Chapter

Metabolic Syndrome as the First Stage of Eldership; the Beginning of Real Aging

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Abstract

The history of active worldwide scientific research on mechanisms of aging and the age-associated diseases counts more than five decades. Of these, among the numerous theories of aging, at least 50 years dominated the free radical theory of aging. Since mitochondria were found to be the major producers of free radicals, the research on aging became largely centered on mitochondria. At the end of 80s of the 20th century, physicians have established a new nosological entity named "Metabolic syndrome" comprising several simultaneously existing symptoms and risk factors, which increase with age to 47% in men and 64% for women. The diagnosis of metabolic syndrome (MetS) requires simultaneous presence of at least three out of five medical conditions: visceral obesity, hypertension, high blood sugar, insulin resistance, low serum high-density lipoprotein accompanied with high serum triglycerides. However, from the beginning of the definition of MetS there was, and still is, a rather lovely debate, which of the symptoms must be considered as the main one. In spite of the enormous number of publications on both mechanisms of aging and MetS, there was relatively small progress in understanding the fundamental processes in these closely related problems. On the contrary, the mitochondrial free radical theory was found to be wrong in its current paradigms. In this Chapter we will discuss recent discoveries and hypotheses which open new perspectives in both theoretical and practical approaches to the problems of aging and MetS. We will show how aging and development of MetS are closely related to each other and the normal ontogenesis of human beings. Why men and women have different rates of aging and mechanisms of transition to MetS. We state that MetS is not just a cluster of symptoms, but one of the last steps of individual ontogenesis, namely the first step of eldership when the aging rate may increase manifold.

Keywords: aging, eldership, energy metabolism, fatty acid oxidation, isoprostane lipid peroxidation, mitochondrial DNA, metabolic syndrome, obesity, ontogenesis, oxidative stress, perhydroxyl radical, reactive oxygen species, ROS, superoxide radical, type 2 diabetes

1. Introduction

Everybody wants to live a long and healthy life. However, the universal laws of the Irreversible Thermodynamics drive changes in our bodies from the moment of birth,

when a human baby has maximum information in his genes and minimum entropy in his body, through a series of consecutive changes to the last stage, when a human body has much less information left in the remaining old genes and maximum entropy in his body, which finally fails and the person dies.

We intentionally started our Chapter by mentioning the genetically predetermined stages of development of the human organism because, as it happened, the concept of ontogeny was somehow lost during the decades of research on mechanisms of aging and metabolic syndrome. As we will see, this approach gives completely different perspectives on the problems from the point of view of normal postembryonic ontogenesis.

The problems of aging and life longevity are not just medical problems, but are complex of fundamental biological problems, which comprise evolution, ontogenesis, genetics, epigenetics, and interactions with the environment. For this reasons, researchers studied aging and longevity starting from simple organisms like yeast and worms, then more complex laboratory animals, and even species like crocodiles and birds. Humans are, probably, much less studied in this respect than, say, mice and rats. To this day there are dozens of aging theories, which reflect the complexity of the problem. We mention only few of the relatively recent theories of aging: The heterochromatin loss model [1, 2]; adult stem cell and mesenchymal progenitor theory [3]; hormonal regulation of longevity in mammals [4]; telomere hypothesis of aging [5, 6]; epigenetic theory [7], and finally, currently the most popular and experimentally developed the free radical hypothesis of aging [8–21]. All theories of aging and longevity are interrelated, but so far, there is no generalizing theory. Therefore, we will start our discussions on human aging mechanisms from the currently most important theory of aging: the mitochondrial free radical theory.

2. The mitochondrial free radical theory of aging (MFRTA)

Initially proposed by Harman [8], the "Free Radical Hypothesis of Aging" was later transformed to the "Mitochondrial Free Radical Theory of Aging" (MFRTA), because mitochondria were found to be the main source of free radicals [9–14, 19, 22]. Collectively, the free radicals derived from oxygen were named "reactive oxygen species" (ROS).

In order to understand the logic of the worldwide research on aging development, it would be useful to look back on the intellectual and scientific background existing 40–50 years ago. After discovery that mitochondria generate oxygen radicals [23, 24], there was an excitement in finding the "universal mechanism" of all diseases. Therefore, for a long-time research on biological effects of ROS was titled "oxidative stress", of which aging was only one of many damaging effects of ROS. For some time, it was not realized that organs and tissues age at different pace, and that environmental conditions, such as radiation, air contamination or industrial pollutants may contribute to the aging process.

Importantly, for a long time any clear-cut specific markers of systemic aging were not known, except mutations of mitochondrial DNA (mtDNA). There is a strong parallelism between production of ROS and mutations of mtDNA [13, 14, 25]. Until recently, this parallelism was explained by a belief that hundreds of "naked" circular mtDNA molecules lie in the mitochondrial matrix and thus mtDNA is an easy target for free radicals. The accumulation in cells of mtDNA damaged by ROS progressively inactivates the DNA templates necessary to repair damaged mitochondria. As a result, the accumulation of mtDNA mutations acts as the aging clock [25].

Understandably, mutations of mtDNA became one of the most important factors in explaining mechanisms of aging, age-associated diseases and practically most major human diseases [25–29]. Only recently it was discovered that mtDNA are protected by the proteinaceous "shield", nucleoids, and that there is no proof for the free radical direct effects on mtDNA [30–33].

Production of ROS occurs not only in organs and tissues, but also in blood cells where radicals evidently have functions different from those in parenchymal cells. The "respiratory burst" of phagocytic cells, when they come in contact with bacteria or immune complexes, is important source of superoxide radicals ($O_2^{\bullet-}$). Phagocytic cells include neutrophils, monocytes, macrophages and eosinophils known to produce large amounts of $O_2^{\bullet-}$ [34].

In some cells, particularly in hepatocytes, the major source of ROS may be of extramitochondrial origin [35]. In the liver, both $O_2^{\bullet-}$ and H_2O_2 are produced during metabolism of xenobiotics by the microsomal P-450-monoxygenases [36], and in the course of catabolism of purine nucleotides and nucleosides by xanthine oxidoreductase [37]. Liver peroxisomes also produce large amounts of hydrogen peroxide during catabolism of very long chain fatty acids and polyunsaturated fatty acids [38]. However, in the liver both mitochondria and peroxisomes possess high catalase activity, which neutralizes hydrogen peroxide [38, 39]. In addition to catalase and superoxide dismutase (isoforms 1 and 2) high activities, liver has high activities of glutathione S-transferase (GST) and glutathione peroxidase [40, 41], and Prohibitin-1 [42], which enhance the liver's antioxidant system. For this reason, liver is relatively protected from deleterious effects of ROS, has high regenerative capacity [43] and, therefore, the rate of aging of this organ is much slower, as compared with other organs [44, 45]. Kidneys also rarely create problems for elderly people because they work constantly at a relatively even pace. In the actively working organs production of ROS is minimal [21]. The fastest rates of aging occur in those organs, which have a wide range of workloads, such as skeletal muscles, brain and heart. These organs have very high capacities in respiratory activity and ATP production to satisfy the organ's energy demands at high workloads. These organs usually have increased ROS production at lower workloads or at rest [21].

Thus, aging is not an evenly distributed over the body process. In addition, mechanisms of aging are distinct in different organs and tissues, and the causing aging oxidants in various organs and tissues can also be different. In order to clarify the last statement and for the sake of the following discussion on the shortcuts of the current paradigm of the MFRTA, we give a brief description of the major biological and environmental radicals and biologically active molecules.

2.1 Superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)

Superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are quantitatively the main species of ROS that are produced constantly by the mitochondrial respiratory chain [21, 22]. Superoxide radicals serve also as a source for other ROS: hydroperoxyl radical, peroxynitrite, lipid radicals. The proportion of H_2O_2, produced at the sites of the respiratory chain is small [21], but the superoxide in many tissues rapidly dismutate to hydrogen peroxide by very high activities of superoxide dismutases in the cytosol (Cu,Zn-SOD1) and mitochondrial matrix (Mn-SOD2) [22, 46]. At the beginning of the mitochondrial free radical theory, superoxide radical was regarded as very dangerous [34]. However, soon it was realized that after $O_2^{\bullet-}$ leaves the membrane's lipid phase, small and negatively charged superoxide anion instantly acquires the hydration shell and thus loses most of its chemical activity [47]. In addition, due to the high activities of SOD1 and SOD2, present at micromolar concentrations, the

superoxide radical half-life is very short (milliseconds) [22]. For this reason attention was shifted to other radicals, which can be formed from the superoxide and hydrogen peroxide. Nevertheless, $O_2^{\bullet-}$ can directly damage enzymes, which contain 4Fe-4S clusters by knocking out one Fe^{2+} atom and turning the 4Fe-4S cluster to inactive 3Fe-4S. A typical enzyme is aconitase, which is sensitive to inhibition by superoxide.

2.2 Hydroxyl radical ($^{\bullet}OH$)

Hydrogen peroxide (H_2O_2), which by itself is a rather harmless chemical, in the presence of transition metal ions Fe^{2+} and Cu^{1+} produces highly aggressive hydroxyl radical ($^{\bullet}OH$) [34]. However, $^{\bullet}OH$ is so active that it instantly reacts with any molecule it encounters (except water). For this reason $^{\bullet}OH$ half-life is only 10^{-9} sec [48] and under normal conditions it is not as harmful as initially believed. This radical is dangerous when formed in very large quantities, such as after exposure to radiation, or high concentration of H_2O_2 and transition metal poisoning. The latter situation is often observed in the experiments *in vitro*, but hardly takes place *in vivo,* since transition metals in the cells are always chelated.

2.3 Nitric oxide ($^{\bullet}NO$) and peroxynitrite ($OONO^-$)

Tissues, such as blood vessels endothelium, neurons and others, which possess tissue-specific nitric oxide synthases (eNOS), produce a free radical nitric oxide ($^{\bullet}NO$), an important cellular signaling molecule involved in many physiological and pathological processes. Formation of one molecule of $^{\bullet}NO$ requires two molecules of O_2, and since the $^{\bullet}NO$ half-life *in vivo* is about 7 sec., for the maintenance of a steady-state concentration of $^{\bullet}NO$ at 1 μM, the consumption of oxygen is very high, about 120 nanomol O_2 per 1 gram of tissue per1 minute [49]. For this reason, the physiologically sufficient steady state levels of $^{\bullet}NO$ can be formed only in the oxygen rich cells, like vascular endothelium, or well vascularized organs like brain. $^{\bullet}NO$ is a powerful vasodilator. The biological effects of $^{\bullet}NO$ can be inhibited due to its fast reaction with superoxide radical yielding a rather toxic product peroxynitrite ($OONO^-$) [50]. Peroxynitrite is a strong oxidant and nitrating agent, it interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. *In vivo*, peroxynitrite generation represents an important pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders [49, 51]. Peroxynitrite plays an important role in pathologies and aging of some organs and tissues, such as blood vessels and neurons, but being a rather strong anion, it hardly contributes to the systemic mechanism of aging, which we will discuss a little later. Nitric oxide is hydrophobic, but chemically weak radical, and, thus, also can be excluded from the systemic aging mechanism, which occur in the lipid phase of the inner mitochondrial membrane.

At this point, we have not discussed the protonated form of superoxide radical ($O_2^{\bullet-}$), namely hydroperoxyl radical ($^{\bullet}HO_2$) because it will be done in conjunctions with the description of polyunsaturated fatty acids (PUFA) autoxidation, named "Isoprostane Pathway of Lipid Peroxidation" (IPLP), which we propose as the main mechanism of aging in people with Metabolic syndrome.

2.4 Environmental biologically active molecules and radicals

Unlike the above described ROS, which are formed in the body and relatively well-studied, the biological effects of sun radiation and air pollutants involving

singlet oxygen and ozone are less known. However, they are the major causes of accelerated aging of skin and lung epithelium. For this reason, we provide a brief description of toxic effects of these ROS.

2.4.1 Singlet oxygen

Singlet oxygen is the common name for the two metastable states of molecular oxygen, but the singlet $O_2{}^1\Delta g$ is the most active in biological systems. It has no unpaired electrons and therefore is not a radical, but upon excitation, one of the O_2 electrons shifts to a higher and unstable orbit, which makes it chemically more active than regular triplet O_2. *In vivo* O_2 to $O_2{}^1\Delta g$ excitation occurs when the sunlight fells on human skin containing several biological pigments, such as porphyrins or flavins. This is the reason of early skin aging and skin damages in people with abnormal porphyrin metabolism. Formation of singlet O_2 by sunlight occurs also *in vivo* in both lens and retina of the mammalian eye, which causes cataract and loss of vision [52]. Thus, wearing dark glasses and a hat, even in not very bright weather, will protect eyes from cataracts early development and skin from early aging. Studies of plants suggested that in photosynthetic systems one of the functions of polyenes and carotenoids is to protect plants from damages caused by singlet oxygen. Therefore, a carrot salad dressed with vegetable oil may have protective effect against complications caused by bright sunlight.

2.4.2 Ozone (O_3)

Ozone (O_3) is an allotrope of oxygen and is much less stable than normal O_2. It forms under the influence of ultraviolet light and during atmospheric electrical discharges (lightning). Even at low concentrations ozone causes damages to respiratory tracts of experimental animals and organic materials, such as latex and various types of plastic. The ozone's half-life depends on temperature, humidity and air circulation. In a closed room with running fan, ozone's half-life is about 24 hours [53]. That is, in a laboratory room, an instrument, like spectrophotometer or spectrophluorometer without special device for burning ozone (for example, instruments from Perkin Elmer), after several hours of work may create a concentration of ozone high enough to cause a headache and errors in experimental results due to accumulation of peroxides in water solutions.

2.4.3 Nitrogen dioxide ($^{\bullet}NO_2$)

For the human's health it is important that in industrial areas with chemical factories and coal power stations the air may be polluted with nitrogen dioxide that may damage skin and respiratory organs. $^{\bullet}NO_2$ induces oxidation, as well as cell's membrane proteins nitration. Nitrated biological products, for example, tyrosine-containing proteins and nitrolipids are often found in the body. It is likely, that $^{\bullet}NO_2$ has been involved in the formation of these products [54].

2.4.4 Peroxynitrates

Nitrogen dioxide quickly reacts with other radicals. This is one of nitrotyrosines formation important mechanisms. During reaction $^{\bullet}NO_2 + O_2^{\bullet-} \rightarrow O_2NOO^{\bullet}$ very rapidly forms peroxynitrate: It contributes to skin and lung damage mechanisms during their contact with air polluted with nitrogen dioxide [54].

3. Crisis of the mitochondrial free radical theory of aging (MFRTA)

From the described above different free radicals and environmental pollutants, we see that all of them have the potential ability to cause damages to cellular and mitochondrial functions, although by different mechanisms. Some of the biologically active molecules and radicals show clear tissue and organ specificities. For example, singlet oxygen damages predominantly skin and eyes; ozone and nitrogen dioxide - lung epithelium, nitric oxide and peroxynitrite - vascular endothelium and neuronal cells. However, none of the above ROS is directly related, with the exception of the superoxide radical, to the inevitable aging mechanism [55].

After decades of research a vast amount of accumulated knowledge revealed serious inconsistencies between the data obtained and the MFRTA, which call into question the correctness of the free radical theory in its current paradigms. We refer the reader to excellent reviews on this topic [16, 18, 56–58]. Taking into consideration the controversies regarding various species, in this Chapter we will focus only on those inconsistencies that directly relate to the topic of humans and mammalians aging mechanism. Animals are often used for modeling of aging mechanisms [59–62]. We shall discuss the following most important for MFRTA facts, which undermine this theory.

3.1 MtDNA are protected from the direct effects of reactive oxygen species

Recently, it has been established that neither of the above listed ROS and biologically active molecules are capable to cause directly mutations of mtDNA, which was and still is for many researchers the main hallmark of the aging process and it is considered as the main pathogenic mechanism of many diseases [30–33]. From the beginning of free radical theory of aging, mutations of mtDNA were the only reliable markers of oxidative stress. As a matter of fact, the MFRTA itself arose on the basis that changes in the production of ROS were always accompanied by parallel changes in the number of mtDNA mutations [13, 14, 25]. Recently, however, it was concluded that there was no reliable evidence for the direct involvement of ROS in mtDNA mutations [31, 57]. There are two main reasons for this conclusion. First, the commonly studied radicals are not active enough to cause mutations [55]. Secondly, mtDNA are encased into a protein coating of nucleoid, which prevents direct contact of mtDNA and radicals [31].

3.2 Antioxidant supplementation interventions do not increase longevity

Antioxidant supplementation interventions do not increase longevity, as would be predicted by the MFRTA. This Antioxidant Paradox is considered as the strongest evidence against the MFRTA; it comes from studies that manipulate antioxidant levels. Many studies have shown that administration of low-molecular weight antioxidants failed to extend longevity [reviewed in 16, 56, 57, 63]. Barja (2014) suggested that the lack of antioxidants to exert effects on longevity could be explained by the spatial separation: a free radical, which causes aging, acts in the lipid phase of membranes, while antioxidants exerts their effects mostly in the water phase of cells [16]. Barja (2014) also summarized the numerous studies of life longevities on various species: “Only two parameters currently correlate with species longevity in the right sense: the mitochondrial rate of reactive oxygen species (mitoROS) production and the degree of fatty acid unsaturation of tissue membranes” [16]. As we will see, these are very correct suggestions.

3.3 The rate of $O_2^{\bullet-}$ production controls lifespan independently of SOD

Muller (2000) has stressed that SOD activity interspecies variation is not correlated with the maximal life span (MLSP) in mammals and the activity of other antioxidant enzymes negatively correlates with MLSP. However, there is a strong negative correlation between longevity and the $O_2^{\bullet-}$ and H_2O_2 production rates by isolated mitochondria from diverse mammalian species. The longevity depends not on the amount of superoxide in a cell, but on the rate of its production [18]. To explain this unexpected observation, Muller suggested that a significant fraction (between 10% and 50%) of $O_2^{\bullet-}$ is not produced as aqueous $O_2^{\bullet-}$ but instead is produced as lipid-phase $HO_2^{\bullet}$ in the inner mitochondrial membrane. In other words, Muller has proposed that it is not the $O_2^{\bullet-}$ in the water phase, but its protonated form - hydroperoxyl radical ($HO_2^{\bullet}$) in the membrane lipid phase exerts damaging effects on longevity, where the radical cannot be affected by a superoxide dismutase. Muller suggested that hydroperoxyl radical may initiate lipid peroxidation and the formation of peroxynitrous acid [18]. Unfortunately, the proposal that the hydroperoxyl radical is the aging main cause remained unnoticed for the next two decades, as well as the earlier similar attempts of other researchers before Muller [48, 63].

4. Hydroperoxyl radical $HO_2^{\bullet}$ as the systemic aging cause

Here we present in brief our current views on the mechanisms of the hydroperoxyl radical formation and its damaging effects, which we consider as the systemic aging main mechanism. The details of the mechanism are presented in recent publications [55, 64–67].

5. Properties of the hydroperoxyl radical or perhydroxyl radical ($^{\bullet}HO_2$)

Lipid phase of the mitochondrial membrane has 4–5 fold higher concentration of oxygen than the cells' water in the cytosol. When O_2 acquires an electron from the respiratory chain and becomes $O_2^{\bullet-}$, it must quickly leave the lipid phase of the membrane. However, before $O_2^{\bullet-}$ riches the bulk of the matrix or cytosol, it crosses the thing layer of the structured water near the charged surfaces of the membrane. The inner leaflet of the inner mitochondrial membrane contains approximately 80–90% of total cardiolipin (CL), which, together with phosphatidylethanolamine (PEA), accommodate respiratory complexes and ATP-synthase into the mitochondrial cristae sharp curves [67]. Since CL bears strong negative charge, at some arears of the inner membrane aggregates of CL form areas with strong negative charge, called antennae, which attract protons [reviewed in 66, 68]. For this reason, the thin layer of structured water near the charged surfaces of the inner mitochondrial membrane has up to three units more acid pH than the bulk of a compartment. This is a very important issue, because 1000 times higher concentration of H^+ increases the probability of the hydroperoxyl radical formation in the $O_2^{\bullet-} + H^+ \leftrightarrow {}^{\bullet}HO_2$ reversible reaction (pK_{α} of the reaction is 4.8) [68]. Highly hydrophobic $^{\bullet}HO_2$ returns back into the membrane's lipid phase. The described mechanism explains why the aging process depends only on the rate of superoxide formation, but not on the concentration of $O_2^{\bullet-}$ and the activities of SODs. Hydroperoxyl radical is a much stronger oxidizing agent than superoxide radical, and has a specific propensity to abstract H atoms from α-tocopherol and,

particularly, from the polyunsaturated fatty acids, such as arachidonic acid (C20:4 n6) and docosahexaenoic acid (C22:6 n3) [69].

For unknown reason, the perhydroxyl radical for a long time was almost completely excluded from the oxidative stress literature [61, 70]. Mitchell (2000) proposed perhydroxyl radical damaging mechanism through formation of peroxynitrite radical in the membrane [18]. This possible situation has been discussed by Gebicki and Bielski [69]. These authors indicated that although both $^{\bullet}NO$ and $^{\bullet}HO_2$ are hydrophobic radicals, the radicals are spatially separated since $^{\bullet}NO$ is normally present in the blood vessel endothelium, and thus the mitochondrial hydroperoxyl radicals have little chance to meet nitric oxide, and even if this might happen, the negatively charged $ONOO^-$ hardly could be a systemic damaging factor because it is immediately excluded from the membrane's lipid phase [69]. Bielski et al. (1983) studied reactions of $^{\bullet}HO_2$ with linoleic (C18:2), linolenic (C18:3) and arachidonic acids (C20:4) in water/ethanol solutions [68]. The obtained kinetic parameters of the reactions indicate that $^{\bullet}HO_2$ reacts with the double allyl hydrogens of polyunsaturated fatty acids, and the more double bonds was present in a PUFA, the more active was the reaction. The abstraction of H atoms by $^{\bullet}HO_2$ was exothermic, which indicates that it is irreversible and highly probable, when $^{\bullet}HO_2$ encounters a PUFA. Since the reactions were performed in the water/ethanol solution, H_2O_2 formed cleaved heterolytically ($2H_2O_2 \rightarrow H_2O + O_2$), and the final products of the fatty acids with $^{\bullet}HO_2$ reactions were stable hydroperoxides without much variation in their structure [68].

6. The isoprostane pathway of lipid peroxidation (IPLP)

When $^{\bullet}HO_2$ reacts inside the lipid phase of the membrane with a PUFA, which is still part of a phospholipid, the products are a racemic mixture of a very large number of various stereo- and positional isomers [71]. Many of these products are similar to enzymatically produced prostaglandins, and were named Isoprostanes (IsoPs). For this reason, this type of PUFA autoxidation was named the Isoprostane Pathway of Lipid Peroxidation (IPLP) [71–73]. IsoPs possess potent biological activity and thus may convey abnormal cellular signaling and inflammation [71, 74]. Furthermore, many products of IPLP are very toxic, such as γ-ketoaldehydes. They form adducts with primary amines of the lysine-containing proteins and phosphatidylethanolamine (PEA). The most active among γ-ketoaldehydes are isolevuglandins (IsoLG) produced from arachidonic acid can be only detected as adducts with proteins or ethanolamine of PEA [74]. In addition to arachidonic acid the most common PUFA among phospholipids, other PUFA such as eicosapentaenoic acid (20:5 n3) and docosahexaenoic acid (22:6 n3), have been found as substrates for the IPLP [75]. Because docosahexaenoic acid (DHA) is present in a larger quantity in neurons, the products of IPLP were correspondingly named neuroprostanes and neuroketals. From arachidonic acid, which has four double bonds, the racemic mixture may contain up to eight hundreds of different products, whereas the products number from the containing 6 double bonds docosahexaenoic acid may be more than one thousand [71–73].

IsoPs and the cyclooxygenase derived prostaglandins (PGs) have a number of distinctions in their origin and properties, which have been discussed in a number of publications [65, 71, 76, 77]. Here we briefly list the most important distinctions: 1) The side chains of normal PGs are almost always oriented *trans* to the prostane ring whereas the products of IPLP have mostly the side chains with *cis* orientation [71, 76]; 2) The IsoPs are formed *in situ* from PUFA, which

are esterified to phospholipids, while PGs are generated exclusively from the free AA and DHA [77]. 3) The products of IPLP are the racemic mixture of molecules with a very large number of possible stereo- and positional isomers, whereas the products of the enzymatically produced prostaglandins have mainly one optical isomer [71, 76].

Figure 1A very schematically presents the pathways of arachidonic acid oxidation, and **Figure 1B** illustrates the suggested mechanism of AA oxidation by $^{\bullet}HO_2$ [65]. The key event after abstraction of the first H atom is that H_2O_2 formed under hydrophobic condition undergoes homolytic cleavage with formation of two molecules of the hydroxyl radicals $H_2O_2 \rightarrow 2\ ^{\bullet}OH$, which instantly subtract another two H atoms from the same PUFA with formation of two molecules of H_2O. The remaining molecule of the AA has completely disarranged double bonds, becomes extremely unstable and quickly attaches randomly two O_2 molecules, and undergoes intramolecular transitions with formation of one out of many possible positional and stereoisomers. Thus $^{\bullet}HO_2$ converts into 2 H_2O and AA loses two out of 4 double bonds, and becomes one of hundreds isoprostanes. The differences between IPLP and the "classical" lipid peroxidation have been discussed in [65, 79].

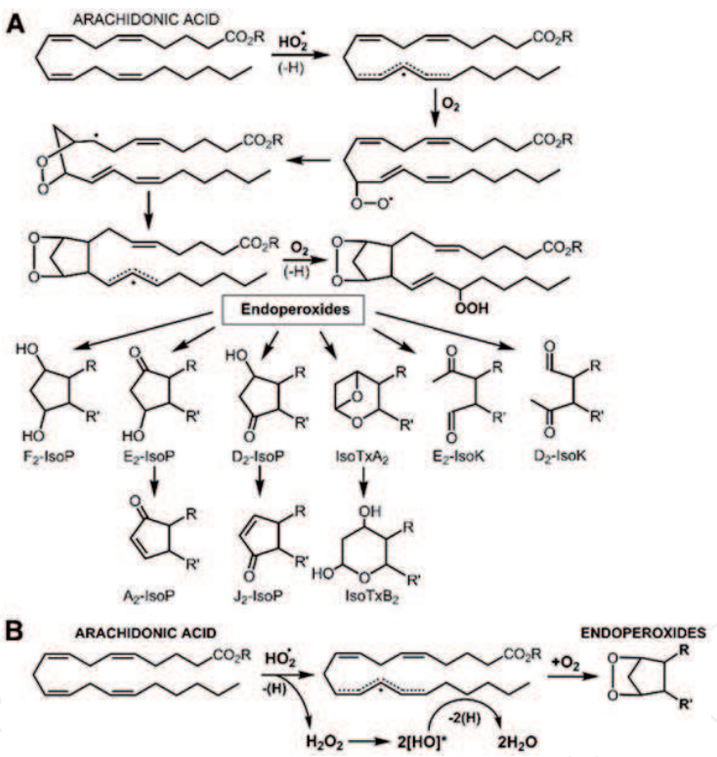

Figure 1.
Autoxidation of arachidonic acid with transformation of the molecule into various ring structures. (A) this part of the figure was adapted from the article [78] and shows different intermediary metabolites during autoxidation of arachidonic acid (AA). $HO_2^{\bullet}$ is the only candidate for initiation of the isoprostane autoxidation of PUFA [65]. (B) the suggested sequence of transformations of $HO_2^{\bullet}$ and AA in the course of isoprostane lipid peroxidation (IPLP) [65]. After abstraction of the first H atom from a molecule of AA, $HO_2^{\bullet}$ turns into H_2O_2, which in the hydrophobic milieu undergoes homolytic cleavage into two molecules of $^{\bullet}OH$ radicals, which instantly subtract the next two H atoms from the AA with formation of two molecules of H_2O and the remains of the AA has completely disarranged double bonds. A very fast abstraction of three H atoms from any of the four double bonds leads to the fact that the residue of the AA becomes extremely unstable and quickly attaches two O_2 molecules in a random way and undergoes intramolecular transitions with formation of one out of many possible positional and stereoisomers. The more PUFA has double bonds, the more possible isomers for the end product of the reaction. Abbreviations: F_2-IsoP, E_2-IsoP, D_2-IsoP – Isoprostanes with, correspondingly, prostane rings F_2, E_2, D_2 or A_2 and J_2; IsoTxA$_2$ and IsoTxB$_2$ – Isothromboxanes with rings A_2 и B_2, correspondingly, which were formed from prostaglandins H_2 (PGH_2); E_2-IsoK и D_2-IsoK – Isoketals with rings E_2 и D_2.

7. IPLP as the mechanism of aging

It has been demonstrated by researchers from the Vanderbilt University that IsoPs are the most early and reliable markers of lipid peroxidation *in vivo*, and recent studies provided valuable information about participation of IPLP in pathogenesis of numerous human diseases [80–82]. According to our model of IPLP initiation by $HO_2^{\bullet}$, the hydroperoxyl radical upon encounter with a PUFA produces one of many variants of isoPG, γ-ketoaldehyde or isolevuglandins. When reacting with fatty acids with two unsaturated bonds, such as linoleic acids of cardiolipin, $HO_2^{\bullet}$ produces corresponding hydroperoxides.

The different toxic products of the IPLP evidently cause numerous and different lesions to mitochondria gradually causing wear and tear of mitochondrial and cellular functions. We distinguish two types of direct lesions to mitochondria: one dysfunctions type is caused by oxidation of CL and PEA, which result in structural changes of respirosomes and ATP-synthase complexes. The second dysfunctions type is caused by direct damages by toxic products, like isolevuglandins, which directly form adducts with PEA and lysine of proteins. This type of damages may explain mtDNA replication damages [33, 34, 83]. Anderson et al. [83] have shown that mtDNA replicase *exo* domain, Pol gamma, is far more sensitive to oxidation than *pol* domain. The authors suggested that under oxidative conditions, exonuclease activity therefore declines more rapidly than polymerase. The oxidized Pol gamma becomes editing-deficient, displaying a 20-fold elevated mutations than the unoxidized enzyme [83]. PEA may be damaged by both pathways: via PUFA at C2 autoxidation, and via formation of adducts of ethanolamine with IsoLG produced upon activation if IPLP by $HO_2^{\bullet}$. Of note, most of the ROS have very short lifetime (seconds) but IsoLG produce rather stable adducts (lifetime days), which can accumulate with age and, therefore, contribute to the development of age-associated conditions.

8. The importance of fatty acids oxidation for increased rate of ROS production

Mammalian tissues mitochondria generate superoxide and hydrogen peroxide (ROS) from 11 different sites depending on substrates used and the redox state of the electron transport chain [21]. All mitochondrial ROS production sites have distinct properties [21, 84]. They can be divided into two groups: six sites operate at the redox potential of the NADH/NAD^+ isopotential pool, about −280 mV, and five sites operate at the redox potential of the ubiquinol/ubiquinone (QH_2/Q) isopotential pool, about +20 mV [21, 84].

Much of the published literature on contribution of separate respiratory complexes in generation of ROS have potential problems for several reasons: first, the authors often used inhibitors of the respiratory chain, which is far from situation *in situ*, secondly, most authors used a single substrate, whereas *in situ* mitochondria metabolize several substrates simultaneously [85–88]. Most importantly and, with few exceptions, authors never used fatty acids as substrates for mitochondrial respiration. Brandt and colleagues provided evidence that the highest rates of ROS production ($O_2^{\bullet-}$ + H_2O_2) are observed during β-oxidation of fatty acids [21, 84, 89]. During β-oxidation of fatty acids the membrane's pool of CoQ becomes fully reduced to $CoQH_2$ and this may reverse the transport of reducing equivalents at the level of succinate dehydrogenase (SDH), also known as Complex II, and thus activate the reverse electron transport and involve the sites of respiratory Complexes I and III, and SDH (Complex II) in production of ROS [21, 89].

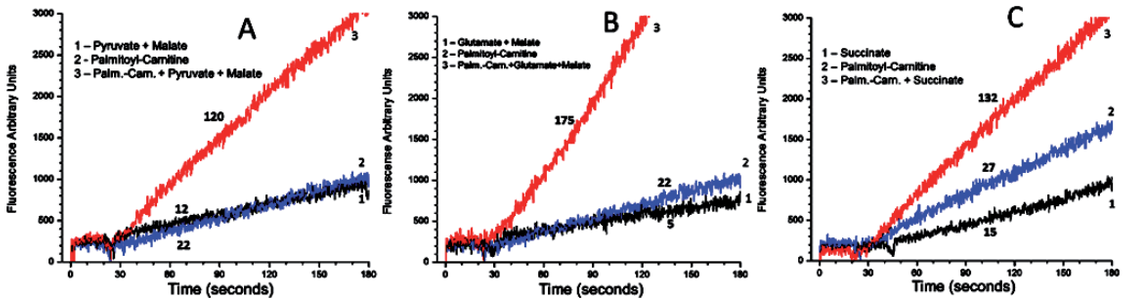

Figure 2.
*Production of superoxide radicals by rat heart mitochondria oxidizing palmitoyl-carnitine. Designations: 1. Supporting substrate only; 2. Palmitoyl-carnitine only, and 3. Palmitoyl-carnitine + supporting substrate. Substrates: **Figure 2A** - pyruvate 2.5 mM + malate 2 mM, **Figure 2B** - glutamate 5 mM + malate 2 mM, and **Figure 2C**. – Succinate 5 mM. Experimental conditions are described in [88]. The incubation medium contained: Amplex red 2 μM, horse radish peroxidase 2 units, substrates as indicated above, volume 1 ml. The reaction was initiated by addition of 50 μg of mitochondria. Initial rates were measured for 3 minutes. Numbers at the traces are the rates of H_2O_2 production in picomol H_2O_2/min/mg protein RHM. The rates were corrected for the time control rate with RHM incubated without added substrates. The figure was taken from [88].*

It has been recently shown that active oxidation of palmitoyl-carnitine by mitochondria in all metabolic states absolutely requires the simultaneous presence of any of the other mitochondrial metabolites such as: pyruvate, succinate, malate or glutamate, which were designated as "fatty acid oxidation supporting substrates" [87, 88]. In the presence of supporting substrates, fatty acids are actively oxidized not only by the isolated heart mitochondria [88], but also by the brain synaptic mitochondria [87], which breaks the old myth that the brain's energy metabolism is supported almost exclusively by glucose [reviewed in 87].

Most importantly, active oxidation of fatty acids in the presence of supporting substrates results in a manifold increase in ROS production in resting mitochondria (**Figure 2**). Earlier, we have proposed that oxidative damages and thus accelerated aging, are more common for organs, which have a wide range of physiological activities, such as heart, skeletal muscles and brain [65, 88]. When these organs are at low workloads or at rest, the very efficient oxidation of fatty acids may redirect excessive electrons to generation of ROS.

9. OK, MRFTA is valid, but what is aging?

In the sections above, we presented evidence that in spite of complications of the MFRTA based on old paradigms, the latest discoveries clearly support the essence of this theory, and the introduction of the perhydroxyl radical as a new mediator of oxidative stress resolve most of the contradictions. However, MFRTA still requires further consideration and we have to find answers to simple questions: what is aging and when the aging begins?

From the beginning, researchers working on MFRTA held the point of view that aging is a pathological process caused by oxidative stress that affects our health, and over time, we succumb to a myriad of age-related pathologies and eventually die [12, 58]. But is aging simply the process of errors accumulation? The Dictionary of Science and Technology designates aging as "the process of growing older or changing over time" [90]. In other words, a person after birth goes through a series of genetically controlled transitions, which are called postembryonic ontogenesis [91]. From the point of view of a human ontogeny, we can roughly divide a person's individual life into five periods: infancy, childhood, adolescence, reproductive period and post-reproductive, or aging period. For several reasons, we hardly expect that during infancy, childhood and adolescence "a myriad of age-related pathologies"

might be accumulated [58]. We will consider the last two periods: "reproductive", which comprises the ages from 20 to 50, and the "aging period", which begins after the age of 50 [92].

Among recent definitions of aging there were few in more general terms: "Aging is characterized by a gradual decline in various health parameters across multiple biochemical, physiological and behavioral systems" [93]. Correspondingly, some researchers started looking for a subset of aging individuals with lack of resilience within these general physiological systems, a condition termed frailty. Frailty has been defined in broad terms as an age-associated syndrome characterized by increased vulnerability to external influences, a diminished capacity to respond correctly to stressors and as an overall loss of fitness. In general, frail individuals are at a greater risk of falls, dependency, disability, institutionalization, hospitalization and mortality [94]. Frailty can be measured in relation to the accumulation of deficits using a frailty index. A frailty index can be developed from most aging databases [95, 96].

In another parallel world of Science, a large group of researchers study the metabolic syndrome, a nosological entity established at the end of 80s of the last century. The metabolic syndrome (MetS) was defined as a condition of simultaneous existence of several risk factors, including obesity, insulin resistance, atherogenic dyslipidemia and hypertension, which are interrelated, age-dependent, and share underlying mediators and metabolic pathways [97]. Undoubtedly, both concepts of frailty and metabolic syndrome are important from a medical perspective, but apparently did little for understanding the mechanisms of aging. Due to intensive research on both concepts, however, there has been made a great discovery: the rates of aging and energy metabolism are sex-specific. In our opinion, these discoveries are crucial for understanding the mechanisms of aging as the process of transition of an individual from the reproduction period to the last stage of ontogeny – aging.

10. Sex-specific differences in the rates of aging and longevity

Most animals and plants are sexual, in spite of the reproductive advantages experienced by asexual variants. Evidently there where selective forces that gave an advantage to sexuality and genetic recombination at either the population or individual level. The effect of sex and recombination increases the efficiency of natural selection, which is a major factor favoring evolution [98, 99]. It has been experimentally shown that sex increases the rate of adaptation to a new harsh environment, but has no measurable effect on fitness in a new benign environment where there is little selection [100]. Nonetheless, we are still far from a definitive answer to the question of why sexual reproduction is so common [99]. Recently, the hypothesis has been put forward that the internal production of ROS 2 billion years ago started the eukaryotic sex (re)evolution [101]. It has also been stressed that earlier theoretical works on sexual reproduction ignored important complexities that face natural populations, such as genetic drift and the spatial structure of populations [102].

The data accumulated show that in many species, including humans, females have slower rate of aging and longer life span than males [99, 103–105]. We suggest that this observation has important general biological goals for a female: bearing and raising a new generation despite any external difficulties and metabolic restrictions. These goals demand that females have to be efficient enough, but not superefficient from the metabolic (thermodynamic) point of view, resilient to harsh environmental conditions, and, in accord with the MFRTA, have lower rate of oxidative stress. Numerous studies of various species showed that in general females

have slower production of ROS than males [99]. As always, there are some exclusions from the general rule, but again they hold the same conclusion: the longer living gender produces less ROS [106].

In the next sections we will discuss possible mechanisms underlying the slower aging and slower rates of ROS production in the longer living females.

11. Sex-specific differences in the rates of fat utilization for the sake of energy metabolism

Laboratory animals are indispensable part of biomedical research and widely used for modeling physiological and pathological situations in humans [107]. For ethical and technical restrictions, it is impossible to study many biomedical problems on humans, whereas animal research provides a degree of experimental control and precision not usually feasible in studies using human subjects [108]. Meanwhile, the animals used in most experiments were males, because researchers usually avoid using females for the reason of their reproductive cycles and hormone fluctuations that may affect the results of their studies [109]. For these reasons research on sex differences has begun relatively recently, but today the related literature is enormous. Human studies on metabolic differences between men and women were stimulated largely due to the progress of the sport medicine. Here, we will discuss only those works, which have direct relation to our subject under discussion: what sex metabolic differences underlie the fact that females live longer than males in many species, including humans [108].

Evidently, the sex differences in the body structure and metabolism depend on the stage of a person's ontogeny. Vijay et al. (2015) studied sexual differences in the expression of mitochondria-related genes in rat heart at different ages that correspond to different stages of the rat's reproductive capacities [110]. The authors studied the whole genome expression profiling in the hearts of young (8-week), adult (21-week), and old (78-week) male and female Fischer 344 rats, and the expression of 670 unique genes related to various mitochondrial functions was analyzed. A significant ($p < 0.05$) sexual dimorphism in expression was observed in young animals for 46, adult for 114 and old rats for 41 genes, respectively [110]. Importantly, in young and adult hearts, sexual dimorphism was not noted in genes encoding oxidative phosphorylation. Adult males showed higher expression of genes associated with the pyruvate dehydrogenase complex as compared to females. In old rats a majority of genes involved in oxidative phosphorylation had higher expression in females. This clearly shows that sexual dimorphism largely depends on the stage of ontogeny. Other studies demonstrated better preservation of myocardial mass and a greater cardiac contractility in women than men during aging [111]. The better heart health in aged women might be the result of either genetically predetermined factors or less oxidative damages and slower aging as compared to men.

It is a well-known observation that women generally have a higher amount of body fat than men. Distribution of fat is also different: women store more fat in the gluteal-femoral region, whereas men have more body fat in the abdominal (visceral) region [112, 113]. Importantly, that visceral fat accumulation is accompanied with multiple endocrine perturbations, including elevated cortisol and androgens in women, as well as low growth hormone and, in men, testosterone secretion. The consequences of the hormones effects will be more expressed in visceral than subcutaneous adipose tissues, because omental fat has higher cellularity, innervation and blood flow. Furthermore, the density of cortisol and androgen receptors seems to be higher in visceral fat than in other regions of adipose tissue [114]. In addition, there are epidemiological and metabolic associations between centralized (visceral)

fat accumulation and disease [114]. This is an important fact because visceral obesity is a common symptom for men and women with metabolic syndrome.

Physiological experiments with oral administration of triglycerides, labeled with a small amount of oleic acid, revealed the following regional differences in the order of lipid uptake: omental = retroperitoneal > subcutaneous abdominal > subcutaneous femoral adipose tissues in men, with a similar rank order for half-life of the triglyceride, indicating also a turn-over of triglycerides in that order. Testosterone amplifies these differences in men. In premenopausal women, the visceral fat accumulation is smaller than in men, and subcutaneous abdominal has a higher turnover than femoral adipose tissue [114]. Among regional gender differences of fat metabolism, there is an interesting evidence that *in vivo*, catecholamine mediated leg free fatty acid release is lower in women than in men, whereas free fatty acid release from the upper body depots is comparable [112].

These experiments *in vivo* indicate that sex variations in fat metabolism are controlled by sex-specific hormones. This presumes that in the post-reproductive stage of ontogenesis, the sexual dimorphism in fat metabolism should be weaker or absent. Results of studies *in vitro* also indicate that this difference is diminished at the menopause, and may be restored by estrogen therapy. This suggest that the functional effects of estrogens in women are similar to those of testosterone in men. As we will show in the next section, the effects of both hormones are targeted on substrate oxidations by mitochondria, and, thus, on the rates of ROS production. However, the mechanism of estrogen on fat metabolism might be indirect because human adipose tissue does not possess specific estrogen and progesterone receptors [114].

As regards protein metabolism, no gender differences in the basal level net muscle protein balance have been found [115]. In general, testosterone increases muscle protein synthesis and net muscle protein balance, resulting in increased muscle mass. At young age, boys and girls have similar amounts of testosterone. At puberty testosterone levels increase much more dramatically in males, as does muscle mass. Furthermore, although no evidence exists in humans, the in-vitro and rat data suggest that ovarian hormones inhibit muscle protein synthesis [115].

12. Sex differences in substrate utilization during physical activities

The indirect effect of the sex hormones on fat metabolism is supported by the data on sexual dimorphism in utilization of fatty acids during physical activities, which demonstrate that the proportion of energy derived from fat during exercise is higher in women than in men [112, 116]. Carter et al. (2001) investigated the effect of endurance training on whole body substrate, glucose, and glycerol utilization during 90 min of exercise in males and females [116]. First, during submaximal physical loads females show a lower respiratory exchange ratio (RER) than males, which indicates on a proportionately lower carbohydrate and higher fat oxidation [116, 117]. In comparison with females, exercising males had a greater increase in leucine oxidation but not lysine levels, which indicated that during intensive physical activity males increase their need for amino acids to fuel energy needs. Under the same conditions, females responded by increased mobilization of fat, thereby requiring less alternative fuels such as carbohydrate and amino acids [116–118]. The overall conclusion of these experiments was that females oxidize a greater proportion of fat and less carbohydrates and amino acids as compared with males. Thus physiologists support our finding that fatty acids oxidation requires simultaneous presence of other mitochondrial metabolites derived from carbohydrates or

proteins [88]. Because women have lesser consumption of supporting substrates, the rate of fatty acids oxidation should be also diminished. This might explain why, in general, women demonstrate lower levels of physical performance during endurance sports and produce less ROS [119–121].

Knowing the fact that mitochondria oxidize fatty acids only in the presence of supporting substrates [87, 88], the data presented above suggest that females require less or different supporting substrates for effective oxidation of fatty acids. We can, therefore, predict that the isolated skeletal muscle or heart mitochondria from females must be different from males in terms of the type and requirement of supporting substrates, and also produce ROS at a slower rate. Unfortunately, until now we have been the only ones who have studied the oxidation of fatty acids in the presence of various supporting substrates, but we used male rats only in our experiments [87, 88]. **Figure 3** illustrates that the rates of ROS production strongly depend on the type of supporting substrates, namely pyruvate, glutamate and succinate, and their various mixtures [55, 87, 88]. Thus the *in vitro* experiments with the isolated mitochondria from different organs require further investigation in order to elucidate molecular mechanisms of the sex diversity at the mitochondrial level. Since males showed higher expression of genes associated with the pyruvate dehydrogenase complex, as compared to females [111], and consume more carbohydrates and amino acids during endurance training [116–118] we can suggest that women's higher longevity and slower rate of aging is associated with the less efficient oxidation of fatty acids and thus slower rates of oxidative stress. However, this conclusion evidently regards only women at the reproductive stage, when, according to Ventura-Clapier et al. (2020), women are "protected" [122]. These authors also stated that "apart from comparisons between males and females, there is a crucial need for studying the female physiology and woman pathology. In particular the biological step that constitutes menopause in women appears to be the border between "female protection" and "female susceptibility" to cardiovascular diseases, which needs to be deciphered further".

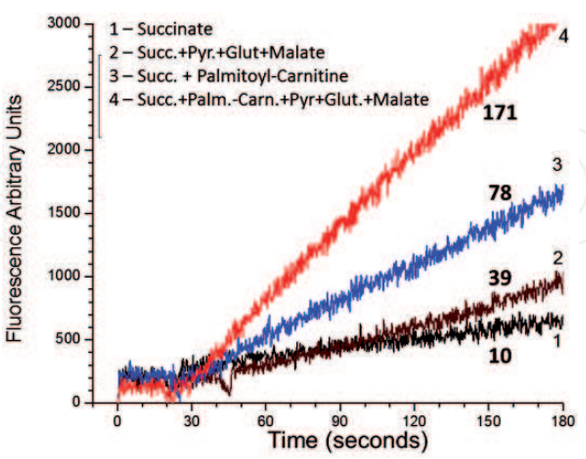

Figure 3.
Effect of substrate mixtures on production of ROS by isolated rat heart mitochondria oxidizing palmitoyl-carnitine. The incubation medium contained: Amplex red 2 μM, horse radish peroxidase 2 units, volume 1 ml. The reaction was initiated by addition of 50 μg of mitochondria. Initial rates were measured for 3 minutes. Substrates: 1. Succinate 5 mM; 2. Succinate + pyruvate 2.5 mM + glutamate 5 mM + malate 2 mM; 3. Palmitoyl-carnitine 50 μM + succinate; 4. Palmitoyl-carnitine + succinate + pyruvate + glutamate + malate. Numbers at the traces are the rates of H_2O_2 production in picomol H_2O_2/min/mg protein RHM. The rates were corrected for the time control rate with RHM incubated without added substrates. The figure was taken from [55].

13. Metabolic syndrome (MetS) as the post-reproductive stage of ontogenesis

Long time ago physicians noticed that many patients have common features of external appearances and biochemical indices of abnormal metabolism, which included insulin resistance, obesity, atherogenic dyslipidemia and hypertension. By the end of 80s of the 20th century the term "Metabolic syndrome" has been accepted and its own diagnostic code: 277.7 has been assigned by the International Classification, 9th Division, Clinical Modification (ICD-9-CM). However, the current definitions of the metabolic syndrome (MetS) give no clues to the essence of MetS and are mostly just listings of symptoms, for example: "The metabolic syndrome is a constellation of metabolic disorders including obesity, hypertension, and insulin resistance, components, which are risk factors for the development of diabetes, hypertension, cardiovascular, and renal disease" [123, 124]. This "clustering" or "constellation" of risk factors were considered to share underlying causes, mechanisms and features. The diagnosis of MetS is accepted only when at least three out of five symptoms are present in a patient [125, 126]. It was early recognized that people with isolated components, but who do not fit the definition of metabolic syndrome, are not at as high a risk for type 2 diabetes (T2D) or cardiovascular diseases (CVD). For example, people with isolated hypertension or isolated hyperlipidemia are at risk of CVD, or people with isolated obesity are at risk for T2D, but less so than people who meet multiple criteria of MetS [123].

It was expected that a comprehensive definition for the metabolic syndrome and its key features will facilitate research into its causes and lead to pharmacologic and lifestyle treatment approaches [127]. However, analysis of the tremendous amount of publications regarding MetS revealed that more than 30 years after defining MetS, there is still no deep understanding how and why MetS develops. Much of the literature can be roughly divided into supporters and opponents of considering insulin resistance as the primary symptom for the diagnosing MetS [123, 128–130]. These discrepancies were reflected in the criteria for diagnosing MetS provided by several Institutions, which were thoroughly reviewed in [123]. The discussions become heated by publications that ethnic and racial factors may greatly affect the criteria for diagnosis of MetS [127, 131].

14. The origin of MetS

The results of genetics studies on the potential hereditary predisposition to MetS were analyzed by Stančakova and Laakso [132]. They concluded that there is only a limited evidence for common genetic background explaining the clustering of the metabolic trait. Instead, the existing evidence suggests the importance of epigenetic mechanisms [132]. This conclusion supports our earlier suggestion [55, 66, 67] that the external appearances and metabolic features of MetS reflect the genetic properties of our distant ancestors. So far, however, aging and MetS have been commonly regarded as the result of accumulation of different kinds of damages caused by oxidative stress and/or improper life style [8–11, 133–135].

As we have stressed earlier, all human beings after birth undergo changes during postembryonic ontogenesis. One of the theories of aging suggests that with the advanced age, the loss of heterochromatin results in altered gene expression [1, 2]. The epigenetic alterations resulting from global heterochromatin loss may be at the root of the various molecular events associated with aging and may tie together the various models of aging [2]. However, the process of ontogenesis in humans suggests that each transition to a new stage switches conversion of a new

portion of heterochromatin into euchromatin, and new genes begin to work, and then during transition to a the next stage, the "previous" portion of euchromatin does not turn back into heterochromatin, but becomes lost. Therefore, the so called "general loss" of the heterochromatin with advancing age simply reflects the advancement of individual ontogeny. Evidently, when men and women enter the post-reproductive stage of ontogenesis, they have lost much of the heterochromatin that was present in a newborn baby. The genes that govern the post-reproductive stage were not the subject for natural selection, and therefore they are the same, or almost the same, genes that our distant ancestors had. This can be proved by many qualities in our bodies that appear after the age of 55: bulky body structure, dark spots of myelin in the skin, hair distribution, etc. Evidently, after transition to the post-reproductive stage many metabolic features also become distinct from the previous stages. There is a lot of evidence that elderly people of the northern Europe and Siberia acquire external and metabolic features common to the people living in the Northern Polar Regions. Inhabitants of the North, for example Eskimos, Dolgans, Innuits, do not consume a lot of plant foods rich in carbohydrates. Their diet is based on meat, animal fat and fish.

Again, the clue to understanding the nature of MetS, we can find in the sex associated differences in the energy metabolism and the transition of men and women from reproductive to the post-reproductive stage of ontogenesis, which is commonly regarded as the stage of aging [92]. This usually occurs between the ages of 50 and 55, when women go through menopause. The sharp changes in appearance and metabolism are particularly evident in women during and after the menopause, which increases the risk of MetS by 60% [136]. It is important, that the occurrence of MetS in the post-menopause period does not depend on the body mass index (BMD) and physical activity [137], but may depend in women on the dynamics of estrogen decline with age [138]. Interestingly, studies on sex hormone replacement in animals have shown that males receiving testosterone showed MetS deterioration, while females with estrogen replacement showed improvement in their MetS symptoms such as decreased hypertension [139]. This agrees with the suggestion that the genetically predetermined transition to the post-reproductive stage during normal ontogenesis, which is accompanied by changes in the hormone status, is the major natural cause of MetS. Thus some features of MetS, namely insensitivity to insulin and gain of fat, particularly visceral obesity, simply reflect a new type of metabolism. From this point of view, T2D may result from the excessive consumption of unnecessary carbohydrates at the post-reproductive stage.

15. Sex differences in the transition from reproductive to post-reproductive stage

In one of the previous sections we provided evidence that women oxidize fatty acids, which are the predominant energy source at all ages, at a slower rate in comparison with men, and thus probably produce ROS at a slower rate [116–118]. Olivetty et all. [111] studied changes in mononucleated and binucleated myocytes with age in enzymatically dissociated cells. The age interval examined varied from 17 to 95 years. The authors have found that in the course of aging women's hearts preserved the ventricular myocardial mass, aggregate number of mononucleated and binucleated myocytes, average cell diameter and volume. In contrast, in the men's hearts the authors observed nearly 1 g/year loss of myocardium, and this phenomenon accounted for the loss of approximately 64 million cells. These detrimental events involved the whole male's heart. In the remaining cells, myocyte cell volume increased at a rate of 158 microns3/year in the left and 167 microns3/year

in the right ventricle. And these changes in the men's hearts were linear from the age of 17 to 95, whereas in women the structural properties of the heart remained unchanged [111]. Thus, it seems that women enter the post-reproductive stage with relatively "young" heart, whereas in men the aged heart lost many cells and the remaining cells increased their volume, which is a disadvantage for the heart's energy metabolism.

16. Features of fatty acids metabolism that increase MetS symptoms and accelerate aging

Among mammalians the human females have a unique duration of post-reproductive longevity [140], which is probably to a large degree associated with the metabolic "protection" that caused slower rate of aging at the reproductive period [122]. There are several reasons to argue that both the accelerated rate of aging of men and the relatively slow aging of women, as well as other sex differences in metabolism and physical performance, are based on the sex differences in fatty acids metabolism. Regardless of age and gender, fats are the major source of energy, carbon and hydrogen for the anaplerotic reactions. **Table 1** shows the relative amounts and times of consumption of the three main sources of mitochondrial substrates for obtaining energy and intermediary metabolites for the growth and maintenance of the body.

Table 1 shows that carbohydrates stores are small and must be constantly replenished by gluconeogenesis in the liver. Amino acids reserves are practically absent and they are constantly formed due to the digestion of food proteins, as well as in anaplerotic reactions in mitochondria. Carbohydrates are too precious to be used for obtaining energy. Much of glucose, particularly at young age, is used for the synthesis of RNA and DNA, purine and pyrimidine nucleotides. Only erythrocytes, which have no mitochondria utilize glucose for obtaining ATP by glycolysis and NADPH for reducing glutathione. There is an old myth that brain consumes only glucose for supporting its energy needs. However, most of the lactate and neuromediators glutamate and γ-aminobutyric acid, which are also used by synaptic mitochondria as energy substrates, are synthesized by the astrocytes from the carbon atoms of fatty acids and for the expense of energy derived during

Source of Energy (Caloric value– kcal/g)	Storage amount (time of consumption)
Carbohydrates:	Total 4–5 grams (20–30 min)
Blood glucose &	100–120 gram (1–3 hrs.)
Glycogen (CV = 3.81)	
Amino acids	Released during catabolism of food, damaged tissue proteins
(CV = 3.12)	and anaplerotic reactions. The content is highly dynamic.
Acyl Fatty Acids	
(CV = 9.3)	Fat (Kilograms) days

Table 1.
It is shown that carbohydrates stores are small and must be constantly replenished by gluconeogenesis in the liver. Amino acids reserves are practically absent and they are constantly formed due to the digestion of food proteins, as well as in anaplerotic reactions in mitochondria. Carbohydrates are too precious to be used for obtaining energy. Much of glucose, particularly at young age, is used for the synthesis of RNA and DNA, purine and pyrimidine nucleotides. Only erythrocytes, which have no mitochondria utilize glucose for obtaining ATP by glycolysis and NADPH for reducing glutathione. There is an old myth that brain consumes only glucose for supporting its energy needs. However, most of the lactate and neuromediators glutamate and γ-aminobutyric acid, which are also used by synaptic mitochondria as energy substrates, are synthesized by the astrocytes from the carbon atoms of fatty acids and for expense of energy derived during β-oxidation of fatty acids. Synaptic mitochondria also gladly oxidize fatty acids in the presence of supporting substrates [85, 87].

β-oxidation of fatty acids. Synaptic mitochondria also gladly oxidize fatty acids in the presence of supporting substrates [85, 87].

The energetic efficiency of β-oxidation of fatty acids in the presence of supporting substrates is the only combination of substrates capable to support the highest rates of oxidative phosphorylation in the heart during maximal physical loads. The efficiency is achieved by the reduction of not only NADH/NAD^+ system in mitochondria, but also by reduction of the membrane pool of ubiquinol/ubiquinone. Therefore, during β-oxidation of fatty acids, electrons enter the respiratory chain not only from Complex I, but mainly through complexes II and III. However, when the energy demands by the organ's functions diminish, the excess of energy in mitochondria may redirect electrons for production of the superoxide radicals, and thus $HO_2^{\bullet}$ [66, 88]. Oxidation by mitochondria of the NAD-dependent substrates cannot provide high rates of ATP and ROS production because NADH-dehydrogenase activity of Complex I is the rate limiting step [141].

Brandt [21] observed that the rate of ROS production may be increased, when mitochondria have abundant supply of substrates and low level of ATP consumption (low functional load), and diminish when consumption of energy is high, or the substrate supply is limiting. This explains why the symptoms of MetS strongly depend on the life style. This is probably the main reason why men start aging faster and earlier then women. At the age of 45–50, many men reduce physical activity, eat too much and abuse alcohol, which dramatically accelerates ROS production.

After menopause, the women's hormonal status becomes closer to that of men, and therefore they also must utilize fatty acids as the main substrates for energy production. At the post-reproductive stage of ontogenesis, we can assume that both men and women have metabolic pattern similar to their distant ancestors, who did not consume a lot of carbohydrates. This may explain the origin of the insulin resistance at MetS. This is not a pathology rather than a new physiological reality due to metabolic reprogramming at the post-reproductive stage. With low insulin sensitivity, most consumed carbohydrates are directed to the synthesis of lipids, which accelerates obesity, first of all visceral obesity. Excessive food consumption plus lower physical and mental activities accelerate production of superoxide radicals, and, thus, perhydroxyl radicals. This accelerates IPLP yielding harmful products, which have proinflammatory activities, cause damages to proteins, cardiolipin and PEA resulting in mitochondrial dysfunctions and accelerating aging.

For a long time, these gradually accumulating various functional disorders and structural damages are not accompanied by specific clinical manifestations. In people predisposed to an earlier aging, the clinical symptoms may be unspecific and look like frailty. With time, the accumulated wear and tear will cause development of clinical symptoms, like acute heart failure, Alzheimer's disease, or something else, and finally death.

Literature show diminished fatty acids oxidation and developments of MetS symptoms in the females without estrogen that can be normalized by administration of estrogen [142, 143]. We do not think that those publications contradict to our conclusions presented in this Chapter, because those experiments have been done on young animals 7–8 weeks old.

17. Conclusions

In this Chapter we have presented evidence that activation of IPLP by hydroperoxyl radical, protonated form of superoxide radical, provides explanation to the slow and inevitable mechanism of aging and resolves many objections against MFRTA in the current paradigms. We also have shown that focusing only on the

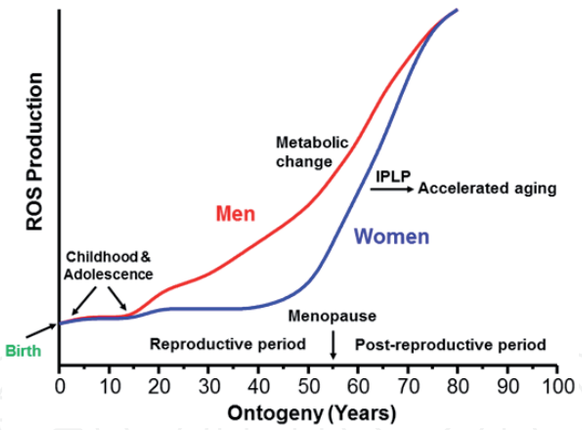

Figure 4.
A schematic presentation of approximate differences between men and women in ROS production during ontogeny. The figure was created based on the data presented in Refs. [111, 112, 116–118, 122, 138].

damages, which accompany aging, is not very helpful, because it gives no answers on why and when aging actually starts, and why women age slower and live longer than men. We have explained the idea that aging is, first of all, the process of development in time, when men and women go through a number of genetically predetermined stages. Because men and women have different biological roles, they also have different metabolic strategies. Fatty acids at all stages of ontogeny are the main substrates for provision of energy and intermediate metabolites for the growth and maintenance. The energetic efficiency of β-oxidation of fatty acids is controlled by the type of mitochondrial metabolites that oxidize simultaneously with fatty acids. However, this results in a significant increase in oxidative stress. We suggest that sex hormones determine the type and quantity of supporting substrates, which result in different rates of energy production and oxidative stress. Women consume more fatty acids with lower efficiency, and thus age at a slower pace. When men and women enter the post-reproductive stage of the ontogeny, the type of metabolism also changes because this last stage of ontogeny is controlled by ancient genes of our distant predecessors. In **Figure 4** we summarized the available information as a scheme, which shows the approximate differences between men and women in ROS production during ontogeny. The metabolic syndrome, which usually begin developing after the age of 45 in men and 55 in women, reflects two main events: the transition to the post-reproductive stage of ontogeny, and the new type of metabolism. Because fatty acids become the major substrates for the energy production in all organs, the rate of ROS production, and, consequently, the rate of aging may increase dramatically. The specific symptoms of the MetS prevailing in particular individuals will depend on the genetic background of their ancestors and the life style.

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