Chapter 14

Aging, Oxidative Stress and Antioxidants

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Additional information is available at the end of the chapter

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1. Introduction

Aging is an extremely complex and multifactorial process that proceeds to the gradual deterioration in functions. It usually manifest after maturity and leads to disability and death. Traditionally researchers focused primarily on understanding how physiological functions decline with the increasing age; almost no research was dedicated to investigation of causes or methods of aging intervention. If scientists would discover a drug for healing all major chronic degenerative diseases, the average lifetime would be increased for just 12 years. People would still die from complications connected with the aging process.

Defects formed in human body as a consequence of the aging process start to arise very early in life, probably *in utero*. In the early years, both the fraction of affected cells and the average burden of damage per affected cell are low [1]. The signs of aging start to appear after maturity, when optimal health, strength and appearance are at the peak. After puberty, all physiological functions gradually start to decline (e.g. the maximum lung, heart and kidney capacities are decreased, the secretion of sexual hormones is lowered, arthritic changes, skin wrinkling, etc). The precise biological and cellular mechanisms responsible for the aging are not known, but according to Fontana and Klein [2], "they are likely to involve a constellation of complex and interrelated factors, including [1] oxidative stress–induced protein and DNA damage in conjunction with insufficient DNA damage repair, as well as genetic instability of mitochondrial and nuclear genomes; [2] noninfectious chronic inflammation caused by increased adipokine and cytokine production; [3] alterations in fatty acid metabolism, including excessive free fatty acid release into plasma with subsequent tissue insulin resistance; [4] accumulation of end products of metabolism, such as advanced glycation end products, amyloid, and proteins that interfere with normal cell function; [5] sympathetic nerve system and angiotensin system activation as well as alterations in neuroendocrine systems; and [6] loss of post-mitotic cells, resulting in a decreased number of neurons and muscle cells as well as deterioration in structure and function of cells in all tissues and organs".

In recent years, oxidative stress has been implicated in a wide variety of degenerative processes, diseases and syndromes, including the following: mutagenesis, cell transformation and cancer; heart attacks, strokes, atherosclerosis, and ischemia/reperfusion injury; chronic inflammatory diseases, like rheumatoid arthritis, lupus erythematosus and psoriatic arthritis; acute inflammatory problems; photooxidative stresses to the eye, e.g. cataract; neurological disorders, such as certain forms of familial amyotrophic lateral sclerosis, certain glutathione peroxidase-linked adolescent seizures, Parkinson's and Alzheimer's diseases; and other age-related disorders, perhaps even including factors underlying the aging process itself [3].

2. Aging theories

Scientists estimated that the allelic variation or mutations in up to 7,000 relevant genes might modulate their expression patterns and/or induce senescence in an aging person, even in the absence of aging specific genes [4, 5]. As these are complex processes they may result from different mechanisms and causes. Consequently, there are many theories trying to explain the aging process, each from its own perspective, and none of the theories can explain all details of aging. The aging theories are not mutually exclusive, especially, when oxidative stress is considered [6].

Mild oxidative stress is the result of normal metabolism; the resulting biomolecular damage cannot be totally repaired or removed by cellular degradation systems, like lysosomes, proteasomes, and cytosolic and mitochondrial proteases. About 1% to 4% of the mitochondrially metabolized oxygen is converted to the superoxide ion that can be converted subsequently to hydrogen peroxide, hydroxyl radical and eventually other reactive species, including other peroxides and singlet oxygen that can in turn, generate free radicals capable of damaging structural proteins and DNA [7, 8, 9, 10, 11]. Since extensive research on the relation between polymorphisms likely to accelerate/decelerate the common mechanisms of aging and resistance to the oxidative stress has been neglected in almost all scientific studies, the data do not allow us to conclude that the oxidative theory supports the theory of programmed aging so far [7]. However, the most recent studies support the idea that oxidative stress is a significant marker of senescence in different species. Resistance to oxidative stress is a common trait of long-lived genetic variations in mammals and lower organisms [5, 12]. Theories on aging process can be divided into programmed and stochastic.

2.1. Free radical theory, oxidative stress theory and mitochondrial theory of aging

Denham Harman was first to propose the free radical theory of aging in the 1950s, and extended the idea to implicate mitochondrial production of reactive oxygen species in 1970s, [13]. According to this theory, enhanced and unopposed metabolism-driven oxidative stress has a major role in diverse chronic age-related diseases [13, 14, 7]. Organisms age because of accumulation of free radical damage in the cells. It was subsequently discovered that reactive oxygen species (ROS) generally contribute to the accumulation of oxidative damage to cellular constituents, eventhough some of them are not free radicals, as they do not have an

unpaired electron in their outer shells [15, 16]. Consistently, aged mammals contain high quantities of oxidized lipids and proteins as well as damaged/mutated DNA, particularly in the mitochondrial genome [13, 14]. In support of a mitochondrial theory of aging, the mitochondrial DNA damage increases with aging [17, 18]. Thus, a modern version of this tenet is the "oxidative stress theory" of aging, which holds that increases in ROS accompany aging and lead to functional alterations, pathological conditions, and even death [19].

The oxygen consumption, production of ATP by mitochondria and free-radical production are linked processes [20, 21]. Harman first proposed that normal aging results from random deleterious damage to tissues by free radicals [14] and subsequently focused on mitochondria as generators of free radicals [13]. Halliwell and Gutteridge later suggested to rename this free radical theory of aging as the "oxidative damage theory of aging" [22], since aging and diseases are caused not only by free radicals, but also by other reactive oxygen and nitrogen species.

Increases in mitochondrial energy production at the cellular level might have beneficial and/or deleterious effects [23]. Increased regeneration of reducing agents (NADH, NADPH and $FADH_2$) and ATP can improve the recycling of antioxidants and assist the antioxidant defence system. On the other hand, enhanced mitochondrial activity may increase the production of superoxide, thereby aggravating the oxidative stress and further burdening the antioxidant defence system. The mitochondria are the major source of toxic oxidants, which have the potential of reacting with and destroying cell constituents and which accumulate with age. The result of this destructive activity is lowererd energy production and a body that more readily displays signs of age (e.g., wrinkled skin, production of lower energy levels). There is now a considerable evidence that mitochondria are altered in the tissues of aging individuals and that the damage to mitochondrial DNA (mtDNA) increases 1,000-fold with age [24].

The mutation rate of mitochondrial DNA is ten-times higher than that of nuclear DNA. Mitochondrial DNA (mtDNA) is a naked, mostly double-stranded, circular, and is continuously exposed to ROS. It is replicated much faster than nuclear DNA with less proofreading and efficient DNA repair mechanisms [25]. Thus, mtDNA is more vulnerable to attack by ROS. Damaged mitochondria can cause the energy crisis in the cell, leading to senescence and aging of tissue. Accumulation of damage decreases the cell's ability to generate ATP, so that cells, tissues, and individuals function less well. The gradual loss of energy experienced with age is paralleled by a decrease in a number of mitochondria per cell, as well as energy-producing efficiency of remaining mitochondria.

A major effect of mitochondrial dysfunction is an unappropriately high generation of ROS and proton leakage, resulting in lowering of ATP production in relation to electron input from metabolism. Leaked ROS and protons cause damage to a wide range of macromolecules, including enzymes, nucleic acids and membrane lipids within and beyond mitochondria, and thus are consistent with the inflammation theory of aging as being proximal events triggering the production of pro-inflammatory cytokines. The age-related increases in the levels of both oxidative damage and mutational load of mtDNA predicted by the mitochondrial theory of aging have been described in multiple species and organ systems [26]. How-

ever, whether this damage affects mitochondrial function or significantly modulates the physiology of aging has remained controversial [27, 28]. As already mentioned, free radicals can damage the mitochondrial inner membrane, creating a positive feedback-loop for increased free-radical creation. Induction of ROS generates mtDNA mutations, in turn leading to a defective respiratory chain. Defective respiratory chain generates even more ROS and generates a vicious cycle. The result is even more damage.

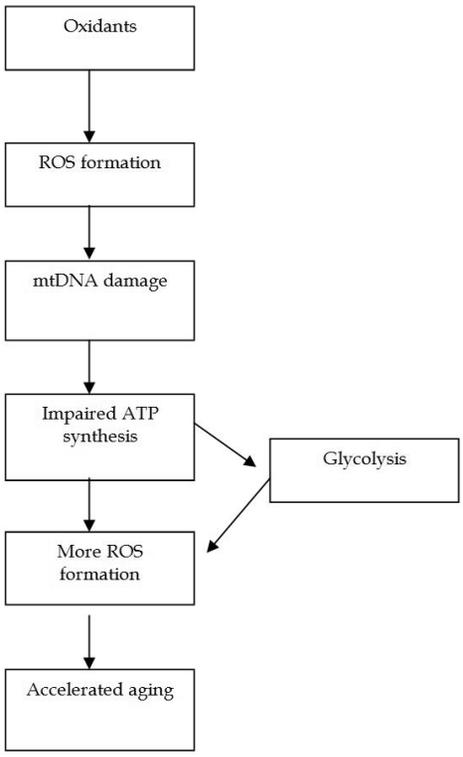

Figure 1. Oxidative stress from endogenous or exogenous sources can trigger the chain reaction, which leads to accelerated aging process of cells and organisms.

On the other hand, the "vicious cycle" theory, which states that free radical damage to mitochondrial DNA leads to mitochondria that produce more superoxide, has been questioned by some scientists since the most damaged mitochondria are degraded by autophagy, whereas the less defective mitochondria (which produce less ATP as well as less superoxide) remain to reproduce themselves [29]. But the efficiency of autophagy to consume malfunctioning mitochondria also declines with age, resulting in more mitochondria producing higher levels of superoxide [30]. Mitochondria of older organisms are fewer in number, larger in size and less efficient (produce less energy and more superoxide).

Free radicals could also be involved in signalling responses, which subsequently stimulate pathways related to cell senescence and death, and in pro-inflammatory gene expression. This inflammatory cascade is more active during aging and has been linked with age-associated pathologies, like cancer, cardiovascular diseases, arthritis, and neurodegenerative diseases [31].

2.2. Other theories of aging

Apart from the free radical theory, the aging is explained by many other theories:

The Telomere shortening hypothesis (also described as "replicative senescence," the "Hayflick phenomenon" or Hayflick limit) is based on the fact that telomeres shorten with each successive cell division. Shortened telomeres activate a mechanism that prevents cell division [32]. The telomere shortening hypothesis cannot explain the aging of the non-dividing cells, e.g. neurons and muscle cells, thus cannot explain the aging process in all the cells of an organism.

The Reproductive-cell cycle theory states that aging is regulated by reproductive hormones, which act in an antagonistic pleiotropic manner through cell cycle signaling. This promotes growth and development early in life in order to achieve reproduction, however later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence [32].

The Wear and tear theory of aging is based on the idea that changes associated with aging result from damage by chance that accumulates over time [32]. The wear-and-tear theories describe aging as an accumulation of damage and garbage that eventually overwhelms our ability to function. Similar are Error accumulation and Accumulative waste theories; Error accumulation theory explains aging as the results from chance events that escape proofreading mechanisms of genetic code [32], according to Accumulative waste theory the aging results from build-up of cell waste products in time because of defective repair-removal processes. Terman, [33] believes that the process of aging derives from imperfect clearance of oxidatively damaged, relatively indigestible material, the accumulation of which further hinders cellular catabolic and anabolic functions (e.g. accumulation of lipofuscin in lysosomes). The programmed theories (e.g. aging clock theory) propose a time-switch in our bodies that controls not only our process of development but also triggers our self-destruction. The shortening of telomeres would provide such a clock in rapidly dividing cells. The Autoimmune theory of aging is based on the idea that aging results from an increase in antibodies that attack the body's tissues [32].

Mitohormesis theory of aging is based on the “hormesis effects”. It describes beneficial actions resulting from the response of an organism to a low-intensity stressor. It has been known since the 1930s that restricting calories while maintaining adequate amounts of other nutrients can extend the lifespan in laboratory animals. Michael Ristow's group has provided evidence for the theory that this effect is due to increased formation of free radicals within the mitochondria causing a secondary induction of increased antioxidant defense capacity [34]. Finkel et al., [35] stated that the best strategy to enhance endogenous antioxidant levels may actually be oxidative stress itself, based on the classical physiological concept of hormesis (for detailed information on hormesis see paragraph Adaptive responses and hormesis).

Additionally, the Disposable soma theory was proposed [36, 37], which postulated a special class of gene mutations with the following antagonistic pleiotropic effects: these hypothetical mutations save energy for reproduction (positive effect) by partially disabling molecular proofreading and other accuracy promoting devices in somatic cells (negative effect). The

Evolutionary theory of aging is based on life history theory and is constituted of a set of ideas that themselves require further elaboration and validation [38].

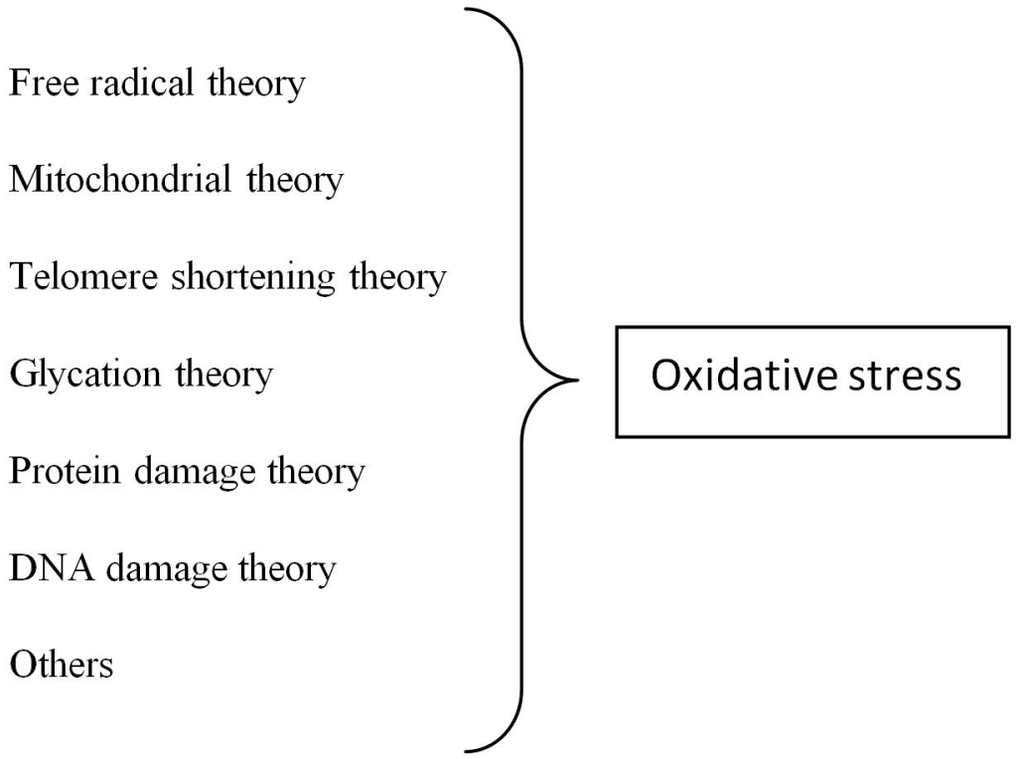

Figure 2. Oxidative stress as the common denominator of majority of aging theories.

Evidence implies that an important theme linking several different kinds of cellular damage is the consequence of exposure to reactive oxygen species [5, 39].

Many of the theories overlap, e.g., ROS can cause DNA damage (free radical theory) and also accelerate telomere shortening (telomere theory), since telomere shortening is accelerated by oxidative stress in vascular endothelial cells [40, 41]. None of the theories explain the aging process, as it may be too complex to be covered by only one theory. Perhaps there is no single mechanism responsible for aging in all living organisms [42]. The definitive mechanisms of aging across species remain equivocal. Diminished capacity for protein synthesis and DNA repair, decline in immune functions, loss of muscle mass and strength, a decrease in bone mineral density as well as a decrease in enzymatic and non-enzymatic antioxidative protections are well established. In essence, aging is progressive accumulation through life of many random molecular defects that build up within the cells and tissues. For this reason, only one "magic bullet" will never be able to prevent or reverse the complex and multicausal process of aging.

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3. The Role of Oxidative Stress on the General Aging Process

In order to understand strategies to reduce oxidative stress and aging, it is first important to briefly explain reasons for oxidative stress formation. Oxidative damage is a result of the intrinsic and extrinsic ROS formation factors. The most important endogenous sources of oxidants are mitochondrial electron transport chain and nitric oxide synthase reaction, and the non-mitochondrial soruces: Fenton reaction, reactions involving cytochromes P450 in microsomes, peroxisomal beta - oxidation and respiratory burst of phagocytic cells [6]. Free radical reactions have been implicated also as the consequence of exposure to many environmental pollutants, e.g. cigarette smoke, alcohol, ionizing and UV radiations, pesticides, ozone, etc. Oxidative stress is the direct consequence of an increased generation of free radicals and/or reduced physiological activity of antioxidant defenses against free radicals. The degree of oxidative stress is proportional to the concentration of free radicals, which depends on their formation and quenching.

Causes of increased free-radical production include [43]:

Endogenous

- elevation in O_2 concentration
- increased mitochondrial leakage
- inflammation
- increased respiration
- others

Exogenous

- environment (pollution, pesticides, radiation, etc.)
- smoking
- poor nutrition
- disorders and chronic diseases
- chronic inflammation
- lifestyle
- strenuous excercise
- psychological and emotional stress
- others

Causes of decreased antioxidant defense include:

- reduced activity of endogenous antioxidative enzymes
- reduced biokinetics of antioxidant metabolism
- reduced intake of antioxidants
- reduced bioabsorption of antioxidants
- others

Oxidative stress is caused mainly by:

- mutation or reduced activity of enzymes (catalase, SOD, glutathione peroxidase)
- decreased intake of exogenous antioxidants from food
- increased metal ion intake (e.g., Fe, Cu, Cr)
- easiliy peroxidized amino acids (e.g., lysine)
- increased triplet oxigen ($^3O_2$) concentration
- increased physical activity of an untrained individual
- ROS from ionizing radiation, air pollution, smoking
- chronic inflammation

Excessive generation of free radicals may overwhelm natural cellular antioxidant defenses, leading to oxidation and further functional impairment. There is an oxidative damage potential, as there is a constant free radical formation in small amounts, which escape the cell defense.

The reduction of oxidative stress can be achieved on three levels [44]: i) by lowering exposure to environmental pollutants ii) by increasing the levels of endogenous and exogenous antioxidants in order to scavenge ROS before they can cause any damage; or iii) lowering the generation of oxidative stress by stabilizing mitochondrial energy production and efficiency - reducing the amount of ROS formed per amount of O_2 consumed.

4. Defenses against ROS and strategies to reduce oxidative stress

Generation of ROS and the activity of antioxidant defenses are balanced *in vivo.* In fact, the balance may be slightly tipped in favor of ROS so that there is continuous low-level oxidative damage in the human body.

Besides the endogenous and exogenous antioxidative protection, the second category of defence are repair processes, which remove the damaged biomolecules before they accumulate to cause altered cell metabolism or viability [45].

4.1. Primary Antioxidant Defenses

Superoxide Dismutase (SOD)

SODs are a group of metalloenzymes, which catalyze the conversion of superoxide anion to hydrogen peroxide and dioxygen [46]. This reaction is a source of cellular hydrogen peroxide.

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

Catalase

Hydrogen peroxide formed by SOD, from other metabolic reactions or from the non-enzymatic reaction of the hydroperoxyl radical, is scavenged by a ubiquitous heme protein catalase. It catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen [47].

$$2\,H_2O_2 \rightarrow O_2 + 2H_2O \quad (2)$$

One antioxidative role of catalases is to lower the risk of hydroxyl radical formation from H_2O_2 via Fenton reaction catalyzed by chromium or ferrous ions.

Glutathione Peroxidase (GPx)

All glutathione peroxidases may catalyze the reduction of H_2O_2 using glutathione (GSH) as a substrate. They can also reduce other peroxides (e.g., lipid peroxides in cell membranes) to alcohols.

$$ROOH + 2\,GSH \rightarrow ROH + GSSG + H_2O \quad (3)$$

GPx is responsible for detoxification of low H_2O_2 amounts, while in higher H_2O_2 amounts, catalase takes the leading part in cellular detoxification [15].

Glutathione-Related Systems

In addition to enzymatic defenses described above, there is an intracellular non-enzymatic defense system to protect cellular constituents against ROS and for maintaining the redox state. Glutathione (GSH) is the most abundant intracellular thiol-based antioxidant, present in millimolar concentrations in all aerobic cells, eukaryotic and prokaryotic [48]. It is a sulfhydryl buffer, detoxifies compounds through conjugation reactions catalyzed by glutathione S-transferases, directly, as in the case with peroxide in the GPx-catalyzed reaction [47] or with Cr(VI) [49]. GSH is capable of reacting with Cr(VI) to yield Cr(V), Cr(IV), GSH thiyl radicals and Cr(III)-GSH complexes [50, 51]. The ratios of reduced-to-oxidized glutathione (GSH/GSSG) in normal cells are high (> 10 : 1), as the enzyme, glutathione reductase, help to reduce oxidized glutathione in the following reaction:

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \quad (4)$$

The NADPH required is from several reactions, the best known from the oxidative phase of pentose phosphate pathway [15]. Both, glutathione reductase and glucose-6-phosphate dehydrogenase are involved in the glutathione recycling system [52].

4.2. Secondary Antioxidant Defenses

Although efficient, the antioxidant enzymes and compounds do not prevent the oxidative damage completely. A series of damage removal and repair enzymes deal with this damage. Many of these essential maintenance and repair systems become deficient in senescent cells, thus a high amount of biological "garbage" is accumulated (e.g., intralysosomal accumulation of lipofuscin) [53, 54]. Age-related oxidative changes are most common in non-proliferating cells, like the neurons and cardiac myocites, as there is no "dilution effect" of damaged structures through cell division [33]. The ability to repair DNA correlates with species-specific lifespan, and is necessary, but not sufficient for longevity [55]. There is an age-related decline in proteasome activity and proteasome content in different tissues (e.g. rat liver, human epidermis); this leads to accumulation of oxidatively modified proteins [56]. Proteasomes are a part of the protein-removal system in eukaryotic cells. Proteasome activity and function may be decreased upon replicative senescence. On the other hand, proteasome activation was shown to enhance the survival during oxidative stress, lifespan extension and maintenance of the juvenile morphology longer in specific cells, e.g. human primary fibroblasts [57]. The total amount of oxidatively modified proteins of an 80-year-old man may be up to 50% [58]. Besides, elevated levels of oxidized proteins, oxidized lipids, advanced DNA oxidation and glycoxidation end products are found in aged organisms [7, 59, 60]. Torres and Perez [61] have shown that proteasome inhibition is a mediator of oxidative stress and ROS production and is affecting mitochondrial function. These authors propose that a progressive decrease in proteasome function during aging can promote mitochondrial damage and ROS accumulation. It is likely that changes in proteasome dynamics could generate a prooxidative conditions that could cause tissue injury during aging, *in vivo* [61].

Numerous studies have reported age-related increases in somatic mutation and other forms of DNA damage, indicating that the capacity for DNA repair is an important determinant of the rate of aging at the cellular and molecular levels [62, 63]. An important player in the immediate cellular response to ROS-induced DNA damage is the enzyme poly(ADP-ribose) polymerase-1 (PARP-1). It recognizes DNA lesions and flags them for repair. Grube and Burkle [64] discovered a strong positive correlation of PARP activity with the lifespan of species: cells from long-lived species had higher levels of PARP activity than cells from short-lived species.

The DNA-repair enzymes, excision-repair enzymes, operate on the basis of damage or mutilation occurring to only one of the two strands of the DNA. The undamaged strand is used as a template to repair the damaged one. The excision repair of oxidized bases involves two

DNA glycosylases, Ogg1p and Ntg2p to remove the damaged bases, like 7,8-dihydro-8-oxoguanine, 2,6-diamino-4-hydroxy-5-n-methylformamidopyrimidine, thymine glycol, and 5-hydroxycytosine (reviewed in 65). Lipid peroxides or damaged lipids are metabolized by peroxidases or lipases. Overall, antioxidant defenses seems to be approximately balanced with the generation of ROS *in vivo*. There appears to be no great reserve of antioxidant defenses in mammals, but as previously mentioned, some oxygen-derived species perform useful metabolic roles [66]. The production of H_2O_2 by activated phagocytes is the classic example of the deliberate metabolic generation of ROS for organism's advantage [67].

4.3. Exogenous Antioxidant Defenses: Compounds Derived from the Diet

The intake of exogenous antioxidants from fruit and vegetables is important in preventing the oxidative stress and cellular damage. Natural antioxidants like vitamin C and E, carotenoids and polyphenols are generally considered as beneficial components of fruits and vegetables. Their antioxidative properties are often claimed to be responsible for the protective effects of these food components against cardiovascular diseases, certain forms of cancers, photosensitivity diseases and aging [68]. However, many of the reported health claims are based on epidemiological studies in which specific diets were associated with reduced risks for specific forms of cancer and cardiovascular diseases.The identification of the actual ingredient in a specific diet responsible for the beneficial health effect remains an important bottleneck for translating observational epidemiology to the development of functional food ingredients. When ingesting high amounts of synthetic antoxidants, toxic pro-oxidant actions may be important to consider [68].

4.4. Adaptive responses and hormesis

The adaptive response is a phenomenon in which exposure to minimal stress results in increased resistance to higher levels of the same stressor or other stressors. Stressors can induce cell repair mechanisms, temporary adaptation to the same or other stressor, induce autophagy or trigger cell death [69]. The molecular mechanisms of adaptation to stress is the least investigated of the stress responses described above. It may inactivate the activation of apoptosis through caspase-9, i.e. through the intrinsic pathway, one of the main apoptotic pathways [70, 117]. Early stress responses result also in the post-translational activation of pre-existing defenses, as well as activation of signal transduction pathways that initiate late responses, namely the *de novo* synthesis of stress proteins and antioxidant defenses [65]. Hormesis is characterized by dose-response relationships displaying low-dose stimulation and high-dose inhibition [71]. Hormesis is observed also upon the exposure to low dose of a toxin, which may increase cell's tolerance for greater toxicity [35]. Reactive oxygen species (ROS) can be thought of as hormetic compounds. They are beneficial in moderate amounts and harmful in the amounts that cause the oxidative stress. Many studies investigated the

induction of adaptive response by oxidative stress [72, 73, 74, 75]. An oxidative stress response is triggered when cells sense an increase of ROS, which may result from exposure of cells to low concentrations of oxidants, increased production of ROS or a decrease in antioxidant defenses. In order to survive, the cells induce the antioxidant defenses and other protective factors, such as stress proteins. Finkel and Holbrook [35] stated that the best strategy to enhance endogenous antioxidant levels may be the oxidative stress itself, based on the classical physiological concept of hormesis.

The enzymatic, non-enzymatic and indirect antioxidant defense systems could be involved in the induction of adaptive response to oxidative stress [76, 77, 78, 79, 80, 81]. It was observed, that a wide variety of stressors, such as pro-oxidants, aldehydes, caloric restriction, irradiation, UV-radiation, osmotic stress, heat shock, hypergravity, etc. can have a life-prolonging effect.The effects of these stresses are linked also to changes in intracellular redox potential, which are transmitted to changes in activity of numerous enzymes and pathways. The main physiological benefit of adaptive response is to protect the cells and organisms from moderate doses of a toxic agent [82, 69]. As such, the stress responses that result in enhanced defense and repair and even cross protection against multiple stressors could have clinical or public-health use.

4.5. Sequestration of metal ions; Fenton-like reactions

Many metal ions are necessary for normal metabolism, however they may represent a health risk when present in higher concentrations. Increased ROS generation has been implicated as a consequence of exposure to high levels of metal ions, like, iron, copper, lead, cobalt, mercury, nickel, chromium, selenium and arsenic, but not to manganese and zinc. The above mentioned transition metal ions are redox active: reduced forms of redox active metal ions participate in already discussed Fenton reaction where hydroxyl radical is generated from hydrogen peroxide [83]. Furthermore, the Haber-Weiss reaction, which involves the oxidized forms of redox active metal ions and superoxide anion, generates the reduced form of metal ion, which can be coupled to Fenton reaction to generate hydroxyl radical [15].

Fenton reaction

$$\text{Metal}^{(n+1)} + \text{H}_2\text{O}_2 \rightarrow \text{Metal}^{(n+1)+} + \text{HO}^{\cdot} + \text{OH-} \quad (5)$$

Haber-Weiss reaction

$$\text{Metal}^{(n+1)+} + 2\text{O}_2^{-\cdot} \rightarrow \text{Metal}^{(n+1)} + \text{O}_2 \quad (6)$$

Redox cycling is a characteristic of transition metals [84], and Fenton-like production of ROS appear to be involved in iron-, copper-, chromium-, and vanadium-mediated tissue damage [85]. Increases in levels of superoxide anion, hydrogen peroxide or the redox active metal

ions are likely to lead to the formation of high levels of hydroxyl radical by the chemical mechanisms listed above. Therefore, the valence state and bioavailability of redox active metal ions contribute significantly to the generation of reactive oxygen species.

- The consequence of formation of free radicals mediated by metals are modifications of DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno and/or propano adducts). The unifying factor in determining toxicity and carcinogenicity for all these metals is the abitliy to generate reactive oxygen and nitrogen species. Common mechanisms involving the Fenton reaction, generation of the superoxide radical and the hydroxyl radical are primarily associated with mitochondria, microsomes and peroxisomes. Enzymatic and non-enzymatic antioxidants protect against deleterious metal-mediated free radical attacks to some extent; e.g., vitamin E and melatonin can prevent the majority of metal-mediated (iron, copper, cadmium) damage both in *in vitro* systems and in metal-loaded animals [86, 87].

Iron Chelators

A chelator is a molecule that has the ability to bind to metal ions, e.g. iron molecules, in order to remove heavy metals from the body. According to Halliwell and Gutteridge [22] chelators act by multiple mechanisms; mainly to i) alter the reduction potential or accessibility of metal ions to stop them catalysing $OH^{\cdot}$ production (e.g. transferrin or lactoferrin) ii) prevent the escape of the free radical into solution (e.g. albumin). In this case the free radicals are formed at the biding site of the metal ions to chelating agent. Chelators can be man-made or be produced naturally, e.g. plant phenols. Because the iron catalyzes ROS generation, sequestering iron by chelating agents is thought to be an effective approach toward preventing intracellular oxidative damage. Many chelating agents have been used to inhibit iron- or copper-mediated ROS formation, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepenta-acetic acid (DETAPAC), N,n'-Bis- (2-Hydroxybenzyl)ethylenediamine-N,n'-diacetic acid (HBED), 2-3-Dihydroxybenzoate, Desferrioxamine B (DFO), deferasirox (ICL 670), N,N'-bis-(3,4,5-trimethoxybenzyl) ethylenediamine N,N,-diacetic acid dihydrochloride (OR10141), phytic acid, PYSer and others (for details see 22).

Desferrioxamine can react directly with several ROS and is used as iron(III) chelator for prevention and treatment of iron overload in patients who ingested toxic oral doses of iron [22]. Also, the intracellular protein ferritin plays a role in cellular antioxidant defense. It binds nonmetabolized intracellular iron, therefore, aids to regulation of iron availability. In this way it can decrease the availability of iron for participation in Fenton reaction and lipid peroxidations. Body iron burden can be assessed by using a variety of measurements, such as serum ferritin levels and liver iron concentration by liver biopsies [for detailed information see 88, 89, 90].

4.6. Stabilizing mitochondrial ROS production

Oxidative stress and oxidative damage accumulation could be decreased by regulating the electron leakage from electron transport chain and the resultant ROS production [44]. Nutritional and lifestyle modifications may decrease mitochondrial ROS formation, e.g. by caloric restriction (CR), sport activities and healthy eating habits. The anti-aging action of caloric restriction is an example of hormesis [91, 92, 93]. The works of Yu and Lee [94], Koizumi et al. [95] and Chen and Lovry [96] imply that food restriction (energetic stress) increases the overall antioxidant capacity to maintain the optimal status of intracellular environment by balancing ROS in CR thus promotes the metabolic shift to result in more efficient electron transport at the mitochondrial respiratory chain [97]. In this way, the leakage of electrons from the respiratory chain is reduced [98, 99]. There are reports of slower aging by intermittent fasting without the overall reduction of caloric intake [100, 101]. Since it is extremely hard to maintain the long-term CR, the search is on for CR mimetics. These are the agents or strategies that can mimic the beneficial health-promoting and anti-aging effects of CR. Several compounds have been tested for a potential to act as CR mimetic; such as plant-derived polyphenols (e.g., resveratrol, quercetin, butein, piceatannol), insulin-action enhancers (e.g., metformin), or pharmacological agents that inhibit glycolysis (e.g., 2-deoxyglucose) [102].

Mitochondrial uncoupling has been proposed as a mechanism that reduces the production of reactive oxygen species and may account for the paradox between longevity and activity [103]. Moderate and regular exercise enhances health and longevity relative to sedentary lifestyles. Endurance training adaptation results in increased efficiency in ATP synthesis at the expense of potential increase in oxidative stress that is likely to be compensated by enhanced activities of antioxidant enzymes [104] and proteasome [105]. Exercise requires a large flux of energy and a shift in substrate metabolism in mitochondria from state 4 to state 3. This shift may cause an increase in superoxide production [106]. Indeed, a single bout of exercise was found to increase the metabolism and oxidative stress during and immediately after exercise [107, 108, 109]. While a single bout of exercise of sedentary animals is likely to cause increased detrimental oxidative modification of proteins [110], moderate daily exercise appears to be beneficial by reducing the damage in rat skeletal muscle [105]. Organisms exposed to oxidative stress often decrease their rate of metabolism [111, 112]. Metabolic uncoupling may reduce the mitochondrial oxidant production [113]. It may account for the paradox between longevity and activity [103]. Heat is produced when oxygen consumption is uncoupled from ATP generation. When the mitochondria are uncoupled and membrane potential is low animals might produce less free radicals when expending the most energy [114]. Postprandial oxidative stress is characterized by an increased susceptibility of the organism toward oxidative damage after consumption of a meal rich in lipids and/or carbohydrates [115]. The generation of excess superoxide due to abundance of energy substrates after the meal may be a predominate factor resulting in oxidative stress and a decrease in nitric oxide. A mixture of antioxidant compounds is required to provide protection from the oxidative effects of postprandial fats and sugars. No specific antioxidant can be claimed to be the most important, as consumption of food varies enormously in humans. However, a variety of polyphenolic compounds derived from plants appear to be effective dietary antioxidants, especially when consumed with high-fat meals [116].

5. Conclusion and perspectives

In conclusion, excessive production of ROS and reduced antioxidant defence with age significantly contribute to aging. It seems that oxidative damage is the major cause and the most important contributor to human aging. Antioxidant defense seems to be approximatly balanced with the generation of oxygen-derived species in young individuals, however, there is an increase of oxidative stress later in life. Then the approaches to lower the increased ROS formation in our bodies could be implemented by avoiding the exposure to exogenous free radicals, by intake of adequate amounts of antioxidants and/or by stimulating the damage-repair systems of the cells [44 and references within].

Developing natural or pharmacological agents capable of increasing the antioxidative protection and/or modulating the endogenous defense and repair mechanisms may potentially improve health, increase longevity and contribute to treatment of degenerative age-related diseases, such as cardiovascular and neurodegenerative disorders and cancer. The lifestyle changes, e.g. regular physical activity, increased intake of fruits and vegetables, and reduced calorie intake may improve health and increase cellular resistance to stress. Synthetic antioxidant supplements may help to correct the high levels of oxidative stress that cannot be controlled by the sinergy of endogenous antioxidant systems.

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