reduced work output that contribute to poor quality of life (Ahad and Ganie 2010). Insufficient formation of

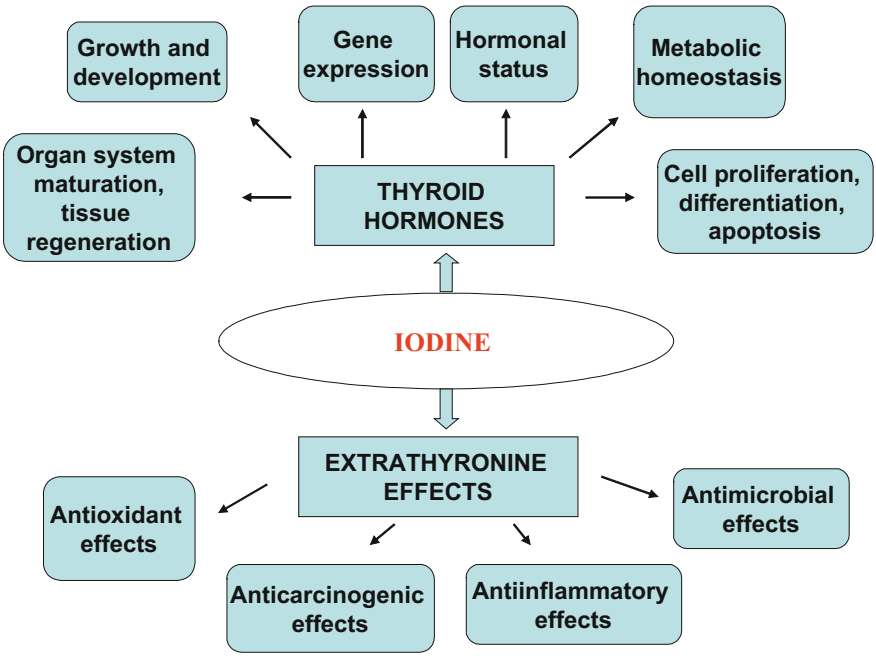

Fig. 10.1 Effects of iodine, associated with its beneficial influence on health

thyroid hormones, particularly in older persons, is often accompanied by hypertension, cardiac insufficiency, adverse lipid profile, insulin resistance, and endothelial dysfunction (Stabouli et al. 2010; Sara et al. 2015). These metabolic alterations create an increased risk of atherosclerosis, cardiovascular diseases, diabetes, cognitive impairment and depression. Thus, iodine is important for the prevention of disorders that arise mainly in adult and older age and are linked to higher mortality in older persons.

There are experimental and clinical data suggesting the association of hypothyroidism with obesity (Knudsen et al. 2005; Longhi and Radetti 2013). This is due to involvement of thyroid hormones in the regulation of basal metabolism and thermogenesis, lipid and carbohydrate metabolism and fat oxidation. Overweight is often accompanied by elevated TSH level (Laurberg et al. 2012). In addition, there is an inverse correlation between free thyroxine values and body mass index, even when fT_4 values remain in the normal range (Knudsen et al. 2005). Obesity, in turn, is associated with the presence of insulin resistance (Cali and Caprio 2008), contributes to the pathogenesis of diabetes and cardiovascular diseases, hence, increases the health risks and worsens the quality of life of people with iodine-deficiency.

Hypothyroidism, including its subclinical form, also has cognitive and neuropsychiatric consequences in adult and older people. In particular, subclinical hypothyroidism may be a predisposing factor for depression, cognitive impairment, and dementia (Davis et al. 2003; Resta et al. 2012; Joffe et al. 2013). Older adults,

particularly women, may be more vulnerable to the effects of subclinical hypothyroidism, given age-related changes to the hypothalamic-pituitary-thyroid axis, and some studies suggest the possible association between subclinical hypothyroid status and depression and cognitive decline in the elderly (Chuiere et al. 2007; Joffe et al. 2013). However, there are reports suggesting that conditions of hyperthyroidism can also lead to mental deficiency in older persons (Kalmijn et al. 2000). In particular, the findings of Kalmijn et al (2000) have shown that persons with subclinical hyperthyroidism, manifested by the reduced TSH levels, had a more than threefold increased risk of dementia and Alzheimer's disease.

It is suggested that an adequate intake of iodine is necessary to prevent several types of cancer, while iodine deficiency increases the risk of thyroid and extrathyroidal cancers (breast, endometrial, ovarian, and stomach cancers) (Stadel 1976; Venturi et al. 2000; Gołkowski et al. 2007; Aceves et al. 2013; Zimmermann and Galetti 2015; Rappaport 2017). Iodine supplementation has been shown to diminish tissue neoplasia and can ameliorate physiopathologies of several organs that take up iodine, primarily the thyroid, mammary, and prostate glands and potentially the pancreas and gastric system (Anguiano et al. 2007; Aceves et al. 2013). This especially refers to the molecular iodine, which, if ingested in milligram amounts, has a suppressive effect on both benign and cancer neoplasias (Aceves et al. 2013). Clinical and experimental data on the antitumor effects of iodine are consistent with epidemiological reports suggesting a direct association in the Japanese population between the low incidence of breast and prostate pathologies and moderately high dietary intake of iodine (Cann et al. 2000; Rappaport 2017).

The limits for iodine intake of 1–2 mg per day have been established by international agencies, given the fact that individuals with underlying thyroid pathologies can develop the hyper- or hypothyroidism if they are exposed to doses higher than 1.5 mg/day (Aceves et al. 2013). However, the available data suggest that only very high doses (>30 mg/day), mainly as iodide, consumed by humans lead to hypothyroidism and goitre, while low (1.5–8 mg/day) and intermediate doses (10–32 mg/day), ingested from various sources, are well-tolerated in euthyroid persons, maintaining levels of thyroid hormones and TSH within normal limits (Bürgi 2010; Aceves et al. 2013; Leung and Braverman 2014). It has been also shown that iodine may act as an antioxidant in the whole organism if this element is ingested at concentrations higher than 3 mg/day (Aceves et al. 2013).

In this aspect, the traditional diet of residents of Japan, which includes iodine-rich products of marine origin, attract considerable attention. Seaweed, which is widely consumed in Japan and other Asian countries, contains a large amount of iodine in the forms of iodide, I_2 and iodate, and the average iodine intake in the Japanese population is 1200–5280 μg/day (Cann et al. 2000; Teas et al. 2004; Zava and Zava 2011). At the same time, despite the high level of iodine consumption in food, Asian population does not differ from the rest of the world in the prevalence of thyroid disorders (Kamangar et al. 2006; Aceves et al. 2013). The studies, conducted in Japan, have shown that normal subjects can maintain normal thyroid function, even if they consume several milligrams per day of dietary iodine (about 30 mg/day); moreover,

the incidences of nontoxic diffuse goitre and toxic nodular goitre is markedly reduced with high dietary iodine (WHO 1996).

In Japan, the average life expectancy of the population is 83 years, that is, higher than in other countries, an extraordinarily low incidence of certain cancers, low infant mortality and a lower mortality rate from cardiovascular diseases compared to residents of other countries (WHO 2010). The main dietary difference that distinguishes Japan from other countries is high consumption of iodine, with seaweed the most common source (Zava and Zava 2011). Given the involvement of iodine in the metabolic processes that underlie the normal functioning of the cardiovascular system, cancer prevention, the maintenance of a healthy pregnancy and infant health, it can be assumed that statistic indices of Japanese people health may be related to the high level of iodine consumption.

On the other hand, the available data suggest that thyroid activity changes with age, but the complex manner of its changes makes a real challenge an understanding its role in aging, as well as the level of iodine required by organism in older age. However, there is common agreement around the fact that some kind of reduced thyroid function tend to associate with increased longevity in a number of species (Gesing et al. 2012; Jansen et al. 2015). Studies conducted in people of the older age suggest that maintaining an elevated TSH level and lower concentrations of thyroid hormones in the serum might be favourable in the oldest-old persons and is related to longevity. In particular, studies on thyroid disease-free population of Ashkenazi Jews, characterized by exceptional longevity (centenarians) have shown the higher serum TSH level in these individuals (median age, 98 year) in comparison to the control group consisted of younger Ashkenazi Jews (median age, 72 year) and to another control group (median age, 68 year) from the U.S. National Health and Nutrition Examination Survey (NHANES) program (Atzmon et al. 2009). The authors have observed an inverse correlation between fT4 and TSH levels in centenarians that may suggest a potential role of decreased thyroid function in lifespan regulation, leading to extended longevity. The association of higher TSH with familial longevity was shown in nonagenarians from the Leiden Longevity Study (Rozing et al. 2010). It has been also found that nonagenarians from families with the lowest family mortality history score had relatively lower levels of thyroid hormones (Rozing et al. 2010). In animal studies, a reduced thyroid function with low thyroid hormone levels also appears to be associated with extended longevity.

Although greater longevity has been associated with higher TSH and lower levels of thyroid hormones, mechanisms underlying TSH/TH differences and longevity remain unknown. The study conducted by Jansen et al. (2015) have shown that offspring of nonagenarians have increased TSH secretion but similar bioactivity of TSH and similar thyroid hormone levels compared to controls as well as similar resting metabolic rate and core body temperature. Hence it is possible that pleiotropic effects of the hypothalamic-pituitary-thyroid axis may favour longevity without altering energy metabolism (Jansen et al. 2015).

Taking into account the above mentioned data, the peculiarities of iodine metabolism in older age are of particular interest. However, to date, there are no particular recommendations on the level of iodine intake in the older people.

10.12 Conclusions

Chemical element iodine is unevenly distributed in an abiotic environment with a relatively low concentration in soils and terrestrial vegetation and a high level of bioconcentration in marine organisms. In humans and vertebrate animals, iodine is a vital micronutrient, required primarily for the processes of hormonogenesis in the thyroid gland. Being an indispensable component of thyroid hormones, iodine is involved in the regulation of growth and development of the organism, in maintaining the metabolic balance and in controlling the key intracellular processes underlying human health and longevity. Iodine is of crucial importance during the early stages of ontogenesis, since thyroid hormones participate in the regulation of neurodevelopment, maturation and formation of functions of organ systems. In periods of childhood and adolescence, iodine is necessary for growth, physical development and cognitive function. An adequate iodine status is also a key factor in the prevention of thyroid disorders in adulthood and is important for the maintaining proper functioning of the endocrine system, central nervous system, cardiovascular, gastrointestinal, haemopoietic and immune systems throughout life. Participation of iodine (as a component of thyroid hormone) in the control of gene expression, the mechanisms of cell proliferation, differentiation and apoptosis underlies its regulatory effects in human organism. Iodine deficiency in the organism at any stage of development is associated with a wide spectrum of metabolic and functional alterations secondary to an insufficient synthesis of thyroid hormones, and is often manifested by goitre. The severe iodine deficiency can lead to hypothyroidism, which is associated with profound changes in the hormonal status and adversely affects virtually all metabolic processes; moreover, it can contribute to the development of tissue malignancies.

In addition to the effects of iodine in the composition of thyroid hormones, this element also exhibits direct antioxidant and anti-inflammatory effects and can be involved in the prevention of benign an malignant tumours, including several types of thyroid and breast cancers. Therefore, adequate intake of iodine is necessary to maintain the hormonal balance and metabolic homeostasis and contributes to the prevention of thyroid dysfunction, metabolic disorders and tissue neoplasia. However, iodine deficiency is one of the most prevalent micronutrient deficiencies worldwide, and its elimination is still a public health problem in many countries.

Along with emphasizing the need for iodine for health and adequate thyroid function throughout life, it should be noted, that thyroid activity changes with age, and alterations in its function can be associated with increased longevity.

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Chapter 11
The Nadir Range of the U-Shaped Curve

Yosef Dror, Shmuel M. Giveon and Felicia Stern

Abstract Vitamins, micronutrients and electrolytes are essential components of optimal cell function. Reliable optimal range of their serum concentrations should be set according to the lowest relative risk (RR) of morbidity and mortality. Optimal concentrations and adjustment to nadir ranges might minimize the RRs of a long list of both non-communicable diseases (NCDs or chronic diseases) as well as communicable diseases, and consequently affect life expectancy and decrease disability. Efforts should be made to define optimal serum concentrations of these essential nutrients. The present dietary recommended intakes (DRIs) for the micronutrients are mostly based on data extracted from a "healthy population", and "normal" values are defined as a mean ±2 SD and not by the lowest morbidity/mortality. Definition according to the nadir ranges of serum concentrations of these nutrients, however, might better predict nutrient requirements than the outdated DRI methodology. Most metabolites have a narrow reliable nadir range for their optimal activity. The concept of the optimal nadir ranges is well demonstrated by examples of U-shaped curves presented here. Thus, optimal nutrient concentrations should be redefined by further studies, based on comprehensive data.

Keywords Nadir · U-shaped curve · Micronutrient · Electrolyte · Microelement Vitamin · Clinical endpoint · Non communicable diseases · NCD

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11.1 Introduction

Microelements, vitamins and some other micronutrients are involved in all metabolic pathways within every cell of the human body. Any metabolic process that is activated by micronutrients, requires a narrow concentration range for all of its metabolites to acquire optimal activity. Lower or higher concentration of any micronutrient reduces optimal activity of the metabolic cycle, eventually leading to a decrease in the capacity to support the immune system and to maintain optimal health status. All the micronutrients are derived from food and supplements; therefore, their serum concentration might be controlled by follow-up of their intake. The main micronutrients comprise microelements (iron, zinc, copper, fluorine, selenium, chromium, iodine, molybdenum), vitamins (A, D, E, K, C, B6, and B12, thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), biotin, and folic acid), methyl donors (choline and betaine), and other antioxidants (such as flavonoids, sterols, tocols, carotenes, ferulic acid and other phenols). Not all of the micronutrients are traditionally defined as essential. Nonetheless, for almost all of these nutrients, a nadir range might ensure the optimal activity of the metabolic cycle. The notion of the optimal concentration range for each compound in a biochemical reaction has been well established, but not for vitamins and microelements.

Serum concentration of many micronutrients often runs below the optimal range because of insufficient intake; concomitantly, however, many subjects in the Western societies consume uncontrolled amounts of the micronutrients, due to aggressive advertisement. Therefore, some of the serum concentrations run beyond the nadir ranges, because of excessive intake. The nadir range for each metabolite should be determined at the lowest relative risk (RR) for morbidity and mortality. The nadir range is statistically distinct from the higher and the lower serum concentrations of the micronutrient. At the lower and the higher concentrations, higher values of RR are observed.

At different physiological states, such as gender, age, BMI, growth or survival, the nadir range might differ. Some of the serum nutrients (such as sodium, potassium, magnesium, phosphate, copper, zinc, vitamin D, folic acid, and vitamin B12) are routinely assayed and included in the electronic health records (EHR), but without well-established cut-off values. The normal value ranges, such as those published by Harrison's Principles of Internal Medicine (Jameson et al. 2018), Mayo Clinic laboratories (MayoClinic 2017) and Lang Current Medical Diagnosis and Treatment (Papadakis et al. 2018) are too wide, and therefore involve RRs much higher than '1'.

The adjustment of micronutrient concentrations to the nadir ranges might significantly delay and even reduce the incidence of many NCDs (chronic diseases), provided that their nadir values are available.

11.1.1 Dietary Reference Intakes (DRI)

The cut-offs of intakes, which cause overt deficiency and toxic symptoms (multiplied by coefficients of uncertainty) were used to establish the DRIs, and these recommendations are still applied (Fig. 11.1) (Sheffer and Taylor 2008). DRIs were essentially set according to experimental studies, in which classical nutrient deficiencies or toxicities, based on daily intake, were observed (Sheffer and Taylor 2008). Widespread micronutrient lab tests, derived from an extensive EHR, encompassing data regarding the prevalence and incidence of NCDs, may enable calculation of the nadir ranges of various metabolites.

11.1.2 DRI Evaluation by the Current Methodology Has Some Major Disadvantages

a. Evaluation of the dietary intake by dietary questionnaires does not estimate the individual effects of the absorption and the degradation (turnover) of the micronutrient, while serum concentration does.
b. Dietary questionnaires regarding intake are often inaccurate and require a tedious task of measurements.
c. The uncertainty coefficients for the evaluation of the recommended dietary allowance (RDA) and the upper tolerable upper intake level (UL) are not evidence-based.

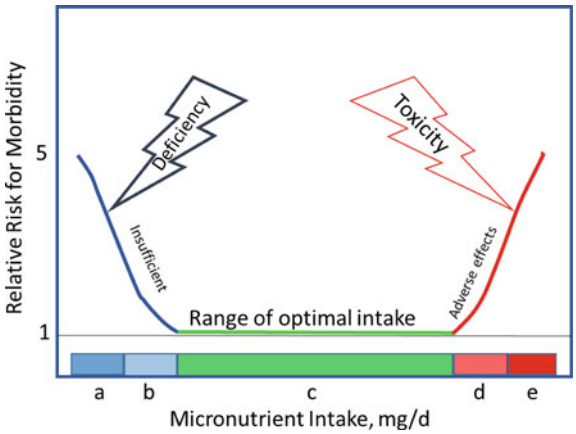

Fig. 11.1 Basic scheme for DRI estimation. The relative risk (RR) response curve for the effect of a supplemented nutrient on the endpoint (disappearance of deficiency/appearance of toxicity). The response curve has 5 ranges from left to right: a the deficient range where overt symptoms are observed; b the range where overt symptoms disappear; c the range of optimal intake; d excessive intake without adverse effects; e the range where overt toxic symptoms are observed

11.1.3 Nadir Range and the Relative Risk of Morbidity/Mortality

The plot of the morbidity + mortality versus serum metabolite shows a U-shaped curve with a nadir range. Some nutrients have wide nadir ranges, but commonly the optimal range is quite narrow. The RR for the morbidity + mortality for a specific nutrient serum concentration is calculated as the ratio of (morbidity + mortality incidence at a specific range of nutrient serum concentration)/(incidence at the nadir range, namely at the mid-range of the U-shaped curve). The lowest risk value is considered as '1' (reference value), while other values are respectively calculated. The highest RR is observed at the extreme serum concentrations (the lowest and the highest) (Fig. 11.2). In some studies, the highest RR is considered as the reference value of '1' and the other values are calculated respectively. For a comparison between some studies, the first approach is more convenient.

11.1.4 Micronutrient Status According to Serum Concentration

Presently, the available data for serum nutrient concentrations in the major databases of the EHRs is limited to iron, calcium, phosphate, sodium, potassium, vitamin D, folic acid, and vitamin B12. Partial information is available for zinc, copper, and magnesium, and the scarcest data is available for the vitamins A, E, C, and B6, with less than 1% of the subjects the tests being performed for them. Data for selenium, manganese, iodine, fluorine, chromium, and the vitamins thiamin, riboflavin, niacin, pantothenic acid, biotin, vitamin K, choline, and betaine are almost unavailable, even though the methodology for their examination is available. Of note, the scarce data,

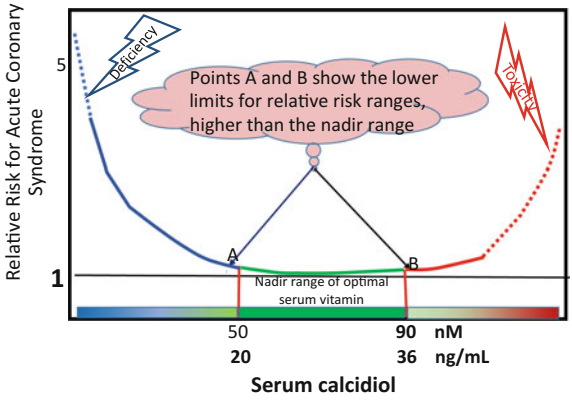

Fig. 11.2 Nadir range for calcidiol. Evaluation of the relative risk (RR) along the calcidiol concentrations shows statistical differences between 20 ng/ml and the descending curve and between 36 ng/ml and the ascending curve, thus defining 20–36 ng/ml (50–90 nM) as the nadir range

which does exist regarding these nutrients, has a major shortcoming, since it is mostly obtained from sick patients and not from the general population.

11.1.5 Specimens for Micronutrient Measurements

Serum is the readiest specimen that is widely used for micronutrient analyses, with the highest prevalence. Within the years, other specimens such as plasma, whole blood, red blood cells, white blood cells, urine, saliva, hair, and nails have been used for nutrient status evaluation. These specimens are often used for many medical tests. For routine nutrient evaluation, however, serum is the preferred one, even though some specimens, such as white blood cells for vitamin C or red blood cells for folate and vitamin B12, are considered as better biomarkers.

11.1.6 The Nutrients for Which Nadir Values of Blood Concentrations May Be Evaluated

Microelements, electrolytes, vitamins and related compounds, such as antioxidants and methyl donors with the medians of the present accepted ranges, listed in Table 11.1, are the target for the nadir range definitions. Most of the presently accepted optimal ranges are derived from big databases of clinical laboratories after careful consideration by medical experts. However, statistical calculation, according to morbidity + mortality, is more accurate for the evaluation of the optimal concentration range than inspection by the experts. The availability of the nutrient test results within the public health system is designated in the table from the highest (1) to the lowest availability (4). Data availability within the large databases can also be similarly designated. We also present the percentage of US population with usual intakes below the Estimated Average Requirement, NHANES 2007–2010 (Kumanyika et al. 2017). Even these data did not estimate the biological biomarkers such as serum concentration, it represents the best available information. Presently, this information may be used as a guideline for establishing optimal concentrations.

Some other microelements, such as silicon, nickel, boron, vanadium, lithium, and tin, may be considered for evaluation and calculation of response curves (WHO 1996). Some of these microelements, such as silicon, have never shown any sign of deficiency, except in experimental animals. Because of low concentrations, low accuracy and reliability, and the interactions with other metals (which are present in blood at much higher concentrations), calculation of the response curves for these elements is problematic and is highly variable. Due to low accuracy of the presently available technology, the U-shaped curves for manganese, chromium, and molybdenum might be incorrect.

Table 11.1 Essential nutrients that might be considered for the evaluation of the optimal serum concentrations, their present accepted median values for adults, accepted ranges from three well-known sources, the prevalence of low intakes in the US and the availability of the data

	Median accepted values	MayoClinic (2017)	Jameson et al. (2018) Harrison's	Papadakis (2018) Lang	Low intake[a], % of subjects	Data availability[b]
Microelements						
	µg/L					
Zinc	960	700–1200	750–1200	500–1500	11	2
Copper	1050	750–1450		700–1400	4	1
Iron	1190	350–1500	410–1410	600–1500	6	1
Selenium	90	70–150	63–160	58–234		3
Iodine	65	42–92				3
Fluorine	26	4–36				4
Manganese	1.35	<2.4		10–12		1
Molybdenum	0.64	0.3–2				4
Chromium	0.4	<0.3				4
Electrolytes						
	mg/L					
Sodium	3230	3105–3335	3130-3360	3130–3270	3	1
Potassium	168	141–203	137–196	137–196		1
Calcium	95	89–101	87–102	82–102	42	1
Phosphorus	37	25–45	25–43	23–47	4	1
Magnesium	19	17–23	15–23	15–25	49	2
Vitamins						
	µg/L					
A	570	320–780	200–1000		40	2
25(OH) D	28	20–50	30–100	14–160	94[c]	1
K	0.52	0.1–2.2	0.13–1.2	0.13–1.2		2
	mg/L					
E	11.2	5.5–17	5–18	5–18	88	2
C	8	4–20	4–10	4–15	38	2
A	0.57		0.2–1			
Niacin (B3)	5.4	0.5–8.5			3	4

(continued)

Table 11.1 (continued)

	Median accepted values	MayoClinic (2017)	Jameson et al. (2018) Harrison's	Papadakis (2018) Lang	Low intake[a], % of subjects	Data availability[b]
	μg/L					
Thiamin (B1)	25	18–48	0–20	25–75	6	3
Riboflavin (B2)	24	1–19	40–240	40–240	2	3
B6 (PLP)	10.7	5–50	5–30	5–30	10	2
Pantothenic acid (B5)	310	37–147				4
Biotin (B7, H)	0.87	0.22–3		0.2–0.5		4
Folic (B9)	7.06	>4	5.4–18	5–25	9	1
B12	0.47	0.18–0.91	0.28–1	0.16–0.95	3	1
Methyl donors						
	mg/L					
Choline	1.01[d]					4
Betaine	3.6[d]					4
Antioxidants						
	mmol/day					
	16[e]					

[a]Percentage of the US Average Requirement, NHANES 2007–2010 Kumanyika et al. (2017)
[b]Availability of the laboratory assay: 1—high; 4—low
[c]As for cholecalciferol
[d]Guertin et al. (2017)
[e]Mancini et al. (2018)

All-cause-mortality incidence and the cause of death extracted from the database of the EHR are essential predictors for an accurate evaluation of the nadir values. Inclusion of these predictors expands the data and may enable further stratification of the RR for distinct population segments.

Currently, the data for methyl donors, antioxidants, and carotenes is limited, even though those nutrients are very important for the nutritional status evaluation and presumably also for nutritional adjustment. Inclusion of blood test results for these nutrients in EHRs databases might be most helpful.

11.1.7 Vitamin D (Calcidiol) as an Example for the Nadir Range Calculation

In a study of 423,000 individuals aged >45 year, a U-shaped curve with a nadir range for serum calcidiol [25(OH)D] versus the combined data of the incidence of acute coronary syndrome (ACS) and all-cause mortality (Fig. 11.2) showed a marked statistical difference between the nadir range and the descending and the ascending curve regions. The nadir hazard ratio was found at serum calcidiol concentration range of 20–36 ng/mL. Analysis including 106,000 observations of ACS did not show higher serum calcidiol concentrations to significantly raise morbidity and mortality. Therefore, the analysis was expanded to include 423,000 observations by incorporating all-cause mortality data, and the study period was extended as well (Dror et al. 2013).

Kaplan-Meier survival curves versus serum calcidiol verified the results. The findings were confirmed by other studies (Sempos et al. 2013; Amrein et al. 2014; Sudfeld et al. 2014; Julian et al. 2016; Gaksch et al. 2017; Tuohimaa and Lou 2012). Such validation by different statistical tools and confirmation by other studies are mandatory for the determination of any formal recommendation to adjust a nutrient status.

Stratification of the calcidiol data by gender did not show differences in the nadir range. However, for some other nutrients, consistent differences between genders were observed (Olsén et al. 2012).

In the cited study (Dror et al. 2013), 61% of the subjects had remarkably low calcidiol blood levels, with a RR for ACS and all-cause mortality (combined data), ranging from 1.08 to 2.8. On the other hand, 3% of the population had blood levels higher than the nadir range, with an average RR of ~1.24. Presumably, the Israeli population is not exceptional, and such values of RR exist in other industrialized societies. Similar RRs might be calculated for at least some of the nutrients listed in Table 11.1. It is still unclear, however, whether adjustment of all out-of-range nutrient concentrations would remarkably decrease the RRs for morbidities + mortality, and decrease the average number of months of disability, particularly on aging.

11.1.8 Adjustment of the Out of Range Values

The out of range serum nutrient concentrations are not considered by the public health systems as health burdens, presumably because there is no sound data on the optimal ranges as they are yet unknown. Calculation of the scarce available data of serum nutrients, found in the EHRs, showed that a considerable part of the population lays outside the accepted ranges. Presently, physicians recommend supplementation after observing low serum micronutrient concentrations, but without a rigid methodology of indication and follow-up. Many people take ‘multivitamin’ supplements, single micronutrients, or any combination of micronutrients at uncontrolled amounts and without a controlled follow-up.

Introduction of the nadir ranges into the routine examination of the patient might provide the caregivers a tool to decrease morbidity and mortality risks caused by low and high intake. Unfortunately, such a routine has never been practiced and the main obstacle for the implementation of out-of-range adjustment is lack of knowledge of the optimal levels of these micronutrients, and how to follow-up such a routine. Adjustments of serum levels of nutrients might be materialized only after future trial and error studies.

11.1.9 Scarce Information in the Medical Literature on Nadir Values

Almost none of the formerly published studies has described U-shaped curve and nadir range of morbidity and mortality with sound statistical evaluation of the difference between the nadir range and the descending or the ascending arms of the U-shaped curve. Most former studies lacked enough statistical power required to estimate the nadir values of morbidity and mortality due to scarce out of range observations of micronutrient blood levels.

11.1.10 Calculation of Nutrient Nadir Ranges

The extensive data, included in the EHR of the public health systems, may allow calculation of the nadir and optimal ranges for the electrolytes, folate and vitamin B12, and validate the calculation made for vitamin D. Additionally, the RR for every serum concentration, outside the nadir range, may be calculated and replace the outdated terms of deficiency, inadequacy, adequacy, adverse effects, and toxicity, which presently are not well-defined. The limited data, in the EHRs of the public health systems, for zinc, copper, vitamins A, E, C, and B6 might be sufficient for a rough estimation of the nadir ranges with a limited statistical support. In a small number of laboratories worldwide, all the other micronutrients listed in Table 11.1, are routinely tested, but without cross-linking with other EHR data. These data do not well-represent the general population, because either sick or wealthy people are checked. Moreover, the interpretation of the results of the assays, which are conducted on a limited scale, has no sound support for defining optimal concentration cut-offs.

11.1.11 Clinical Endpoints

The nadir ranges for the microelements, electrolytes, and vitamins shown below are based on observations made on the following clinical endpoints:

a. All-cause mortality
b. Cancer mortality
c. Hepatocellular carcinoma
d. Colorectal cancer (CRC)
e. Prostate cancer
f. Breast cancer
g. Cardiovascular disease (CVD)
h. Peripheral arterial disease
i. Dementia
j. Cognitive function
k. Unexplained anemia
l. Thyroid nodules
m. Acute respiratory failure
n. Inflammatory markers

11.1.12 Available Nadir Ranges Published in Peer-Reviewed Journals

The available information on U-shaped curves for specific endpoints at certain nutrient concentrations or intakes is scarce. We found only a small number of studies for zinc, copper, iron, selenium, iodine, magnesium, potassium, sodium, calcium and phosphorus. We present also nadir ranges for the vitamins A, B6, B12, C, D, E, folic acid, betaine, and choline. Because vitamins are not within the scope of this book, they are briefly presented. The nadir RR of morbidity + mortality serves as the reference value of '1'.

The collected data on the nadir ranges presented here (except for vitamin D) are definitely not recommended or suggested as practical nadir ranges, because each of them was obtained following a single study, part of the studies comprised a small group of subjects, and the findings were not confirmed by additional studies.

11.1.12.1 Microelements

Zinc

As part of the 2nd National Health and Nutrition Examination Survey (NHANES II) in the US, serum zinc was assayed in >6000 healthy adults. The nadir RR for the lower incidence of cancer death was found at serum zinc concentrations of 860–1030 (average 950) μg/L. At the lower range of <570, and at the higher range of >2300 μg/L, the RRs for cancer death were 1.45 and 1.16, respectively (Wu et al. 2004).

Copper

As part of the NHANES II in the US, serum copper was assayed in >10,000 healthy adults. The nadir RR for the lower incidence of unexplained anemia was found at the 48th percentile of serum copper concentration (~1190 μg/L). At the lower percentile (44th) and at the higher percentile (50th), the RRs for the unexplained anemia were 1.5 and 3.0, respectively (Knovich et al. 2007).

In ~600 middle-class white males, in the Southern California community of Rancho Bernardo residents, the highest cognitive performance was found at plasma copper level of 1300 μg/L with a decreased performance towards the lower and the higher concentrations (Lam et al. 2008).

Iron

As part of the NHANES II in the US, serum iron concentration was assayed in ~6000 healthy adults. The nadir for the lower incidence of cancer death was found at serum iron concentration range of 610–940 (average 780) μg/L. At the lower range of <380, and at the higher range of >1670 μg/L, the RRs for cancer death were 1.15 and 1.53, respectively (Wu et al. 2004).

In ~600 white middle-class males, in the Southern California community of Rancho Bernardo residents, the highest cognitive performances were found at plasma iron concentration of 1500 μg/L. Inverse associations were found for both low and high levels, and were associated with poor cognitive performance (Lam et al. 2008).

Selenium

As part of the NHANES III in the US, serum selenium was assayed in ~14,000 healthy adults. The nadir hazard ratio for the lower incidence of all-cause mortality was found at serum selenium concentration of 140 μg/L (130–150 μg/L). At the lower range of <107, and at the higher range of >168 μg/L, the RRs of all-cause mortality were ~1.3 and 1.20, respectively (Rayman 2012).

As part of the NHANES 2003–2004 in the US, serum selenium was assayed in ~2000 healthy adults aged >40 year. The nadir RR for the lower prevalence of peripheral arterial disease was found at serum selenium concentration of 155 μg/L. At both, the lower range of <117 and at the higher range of >191 μg/L, the RR of mortality was 1.72 (Bleys et al. 2009).

According to several studies, the nadir RR for lower incidence of hepatocellular carcinoma was found at serum selenium concentration of ~155 ng/mL. At both, the lowest concentration of ~130 and the highest concentration of ~170 ng/mL, the RR was ~4 (Fairweather-Tait et al. 2011).

In a review of some studies from the NHANES III, the nadir RR of CVD with the lowest incidence of mortality was found at serum selenium concentration of

125 ng/ml. At both, the lower level of 105 ng/ml and the higher level of 155 ng/ml, the RR of mortality was 1.19 (Stranges et al. 2010).

Iodine

In a Shanghai survey, ~6000 subjects aged 15–69 year were randomly recruited. A nadir range for the lower prevalence of thyroid nodules was found at urine iodine concentration of 266 μg/L. At both, the lower range of <100 μg/L and the higher range of >420 μg/L, the RR was 1.20 (Song et al. 2016).

11.1.12.2 Major Elements (Electrolytes)

Sodium

As part of the NHANES 1999–2004 in the US, serum sodium was assayed in ~15,000 healthy adults. The nadir hazard ratio for the lower incidence of all-cause mortality was found at serum sodium concentration of 3200 mg/L. At both the lower range of <3130 and the higher range of >3270 mg/L, the RR for mortality was ~2 (Mohan et al. 2013).

In a meta-analysis of 4 international prospective studies based on an analysis of ~133,000 people selected from 49 countries in 6 continents, the nadir RR for the lower incidence of death or major CVD events was found in subjects with urinary sodium excretion of ~4500 mg/d. At both, the lower excretion of 3000 mg/d and the higher excretion of 7800 mg/d, the RR for death or major CVD events was 1.18 (Mente et al. 2016).

Potassium

In ~120,000 dialysis patients in Da-Vita facilities, California, the nadir RR for the lower incidence of all-cause mortality was found at serum potassium concentration of 188 mg/L. At both the lower concentration range of <137 and the higher range of >215 mg/L, the RR for all-cause mortality was 1.55 (Torlen et al. 2012).

In ~45,000 hypertensive patients in Denmark, the nadir RR for the lower incidence of all-cause mortality was found at serum potassium concentration of 172 mg/L. At both the lower concentration of 117 mg/L and the higher concentration of 219 mg/L, the RR for all-cause mortality was 3.0 (Krogager et al. 2017).

In 2370 patients with ACS from the West China Hospital at Sichuan, the nadir hazard ratio for the lower incidence of all-cause mortality was found at serum potassium concentration of ~149 mg/L (108 to <149). At the lower concentration range of <137 and at the higher concentration of >215 mg/L, the RRs for all-cause mortality were ~2.1 and 1.55, respectively (Peng et al. 2015).

In 2065 Taiwanese elderly, the nadir RR for the lower incidence of all-cause mortality was found at serum potassium concentration of 162 mg/L. At the lower concentration of 121 mg/L and the higher concentration of ~196 mg/L, the RRs for all-cause mortality were 1.36 and 1.63, respectively (Lai et al. 2015).

Calcium

In a meta-analysis of 6 studies comprising ~225,000 subjects, the nadir RR for the lower incidence of CVD mortality was found in subjects with calcium intake of 860 mg/d. At the lower intake range of <500 mg/d and at the higher intake range of >1400 mg/d, the RRs for CVD mortality were ~1.09 and 1.25, respectively (Wang et al. 2014).

In ~2 million subjects selected from a historical cohort examining risk factors and outcomes of incident chronic kidney disease of veterans, who received Veterans Affairs medical services, the nadir hazard ratio for the lower incidence of mortality was found at serum calcium concentration of 92 mg/L (91 to <94). At both the lower range of <85 mg/L and the higher range of >100 mg/L, the hazard ratio for mortality was ~1.3 (Lu et al. 2015).

Magnesium

In an analysis of 40 studies with ~1 million subjects the nadir hazard ratio for the lower incident CVD was found at magnesium intake of 310 mg/d. At the lower intake of 200 and at the higher intake of 420 mg/d, the RRs were 1.13 (Fang et al. 2016).

In the Mayo Clinic Rochester hospital, ~10,000 patients were evaluated. The nadir RR for the lower incidence of in-hospital acute respiratory failure requiring mechanical ventilation was found at serum magnesium concentration of 18 mg/L. At the lower range of <15 mg/L and the higher range of >23 mg/L, the RRs were 1.7 and 1.4, respectively (Thongprayoon et al. 2015).

Phosphorus

In 113,993 patients from the original set of patients with serum phosphate records from the Royal College of General Practitioners Research and Surveillance Centre, database from 135 General Practitioner medical practices with a total population of ~1.2 million from a mix of urban and rural UK locations, the nadir hazard ratio for the lower incident coronary event was at serum phosphorus concentration of 31 mg/L (~24 to ~37). At the lower range of <22 mg/L and the higher range of >39 mg/L, the RRs for the incident coronary event were >1.5 (Hayward et al. 2017).

In ~744,000 users of the Veteran Administration Healthcare System, the nadir hazard ratio for the lower incident dementia was at serum phosphorus concentration of 31 mg/L (>29 to <32). At the lower range of <29 mg/L and at the higher range

of >39 mg/L, the RRs for the incident dementia were 1.09 and 1.14, respectively (Li et al. 2017).

In ~9700 healthy US adults aged 20–80 years drawn from the NHANES III, the nadir hazard ratio for the lower incidence of CVD mortality was in subjects with phosphorus intake of 0.35 mg/kcal. At both, the lower intake of 0.31 mg/kcal and the higher intake of 1.1 mg/kcal, the hazard ratio was ~2 (Chang et al. 2014).

11.1.12.3 Vitamins and Other Nutritional Compounds

Vitamin A

For the lower incidence of all-cause mortality, the nadir is at serum vitamin A concentration of 2.07 μM, with a range of 1.5 (RR = 1.22) to 2.4 (RR = 1.16) (Goyal et al. 2013).

For the lower incidence of hip fracture, the nadir is at serum vitamin A concentration of 2.02 μM, with a range of 1.5 (RR = 1.9) to 2.6 (RR = 2.1) (Opotowsky and Bilezikian 2004).

For the lower incidence of hip-fracture, the nadir is at vitamin A intake of 2.14 mg/d, with a range of 1.4 (RR = 1.7) to 2.9 (RR = 1.8) (Wu et al. 2014).

For the lower incidence of death from stroke, the nadir is at vitamin A intake of 2.05 mg/d, with a range of 0.8 (RR = 1.33) to 4.4 (RR = 1.13) (Yochum et al. 2000).

Vitamin D

For the lower incidence of ACS and all-cause mortality (combined data), the nadir is at serum calcidiol concentration of 20–36, ng/mL, with a range of 4 (RR = 2.1) to 42 (RR = 1.2) (Dror et al. 2013).

For the lower mortality incidence, the nadir is at serum calcidiol concentration of 24–48 ng/mL, with a range of 6.5 (RR = 2) to 52 (RR = 1.6) (Sempos et al. 2013).

For the lower incidence of prostate cancer, the nadir is at serum calcidiol concentration of 26 ng/mL, with a range of 16 (RR = 1.35) to 37 (RR = 1.3), ng/mL (Kristal et al. 2014).

For the lower presence of inflammatory markers, the nadir is at serum calcidiol concentration of 18 ng/mL, with a range of 5 (RR = 2.3) to 45 (RR = 2.3) (Mellenthin et al. 2014).

Vitamin E

For the lower incidence of all-cause mortality, the nadir is at serum vitamin E concentration of 24.1 μM, with a range of 18.7 (RR = 1.23) to 32.2 (RR = 1.10) (Goyal et al. 2013).

For the lower incidence of death from stroke, the nadir is at serum vitamin E concentration of 22 μM, with a range of 4.9 (RR = 1.7) to 238 (RR = 1.56) (Yochum et al. 2000).

Vitamin B6

For the lower incidence of breast cancer, the nadir is at serum vitamin B6 concentration of 1.88 μM, with a range of 1.25 (RR = 1.19) to 2.8 (RR = 1.22) (Basset et al. 2013a).

For the lower incidence of colorectal cancer, the nadir is at serum vitamin B6 concentration of 2.3 μM, with a range of 1.3 (RR = 1.5) to 3.9 (RR = 1.47) (Basset et al. 2013b).

For the lower incidence of colorectal cancer, the nadir is at serum PLP concentration of 53 nM, with a range of 27 (RR = 1.18) to 140 (RR = 1.18) (Gylling et al. 2017).

Folate

For the lower incidence of prostate cancer, the nadir is at serum folate concentration of 11.8 nM, with a range of 10.8 (RR = 1.09) to 18 (RR = 1.25) (De Vogel et al. 2013).

For the lower incidence of colorectal cancer, the nadir is at folic acid intake of 480 μg/d, with a range of 60 (RR = 1.43) to 1100 (RR = 1.07) (Gibson et al. 2011).

For the lower incidence of breast cancer, the nadir is at folic acid intake of 320 μg/d, with a range of 150 (RR = 1.19) to 550 (RR = 1.10) (Chen et al. 2014).

For the lower incidence of mild cognitive impairment, the nadir is at folic acid intake of 345 μg/d, with a range of 200 (RR = 1.6) to 750 (RR = 1.30) (Agnew-Blais et al. 2015).

For the lower incidence of breast cancer, the nadir is at folic acid intake of 286 μg/d, with a range of 224 (RR = 1.25) to 422 (RR = 1.24) (Basset et al. 2013b).

For the lower incidence of Autism Spectrum Disorder (ASD) in the offsprings of pregnant women, the nadir is at maternal plasma folate concentration of 13.5 to 45 nM with a range of <13.5 (RR = 1.1) to >45 (RR = 1.5) (Raghavan et al. 2017).

Vitamin B12

For the lower incidence of mild cognitive impairment, the nadir is at vitamin B12 intake of 6.9 μg/d, with a range of 4 (RR = 1.4) to 13 (RR = 1.27) Agnew-Blais et al. 2015).

For the lower incidence of ASD in the offsprings of pregnant women the nadir is at maternal vitamin B12 plasma concentration of 200–600 pM with a range of <200 (RR = 1.9) to >600 (RR = 3) (Raghavan et al. 2017).

Table 11.2 A summary of the endpoints, the available nadir ranges, the RRs at the lower range, and the RRs at the higher range

Microelements/ electrolyte	End-point	Concentration; intake	Nadir	Concentration at the lower and at the higher ranges		References
				Lower range (RR)	Higher range (RR)	
Microelements						
Zinc	Cancer mortality	Serum μg/L	950	<570 (1.45)	2300 (1.16)	Wu et al. (2004)
Copper	Unexplained anemia	Serum μg/L	1190	44%tile (1.5)	50%tile (3)	Knovich et al. (2007)
	Cognitive performance	Serum μg/L	1300 Highest	(Lower)	(Lower)	Lam et al. (2008)
Iron	Cancer mortality	Serum μg/L	780	<380 (1.15)	>1670 (1.53)	Wu et al. (2004)
	Highest cognitive performances	Serum μg/L	1500	(Lower)	(Lower)	Lam et al. (2008)
Selenium	All-cause mortality	Serum μg/L	140	<107 (1.3)	>168 (1.2)	Rayman et al. (2012)
	Peripheral arterial disease	Serum μg/L	155	<117 (1.72)	>191 (1.72)	Bleys et al. (2009)
	Hepatocellular carcinoma	Serum μg/L	155	130 (4)	170 (4)	Fairweather-Tait et al. (2011)
	All-cause mortality	Serum μg/L	125	105 (1.19)	155 (1.19)	Stranges et al. (2010)
Iodine	Thyroid nodules	Urine μg/L	266	<100 (1.2)	>420 (1.2)	Song et al. (2016)
Electrolytes						
Sodium	All-cause mortality	Serum mg/L	3200	<3130 (2)	3270 (2)	Mohan et al. (2013)
	mortality + major CVD	Urine mg/d	4500	3000 (1.18)	7800 (1.18)	Mente et al. (2016)
Potassium	All-cause mortality	Serum mg/L	188	<137 (1.55)	>215 (1.55)	Torlen et al. (2012)
	All-cause mortality	Serum mg/L	172	117 (3)	219 (3)	Krogager et al. (2017)

(continued)

Table 11.2 (continued)

Microelements/ electrolyte	End-point	Concentration; intake	Nadir	Concentration at the lower and at the higher ranges		References
				Lower range (RR)	Higher range (RR)	
	All-cause mortality	Serum mg/L	149	<137 (2.1)	>215 (1.55)	Peng et al. (2015)
	All-cause mortality	Serum mg/L	162	121 (1.36)	196 (1.63)	Lai et al. (2015)
Calcium	CVD mortality	Intake mg/d	860	<500 (1.09)	>1400 (1.25)	Wang et al. (2014)
	All-cause mortality	Serum μg/L	92	<85 (1.3)	>100 (1.3)	Lu et al. (2015)
Magnesium	CVD	Intake mg/d	310	200 (1.13)	420 (1.13)	Fang et al. (2016)
	Acute respiratory failure	Serum mg/L	18	<15 (1.7)	>23 (1.4)	Thongprayoon et al. (2015)
Phosphorus	Cardiac event	Serum mg/L	31	<22 (>1.5)	>39 (>1.5)	Hayward et al. (2017)
	Dementia	Serum mg/L	31	<29 (1.09)	>39 (1.14)	Li et al. (2017)
	CVD mortality	Intake mg/kcal	0.35	0.31 (~2)	1.1 (~2)	Chang et al. (2014)
Vitamins and other nutritional compounds						
Vitamin A	All-cause mortality	Serum μM	2.07	1.5 (1.22)	2.4 (1.16)	Goyal et al. (2013)
	hip-fracture	Serum μM	2.02	1.5 (1.9)	2.6 (2.1)	Opotowsky and Bilezikian (2004)
	hip-fracture	Serum μM	2.14	1.4 (1.7)	2.9 (1.8)	Wu et al. (2014)
Retinol equivalent	Death from stroke	Intake mg/d	2.05	0.78 (1.33)	4.4 (1.13)	Yochum et al. (2000)
Vitamin D (Calcidiol)	mortality + acute coronary syndrome	Serum ng/ml	20–36	4 (2.1)	42 (1.2)	Dror et al. (2013)

(continued)

Table 11.2 (continued)

Microelements/ electrolyte	End-point	Concentration; intake	Nadir	Concentration at the lower and at the higher ranges		References
				Lower range (RR)	Higher range (RR)	
	mortality	Serum ng/ml	24–48	6.5 (2.5)	52 (1.6)	Sempos et al. (2013)
	cancer prostate	Serum ng/ml	26	16 (1.35)	37 (1.3)	Kristal et al. (2014)
	Inflammatory markers (CRP)	Serum ng/ml	18	5 (2.3)	45 (2.3)	Mellenthin et al. (2014)
Vitamin E	All-cause mortality	Serum μM	24.1	18.7 (1.23)	32.2 (1.10)	Goyal et al. (2013)
	Death from stroke	Intake mg/d	22	4.9 (1.7)	238 (1.56)	Yochum et al. (2000)
Vitamin B6	Breast cancer	Serum μM	1.88	1.25 (1.19)	2.8 (1.22)	Bassett et al. (2013b)
	Colorectal cancer (CRC)	Serum μM	2.3	1.3 (1.5)	3.9 (1.47)	Bassett et al. (2013a)
PLP (vitamin B6)	Colorectal cancer (CRC)	Serum mg/d	53	27 (1.18)	140 (1.18)	Gylling et al. (2017)
Folate/folic acid	Prostate cancer	Serum mg/d	11.8	10.8 (1.09)	18 (1.25)	De Vogel et al. (2013)
	Colorectal cancer (CRC)	Intake μg/d	480	60 (1.43)	1100 (1.07)	Gibson et al. (2011)
	Breast cancer	Intake μg/d	320	150 (1.19)	550 (1.10)	Chen et al. (2014)
	Mild cognitive impairment	Intake μg/d	570	200 (1.30)	800 (1.14)	Agnew-Blais et al. (2015)
	Breast cancer	Intake μg/d	286	224 (1.25)	422 (1.24)	Bassett et al. (2013b)
	Autism Spectrum Disorder (ADS) in offsprings	Plasma nM	13.5–45	<13.5 (1.1)	>45 (1.5)	Raghavan et al. (2017)

(continued)

Table 11.2 (continued)

Microelements/ electrolyte	End-point	Concentration; intake	Nadir	Concentration at the lower and at the higher ranges		References
				Lower range (RR)	Higher range (RR)	
Vitamin B12	Mild cognitive impairment	Intake μg/d	6.9	4 (1.44)	13 (1.27)	Agnew-Blais et al. (2015)
	Autism Spectrum Disorder (ADS) in offsprings	Plasma pM	200–600	<200 (1.9)	>600 (3)	Raghavan et al. (2017)
Vitamin C	Death from stroke	Intake mg/d	281	82 (1.38)	680 (1.70)	Yochum et al. (2000)
Betaine	Colorectal cancer (CRC)	Intake mg/d	245	117 (1.38)	320 (1.27)	Lu et al. (2015)
Total antioxidant capacity	Diabetes	Intake mmole/d	15.6	8 (1.33)	30 (1.1)	Mancini et al. (2018)

CVD Cardiovascular disease

Vitamin C

For the lower incidence of death from stroke, the nadir is at vitamin C intake of 281 mg/d, with a range of 82 (RR = 1.4) to 680 (RR = 1.7) (Yochum et al. 2000).

Betaine

For the lower incidence of colorectal cancer, the nadir is at betaine intake of 245 mg/d, with a range of 117 (RR = 1.38) to 320 (RR = 1.27) (Lu et al. 2015).

Total Antioxidant Capacity of Food (Estimated by Using Ferric Ion Reducing Antioxidant Power)

for the lowest risk of diabetes type 2 incidence, the nadir is at total antioxidant capacity of 16 mmol/d with a range of 8 (RR = 1.33) to 30 (RR = 1.1) (Mancini et al. 2018).

The data for the endpoints, the available nadir values, the RRs at the lower range and the RRs at the higher range are summarized in Table 11.2.

11.2 Conclusions

1. Adjustment of serum micronutrient concentrations might have a considerable influence on the health status of the population. Such an adjustment is mostly crucial for patients with a history of major non-communicable-diseases (NCDs) .
2. Microelement serum concentrations data, well recorded in electronic health records (EHRs), is essential for the evaluation of optimal microelement status.
3. The current normal ranges of the microelements (and other nutrients) are not supported by sound data. Some of these ranges presumably cover concentrations with elevated relative risks (RRs).
4. Serum concentration of the microelements (and other nutrients) are the best biomarkers for the evaluation of the optimal micronutrient status.
5. The optimal and the nadir ranges of the micronutrients may be determined by using serum concentrations and the endpoint parameters of the EHRs.
6. Presently, for most of the microelements, only a limited number of assays of serum concentrations in the population are available.
7. Adjustment of the micronutrient status must be strictly followed-up.

The authors declare no conflict of interests.

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