Healthy Ageing and Longevity 15
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Ufuk Çakatay *Editor*

Redox Signaling and Biomarkers in Ageing

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Healthy Ageing and Longevity

Volume 15

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

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In memory of my beloved mother who passed away June 2020 and other victims of COVID-19

Ufuk Çakatay

As the poet laureate Louise Glück said in The Wild Iris:
"You who do not remember passage from the other world I tell you I could speak again: whatever returns from oblivion returns to find a voice:
from the center of my life came a great fountain, deep blue shadows on azure seawater."

Preface

This multi-chapter book is dedicated to present age-related alterations in redox signaling networks and their diagnostic biomarkers in aging cell. Establishing sensitive and specific biomarkers of dynamic redox homeostasis is crucially important in the development of effective anti-aging and senolytic interventions. Recent years have seen tremendous advances in the understanding of redox signaling events which underline the process of aging and age-related pathologies. A major challenge in biological aging research is to develop reliable biomarkers to determine the consequences of disrupted redox signaling networks long before the clinical diagnosis of age-related diseases is made.

Activation of redox-sensitive transcription factors orchestrates the expression of antioxidant and cytoprotective genes. Impaired redox status-related transformations of aging cells have gained immense interest in recent times. Although oxidation is a natural metabolic process, an imbalance in oxidants and antioxidants can lead to oxidative stress, which damages cellular molecules, contributing to inflammatory conditions, diabetes, neurodegenerative diseases, and cancer. Novel redox-sensitive biomarkers for the evaluation of aging-induced proteinopathies such as amyloid ß and tau proteins in Alzheimer's disease, α-synuclein in Parkinson's disease, and islet amyloid polypeptides in type 2 diabetes mellitus recently drew the attention of researchers in the field of Gerontology. Therefore, we have chosen to concentrate on aging-induced aberrant redox signaling networks, their biomarkers, and pathological consequences in this book.

Due to the inherent relationship between impaired metabolic activities and oxidative stress, the temporal interaction between intermediary metabolism and disturbed redox metabolism can lead to greater susceptibility to age-related diseases, such as cardiovascular diseases, hypertension, and diabetes. This knowledge could be key to continued research toward improving medication regimens such as in cancer and cardiovascular therapies, and procedural outcomes for patients. Inside this textbook, readers will find comprehensive perspectives on the association between redox homeostasis and the aging process both at the molecular and clinical levels. Therefore, we hope that this textbook will be of interest to a wide group of researchers and clinicians alike.

This book brings together current research evidence and knowledge on redox signaling and biomarkers in aging in chapters written by leading experts in this area from all around the world. I would like to thank all our valuable contributors who provided us with excellent chapters making possible the compilation of this textbook.

Istanbul, Turkey Ufuk Çakatay

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Dr. Ufuk Çakatay is a Tenured Professor of Medical Biochemistry and a Senior Scientist at Cerrahpaşa Faculty of Medicine, Istanbul University-Cerrahpasa, in Istanbul, Turkey. His research interests include the optimization of various bioanalytical methods to measure the levels of redox status biomarkers in aging tissues as well as anti-aging interventions, establishment experimental animal models for aging studies, and redoxtasis in age-related disorders.

He has a long-standing interest in oxidative protein damage and proteostasis biomarkers. In addition, Prof. Çakatay has a deep interest in mentoring medical students and fostering their research career. Professor Çakatay has authored more than 100 publications in several leading journals, which include research papers, book chapters, editorials, and invited reviews on free radical biology, aging, diabetes, and biological activity of alpha-lipoic acid. He has served as a guest editor, referee, or member of the editorial board for more than 50 journals. He has received various prestigious awards such as the Top Reviewer Award from Elsevier Science Publishing and previously served as a committee member in Istanbul University's Committee on Animal Research and Ethics.

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Part I
Redox Dynamics

Chapter 1
Redox Dynamic Homeostasis and Aging

Volodymyr I. Lushchak

Abstract Recently, general principles of existence and functioning of the redox code were proposed by Jones and Sies (Jones and Sies Antioxid Redox Signal 23:734–746, 2015). These principles include: (1) use of reversible electron acceptors/donors NAD^+ and $NADP^+$ operating at near equilibrium states to provide energy resources for anabolism, defense and signaling; (2) cysteine-based kinetically controlled protein redox switches in the proteome which may change tertiary protein structure; (3) redox-based sensing of signals and their transmission through regulatory pathways; and (4) systems of adaptive responses to environmental stimuli involving multilevel redox networks. In the same 2015, D. Jones proposed the so-called redox theory of aging which states that aging is connected with a decline in the plasticity of genome-exposome interaction followed by a number of functional consequences described as hallmarks of aging. The whole continuum of components participating in redox reactions and providing all features of living organisms has been called "redoxome". In this chapter, principles of the redox code or redoxome functioning are analyzed through the prism of lifespan with special attention to aging. The decline in the plasticity at the aging of organisms may be connected with an increased oxidation state of intracellular milieu resulting in loss of adaptability due to irreversible oxidation of cysteine-based redox sensors. Potential interventions to restore adaptive potential in the aged organisms such as physical activity and dietary restriction or their mimetics are discussed.

Keywords Adaptability · Glycolysis · Intermediary metabolism · Lifespan · $NAD(P)^+$/NAD(P)H ratio · Oxidative phosphorylation · Oxidative stress · Reductive stress · Reactive oxygen species · Spatiotemporal organization

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1.1 Introduction

In a seminal article "Redox theory of aging" Jones (2015) wrote, "The redox theory of aging is that aging is a decline in plasticity of genome-exposome interaction that occurs as a consequence of execution of differentiation and exposure memory systems. This includes compromised mitochondrial and bioenergetic flexibility, impaired food utilization and metabolic homeostasis, decreased barrier and defense capabilities and loss of reproductive fidelity and fecundity". He described the functioning of living organisms as preparation and processing of energy sources (carbohydrates, lipids and proteins) which along with some other components of food serve as *environmental reductants*. These processes provide energy in the form of reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide ($FADH_2$), $\Delta\psi$ and ATP with or without utilization of oxygen and other *environmental oxidants*. Realization of all functions of living organisms lies at the interaction interface between these two groups of environmental reductants and oxidants, the processes in-between them provide all features of your Majesty Life. The critical question from the aging point of view is: what is going wrong within this interface during the life which ultimately leads to aging and culminating in death?

In the fundamental work "The redox code" Jones and Sies (2015) described the redox code as "a set of principles that defines the positioning of the nicotinamide adenine dinucleotide (NAD^+, $NADP^+$) and thiol/disulfide and other redox systems as well as the thiol redox proteome in space and time in biological systems". Earlier, the above-described processes I tried to put in the frames of oxidative and reductive stresses, i.e. whole continuum of redox proceses in living organisms (Lushchak 2011). However, due to limited technical possibilities a decade ago, it was impossible to create an unified vision of the whole picture of the redox continuum despite it was absolutely clear that processes involving NAD(P), –SH and reactive oxygen species (ROS) are interconnected and in some ways they are related with aging. And now fragmentary knowledge can be placed in the system and insufficient details may be deduced from accumulated to date information. So, it seems to date the puzzles are organized in a logical way to give a whole picture.

In this chapter, being equipped with a general picture of the redox milieu, I aim to discuss available information on spatiotemporal organization of the redox processes during a lifetime with special attention to aging and potential approaches to slow down or even return partially naturally occurring aging process.

1.2 Lifespan Trajectories

Virtually all modern aging theories and hypotheses involve a question on "accumulation of damaged molecules" (López-Otín et al. 2013; Jones 2015; Garaschuk et al. 2018; Lushchak 2021). It principally does not matter which type of damaged

molecules is accumulated or which reactive species cause these damages. Probably, the most fundamental reasons for aging have been collected in the excellent paper called "The Hallmarks of Aging" (López-Otín et al. 2013). The authors listed nine hallmarks as common indicators of aging in different living organisms: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication. All of them are connected in different ways with the accumulation of damaged substances. Very few types of damaged molecules and their aggregates cannot be removed by certain treatments due to which we must speak not about the accumulation of damages but rather about dynamics of the markers of aging, i.e., about the balance between their formation and elimination. Such a point of view may be placed in the frames of the steady state concept, where the actual levels of certain components of living organisms are determined by such balance. Obviously, we have to recognize or even emphasize that the steady state levels of damages increase with aging.

Figure 1.1 shows the relationship between lifespan and levels of damages presented by the different trajectories. As above mentioned, in this paper, steady-state levels of any damaged molecules or their complexes will be mainly discussed. Up to middle age, the levels of damages increase slowly, but starting from half of life and especially at the last trimester of life, the amount of damaged molecules increases fast (Levine and Stadtman 2001). It culminates in cell or organism death. Three principally different life trajectories are depicted in Fig. 1.1: normal aging (yellow solid line), delayed aging (green solid line) and accelerated aging (red solid line). Clearly, such differentiation is rather artificial to a big extent but may be useful for the description. So, normal aging is a type of aging characteristic for the average population or cohort under inspection. Accelerated aging may result from certain

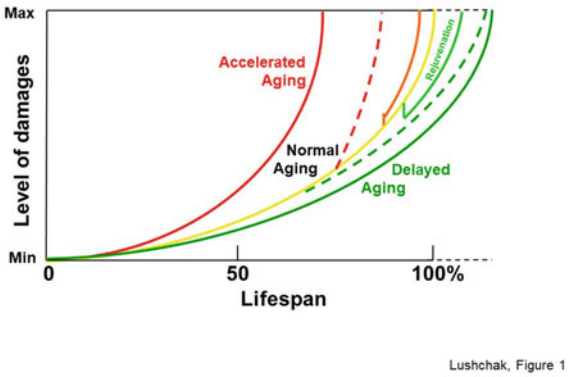

Fig. 1.1 Lifespan trajectories of the organisms dependently on the rate of accumulation of damages. There are three principally different life scenarios or lifespan trajectories: normal aging, accelerated aging and delayed aging with the potential to change them. A detailed description is in the text

more or less specific diseases such as Werner syndrome or from life under the pressure of chronic stresses or frequent acute stresses of various nature. Delayed aging may result from a very accurate following of a healthy lifestyle from birth to death. However, it is not a common situation, and in most cases (at least in human case) commonly serious attention to their health is not paid which provides an average scenario, i.e., normal lifespan. In reality, generally, humans start to pay serious attention to their health after life equator or even in the advanced age. Therefore, the question here is: can be life trajectory changed if influencing is started at a late age? Yes, of course, and in both directions! Certainly, it is more attractive and interesting to talk about the extension of life- and especially healthspan. In this context, we can address different kinds of rejuvenation strategies and there are a number of techniques to do so. That to a different extent may be manipulated by starvation, physical activity, diets, etc. At the cellular level rejuvenation can be achieved by mitoptosis in concert with broader autophagy, biogenesis of mitochondria, activation of stem cells and in some cases dedifferentiation of cells to enhance the population of stem cells (López-Otín et al. 2013). Certain interventions in middle age may shift lifespan trajectory without substantial efforts (green dashed line). But in advanced age, the shift of lifespan trajectory would need more radical interventions and some sort of rejuvenation treatment in order to at least partially reverse the aging process which is reflected by the sharp jump of the trajectory (light green dashed line). That was a discussion about the potential extension of life- and healthspan. Virtually the same events can be applied to facilitate the aging process just with the opposite logic. At following normal lifespan, some external challenges may worse general or specific conditions and would shift lifespan trajectory from normal to accelerated one (red dashed line). In the common case, such shift may be rather sharp caused by chronic stress, moving to other climate conditions or disease (light red dashed line).

Because the key questions in many cases are related to potential approaches to change lifespan trajectories, I would try to respond to it at the end of the chapter after analysis of molecular mechanisms related to the functioning of redoxome during lifespan. The term “redoxome” will be used to describe the whole continuum of components of living organisms participating in redox reactions that provide all properties of living organisms. But first, there is a need to describe in some details critical groups of redox processes characterized to date.

1.3 General Overview of Redox Homeostasis

Living organisms as open thermodynamic systems need constant inflow of energy and other resources from the environment and release of the end products of metabolism. In-between these processes, there is a huge plethora of processes directed to perform all aspects of life starting from “simple” mechanical responses to environmental stimulus up to complex processes such as reproduction and behavioral reactions, learning and memory. For this, external resources have to be converted into the “edible” form of ATP as a universal energy currency and simple compounds, namely amino acids,

monomeric carbohydrates, which can be used to build all components of the organisms. Therefore, big complex molecules such as polysaccharides, proteins, lipids and nucleic acids are usually externally hydrolyzed to join a pool of simple molecules in order to enter intracellular catabolism. Anaerobic production of energy (glycolysis) is low efficient, and therefore, living organisms involve aerobic mechanisms to produce ATP as a more efficient way to produce ATP than anaerobic ones.

Most resources needed by the living organisms are available in the environment in the form of biopolymers and before absorption by the cell they have to be hydrolyzed to simple monomers. These processes are usually extracellular for which organisms have to produce and excrete into the environment specific hydrolases. Further, produced "simple" molecules can be either used by the cell directly (in unicellular organisms) or transported through the body (in metazoans). The general scheme of operation of living organisms is given in Fig. 1.2. On the left side of the figure,

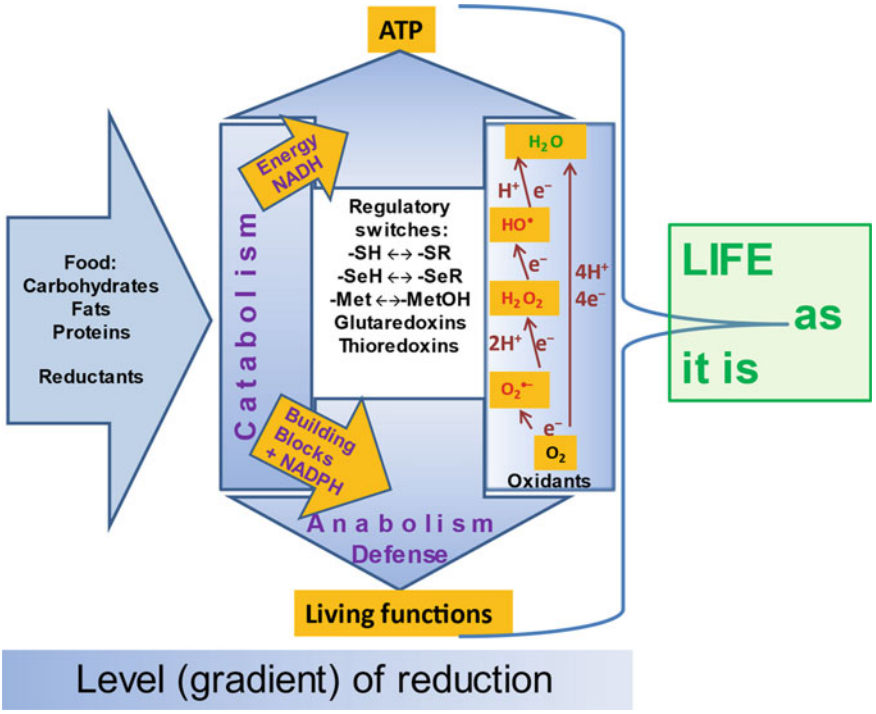

Fig. 1.2 The continuum of redox processes or redoxome in living organisms. It is represented by the two marginal sides: highly reduced left one and highly oxidized right one. Food stuff is represented by diverse components and in concert with other environmental reductants fuel cellular catabolism to provide energy and reductive equivalents for the organisms. At the right side, oxygen is represented along with its reduced form used to oxidize processed food components. At interface between two poles, there is a plethora of redox processes which along with catabolism providing formation, maintaining and adaptation of the organisms to environmental challenges. A detailed description is in the text

preparation extracellular phase (actually the first catabolic phase) is shown in the arrow. After absorption by the cell, "simple" organic molecules derived from carbohydrates, proteins, fats (lipids) and nucleic acids are used in catabolism to feed two main groups of processes: energy production and catabolism for building and maintaining of cellular structure which in concert supports and provides the life in all its aspects.

In order to produce energy, catabolism provides entrances of absorbed resources in glycolysis, pentose phosphate pathway (PPP), and tricarboxylic acid cycle (TCA) which further may be coupled with oxidative phosphorylation (OxPhos). The energy is produced in several forms such as electrochemical gradient, ATP and other macroergic compounds. ATP is believed to play a central role in energy homeostasis being a change coin. It is produced in two processes: glycolysis and OxPhos. Glycolysis is an anaerobic process with low energy yield, whereas OxPhos is an aerobic process with high ATP yield. In OxPhos, electrons from any substrate are used for four-electron reduction of molecular oxygen, and released energy is first converted in electrochemical gradient at the inner mitochondrial membrane. At the final stage, the energy in the form of an electrochemical gradient is converted to a chemical form as a macroergic compound ATP. However, there is a dark side of aerobic ATP production. Some portion of consumed O_2 (<5%) is reduced not via four-electron scheme but via sequential one-electron reduction and partially reduced oxygen forms are generated as side products in electron transport chain (ETC) (Fig. 1.2, right part). They are superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) which collectively are called reactive oxygen species (ROS) due to their higher chemical activity than that of molecular oxygen (Lushchak 2014). Of them, $O_2^{\bullet -}$ and $HO^{\bullet}$ are free radicals, whereas H_2O_2 is not a free radical.

It should be added here that some ROS amounts are produced as side products other than mitochondrial ETC and by certain oxidases. The formation of ROS as side products is mostly either uncontrolled or poorly controlled processes. But one of the oxidases occupies a special position in this context. It is NADPH oxidase located in the cytoplasmic membrane (Fukai and Ushio-Fukai 2020). This enzyme produces $O_2^{\bullet -}$ in highly regulated, controlled manner. It was discovered in white blood cells as a key component of the immune defense system to combat diverse invaders and later NADPH oxidases were found in many other cell types. In most cell types, $O_2^{\bullet -}$ produced by NADPH oxidases is converted to H_2O_2 which also plays the role of signaling molecule.

Since ROS may cause damage to the living organisms, they have evolved complex multilevel defense systems against ROS and other reactive species. The latter include reactive nitrogen species, reactive carbonyl species and a number of other reactive species. The right part of Fig. 1.2 shows ROS formation and its interconversions. The system combating ROS formally is grouped in low and high molecular mass antioxidants. The first group includes vitamins A, C, and E, carotenoids, anthocyanins, uric acid, and bilirubin with glutathione which occupies a very special position in this group (Lushchak 2011,2012,2014). Glutathione is produced and maintained in a reduced state in a tightly controlled manner by coordinated operation of several

systems. High molecular mass antioxidants are represented mainly by antioxidant and associated enzymes (Lushchak 2011,2014).

The left side of Fig. 1.2 is represented by the compounds with high chemical energy and high amount of reductive equivalents, whereas the right part possesses high oxidative potential. Burning of organic compounds in the oxygen atmosphere results in high energy release in heat form. Formally, living aerobic organisms also "burn fuel" but in a highly controlled multistep manner. Gradual multistep release of energy from the substrates makes possible its conversion in "edible" forms such as ATP. Obviously, life is characterized by high organization and capability to respond to diverse challenges in a highly controlled and coordinated manner. So, aerobic life poses the question: how to adjust internal processes to environmental requirements? Of this huge complex problem, in this chapter, I will focus only on molecular mechanisms of response and adaptation of redox systems. There are several molecular machines that are involved in such processes and the cysteine-based system seems to occupy a central position in this case and is the best studied to date.

The left side of Fig. 1.2 depicts catabolic processes related to the production of NAD(P)H and the right side represents highly oxidative O_2 based processes accompanied by ROS generation. These two groups of processes met in the central part of Fig. 1.2, where cysteine-centered reversible redox processes are shown. These specific cysteine-based processes are mainly responsible for the coordination of adaptive responses of organisms to ROS-related and many other challenges. The involvement of other regulatory switches such as selenocysteine, methionine, glutharedoxin and thioredoxin centered is shown, but not highlighted here in order to keep the chapter focused on cysteine-based processes.

The adaptive response is realized via the operation of signaling regulatory cascades which include sensors, transducers and effectors. In the case discussed here, cysteine residues of diverse proteins play a central role in ROS sensing. The best-studied such systems are bacterial OxyR, yeast YAP1/Gpx3 and animal Nrf2/Keap1 systems (Lushchak 2011). Despite OxyR and YAP1 systems played key roles in the deciphering of molecular mechanisms of ROS-initiated redox signaling, this chapter will be focused only on the animal Nrf2/Keap1 system.

Jones and Sies (2015) have identified four principles of the redox code by which biological systems are organized: (1) Use of reversible electron acceptors/donors NAD^+ and $NADP^+$ operating at near-equilibrium states, which provide energy in ATP and other forms; NAD^+ is associated with catabolism and energy production, whereas $NADP^+$ is associated with anabolism, defense and signaling. (2) The metabolism is linked with the structure of proteins through kinetically controlled redox switches in the proteome, which determines the tertiary structure, intermolecular interactions, trafficking, activity and functioning. In most studied cases, such events are centered around sulfur-containing switches which sensitivity to oxidation/reduction depends on the microenvironment and may vary over several orders of magnitude and which determines their specificity in the biological milieu. (3) Spatiotemporal sequencing in differentiation and other life processes are related with redox sensing based on activation/deactivation (oxidation–reduction) cycles of redox metabolism, particularly with involving of hydrogen peroxide and other ROS. (4) The system of adaptive

responses to the environment, from microcompartments up to whole organisms, is based on multilevel redox networks. Such adaptive redox networks are responsible for maintaining normal states of organisms, providing health and if disturbed may lead to disease and organism failure. Further, these principles will be imposed on the aging process.

1.4 NAD(P) and NAD(P)H Pairs as Common Redox Denominators

Catabolism provides resources for the generation of energy and building blocks for living organisms. Two principal compounds are critically important here: NAD^+ and $NADP^+$. They can be in the oxidized forms as it is given above or in the reduced one abbreviated as NADH and NADPH, respectively. They both are reduced using the reductive power of energetic substrates such as carbohydrates, fatty acids and to smaller extent amino acids. Importantly, both NAD^+ and $NADP^+$ are used predominantly in principally different processes. The couple NAD^+/NADH operates near thermodynamic equilibrium which can be described by the Nernst equation (Jones and Sies 2015). Under normal conditions, bulk NADH generated is used at OxPhos to produce ATP in mitochondria. It should not be also forgotten that NAD^+ can be converted into $NADP^+$ via phosphorylation at 2′ position by nicotinamide phosphoribosyltransferase (NAMPT, EC 2.4.2.12) for ATP expenses.

It is important to mention that certain NAD^+ portion is also permanently removed from the pool and used by specific enzymes which are related to defense systems. There are two big groups of reactions where NAD^+ participates in nonreductive and reductive ones. In the first group, it takes part in number of processes such as cellular signaling, DNA repair, posttranslational modifications of proteins, mitochondrial metabolism, inflammatory responses, senescence and apoptosis (Navas and Carnero 2021). In these nonreductive processes, NAD^+ serves as a substrate for a number of enzymes such as sirtuins (EC 2.3.1.286), poly (ADP-ribose) polymerases (PARPs, EC 2.4.2.30) and cyclic ADP-ribose synthases or ADP-ribosyl cyclase (cADPRS, EC 3.2.2.6). The enzymes mentioned above use NAD^+ as a donator of ADP-ribose, whereas nicotinamide is released and can be recycled. Nonreductive processes involving NAD^+ are not in the focus of this chapter, whereas redox ones involving this compound would be analyzed with more details and with dynamics of their relative concentrations (NAD^+/NADH).

The second described here redox pair $NADP^+$/NADPH is an important counterpart of the NAD^+/NADH couple and directly or indirectly provides reductive power for biosynthetic processes in anabolism, for example, for fatty acid biosynthesis, ribonucleotide dehydration and other anabolic processes. Two large groups of $NADP^+$-related processes are defense systems against reactive species and maintaining balance in the cysteine-based redox-sensitive systems.

The components of $NADP^+$/NADPH pair are produced by the organism from many precursors and the processes leading to their formation have been described in detail (Navas and Carnero 2021). The balance between reduced and oxidized forms $NADP^+$/NADPH is maintained by several mechanisms. They were described by me in details earlier (Lushchak 2014) due to which here they will be just briefly listed. Probably most of cellular NADPH is produced by two enzymes of pentose phosphate pathway (PPP), namely, glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.43). In several tissues, particularly, in the brain, malate dehydrogenase (oxaloacetate-decarboxylating $NADP^+$, EC 1.1.1.40) or NADP-malic enzyme (NADP-ME) also produces substantial NADPH amounts. $NADP^+$-dependent isocitrate dehydrogenase (IDH, threo-DS-isocitrate: $NADP^+$ oxidoreductase (decarboxylating, EC 1.1.1.42) also can be responsible for the reduction of certain $NADP^+$ amounts to NADPH.

The cytosolic redox poise of [NADH]/[NAD^+] pair is −241 mV, whereas the mitochondrial potential of this couple is much more negative something about −318 mV (cited after (Jones and Sies 2015)). Such difference provides better conditions in mitochondria to produce ATP. It is very important to note that substantial part of NADH is bound and cannot be used to produce ATP in mitochondria (Dong et al. 2019). In mouse hippocampal/cortical neurons, total amount of NAD(P)H levels and redox state in neurons enhanced until middle age, followed by a decline in old animals (Ghosh et al. 2012). The changes in redox homeostasis as a critical aging hallmark, i.e. [NAD^+]/[NADH] ratio, were confirmed in many works where age-related decline in NAD^+ concentration was found (Braidy et al. 2011; Ghosh et al. 2012; Zhang et al. 2020).

It is critically important to note that *changes in a reversible early oxidized redox state preceded ROS-promoted damages to macromolecules in aging mice* (Ghosh et al. 2012). Utilization of 3xTg mouse model of Alzheimer's disease in this work also demonstrated that both the above mentioned changes also preceded the onset of cognitive deficits.

Relatively to NAD^+/NADH, $NADP^+$/NADPH system operates at more negative redox potential, which is −393 mV in the cytosol and −415 mV in the mitochondrial matrix (cited after (Jones and Sies 2015)). As mentioned above, this system is equilibrated by NADP-dependent dehydrogenases such as G6PDH, 6PGDH, NADP-ME and IDH. Both, $NADP^+$ and NAD^+ systems are interconnected by transhydrogenase (EC 1.6.1.1), which may operate as a sensor that determines the positioning of either NAD^+ and $NADP^+$ systems at the near-equilibrium steady state. A delicate balance between operation of $NADP^+$/NADPH and NAD^+/NADH based pairs is provided to distribute or redistribute resources between catabolic and anabolic processes which are related to all living processes. Just one small difference such as phospho/dephospho group in 2′C position in the structure of these nicotinamide dinucleotides provides their involvement in so many different groups of processes. It should be also memorized that NADPH homeostasis is related to ROS-related processes in two ways: ROS production and ROS combating.

The homeostasis of $NADP^+$ and NADPH also changes with aging: the concentrations of $NADP^+$ decreased and NADPH concentrations increased at the aging of rats, which resulted in a decrease of $NADP^+$/NADPH ratio (Braidy et al. 2011).

There is a possibility to influence the distribution of resources to produce either more NADPH or NADH in connection with intermediary metabolism. In our recent work, we have found that in middle-aged mouse forebrain, a potential to produce NADPH via PPP was increased in both, males and females, which was evidenced by higher activity of key PPP enzyme glucose-6-phosphate dehydrogenase and no further changes took place with age advancing (Bayliak et al. 2021). At the same time, the activity of key glycolytic enzyme phosphofructokinase dramatically decreased in the middle age and did not change after that at aging. This work has disclosed molecular mechanisms which may be responsible for redistribution of glucose-derived intermediates between glycolysis and PPP at aging (Garaschuk et al. 2018; Lushchak 2021). Moreover, such changes may redirect resources between glucose utilization for energy production and operation of anti- and prooxidant systems such as antioxidant enzymes and NADH-oxidases, respectively.

1.5 Intermediary Metabolism is Linked with Protein Structure Through Kinetically Controlled Processes

Despite the existence of a number of redox sensitive groups in proteins described to date, here I will concentrate on the thiol group of cysteine residue due to its highly investigated state and well-established roles in the regulation of most organisms' functions. The reactivity of protein cysteine residues in redox processes may differ in orders dependently on the microenvironment. Their capability to enter reversible oxidation was adapted to play the role of sensors of cellular redox state. How these switches do work and what are their functions? Basically, the switches link intermediary metabolism with the structure of specific proteins through kinetically controlled oxidation and reduction reactions (Jones and Sies 2015). Reversible oxidation of certain thiol groups changes the tertiary and, in some cases, the quaternary structure of proteins, and dependently on their functions it affects their operation. In opposite to $NAD(P)^+$-linked systems, which are involved in catalytic reactions in metabolism and energy production, regulatory thiol ones do not possess catalytic functions. They are responsible for protein folding, regulation of diverse activities including intra- and intermolecular interactions.

Protein thiols are constantly oxidized and this process is counterbalanced by central thiol antioxidants such as glutathione (GSH) and thiodredoxins (Trx). Steady-state redox potentials of these antioxidants show that they are not in thermodynamic equilibrium with NADPH/$NADP^+$ pair (Jones and Sies 2015). Both systems, GSH and Trx, depend on NADPH level and via several dehydrogenases are related with intermediary and energetic metabolisms. So, NADPH availability may directly affect

ratio of reduced and oxidized forms of GSH and Trx from one side, and intermediary with energetic metabolism from the other side.

In animals, NF-kB, p53, AP-1, glucocorticoid receptor and Keap1/Nrf2 are typical representatives of regulators of cellular redox-operated signaling pathways. Particularly, thiol groups of Keap1 enter oxidation which prevents their interaction with Nrf2 resulting in an increased level of the latter, its entering into the nucleus and upregulation of expression of a number of genes encoding proteins responsible for enhanced efficiency to combat ROS by antioxidants and invaders by the immune system (Vasileva et al. 2020; Cuadrado et al. 2020).

The GSH system with its specific and nonspecific reductases and dehydrogenases operates complementary to Trx one (Jones and Sies 2015). It functionally links redox processes with the intermediary metabolism. Glutathione peroxidases (EC 1.11.1.9) with glutathione-S-transferases (EC 2.5.1.18.) use GSH to reduce disulfides and peroxides, whereas glutathione reductase (EC 1.6.4.2) reduces oxidized glutathione (Lushchak 2012). Homeostasis of NAD^+/NADH and $NADP^+$/NADPH pairs functionally links redox processes with intermediary metabolism and redox sensors to regulate adaptive responses to environmental challenges.

Peroxiredoxin reduces H_2O_2 oxidizing Trx which in concert with Trx reductase using reductive power of NAPDH makes the system reduced to prepare to new reduction rounds (Yoshida et al. 2003). Thioredoxin also reduces disulfide bonds in diverse proteins. Actually, Trx, the small protein with a molecular mass of about 12 kD, along with the GSH system, forms a powerful antioxidant system tightly regulated by the cell and heavily involved in adaptive responses to environmental challenges. Despite in this chapter, reversible oxidation of protein methionine residues is not in the focus, it cannot be avoided to mention it here. Interestingly, methionine sulfoxide reductase A (MsrA, EC 1.8.4.6) which catalyzes the reduction of oxidized methionine in proteins by converting methionine sulfoxide to methionine completely dependent on the Trx redox system (Yoshida et al. 2003; Jiang and Moskovitz 2018). Involvement of MsrA in the regulation of aging was demonstrated with *msra* transgenic fruit fly *Drosophila* which had a markedly extended lifespan and significant resistance to paraquat-induced oxidative stress (Ruan et al. 2002).

1.6 Spatiotemporal Organization of Redox Signaling

Last years, a number of genetically encoded ROS sensors were introduced and they helped to visualize the spatiotemporal organization of redox signaling. Being produced by the so-called nonphagocytic NADPH oxidase, ROS mainly in the form of H_2O_2 regulate the number of physiological processes in living organisms. For example, with *Caenorhabditis elegans,* it was shown that at larval stages H_2O_2 level was higher than that in adults and at adulthood H_2O_2 level increased at aging (Knoefler et al. 2012). Moreover, at the development of *C. elegans* dynamics of H_2O_2 levels accompanied selective oxidation of protein thiol groups. Later, the Chinese group found that in young *C. elegans* exposure to paraquat enhanced ROS level

(Meng et al. 2017). Regeneration after amputation of tails in tadpole (Love et al. 2013) and zebrafish *Brachidanio rerio* (Niethammer et al. 2009) demonstrated H_2O_2 waives and its concentration gradient at wound places and changed levels at wound healing.

It is now becoming evident that H_2O_2, which is constantly produced by nearly all cells, contributes to bona fide physiological processes. However, little is known regarding the distribution and functions of H_2O_2 during embryonic development. To address this question, a dedicated genetic sensor spatiotemporal pattern of H_2O_2 levels during zebrafish morphogenesis was studied (Gauron et al. 2016). At stages of somitogenesis and organogenesis, H_2O_2 levels were highest and gradually decreased at maturation. The distribution of H_2O_2 seemed was mainly controlled by enzymatic degradation. Interestingly, H_2O_2 was heterogeneously distributed in different regions of the developing brain and served as an axonal guidance. Finally, the authors concluded that at physiological levels of H_2O_2 regulated axonal growth through the modulation of the Hedgehog pathway (Gauron et al. 2016). Unfortunately, such kind of studies has not been available in the literature regarding aging which would be very interesting to get in future.

Local production of H_2O_2 may represent a small portion of the total amount. However, at the focal point, it may reach relatively high H_2O_2 concentrations due to which it may turn on redox signal in the close vicinity to the place of production. This provides specificity of local H_2O_2-based signaling. Spatial propagation of the signal may be limited by the H_2O_2-degrading enzymes like peroxidases or peroxiredoxins. Certain enzymes from these groups with high reactivity to ROS themselves may be sensors for reactive species. For example, in yeasts glutathione peroxidase 3 (Gpx3) is believed to be a direct ROS sensor that further transfers the signal to YAP1 protein which upregulates expression of the target genes (Lushchak 2010). This provides from one side high sensitivity to local H_2O_2 and, from the other side, provides highly specific transmission of such signal along regulatory cascades. In the yeast case, Gpx3 oxidation signal directly and specifically transduces to Yap1 which further coordinates yeast adaptive response to oxidants. In some cases, the primary ROS-receptor may have several targets with reactive cysteine or methionine residues. Such organization may create a specific spatiotemporal vector signaling net.

Further specificity may be provided by the microenvironment. For example, reactions with the involvement of thiol groups like cysteine residues are highly sensitive to pH, presence of different metal ions, etc. In many cases, cellular organelle structure adds specificity. For example, in the endoplasmic reticulum, specific oxidase system called protein disulfide isomerase (PDI, EC 5.3.4.1) uses molecular oxygen to introduce disulfide bonds in the polypeptides like insulin (Laporte et al. 2020).

The local and temporally processed signaling form vector transduction of signals, for example, between cytosol and nucleus or mitochondria. In yeasts, YAP1 oxidation and temporary accumulation of oxidized YAP1 in the nucleus clearly form a chain of oxidative signal transduction from a place of Gpx3 oxidation by H_2O_2 to YAP1 followed by transfer of the latter into the nucleus where it upregulates expression of many target genes (Lushchak 2010). Covalent modification of Keap1 protein by

ROS or electrophiles prevents its interaction with Nrf2 associated with proteasomal degradation of the latter (Lushchak 2012,2014). This results in Nrf2 accumulation in the nucleus and upregulation of the expression of the target genes.

Unfortunately, to date, there is no available information on age-related changes in spatiotemporal organization of redox signaling and it clearly provides the avenue for future studies.

1.7 Adaptability to Environmental Challenges

Adaptability to environmental challenges is an inevitable part of life. An adequate stimuli response of redox systems from microcompartments through the subcellular level to cellular, tissue/organ and whole organismal level forms system response. The adaptation process needs adjustment of energy supply and building blocks in concert with redox networks which is a crucial point not only for survive, but also for successful propagation and development of higher mental functions. Jones and Sies (2015) gave an excellent analysis of series of publications on the operation of circadian redox clocks concerted with a diurnal variation of redox control in eukaryotes with central positioning of H_2O_2 and thiol redox states. In general, levels of H_2O_2 in space and time are subjected to the circadian variation in connection with the activity of peroxiredoxins, the enzymes which in concert with glutathione-dependent peroxidases and H_2O_2, are directly involved in redox signaling and control of the thiol proteome (Jones and Sies 2015). Interestingly, in human plasma the ratio Cys/CysSS also is subjected to diurnal variation which is linked to the timing of food intake (Blanco et al. 2007). Generally speaking, the adaptive mechanisms are related to food availability, avoiding competition or predation, and are directed to maintain homeostatic control of energetics, metabolism and proteomic structure. An adaptive response includes a whole spatiotemporally organized system of events from locally produced ROS, mainly based on H_2O_2, in a tightly controlled manner through concerting with targets and H_2O_2 eliminating enzymes to meet internal and environmental challenges.

It is important to underline, that many of the studied parameters of redoxome are changed during lifespan. For example, NAD^+ levels, oxidative steady state of thiol and other redox systems protome shift to the more oxidized state during aging (Blanco et al. 2007). According to this, it is clear that the capability of organisms' redoxome to respond adequately to environmental challenges provides a mechanistic basis for adaptive response. Since the responses are initiated by oxidation of thiol-based sensors and with aging, redox balance is shifted to more oxidized state readiness of the system to respond to environmental challenges may decrease with the aging. This idea echoes with the proposed concept "Redox-stress Response Capacity" "which suggests cells or organisms are capable of generating dynamic redox responses to activate cellular signaling and maintain cellular homeostasis" and such capacity is decreased with aging (Meng et al. 2017).

The general picture of adaptive redox homeostasis (which is not necessarily related to ROS involvement) includes local ROS generation (H_2O_2 seems is central here), Cys-based ROS sensing and transduction of the signal in cellular response adequate to challenge type, duration and its intensity. In the case of ROS-initiated challenge, under activation of Keap1/Nrf2 system thiol groups of Keap1 protein are oxidized which results in translocation and accumulation of Nrf2 in the nucleus and upregulation of about 200 genes some products of which are clearly involved in the prevention of ROS generation, neutralization and removing of ROS-damaged molecules (Garaschuk et al. 2018; Vasileva et al. 2020).

Recently Davies (2016) proposed the concept of "adaptive homeostasis" which means that organism can potentially react rapidly to multiple transitory fluctuations in environmental hazards changing its resistance and minimizing energy cost. Later, the concept was extended to aging process (Pomatto and Davies 2017) and argue a decline in adaptive homeostasis with aging, especially in the last third of the lifespan. This clearly well corresponds to described two decades ago lifespan dynamics of ROS-promoted oxidation of proteins (Levine and Stadtman 2001). The concept is based on the fact that diverse exposure of number organisms to such stresses as oxidative, heat, cold, metabolic, environmental, glucose, hypoxia, exercise, caloric restriction, osmotic, mechanical, emotional, psychological, etc., in young organisms induce a strong adaptive response, whereas in older organisms, they induce weakly or not induce response at all (Pomatto and Davies 2017). Significant loss of adaptive response capability is a clear hallmark of the aging process.

1.8 Conclusive Remarks and Perspectives

Redoxome is defined as the redox state of a full set of all redox reactions in the organism. It is a steady state situation, which is provided by a net continuum of compounds capable to enter redox reactions. This is based on the balance between reduced and oxidized states of all components of living organisms and which provides living functions of the organisms via the production of energy equivalents and building blocks. This well corresponds to the redox theory of aging stating that aging is a decline in the plasticity of genome-exposome interaction (Jones 2015).

As mentioned above, during lifespan redox state of the organism is shifted to an oxidized one. Initially in this chapter, it was stated that all redox pairs responsible for the coordination of organisms' response to redox challenges of cysteine residues of peptides are best characterized to date. Actually, the operation of this couple is determined by the ratio of the concentration of the oxidized or more broadly modified thiol groups to the concentration of reduced thiol groups, which can be given as a ratio [–SR]/[–SH]. Again, I focused on them because they are well known as critical components for redox sensing in a number of regulatory proteins. The ratio [–SR]/[–SH] is increased over lifespan relatively slowly up to half of life and more quickly at the second part of life which is better pronounced in the last trimester of the life (Jones 2015). This virtually absolutely corresponds to the described two decades

earlier lifespan dynamics of oxidative modification of proteins (Levine and Stadtman 2001).

Thiol groups of sensor proteins such as one OxyR in bacteria, Gpx3/Yap1 in yeasts, and NF-kβ and Keap1 in animals are known to be responsible for the launch of regulatory redox cascades (Lushchak 2011). It is well known also that the capability of the organisms to respond to redox stimulus is decreased with the age (Pomatto and Davies 2017). Unfortunately, it was impossible to find in the literature how this capability to stimulation or "stimulability" is changed over the whole lifespan because only certain points have been characterized from this point of view. Obviously, that can be an interesting topic for future studies. It may exactly correspond to the mentioned parameter if one would follow the reductive state of the –SH groups exactly involved in the regulation of the redox adaptive response. However, there are many cysteine-containing redox sensor proteins involved in the organisms' response to stresses. In other words, this is a multicomponent system, and therefore, obviously registered response in the ratio and stimulability in practice may not be synchronic. Deviation from synchronic responses of two groups of processes depends on many circumstances and may be the avenue for future studies.

Now the question is: may lifespan trajectories differ from "standard" or normal trajectory? Yes, there are accelerated and delayed aging trajectories described already in the first part of this chapter. Naturally, the next question is: may lifespan trajectories be changed and if "yes", in which way? However, as it was noted above, the possibility to change lifespan trajectory at the last stage of life is the most intriguing and practical. In other words, one may mean the so-called rejuvenation. At the last third part of life, the trajectory potentially can be changed by radical influence on redoxome, whereas earlier more soft changes may cause such shift (Fig. 1.1). That means that the ratio [–SR]/[–SH] should be in some way sharply decreased in the last half or trimester of life. To get this, the balance between oxidized and reduced thiol groups responsible for adaptive response to redox stimulus must be sharply shifted to reduced one. There is no available information on such events but it can be deduced from the data showing that many rejuvenation procedures include or are related to activation of autophagy and removing certain amounts of oxidized components of living organisms such as ROS-modified proteins. Certainly, some of these proteins may be ones with terminally oxidized –SH groups where sulfur is in oxidized state VI (Lushchak 2012). It is known that from discrete valence states II and IV, sulfur can be reduced to –SH state by thioredoxins and sulfiredoxins, respectively (Lushchak 2012). To date, there is no known living organism system to reduce sulfur in the valence state VI. This state may be also called irreversibly oxidized (in conditions of living organisms). Therefore, the proteins with terminally or maximally oxidized cysteine residues involved in redox signaling must be just removed. Usually, it is realized via proteolysis. Such regulatory proteins must be replaced by newly synthesized ones in order to keep the organism functioning. This scenario may restore the ratio [–SR]/[–SH], i.e., decrease it and the organism may enter a new lifespan trajectory which would be shifted to the right (in "green zone"), i.e., it will extend lifespan even in the case when it started at late or even advanced age. The shift in the ratio [–SR]/[–SH] would restore the capability of the organisms

to respond adequately to environmental redox stimulus. This increases plasticity or restores adaptive homeostasis of the organism (Pomatto and Davies 2017) which declined before the rejuvenation procedure. A very similar logic can be applied for shortening of lifespan due to change of lifestyle with shifting of the trajectories to the left, i.e., "red zone" (Fig. 1.1).

In conclusion, it should be said that accurate portraiture of redoxome during lifespan with identification of key regulatory players may disclose targets, or probably set of targets for combating age-related changes and age-related diseases. This can be a prosperous strategy to extend healthspan, i.e., being free from a serious disease or leading to death. There are already some clues how to achieve that even if start to care about health at advanced age.

The slow increase of portion of ROS-modified proteins which is better expressed in the last trimester of life has been established two decades ago (Stadtman and Levine 2001). Later, a similar shift was documented for thiol residues of the proteins and low molecular mass thiol-containing compounds such as cysteine and glutathione (Blanco et al. 2007). Similarly, an adaptive potential also demonstrates a significant decline in the last third of life (Pomatto and Davies 2017). So, it seems that at aging, decrease in the capability of living organisms to adapt to environmental challenges at least in the redox field is closely related to the more oxidized state of proteins particularly their cysteine residues. Only autophagy is the known mechanism to remove efficiently irreversibly oxidized proteins, but its efficiency also decreases with aging (Garaschuk et al. 2018). Therefore, there is a reason to increase autophagy efficiency in the aged organisms. Dietary restriction has been identified as the only unequivocally accepted scheme to extend lifespan significantly for most organisms investigated to date. This approach stimulates autophagy and causes some sort of rejuvenation. But very few people would like to subject them to dietary restriction due to which there is a significant interest in compounds called dietary restriction mimetics (Vaiserman et al. 2016). These compounds may partially model dietary restriction, but none of known to date mimetics provides a full set of parameters found at this state, and moreover, all of them have some side effects. Therefore, only dietary restriction and better in combination with professionally controlled physical activity may provide healthy aging. In addition, from a redox point of view, the NAD^+ system looks like an attractive target for intervention due to which NAD^+ precursors such as nicotinic acid may be potentially used as a complementary to dietary restriction strategies (Mouchiroud et al. 2013).

Finally, the aging-related shift of redox homeostasis to a more oxidized state decreases the adaptive potential of organisms (Pomatto and Davies 2017). This takes place because many critically important biomolecules (e.g., proteins, fatty and nucleic acids) lose their functionality when oxidized. The only reliable strategy to restore redox balance or redoxome and achieve a younger phenotype is to replace such oxidized biomolecules with newly synthesized ones. Maintaining or even restoration of "younger redox phenotype" or rejuvenation needs energy which production is decreased with aging partially due to uncoupling of mitochondria because of their damage by ROS. So, direct or indirect interaction between energy-producing systems and redox homeostasis from point of view of such interactions is an appearing field

of research (Garaschuk et al. 2018; Bayliak et al. 2021; Lushchak 2021). It may help to disclose molecular mechanisms of the system response to age-related changes and identify potential target candidates for health-promoting interventions.

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Chapter 2
Reliability and Longevity of Biological Systems: The Free-Radical Redox Timer of Aging

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Abstract The system approach, based on the engineering theory of reliability, integrates the concept of aging program and the free radical theory of aging into a unified pattern. The main line of assuring the high system reliability is preventive maintenance, i.e., unreliable elements should be timely replaced for novel ones ahead of the phase of their wear-out begins. This prophylaxis of failures is controlled via the longevity-assurance structures (supervisors) of the highest level of hierarchy which operate with limited (genetically preset) reliability. The stochastic malfunctions of the mitochondrial electron transport nanoreactors, which produce the anion-radicals of oxygen ($O_2^{\bullet -}$, "superoxide radical") as a by-product of oxidative phosphorylation, are of first importance. In reality, this radical is not an oxidant but a reducing agent. As the reducing agent, it affects NADH/NAD^+ ratio, thereby impacting the epigenetic sirtuin regulators of metabolic repair and renewal processes. As a consequence, the oxidative-stress products and other metabolic slag accumulate with time, resulting in the impetus to autophagic or apoptotic cell death and age-associated clinical disorders. On this basis, the universal features of aging, the exponential growth of mortality rate with time (Gompertz law of mortality), and the correlation of longevity with the species-specific resting metabolism (Rubner scaling relation) are explained. Thus, from the reliability point of view, aging occurs as an inevitable consequence of the genetically preset deficiency in reliability of the biomolecular constructs, while the free radical redox timer, located presumably in cells of the hypothalamus, serves as an effective stochastic mechanism of realization of the genetical deficiency in system reliability of an organism as the whole.

Keywords Longevity · Aging · Systems biology · Reliability · Robustness · Free radicals · Sirtuins · Prophylaxis · Preventive maintenance

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2.1 Introduction

Aging is a universal process to which all organisms, both multi-cellular and unicellular, are subjected (Finch and Hayflick 1977; Anisimov 2008). Few doubt the endogeneity of aging: poisoning by the products of metabolism of intestinal microflora, Moon gravitation, the position of the planets, and other exogenic causes influence aging, if at all, only as secondary factors. Opinions on the issue of whether aging is programmed or stochastic differ more radically. Some researchers consider aging as the last stage of a genetic program of ontogenesis and believe that there are special "genes of aging" which regulate aging and death. Other researchers believe that aging is a stochastic process, the cumulative side result of the pleiotropic action of a large number of regulatory genes, whose useful action manifests itself in the reproductive period and whose harmful influence becomes apparent only after the reproductive period ends.

In this research direction, the free radical hypothesis of aging has determined the most heuristic lines of investigations. The studies in free radical biology and medicine were stimulated, in the mid-twentieth century, by awarding the Nobel Prize in Chemistry to Cyril Hinshelwood and Nikolay Semenov in 1956 for the studies of free radical mechanisms of chain radical reactions. The idea that free radicals of oxygen are responsible for oxygen toxicity was stated by Rebecca Gershman and Daniel Gilbert in 1954 (quoted from Gilbert 1981). Denham Harman was the first who conceptually stated the free radical hypothesis of aging. "Free radical reactions, however, initiated, could be responsible for the progressive deterioration of biological systems with time" (Harman 1956).

Following the memories of Harman (1992), his interest in aging had been sparked in December 1946, when his wife Helen had called to his attention an article published in a popular lady magazine. The article, "Tomorrow you may be younger", written by William L. Laurence, science editor of the New York Times, was concerned with the book of Alexander A. Bogomolets, "The prolongation of life", published in English in New York in 1946 (Bogomolets 1946; first published in Kiev, Ukraine (former Soviet Union) in 1940). When Harman was employed in 1954 to work in the Donner Laboratory of the University of California in Berkeley, his task was the searching of anti-radiation protectors, i.e.—the inhibitors of the free radical reactions to protect the organic materials from ionizing radiation. Harman was the first who had discovered that the antioxidant, radiation protector 2-mercaptoethylamine, essentially prolongs the life spans of laboratory mice (Harman 1957). Since then, the beneficial effects of the antioxidants were experimentally proved over and over again (see refs. in Harman 2003; Koltover 2010, 2018b). However, neither natural antioxidants, like vitamin E and flavonoids, nor synthetic antioxidants are able to serve as efficient scavengers of oxygen free radicals in vivo, since the rate constants of the antioxidants in the reactions with oxygen free radicals are negligibly small as compared with the natural antioxidant enzymes (Koltover 2009, 2010; Forman et al. 2014). Accordingly, it has been questioned whether or not the free radical hypothesis itself, on its own, is

comprehensively able to explain the inevitability of aging (Gladyshev 2014; Halliwell 2020).

At the end of the twentieth century, a new approach to the problem of aging was developed on the basis of the theory of reliability (Koltover 1981, 1982, 1983, 1992, 1997, 1999). This chapter is designed to show that the system theory of reliability allows to integrate the programmed events and the stochastic events into a single united theory. In addition, this review presents the data that antioxidants provide preventive protection from free radicals via the beneficial effects of the antioxidants on the system of neuro-hormonal regulation along with their system effects on microbiota.

2.2 Reliability of Biological Systems: Historical Synopsis and Terminology

In engineering, reliability is defined as the ability of a device to perform the preset function for the given time under the given conditions. The foundations of the mathematical theory of reliability were laid in the 1950s due to the needs of aeronautic machinery and problems of communication, management, etc. (Bazovsky 1961; Lloyd and Lipov 1962). Like in engineering, all biological systems are constructs or devices. Each of them is synthesized and assembled according to the information plane, i.e.—the special genetic program, with the purpose to perform the preset functions. Biological systems perform their functions in the presence of a great number of random factors which disturb all functional strata, from biomolecules up to and including ecosystems. Therefore, similar to technical devices, biological constructs are not perfectly reliable in operation. For each and every device, normal acts of operations alternate with stochastic (random) malfunctions or failures. The field of systems biology, in dealing with the problem of reliability, incorporates the theoretical and experimental investigations of: classification and systematization of failures in biological systems; mechanisms of failures of biomolecular nanoreactors and mechanisms of realization of the failures in functional breaks; investigations of renewal processes; elaboration of methods for testing the reliability and predicting failures in biological systems (Koltover 2019).

The problem of reliability of biological systems was first formulated and put forward in the works of D.M. Grodzinsky and his colleagues (Grodzinsky et al. 1987; Koltover 1981, 1997; Kutlakhmedov et al. 2003, 2006). The regular conferences on the reliability of biological systems, starting from the first one in 1975, in Kiev, Ukraine (former USSR), and the conference books had spurred the studies on the reliability of biological systems in the former USSR and beyond the former "iron curtain" (Dimitrov 2010; Doubal 1982; Finkelstein 2005; Gavrilov and Gavrilova 2001; Steinsaltz and Goldwasser 2006; Witten 1983). The special Scientific Commission on Reliability of Biological Systems (chairman D. M. Grodzinsky, vice-chairmen V. K. Koltover, and Y. A. Kutlakhmedov) had been organized in 1978, at the Scientific

Council on Biological Physics of the USSR Academy of Sciences, in order to deal with the problems of reliability of biological systems.

Not long ago, a new wave of analogous research has been spurred under the style of "robustness" (Kitano 2004; Larhlimi et al. 2011; Kriete 2013). This term, "robustness", is now often used in articles and databases instead of the term "reliability". Meanwhile, the term "reliability", but not "robustness", is generally received in the scientific literature on engineering, communication, and so on, see, for example, the journal "Reliability Engineering & System Safety" (Elsevier Sci. Ltd., sited in WEB of Sciences, Q1). The term "reliability", not "robustness", has been used in the pioneering works on biological reliability (Grodzinsky, ed. 1977, 1980; Grodzinsky et al. 1987; Koltover et al. 1980; Koltover, 1981, 1982, 1997, 1999). Reliability in engineering is determined as the probability to work without failures during a given period of time. As a matter of fact, it is the probability to work robustly, i.e., without failures. It might be said that reliability is robustness exerted in the course of work. As for the term "robustness", it is slang smacked of the terms like "serviceability" or "Robusta coffee". There is the mathematical theory of reliability, and all mathematicians consider this theory as a part of probability theory (Gnedenko et al. 1965). Hence, there is no need to contrive "biological robustness" or any other quasi-novel approaches to the problem of reliability of biological systems. As said by Immanuel Kant, "in any partial doctrine of nature, one can find as much genuine science as there is mathematics in this doctrine and no more".

2.3 Longevity and Reliability: Genetics Determinants and Mathematical Theory of Reliability

Despite the complexity and phenotypic variety of living organisms, aging is governed by some common quantitative laws or, more precisely, "patterns of relationship". First, there is the so-called species-specific maximal lifespan potential. Indeed, it is common knowledge that there are neither mice nor rats exceeding 3–4 years of age and that a human lifespan does not exceed ≈120 years provided we take reliable data into account, not sensational press reports or legends (Anisimov 2008; Dong et al. 2016; Novoselov 2020). The limited lifetime of diploid cell strains in vitro is also a well-known phenomenon. For example, human fibroblasts in vitro die or mutate into cancer cells after performing about 50 doublings. American biologist Hayflick discovered this effect in 1961 and Russian biologist Olovnikov explained the Hayflick's limit suggesting the mechanism of under-reparation (incomplete copying) of telomere ends of DNA (Finch and Hayflick 1977; Anisimov 2008). According to Olovnikov's theory of marginotomy, every cell division is accompanied by reduction of the telomere ends of cell chromosomes. In essence, it means that the cell division stops as soon as the telomere circumcision runs up to the limit fatal level (Olovnikov 1996).

Another "pattern of relationship" is the correlation, for placental mammals, between the lifespan potential and the rate of the basal oxygen consumption

$$T \cdot V_0 = 4.12 \cdot 10^{10} \cdot B^{1.37}, \tag{2.3.1}$$

where T is the maximal lifespan value of the species (in sec), V_0 is the basal oxygen consumption (resting metabolic rate, in ml/sec), B is the brain mass (in kilograms). This correlation, first discovered by Rubner in 1883, had been confirmed since then for different mammalian and non-mammalian species as the universal scaling relation (Schmidt-Nielsen 1984).

The growth of mortality rate with age obeys the universal kinetics. Namely, if $n(t)$ is a number of people alive at age t, Δn is a number of those who died during the time interval Δt (usually taken to be 1 year), then the mortality rate

$$h(t) = -\Delta n(t)/n(t)\Delta t = h_0 \exp(\gamma t), \tag{2.3.2}$$

where parameters h_o and γ are independent of time. This is the so-called Gompertz law of mortality that had been confirmed for people (of age approximately from 35 to 90 years), other mammals, flies, mollusks (Sacher 1977; Koltover 2019). Moreover, it has been shown that the aging of prokaryote cells, *Acholeplasma laidlawii*, in the stationary phase of growth, namely—the loss of viability measured as their ability to form macrocolonies, follows the same kinetic pattern (Kapitanov et al. 1985). Noteworthy, the cited work (Kapitanov et al. 1985) has been the first one where it was demonstrated that the cell viability in cellular cultures declines according to Gompertz law.

In contrast to the so-called "robustness", there are many mathematical models in the engineering theory of reliability (Bazovsky 1961; Gnedenko et al. 1965; Lloyd and Lipov 1962). The Gompertzian mortality function corresponds to the so-called Type-1 asymptotic distribution for the minimum value, known from the statistics of extremes. Namely, the hazard function for a complex physical system consisted of a large number of components increases exponentially whenever the components are connected in series and are all subjected to a "wearing-out" process in which the risk of failure increases progressively (Gumbel 1962). This limit theorem of the statistics of extremes makes the Gompertzian mortality law appear as if it is almost universally valid, much like the central-limit theorem makes the normal distribution appear as the very appropriate model in the theory of errors.

It is generally known that any organism is a hierarchical structure in which a relatively small number of key elements, which manage a large number of executive elements, can be distinguished. The template principle of organization of living systems implies that information DNA structures are of the first operation importance in the cell hierarchy. A multi-cellular organism is governed by genes of a special anatomically isolated group of cells, like the specialized neurons of the hypothalamus in animals. Furthermore, from the mathematical theory of reliability, it is known that

the effectiveness of operation of a complex system is determined mainly by the reliability of the system's governing elements.

Following this line, the system reliability approach to the problems of mortality and longevity was developed in our papers (Koltover 1981, 1982, 1983, 1988, 1992, 1997, 2011, 2014, 2016, 2017a, b, 2019, 2020). This approach is based on the simple general principles. The 1st one is the template principle of organization of living systems implying that information structures rank first in cell hierarchy ("original idiotype" of the molecular design or the template principle of organization of living systems). Any organism works like a system of biomolecular constructs designed in accordance with the genetic program *(information plan)* in order to perform the preset programmed functions (*purpose*). The 2nd principle is that all biomolecular constructs operate with limited reliability, namely, for each and every biological device, starting from enzymes, normal operation acts alternate with accidental malfunctions. The 3rd principle states that preventive maintenance replacement of functional elements in cells and tissues is the main line of assuring high system reliability. Bioconstructs need prophylaxis, just as in engineering. Following the preset genome pattern, unreliable elements should be timely replaced for novel ones ahead of the phase of their wear-out begins. In essence, it is the metabolic turnover. The 4th principle states that there is a finite number of critical elements which perform the supervisory functions over the organism's repair and renewal processes, i.e.—over the metabolic turnover. Since these critical elements of the highest hierarchy level exert control over the system reliability, they can be called "supervisors" or "longevity-assurance structures" (LAS). The 5th principle states that the supervisors also operate with the limited, namely, genetically preset reliability so that stochastic damages are accumulated with time in "the power structure" up to the preset threshold dysfunction levels. As a result, each organism has a limited lifespan.

Following this reliability-theory approach, the simple mathematical model of aging was suggested first in our papers (Koltover 1981, 1982). It was taken that LAS accumulate stochastic flaws resulting in the disarray of their functions. The account was also taken of another widespread peculiarity of living systems, i.e.—the existence of threshold values for the most important functional parameters. According to this general idea, there is to be an upper limit value, m_c, at which LAS fails. The set of initial flaws, m_j ($j = 1, 2, \ldots, N$), represents a random sample of the exponential, though truncated, distribution with the density function

$$f(m) = \alpha \exp(-\alpha m)/[1 - \exp(-\alpha m_c)], \tag{2.3.3}$$

where $\alpha > 0$ is a parameter of this distribution, and $0 \leq m \leq m_c$. To underlay this hypothetical density function, some simple arguments were brought into account. As the flaws to LAS occur as rare events, the probability of getting an extensive flaw should obviously be less than the probability of getting a smaller one. Then, the exponent was used as the simplest asymmetric distribution known from mathematical statistics. Similar truncated exponential distributions have been known for many years ago as the simplest asymmetrical distributions in the physics of linear polymer

chains (Flory 1953). The organism has been assumed to perish the moment that any of the LAS develops the threshold dysfunction, i.e., the expected lifespan

$$\tau = \min \tau_j \tag{2.3.4}$$

Here $\tau_j = b(m_c - m_j)$, where $b > 0$ is the reciprocal of the dysfunction growth rate in LAS with time. As a matter of fact, the lifespan of the organism is determined by the weakest link's longevity. Then, the survival function is given by the smallest value of the random sample of size N

$$R(t) = \left\{1 - \left[exp(\gamma t) - 1\right]/\left[exp(\gamma T) - 1\right]\right\}^N \tag{2.3.5}$$

where N is a number of LAS, $T = bm_c$ and $\gamma = \alpha/b$. For not very high values of time, the following approximation can easily be derived:

$$R(t) = \exp\{(h_0/\gamma)[1 - \exp(\gamma t)]\}, \tag{2.3.6}$$

with the relevant expression for mortality rate being

$$h(t) = h_0 \exp(\gamma t), \text{ where } h_0 = \gamma N/[\exp(\gamma T) - 1]. \tag{2.3.7}$$

Here is the Gompertz law of mortality. Hence, this law gets its explanation in the context of the reliability-theory approach stated above. The limit lifespan T has appeared as the direct result of the existence of the limit dysfunction, m_c, for the LAS. Formally, this limit is the lifespan of an "ideal" organism with no flaws at $t = 0$.

All the hypothetical LAS are differently fallible since they are initially flawed at statistically varying degrees. However, they were postulated to have the same values for the reliability characteristics (α, b, m_c). This was certainly assumed for the sake of mathematical simplicity. However, an evolutionary mechanism could be proposed to support this assumption. The arrangement of the appropriate level of reliability for these structures falls into the basic cell maintenance processes of defense, restoration, and renewal, because each of them is vitally important. All processes of this kind are metabolically expensive. Meanwhile, the energy budget of a cell is limited. If the organism perishes the moment that any one of LAS fails, there is no use in natural selection making some of them more reliable than others. The acquisition of greater maintenance for any one, than it is necessary for others, should obviously require an excess cost.

If we take that the maximum lifespan for human populations, on the average, is about 95 years, the magnitude of γ varies from 0.0612 to 0.119 years^{-1} and the magnitude of h_0 varies from 0.820×10^{-3} to 0.022×10^{-3} years^{-1} (Sacher 1977), then, using the expression for h_0, we find that $N \approx 5 - 15$. It is worthy to note that this estimation corresponds, by the order of magnitude, to the number of the so-called

"longevity-assurance genes" which have been recently discovered in nematodes, yeasts, flies, mice, and other organisms (Longo et al. 2012; Yashin et al. 1985). It has been assumed that "the power structure", other words "supervisors" or LAS in organisms of humans and animals are the longevity-assurance genes located in the special cells of, most probably, suprachiasmatic nucleus of the hypothalamus.

There are reports on the deceleration of the mortality-rate functions in cohorts of *Drosophila* flies at an advanced age. Similar findings for humans were taken up in the literature, notwithstanding the fact that the statistical data on mortality at geriatric ages are poor (Yashin et al. 1985; Smith et al. 2008). A qualitative attempt to highlight this limitation of the classical Gompertzian approach was undertaken by assuming a simple mixture of a few homogeneous populations (Koltover 1983). The first quantitative stochastic model of mortality and aging in heterogeneous populations was suggested in (Yashin et al. 1985). In succeeding years, the question of how inhomogeneity, i.e.—a genetic or phenotypic variability of populations, may affect the behavior of the reliability model was examined quantitatively (Koltover 1988; Koltover et al. 1993). Namely, the parameters T and γ were averaged over the ensemble assuming the normal distributions for these parameters with the respective probability density functions. At the advanced time values, the mortality rate function generated from the heterogeneous model may accelerate its run, slow it down, display a maximum or level it off depending upon the extent of heterogeneity of the parameters, thereby behaving in quantitative agreement with the mortality rate curves of real populations (Koltover et al. 1993; Koltover 2014, 2016). At this, the quantitative estimations of N obtained from the death statistics of the heterogeneous populations exceed the previous estimation obtained for the case of homogenous population. The origin of this diversity is quite obvious. Individuals of a homogeneous population, being alike, die from a limited number of similar pathological reasons. However, it is not the case for heterogeneous populations. In this case, on the "tails" of the distribution function, there are different individuals, from a centenarian who dies at the age of older than 100 years to a short-lived person who carries a mutant fatal gene in one of his 46 chromosomes. In part, the life-tables of the 1969–1973 calendar periods for Swedish men, in the age range from 35 to 105 years, have been computed and the set of the fitting parameters, $T_0\,(\pm\sigma_T) = 120\,(\pm 0.3)$ years, $\gamma_0\,(\pm\sigma_\gamma) = 0.095$ (± 0.001) years^{-1} and $N = 46$, gave an agreement between the reliability model and the overall mortality data with an accuracy of 13% (Koltover et al. 1993).

Based on this reliability-theory approach, one could formulate a more complex mathematical model to take into account the preset pattern of the longevity-assurance genes, their functional interrelations (feedbacks), and other non-linear effects. It is to be hoped that a non-linear reliability-theory model will explain even so sophisticated cases as the demographic profiles of *D. melanogaster* strains (Smith et al. 2008) and justifying Gompertz curves of mortality of human populations via the generalized Polya process of shocks (Cha and Finkelstein 2016).

2.4 Free-Radical Redox Timer of Aging: Stochastic Realization of the Genetic Program

It is generally known that the energetic demands of every operation in living systems are met by molecules of adenosine triphosphate (ATP), most of which are synthesized in mitochondria. For human beings, oxygen consumption in the resting metabolism is ≈0.280 L/min (≥400 L/day). Up to 98% of oxygen, consumed by cells, is used in mitochondria during oxidative metabolism to produce ATP. In mitochondria, there are the special biomolecular constructs, the electron-transport nanoreactors (ETN). The normal functioning of the ETN lies in the transport of electrons from the oxidation substrates, NADH or succinate, to cytochrome oxidase and then to oxygen. The oxygen molecule, O_2, taking two electrons, one after another, is reduced into water while the energy, which is released at the substrate oxidation, is used for ATP synthesis (Nelson and Cox 2008).

In objective reality, however, mitochondrial nanoreactors are not perfect in operation. Similar to the so-called recurrent failures or malfunctions in engineering, the normal elementary acts of electron transfers in ETN alternate with the accidental malfunctions which result in the formation of anion-radicals of oxygen, $O_2^{\bullet-}$. In biomedical literature, this radical is called "superoxide radical" that triggers "reactive oxygen species" (ROS) in cells and tissues and "oxidative stress" (McCord and Fridovich 1969, 2014). Among the generators of $O_2^{\bullet-}$ in cells and tissues, there are also NAD(P)H-cytochrome-C reductase and cytochrome P-450 of endoplasmic reticulum, xanthine oxidase, catecholamine and other biogenic amines, mono- and diamine oxidases, aldehyde oxidases, oxidases of D-aminoacids, D-galactosidase, lipoxygenase, nitric oxide synthase, leukoflavines, hemoglobin, and myoglobin, ascorbate, NAD(P)H-oxidase of phagocytes, and other so-called NAD(P)H oxidases (NOX) enzymes (Brown and Griendling 2009). However, in eukaryotic cells, the main bulk of $O_2^{\bullet-}$ is formed as the by-product of respiration in cellular mitochondria. Moreover, the $O_2^{\bullet-}$ generation is increased when the normal operation of mitochondria is disrupted. For example, the drastic increase in the generation of $O_2^{\bullet-}$ has been detected in the experiments with isolated heart mitochondria, after the mitochondria were exposed to hypoxia or ischemia (Nohl et al. 1993; Nohl and Koltover 1994).

To protect cell structures from $O_2^{\bullet-}$ and its toxic chemical products, there is the special defense enzyme, superoxide dismutase (SOD) which catalyzes the reaction of dismutation of $O_2^{\bullet-}$ into H_2O_2 and oxygen. There are three kinds of SOD, mitochondrial Mn-SOD, cytosolic Cu, Zn-SOD, and periplasmatic Fe-SOD. SOD enzymes work in cooperation with other antioxidant enzymes, catalase and glutathione peroxidase, which catalyze the decomposition of H_2O_2 (Nelson and Cox 2008). However, like other biomolecular constructs and systems, the enzyme system of antiradical defense operates with limited reliability. The elementary acts of occurrence of $O_2^{\bullet-}$ as the by-products of oxidative metabolism and the elementary acts of disappearance of $O_2^{\bullet-}$ in the dismutation reaction are stochastic events. Accordingly, the $O_2^{\bullet-}$ radicals can slip through the SOD defense system.

We have analyzed the stochastic dynamics of this system by a mathematical "Birth and Death" model often used in the mathematical reliability theory. The calculations, based on the experimental data from the available literature, show that the probability of slipping of $O_2^{\bullet-}$ through the mitochondrial SOD is about 1.9×10^{-5}, i.e., about 2 radicals from every 100 000 may penetrate the defense system (Koltover 1981, 1982, 1983). Noteworthy, that $O_2^{\bullet-}$ can penetrate through lipid membranes (Gus'kova et al. 1984). Hence, with the intense electron transport fluxes in mitochondria and outside, the probability of the $O_2^{\bullet-}$ induced free radical damages in cells can be high enough.

In biochemistry, this radical is heighted, over half a century, as the "superoxide radical". In itself, $O_2^{\bullet-}$ is not an oxidant but, on the contrary, it is a reductant. Armed with one uncoupled electron, this radical is able to donate it into other molecules (Koltover et al. 1978; Koltover 2010). However, some oxidants are really formed as the reaction products of $O_2^{\bullet-}$. First, hydrogen peroxide (H_2O_2) is formed as the product of the reaction of dismutation of the $O_2^{\bullet-}$ radicals (Nelson and Cox 2008; Koltover and Skipa 2021):

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \Rightarrow H_2O_2 + O_2 \tag{2.4.1}$$

Next, $O_2^{\bullet-}$ reacts with H_2O_2 with the formation of $OH^{\bullet}$ radical

$$O_2^{\bullet-} + H_2O_2 \Rightarrow OH^- + OH^{\bullet} + O_2 \tag{2.4.2}$$

This reaction, called the reaction of Haber–Weiss, is accelerated in the presence of ions of variable valence, like iron (Wardman and Candeias 1996)

$$O_2^{\bullet-} + Fe^{3+} \Rightarrow Fe^{2+} + O_2 \tag{2.4.3}$$

$$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + OH^{\bullet} + OH^- \tag{2.4.4}$$

Meanwhile, it is generally known that $OH^{\bullet}$ radical is a very strong chemical oxidant that initiates free radical reactions of oxidation of lipids and proteins, oxidative degradation of DNA with the formation of 8-oxo-7,8-dihydroguanine, and so on. Besides, there are the reasons to believe that $O_2^{\bullet-}$ can react with the nitric oxide ($NO^{\bullet}$) radical with the formation of peroxynitrite anion ($ONOO^-$)

$$O_2^{\bullet-} + NO^{\bullet} + H^+ \Rightarrow O = N - O - OH \tag{2.4.5}$$

$$O = N - O - OH \Rightarrow O = NO^{\bullet} + OH^{\bullet} \tag{2.4.6}$$

$$O = N - O - OH \Rightarrow NO_3^- + H^+ \tag{2.4.7}$$

Similar to $OH^{\bullet}$ radical, peroxynitrite is also considered as a strong oxidant that mediates oxidative damage effects of $O_2^{\bullet-}$ (along with nitric oxide) in cells and tissues (Crow and Beckman 1995; Shiva 2013; Winterbourn 2008).

Up to 5% of oxygen, consumed by cells and tissues of aerobic organisms, is transformed into the oxygen anion-radical $O_2^{\bullet-}$, hydroxyl radical $OH^{\bullet}$, hydrogen peroxide H_2O_2, lipid peroxide radical $LO_2^{\bullet}$, lipid peroxides LOOH, peroxynitrite and other "reactive forms of oxygen" (ROS). That is why the $O_2^{\bullet-}$ radical may, in general ("de jure"), be considered as the superoxide radical since it does initiate the reactions in which the strong chemical oxidants are produced.

Now, let us consider the case of damages initiated by $O_2^{\bullet-}$ radicals as the by-products of the mitochondrial malfunctions. The rate of the free radical malfunctions should be proportional, in the first approximation, to the rate of oxidative metabolism, while the rate of the age-dependent changes in LAS should be proportional both, to probability of free radical failures and probability that the free radical failures provoke the functional violations in the supervisor LAS. Thus, in the case of the damages in LAS caused by the $O_2^{\bullet-}$ radicals, the equation for the maximum (limit) lifespan T follows from the Eq. (2.3.4)

$$T = bm_c \approx m_c/[(qV/E)u + D] \tag{2.4.8}$$

In this equation, m_c is a threshold value of the functional violations in the supervisors, q is probability of the malfunction in mitochondrial nanoreactors leading to the occurrence of $O_2^{\bullet-}$, V is respiration rate, E is the activity of SOD in LAS, u is the probability for the free radical failures to provoke damages, i.e., functional violations (Koltover 1982, 1983). The parameter u takes into account that deleterious effects of $O_2^{\bullet-}$, which slipped through the enzyme antioxidant defense, are of no concern, if the preventive replacement of the damaged "biological constructs" is properly maintained in cells and tissues. D is an index to incorporate other damage factors that are not associated with oxygen free radicals.

As a matter of fact, the latter equation explains the universal quantitative law of aging, the so-called "Rubner scaling relation" (see Eq. 2.3.1). This equation predicts that it should be a linear correlation between the reciprocal of the species-specific maximum lifespan ($1/T$) and the ratio of the respiration rate (V) to the SOD activity (E), i.e.

$$1/T = A(V/E) + B \tag{2.4.9}$$

Using the data on the SOD activity in brain, liver, and heart tissues from men and animals of 13 species (Tolmasoff et al. 1980), and using the literature data on the species-specific oxygen consumption rates, we have plotted the reciprocal maximum lifespan potential ($1/T$) versus the ratio of V/E. Figure 2.1 demonstrates the plots, i.e.—the correlation graphs between the reciprocal maximum lifespan potential and the ratio of V/E.

In agreement with the prediction of our theory, the straight lines have been obtained, and the relevant correlative equations:

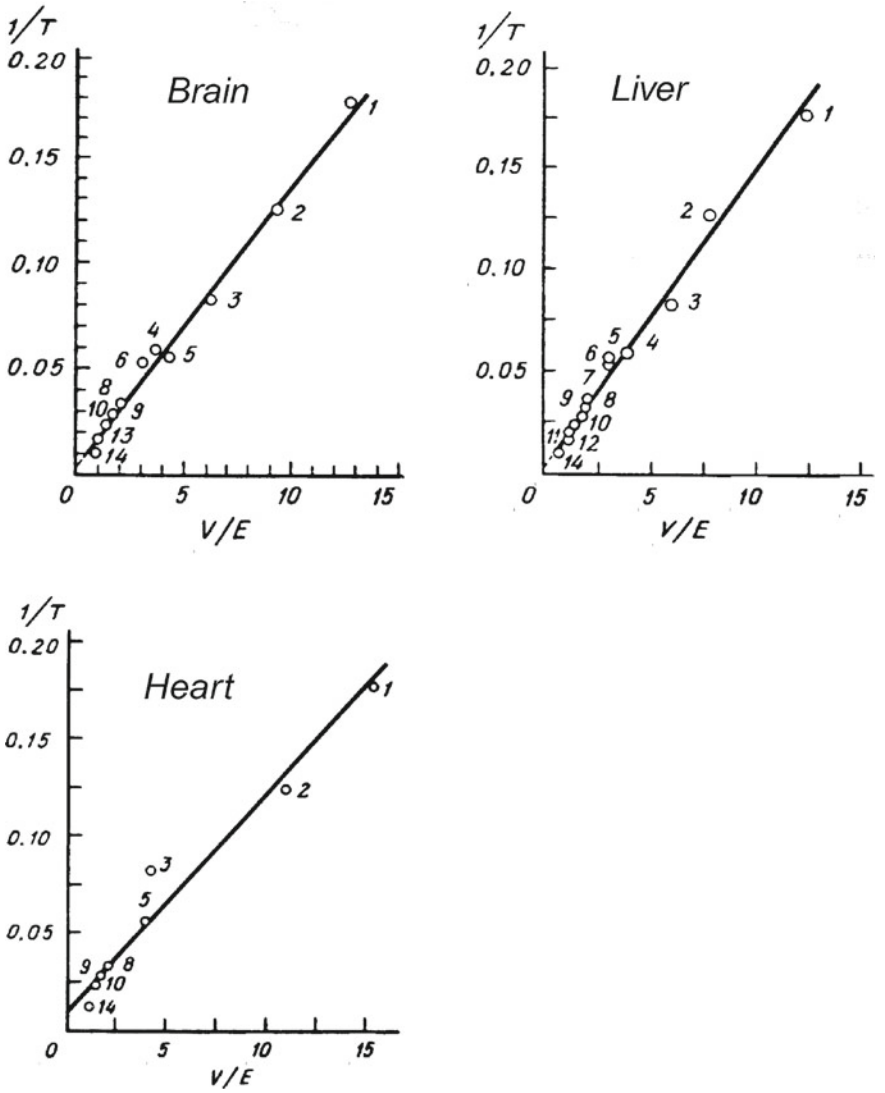

Fig. 2.1 Correlations graphs between the reciprocal maximum lifespan potential (*1/T*) of different mammalian species and the ratio of species-specific metabolic rate to superoxide dismutase activity (*V/E*) for brain, liver, and heart plotted according to the mathematical model based on the theory of reliability. Numbers correspond to the species: 1—house mouse (*Mus musculus*), 2—deer mouse (*Peromyscus maniculatus*), 3—common tree shrew (*Tupaii glis*), 4—squirrel monkey (*Saimarii scuireus*), 5—bush baby (*Galago crassicaudatus*), 6—moustache tamarin (*Saguinus mystak*), 7—lemur (*Lemur macaca fulvus*), 8—African green monkey (*Cercopithecus aethiops*), 9—rhesus monkey (*Macaca mulatta*), 10—olive baboon (*Papio anubis*), 11—gorilla (*Gorilla gorilla*), 12—chimpanzee (*Pan troglodytes*), 13—orangutan (*Pongo pygmaeus*), 14—man (*Homo sapiens*). Compiled from (Koltover 2017b)

$$\text{Brain}: 1/T = (0.0132 \pm 0.0002)(V/E) + (0.004 \pm 0.002)\ (r = 0.997), \tag{2.4.10}$$

$$\text{Liver}: 1/T = (0.0144 \pm 0.0003)(V/E) + (0.005 \pm 0.002)\ (r = 0.997), \tag{2.4.11}$$

$$\text{Heart}: 1/T = (0.0110 \pm 0.0009)(V/E) + (0.011 \pm 0.006)(r = 0.981). \tag{2.4.12}$$

By using the free coefficient D, it was estimated that the longevity of the human brain could reach 250 years, should the reliability of the antioxidant defense be absolutely perfect. This allows suggesting that the longevity-assurance structures (LAS) are located in the brain. By using the free coefficient D of the relevant equations for liver and heart, the limit longevity values were estimated to be 200 years for liver and only 100 for heart. Although these estimations are illustrative, it should be emphasized that the $O_2^{\bullet-}$ radical ("superoxide") does play a large role in the pathogenesis of cardiovascular system.

Chemically, however, $O_2^{\bullet-}$ is a reductant. So, the question arises, which way should go the $O_2^{\bullet-}$ to bring the bale in cells and tissues? The recent discovery of sirtuins can provide the answer. The sirtuins, from SIRT1 to SIRT7, were found to be the key regulators of many important cellular processes (Imai et al. 2000; Tissenbaum and Guarente 2001). Such metabolic functions as gluconeogenesis, fatty acids oxidation, cholesterol scavenging, fat storage, mitochondrial activity, thermogenesis, insulin secretion, formation of muscular tissue (myogenesis), and fat cells (adipogenesis) are mostly regulated by SIRT1. Some other metabolic functions like adipogenesis are also regulated by SIRT2, insulin secretion—by SIRT4, glucose homeostasis—by SIRT6, etc. The inspiring results on the sirtuin-dependent lifespan prolongation have been demonstrated in experiments with yeast *Saccharomyces cerevisiae* (Imai et al. 2000), nematodes *Caenorhabditis elegans* (Tissenbaum and Guarente 2001), flies *D. melanogaster* (Wood et al. 2018), and with mammals too. In mice, the directed overexpression of SIRT1 in the hypothalamus resulted in lifespan increase (Satoh et al. 2013). The lifespan of the transgenic mice with overexpression of the SIRT6 also appeared to be 16% longer compared to the control mice (Kanfi et al. 2012). Moreover, the cross-over interactions between the sirtuin expression and longevity have been revealed for humans (Houtkooper et al. 2012; Watroba et al. 2017; Lee et al. 2019). Meanwhile, all sirtuins have been identified as the NAD^+-dependent protein deacetylases. As such, as the NAD^+-dependent enzymes, they are highly sensitive to the redox state of their environment. Moreover, the expression of the sirtuin genes crucially depends on the redox state of their environment (Koltover and Skipa 2021, and Refs. therein).

One can suggest that $O_2^{\bullet-}$, as the powerful reducing agent, is capable to significantly affect the ratio of $NADH/NAD^+$, thereby provoking the unfavorable changes in the activity of the NAD^+-dependent sirtuin system. As a result, it slows down the processes of repair and renewal of biomolecular constructs. The evident consequence of this will be accumulation of free-radical products, along with other metabolic slag,

in cells and tissues with the resulting impetus to autophagic, apoptotic or necrotic cell death and, thereby, age-associated clinical disorders. As a matter of fact, the $O_2^{\bullet-}$ radicals are targeted onto the NAD^+-dependent sirtuin system, which performs, in its turn, the function of the biological amplifier of the free radical malfunctions. Thus, the discovery of the sirtuins put novel light onto the free radical redox timer of aging and longevity.

2.5 Antioxidant Therapy of Aging: Reliability-Theory Overlook

According to the parameters of Eq. (2.4.8), there are several ways of prolongation of longevity. For example, it is known that human fibroblasts can make no more than 50 duplications before either dying or mutating into cancer cells, the so-called "Hayflick limit". However, researchers have succeeded in exceeding this limit by 20 or more duplications through the introduction into normal cells of a gene that encodes telomerase—the enzyme which completes the building of incompletely repaired telomere ends of the chromosome (Olovnikov 1996). In the context of Eq. (2.4.8), it means the significant increase of the parameter m_c, i.e.—the admissible threshold for accumulation of damages in the critical "longevity-assurance structures". Furthermore, there are the data on prolongation of life of animals of various species, from fishes to primates, by the method of the caloric-restriction diet. There are grounds to believe that, in this case, the total consumption of oxygen by the organism decreases (Anisimov 2008, and Refs. therein). Meanwhile, the lower the consumption of oxygen by the organism (parameter V in Eq. 2.4.8), the fewer oxygen radicals are formed as the by-products of respiration. Furthermore, the experiments on prolonging of life of the transgenic mice by intensifying the expression of SOD and catalase have been successful (Schriner et al. 2005). In this case, the obvious growth of efficiency of the enzyme antioxidant defense takes place, i.e.—the increase in the parameter E. in Eq. (2.4.8).

There are also results that do not support the hypothesis that enhanced mitochondrial SOD or catalase activity promotes longevity. For example, the simultaneous overexpression of multiple copies of Mn-SOD and ectopic catalase transgenes in the mitochondria of *D. melanogaster*, does not extend but diminish the lifespan of the transgenic fruit flies (Mockett et al. 2010). One might deduce that these results do not support the free radical hypothesis. Moreover, one might be entitled to doubt whether the free radical theory of aging is alive (Gladyshev 2014). However, coming back to Eq. (2.4.8), one can see that the length of lifespan is depending not only upon the activity of SOD, but upon some other parameters too. Among them, there are the respiration rate (V), the probability (q) of the malfunctions in the mitochondrial electron-transport nanoreactors (ETN), which lead to the occurrence of $O_2^{\bullet-}$, and the probability (u) that the free radical failures will provoke any structural damages and functional violations. The last one is quantitative characteristics of efficiency

of the reparation systems, including DNA polymerase, etc. None of these factors, except on the activities of the antioxidant enzymes, had been examined in the cited works. Meanwhile, the transgenes can considerably increase the probability of the free radical malfunctions in the ETN, so that the $O_2^{\bullet-}$ fluxes far exceed the defense potential of the antioxidant defense as well as the defense potentials of other enzymes of reparation and restore.

Another point of the agenda is the application of chemical antioxidants for the antiradical defense of cells and tissues. By definition, in chemistry, antioxidants represent a broad class of compounds, both synthetic and natural, molecules of which are capable to react with active free radicals with the formation of inactive radicals of the antioxidant and, thereby terminate free radical chain reactions (Obukhova and Emanuel 1983; Koltover 2010). Synthetic antioxidants for anti-aging therapy had been first successfully used by Harman, who had discovered that radiation protector 2- mercaptoethylamine prolongs the mean lifespan of C3H female mice by 26% and LAF_1 male mice by 29.2% (Harman 1957). Later, it was revealed that antioxidant 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene or BHT in English literature and dibunol or ionol in Russian literature) retards the development of leucosis in mice (Emanuel and Lipchina 1958). By adding BHT to food, it was possible to extend the lives of *Drosophila* flies by 25% (Sharma and Wadhwa 1983) and mice of some lines by 25–30% (Clapp et al. 1979). The addition of the water-soluble antioxidant, 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride, to drinking water extends the average lifespan of *Drosophila* flies and SHK mice by 24 and 38%, respectively (Obukhova and Emanuel 1983).

Most of the natural antioxidants are the substituted phenols or polyphenols of plant origin which, owing to their hydroxyl groups, inhibit the free radical chain reactions of oxidation in model systems (in vitro), for example, the reaction of oxidation of linolenic acid. These are flavonoids, in particular, quercetin, flavones, and resveratrol which is especially abundant in grapes and red wine, simple catechols which are present in large amounts in green tea, and catechol oligomers present in high concentrations in grapes and cocoa beans, as well as carotenoids, tannins, anthocyanins, coumarins, and hydroxycinnamic acid derivatives (Baur and Sinclair 2006; Koltover 2017a, 2018b). Currently, the highest geroprotector effect was found for resveratrol (*trans*-3,4′,5-trihydroxystilbene). This polyphenolic compound has provided an extension of maximum lifespan by 59% in the experiments on fishes and an extension of average lifespan by 30% in the experiments on mice on a fatty diet (Baur and Sinclair 2006). It is noteworthy, however, that the classical natural antioxidant, vitamin E, has turned out to be inefficient in analogous biomedical testing (Driver and Georgeou 2003).

At the present time, the free radical chemical mechanism of action of antioxidants in living systems does not seem as unambiguous as half a century ago. In vivo, contrary to the popular opinion, neither natural antioxidants, like vitamin E, ascorbic acid or flavonoids, nor synthetic antioxidants like the water-insoluble butylated hydroxytoluene or the water-soluble 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride, are able to operate by the simple antiradical way, i.e.—as the free radical scavengers. Actually, the rate constants (k) and the real concentrations of

the antioxidants are negligibly low to compete for reactive oxygen species with the specialized antioxidative enzymes. All types of SOD catalyze the reaction of dismutation of $O_2^{\bullet-}$ into H_2O_2 and oxygen, thereby scavenging $O_2^{\bullet-}$, with $k \approx 10^9$ L mol^{-1} s^{-1} (McCord and Fridovich 2014). Meanwhile, the k values for the reactions of ascorbic acid and other antioxidants, including 5,7,8-trimethyltocol (water-soluble derivative of vitamin E) with the $O_2^{\bullet-}$ radical do not exceed 10^5 L mol^{-1} s^{-1}, while those for hydroxypyridine antioxidants are no more than 10^2 L mol^{-1} s^{-1} (Koltover 2010, 2018b). The rate constant for the reaction of the antioxidant α-tocopherol with the highly reactive hydroxyl radical ($OH^{\bullet}$) can be as high as $8 \cdot 10^{10}$ L mol^{-1} s^{-1}. However, the $OH^{\bullet}$ radical is known to react with any organic molecules as a strong oxidant with the rate constants close to the diffusion limit, $>10^{10}$–10^{11} L mol^{-1} s^{-1} (Koltover 2009, 2010, 2018b). Therefore, none of the so-called antioxidants can compete for the hydroxyl radical in vivo with other organic molecules, lots of which are always present around this radical in considerably greater numbers than the molecules of any antioxidants. Of course, the peroxyl radicals $RO_2^{\bullet}$ can appear in reactions of $OH^{\bullet}$ radicals with lipids. In vivo, however, $RO_2^{\bullet}$ and other products of peroxidation arise mainly as secondary products in the reactions that accompany cell death on apoptosis and autophagocytosis during utilization of the cellular waste by lysosomes and peroxysomes. The rate constants for reactions of synthetic and natural antioxidants with $RO_2^{\bullet}$ in model reactions may range up to about 10^6 L mol^{-1} s^{-1}. However, the antioxidants are unlikely to be highly necessary for scavenging the active radicals in the catabolic processes. Thus, manifold effects of antioxidants in vivo can hardly be interpreted on the basis of simple chemical analogy with the action of the same antioxidants as radical scavengers in vitro.

Meanwhile, from the general theory of reliability, it is known that the most efficient way to increase the reliability ("robustness") of any complex system, be it a technical system or a biological system, is prophylaxis, i.e.—the well-timed prevention of malfunctions (failures) of functional elements from which the system is constructed (Bazovsky 1961; Koltover 2019). Following this reliability-theory guideline, a special series of experiments was performed by our group, in which it has been shown that antioxidants in vivo do provide preventive protection from the oxygen-free radicals. At this, the particular protection mechanisms are different for antioxidants of different types.

According to Eq. (2.4.8), an increase in lifespan can be achieved by increasing the reliability of mitochondrial nanoreactors, i.e., by decreasing the probability (q) of the formation of radicals. Apparently, this is the way in which BHT (butylated hydroxytoluene) acts. It was shown in the experiments with rats that the injections of BHT lead to the increase in oxygenation of the myocardium (Koltover et al. 1984; Koltover 1995). Meanwhile, the state of hypoxia is characteristic of the myocardium and other tissues of old animals. It is also known that, due to hypoxia, the heart mitochondria are damaged and turn into generators of intensive fluxes of the $O_2^{\bullet-}$ radicals (Nohl et al. 1993; Nohl and Koltover 1994). It stands to reason that BHT, increasing the myocardium oxygenation, possesses the antioxidant effect but it does it not directly, through the interception of radicals, but indirectly, by decreasing the level of generation of the oxygen radicals as by-products of respiration.

Figure 2.2 represents the results of measurements of corticotropin, thyrotropin, oxycorticosteroids, and L-3,3',5-triiodothyronine in the blood plasma of rats after the injection of BHT (Froliks et al. 1990). One can see that BHT induces the substantial shift in the activity of adenohypophysis gland which is the source of corticotropin and thyrotropin hormones, and this is accompanied by the relevant shifts in the activity of peripheral endocrine glands, the adrenal cortex (the source of corticosteroids), and the thyroid gland (the source of triiodothyronine). It means that the injections of BHT produce dramatic hormonal changes in the animal's blood, i.e., the increase of corticotropin and corticosteroids along with the decrease of thyrotropin and triiodothyronine in the blood plasma of rats after the BHT administration. It is common knowledge that the release of corticotropin into blood, followed by an increase in the synthesis of corticosteroids and a decrease in the synthesis of thyroid hormones, is a significant phase of the system's adaptation to stress. In essence, BHT increases the reliability of the mitochondrial electron-transport nanoreactors. This preventive antioxidant effect is realized through the hormonal regulation of redox-homeostasis via the hypothalamus–pituitary–adrenal gland axis. Thus, there are reasons to suggest that BHT, being regularly introduced into the animal diet, "trains" the neuro-hormonal system as a mild stress factor, thereby increasing the system reliability (adaptive capabilities) of the organism.

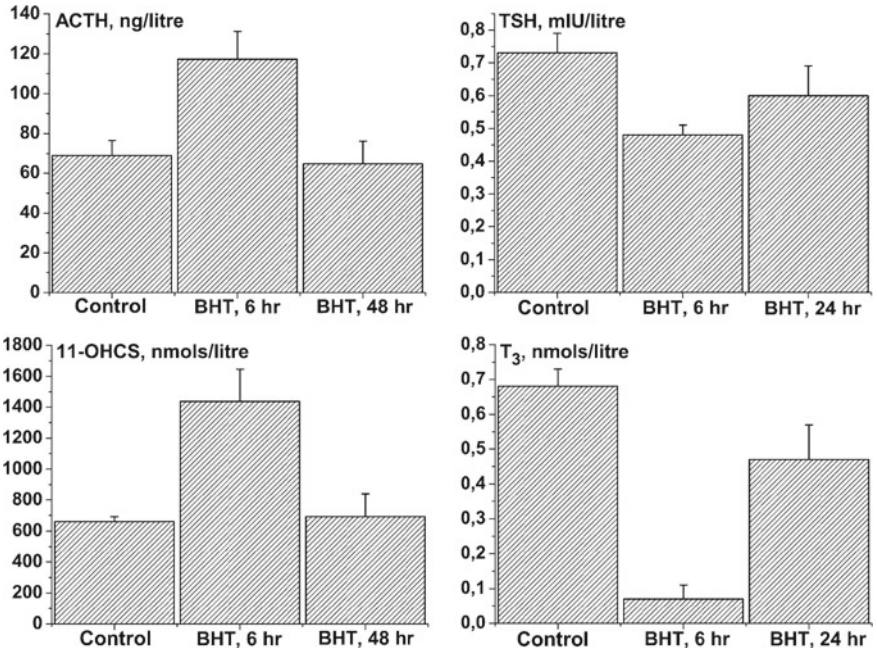

Fig. 2.2 Concentrations of corticotropin (ACTH), thyrotropin (TSH), 11-oxycorticosteroids (11-OHCS) and L-3,3',5-triiodothyronine (T_3) in the blood plasma of rats (adult, 4–6 months, male Wistar) in control and after injection of antioxidant BHT (Froliks et al. 1990)

Mechanisms of antioxidant activity of natural antioxidants have been revised too. For example, flavonoids can provide preventive protection against oxygen radicals by inducing the biosynthesis of specific antioxidant enzymes. Indeed, the induction of synthesis of SOD and catalase was detected in blood erythrocytes of humans who received *Protandim* (extracts from five medical plants) as the food additive (Nelson et al. 2006). These authors concluded that modest induction of the antioxidant enzymes, SOD and catalase, may be a much more effective approach to the problem of defense from free radicals than supplementation with antioxidants "that can, at best, stoichiometrically scavenge a very small fraction of total oxidant production" (Nelson et al. 2006). Noteworthy, that resveratrol activates the expression of the sirtuin proteins thereby providing, in part, the increase in expression of mitochondrial SOD in vivo (Refs. in Houtkooper et al. 2012).

Considering that the expressions of SOD and other antioxidant enzymes in humans and animals are under the hormonal control, flavonoids also seem to make their preventive maintenance defense through hormonal regulation mechanisms. Indeed, in the experiments with *Macaca mulatta* monkeys, it was found that the diurnal changes (circadian rhythms) in the SOD activity in erythrocytes tightly and positively correlate with the diurnal changes in the levels of cortisol and dehydroepiandrosterone sulfate (DHEAS) in blood plasma (Goncharova et al. 2006). For young animals, the values of the correlation coefficient were 0.92 ± 0.09 (cortisol *versus* SOD) and 0.99 ± 0.02 (DHEAS *versus* SOD). With aging, the circadian rhythms of SOD, cortisol, and DHEAS are smoothed out although the correlation between the diurnal changes in cortisol and in SOD still maintains even for old animals. These results, like the above-mentioned experiments with BHT, testify that corticosteroid hormones do play an essential role in the regulation of SOD activity. One way or the other, the timely introduction of antioxidants, the natural or synthetic ones, provides beneficial physiological effects through the prophylactic reliability maintenance against reactive forms of oxygen via the neuro-hormonal system, in essence, through the "longevity-assurance structures".

Furthermore, there are more and more data indicating that the therapeutic effects of many pharmaceutical drugs are due to their beneficial action not only on the cells and tissues of the host organism, but also on gastric and intestinal microbiota too. Most of the flavonoids, which are traditionally regarded as natural antioxidants, refer to the extensive class of physiologically active compounds long known as phytoalexins. Moreover, the phytoalexins are synthesized in plant tissues just for fighting, like antibiotics, against bacterial and fungal infections and for acting as inhibitors of transcription and translation of particular proteins in the cells of the infecting organisms (Bailey and Mansfield 1982). Meanwhile, the number of microbiota cells in the gastrointestinal tract, on the skin, and in some other organs and tissues nearly exceeds the number of cells of the host organism. Of even greater importance is that the microbial cells produce physiologically active substances that markedly affect all organs and tissues including the immune system. More and more increasing number of experimental data are coming that the microbial metabolites promote metabolic benefits in the brain cells via gut-brain neural circuits, through the hypothalamus–pituitary–adrenal gland axis and so on (Heintz and Mair 2014;

Espin et al. 2017; Muller et al. 2020). In view of the advances in systems biology, one can suggest that the so-called antioxidants, both natural and synthetic ones, attack the organism's microbial population. In high doses, these substances are toxic, as implied, because of their deleterious effects on the microbiota. In low doses, however, the same compounds produce favorable effects on the organism's microbiota, in a hormetic-like fashion, thereby increasing the system reliability and lifespan of the organism.

Thus, over the years, more and more experimental results indicate that the true mechanisms of the antioxidant effects are to be studied in the ways of systems biology instead of free radical chemistry. Last years, such terms as "polyphenols", instead of antioxidants, and "redox regulation/redox signaling pathways", instead of oxidative stress, came into use, at last (Forman et al. 2014; Halliwell 2020). Yet, the paradigm that the antioxidants directly intercept free radicals in vivo in the same manner as in vitro had a very long-lived existence. "It is harder to overcome old ideas, rather than create the new ones" (Keynes 2007).

2.6 Conclusions and Outlook

Beyond the doubts, the free radical theory of aging is not dead. It is alive, owing to the system theory of reliability. The system approach, based on the engineering theory of reliability, integrates the concept of aging program and the free radical theory of aging into a unified pattern. Like in engineering, the main line of assuring the high system reliability is preventive maintenance, i.e., unreliable elements should be timely replaced for novel ones ahead of the phase of their wear-out begins. The prophylaxis of failures, i.e.—the metabolic turnover, is controlled by the longevity-assurance structures of the highest level of hierarchy which perform the supervisory functions over the metabolic turnover. The longevity of an organism as the whole is predetermined, mainly, by the genetically preset reliability of the supervisors. The stochastic malfunctions of the mitochondrial electron transport nanoreactors, which produce the anion-radicals of oxygen ($O_2^{\bullet -}$) as by-products of oxidative phosphorylation, are of first importance. As the reducing agent, this radical affects NADH/NAD^+ ratio, thereby impacting the epigenetic sirtuin regulators of metabolic repair and renewal processes. As the consequence, oxidative-stress products and other metabolic slag accumulate with time, resulting in the impetus to autophagic or apoptotic cell death and age-associated clinical disorders. On this basis, the universal features of aging, the exponential growth of mortality rate with time (Gompertz law of mortality), and the correlation of longevity with the species-specific resting metabolism (Rubner scaling relation) are naturally explained. Thus, aging occurs as an inevitable consequence of the genetically preset deficiency in reliability of the biomolecular constructs while the free radical redox timer, located in the specialized cells of the central nervous system (presumably hypothalamus), serves as the effective stochastic mechanism of realization of the genetically preset deficiency in the system reliability of an organism as the whole. Thus, the system reliability approach

serves as a heuristic methodology in search of realistic mechanisms of aging and ways of prolongation of longevity.

Acknowledgements Boris V. Gnedenko, an outstanding mathematician from Kiev, was one of the Founding Fathers of the mathematical theory of reliability (Gnedenko et al. 1965). The first conference on reliability of biological systems had been held in Kiev, 1975. The first papers and books on reliability of biological systems had been published in Kiev (Grodzinsky, ed 1977, 1980; Grodzinsky et al. 1987). The reliability-theory approach as a methodology for searching the realistic mechanisms of antioxidant therapy had been first applied in collaboration with my colleagues and friends in Kiev (Gorban and Koltover 1985; Frolkis et al. 1985, 1990; Koltover et al. 1980, 1984). The first doctor dissertation on reliability and aging of biological systems had been successfully defended in Kiev at Bogomolets Institute of Physiology (Koltover 1988). Thus, located at the crossroads of Western Europe and the Orient, Kiev, had spurred the studies on reliability ("robustness") of biological systems. It confirms the old saying of the Middle Ages that "Teaching comes from Kiev."

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Compliance with Ethical Standards This article is a review of the cited papers which were published previously. This article does not contain any studies with human participants or animals performed by any of the authors. I confirm that all experiments involving humans or animals, which are cited in this article, were conducted by respecting the corresponding ethical guidelines and that informed consent was obtained in case humans were involved.

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Chapter 3
Disturbances in Redox Homeostasis in the Ageing Brain

S. Asha Devi and T. B. Basavaraju

Abstract The brain normally adapts to various extents of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within its complex network of neurons and glial cells and, thereby, maintains the neuronal circuits. Although the neuron is the highest consumer of O_2 and glucose, it has compromised responses to oxidative stress and nitrative stress, resulting in overwhelming levels of oxidants in the ageing brain. More specifically, the neurons face challenges posed by high levels of free radicals, alterations in mitochondrial metabolism, and calcium signalling, accompanied by overloading of iron at various sites in the brain. The brain has the least antioxidant defences against oxidants, compared to the heart, and previous studies from our group have revealed that the ageing brain is challenged with an accumulation of several oxidised products, resulting in neuronal degeneration and deficits in cognitive functions. Here, we review the findings on redox homeostasis in an ageing brain, which when disturbed by ROS and RNS, paves the way to neurodegenerative diseases that also influence the longevity of the patient. The possibilities of certain non-invasive interventions in offering protection against OS-mediated redox dyshomeostasis in the aged brain are also addressed.

Keywords Ageing · Free radicals · Lipid peroxidation · Oxidative stress · Protein oxidation · Proteomics · Redox signalling

3.1 Introduction

In the human body, several redox reactions occur that are characterised by the systematic transfer of electrons between the molecules. Redox homeostasis is disturbed when an imbalance occurs between the oxidants and the antioxidants. Oxidants have unpaired electrons and consist of several free radicals (FRs) that include reactive oxygen species (ROS) (Lushchak 2014) and reactive nitrogen species (RNS) (Ye

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et al. 2015). Although these reactive species are necessary as signalling messengers for the physiological functions of the body (Egea et al. 2017), they are harmful in overwhelming levels that lead to oxidative stress (OS), which initiates various diseases, such as renal, cognitive disorders, cancer, vascular diseases, and diabetes.

In this chapter, we will review the significance of redox equilibrium in the normal healthy brain, and then focus on the different sources of ROS, followed by disturbances in the equilibrium with ageing. We will further discuss how age-related diseases of the brain can be related to a disturbed redox network with ageing. Finally, we will go into non-invasive approaches for decelerating brain ageing and the prime neurodegenerative diseases.

3.2 Redox Homeostasis in the Brain

The brain has highly differentiated cells that are located in different anatomical regions and it needs approximately 20% of the body's oxygen for its regular functions (Schönfeld and Reiser 2013). The brain acquires energy through the oxidation of glucose, yielding a high ATP turnover in the neurons and non-neurons. The neurons rely heavily on mitochondria that aid in supporting synaptic plasticity, and neurotransmitter synthesis (Mattson et al. 2018) and redox homeostasis. Major biomolecules such as proteins and lipids are subjected most to oxidative damage at their structural and functional levels. Incidentally, high levels of ROS such as hydroxyl radicals and peroxynitrites react with proteins, lipids, and DNA, while low levels of ROS, superoxide, and hydrogen peroxide react with transition metals, such as iron in the Fenton and Haber-Weiss reaction.. In the mitochondria, iron-sulphur aggregates that are present in enzymes, such as aconitase, are vulnerable to superoxide attacks. Thus, they release free hydrogen peroxide and iron, which produce more hydroxyl radicals, leading to OS culminating in cell death (Esposito et al. 2013). Nevertheless, the brain has enzymatic and non-enzymatic antioxidants that fight against a redox imbalance (Flohé 2016; Rodriguez-Rocha et al. 2013). NADPH oxidase (NOXs) is an important source of H_2O_2 because of their tightly regulated production of superoxide/H_2O_2 (Brandes et al. 2014). It has been demonstrated that at a steady state, the H_2O_2 level is at 10^{-7} M–10^{-8} M (Chance et al. 1979), with a physiological/eustress response at slightly deviated levels, and a pathological state at high levels (Sies 2017).

Vitamin E (α-tocopherol), a non-enzymatic antioxidant can convert FRs into less reactive compounds, thereby protecting against cytotoxicity through the inhibition of lipid peroxidation (Brigelius-Flohé and Traber 1999). It can also aid in the continuance of complex systems, without their destruction (Zing 2007), although in vitro studies on the mouse cerebral cortex have demonstrated cytotoxicity of high doses of vitamin E, with low neuronal survivability (Kan et al. 1991). However, studies from our laboratory have suggested that vitamin E (α-tocopherol), along with vitamin C (ascorbic acid), is more effective against an accumulation of FRs in the frontoparietal cortex of rats subjected to stress (Asha Devi et al. 2012). Although the brain has a

high content of oxidizable polyunsaturated fats (PUFA) and high oxygen consumption, its weak antioxidant defences make it most vulnerable to OS and nitrative stress (Cobley et al. 2018). This is of immense concern from the point of neuronal and glial redox homeostasis. The extent of cognitive performance in ageing rodents has been observed to depend on the severity of the oxidative damage (Candelario-Jalil et al. 2001). Neurons, in contrast to glial cells, produce more ROS since their metabolism largely depends on mitochondrial oxidative phosphorylation (Lopez-Fabuel et al. 2016). In the neurons, GSH has a prominent role in the detoxification of ROS (Quintana-Cabrera et al. 2012). However, this declines in neurons with ageing. Therefore, deficient GSH levels and increased ROS levels in neurons with ageing are partially accountable for the onset of neurodegenerative diseases, in contrast to the glia (Halliwell 2011; Bolaños et al. 2009; Levy et al. 2009).

3.2.1 Oxidation of Proteins in the Brain

The major end-products of protein oxidation are an accumulation of protein carbonyls, lessened thiol (P-SH) levels, and nitrated proteins (Dean et al. 1997). Proteasomes are protein complexes that are required for the regulation of proteins in the cells. They constitute one of the main cellular proteolytic mechanisms. Systems that are of importance in the oxidation of proteins in mammals are first, the thioredoxin (Trx)/Trx reductase (TrxR) system that is involved in the formation of disulphide bridges and sulphenic acid reduction. Firstly, TrxR reduces disulphide containing Trx via the NADPH-dependent reaction in the cytosol and mitochondria as well (Moriarty-Craige and Jones 2004; Nakamura 2003). Secondly, the glutathione system constituted by glutaredoxin (Grx)/glutathione (GSH)/glutathione reductase (GR) system can reduce disulphide bridges.

Interestingly, Trx is expressed in response to different OS conditions and regulates intracellular signal transduction and scavenge radicals. Failure of this mechanism results in disulphide stress. Conversely, under non-oxidative conditions, TrxR reduces the cysteine modifications, thereby restoring the original protein activity (Ahmad et al. 2017).

Although the oxidised proteins are destroyed by the previously mentioned proteasome complexes, they form high molecular weight non-degradable complexes that accumulate in ageing brain cells.

3.2.2 Lipid Oxidation in the Brain

Lipid peroxidation generates a disturbance in membrane organisation, leading to partial modifications to DNA and proteins, leading to changes such as high membrane rigidity and partial destruction of membrane receptors (Anzai et al. 1999). The products of lipid peroxidation are efficient redox signalling mediators (Zmijewski et al.

2005). The brain is a major source of ROS. Further, the PUFAs arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) are particularly susceptible to OS because of their unsaturated double bonds (Chen et al. 2008; Uchida 2003). Besides aldehyde formation, lipid hydroperoxyl radicals undergo endocyclisation to generate large amounts of fatty acid esters, isoprostanes, and neuroprostanes (Musiek et al. 2005; Roberts et al. 2005). Importantly, F_2- and F_4-Neuroprostanes hydrolyse to free isoprostanes and neuroprostanes that serve as potential biomarkers in body fluids. Apart from these, the F_2-NPs and F_4-NPs can also be converted to isochetals and neurochetals, which are toxic to cells (Montuschi et al. 2007). Hence, the determination of PUFAs levels and their metabolites are indicative of the extent of neuronal redox disturbance, leading to brain dysfunction that is manifested in terms of a sizeable number of neurodegenerative disorders associated with ageing.

3.3 Redox Alterations and ROS-Mediated Signal Transduction Mechanisms in the Ageing Brain

Ageing per se results in an overproduction of ROS and RNS in regions of the brain, resulting in a disturbance in redox homeostasis. Studies have shown that redox homeostasis is achieved not only through the components of ROS and RNS, but also through specific redox signalling pathways that determine longevity and age-related disorders. ROS is distinguished from other signalling molecules, wherein the functions of proteins are regulated through non-covalent binding of ligands to their receptors. ROS often operate through chemical interactions with specific amino acid residues like cysteine in target proteins, leading to covalent modifications (Nathan 2003). Considerable experimental evidence has documented redox signalling pathways with a high level of specificity.

It is known that mitochondrial dysfunction can lead to age-related pathologies and that ROS levels are increased in senescence and premature ageing (Kim et al. 2016; Li et al. 2016). During ageing, the brain is more vulnerable to OS because of the overproduction of FRs, an increase in the generation of iron (a transition metal), leading to hydroxyl radicals and weakening endogenous antioxidant defence mechanisms and reduced ATP synthesis (Riera and Dillin 2015; Jovanovic and Jovanovic 2011). Further, neuronal death, as a consequence of altered redox homeostasis, is responsible for the ageing of the brain, together with the incidence of neurodegenerative pathologies. Particularly, glial gene expression is greater than neuron gene expression with ageing (Soreq et al. 2017). Unlike the adult brain, ageing is associated with an imbalance in the redox state and is largely attributed to insufficient antioxidant defences in the brain (Fig. 3.1). Thus, changes in redox homeostasis with ageing represent a hallmark for the lowered cognitive performance that constitutes changes in synaptic function and intracellular calcium regulation, as evidenced in humans, primates, and rodents (Foster et al. 2012; Bergado et al. 2011; Burke et al. 2010).

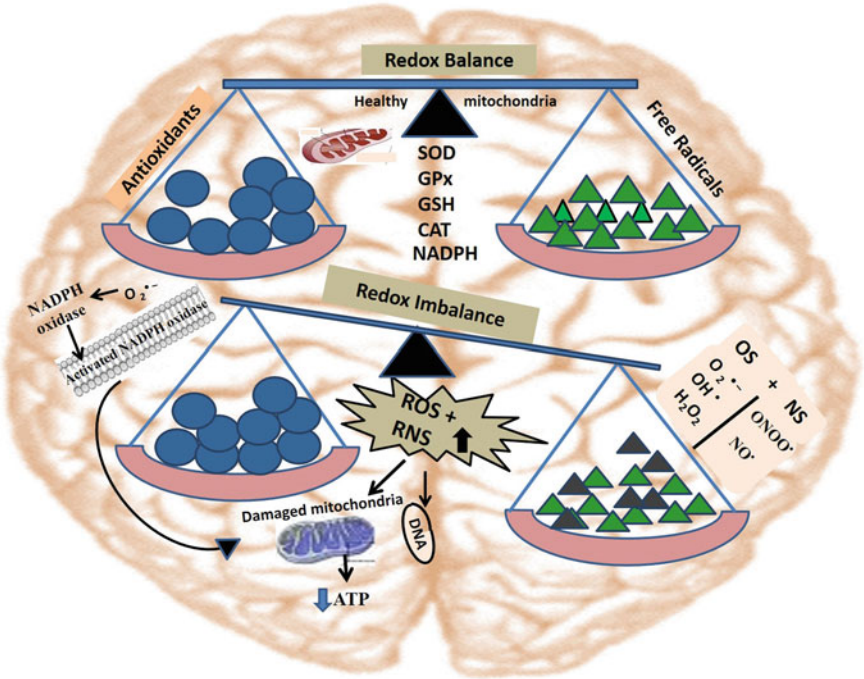

Fig. 3.1 Schematic diagram reflecting redox status in the ageing brain. The upper part shows free radicals in balance with antioxidant defences in the young/adult normal brain. The lower part indicates some of the main contributors to redox imbalance with ageing in the brain

Proteomic studies on signalling pathways have furthered our understanding of protein interactions in neurons (Grant and Blackstock 2001). Proteomic analyses in neonatal mice and mice at different stages of ageing have contributed to our knowledge on the mechanisms of brain ageing. Most of the proteins concerned are associated with normal metabolism, transport, signalling, stress responses, and cognitive functions, and are ultimately accountable for the increased apoptosis of neurons and glia with ageing brain (Yang et al. 2008).

Synaptic plasticity (SP) is important for neuronal function and for information processing through the synaptic junctions that are ultimately responsible for memory encoding. Presynaptic and postsynaptic polarisations and depolarisations are mediated through neurotransmitter release and ion channel activities. All of these render synaptic plasticity in the central nervous system (Bailey et al. 2015). For instance, N-methyl-D-aspartate receptor (NMDAR) activation responsible for hippocampal plasticity produces secondary messengers, including cAMP, NO, and calcium. Oxidative stress alters synaptic plasticity through NMDAR activation (Kishida et al. 2005). The concentration and duration of the oxidant stimuli, in particular formation of $O_2^{\bullet-}$, are pivotal factors for the induction of long-term potentiation (LTP) (Kamsler and Segal 2003; Knapp and Klann 2002). Studies by Thiels et al. (2000) and Tejada-Simon

et al. (2005) on $O_2^{\bullet-}$, a signalling molecule for a normal neuronal function, further emphasise the presence of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) in brain regions, being a key enzyme source of $O_2^{\bullet-}$ required for LTP and memory in the mouse brain. Hence, $O_2^{\bullet-}$ has a physiological function, as well as a pathophysiological role. In comparison to $O_2^{\bullet-}$, H_2O_2 is less toxic (Toledano et al. 2010) and better for signalling purposes, partially due to its longer half-life, 10^{-3} s in cells (Gadjev et al. 2008).

Experimental evidence in rodent models of ageing has related lowered spatial memory retrieval with elevated H_2O_2 levels in the hippocampus (Abhijit et al. 2018; Asha Devi et al. 2011). Hippocampal injuries in primates (Lavenex and Lavenex 2009) and humans (Parslow et al. 2005; Holdstock et al. 2000) are correlated with a loss of spatial memory. Furthermore, altered contextual memory is associated with intracellular calcium modulation that corroborates with an inability to acquire, store, and retrieve information. Hence, consideration of such factors is of importance while designing pharmaceutical drugs for cognitive insufficiency. Table 3.1 represents the effects of age on some of the redox parameters in human and experimental animal brains. Here, we briefly outline the role of mitochondrial ROS (mtROS)-mediated signalling in the ageing brain. However, enzymes located outside the mitochondrion can also produce ROS.

These include NOXs, xanthine oxidase, monoamine oxidase, and peroxisomal enzymes. NOXs are the only enzymes whose specific function relates to superoxide or hydrogen peroxide production (Drummond et al. 2011), and whose activities increase during neuroinflammation, ageing (Dugan et al. 2009; Lambeth 2004; Navarro and Boveris 2007), and in neurodegenerative diseases. NOX1 and NOX4 are expressed in neurons and aid in synaptic plasticity and cerebral blood flow, while NOX2 is located in microglia and forms a major source for $O_2^{\bullet-}$-related neuroinflammation (Cheret et al. 2008; Mander and Brown 2005). In fact, ROS levels are modified as a result of interaction between NOXs and mtROS (Dikalov 2011). Neurons and glia are more vulnerable to changes in ROS levels due to their higher sensitivity to oxidative injury or lesser sensitivity to altered ROS signalling. Therefore, these differential responses of brain cells to altered redox signalling, explain the region-specific oxidative damage in neurodegenerative diseases.

An increase in H_2O_2 activates glucose-6-phosphate dehydrogenase to form glyceraldehydes-3-phosphate dehydrogenase with disulphide bonds incorporated between cysteines. Glucose metabolism occurs through the hexose monophosphate pathway rather than glycolysis and the shift generates NADPH for redox reactions (Foley et al. 2016; Raiser et al. 2007). H_2O_2, which is released in high levels, is recognised as a signalling molecule (Floyd and Hensley 2002). Notably, it has high infusibility across membranes and the ability to oxidise cysteine residues of redox proteins (D'Autreaux and Toledano 2007). In fact, few cysteine residues occurring as thiolate anions (S^-) are oxidised by H_2O_2 to the sulphenic form (SO^-), which interferes with cellular signalling by modifying protein structure and subsequently its activity. Additionally, oxidisation leads to the generation of sulphuric (SO_2^-) and sulphonic (SO_3^-) acids, the latter being an irreversible modification. However, the brain has protective glutaredoxin (GRX) and thioredoxin (TRX) that prevent

Table 3.1 Oxidative stress-related redox imbalance in the normal ageing brain

Features of oxidative stress in the brain	Subjects/Organism	Effects on age	Reference
Intracellular Ca^{2+} homeostasis	Young, middle-age and aged wild type C57BL6J mice	Disturbances in Ca^{2+} homeostasis leading to cognitive deficits in normal aging and degenerative diseases	Uryash et al. (2020)
Total antioxidant capacity is associated with changes in serum HCY and memory deficits	Normal elderly humans (62–78 years of age)	Subjects show decreased TAC levels that correlate to lower glucose passage in the temporal cortex	Palomar-Bonet et al. (2020)
Mitochondrial dysfunction, impaired iron homeostasis, epigenetic modifications	Humans	A progressive decline in metabolism with ageing and aging and neurodegeneration	Vanni et al. (2019)
Glia metal deposition glial distribution	Normal 2-, 6-, 19- and 27-month old C57Bl/6 J mice	Dystrophy of glial dystrophy, disruption of metal homeostasis with age	Ashraf et al. (2019)
Proteomic changes in the dentate gyrus (DG) and spatial memory	Impaired and unimpaired aged male rats	Peroxiredoxin 6 levels different in impaired and unimpaired rats	Lubec et al. (2019)
ROS and neurological disorders with age	Designed Glutamate-cysteine ligase (GCL) knockdown (CK2a-Cre/shGC LSFL) mice	Modest glutathione and redox stress in the HC, neuronal dendrite disruption and glial activation in CA1 layer only. Improved cognition	Fernandez-Fernandez et al. (2018)

(continued)

the formation of SO_3^- by intermediate disulphide (S–S) or sulphenic-amide (S–N), whereby the oxidised protein is reduced.

Table 3.1 (continued)

Features of oxidative stress in the brain	Subjects/Organism	Effects on age	Reference
Cognitive abilities for hippocampal-dependent tasks	2- and 23-months old rats	Increased oxidative stress, reduced glutathione NMDAR hypofunction and altered synaptic transmission. Cellular redox metabolism contribute to memory dysfunctions in the old	González-Fraguela et al. (2018)
Iron and copper –related neuronal death	In vitro model HT22 mouse hippocampal neurons of cell death	Increases in ROS production and cell death are similar. Copper is more effective than iron in reducing GSH level	Maher et al. (2018)
Redox proteomics and target proteins modified by NKT (neuroketals) adducts	Middle-aged and old Humans	Lipoxidized proteins in the middle-aged affect energy metabolism, cytoskeleton, proteostasis, neurotransmission	Domínguez et al. (2016)
GSH changes in microglia by γ-glutamylcysteine synthase inhibitor BSO	Human microglia, astrocytes, and cell lines- THP-1 and U373	Inhibition of GSH synthesis elicits neuroinflammatory response in microglia and astrocytes, Ca^{2+} influx through TRPM2 channels. Has relevancy to neuronal death	Lee et al. (2010)
Redox proteomics in different brain regions	12- (aged) and 28- (senescent) month old male Wistar rats	Proteins related to mitochondrial function and energy metabolism are oxidized in the different brain regions	Perluigi et al. (2010)

(continued)

Table 3.1 (continued)

Features of oxidative stress in the brain	Subjects/Organism	Effects on age	Reference
Total antioxidant capacity, in different cerebral regions of the aging rat (cortex, striatum, hippocampus and the cerebellum	Young (2 months-old), mature adult (6 months-old) and old (24 months-old) male Wistar rats	Age-related variations of total antioxidant defenses in brain may predispose structures to oxidative stress-related neurodegenerative disorders	Siqueira et al. (2005)
Differential expression of proteins in the brain	12-mo and 4-mo-old senescence-accelerated mouse (SAM), SAMP8	Aged SMP8 mice have oxidatively altered proteins correlate with learning and memory deficits	Poon et al. (2004)
Oxidative stress markers are related to mitochondrial function	Young (7-month old), adult (13-month old), and old (18-month old) mice	Accumulative toxicity from oxyradicals account for the altered molecular mechanisms resulting in cognitive and behavioral deficits	Navarro et al. (2002)

3.4 Redox Imbalance in Neurodegenerative Diseases

It has been noted that an age-related imbalance in redox homeostasis at different sites in the brain is a potent risk factor for the development of neurodegenerative diseases, with a high risk in the elderly. The progression of disturbed redox homeostasis in the brain is also a result of ROS and Ca^{2+} interactions (Singh et al. 2019). Some of the proinflammatory mediators, such as IL-1β, IL-6, and TNF-α, can cross the blood–brain barrier and mediate the production of increased levels of ROS (Dugan et al. 2009; Banks et al. 1994). Furthermore, increased inflammatory signalling is initiated by increased ROS levels and the subsequent activation of stress kinases, Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinase (MAPK) (Hsieh and Yang 2013). The interplay between calcium signalling and ROS in various diseases has been reviewed in detail by Madreiter-Sokolowski and colleagues (2020).

Parkinson's disease (PD), a movement disorder in elderly subjects is linked with increased lipid peroxidation, 4-hydroxynonenal, and malondialdehyde. Increased protein oxidation in the substantia nigra (SN) contributes to the loss of dopaminergic neurons and accumulation of alpha-synuclein in protein aggregates, as Lewy bodies in the cytosol of surviving neurons (Chinta and Andersen 2008; Braak et al. 2003;

Dauer and Przedborski 2003). Studies have also demonstrated that nitrated alpha-synuclein induces microglia to secrete an excess of ROS by altering ion channels (Thomas et al. 2007), and oxidation of alpha-synuclein is predominantly seen in Lewy bodies (Vila and Przedborski 2004). Interestingly, overexpression of α-synuclein in small animal models such as rats and mice causes neuroinflammation and prolonged microglial activation. These changes are related to the expression of iNOS and NOX that generate $^{\bullet}NO$ and $O_2^{\bullet -}$, respectively (Gao et al. 2011). Such immunoactive catalytic subunits, NOX1, NOX2, and NOX4 have been reported in the brains of PD patients. In addition, genetic inactivation of the NOXs exerts a neuroprotective effect against pathology in experimental models of the disease (Belarbi et al. 2017). Further, increased oxidative stress in PD is also related to increased iron levels in the SN and a flipped ratio of Fe(III)/Fe(II) from 1:2 to 1:2 (Berg et al. 2004). Although, iron may have a role in many neuropathological diseases including PD, experimental evidence has indicated that iron chelators can alleviate the symptoms of PD, supporting the case for iron in degenerative processes in PD (Kaur et al. 2003). In reality, oxytosis may be a coalescence of ferroptosis and a type of damage that is induced by glutamate, since the depletion of GSH in ferroptosis can occur, due to elevated levels of iron (Latunde-Dada 2017).

Amyotrophic lateral sclerosis (ALS) affects the upper motor neurons in the cerebral cortex and the lower motor neurons in the brain stem and spinal cord. Amyotrophic lateral sclerosis is caused by excessive ROS, with weakened antioxidant defence and mitochondrial dysfunction leading to oxidative stress. Normal ageing with a structural and functional decline in the motor neurons is thought to directly or indirectly lead to motor neuron pathology in ALS. According to a large amount of evidence, increased oxidative damage to macromolecules, and the DNA of post-mortem neuronal tissue (Simpson et al. 2010), cerebrospinal fluid (CSF) (Ihara et al. 2013), and urine (Mitsumoto et al. 2008) samples from ALS patients suggest the oxidative stress mechanisms in the central nervous system (CNS). In addition, the accumulation of lipofuscin in the motor neurons of ageing animals and humans (Maxwell et al. 2018; Rygiel et al. 2014) indicates the importance of lipofuscin in the progression of the disease.

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is a primary cause of death in the elderly population due to dementia. It is characterised by the loss of functional proteosomes, oxidative stress, and biometal redistribution, aside from genomic instability, mitochondrial disorder, altered neuronal synapsis, amyloid plaque deposits in the brain, tau neurofibrillary tangles (NFTs), and cognitive deficits (Dhakal et al. 2019). Studies have indicated that serum markers of inflammation at low levels (Singh and Newman 2011; Howcroft et al. 2013) may indicate AD and memory deficits during ageing (Macchi et al. 2014; Gimino et al. 2008). An imbalance between the generation and elimination of ROS sets forth β-amyloid-mediated cytotoxicity that leads to a significant neuronal loss and cognitive impairment, a hallmark of AD. However, insufficient antioxidant mechanisms in AD brain do not provide the necessary protection against ROS. This is unlikely in normally ageing healthy individuals, wherein the ROS generation is impeded by potent antioxidant that maintains redox homeostasis (Polis and Samson 2021). Non-enzymatic antioxidants, such as

the lipophilic radical scavenger α-tocopherol or the hydrophilic compound ascorbate (vitamin C), can directly interact with ROS at the molecular level (Finke and Holbrook 2000).

Although it is known that neurogenesis progressively declines in ageing animals and humans, the fact that the brains of centenarians and AD patients have newborn cells is supported by evidence on the neuronal progenitors in the AD brain, in contrast to healthy aged controls. Studies suggest that, in AD patients, there are impaired mechanisms that contribute to redox imbalance (Moreno-Jiménez et al. 2019). Therefore, it is essential to prevent OS in newborn cells and devise therapeutic strategies to overcome the AD-associated cognitive decline, perhaps more vigorously from middle-age onwards.

3.5 Redox Imbalance and Interventions for Healthy Brain Ageing

3.5.1 Antioxidants

With healthy ageing, the brain is challenged in maintaining redox homeostasis and free radical generation in the neurons (Chong et al. 2012). Co-administration of vitamin C and vitamin E has been reported to reduce ROS in the hippocampus and cortex of ageing rats that were subjected to low temperature-induced oxidative stress (Asha Devi and Manjula 2014). A well-known function of vitamin E is its neuroprotective role to counteract the excessive accumulation of ROS (Harrison et al. 2014). Vitamin C can act synergistically with vitamin E since the α-tocopherol is regenerated from tocopheroxyl radical (Dolu et al. 2015; Mocchegiani et al. 2014) and improve cognitive ability in elderly humans (Masaki et al. 2000). Vitamin E inhibits several AD-relevant enzymes including NOX, 5-LOX, and COX2, all of which have been linked to neuroinflammation and oxidative damage (Block 2008). The binding of activated nuclear factor like 2 (Nrf2) with antioxidant response element (ARE) in the nucleus increases the expression of many target antioxidant genes (Fig. 3.2). However, the binding capacity of activated Nrf2 with ARE in the nucleus is reported to be impaired in aged rats (Suh et al. 2004). Interestingly, these vitamins are effective in prevention against neurodegeneration linked with age (Sun et al. 2018; Ramis et al. 2016).

3.5.2 Exercise

The importance of Nrf2 signalling as one of the reactive pathways to oxidative stress and exercise will be emphasised here. Proteomic studies have revealed that proteins that are relevant to energy metabolism and synaptic plasticity are elevated in the

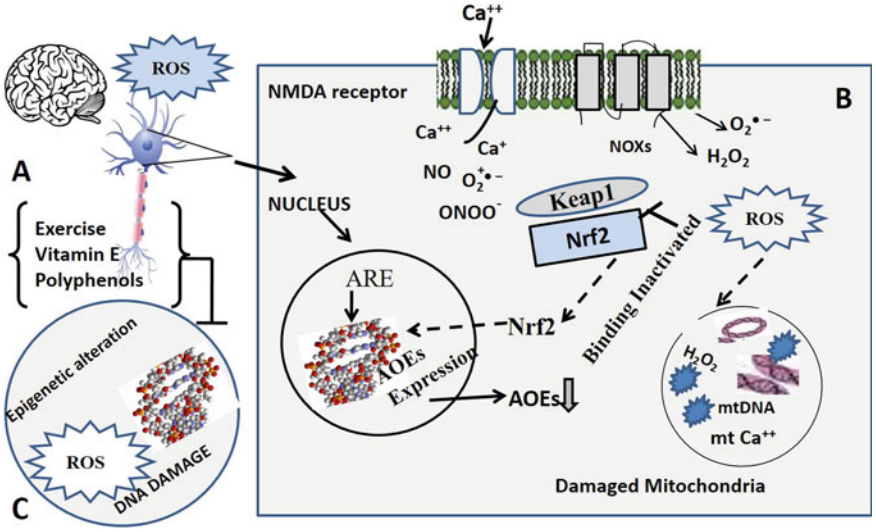

Fig. 3.2 Schematic diagram outlining the mechanisms of ROS injury in neurons of ageing brain. Following a large amount of ROS generation, neuron experiences oxidative stress (**a**). High levels of Ca^{2+} influx are seen through NMDA receptor and excess amounts of RNS ($ONOO^-$) and ROS ($O_2^{\bullet-}$) are generated that result in lipid peroxidation and protein oxidation. The NOX1 is a key source of H_2O_2 and $O_2^{\bullet-}$ and the binding of Keap1 and Nrf2 is less activated. Nrf2 has lesser influence (dashed arrow) on nuclear DNA. This is followed by alleviated levels of expression of genes for antioxidant enzymes (AOEs) leading to lesser availability of AOEs in the cytoplasm. Mitochondrial DNA (mtDNA) disruption occurs from the excess of H_2O_2 and Ca^{2+} accumulation. ARE, Antioxidant response element, an enhancer sequence in the genes is less impacted by low levels of Nrf2 under high ROS levels (**b**). Dietary vitamin E / polyphenols and exercise can alter nuclear epigenetic changes during oxidative stress through their antioxidant property (**c**)

cortex and hippocampus of exercised rats (Anand et al. 2014; Kumar et al. 2012). Furthermore, voluntary free wheel exercise for five days in adult male Sprague–Dawley rats has also been observed to up-regulate ATP synthesis and transduction, glutamate turnover, synaptic plasticity-related proteins, cytoskeletal proteins, internexin, and molecular chaperones (Ding et al. 2006). Furthermore, survival pathways are enhanced, with reduced apoptosis and inflammatory markers in the hippocampus to swim exercise in the aged Sprague–Dawley rats, suggesting additional benefits derived from exercise (Lin et al. 2020). A six-month exercise initiated in middle-aged humans has been reported to be an efficient approach to increase hippocampal firing while encoding a virtual environment (Holzschneider et al. 2012). Interestingly, regular swimming exercise, along with dietary vitamin E supplementation, has been proposed as an approach for declined spatial learning in middle-aged and old rats, by reducing hydrogen peroxide alongside other responses, such as increases in Mn-SOD and decreases in lipofuscin-like substances, and protein oxidation markers in the brain regions (Jolitha et al. 2006; Asha Devi and Prathima 2005; Asha Devi and Ravi Kiran 2004). These studies emphasise the efficiency

of combining vitamin E supplementation with exercise for alleviating ROS in the brain, while improving synaptic plasticity, to prevent brain redox disturbances during ageing. Exercise-mediated neurogenesis is partly facilitated by ROS generation. Furthermore, exercise impacts the immune system and alters the production of cytokines, including IL-6, IL-1, TNF-α, IL-18 , and IFN-γ. These cytokines modulate synaptic plasticity and neurogenesis (Radak et al. 2016). Under homeostatic conditions, there is a large amount of Nrf2 transcription factor since it is degraded by keap-1 dependent ubiquitination (McMahon et al. 2003). The binding between Nrf2 and keap-1 is inhibited by the Neh2 site of Nrf2 in an oxidative state, thereby increasing Nrf2 levels. As a result of these mechanisms, an upshot of transcribed genes for antioxidants is seen (Vargas-Mendoza et al. 2019). It has been documented that regular exercise can induce neurogenesis in the neural stem cells in the hippocampus, and these new neurons are incorporated into the dentate gyrus wherein neural circuits are formed due to raised oxidative stress. Incidentally, oxidative stress in the hippocampus during regular exercise training is alleviated by antioxidant treatments in adult (Walton et al. 2012) and aged rodent models.

3.6 Conclusions

There has been significant progress and development of several natural and synthetic therapeutic agents for attenuating redox imbalance during brain ageing. However, when taking into consideration the redox imbalance and its destructive impact on the ageing brain, diverse approaches comprising nutritional antioxidants, exercise, and pharmacological interventions are promising against the disturbance of normal functions in the ageing brain. In this context, mtROS has a pivotal role in altered redox signalling with ageing, and related neurodegenerative diseases. Therefore, antioxidants combined with exercise training may be an effective non-invasive therapeutic approach against mtROS-induced redox dyshomeostasis and cognitive insufficiency.

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Chapter 4
Impaired Redox Homeostasis and Cardiovascular Aging

Bahadir Simsek and Junaid Afzal

Abstract Cardiovascular diseases are the leading cause of death in the world. The prevalence of cardiovascular diseases has a strong positive correlation with aging. Impaired redox homeostasis is one of the hallmarks of aging. In the present chapter, we outline the main mechanisms in redox homeostasis and explain how increased oxidative stress might increase susceptibility to the development of cardiovascular diseases by contributing to the pathogenesis of hypertension, atherothrombosis, atherogenesis, ischemia–reperfusion injury, and diabetes and obesity. In addition, we outline the involvement of the cardiovascular cell population: cardiomyocytes, endothelial cells, monocytes, and fibroblasts in redox homeostasis and explain the free radical theory of aging and damage theory and how cardiomyocytes are more susceptible to aging and oxidative stress due to their post-mitotic status and high-energy demand. Furthermore, we provide a brief overview of several anti-inflammatory and/or antioxidative substances such as statins, colchicine, canakinumab, and vitamins and briefly present the evidence from experimental studies and randomized controlled trials regarding the utility of these substances in the prevention and/or treatment of cardiovascular diseases.

Keywords Aging · Redox homeostasis · Cardiovascular disease · Antioxidative substances · Anti-inflammatory agents · Canakinumab · Colchicine · Vitamins

4.1 Introduction

Improvements in medical innovation, public health, and economics combined with decreasing fertility are leading to an aging human population. United Nations World Population Aging report highlights that in 2019, there were 703 million people at

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or over the age of 65 years. This number is expected to double to 1.5 billion in 2050. Moreover, the percentage of people over the age of 65 years is projected to grow from 9% in 2019 to 16% in 2050 (United Nations Department of Economic and Social Affairs, Population Division 2020). Even though major improvements in cardiovascular care led to significant improvements in mortality from major cardiovascular events especially in developed countries, cardiovascular diseases (CVD) continue to be the leading cause of death and disability in the world, and the CVD burden is increasing in developing countries. The fact that CVD are more common in the elderly and are the leading cause of disability-adjusted-life-years in our aging population necessitates a better understanding of cardiovascular aging to combat this epidemic.

One of the theories explaining why/how we age is called the free radical theory of aging. Proposed by Denham Harman in the 1950s, this theory proposed that aging results as a consequence of reactive oxygen species (ROS) reactions (Harman 1992). This theory claims that slowing the rate of free radical reactions, such as by caloric restriction (CR), would increase the lifespan of an organism. This theory provided the groundwork for hundreds of studies, garnered significant support, and helped elucidate the contribution of ROS-induced damage to the aging process. On the other hand, experimental studies did not fully support this theory, where overexpression of antioxidant proteins did not result in an increased lifespan (Mockett et al. 2010). Another problem with this theory is that oxidative damage does not explain the (current) inevitability of aging, which in turn resulted in the proposition of damage theory. Damage theory underlies that free radical ROS-induced damage is not the whole story, but it is one of the contributors to aging and that the imperfectness of each biological process inevitably results in accumulation of by-products, and this results in aging (Gladyshev 2014).

In this chapter, we focus on the redox homeostasis aspect of cardiovascular aging by reviewing the general mechanisms that regulate redox homeostasis in different intracellular compartments and cell types present in the cardiovascular system and their dysregulation with aging. Then, we elaborate on the role of impaired cardiovascular redox homeostasis toward the pathogenesis of cardiovascular events, as well as present recent evidence for current, new, and potential therapies.

4.2 General Mechanisms of Redox Homeostasis

Organisms have the ability to maintain inner stability despite changing external conditions. The modern understanding of this stability was described by Claude Bernard (1813–1878) as "*a fixité du milieu intérieur*". Walter Cannon (1871–1945) developed upon Claude Bernard's work and coined the term "homeostasis" to describe the regulatory processes that keep biological systems in relative balance despite changing environments. Moreover, Claude Bernard proposed that internal stability was needed for the maintenance of life (Billman 2020). One of these homeostatic processes is called redox homeostasis, which refers to the maintenance of a

balance between oxidants such as reactive oxygen species (ROS), Reactive Nitrogen Species, and antioxidants while cellular processes that maintain life take place. Oxidants are produced in cells as a result of cellular metabolism and enzymatic reactions. While ROS can be free radicals (e.g., superoxide or hydroxyl), there are also non-radical oxidants (e.g., hydrogen peroxide—H_2O_2). Despite ROS are known to have detrimental modifying effects on proteins, lipids, and DNA, which can disrupt normal physiology, they are also known to serve as cellular signaling molecules (Auten and Davis 2009). Therefore, the equilibrium between ROS and antioxidant defenses that scavenge ROS becomes particularly important, and the disruption of this balance is known to play a role in the development of various CVD such as hypertension, atherothrombosis, atherogenesis, ischemia–reperfusion injury, and obesity–diabetes. One major example that demonstrates the importance of redox balance is seen in hyperoxia-induced lung injury, which triggers pulmonary edema through direct oxygen toxicity and due to the accumulation of inflammatory mediators in lung tissue (Pagano and Barazzone-Argiroffo 2003). Another example that highlights the physiological role of ROS and its aberration in the development of pathologies is seen in Duchenne muscular dystrophy (DMD). In a healthy myocyte, mechanical stretch produces ROS which modulates ryanodine receptor 2 (RyR2) to enhance Ca^{++} sensitivity and fine-tune the excitation–contraction coupling (Fauconnier et al. 2010; Prosser et al. 2011). The excessive ROS production through this pathway in DMD causes the chronic activation of RyR2 with aberrant Ca^{++} release from the sarcoplasmic reticulum (SR), and this "leakiness" of RyR2 due to impaired cellular redox homeostasis leads to the development of cardiomyopathy and/or arrhythmias (Fauconnier et al. 2010; Prosser et al. 2011).

4.2.1 Reactive Oxygen Species Generation

Some ROS molecules are more reactive and more likely to cause cellular damage (Table 4.1). Hydroxyl, for example, is a very reactive ROS and is produced via the Fenton reaction. It has a strong redox potential with an unpaired electron and there is essentially no enzymatic reaction to remove it. In the vasculature, there are four major sources of ROS production. It is estimated that approximately 90% of cellular ROS is produced in the mitochondria, where electron transport chain (ETC) is located and oxidative respiration takes place (Balaban et al. 2005). Mitochondria contain ETC, where conversion of chemical energy in the form of NADH and $FADH_2$ (electron carriers) generates ATP through several redox reactions.

The flow of electrons (from electron carriers) through ETC subunits causes the efflux of protons from mitochondria to create a proton gradient, which is used to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. In mitochondria, superoxide radicals (O_2^-) are known to be produced in NADPH dehydrogenase (also known as Complex I) and in the ubiquinone–cytochrome b-c1 (also known as Complex III). Even though the exact mechanisms of ROS production through ETC are not well-known, the rate of ROS production in mitochondria likely

Table 4.1 Main reactive oxygen species and their attributes

Main reactive oxygen species (ROS)	Structure	Reactivity	Main production source	Main target(s)	Scavenged by
Superoxide	O_2^-	Moderate	Electron transport chain (Complex I and III)	Iron–sulfur clusters in enzymes/proteins	Superoxide dismutase
Hydroxyl	·OH	Highest known	Fenton reaction	DNA Proteins Lipids	–
Hydrogen peroxide	H_2O_2	Moderate	Dismutation of superoxide	Proteins Lipids	Catalase Glutaredoxins Thioredoxins

depends on several factors such as the activity of mitochondrial Mn-SOD2, the relative concentration of oxygen (O_2), age and hormonal status of the organism, redox potential for accepting or donating an electron in the mitochondrial environment, the concentration of proteins/enzymes that can react with oxygen to form superoxide, competency/abnormalities of ETC subunits, and the number of mitochondria (Semenza 2011; Murphy 2009).

The second source is NADPH oxidase (NOX). This enzyme catalyzes the reaction between oxygen and NAPDH, which results in the production of superoxide. Through experimental studies, it has been shown that in the vasculature, endothelial cells, fibroblasts, leukocytes, and smooth muscle cells possess this enzyme. It has also been shown that in atherosclerotic arteries, the expression of NOX subunits such as NOX2 and NOX4 is increased (Sorescu et al. 2002; Förstermann 2010). Furthermore, in atherosclerosis, phagocytic cells with this enzyme also pass to the subendothelial space.

The third source is xanthine oxidase (XO). This enzyme donates an electron to oxygen and results in the production of superoxide and hydrogen peroxide. Even though data regarding the potential contribution of this enzyme to ROS production in the vasculature are not as strong as other contributors, an inhibitor of XO, oxypurinol, has been shown to decrease ROS production and improve vascular relaxation (Ohara et al. 1993; Förstermann 2010).

The fourth potential source of ROS in the vasculature is endothelial nitric oxide synthase (NOS). NOS converts arginine into citrulline and NO. Notably, this enzyme contains prosthetic groups, such as heme, that actively participate in redox reactions. Increased oxidative stress can modify the NOS enzyme and render it dysfunctional. The resulting dysfunctional NOS could in turn produce superoxide instead of NO and contribute to oxidative stress. In fact, experimental studies in rat aorta have shown that a modified NOS enzyme could turn into a superoxide-producing dysfunctional enzyme (Laursen et al. 2001).

4.2.2 Reactive Oxygen Species Scavenging

Organisms have also developed several enzymatic mechanisms to scavenge ROS and maintain redox homeostasis, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), thioredoxins (Trxs), and glutaredoxins (Grxs) (Table 4.1). Serving as a major antioxidant, SOD catalyzes the reaction that converts superoxide to hydrogen peroxide. There are three main forms of this enzyme in the human body. SOD1 (Cu, Zn-SOD) is located mainly in the cell, SOD2 (Mn-SOD) is mainly in the mitochondria, and SOD3 is extracellular. In the cardiovascular system, SOD3 has a role in the maintenance and production of NO by lowering superoxide. Knockout models of SOD1 or other SOD isozymes have been shown to result in increased susceptibility to ischemia–reperfusion injury and NO inactivation (Iida et al. 2006; Jung et al. 2007). Catalase is another enzyme with antioxidative properties. Catalase converts hydrogen peroxide (a non-radical oxidant) to water and molecular oxygen. Even though the experimental evidence regarding the importance of catalase is not clear, some studies suggested that increased availability of catalase might have protective effects on the cardiovascular system, such as delaying the progression of atherosclerosis (Yang et al. 2004). Glutathione peroxidase is another enzyme involved in redox homeostasis. This enzyme converts hydrogen peroxide to water and hydroperoxides, and several experimental studies suggested that GPx might be protective in ischemia–reperfusion injury, coronary artery disease (CAD), and atherosclerotic disease (Yoshida et al. 1997; Torzewski et al. 2007). Thioredoxins are a group of antioxidant proteins that catalyze dithioldisulphide oxidoreductase reactions. These proteins have two redox reaction capable cysteines separated by a pair of amino acids. This family of proteins includes Trxs and Grxs. Both Trxs and Grxs are reductants, and the major function of these groups of enzymes is reducing the disulfide bonds in their target proteins (Meyer et al. 2009). As well as these enzymatic defenses, non-enzymatic scavengers such as Vitamins A, C, E, and glutathione also possess antioxidative capacity (Birben et al. 2012).

In the remaining sections of this chapter, we will focus on components of the cardiovascular system and explain alterations in redox homeostasis in the process of aging and how they might contribute to CVD development.

4.3 Redox Homeostasis in Cardiovascular System

The cardiovascular system consists of the heart and the vessels. The main cells that compose this system include cardiomyocytes, fibroblasts, monocytes, endothelial cells, and vascular smooth muscle cells. Cardiomyocytes are the specialized muscle cells of the heart that provide the contractile capability. They consist of sarcomeres, which are formed by specialized contractile proteins. Cardiomyocytes are unique in the sense that they are mostly post-mitotic, which makes them especially important from an aging perspective. Moreover, due to their contractile work throughout

life, cardiomyocytes are highly energy-dependent. Because of these two properties, namely being mostly post-mitotic and highly energy demanding, cardiomyocytes are especially susceptible to aging and important targets to investigate and better understand the relationship between redox homeostasis and aging. It is known that CVD increase with aging. Some of the risk factors for CVD include hypertension, atherosclerosis, obesity, and diabetes. Impaired redox homeostasis is believed to play a role in the etiology of these risk factors, exacerbating the development of CVD.

4.4 Impaired Redox Homeostasis and Cardiovascular Disease

4.4.1 Hypertension

Hypertension is one of the strongest risk factors for CVD. The prevalence of hypertension increases with aging, with about 5–10% in the 20–35-year-old age group and up to 80% in people over the age of 70 years. In addition, hypertension is among the leading causes of left ventricular hypertrophy and predisposes the heart to ischemic events, dilatation, heart failure (HF), and arrhythmias (Buford 2016; Mozaffarian et al. 2015). Moreover, controlling hypertension is known to translate into better clinical outcomes (Neal et al. 2000). Increased oxidative stress is commonly observed with aging and has been recognized as a factor contributing to the development of hypertension. Endothelial cells in vasculature produce nitric oxide (NO). NO has a short half-life and either acts in the cell it is produced in or diffuses into the adjacent cells. Nitric oxide through its effect on the tone of vasculature plays a direct role in lowering blood pressure (BP). In vascular smooth muscle cells, by dephosphorylating guanosine triphosphate, NO produces cyclic guanosine 3′ 5′ monophosphate (cGMP), which in turn activates K^+ channels and inhibits Ca^+ entry into the smooth muscle cell, resulting in smooth muscle cell relaxation and vasodilation. Disruption of NO synthesis has been shown to result in arterial stiffness and increased BP. In addition, NO is in the armamentarium in the treatment of pulmonary hypertension. Many studies demonstrated increased oxidative stress in patients with hypertension and in experimental models as well as depleted antioxidative capacity. In several human and experimental animal studies, a positive correlation between increased oxidative stress and increased BP has also been shown (Tanito et al. 2004; Lacy et al. 2000).

Moreover, it was shown that in animal models where ROS-producing enzymes were knocked out, BP was lower compared to wild-type littermates (Gavazzi et al. 2006). Furthermore, experimental studies in rats and mice demonstrated that Angiotensin-II infusion increased the expression of Nox subunits and the generation of ROS (Landmesser et al. 2002; Rodrigo et al. 2011). Therefore, it was shown that the renin–angiotensin–aldosterone system (RAAS) is a known inducer of NOX and triggers ROS production. This is one of the reasons why it is hypothesized that the

beneficial effects of antihypertensive agents such as angiotensin receptor blockers and angiotensin-converting enzyme inhibitors might be partly achieved by improvements in endothelial function and redox homeostasis (Ghiadoni et al. 2003). It is believed that the increase in ROS due to RAAS signaling is mediated by ROS-induced signaling and damage, where mitochondrial localization of NOX4 plays a role (Dai et al. 2012). Furthermore, observed beneficial effects of mitochondrial antioxidants are attributed to this hypothesis (Dai et al. 2012).

Experimental animal studies indicate that some downstream effects of chronic β-adrenergic stimulation are also mediated by ROS. For example, in adult rat cardiomyocytes, chronic β-adrenergic stimulation has been shown to result in mitochondrial membrane depolarization. Mechanistically, this effect was inhibited by antioxidant mimetics such as catalase and Mn-SOD. In addition, β-adrenergic stimulation was also linked to increased cAMP and PKA-dependent ROS increase in the mitochondria of mouse ventricular myocytes (Burgoyne et al. 2012).

4.4.2 Atherothrombosis

The incidence of venous thrombosis increases dramatically with aging. While arterial embolic events are more common in the elderly, attributing the increase in the incidence of arterial embolism to aging per se has been difficult, due to the progression of other risk factors in the elderly such as atherosclerosis and atrial fibrillation (AFib). One of the hypotheses proposed to explain the increase of prothrombotic state in the elderly is increased levels of inflammatory cytokines and plasminogen activator inhibitor-1 in the elderly (Wilkerson and Sane 2002), as well as endothelial dysfunction. Arterial endothelium is a major source of NO production. Nitric oxide inhibits atherothrombosis by preventing platelet activation and adhesion of white blood cells to endothelium. There is evidence to suggest that cGMP, through downregulation of protein kinase C and desensitization of thromboxane A2 receptor, inhibits platelet aggregation and vasoconstriction (Murohara et al. 1995). In addition, NO-induced cGMP regulates the expression of alpha-granule protein P-selectin and the affinity of fibrinogen–platelet binding by inhibiting the conformational changes in glycoprotein IIb/IIIa, which in turn prevents platelet aggregation (Michelson et al. 1996). Moreover, through non-cGMP-dependent mechanisms, NO is believed to inhibit platelet aggregation, expression of cytokine-mediated cell surface molecules, and proliferation of vascular smooth muscle (Walford and Loscalzo 2003; Lubos et al. 2008).

Impaired redox balance in which the balance is tipped toward increased oxidative stress in aging results in a reduction of NO due to the inactivation of NO by ROS, which in turn leads to endothelial dysfunction, platelet aggregation, and atherogenesis (Förstermann 2010). Furthermore, evidence indicates that increased oxidative stress could render endothelial NOS dysfunctional, which no longer produces NO, but produces superoxide radicals instead (Förstermann 2010).

4.4.3 Atherogenesis

Advancing age is a major risk factor for atherosclerotic CVD. Recent studies showed that age-related changes in bone marrow result in skewed differentiation of hemopoietic progenitor cells to the myeloid lineage. In addition, it has also been demonstrated that the development of an age-related phenomenon called clonal hematopoiesis of indeterminate potential (CHIP), in which hemopoietic clones without malignancy or other known clonal diseases are produced, leads to significantly increased atherosclerotic CVD risk (Jaiswal et al. 2014, 2017). Even though how CHIP leads to increased atherosclerotic CVD risk is not clearly understood, genetically reduced IL-6 signaling (involved in the inflammatory response and redox homeostasis) has been shown to abrogate this risk.

Low-density lipoprotein (LDL) exposure and atherosclerotic CVD risk have been one of the most extensively studied subjects in cardiology. As a result of these investigations, through experimental, clinical, and genetic studies, without a doubt, it is established that LDL exposure increases atherosclerotic CVD risk (Ference et al. 2017). The formation of atherosclerotic plaques involves the cholesterol-rich lipids, mainly LDL, which pass the endothelium of arteries, undergo oxidation/modification and retention in the intima of blood vessels, and induce an inflammatory response. The inflammatory response begins once the endothelial cells start secreting adhesion molecules and together with smooth muscle cells attract leukocytes into the arterial wall. Monocytes turn into macrophages, phagocytose the lipid particles, and form foam cells. Continuity of this process combined with the inability of leukocytes to clear the lipid particles from the vessel wall leads to chronic inflammation. The chronic inflammatory response eventually results in the accumulation of fat in smooth muscle cells and the formation of fatty streak, which then evolves into fibrous plaques, fibroatheroma, and atherosclerotic plaques (Insull 2009). Moreover, impaired redox homeostasis and oxidative stress take part in the oxidative modification of subendothelial lipid particles. Oxidation of LDL triggers pro-inflammatory gene transcription such as increased expression of IL-1 and TNF-alpha (Navab et al. 1991). Exposure to these cytokines leads to increased expression of vascular adhesion molecules, which further increases the inflammatory response by attracting more leukocytes to the subendothelial space, leading to a vicious cycle. Furthermore, endothelial dysfunction accompanying this process could lead to decreased synthesis of NO and modification of eNOS, which could exacerbate the impaired redox homeostasis even further (Martinon 2010; Reuter et al. 2010).

4.4.4 Cardiomyocytes, Fibroblasts, and Monocytes

The damage theory of aging states that each biological process generates damage, and even though clearance systems rectify some of this damage, clearance systems

themselves are not fool-proof, and they also cause damage. Therefore, damage accumulates in a cell and unless the cell can divide and dilute this accumulated damage, the function of the cell will be compromised, and eventually, the cell will die. Cardiomyocytes are vital to life and are mostly post-mitotic cells. Hence, their functional continuity is of utmost importance, and loss of which would result in loss of life. Therefore, organisms must have had to evolve mechanisms to protect them, until at least one could create progenies. Aging is associated with structural changes in the heart. The diastolic function of the heart measured by the Doppler echocardiography as E wave is known to gradually compromise during the aging process. This compromise is generally associated with structural changes in the heart caused by increased fibrosis and reduced relaxation of the myocardium caused by impaired SR calcium re-uptake, leading to impaired myocardial relaxation. In addition, impaired myocardial relaxation is thought to trigger an increase in atrial contraction, which then leads to increased atrial pressure and contributes to structural changes in the left atrium, making it more susceptible to the development of AFib (Dai et al. 2012). The aging population, therefore, created problems, such as increased CVD, which evolution did not necessarily have to deal with. Indeed, complex signaling mechanisms and adaptational responses exist in the heart. In the heart, ROS are known to trigger myocardial growth, cellular dysfunction, and matrix remodeling.

Moreover, ROS activate various transcriptional factors and can trigger cardiac hypertrophy (Sabri et al. 2003). For example, in neonatal rat cardiomyocytes, hydrogen peroxide has been shown to stimulate tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, as well as mitogen-activated protein kinases (Wei et al. 2001). ROS have also been shown to play a crucial role in G protein-coupled hypertrophic signaling by angiotensin II. Besides their effects on cardiomyocytes, ROS also affect cardiac fibroblasts and the extracellular matrix. These effects include, but are not limited to, stimulation of cardiac fibroblasts and modification of matrix metalloproteinase (MMP) enzymes, leading to fibrosis and extracellular matrix remodeling. In addition to triggering inflammation in the vessel wall, ROS have also been shown to stimulate pro-inflammatory cytokines in the heart, such as NF-kB. It has been shown that low levels of ROS inhibit apoptosis in the heart, but higher levels seem to stimulate apoptosis in the heart (Kwon et al. 2003), which implies that there is a delicate balance. The delicacy of homeostasis is further supported by other experimental studies where while inhibition of antioxidant enzymes resulted in increased cardiac hypertrophy, overexpression of Trx has been shown to lead to reduced hypertrophy (Yamamoto et al. 2003; Takimoto and Kass 2007).

Recently, the contribution of bone marrow–monocyte–macrophages to atherosclerosis and myocardial damage has been a hot topic. Several studies found a correlation between increased local macrophages and increased atherosclerosis/myocardial damage. Under steady-state conditions, macrophages reside in the heart. While monocytes in blood move to cardiovascular tissue and turn into macrophages, macrophages in the cardiovascular system seem to be able to proliferate in the heart and self-maintain (Sager et al. 2017; Epelman et al. 2014). In fact, in murine atherosclerotic models, it has been shown that macrophage turnover takes place in a matter of weeks and the replenishment is not from the blood/bone marrow, but from

the proliferation of local macrophages. Therefore, local macrophage proliferation has been considered a key target in the prevention of atherosclerosis and CVD (Robbins et al. 2013). Further investigation demonstrated a plausible mechanism that links the LNK/SH2B3 gene, which inhibits differentiation of hematopoietic stem cells to monocytes and neutrophils, to attenuated CVD. The results of these studies implied that under-expression of this gene might result in susceptibility to CVD through increased production of myeloid cells (Swirski and Nahrendorf 2016). Because macrophages are involved in inflammation response (which involves ROS production), they are a part of cardiovascular tissue repair, regeneration, and signaling. Importantly, these processes are vital in the understanding of HF, AFib, and cardiac fibrosis. Cardiac fibrosis is an active process that results as part of the remodeling due to mechanical and chemical stresses exerted on the heart. Macrophages in the heart seem to be diverse and have distinct protein expressions, with some monocyte subsets that seem to predict future cardiovascular risk (Berg et al. 2012). Several subsets of macrophages following acute MI have been detected in the hearts of murine models. For example, it has been shown that Ly-6Chigh monocytes dominate in the early phase of inflammation and Ly-6Clow monocytes play a dominant role later. Because of the involvement of different subsets of macrophages in the repair process, targeted therapies to block subsets of monocytes and in turn limit excess ROS generation, reperfusion injury, and atherosclerotic disease have been under examination (Shahid et al. 2018).

Redox regulation is thought to be involved in fibrosis response and affects multiple cell types. Transforming growth factor-β (TGF-β) is known to be involved in the transformation of interstitial fibroblasts to myofibroblasts. In addition, ROS are involved in the activation of fibrosis response including the activation of the TGF-β pathway and transcription of other growth factors involved in fibrosis. It is postulated that ROS-related activation of calcineurin is involved in TGF-β-induced mesangial cell proliferation. Moreover, inflammatory process and redox signaling are known to activate the expression of endothelial surface adhesion molecules, and this could also result in the transmigration of circulating fibroblast progenitors, contributing to extracellular remodeling, and fibrosis response. Matrix metalloproteinases are involved in the remodeling of the extracellular matrix. Because the activation of MMPs is redox-sensitive, and they are involved in the fibrotic response, it is plausible that ROS would be a player in the remodeling process. Furthermore, NOX proteins, RAAS, and β-adrenergic stimulation have also been shown to increase MMP-2 and MMP-9 activity in cardiomyocytes.

4.4.5 Ischemia–Reperfusion Injury

Myocardial infarction (MI) is the irreversible damage that takes place due to sustained and severe myocardial ischemia, which generally occurs due to the rupture of an atherosclerotic plaque followed by superimposed thrombosis and arterial occlusion. Because the heart needs to beat continuously to sustain life and the myocardial tissue

is highly dependent on oxygen and nutrients, rapid reperfusion is recommended. Despite this need, reperfusion of the ischemic myocardium itself has been shown to induce myocardial tissue loss. Major contributors to reperfusion injury, with the return of arterialized blood, include increased oxidative stress, the opening of the mitochondrial permeability transition pore (MPTP), and calcium overload. Experimental studies demonstrated that myocardial reperfusion injury itself may account for up to 50% of the infarct size (Hausenloy and Yellon 2013; Yellon and Hausenloy 2007). During the time blood supply is cut off, cardiomyocytes have to depend on anaerobic respiration. Anaerobic respiration produces lactate and this results in a drop in pH. The drop in pH then via Na^+-H^+ exchanger leads to increased intracellular Na^+, which in turn activates the $2Na^+$-Ca^{++} channel and leads to increased intracellular Ca^{++}. Because enough ATP cannot be produced via anaerobic respiration to meet the demands of the myocardium, K^+-Na^+-ATPase ceases to function. After this, intracellular pH drops and cellular swelling begins. During this process, MPTP, which is located in the inner mitochondrial membrane, remains closed. On the other hand, once the arterial reperfusion is established, mitochondrial respiration begins and generates ROS. ROS generated during this process and relative ATP depletion combined with rapid correction of intracellular Ca^{++} results in the opening of MPTP and cell death. Therefore, preventing the opening of MPTP has been suggested as a way to limit reperfusion injury. Cyclosporin A, an immunosuppressant that also inhibits MPTP opening, has been shown to reduce infarct size by half in animal models (Hausenloy and Yellon 2013). Moreover, the inflammatory process triggered by ischemia draws in leukocytes, which further increases the release of inflammatory cytokines and ROS production. Hypoxia also affects oxygen concentration in the mitochondria and results in dysfunctional ETC, increased XO, and NOS uncoupling, which leads to increased ROS production. The inflammatory response and ROS generation in this process have also recently been targeted to combat reperfusion injury (discussed later). Even though administration of antioxidative agents at the time of reperfusion has not been consistently positive, several experiments have been carried out to test whether normalization of pH in a gradual fashion as opposed to rapid normalization could decrease reperfusion injury (Fujita et al. 2007; Heusch 2020). The heart, being a metabolically demanding tissue, is particularly susceptible to ischemia–reperfusion injury. It is thought that mitochondrial ROS generation plays an important role in the pathogenesis of ischemia–reperfusion injury. Disappointingly, antioxidative supplementation has been found to be ineffective in the management of ischemia–reperfusion injury (Andreadou et al. 2009). On the other hand, it has been speculated that this could be because ROS generation not only plays a detrimental role, but ROS also have vital roles in a cell. Therefore, investigators have proposed that targeted antioxidative supplementation particularly to mitochondrial ROS generation may have protective effects. Substances such as mitoquinone and mito-phenyl tert-butylnitrone (mito-PBN) have antioxidative properties and have been found to accumulate in mitochondria. In addition, analogs of Mn-SOD may also have benefits in this regard (Kalogeris et al. 2014).

When the blood flow is re-established to the myocardium, inflammatory cytokines from the local tissues lead to leukocyte-endothelial activation. Transmigration of

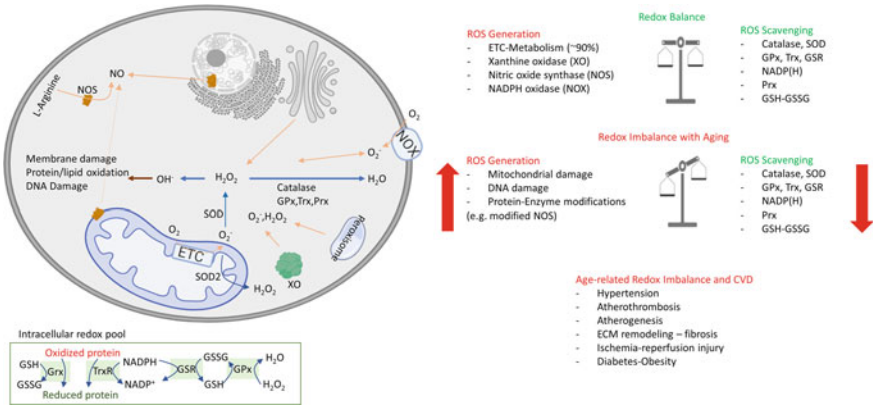

Fig. 4.1 Main redox reactions in a cell and the contribution of redox imbalance brought on by the aging process to cardiovascular disease development. CVD: Cardiovascular diseases, ECM: Extracellular matrix, ETC: Electron Transport Chain, GPx: Glutaredoxin, GSH-GSSH: Reduced and oxidized glutathione, GSR: Glutathione reductase, NO: Nitric Oxide, NOS: Nitric Oxide Synthase, O_2^-: Superoxide, OH^-: Hydroxyl, H_2O_2: Hydrogen Peroxide, Prx: Peroxiredoxin, ROS: Reactive Oxygen Species, SOD: Superoxide dismutase, Trx: Thioredoxin. Created with biorender

monocytes to myocardial tissue also leads to increased ROS production and the introduction of ROS-generating monocyte enzymes (such as NOX) might damage the cell membrane, oxidize membrane lipids, and lead to lysis of cells (Kalogeris et al. 2014) (Fig. 4.1).

In experimental models of acute MI, it was shown that aldosterone-induced Nox2-dependent CaMKII oxidation has been shown to promote MMP-9 activity and be involved in early cardiac rupture, a process that involves phagocytes. Importantly, in $p47^{phox-/-}$ mice (a knock-out model of a cytosolic protein in the Nox complex), this complication was prevented. In the later phases of infarct healing, fibrosis, functional loss, and LV dilatation are commonly observed. Interestingly, $p47^{phox-/-}$ and Nox2-null mice models had less adverse remodeling following MI, implying that functional loss following MI might partly be attributed to ROS and inflammatory response, which is also supported by the positive results of anti-inflammatory and anti-ROS substances in clinical trials (Looi et al. 2008; Doerries et al. 2007). Therefore, as well as antioxidative therapies, several anti-inflammatory agents are also promising in the prevention of ischemia–reperfusion injury.

4.4.6 Diabetes and Obesity

Obesity, metabolic syndrome, and insulin resistance are commonly seen in the aging process. Emerged evidence indicates that inflammation and impaired redox balance, commonly observed in aging, are linked to the development of insulin resistance,

diabetes, and obesity, which are strong risk factors in the development of CVD (Ahima 2009). In addition to this, the opposite of this statement is also true; that is, diabetes and obesity are also known to increase oxidative stress. Therefore, in this topic, cause–effect relationship is generally hard to pinpoint, and there seems to be a vicious cycle in which diabetes and obesity are leading to an environment which results in increased oxidative stress. While in obesity excess supply of nutrients is believed to lead to mitochondrial dysfunction and increased ROS production, increased ROS production is also known to affect post-translational modification of proteins and trigger major transcriptional programs and affect metabolism. Moreover, it is suggested that the pro-inflammatory NF-kB signaling pathway is induced by ROS and inhibited by SOD2 overexpression (Forrester et al. 2018). Complicating the cause–effect relationship further, some evidence also indicates that NF-kB signaling itself also triggers increased oxidative stress (Zhang et al. 2017). Experimental animal studies showed that activation of the NF-kB signaling pathway increases metabolic demand and insulin sensitivity, which could play a protective role in the development of diabetes. On the other hand, other studies reported that upregulation of the NF-kB pathway may lead to hyperglycemia and insulin resistance (Tang et al. 2010; Cai et al. 2005). Importantly, increased ROS levels were also found in obese people without diabetes, as well as in obese mice models. Furthermore, in obese mice, antioxidant supplementation has been shown to prevent the development of diabetes. These findings imply that obesity itself is a contributor to increased oxidative stress, and ROS originating from adipose tissue could in turn trigger an inflammatory state and insulin resistance (Matsuda and Shimomura 2013). The vicious cycle between impaired redox homeostasis and obesity has also been bolstered by other studies which demonstrated that impaired redox homeostasis leads to pre-adipocyte proliferation and differentiation, and further evidence shows that ROS might be involved in the pathways leading to hunger and satiety and, therefore, affect eating behavior and regulate body weight (Savini et al. 2013).

While insulin-like growth factor-1 (IGF-1) signaling is used as a marker in experimental aging models, the role of IGF-1 signaling in humans is complicated due to the fact that its levels decrease with age. Observational studies indicate that there is a correlation between low levels of IGF-1 and increased HF and that growth hormone supplementation might decrease HF in some patients. Stimulation of insulin receptor leads to activation of phosphoinositide-3 kinase and activation of Akt. Akt then inactivates Forkhead Box O (FOXO) in the nucleus by phosphorylation. In *C. elegans*, FOXO transcription factors have been shown to be involved in anti-aging modifications, where increased activation of these transcription factors has been shown to increase lifespan (Kenyon et al. 1993). Moreover, in Drosophila, the overexpression of these transcription factors has been shown to lead to the prevention of age-related decline in cardiac function. The suggested mechanisms of the anti-aging effects of these transcription factors have been thought to include activation of endogenous antioxidants and sirtuin-1, anti-aging effects of which have been demonstrated in many studies.

4.5 Recent Evidence and Potential Future Therapies

To fight against CVD, first, classic risk factors such as diet, smoking cessation, and exercise need to be addressed. Implementing these changes is not always possible, and several medications have been proven to help. One of these medications is statins. Statins have anti-inflammatory and antioxidative effects. Their antioxidative effects are thought to operate via the suppression of oxidation pathways such as the inhibition of myeloperoxidase and NOX and prevention of the formation of NO-derived oxidants (Shishehbor et al. 2003). Moreover, statins are shown to up-regulate antioxidative enzymes including catalase and paraoxonase (Davignon et al. 2004). Statins have been proven to reduce cardiovascular events not only in those with high LDL, but also in people with average LDL levels. Interestingly, while statins have been shown to decrease major cardiovascular events by 20–40% in numerous studies, their observed effect on atherosclerotic plaque size reduction has been much smaller (Libby 2006). This observation is explained by how MIs happen. Myocardial infarctions generally happen not because of the vessel stenosis itself, but due to atherosclerotic plaque rupture followed by atherothrombotic occlusion. Therefore, the stability of the atherosclerotic fibrous cap and its functional state becomes of utmost importance. In fact, the improvement in mortality rendered by statins has been attributed to their anti-inflammatory properties, which are involved in ROS generation and impaired redox balance. Moreover, several pro-inflammatory markers such as C-reactive protein (CRP) have been suggested as a marker to assess the susceptibility of a patient to cardiovascular events. Therefore, it was postulated that if statins have anti-inflammatory properties, they would be expected to drop CRP levels, which was proven by later studies (van de Ree et al. 2003). Furthermore, decreasing CRP levels was shown to correlate with improved clinical cardiovascular outcomes. The contribution of oxidative stress and inflammation to CVD has, therefore, been proven. Following solidification of this link, investigators sought to determine the pathways, the blockage of which could lead to improvements in cardiovascular outcomes. One of these trials tested the blockage of interleukin-1β (IL-1β), a cytokine the downstream actions of which triggers inflammation and ROS generation. This trial was published in 2017 in the *New England Journal of Medicine* (*NEJM*). In their randomized, double-blind trial, investigators enrolled 10,061 patients with previous MI and a high-sensitivity CRP that was greater than 2 mg/L. The intervention was three different doses of canakinumab (50, 150, 300 mg), a monoclonal antibody that targets IL-1β. At the end of 48 months, a dose–response relationship in high-sensitivity CRP was observed in patients who took canakinumab, while lipid levels did not change from baseline. Notably, patients who took 150 and 300 mg of canakinumab had significantly lower primary endpoint events (non-fatal MI, non-fatal stroke, or cardiovascular death). Furthermore, patients who took 150 mg had significantly lower rates of hospitalization for unstable angina requiring urgent revascularization. In this trial, while canakinumab had significantly improved cardiovascular clinical events, patients who took canakinumab also had higher rates of fatal infection. While no difference was observed in all-cause mortality, this trial by measuring clinical hard

outcomes proved that inflammation and oxidative stress were indeed involved in the pathogenesis of CVD and that anti-inflammatory treatment, independent of serum lipid status, might reduce the development of cardiovascular events (Ridker et al. 2017). A follow-up to this study, which was recently published in the *Journal of the American College of Cardiology,* confirmed that the effects of canakinumab were not only limited to the first cardiovascular events, but canakinumab also reduced the total cardiovascular events (Everett et al. 2020).

Colchicine is a drug that has been used in the treatment of several rheumatological diseases such as Behcet's disease, familial Mediterranean fever, and gout. The mechanism of action of colchicine is believed to operate through inhibition of neutrophils, endothelial cells, and platelets, and as a result, colchicine inhibits mitosis and has anti-inflammatory and antioxidative properties. Several studies showed that colchicine may be useful in the treatment of pericarditis and CAD (Gasparyan et al. 2015). In a recent trial published in *NEJM*, investigators tested whether colchicine could reduce CVD risk in patients with chronic CAD. In this randomized, double-blind placebo-controlled trial, 5522 patients were randomized 1:1 to placebo or 0.5 mg daily colchicine. After a median follow-up period of 28.6 months, patients who took colchicine had significantly lower rates of the primary endpoint (composite of cardiovascular death, non-procedural MI, ischemic stroke, or ischemia-driven revascularization). Colchicine also significantly reduced secondary outcomes, which included cardiovascular death, spontaneous MI, or ischemic stroke (Nidorf et al. 2020). This study supplemented a previously conducted *NEJM* study, in which colchicine significantly reduced ischemic cardiovascular events in patients with a recent MI (Tardif et al. 2019).

Furthermore, many substances, including vitamins with antioxidative properties, have been tested to see whether they could improve cardiovascular events. The first studies on the effects of vitamin supplementation and their effects on CVD came overwhelmingly from observational studies, in which groups of people were compared based on their supplemental vitamin intake, dietary intake, or serum vitamin levels (Gey et al. 1987; Kok et al. 1987; Ward et al. 1997; Enstrom et al. 1986). These studies suggested that supplemental vitamins might improve cardiovascular health. On the other hand, because these were observational studies, the likelihood of confounding factors could not be fully addressed. Therefore, these were followed by two dozen or so randomized controlled trials (RCTs), including for Vitamin A, C, and E, which are vitamins with antioxidative effects. Overall, these RCTs did not find tangible evidence to suggest that antioxidative vitamin supplementations might improve cardiovascular health, which meant that the previous observational studies were likely confounded by other factors, such as differences in lifestyle and health-conscious behavior observed in those who were taking vitamin supplements (Lee et al. 2005; Sesso et al. 2008; Burgoyne et al. 2012).

Another method that has been shown to increase lifespan in several model organisms and non-human primates is CR. It is suggested that CR preferentially increases mitochondrial metabolism. Increased mitochondrial metabolism induced by CR is expected to increase oxidative stress. While increased oxidative stress would be expected to have detrimental effects, experimental studies indicated that glucose

restriction extends lifespan in many organisms, including non-human primates. Furthermore, supplementation of N-acetylcysteine, an antioxidant, has been shown to negate the life-prolonging effects of CR. Hence, oxidative stress likely plays a key role in cellular stress resistance. The concept that small amounts of increase in oxidative stress by exercise or CR might actually improve longevity by inducing cellular adaptations to stress is known as mitochondrial hormesis (Ristow and Schmeisser 2014; Dai et al. 2012).

One of the mechanisms of hormesis is thought to be the activation of adenosine monophosphate-activated protein kinase pathway (AMPK), which is involved in the regulation of growth and metabolism and has recently been shown to be involved in autophagy (Mihaylova and Shaw 2011). Caloric restriction is also thought to be involved in the inhibition of the mammalian target of the rapamycin (mTOR) pathway, which is a regulator of a wide array of processes, including cell metabolism, cellular growth, and autophagy. Activation of mammalian target of rapamycin complex (mTORC1) pathway is associated with disease processes such as tumor formation, insulin resistance, and diabetes and obesity. This pathway is also closely associated with metabolic changes and insulin levels. Insulin and mTORC1 are involved in a feedback mechanism. While insulin leads to activation of lipogenesis, lipid storage, and glycogen synthesis, it also activates Akt, which then activates mTORC1 and inhibits the activation of Akt by insulin. In addition, mTORC1 also activates lipogenesis and lipid storage.

While CR is an unlikely method to be widely applicable, and vitamin supplementation and other antioxidant therapies generally yielded negative results, several more specific antioxidative substances seem to offer promising results. One of these is a combined NOX3 and NOX4 inhibitor, which was found to have promising effects in a liver fibrosis model (Aoyama et al. 2012). Because Nox activity is known to be involved in several aforementioned pathophysiologic mechanisms, other substances thought to inhibit several combinations of Nox isozymes are also being tested clinically. In addition, to prevent decoupling of NOS, oral administration of BH4 and allosteric NOS inhibitor is also being tested. More specific intervention such as a mitochondrial antioxidant peptide called SS-31 was found to have positive effects in an experimental HF model (Dai et al. 2011). Another substance called MitoQ has also shown some promising effects in hypertension-induced cardiac hypertrophy, cardiac ischemia–reperfusion injury as well as liver injury (Gane et al. 2010; Dai et al. 2011). In addition to preventing ROS formation, one might try increasing the ROS scavenging capacity of the organism. Even though whether this would be feasible in humans is not clear at the moment, experimental studies in animal models have found promising effects for several interventions including gene therapy targeting Trxs, an activator of a transcription factor called erythroid-derived 2 (involved in the expression of antioxidative proteins), as well as several substances targeting the pathways discussed above, such as CaMKII (Burgoyne et al. 2012).

4.6 Conclusion

Impaired redox homeostasis is a major problem in aging and plays a significant role in the development of CVD with a direct contribution to the pathophysiology of leading causes of CVD such as hypertension, atherothrombosis, atherogenesis, ischemia–reperfusion injury, and diabetes and obesity. Anti-inflammatory and antioxidative drugs such as statins and colchicine have been proven to significantly decrease CVD development and improve clinical outcomes. While CR or intermittent fasting might lead to increased lifespan, CR is unlikely to be a popular method to prolong lifespan society. Whereas vitamin supplementation and antioxidative substances such as lipoic acid have been associated with improved outcomes in observational studies, in well-designed RCTs, these substances did not result in improved outcomes. While several cytokine inhibitors, such as IL-1β inhibitor canakinumab, have also been shown to attenuate the development of atherogenesis and improve cardiovascular outcomes, vitamins with antioxidative properties do not seem to have palpable clinical effects in the general population. Future mechanistic studies looking into the causal relationship between impaired redox homeostasis and the development of CVD are needed to better understand and separate correlation and causation and the order of development of disease and impaired redox homeostasis. Moreover, RCTs investigating selective inhibitors of several subsets of monocytes (Ly-6Chigh), enzymes (NOX, Trxs), specific signaling pathways (CaMKII), and transcription factors (erythroid-derived 2) as well as supplementation with mitochondria-specific novel antioxidative substances (SS-31, MitoQ) might be promising in the optimization of management of major cardiovascular events. Before RCTs can prove the efficaciousness and safety of these interventions in humans, the utilization of these interventions should be reserved only for well-designed RCTs.

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Chapter 5
Redox Homeostasis in Skeletal Muscle Aging

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Abstract Aging and age-related complications are the major social burden that contributes to disability and other poor outcomes at a higher prevalence. However, no clear attempt has been made so far in terms of diagnosis and treatment. Despite considering several theories, the reactive oxygen species (ROS) production is the one that implicates in inducing aging and age-related diseases. Nevertheless, within certain concentrations, ROS can effectively regulate various age-related signaling pathways such as PGC-1 alpha, MAPK, and mTOR. Further, the optimal ROS concentration could provide better adaptation to the cells during initial oxidative stress response which could ultimately improve the antioxidant defense system and support against age-related complications. But studies have shown that removal of ROS during antioxidants treatment or genetic increase in these antioxidants did not improve the life extension which inconclusively provides debate of ROS-induced aging and diseases. Skeletal muscle loss is one of the major hallmarks of aging and ROS, and oxidative stress is considered to be the main cause of skeletal muscle aging. This chapter discusses how ROS can influence the aging of skeletal muscle including ROS-targeted signaling in aging and age-related complications.

Keywords Aging · Redox homeostasis · Oxidative stress · Skeletal muscle aging

5.1 Introduction

Although it is hard or nearly impossible to ignore aging and age-related complications, the only alternative option that we have is care and cure that can possibly help to improve life quality and life expectancy. According to WHO, the life expectancy of global population is increased up to 72 years in 2016. This is because of the remarkable development in the medical system and improvements in the living standards of

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the elderly. Simultaneously, this can place the population at risk of various chronic, non-communicable, and even communicable diseases, and these are the major threat of death and disability. Indeed, age-related complications have become a major life threat in the twenty-first century. Therefore, understanding the age-related mechanism and finding treatment for age-related complications are in immediate need of attention and are regarded as the most important socioeconomic challenge for the next decades. Aging is defined as progressive deterioration of physiological functions that impairs the organism to maintain homeostasis, increasing susceptibility to diseases and death (Scicchitano et al. 2018). As skeletal muscle is the largest organ in the body comprising approximately 40% of its mass, its role in either preventing or causing age-related diseases is crucial. Considering skeletal muscle's major role in movement, posture, and energy metabolism, its dysfunction is likely to be increased with aging in several ways such as altering muscle contraction and relaxation and failure of adaptive response to pathological consequences. As a result, these scenarios have a greater impact on the quality of life and increase the dependency and frailty (Degens 2010; Viña et al. 2016).

The disuse leads to loss of skeletal muscle mass and function and consequent sarcopenia. This is directly linked to adverse outcomes, including behavioral, cognitive, and metabolic changes, which are intrinsically related to several chronic diseases (Eriksen et al. 2016; Picca and Calvani 2021). While disuse is a significant contributor to age-related muscle wasting (Larsson et al. 2019). In contrast, physical exercise is an essential contributor to prevent, reverse or minimize this situation (Nascimento et al. 2019). The muscle redox profile changes are also believed to play a significant role in aging (Radak et al. 2019). The imbalance in muscle redox influences numerous other cellular processes such as inflammation, mitochondrial function, protein synthesis, which are also influenced by physical exercise (Powers et al. 2020). The redox changes can result in the loss of muscle's ability to control the generation of reactive oxygen/nitrogen species (RONS). Within this scenario, several studies have revealed the role of physical exercise in regulating this system by having hormesis-like benefits where low doses of a stressor to the organism can bring about benefic results to the long-term welfare of the individuals (Ji et al. 2006; Radak et al. 2019). The results found in this regard are highlighted in this chapter. However, there is still conflicting information that needs further investigation.

5.1.1 ROS Production and Diffusion in Skeletal Muscle

ROS can generate at multiple subcellular levels in the skeletal muscle including mitochondria which is considered to be the major source of oxidants production. However, other sources of ROS production such as NADPH oxidase (NOX), xanthine oxidase, and phospholipase (PLA2) can further contribute to producing ROS which subsequently increases its concentration and perturbs the steady-state level of several enzymatic antioxidants in the skeletal muscle (Thirupathi et al. 2019). Cytosolic

NADPH oxidase (NOX2) can produce ROS in the sarcoplasmic reticulum, transverse tubule, and the sarcolemma of skeletal muscle (Sakellariou et al. 2013), while xanthine oxidase produces ROS during oxidative conversion of xanthine to uric acid in a calcium-dependent protease activation process, and PLA2 plays a crucial role in the formation of ROS in the skeletal muscle through the arachidonic acid pathway (Prunonosa Cervera et al. 2021). Further, the diffusion rate of ROS from the site of production is ambiguous due to its stability; particularly, how it can diffuse from one site to another site needs careful evaluation. ROS from other sources of skeletal muscle possibly enters into mitochondria in two ways. One way is that it can use anion exporters for penetration for further reduction. The other way is that it can induce RONS through Fenton reaction in the skeletal muscle. Next, H_2O_2 is the stable molecule that relatively penetrates longer distance within the skeletal muscle where it can influence various redox-sensitive molecules and signaling pathways, and its cytotoxicity status depends on the reactive metal such as Cu^+ and Fe^{2+}, within the skeletal muscle, resulting in the formation of hydroxyl radical (Schubert and Wilmer 1991; Ji 2015; Lindsey and Tarr 2000). For example, Fe cluster enzymes such as fumarases and aconitase can contribute effectively to the production of hydroxyl radical through Fenton reaction (Thirupathi et al. 2019).

5.1.2 ROS in the Skeletal Muscle Aging

Free radical-induced aging theory is one of the most studied concepts in the skeletal muscle aging in the last few decades (Harman 1956). Indeed, this theory has its own agreements and disagreements in several ways. For example, protein oxidation and nitration are often linked with oxidative damage and have been used as crucial biomarkers for the measurement of oxidative stress in aging and diseases. However, evidence has shown that protein modifications are necessary for cellular functions under stress conditions. These contradictory results impede the ROS role in understanding skeletal muscle aging and diseases, but within certain concentrations, ROS can increase skeletal muscle aging or possibly have a contrasting role to prevent aging. Further, ROS can activate several cellular signaling pathways including adaptation and biogenesis in the skeletal muscle (Kregel and Zhang 2007).

The source of ROS production is a major player in the acceleration of skeletal muscle aging. Indeed, skeletal muscle is one of the largest mitochondrial content tissues, and mutation in the mitochondria due to increased concentration of ROS is the main contributor of skeletal muscle aging (Hiona et al. 2010). The failure of ROS scavenging can damage mitochondrial DNA. Consequently, mitochondrial respiration impediment can further increase ROS which ultimately ramps up aging and age-related diseases. Studies have shown the correlation between aging and mitochondrial respiration dysfunction and mtDNA deletions and mutations in the single muscle fibers (Cormio et al. 2000; Cortopassi and Arnheim 1990), whereas other studies have reported no age-linked mitochondrial respiration dysfunctions

(Rasmussen et al. 2003). These inconsistent results question whether excess mitochondrial ROS and following dysfunction is the real contributor of skeletal muscle aging or are any other factors that are associated with this such as physical inactivity or nutrition (Gianni et al. 2004). But failure to control ROS within the threshold value could be a clear indicator of affecting various subcellular organelles' function of skeletal muscle. For example, reduced mitochondrial population due to ROS could reduce the muscle mass and force production decrement, and this is linked with aging (Zorov et al. 2014). However, we are not sure whether ROS is the primary cause of aging or the consequence of ROS remains to be explored.

5.1.3 ROS-Induced Signaling and Skeletal Muscle Aging

Life genesis and further survival are based on how cells can potentially balance redox status. Until the 1990s, it was believed that ROS is toxic to cells and their organelles, but its role as regulating or being as signaling in energy homeostasis and biogenesis proved that ROS could help in normal aging within a certain concentration. Indeed, obtaining gradual aging depends on those signaling, and failure or changes in such signaling pathways aggravates skeletal muscle aging. And the expression of antioxidants cycle has been largely affected during skeletal muscle aging including superoxide mutase (SOD), catalase (CAT), and glutathione (GSH), and this can increase ROS generation robustly. But factors like exercise can optimize this situation to control ROS formation within the threshold value. For example, exercise-induced ROS formation can target redox-sensitive proteins such as γ-glutamylcysteine synthetase, which is a rate-limiting enzyme of GSH synthesis (Gomez-Cabrera et al. 2008). But aging can affect this situation which can ultimately increase the ROS concentration above the threshold level (Fig. 5.1). Other important signaling pathways such as nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), and mitogen-activated protein kinases (MAPKs) have been linked with the ROS formation, and these pathways are directly involved in apoptosis. Tp53-induced glycolysis and apoptosis regulator (TIGAR) can regulate ROS through GSH cycle for lowering ROS-induced apoptosis (Bensaad et al. 2006). Further, ROS scavenging inhibits important signaling pathways such as MAP kinase and following activation of NF-κB, indicating the role of ROS as signals (Gomez-Cabrera et al. 2008), and these pathways are the key mediators of skeletal muscle aging (Tilstra et al. 2011; Morsch et al. 2019).

Next, ROS can affect the signaling of mitochondrial biogenesis in the skeletal muscle. For example, ROS can trigger a peroxisome proliferator-activated receptor gamma coactivator-1α(PGC-1α) mediated pathway which can induce mitochondrial biogenesis and regulate energy homeostasis (Thirupathi et al. 2019). Further, several signaling kinases are mediated through PGC-1α activation such as 5' AMP-activated protein kinase (AMPK) and calcium-/calmodulin-dependent protein kinase (CaMK), and these are important signaling pathways in energy homeostasis and skeletal muscle aging (Thirupathi et al. 2019; Chin 2004). However, increased concentration of ROS

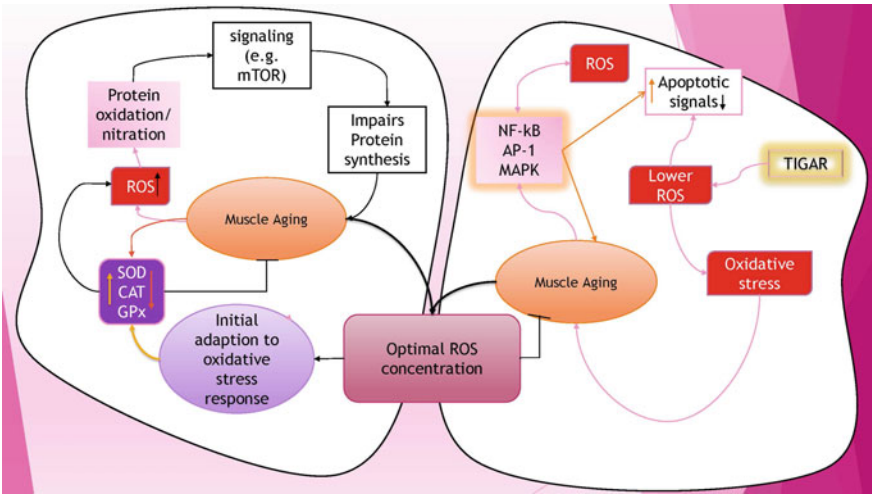

Fig. 5.1 The schematic diagram represents that TIGAR can reduce ROS and further oxidative stress resulting in the decrease in apoptotic signals. Optimal concentration of ROS can provide initial adaptation against oxidative stress response and further increase in antioxidants to reduce muscle aging. Muscle aging increases the ROS concentration and protein oxidation and nitration which impairs the protein synthesis leading to muscle aging

in the mitochondria can alter the entire scenario and aggravate muscle aging. For example, ROS can stimulate muscle wasting and aging through NF-κB and forkhead box O3 (FoxO3) autophagic pathway (Morsch et al. 2019). And even ROS have an effect on inducing apoptosis pathways, e.g., increased ROS can dysregulate the sarcoplasmic reticulum Ca^{2+} flux, resulting in caspase 7 activation. Even, increased ROS can alter mitochondrial permeability transition pores which facilitates the release of proapoptotic proteins in the skeletal muscle. And ROS can induce the release of endonuclease G (Endo G) and/or apoptosis-inducing factors, and these are able to induce DNA fragmentation in the skeletal muscle (Dupont-Versteegden et al. 2006). Redox status is important for oxidation within protein which further alters enzymatic reaction and gene transcription. For example, pathway like nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1(Nrf2-Keap1) has been reported to prevent various age-related phenotypical changes in the skeletal muscle, and this signaling pathway is required for the oxidation of cys 151 residue within the Keap1 to prevent Nrf2 ubiquitylation, thereby increasing Nrf2 stability and further translocation to the nucleus. In contrast to this scenario, oxidation of methionine can be crucial in the development and progression of several aging-related diseases. Nrf2 regulates the proteostasis, and loss of Nrf2 signaling is the key player in driving age-related diseases. Further, Nrf2 attributes to telomere attrition through driving telomerase reverse transcriptase (Ahmad et al. 2016). Therefore, loss or reduction of Nrf2 and further increase of oxidative stress can contribute to several

hallmarks of aging including influencing genomic stability, epigenetic alteration, and mitochondrial dysfunction.

5.1.4 Physiological Level of ROS in the Skeletal Muscle

ROS can modulate a number of physiological functions in the skeletal muscle by acting as secondary messengers, and H2O2 having a higher advantage of being signaling because of its stability and membrane permeability. The evidence has shown that a single period of muscle contraction can increase the ROS formation, which facilitates various physiological responses such as activating muscle adaptation signaling and antioxidant response (Thirupathi et al. 2019). However, this effect is varied at different physiological levels of ROS, and this can be determined according to the tolerable limit of ROS within the cells, tissues, or even different organisms. For example, a higher increase in ROS and further interaction between ROS redox-sensitive proteins can increase the ROS regulatory enzymes such as SOD and inducible nitric oxide synthase (iNOS), thus facilitating muscle adaptation, while other studies have reported that a low level of SOD is not linked with aging in the experimental animals, and this may be due to different tolerable level of ROS by the experimental animals (Lennicke and Cochemé 2020). Also, the disruption of iNOS has been implicated with insulin resistance in skeletal muscle aging, and ROS could be a major factor for this insulin resistance. But, within certain physiological levels, ROS can induce insulin signaling and increase glucose uptake, but a higher level of ROS can upregulate the miR200a which acts on 3′UTR of insr and isr1, thus restricting insulin signaling resulting in the impairment of insulin-dependent glucose uptake in the skeletal muscle. Although a higher level of oxidative damage can increase skeletal muscle aging, a lower level of oxidative damage due to metabolic adaptation can increase longevity (Zainal et al. 2000; Redman et al. 2018). And even low level of ROS in patients with chronic hereditary diseases like lupus erythematosus and chronic granulomatous is more susceptible to aggravating these diseases, suggesting that low level of ROS is not enough to maintain physiological processes, leading to interference in health and longevity. Therefore, defining and determining the physiological level of ROS need to be addressed with several caveats. ROS formation occurs all the time at the cellular level, having a lot of merits and demerits such as interfering with signaling events and damaging cellular structures simultaneously (Luo et al. 2020). For instance, protein oxidation and its consequences lead to a lot of damages in skeletal muscle aging, while cystine oxidation-based signaling is involved in reverse age-related complications (Luo et al. 2020).

5.1.5 Oxidative Products in the Skeletal Muscle Aging

Oxidation products accumulation is one of the hallmarks of age-related complications including muscle dysfunction and atrophy. Oxidation within proteins can produce reactive aldehydes and ketones like lysine and proline oxidation, which cannot be modified, which means the affected protein oxidation by-products can undergo proteasomal degradation. But sometimes these by-products can cross-react with other proteins and accumulate to cause age-related diseases, resulting in the failure of protein synthesis. One example is the oxidation of lysine interference with the mTOR signaling in the fast-twitch and slow-twitch fibers impairing the protein synthesis (Edman et al. 2019). It has been recently reported that heat-shock proteins can prevent the accumulation of oxidation products even against the first contraction of skeletal muscle-induced ROS through upregulating SOD and CAT (Jackson and McArdle 2011; Durham et al. 2008). For example, overexpression of HSP70 in the skeletal muscle of transgenic mice prevents the ROS-induced muscle damage in the old mice by increasing SOD and CAT (Broome et al. 2006), and proteasome could be effectively involved in the removal of oxidation products in the skeletal muscle aging. However, studies have shown that proteasome is implicated with the total protein turnover and oxidized protein removal, and its role in aging muscle is declined (Farout and Friguet 2006). Further, genetic deletion of CuZnSOD in mice and the following elevation of oxidative stress accelerates age-related muscle atrophy by activating proteolytic systems, suggesting the role of ROS and oxidative stress in regulating proteolysis (Jang et al. 2020). Since mitochondria are a major source of ROS, ATP-stimulated Lon protease is thought to be a major player in degrading oxidized proteins, and oxidized aconitase is degraded by the Lon protease (Marcillat et al. 1988; Bota et al. 2002). Moreover, the level of Lon protease is attenuated during the aging of both mice and rat skeletal muscles, and this may be due to age-related impairment of Lon protease function (Bakala et al. 2003; Bota et al. 2002; Delaval et al. 2004). This further contributes age-related accumulation of oxidized mitochondrial proteins and mitochondrial dysfunction. Other oxidative products such as lipofuscin and advanced glycosylation end-products induced by carbohydrates, protein, and lipid oxidation play a major role in age-related morbidities including centronuclear myopathies. ROS can reduce the DNA damage response and DNA repair in the skeletal muscle by inducing double-strand break or inter-strand cross-links which are directly attributed to increasing aging and age-associated diseases.

5.2 Conclusion

Although aging is certain; at least, understanding age aggravation factors can shelter against age-related complications. In this regard, ROS formation and oxidative stress in the skeletal muscle could be major drivers for altering or inducing age-related

pathological conditions. Indeed, oxidants are necessary for either producing adaptation to cells or being as signaling or regulating signaling pathways to impede aging and age-related diseases. Therefore, reckoning of preventing ROS can merely provide a negative impact to cells, while managing optimum ROS concentration is difficult since aging cells are more susceptible or having an increased concentration of ROS. However, some factors including nutrition and physical activity could partially alleviate this situation, but fixing the exact amount of food intake and further physical activity setup has several limitations. For example, setting up physical activity in aging has inconclusive concepts in terms of duration and intensity, which are mostly resulting in increased ROS concentration. Therefore, several ways need to be constructed including the beneficial limit of ROS concentration, signaling, and skeletal muscle adaptation to aging.

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Compliance with Ethical Standards

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Chapter 6
Aging and Exercise-Induced Reactive Oxygen Species

Eda A. Ağaşcioğlu and Anand Thirupathi

Abstract Aging is multidimensional and the loss of physical activity may be an important reason in a number of age-related diseases. Exercise seems to be a required challenge for the modern urban society in order to maintain a healthy life. An individual's capacity to utilize oxygen is an indicator of their Reactive Oxygen Species (ROS) generation rate. The common feature of all age-related disorders is the reduced quantity and quality of mitochondria due to the overproduction of ROS. Regular aerobic exercise induces the biogenesis and functionality of mitochondria via various pathways such as peroxisome proliferator-activated receptor-γ coactivator 1 alpha (PGC-1a) during aging. According to recent findings revealing that the sources of aging and exercise-induced ROS are both mitochondrial and extramitochondrial. Although going over of the physiological limits of ROS has detrimental effects, it allows the cells to adapt to exercise-induced stress within individual physiological limits. Thus, regular antioxidant supplementation might be beneficial for the elderly with regards to exercise performance. Albeit redox homeostasis is fundamentally related to aerobic type of exercise, anaerobic exercise has proven to be beneficial during aging. Regular exercise of low intensity and short duration, that targets endurance, strength, balance, and flexibility, would be effective in enhancing quality of life and aging longevity.

Keywords Aging · Exercise · Reactive oxygen species · Mitochondria · Antioxidants · PGC1a · Nrf2

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6.1 Introduction

Life expectancy and advancement in the healthcare system have been improving coordinately for the last 200 years, and the current research has been targeting a further improvement in quality of life particularly in the context of elderly mortality (Madreiter-Sokolowski et al. 2020). Although the advancement in health systems, including vaccination development can lead to a healthier lifestyle, the prevalence of age-related complications remains high. The misdiagnosis of health problems being age-related for the general population by the scientific community is among the factors that hinder the understanding of the aging process and diseases. Recent papers have recommended that external methods such as behavioral changes can influence delayed aging and diseases. Exercise is one of the main behavioral strategies that can delay aging and various age-related chronic diseases including cardiovascular diseases, obesity, diabetes and neurodegenerative diseases such as Alzheimer's or Parkinson's diseases (Ezzati et al. 2008; Booth et al. 2012; Jaul and Barron 2017). However, there is no specific exercise-linked mechanism that delays aging and age-related diseases that has been so far established. This demands us to focus on other mechanistic theories of how aging processes are regulated. The most studied theory so far in aging is the oxidative stress theory of aging first postulated by Denham Harman in the 1950s which is otherwise called the free radical theory of aging, an idea that tells that production of oxygen-free radicals during the metabolic process can regulate the aging phenomenon through inducing oxidative damage in the cellular structures. However, recent studies contradict this concept that within a certain level of ROS can regulate various signaling which leads to a delay in the aging processes and diseases. During oxidative metabolism, the superoxide radical anion (O_2^-) formation is the initial formation that further produces various ROS either by chemical reactions or by cellular enzymes resulting in redox shifts. For the human coping system to deal with oxidative stress, powerful antioxidants including superoxide dismutase (SOD), catalase (CAT), and glutathione system (GSH) can intoxicate ROS or make O_2^- to become less reactive ROS or inert products. These less reactive ROS such as hydrogen peroxide (H_2O_2) may function as a signal or mimic some important signaling including age-related signaling. For example, a common oxidative product of methionine named methionine sulfoxide increases during aging, and it can be restored by the enzyme called methionine sulfoxide reductases (MSR) A and B. The overexpression of MSR is linked with an increased stress resistance and extended lifespan, but the expression of these antioxidants during aging may be limited, which demands additional factors that could induce this enzyme or keep the oxidative stress at the physiological level. Physical exercise is one of the factors that can maintain cells under a physiological oxidative stress level.

Advanced technologies have generally increased a physically inactive lifestyle. Exercise is a required challenge for the elderly in order to keep up homeostasis which demands an integrated organ systems response, supporting the form, structure, and functions of the body. Exercise is a keyway to sustain physical activity at an optimum level. The general types of exercise are strengthening, stretching, balance, and aerobic

exercise. Being healthy necessitates all of these. However, aerobic exercise has a special emphasis on aging and the metabolism of free radicals. Aerobic exercise, i.e., endurance exercise, is any type of repetitive activity which provides work to the lungs, cardiac, and skeletal muscles. In other words, it requires a higher rate of oxygen consumption and mitochondrial activity, which facilitates higher ROS production, while exercises like strengthening can produce site-specific oxidative stress, which may not reflect on the entire organism resulting in ROS-mediated signaling failure. The failure in the implementation of exercise regimens for the general society of aging people leads to inactivity induced age-related diseases. Mismanaging exercise regimens such as timing, duration, intensity, and individual training status may additionally lead to inducing pathological changes in the cells instead of maintaining ROS homeostasis. This scenario allows any organism to become more prone to age-related disorders and apoptosis instead of delaying aging. Focusing on all these aspects not only provides better management of increasing muscle strengthening and gait training, but also provides protection against aging and age-related diseases.

6.2 Aging, Exercise, and Free Radicals

Accelerated free radical generation may occur as a response to acute and habitual exercise. Previous studies investigated that physical activity positively affects the quality of life via decreasing age-related physical inactivity, frailty, and supporting mental performance in aged rats but has no effect on extending their life span (Deepa et al. 2017; Cao et al. 2012). Also, it was demonstrated that exercise does have health benefits, due to the resulting optimal physiological rate of ROS formation.

In addition, excessive amounts of antioxidants supplementation have been widely preferred to reduce the detrimental effects of free radicals generated during exercise. However, scientific findings have revealed contrary results. It has been demonstrated that a megadose of vitamin C supplementation in training inhibits exercise adaptation, mitochondrial biogenesis, and the synthesis of antioxidant enzymes (Gomez-Cabrera et al. 2008). Later, Ristow et al. (2009) stated that antioxidant intake during exercise hindered exercise-related physical performance enhancement and overall health improvement.

6.3 Exercise-Induced Extramitochondrial ROS

Albeit mitochondria are generally accepted as the source of free radical formation in aging, recently scientists believe that this radical-centered aging theory has lost its importance. Aging is probably a multidimensional cause process. Mitochondria are also considered as the ROS formation center during exercise. Indeed, recent scientific findings suggest that there are other sources of free radical formation in exercise. Even the relationship between free radical metabolism and exercise during

aging still requires further studies, while the beneficial effects of exercise on all physiological systems is unquestionable. The most studied tissues related to ROS formation in exercise are the heart, lungs, white blood cells, and skeletal muscles (Gomez-Cabrera et al. 2009; Powers and Jackson 2008). Superoxide and nitric oxide (NO) are the radicals mainly formed in cells and both can potentially react to generate a series of other ROS formations and reactive nitrogen species (RNS). As mentioned above, there may be other sources for ROS formation outside of mitochondria in exercise, such as nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase (NOX) from transverse tubules (T-tubules), the sarcolemma, the sarcoplasmic reticulum, and neutrophils secondarily, via myeloperoxidase. As the family members of NOX are differentially expressed among tissues, NOX1 to NOX5 and DUOX1 and DUOX2 make up the NOX family while DUOX1 and DUOX2 are homologs of NOX2, which are composed of the NOX-like region at the C-terminal half, two EF-hands, a membrane-spanning region, and a peroxidase-like domain at the N terminus. Therefore, they are called dual-oxidase, DUOX (Katsuyama et al. 2012). NOX1, NOX3, and NOX5 form a superoxide, while DUOX1, DUOX2, and NOX4 seem to mainly produce H_2O_2 (Katsuyama 2010). NOX2, NOX4, and their partner proteins (p22phox, p67phox, p47phox, and p40phox) are expressed in skeletal muscle (Cheng et al. 2001) and are found in T-tubules, the sarcolemma, and the sarcoplasmic reticulum while NOX4 also exists in mitochondria (Sakellariou et al. 2013). The flavin adenine dinucleotide (FAD) and NADPH-binding sites and six transmembrane alpha-helices with cytosolic N- and C-termini exist in NOX isoforms (Bedard and Krause 2007) which seem to play significant roles in the skeletal muscle fibers' adaptation to exercise in response to physiological stimuli (Sakellariou et al. 2013). In contrast, the overactivity of NO synthase (NOS) isoforms in glycolytic muscle causes atrophy in experimental rats (Cunha et al. 2017). In this process, the NF-κB was activated and the p38 phosphorylation was increased while on the other hand, aerobic exercise training diminished p38 phosphorylation and NF-κB activation. The aforementioned aerobic exercise was of a long-term, low-intensity exercise in which the mitochondrial energy system was used more actively. This type of exercise might cause ROS formation and the activation of upper cascade molecules of PGC1a, which might lead to a PGC1a expression. The PGC1a might have activated the expression of antioxidant enzymes with the genes involved in mitochondrial biogenesis in oxidative muscle as the antioxidant enzymes' activities are very low in glycolytic muscle fibers. The antioxidants expressed in slow-twitch muscles by way of aerobic exercise detoxify over-formed ROS in the cellular environment since a superoxide is very labile and reduced to H_2O_2. This non-radical, weak oxidant is more stable and can diffuse across cell membranes. H_2O_2 also generates the hydroxyl radical (OH·) by the Fenton Reaction if there is free iron or transition metals in the cellular environment. Iron is freely present in skeletal muscle at a rate of 10–15% and is mostly found in mitochondria and myoglobin while OH·, which is highly reactive, is known to cause damage to lipids, proteins, and DNA. Nevertheless, H_2O_2 is considered as second messenger due to its feature of being more stable and more suitable for intercellular transfer.

The necessity of phospholipase A2's function has been shown in both tiring and repetitive muscle contractions (Gong et al. 2006). Phospholipase A2 enzyme enhances intercellular ROS formation while also causing the activation of NAD(P)H oxidase enzymes and is effective in separating arachidonic acids from membranes. Arachidonic acids are a substrate for ROS-forming enzyme systems like cyclooxygenase and lipoxygenase. There are two types of phospholipase A2 enzyme isoforms in skeletal muscles: calcium-dependent and calcium-independent. Both of these isoforms are active in the formation of ROS in muscle contraction while only calcium-independent isoform supports the formation of cytosolic ROS (Zhao et al. 2002), only calcium-dependent isoform plays a role in generating mitochondrial ROS in skeletal muscle (Zhao et al. 2002). Furthermore, calcium-independent phospholipase A2 is found in the myotubes of adult mice tissues lile, diaphragm, soleus, extensor digitorum longus, gastrocnemius, and heart. The expression and protein levels of phospholipase A2 muscular cells exhibit no difference in levels among rat muscles. Phospholipase A2 was reported to arrange cytosolic oxidant activity and contractile function in murine skeletal muscle cells while the blockage of phospholipase A2 caused reduced cytosolic oxidant activity in myotubes and intact soleus muscle fibers. As a result, a diminished soleus muscle function and depressed force production with a rightward shift in the force–frequency relationship was observed. The scientists also reported all these changes could be repeated by the depletion of superoxide anions (Gong et al. 2006). The findings of this research show that calcium-independent phospholipase A2 regulates the oxidant activity of skeletal muscle by supporting the formation of ROS, and thus, it might affect muscle contraction and fatigue during physical activity.

The xanthine oxidase (XO) is the most ROS productive enzymatic system among the other enzyme systems and exists in most tissues and organs as well as in the vascular endothelium. XO delivers electrons to molecular oxygen and forms $O_2 \cdot -$ by a one-electron reduction or H_2O_2 by a two-electron reduction. Then, hydroxyl radicals can be generated in the presence of iron or other transition metals. Vida et al. (2011) investigated the activity and expression of the XO in the liver, kidney, and thymus of different age groups of mice, including long-lived ones. A higher activity and expression of XO were determined in all the tissues of old mice. The inhibition of XO by allopurinol revealed that XO may have a special relevance in the formation of H_2O_2 in older animals. Furthermore, among the other ROS generating enzymes, XO had the highest activity and expression amongst all tissues, therefore XO might play an important role in the etiology of aging. Additionally, when Lee et al. (2009) reviewed ischemia–reperfusion and heart failure in association with XO, they reported the inhibition of XO diminishes the formation of ROS as well as uric acid production in ischemia and reperfusion injury. During exercise, hypoxia occurs at tissue level for short periods of time so, XO might be more effective during these times as XO was informed as an oxidant producer in high-intensity intermittent exercise (Gomez-Cabrera et al. 2006). This type of exercise fundamentally meets its energy requirement from anaerobic glycolysis. Low levels of ATP results in an accumulation of hypoxanthine and xanthine and conversion of xanthine dehydrogenase to XO. This process is a step for O_2 generation in the replenishment of oxygen to a relatively

hypoxic muscle (Kaminsky and Kosenko 2009). By inhibiting XO by allopurinol, a reduction in muscle oxidative stress after strenuous exercise in both rats and humans was observed (Gomez-Cabrera et al. 2010), though human skeletal muscle has low levels of xanthine dehydrogenase or oxidase (Hirschfield et al. 2000). These outcomes suggest that XO might generate ROS effectively in anaerobic exercise. Moreover, the decrease in lung capacity and the consumption of less oxygen during aging may be one of the reasons why this enzyme is more effective in ROS production in the elderly.

6.4 Exercise-Induced NO

Nitric oxide, which is a highly diffusible gas, is synthesized via NO synthase's enzymes in a number of cell types. There are three NOS isoforms: neuronal NOS (nNOS/NOS1); endothelial NOS (eNOS/NOS3); and inducible NOS (iNOS/NOS2). L-Arginine is converted into NO and citrulline via these NO synthases. NO reacts with oxygen to form nitric dioxide and reacts with superoxide to generate peroxynitrite which is a strong oxidizing agent. Albeit, NO is synthesized in various cells, it has an important role in the cardiovascular system, nervous system, and skeletal muscle contraction. NO diminishes leukocytes adhesion, aids vasodilation, and lessens thrombosis and apoptosis. Arterial hypertension, atherosclerosis, heart failure, and neurodegenerative diseases have endothelial dysfunction, which are related to a reduction in NO bioavailability in aging while eNOS expression and activity decrease in aged vascular system. Indeed, aged endothelial dysfunction is related to a higher expression of proinflammatory enzymes, i.e., cyclooxygenase-2 (COX-2) and iNOS. Augmented NO synthesis is based on iNOS, which reacts with ROS to produce RNS and deforms post-translational modification of proteins. All these result in a higher vasoconstriction tonus in the arteries of the elderly (Novella et al. 2013).

All three NOS enzymes are found in skeletal muscle. The eNOS and nNOS are Ca^{2+}-dependent isoforms, while iNOS is Ca^{2+}-independent. These three enzymes are normally responsible for NO synthesis at a lower physiological level. NO, like ROS, shows its toxic effects depending on its levels. When NO is expressed in high levels in the cellular environment, it shows its toxic effects. The nNOS isoform is expressed at a higher ratio in fast-twitch muscle fibers when compared with slow-twitch fibers (Reid 1998). The iNOS expression rate increases with respect to aging. In other words, iNOS's role in muscle contractile function decreases while increasing its inflammatory role (Song et al. 2009). It is informed that NO synthesis increases during muscle contraction, while muscle fibers form lower physiological levels of NO during inactivity (Pye et al. 2007). Moreover, NOS inhibitors lead to an increase of muscle force production, though NO providers cause a depression in muscle force generation during submaximal tetanic contractions (Andrade et al. 1998). Song et al. (2009) investigated the effects of endurance exercise on NOS enzymes in the soleus and white gastrocnemius muscles in aged rats. In aged, sedentary rats, the protein

level and enzyme activity of iNOS was found to be high in both fast- and slow-twitch muscles while the protein level of nNOS was found to be lower in each muscle, the protein level of eNOS was only found to be lower in the white gastrocnemius. In addition, after endurance exercise, an increase in the protein level of nNOS was detected in both muscles, while an increase in the protein level in the fast-twitch muscle was detected only in the eNOS enzyme. When the eNOS and nNOS enzyme activities were evaluated together after exercise, only in the white gastrocnemius muscle was an increase found. No significant change in the protein level and enzyme activity of iNOS were found in both muscles from endurance exercise (Song et al. 2009). This study was supported by another research. Chung et al. (2001) reported that a caloric restriction diminished age-related augmentations in the proinflammatory mediator cytokines, iNOS, and NF-kB in the kidney, heart, and brain. In summary, the findings show that there is an increased iNOS expression in the brain, skeletal muscle, and endothelial cells, though there is a decreased expression of nNOS and eNOS in these tissues. Unlike nNOS and eNOS, that are responsible for lower levels of NO synthesis, iNOS is responsible for a higher ratio of NO synthesis. In the study of Song et al. (2009), endurance exercise increased the expression of the nNOS and eNOS ratios in the fast-twitch muscle, while there was no change in the expression level of these enzymes in the slow-twitch muscle. These findings show that different mechanisms may be more effective in the slow-twitch muscle during aging.

6.5 Aging, Exercise, and Nrf2 Signaling

Redox shift, inflammation, and mitochondrial dysfunction alter the way of signaling pathways from the way they should behave in aging cells which finally contribute to degrading the molecular process and manifest age-related pathologies. Studies have reported that ROS can mediate to crosstalk these signaling pathways (Silva-Palacios et al. 2018; Yanar et al. 2020). However, how this is orchestrated during aging is not well established particularly at the transcriptional level of the antioxidants' expressions. The transcription factor nuclear erythroid-2-p45-related factor-2 (Nrf-2) is not only the master regulator of antioxidants, but it can also contribute to regulate several cytoprotective genes (approximately 250) including detoxifying enzymes and drug transporters (Silva-Palacios et al. 2018). Generally, Nrf-2 is coupled with the cysteine-rich Keap-1 protein which is associated with the Cullin-3 (Cul-3) and Ring-box protein. This scenario leads to Nrf2 ubiquitination and proteasomal degradation. ROS exposure induces the cysteine oxidation within the Keap-1 (Cys 151, Cys273, Cys288), which results in the release of Nrf2 and the further re-establishment of redox status. The activation of Nrf2 is not only dependent on ROS exposure, but also depends on aging. Nrf2 expression declines with aging and this decrease may be related to the decrease of Nrf-2 target genes such as quinone 1 (NQO1) and heme-oxygenase 1 (HO-1) (Done et al. 2016). Furthermore, an external stimulus can activate Nrf2 and extend its half-life. Exercise is a known external stimulus that induces various stresses on the skeletal system such as oxidative, mechanical,

and thermal stresses. Therefore, it is clear that exercise can induce a redox balance; however, how the exercise protocol can activate Nrf2-ARE pathway and what is the duration or intensity necessary to activate the Nrf2-ARE pathway remains unknown. Yet, studies have shown that Nrf2 activation depends on exercise duration (>60 min of exercise) (Li et al. 2015; Wang et al. 2016). However, the duration could be varied even more during the condition of aging as elderly individuals may respond poorly to this redox balance. Consequently, Nrf2 accumulation and its stability becomes lower with aging, suggesting that there is an important role to Nrf2 and biological aging (Yates et al. 2007). It was observed that acute exercise impaired the Nrf2 nuclear accumulation and its downstream antioxidant genes in the aging animals, but moderate 6-week exercise restores the Nrf2 accumulation and antioxidant genes (Gounder et al. 2012). However, studies have shown that exercise only influences the gene expression rate during aging, and does not impact the level of protein response such as SOD1 and HMOX to exercise (Done et al. 2016). This may be due to having a defect in the sampling collection which will delay in the detection of protein after the exercise stimulus, suggesting that the change in the findings of the protein synthesis need several hours or even days or weeks after exercise. Nonetheless, exercise's influence on mRNA expression within hours of exercise is assumed to be as a response to aging (Done et al. 2016). Therefore, focusing on exercise duration and the number of stimuli that influence Nrf2 expression and by following its target genes in the subjects will pave the way to developing exercise as non-pharmacological approach to preventing aging and diseases (Fig. 6.1). The Nrf-2 and further antioxidant activation via the redox balance during exercise is demonstrated in Fig. 6.1.

6.6 Exercise-Induced Mitochondrial ROS

The mitochondria control cell cycles, various aging processes, redox homeostasis, and apoptosis. Exercise is one of the non-pharmacological interventions for alleviating age-related impaired redox status. Oxidative metabolism in the mitochondria is the major source of ROS along with producing ATP through oxidative phosphorylation. Approximately 0.2–2% of molecular oxygen undergoes a one-electron reduction into superoxide radicals in the respiratory complexes I and III. This is further converted into singlet oxygen or H_2O_2.

An increase in mitochondrial ROS is linked with oxidative damage and a loss of mitochondrial membrane potential which further releases pro-apoptotic factors and induces apoptosis. ROS is also linked with telomere shortening and DNA damage. Biological aging is characterized by the increase in mitochondrial ROS, mutation in the mitochondrial genome, morphological abnormalities in the mitochondria, and ATP synthesis impairment. As mentioned above, exercise can be the rejuvenating method that can alter ROS-mediated intracellular signaling, decrease the energy ratio (NAD/NADH and AMP/ATP), and increase the release of several circulatory factors which all coordinately activate mitochondrial biogenesis pathways such as PGC-1a,

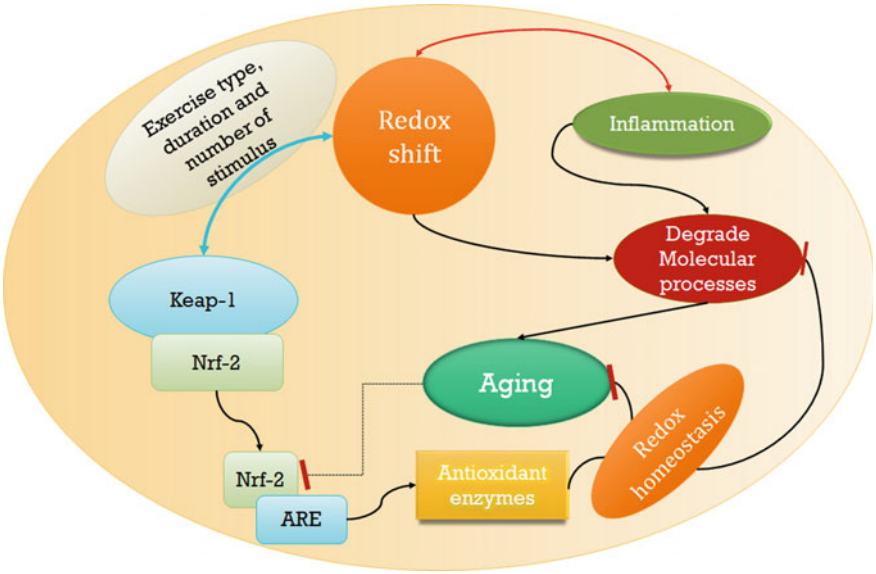

Fig. 6.1 Schematic illustration represents that an exercise-induced redox shift activates the Nrf-2 and a further antioxidant response, which results in a regulation of the molecular processes and delays aging and prevents diseases. Nrf2: nuclear erythroid-2-p45-related factor-2; ARE: antioxidant response element; Keap-1: Kelch-like ECH-associated protein 1

SIRT1, and AMPK and antioxidant defense genes including Nrf2-Keap-1. Further, exercise strengthens the immune system and contributes to the removal of damage which ultimately increases the functional capacity of the organs. Aerobic exercise is the gold-standard method to increase mtDNA replication, protein expression, enzymatic antioxidants, and ATP synthesis. For example, Viña et al. (2009) reported that aerobic training improves mitochondrial biogenesis (PGC-1a and Nrf1) and increases the respiratory complex capacity during aging. One of the important things to consider is that the reduction in mitochondrial content is associated with aging or the increased inactivity that comes with aging. Furthermore, whether exercise has an important role in maintaining mitochondrial content and function or whether exercise can aid in the recovery of mitochondrial content during aging is not known. Indeed, scientific findings show that aerobic or anaerobic exercise training is effective in preserving both mitochondrial content and function and has positive effects on the quality and quantity of mitochondria in all age groups. Indeed, prolonged moderate-intensity aerobic exercise at 50–70% of VO_2max for 12 weeks showed an increased mitochondrial quality, NADH oxidase, and succinate oxidase enzyme activity levels in the elderly (Menshikova et al. 2005). In a similar manner, regular anaerobic exercise, i.e., strength training, enhanced mitochondrial quality with potentially increased efficiency in mitochondrial complex I and complex II with respect to maximum electron transfer (Porter et al. 2014). Furthermore, progressive strength exercises at 50–75% of one-repetition maximum decrease mtDNA deletions in older

adults (Tarnopolsky 2009). Tarnopolosky also reported that strength training partially activated satellite cells, which fused with the myofiber and brought in undamaged wildtype mtDNA. These studies illustrate that both aerobic and anaerobic exercise models have positive effects on mitochondrial content and function. Both types of exercise in these studies are of moderate intensity: Aerobic exercise is at 50–70% of VO_2max while anaerobic exercise is at 50–75% of one-repetition maximum. It is expected that ROS formation rates in these exercise types might have been at a lower level. Radak et al. (2005) proposed to apply the hormesis theory to ROS, which appears to plateau during exercise. ROS at lower levels has a modulation effect on the cellular environment like as in signaling, receptor stimulation, and enzymatic stimulation, though an over-generated ratio of ROS damages macromolecules and may result in apoptosis or necrosis.

6.7 Aging, Exercise, and PGC1a Signaling

PGC-1a can be regulated by multiple signaling pathways during endurance exercise. Silent mating type information regulation 2 homolog 1 (SIRT1), AMP-activated protein kinase (AMPK), calcineurin A, Ca^{2+}/calmodulin-dependent protein kinases, p38 MAPK, NO, and ROS can activate PGC-1a (Erlich et al. 2016; Philp and Schenk 2013; Canto et al. 2009; Serrano et al. 2001; Puigserver et al. 2001). The activation of PGC-1a during exercise is also partially aided by β-adrenergic signaling (Miura et al. 2007), while the cAMP and cAMP response element-binding protein (CREB) seems to activate a PGC-1a expression (Herzig et al. 2001). The activation of AMPK is associated with diminished levels of ATP during exercise in muscles. AMPK either directly phosphorylates PGC-1α or activates it via promoting Sirt1 (Canto et al. 2009). Muscle contractions activate the Ca2/calmodulin-dependent serine/threonine protein phosphatase calcineurin A and Ca2/calmodulin-dependent protein kinases (Serrano et al. 2001). p38 MAPK phosphorylates and activates PGC-1a (Puigserver et al. 2001), which seems to be in the center of exercise-induced PGC1a regulation (Fig. 6.2). The regulation of PGC1a during endurance exercise is illustrated in Fig. 6.2.

Muscle contraction causes NO generation via both eNOS and nNOS. Both have been reported to be activated by Ca2/calmodulin and AMPK (Pattwell et al. 2004; Tatsumi et al. 2009; Close et al. 2005). However, PGC-1a activation through NO seems to be blunted during exercise. Exercise induces activity in all three NOS enzymes isoforms (endothelial, neural, and inducible isoforms) and the generation of NO. Boushel et al. (2012) reported that NO competes with oxygen in order to bind to a 3-heme-site of cytochrome oxidase at a lower physiological level and reversibly decreases the oxygen consumption of mitochondria. However, NO forms peroxynitrite at a higher level, which irreversibly inhibits complex I and II of the electron transport chain. On the other hand, the upregulation of the slow-twitch myosin heavy chain (MHC I) during overload exercise necessitates NO (Sellman

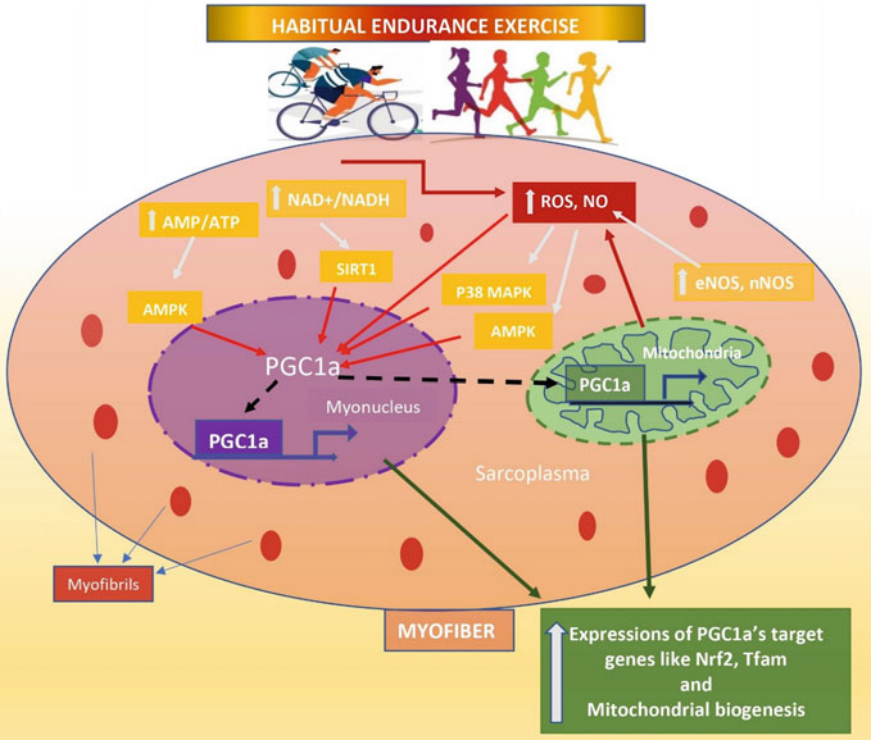

Fig. 6.2 Schematic illustration represents endurance exercise-induced signaling pathways, ROS and RNS activation of the PGC1a, and further gene expressions, which results in regulating mitochondrial biogenesis. ROS: reactive oxygen species; NO: nitric oxide; PGC1a: peroxisome proliferator-activated receptor-γ coactivator 1 alpha; AMPK: AMP-activated protein kinase; SIRT1: silent mating type information regulation 2 homolog 1; Nrf2: nuclear erythroid-2-p45-related factor-2; Tfam: mitochondrial transcription factor A; p38 MAPK: p38 mitogen-activated protein kinases; eNOS: endothelial nitric oxide synthase; nNOS: neural nitric oxide synthase; ATP: adenosine triphosphate; AMP: adenosine monophosphate; NAD: Nicotinamide adenine dinucleotide; NADH: Nicotinamide adenine dinucleotide phosphate

et al. 2006) by the activation of Akt and glycogen synthase kinase-3 (GSK-3) and CnA/NFAT-dependent signaling (Drenning et al. 2008).

Ca2/calmodulin and AMPK activate eNOS and nNOS in skeletal muscle (Tatsumi et al. 2009). In addition, it is reported that NO interacts with AMPK which cooperatively regulate PGC-1α in skeletal muscle cells (McConell et al. 2010). Endurance exercise induces a PGC-1a expression which cannot be hindered by either the deletion of eNOS (or nNOS) genes in mice or the inactivation of NOS enzymes in rats (Wadley and McConell 2007). These outcomes suggest that NO plays to some extent a regulatory role in PGC-1a, but there are some alternative ways to exercise-induce the upregulation of PGC-1a (Fig. 6.2).

6.8 Exercise and Antioxidant Defense

Free radicals are products of normal cellular function and the natural physiological process. They have both beneficial and toxic effects depending on their cellular levels in the metabolism. When free radicals are over-generated, i.e., when they exceed the antioxidant capacity, their harmful effects cannot be avoided. Antioxidants are basically classified into two groups as endogenous and exogenous. Exogenous antioxidants are nutritionally taken by one's diet and/or supplements. Endogenous antioxidants are synthesized by the body, and this group of antioxidants is further divided into two subgroups, enzymatic and nonenzymatic. The former group consists of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT). Nonenzymatic antioxidant group includes glutathione (GSH), protein thiol groups, thioredoxin (TRX), ubiquinone/CoQ10, uric acid, lipoic acid, bilirubin (Kayali et al. 2007, 2009). Some of the free radicals which are formed in the oxidative metabolism may escape from the control of the endogenous antioxidant system. Thus, it may cause oxidative damage to the surrounding mitotic and post-mitotic tissues, and ultimately initiate the molecular aging process (Cakatay et al. 2003). Thavanati et al. (2008) reported that muscular glutathione S-transferase (GST), SOD, and catalase activities were significantly reduced in elderly people. All of this information suggests that elderly individuals are more susceptible to oxidative damage due to their diminished muscular antioxidant defense capacity.

The effects of exercise on antioxidant mechanisms in the elderly are still obscure. Lambertucci et al. (2007a, b) reported that the activities of the CAT, GPX, and Cu,Zn- and Mn-superoxide dismutase (Cu,Zn-SOD) were not changed in the soleus of aged rats, whereas the activities of the Mn-SOD and XO were found to be decreased. Furthermore, the same groups of authors reported that the expression levels of CAT, GPX, and Cu,Zn-SOD were significantly elevated in the soleus of aged rats. There may be many reasons why the outcomes of Lambertucci et al.'s study were unexpected. Some of the possible reasons can be listed as the duration, intensity, and type of exercise, as well as differences in tissue and ages.

As mentioned earlier, exercise-induced ROS formation enhances the expression of PGC1a, which leads to the upregulation of endogenous ROS-scavenger enzymes (St-Pierre et al. 2003, 2006). Moreover, exercise-induced ROS also activates the nuclear factor kappa-B (NF-kB), which has a role in the regulation of antioxidant enzyme coding gene clusters (Lingappan 2018). Furthermore, NF-kB is believed to be an important regulator of muscular adaptation to exercise stress (Cuevas et al. 2005). NF-kB is also reported to modulate redox homeostasis via the upregulation of the expression of antioxidant enzymes (Jarosz et al. 2017). Indeed, elevated muscular antioxidant enzyme activity illustrates a higher tolerance to exercise-induced ROS formation (Pittaluga et al. 2006). An increased muscular antioxidant enzyme activity was reported in aged rodents which undertook a habitual endurance and strength exercise regime (Lambertucci et al. 2007a, b).

Previous studies indicate that the benefits of antioxidant supplements in exercise is controversial as the administration of antioxidants negatively affect the adaptation

process of skeletal muscle to endurance exercise (Morrison et al. 2015; Paulsen et al. 2014). On the other hand, antioxidant supplementation may interfere with the muscular redox-signaling pathways during endurance exercise (Gomez-Cabrera et al. 2015).

The administration of antioxidants may be beneficial for elderly individuals during exercise. It was reported that the administration of resveratrol ameliorates the aging-related insufficiency in physical performance in elderly individuals, who have regular exercise habits (Murase et al. 2009). Moreover, the intake of vitamin C enhances the physical performance capacity in the elderly (Saito et al. 2012). However, Nalbant et al. (2009) reported that the supplementation of vitamin E over a 6-month period has no extra beneficial effects on physical performance as well as aerobic exercise. In brief, both of the exercises' effects on the endogenous antioxidant system and the benefits of exogenous antioxidant supplementation during exercise by the elderly are thought-provoking. Hopefully, future studies will enlighten all these unresolved matters.

6.9 Conclusion

Aging is linked with several degenerative processes which are related with restricted mobility and a compromised quality of life. At the same, finding a target for pharmacotherapy is not fully achievable since aging corresponds to multifactorial targets including cellular and molecular mechanisms. Redox homeostasis management could be the core of either initiating aging and diseases or regulating age-related signaling. A slight increase in the level of ROS could facilitate the adaptation of cells to a better homeostatic status through activating various redox sensitive signaling mechanisms, by having an effective antioxidant defense system in the cell since the redox balance is significantly altered in the elderly as aging cells are prone to an impaired redox homeostasis and aberrant signaling. Exercise is one of the non-pharmacological approaches that induces an antioxidant enzyme expression and scavenger activity. It was proven that exercise could produce an over formation of ROS. The mismanagement of exercise protocols is linked with an unregulated ROS production and cellular damage. With advancements in techniques developed for the assessment of ROS, exercise-induced ROS sources (including its further pleiotropic effect on cellular signaling), metabolic functions, transcriptional factors, we now know that exercise-induced ROS aids in the cellular adaptations to exercise.

However, ROS has complex interactions with metabolome, which may be the core of several degenerative diseases and disorders. Also, the overall effects of exercise on ROS-aging cell interaction still remain a mystery. For example, we still do not know at what exercise-induced production rate of ROS would be beneficial to the overall redox status and how it could be maintained in aging cells. Exercise professionals need to design exercise regimens carefully with respect to the regulation of the redox status which occurs during aging as the regulation of exercise needs added attention for establishing an optimum redox status.

Compliance with Ethical Standards

Conflict of Interest: All authors declare they have no conflict of interest.

Ethical Approval: This article does not contain any studies performed by any of the authors with human participants or animals.

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Part II
Redox Signalling and Subcellular Ageing

Chapter 7
Redox Signalling, Autophagy and Ageing

Fatma Hussain, Umm-E-Ammara Warraich, and Amer Jamil

Abstract Reactive oxygen species (ROS) transform cell responses through miscellaneous processes. These act as signalling molecules when present in low concentrations or damage cell machinery when present in high levels. ROS-mediated mitochondrial damage leads to lesser oxidative phosphorylation and enhanced cell death. Aberrant cell death and redox signalling are implicated in numerous pathological conditions such as cardiac, neurodegenerative, and metabolic diseases. Cells have adopted an auto-degradation process as a cytoprotective strategy by inhibition of aberrant cell death and oxidation stress along with activation of autophagy. Autophagy, a pervasive degradation or turnover homeostatic mechanism of cell organelles and components, involves differentiation and ageing. Any dysfunction of autophagy can lead to anomalous mitochondrial function and ROS. Redox signalling cascade is related to the etiology of ageing, and the cellular machinery that regulates redox, autophagy and ageing has been elucidated. Redox signalling orchestrates autophagy and ageing processes leading to altered transduction of redox homeostasis. This chapter discusses ageing process, generation of ROS, redox signalling, autophagy types and mechanisms, the interplay between redox signalling, autophagy and ageing.

Keywords Reactive oxygen species · Autophagy · Redox signalling · Ageing

7.1 Ageing

Ageing can be defined as an intrinsic, universal process experienced by all living beings and depicted as the steady accretion of molecular and cellular damage (Alonso-Fernández and De la Fuente 2011; Carmona and Michan 2016). Ageing is a heterochronic and heterogeneous process. The heterochronic nature of ageing

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can be defined as the asynchrony by which different tissues and cells age within an organism (Alonso-Fernández and De la Fuente 2011; Peng et al. 2014). Several prominent deleterious changes may occur because of ageing including telomere shortening, DNA damage, oxidative stress, and inflammatory senescence-associated secretory phenotype (SASP) (Cheon et al. 2019). Hence, ageing is one of the perfect examples of homeostasis deterioration being related to impaired biological systems (Abbas et al. 2017). As a heterogeneous process, ageing may occur across diverse organisms at various rates; even among members of the same species, ageing occurs at variable rates (Alonso-Fernández and De la Fuente 2011; Carmona and Michan 2016; Urtamo et al. 2019).

7.1.1 Ageing Process

Growing old is a process called quasi-programmed i.e., a plan that is sustained and not once switched off. It is a developmental program with aimless continuation even after the completion of assigned tasks, as predicted by evolutionary theory. Ageing is not caused by damage, in fact, damage occurs due to ageing (Blagosklonny 2008). The damage driven by ageing results in the deterioration of an organism's body function, causing several diseases and gradually lessens the stress resistance (Davalli et al. 2016). The biogerontologists demonstrated that both environmental conditions and genetics contribute to the onset of ageing, which can be explained by the fact that 75% of individuals get older due to environmental factors including behavioural patterns, whereas 25% of individuals are accounted by genetics (Fernández-Ballesteros et al. 2013; Labat-Robert and Robert 2015). Therefore, other than genes, ageing is ascribed to socio-environmental conditions as well as behavioural and personal events (Theurey and Pizzo 2018).

7.1.2 Healthy Ageing

The principal challenge is to figure out the basis of healthy ageing. The study of desirable phenotypes of ageing that are healthy ageing and longevity has been mentioned as 'positive biology' (Farrelly 2012; Brooks-Wilson 2013). However, healthy ageing can be defined as warding off the decline of cellular and molecular processes for the longest period of the lifespan. Not surprisingly, increased longevity and healthy ageing have a strong association. The main difference between healthy ageing and longevity is that the former emphasizes health span and the latter is aimed at life span. Hence, both are closely related to each other because a person, who exceptionally has a long lifespan, tends to be healthy for much of his life (Fig. 7.1). This statement can be justified by the fact that certain factors such as stress resistance, protection against age-related diseases, and cellular homeostasis can be promoted by dietary, genetic, and/or pharmacological interventions which ultimately tend to enhance the

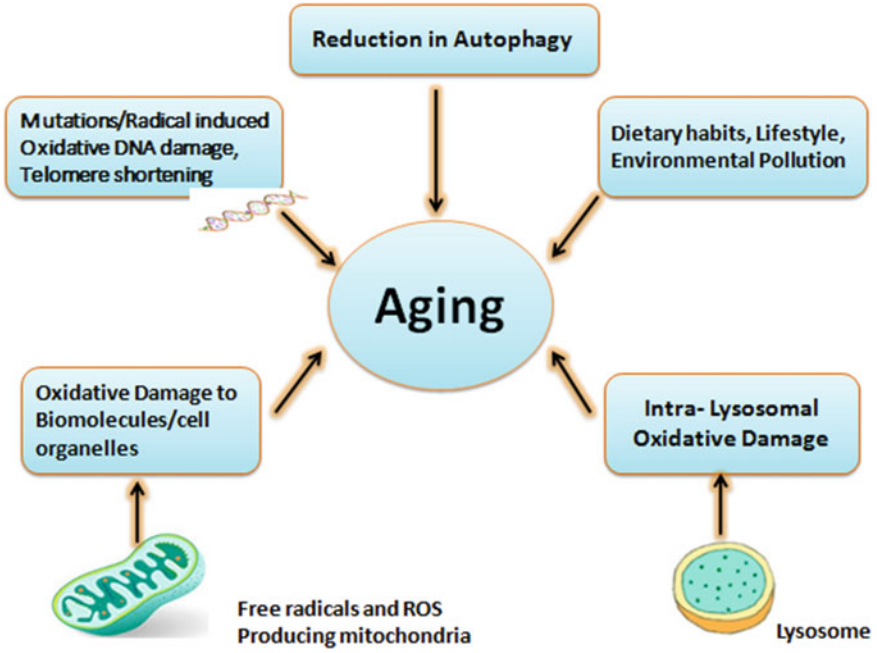

Fig. 7.1 Factors contributing to ageing. Increased ROS/RNS (reactive nitrogen species) generation damage protein, mitochondria and this damage propagate to neighboring organelles (lysosomes), cells and other macromolecules (DNA). Dietary habits, sedentary lifestyle and environmental factors along with declined autophagy further aggravate the ageing process

lifespan and vice versa (Carmona and Michan 2016). The extrinsic skin age process is called photo-ageing, and the skin being an outermost organ is affected not only by ultraviolet radiation but also by other environmental factors including tobacco smoking and air pollution (Gu et al. 2020).

7.1.3 Cellular Ageing

Cellular factors that influence ageing are strongly connected with progressively compromised autophagy (a process that causes degradation of cell debris) (Cheon et al. 2019). ROS along with genetic factors are the major cause of intrinsic ageing (Abbas et al. 2017). In diverse eukaryotic species, autophagy is associated with a vital supervisory position for ageing as it can eliminate impaired molecules and cell organelles (Wang and Xu 2020). The removal of defective mitochondria, a significantly selective pathway of autophagy, is known as mitophagy (Poljšak et al. 2012; Couve et al. 2013; Markaki et al. 2017).

Ageing symptoms can be mitigated by cellular antioxidant systems and restoration or induction of autophagy (Wong et al. 2020). Over the last 30 years, main anti-ageing cures; consumption of polyamine-rich antioxidant, autophagic inducers, and caloric control were found to be effective. Given the ever-increasing ageing population and human lifespan along with the occurrence of cardiovascular disease, it is mandatory to figure out the fundamental autophagy mechanisms. From an autophagic approach, two processes leading to ageing occur simultaneously i.e., mitophagy and augmentation in ROS levels (Ren and Zhang 2018; Barbosa et al. 2019).

7.2 Oxidative Stress and Ageing

How ageing is influenced by reactive oxygen species (ROS) is a mystery in biology (Ewald 2018). The free radicals or ROS are responsible for the development of ageing, as illustrated by theories on ageing that is from programmed cell death to 'natural inevitable cell damage'. The report for ageing research would carefully and accurately be described as "It is the free radicals and oxygen is poisonous when it is present as reactive specie". The remarkably common concept regarding ageing is the free radical concept of age established in earlier work (Gerschman 1954). Oxidative toxicity is instigated by free radicals as these radicals trigger the destruction of cell machinery and tissues. However, the production of free radicals generally arises through metabolic reactions. Thus, the concept inspired of Harman's theory that comprehensive elimination of the so-called detrimental compounds could lessen such impairments, subsequently, slow the ageing progression (Abbas et al. 2017; Pomatto and Davies 2018).

Oxidative damage can be defined as free radical's accumulation due to the overproduction of free radicals that cannot be processed progressively or due to fewer antioxidants bioavailability (Weidinger and Kozlov 2015; Simioni et al. 2018). The decline of antioxidant defending ability as compared to ROS leads to oxidation tension. The surplus ROS can primarily promote inflammation and increased concomitant cytokine synthesis, which can additionally activate the formation of ROS (Pisoschi and Pop 2015; Luo et al. 2020a, b).

Oxidative stress promotes the development of ageing as well as several long-lasting and deteriorating ailments such as inflammation, arthritis, cancer, autoimmune disorders, cardiac disease and neuropathies (Chandrasekaran et al. 2017). Oxidative stress disrupts bio-signalling, due to which different pathophysiological events could occur and successive modifications at different life phases, specifically in the older stage (Szentesi et al. 2019). When acceptable equilibrium among antioxidant-oxidant processes is maintained, then the modifying influence on ROS synthesis and deactivation occurs continuously in normal as well as pathological settings (Davalli et al. 2016).

Since ROS are responsible for molecular damage, certain mechanisms have been evolved by the organisms for the protection against abnormally increased ROS. Both low and acute ROS exposure can trigger protective mechanisms (Ewald 2018).

Recently a novel idea termed "hormesis" has been introduced, conferring that the cellular response for a more deleterious condition may be corrected by small dosages of a stressor. This could enhance cellular fitness and lifespan (Barbosa et al. 2019). This acute or low ROS exposure in model organisms can enhance lifespans such as in rodents, nematodes, flies, and yeast. Low or acute ROS levels can function as a secondary messenger at the physiological level to modify bio-signalling and play a significant role in an adjustment under oxidative stress (Ewald 2018). In this context, the beneficial effect of low levels of ROS is significant as it triggers the homeostatic responses; however, severe impairment or ageing could be due to its unequal accumulation. ROS performs several cellular activities such as inflammation, cell signal transduction, differentiation, cell survival, cell death, immune response, and gene transcription. Therefore, the balance between antioxidants (AOs) and oxidation species is important for a biological role such as adaptation, growth, and regulation (Warraich et al. 2020).

7.2.1 Synthesis of ROS

Reactive oxygen–nitrogen species (RONS) including potent nitrogen and oxygen species are normally generated as by-products during the cellular redox process. Such by-products may be non-radical as well as radical dynamic compounds (Pham-Huy et al. 2008; Powers et al. 2011). Numerous exogenous RONS sources are transition or heavy metals, drugs, radiation, alcohol, air pollution and tobacco. The endogenous RONS sources are lipoxygenase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, angiotensin II and myeloperoxidase (MPO). The main intracellular ROS source is enzymes (Liguori et al. 2018). ROS are produced within several cellular compartments. These compartments include mitochondria during oxidative phosphorylation, NADPH oxidase at the plasma membrane, cyclooxygenases, oxidase, cytoplasm, and lipid oxidation inside the peroxisomes (Campisi et al. 2019). Although all these sources contribute to producing an overall oxidative burden during ageing, maximum production of ROS occurs during oxidative phosphorylation. In addition to these sources, other major endogenous oxidant sources are monoamine oxidase, nitric oxide synthase, cytochrome p450, mitochondrial respiratory chain (RC), several oxidoreductases such as xanthine oxidase and enzymes that activate xenobiotic such as NADPH oxidases by causing inflammatory and infection responses (Warraich et al. 2020).

Oxidant generation from all these sources can vary with pathophysiological conditions and increases with age. However, contact with endogenous sources of oxidants is much more extensive as the internal oxidants are produced continuously during the lifetime (Warraich et al. 2020). The radicals and non-radical compounds that can trigger any deleterious response due to free oxygen are identified as ROS and comprise of superoxide anion (O^{-}), H_2O_2 (hydrogen peroxide), hydroperoxyl (HO_2), hydroxidochlorine (HClO), dioxygen (O=O) alkoxyl radical (R–O·), and hydroxyl radical (HO·). ROS include derivatives of oxygen and are either non-radical or radical

oxidizing agents and/or are easily convertible to radicals (Lambert and Brand 2009; Cui et al. 2012; Genova and Lenaz 2015).

The sequential oxidation reduction reactions of mitochondria generate ATP along with free radicals. Other sources of free radicals are vigorous physical activity and infectious conditions that involve activation of phagocytes (Pham-Huy et al. 2008; Pisoschi and Pop 2015). Free radicals in bio-systems were observed in the 1950s and since then it was believed that these radicals contribute largely to diverse pathological processes and ageing (Lushchak 2015). The interaction of oxygen with certain molecules produces radicals with an outmost shell having unpaired electrons such as O_2 i.e., diatomic oxygen. These potent radicals in a cell are generated by the removal or gain of a single electron and behave as reductants or oxidants (Bailey 2019). The same spin quantum number is carried by electrons when all electrons are not located in the same π^* anti-bonding orbital. This parallel spin is responsible for their less reactivity with non-radical species. Conversion of O_2 (molecular oxygen) into extra active singlet oxygen O_2 (dioxygen) is likely to happen by the supply of energy to reverse the rotation of one electron. Therefore, both electrons either occupy the same π^* orbital by pairing or they may remain in two discrete orbitals. The spin constraint can be controlled by the addition of an electron to oxygen. Non-radicals are synthesized during stress conditions in the body and the normal aerobic metabolism such as H_2O_2 (Genova and Lenaz 2015; Liguori et al. 2018). Numerous forms of ROS possess distinctive characteristics. hiROS (highly reactive oxygen species) damage biomolecules while loROS (less reactive oxygen species) impair cell signalling mechanism (Scialò et al. 2017). The term "ROS physioma" is used for the hiROS family and includes superoxide anion, hydroxyl radical and hydrogen peroxide. Superoxide radicals participate in the generation of other ROS such as H_2O_2 and HO· (Zarkovic 2020).

The manifestation of cellular degenerative characteristics of Friedreich's ataxia (FRDA) is due to mitochondrial ROS generation and is enhanced by the deficient mitochondrial frataxin. The increased level of ROS in FRDA cells is due to the defect produced in complex IV by defective synthesis of heme and the damaged cytochrome c as well as heme in complex IV and III. Thus, heme defect in FRDA results in limited oxidase and cytochrome c action as well as increased mitochondrial ROS, all take place due to defective iron-sulphur centres. Consequently, shortage of functional heme, the presence of defective heme and eventually increased ROS synthesis happens (Napoli et al. 2006).

Several diseases have a strong association with ageing, which are progressed due to the stimulation of a protein pore mPTP (mitochondrial permeability transition pore) on the internal membrane where the development of the voltage-gated channel occurs, stimulated by calcium overloaded mitochondria and ROS. This initiates the production of more ROS by the complete opening of mPTP, and it may also cause matrix DNA to be released into inter-membrane space for the hydrolytic reaction. This results in cell DNA depletion thus contributing to the activation of the ageing (Rottenberg and Hoek 2017). It is assumed that the opening of mPTP is increased by ageing and vice versa. ROS also initiates the stimulation of mPTP. The activated

mPTP causes damage to mitochondria during oxidative reactions, hence further worsening the clinical features of FRDA. Many deleterious properties of dysfunctional mitochondria and ROS towards ageing and longevity are interceded by activated mPTP (Panel et al. 2018).

7.3 Ageing and Mitochondria

In human mtDNA (mitochondrial DNA), 16,659 base pairs are present. Mammalian cells contain several copies of mitochondrial DNA. Human mtDNA codes for rRNA, tRNA, and thirteen different proteins which are crucial for the functional and structural stability of mitochondria (Dröse and Brandt 2012). Frequent studies indicate that the mutations in mitochondrial DNA may affect the production of ATP. Neurodegenerative diseases and early phases of ageing are associated with disturbance in the integrity of mitochondrial DNA. In mitochondria, various metabolic pathways including TCA (tri-carboxylic acid) cycle, beta-oxidation of fatty acids, amino acids oxidation and one-carbon cycle takes place. Mitochondria are responsible for generating 90% of the cell's energy (Warraich et al. 2020).

Ageing at the molecular level is caused by a lifelong accumulation of numerous damages, many of which have yet to be completely elucidated (Table 7.1). Functional abnormalities in mitochondria are considered indicators of ageing (Bolduc et al. 2019). Over time, the mitochondrial DNA destruction and production of ROS occurs which ultimately leads to the cell's inefficiency to recognize the vital function of mitochondria. This information contributed to the formation of MFRTA (mitochondrial free radical theory of ageing) theory although it is controversial to some extent (Son and Lee 2019). Many researchers have updated, extended, and questioned

Table 7.1 Ageing at molecular level

DNA
Somatic and mtDNA mutations
Shortening of telomere ends
Genetic interference (viral or transposon agents)
Epigenetic factors
RNA
Aberrant transcription process
Errors in post-transcriptional modifications
Proteins
Misfolding of specific 3-D structure
Aberrant translation process
Errors in post-translational modifications
Aberrant protein catabolism (turnover)
Membranes
Oxidative damage
Less fluidity
Deficit of integrity

it. Nonetheless, two basic statements were not revised. First; the antioxidant/oxidant imbalance ensues with ageing, which causes the accumulation of damaged macromolecules. Second, oxidative damage causes the degenerative phenotype of ageing. A few studies questioned the second statement, but the first statement is well known that in the elderly, damaged macromolecules accumulate due to the antioxidant/oxidant imbalance (Luo et al. 2020a, b).

MFRTA theory (Lara et al. 2018) is currently termed as OST (oxidative stress theory). Several studies indicate that during ageing, alteration in mitochondria and mtDNA, accelerated structural disintegration, declined phosphorylation during aerobic metabolism, improved synthesis of ROS, and harmful impact on nucleic acid, fats, and protein occur (Haas 2019). An estimated mitochondrial mutation rate is ten times greater than nuclear DNA. The mtDNA has lesser repairing ability and deficiency of histones which influence cancer and ageing (Kammeyer and Luiten 2015).

As concluded from the above review of literature, the mammalian mitochondria are the central places for ROS production and thus the mtDNA is more vulnerable to harm due to their existence adjacent to the ROS production site. It has been demonstrated that mitochondrial DNA contains a large amount of 8-hydroxy-20-deoxyguanosine (a by-product of oxidation) than DNA present in the nucleus (Cui et al. 2012). The decreased mitochondrial function correlates with the emergence of several ailments. However, the general idea is that ROS causes the accumulation of damaged macromolecules which leads to ageing. The number of impaired mitochondria is increased during ageing, and it produces less ATP and more ROS. In cells, the harmful effect due to DNA damage occurs by blocking replication, breaks in DNA strands, transcription, and rearrangement of chromosomes (Warraich et al. 2020).

With the increase in age, the progressive oxidative reactions induce the phenomenon of strand breaks in DNA and mutations in somatic mitochondrial DNA. The RC complex damage occurs by the mutations in mitochondrial DNA which results in a cycle having more alternations in mtDNA and increased synthesis of ROS (Bonomini et al. 2015). Mitochondrial damage and energy crisis due to ROS and oxidation stress cause neurodegenerative disorders and trigger ageing. Destruction of mtDNA is linked with ageing, but it is not clear that which one is directly affecting vascular ageing; mitochondrial DNA damage or mitochondrial dysfunction (Foote et al. 2018; Stefanatos and Sanz 2018).

7.3.1 ROS Production in Mitochondria

Chance and colleagues documented the ROS production in the respiratory chain for the first time in 1966 (Chance et al. 1979). Within the chain, prominent sources of ROS production are the first and third complexes when a direct reaction between electrons derived from NADH, ubiquinone and oxygen or other electron acceptors occurs, resulting in the generation of free radicals. Thus, during the respiration process, ROS are the normal side products but their reaction with fats, proteins and nucleic

acids deteriorates these molecules (Marchi et al. 2012). The isolated mitochondria generate a significant proportion of ROS by two approaches, principally through (i) first complex in a certain condition such as reduced coenzyme Q and high proton motive force (Fp) as there is no ATP synthesis; (ii) when a higher ratio of reduced and oxidized NAD present in the mitochondrial matrix. When active production of ATP occurs by mitochondria, less amount of O_2 (singlet oxygen) is produced, consequently, lower NADH/NAD+ and Fp ratio are present. ROS generation in isolated mitochondria takes place at eleven sites, although only three sites are more relevant for in vivo ROS production: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), and complex III (cytochrome *bc*1 complex). However, the recent investigation indicated that at complex II, the substantial formation of O_2 (singlet oxygen) occurs (Eleutherio et al. 2018).

According to Brand (2016), hydrogen-free radicals are formed in mammalian mitochondria at eleven different sites. Each site is closely related to substrate catabolism and RC and has its unique properties. Sequential action of electrons transport through mitochondrial chain complexes produces a difference of chemical concentration and electric charges. Eventually, proton transport takes place from the matrix to the inter-membrane part. During this transmission, the direct electron transfer to oxygen chiefly at I, II, and III complexes results in the production of superoxide (one electron transport) or hydrogen peroxide (two electrons transport) (Eleutherio et al. 2018).

Surprisingly, ROS generation does not occur at complex IV (cytochrome c oxidase), although it causes reduction of oxygen to water, elaborating that ROS production and electron leakage in RC can be inhibited. ROS generation in RC complexes is significant in the regulation of different biochemical processes such as cellular differentiation, and deterioration in ischemia/reoxygenation injury (Scialò et al. 2017), whereas the ROS also mediates the progression of some cancer types. The ROS formed from the third complex are shared between the mitochondrial matrix and IMS. While ROS generated by complex 1 are transferred to the matrix only, thereby illustrating the adoption of different distribution mechanisms for both the complexes (Reczek and Chandel 2015; Stefanatos and Sanz 2018).

When the electron transfer takes place at complex I (from NADH to CoQ), the generation of ROS may occur at two places, IQ (CoQ attachment position) and IF (FMN position) within the matrix. ROS generation in complex II takes place on IIF position, having an association with succinate dehydrogenase. The small amount of ROS production by complex III is negligible in comparison to ROS produced from CI. The ROS formed by CIII is transported into IMS and matrix. Within IMS and matrix, ROS are converted into H_2O_2, reactions catalyzed by enzymes (superoxide dismutase) SOD2 (matrix) and SOD1 (IMS). This H_2O_2 has a significant function in physiological mechanisms. Superoxide presumably functions at the site of its synthesis is short-lived and has less membrane-penetrability in comparison to the H_2O_2 that is uncharged, extra stable and can pass through aquaporin channels, thus a better multipurpose signalling particle. Though analogous to superoxide molecules, substantial destruction of molecules by H_2O_2 takes place in the presence of free Fe^{2+}.

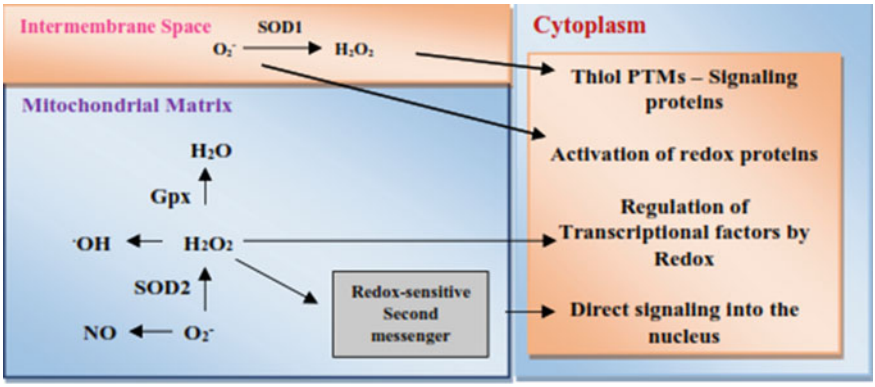

Fig. 7.2 Mitochondrial redox mediated signaling. ROS in mitochondria is produced from the escape of electrons to form superoxide (O_2^-) in complex I and complex III of the electron transport chain. O_2^- is converted to H_2O_2 by SOD1 and SOD2 in intermembrane space and matrix. H_2O_2 is reduced to water by Gpx. Both O_2^- and H_2O_2 are mtROS that act as signaling molecules triggering metabolic processes in the cytoplasm and nucleus. SOD: Superoxide dismutase, Gpx: Glutathione peroxidase, NO: Nitric oxide, PTM: Post-transcriptional modifications

The presence of this cation results in the production of highly active OH-radicals as shown in Fig. 7.2 (Warraich et al. 2020).

The generation of a progenitor ROS i.e., superoxide anion occurs at almost nine mitochondrial sites (Andreyev et al. 2005). Moreover, NADPH oxidases also produce superoxide, which catalyses the controlled formation of O_2 (singlet oxygen) by coupling the electrons derived from NADPH to oxygen. The topological significance of sites generating ROS and variation among various ROS peculiarly superoxide and hydrogen peroxide should be appreciated. For example, superoxide in matrix generating oxidation–reduction bio-signalling at IQ site may vary from the superoxide at complex IIIQo that sends such bio-signalling into the cytosol. Likewise, hydrogen peroxide producing redox signalling from both these will be different (Warraich et al. 2020).

A momentous correlation between ROS production rate, RC complexes action, and mitochondrial membrane potential ($\Delta\Psi m$) exists. If increased production of ROS occurs by the dissipation of mitochondrial membrane potential and respiration is inhibited, then the rate of free radical formation can be reduced by uncoupling which activates the drop in $\Delta\Psi m$ (Angelova and Abramov 2018). Even though in RC a large amount of ROS generation occurs under resting situation, O2 generation also takes place by the proteins in the matrix including enzymes of the citric acid cycle (pyruvate, alpha-ketoglutarate dehydrogenase, aconitase) and by different complexes. Studies have confirmed that the function of RC is disturbed during ageing and the reason might be the weakened antioxidant defence and increased accumulation of ROS from impaired metabolic process. Though, mitohormesis induction at a young age to avoid age-associated illnesses and management of RC activity in

old age for enhancement of lifespan using therapeutics can be a bright scenario to continue (Bouska et al. 2019).

7.4 Redox Signalling

The molecular basis of the ageing process in humans is a complicated unanswered question, although numerous studies support the concept of the altered mitochondrial function being the major regulatory point during ageing (Jang et al. 2018). Throughout the history of scientific research, ROS and redox reactions have been declared potent and harmful agents. However, recently it has been suggested that ROS are involved in the regulation of bio-signalling. Explicit properties of ROS are modified mostly by covalently modified cysteine in specific redox-sensing target proteins. Oxidized cysteine residues then lead to alterable enzymatic modifications. Thus, ROS regulate an array of biological phenomenon such as the generation of inflammatory and growth factor responses. Any disturbance in redox signalling can lead to pathological manifestations (Finkel 2011). Redox biology has emerged from pathological findings and deals with the role of oxidative species in signalling pathways. The earliest studies from the 1930s by McCord and Fridovich on the discovery of superoxide dismutase also described the association between signalling and redox reactions. Initially, most of the researchers focused on damage of biomolecules by free radicals but later on, it was realized that peroxides such as H_2O_2 and lipid oxidation electrophiles are helpers in signal transduction and regulators of some transcription factors (Fig. 7.2). The new field of redox signalling emerged from the combination of signal transduction and redox biology. It is well established that redox signals are changed in ageing, but these signals are important in maintaining normal homeostasis (Forman 2016).

7.4.1 Dual Role of ROS

ROS are toxic products of aerobic metabolism that play a dual role. These are required for many signalling reactions such as programmed cell death. It is plausible to believe that ROS have benefits in bio-system viability and maintenance of specific intracellular ROS level is essential to sustain life (Mittler 2017). ROS perform an important function in the production of blood cells, differentiation and the equilibrium between dormancy and production of relevant stem cells. Suitable ROS levels can be an ideal therapeutic target for the treatment of blood cancers (Samimi et al. 2020).

The most interesting biological paradox in recent scientific history is the fact that ROS are toxic yet signal supporting molecules. Redox mechanisms based on the scavenging chemistry of oxidants have been evolved that facilitate the wellbeing of the living organism during the ageing process (D'Autréaux and Toledano 2007).

7.4.2 Redox Signalling

Signalling systems in living organisms are dependent upon chemicals with free energy, redox atmosphere and ion channels across the membranes. Thiol-sulfhydryl redox systems are controlled in dynamic yet non-equilibrium settings, are constantly oxidized and differ in redox potential within cellular organelles or intracellular compartments. Redox signalling uses "sulphur switch" which means cysteine amino acids that undergo reversible oxidation reactions, covalent incorporation of nitric oxide, reversible binding of glutathione, and the addition of acyl or sulfhydryl groups (Paulsen and Carroll 2013). These redox signalling systems control delivery, biological affinity, movement and sensitivity of signalling proteins, thereby influencing the rate and activities of redox systems. Thio-sulfhydryl reservoirs are oxidized with increasing age, lifestyle, environmental pollution and diseases such as diabetes, obesity and neurodegenerative diseases (Jones 2010).

Foyer and Noctor (2016) stated that considering the limited data available on ROS mediated signalling, some knowledge can be extracted about the stress-triggered signalling process. ROS transmit information in response to internal or external stimuli. It is speculated that cells can rely on ROS to monitor metabolic flux and ROS may become the symbol of sustainability in the context of ever-changing demanding situations. Previously, it was stated by Kamata and Hirata (1999) that biochemical signalling involves the binding of growth factor to receptors to activate tyrosine kinase that stimulates MAP (mitogen-activated protein), PLC-gamma (phospholipase C-gamma) and PI3K (phosphatidylinositol-3 kinase). The message is conveyed to the nucleus for gene expression after activation of many transcription factors. ROS such as H_2O_2 activates tyrosine kinases, triggering a cascade of molecules like MAP kinases and PLC-gamma (Zhang et al. 2016). This alters calcium levels, leading to stimulate or block many transcription factors. Redox molecules either stimulate or suppress some transcriptional factors, applying to double-check regulation of cell signalling. ROS also acts as a secondary messenger in response to the extracellular stimulus by crosstalk between signalling systems and redox systems. Cell death also has redox regulation by oxidation and reduction reactions. Some death signals generate ROS and which in turn activates death machinery. It is confirmed that a cell's destiny is ultimately decided by crosstalk between bio-signalling pathways and redox species (Redza-Dutordoir and Averill-Bates 2016).

ROS can affect cellular proliferation, apoptosis, necrosis, gene expression and signalling processes especially involving mitogen-activating protein kinases cascades (Sies and Jones 2020). Within the human redox pool, damage caused by ROS is mended and key players of redox atmosphere are enzymes and antioxidants that establish a reduced environment by continuous addition of energy (Genestra 2007). The ability of living matter to extract energy from the environment, transform it to another form and use it for growth and reproduction is the basic attribute of life. During aerobic metabolism in mitochondria, oxidative phosphorylation generates ROS as a by-product that can damage cell machinery, though recently these have

been recognized as signal molecules. ROS-controlled bio-signalling processes play important role in homeostasis (Shadel and Horvath 2015).

7.5 Autophagy

Participation of the lysosomal system to degrade intracellular organelles and macromolecules through a process named autophagy and of extracellular components and membrane through a process known as heterophagy or endocytosis is well-known for more than half a century (Kaushik and Cuervo 2018). The autophagy term is derivative of a Greek word with the meaning "to eat itself". Autophagy is a catabolic and well-preserved process that has evolved in all eukaryotic cells during evolution and is a method for transportation of extra and intracellular components from cells to lysosomes, where their degradation and subsequent recycling take place. This may occur because of natural or stress response. The autophagy process was first observed in 1962 by T. Ashford and K. Porter in the cells of the rat liver after receiving glucagon. The name was given in 1963 soon after its discovery by a Belgian biochemist Christian de Duve (Fîlfan et al. 2017; Arensman and Eng 2018; Barbosa et al. 2019). Subsequently, recognition of genes linked to autophagy and autophagy morphology in yeast cells was described in the 1990s by Japanese researcher Yoshinori Ohsumi (Wang and Xu 2020).

Autophagy allows cell survival by inducing mobilization of endogenous macromolecules during periods of nutrient deprivation. It plays a paramount role in a stressful environment by maintaining the homeostasis in cells and metabolic stability during routine degradation, synthesis, and successive replacement of cytoplasmic components (Tan et al. 2014; Arensman and Eng 2018).

7.5.1 Lysosomal System in Autophagy

The contribution of autophagy, in almost all cases, in diverse physiological functions is attributed to its two major functions: as a procedure to eliminate undesirable cell components or as a source of energy. The lysosomal system has the unique ability to degrade and sequester the complete cellular portion, and this ability confers a paramount involvement of autophagy in certain circumstances where widespread cellular transformation is required for example embryogenesis, cell differentiation as well as degradation that occurs during some types of apoptosis (Buratta et al. 2020).

Lysosome, cell's recycling centre is fully devoted to the degradation of various macromolecules both from the intracellular and from the extracellular environment. The lysosomal membrane contains permeases for recycling vital building blocks that are produced from the degraded products (e.g. fatty acids, sugars, cholesterol, and amino acids, etc.) in the cytosol. The lumen of the lysosomes also contains about 40

Table 7.2 Types of Autophagy gene (ATG) proteins

Categories	ATG proteins
1	Type III phosphatidylinositol 3-kinase (PI3K-III), which has Beclin1 (Bcl2 interacting protein), Vps15, Vps34 (type III PI3K), ATG14L (ATG14-like)
2	Unc-51-like kinase 1 (ULK1) complex, that has ULK1, IFP200, ATG13, ATG101
3	ATG9
4	ATG5-ATG10-ATG12 system
5	ATG- WIPI
6	Ubiquitin-like binding protein LC3-II

types of hydrolytic enzymes such as glycosidase, lipases, proteases, phosphatases and nucleases. The degradation of soluble individual proteins, cell organelles as well as particulate structures occurs through lysosomes. This characteristic of the lysosomal system makes it specifically relevant under circumstances when the formation of irreversible aggregates and oligomers starts from damaged proteins (Cuervo 2008; Hubbard et al. 2012). Moreover, the degradation of cytosolic components by lysosomes occurs non-specifically; however, it may also be distinguished between the targets to be degraded with the participation of a degradation label like a chaperone and a complicated process to create a way for the targeted proteins to cross the membrane of lysosomes through a specific translocation complex (Yang et al. 2019).

Autophagy also plays a role in attained (acquired) and inborn (inherited) immunity through digestion, sampling, and by presenting the peptides from both their cellular milieu as well as from the invasive pathogens that get an entry into the cells (Cuervo 2008). Generally, autophagy can target either the degradation of selective cargo or the bulk. However, many studies have elaborated that the process of autophagy specifically targets its cargo for degradation. Examples are the specifically chosen degradation of mitochondria (mitophagy), protein masses (aggrephagy), ribosomes (ribophagy), and lysosomes (lysophagy) (Barbosa et al. 2019; Wong et al. 2020). Several inducers start the process of autophagy such as various stressors, while one of the major stimuli is a nutrient restriction which can rapidly activate the autophagy process along with protein synthesis inhibition (Boya et al. 2013). Several genes linked to autophagy (Atg) that encode proteins involved in autophagy have been reported (Shibutani et al., 2015; Luo et al. 2020a, b). The complexes of ATG proteins primarily have several categories (Table 7.2).

7.5.2 Autophagy Mechanism

There are three steps of autophagy: initiation, formation, and degradation.

Start-up stage: Firstly, the autophagy regulation is mediated by environmental signals including the level of nutrients and exogenous stress agents such as heat

or hypoxia over a multifaceted system of signalling pathways and proteins. The serine-threonine protein kinases are the key proteins in the initiation phase and are characterized by the mammalian target of rapamycin (mTOR). Two mechanisms proceed in different modes for the modulation of autophagy i.e., mechanistic target of rapamycin (mTOR) and nutrient-sensing pathways that include adenosine monophosphate-activated kinase (AMPK). Activation of AMPK induced autophagy during nutrient-depleted conditions drives a high AMP/ATP ratio. Consequently, phosphorylation and stimulation of ULK1, a serine/threonine-protein kinase, occurs that is an activator of autophagy. Conversely, an elevated level of amino acids is detected by mTORC1 a nutrient-sensing complex at the lysosomal membrane containing mTOR, endorses biomass production and cell proliferation actively and suppresses the autophagy process by repressing ULK1 (Gu et al. 2020; Wong et al. 2020).

Formation stage: The next stage requires the participation of the ATG proteins group to control the double-membrane vesicle generation for ingesting the cytoplasmic material (Madeo et al. 2015). The ingestion occurs by the formation of phagophore (Gu et al. 2020). The membrane used for the formation of phagophore originates from various locations including endosomes, mitochondria, endoplasmic reticulum, golgi complex, and plasma membrane (Lapierre et al. 2015). Moreover, certain parts of the plasma membrane having marked ATG16L1 may be delivered to the core of autophagic machinery by recycling endosomes (Madeo et al. 2015). Phagophore causes encapsulation of misfolded proteins or dysfunctional organelles, followed by the formation of autophagosome by extension and edge gradual fusion of phagophore. Beclin-1/Atg6 and Vps34 (vesicular protein sorting 34) are two distinct class III phosphatidylinositol 3-kinase (PI3-kinase) complexes in mammalian systems, involved in the phagophore extension (Itakura et al. 2008). Inhibition of phosphatidylinositol 3-kinases (PI3K) by 3-Methyladenine (3-MA) causes inhibition of autophagy due to blockage of autophagosome formation (Gu et al. 2020; Wang and Xu 2020). A previous study elaborated that the elongation of the phagophore includes ATG6 (Beclin-1), through contact with various binding partners; it likewise comprises a setting for incorporation of autophagy and programmed cell death processes. Many ATG16L1-interacting ATG proteins, including ATG12 and ATG5, participate in the stabilization of incipient phagophores (Madeo et al. 2015).

Degradation stage: Finally, at the third and last step of autophagy, phagophores enclosing the degraded cellular material form vesicular autophagosomes. Autophagosomes at that point merge with lysosomes forming autolysosomes for digestion through the transmission of microtubules cytoskeletal network system. The endomembrane of the autophagosome and components encapsulated by it are degraded by hydrolase present in the autolysosome. The permeases then release the degraded products into the cytoplasm and are reused (Madeo et al. 2015; Gu et al. 2020). The autolysosome formation should be precisely processed as incomplete autolysosome processing can instead result in the production of the residual body having indigestible material. The accumulated unprocessed autophagic vacuoles cause various age-related diseases including neurodegenerative disorders.

This suggests that in aged individuals the cell's potential to effectively manage and accomplish the autophagy is progressively diminished. SNARE (soluble NSF [N-ethyl-maleimide-sensitive Rab7, Dynein, and HSPB1 (heat shock 27 kDa protein 1) are involved in these processes (Lapierre et al. 2015; Wang and Xu. 2020).

7.5.3 *Types of Autophagy*

Three principal types of autophagy (Fig. 7.3) can be differentiated based on the procedure through which cytoplasmic components are carried to the lysosomes and the volume of sequestered substrates (Morgunova et al. 2016). Macroautophagy

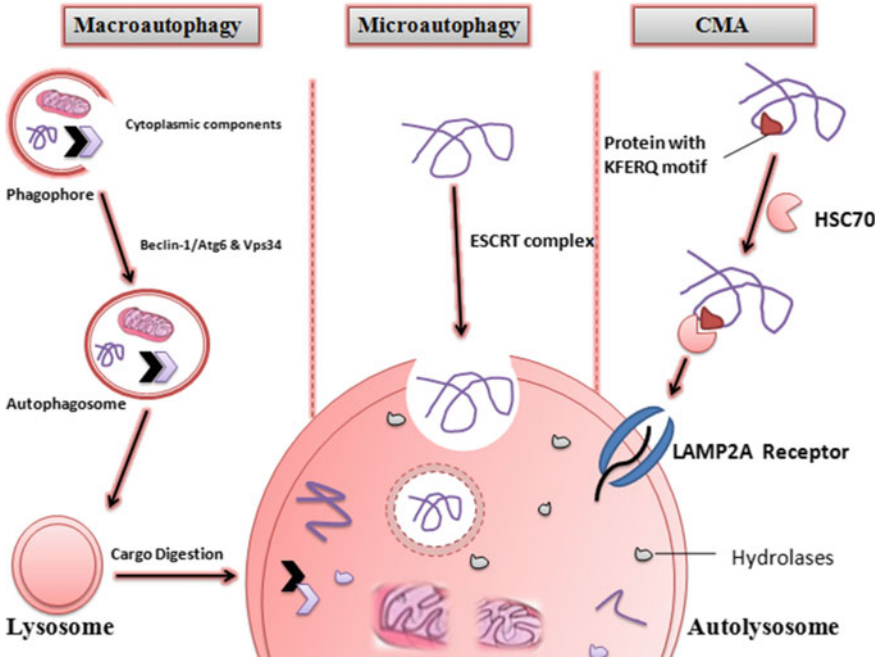

Fig. 7.3 Types of autophagy. Autophagy leads to the degradation of cargo and discharge of breakdown products into the cytosol for reuse. Macroautophagy depends on autophagosomes formation in the cytosol, to remove and transport materials to the lysosome. Autophagy gene Beclin 1, an ortholog of Atg6/vacuolar protein sorting (Vps)-30 protein plays role in positioning proteins before autophagial degradation. CMA (chaperone-mediated autophagy) transports unfolded proteins directly across the lysosomal membrane. Substrate proteins with specific KFERQ sequences are recognized by HSC70 and are transported into lysosomes through binding with LAMP2A protein. Microautophagy comprises the uptake of materials through invagination of the lysosomal membrane. ESCRT, cytosolic proteins together with numerous supporting proteins facilitate membrane bending/budding forming multivesicular bodies. Multivesicular bodies ultimately fuse with the lysosome for the degradation of materials. HSC70: heat shock protein 70 complex, LAMP2A: lysosome-associated membrane protein type 2A, ESCRT: endosomal sorting complexes required for transport

is commonly described as autophagy, the second type is conserved from yeast to mammals, named microautophagy while the third type has only been explained in mammals, termed as chaperone-mediated autophagy (CMA) (Cuervo et al. 2005).

7.5.3.1 Macroautophagy

It is a well-studied and stress-induced type of autophagy where degradation occurs through autophagosome having a vacuole with bilayer and autophagolysosome, a lysosome having single-membrane. A group of proteins is involved for the completion of various steps in macroautophagy such as restraining membrane formation, length extension, growth, the merging of lysosomes, and breakdown (Lőrincz and Juhász 2020). These are generically named Atg proteins and were elucidated for the first time in yeast. Overexpression and Knockdowns of the genes in different organisms encoding these proteins have greatly increased the knowledge about the involvement of macroautophagy in enormous pathological as well as normal physiological processes. Macroautophagy helps a cell to renew the non-nuclear intracellular materials, destroy the worn-out structures and produce the new ones by utilizing the building blocks generated during "**digestion**" (Cuervo et al. 2005; Morgunova et al. 2016).

7.5.3.2 Microautophagy

In mammalian cells, microautophagy has been reported to take place in late endosomes instead of lysosomes, hence named endosomal microautophagy (Sahu et al. 2011). It comprises the transport of components from the cytosol to the endosome with the formation of endosomal membrane invaginations, and this process is facilitated by the ESCRT (endosomal sorting complexes required for transport). Hence, in microautophagy, invaginations are formed by the lysosome, and macromolecules as well as small structures are sequestered without the formation of autophagosomes as in macroautophagy (Macian 2019). Microautophagy is also named basal autophagy because in cells this process is maintained at a constant level. When a cell experiences deficiency of energy it utilizes the process of microautophagy (Morgunova et al. 2016).

7.5.3.3 Chaperone-mediated autophagy (CMA)

In CMA rearrangement of the lysosomal membrane is not required; instead, chaperone proteins are involved in the transportation of "faulty" proteins to the lysosome. Specific "chaperones" recognition and binding to the target substrate molecules result

in their degradation. The specific sequences of amino acid on degraded substrates are required by these "molecular chaperones" (Morgunova et al. 2016; Luo et al. 2020a, b).

Many scientific reports have defined CMA as an extremely degradative and regulated process that involves the participation of HSC70 (heat shock protein 70 complex). Additionally, receptor lysosome-associated membrane protein type 2A (LAMP2A) undergoes multimerization.

The degradation of proteins by CMA must have a KFERQ motif in amino acid sequence, which is essential for the binding of chaperone HSC70. For lysosomal docking, binding of LAMP2A (twelve amino-acid tails in the cytosol) to the HSC70 complex and substrate is required. Moreover, multimerization of LAMP2A is mandatory for substrate transfer inside the lysosome. The multimeric complex releases cytosolic HSC70, after which an HSP90 chaperone present at the membrane of lysosome lumen associates with LAMP2A to stabilize it all through the substrate transfer process. Finally, to end the process of translocation, there is a requirement for a luminal chaperone HSC70 and once inside, the lysosomal enzymes degraded the targeted protein (Barbosa et al. 2019).

CMA is very sensitive and mainly liable for protein breakdown only (Luo et al. 2020a, b). Hence, transportation of only soluble cytosolic proteins occurs this way and for entry to the lysosomes, unfolding of the proteins is necessary (Massey et al. 2006; Kaushik and Cuervo 2018).

7.5.4 Ageing and Autophagy

Researchers have focused to explore the role of lysosomal and autophagy in ageing. Current studies have indicated that autophagy might have an impact on ageing, stress induced by oxidation, inflammation, and astrocytes functionality (Wang and Xu 2020). Almost all cells and tissues experience reduced autophagic activity as an organism ages, and it was supposed to largely contribute to the commencement of several detrimental age-associated ailments and various aspects of the age-related phenotypes (Cuervo 2008). Decreased autophagy is linked with enhanced ageing, while stimulation of autophagy may induce effective age control potentials (Madeo et al. 2010). In yeast, during nutrient deprivation, autophagy is crucial for survival as it provides energy and new nutrients by enabling the recycling of macromolecules (Rubinsztein et al. 2011). Logically, an indication that ageing is accelerated by autophagosomes formation deficiency proposed that health span should be prolonged by the enhancement of autophagic activity, specifically if there was insufficient normal autophagy to respond against cell impairment associated with ageing. Genetic manipulations in various species are specially developed to enhance autophagy capable of extending longevity (Madeo et al. 2015).

One of the earliest proofs that elevated autophagy have a paramount role in enhancing lifespan originates from the inspiring opinion that in *C. elegans*, autophagy

is caused by the failure of insulin-like growth factor pathway and autophagy inhibition is due to essential Atg genes mutation, which hampers the improvement of lifespan (Meléndez et al. 2003). Pyo and collaborators conducted an experimental work to evaluate the association between increased lifespan and genetic overexpression of one Atg. The authors observed a rise in the process of autophagy and anti-ageing features by overexpressing the Atg5 gene in mice as compared to the wild-type mice (Pyo et al. 2013).

Another study was performed in Ana María Cuervo's laboratory that explained the significance of autophagy in ageing. A double transgenic mouse model was generated in aged mice within which for CMA, expression of the lysosome receptor could be modulated. It was concluded that ageing characteristics can be limited at the organ and cellular levels by the enhancement of this receptor (Zhang and Cuervo 2008). Furthermore, expression of the Atg5 showed increased insulin sensitivity, improved resistance to age-related obesity, showing an enhanced metabolism in aged individuals. However, several studies failed to verify that longevity can be achieved by the upregulation of one autophagic factor. Moreover, enhanced lifespan has been shown by many KO (knock-out) mouse models; however, the connection with ageing and the molecular mechanisms behind it are not yet well-defined (Barbosa et al. 2019).

Similar characteristics are shared by an animal model named senescence-accelerated mouse prone 8 (SAMP8) that are non-genetically altered strains of mice (Ma et al. 2011). During ageing, certain remarkable changes related to autophagy were detected in the SAMP8 mice brain, such as reduced autophagy activity and accumulation of ubiquitin-positive proteins (Tan et al. 2014). Dysfunctional management of bio-signalling pathways including AMPK and mTOR resulting in cellular vulnerabilities that are conducive for the progression of neurodegeneration, cancer, and metabolic disease as well as impaired autophagy which ultimately contributes to ageing. However, a reduction in the function of mTORC1 is enough to enhance the life duration in worms, mold, mice, and flies (Wong et al. 2020).

Overexpression of an autophagy stimulator viz. HLH-30 was identified in *C. elegans* to increase the lifetime and is homologous to the transcription factor EB (TFEB) in mammals (Lapierre et al. 2013). An in vitro ageing model in humans indicated that over-expression of certain factors of the autophagy machinery including LC3/ATG8 and ATG12 contributed to the maintenance of mitochondria and long life. This suggests advantageous effects of pro-autophagic protein expression either in humans or within other mammalian cells. Moreover, within the nervous system, initiation of autophagy is of great importance for example in *D. melanogaster*, overexpression of brain-specific LC3/ATG8 was linked to prolonging age (Simonsen et al. 2008) and the absence of polyglutamine stretch of huntingtin in the brain exhibited a rise in autophagy which was linked to enhance longevity (Zheng et al. 2010).

The over-expression of Atg8 leads to longevity in muscle and neurons of adult Drosophila flies. Similarly, neuron-specific autophagy in adult Drosophila is induced in both non-cell and cell autonomously by the overexpression of Atg1, hence also results in extension of lifespan. Concordant with the biological significance of all findings, numerous relevant genes such as Atg5, Atg6, Atg1, Atg7, and Atg8

represent decreased appearance in flies with ageing and in human as well as mice muscle, ATG7 and LC3 proteins levels also decline with age. Generally, age-related and genetic loss of adequate lysosomal and autophagic function associate with the progress of various metabolic as well as anti-amnesic diseases. Examples are the mutations that result in loss of function of enormous genes relevant to autophagy such as Becn1/VPS30/ATG6, Atg5, and Atg7 decrease autophagy and higher accumulation of aggregated, disordered proteins in Alzheimer's disease (Ab and MAPT/tau), Huntington disease (HTT/huntingtin), and Parkinson's disease (SNCA/a-synuclein) (Lapierre et al. 2015).

Most ageing studies in humans are conducted using postmortem tissues, biopsies, peripheral blood samples and different types of cells that can be propagated by employing an artificial culturing medium *in vitro*. In all these cases, individual environmental factors/lifestyle is difficult to control between people, which leads to considerable experimental variability. Moreover, the literature reports additional ageing models that are used for better elucidation about the multi-factorial process of ageing in different species. Some emerging models include the longest-lived (30 years) rodent mole nude rat, the longest-lived mammal named the bowhead whale (200 years), killifish, the shortest-lived (~four months) vertebrate, and bivalve mollusks (life span up to 500 years). Diverse experimental approaches in all of these species have been applied to figure out the biological signals of ageing that range from detection of age-related biomarkers, regulatory proteins, genes/polymorphisms, compounds/metabolites, hormones, and different diets and decreased intake of calorie to intermittent fasting, which may defer the start of age-associated diseases and/or confer resistance as well as modify the process of ageing and longevity to manage with the environmental challenges (Carmona and Michan 2016). Autophagy activation can delay ageing, but at the same time, several studies have highlighted the opposite view. Hence, further work is warranted to assess the exact relationship between ageing and autophagy processes (Gu et al. 2020).

7.5.4.1 Role of Macroautophagy and CMA in Ageing

Alteration in macroautophagy and CMA happens with ageing; consequently, the contribution of autophagy to longevity has been supported by both vertebrate and invertebrate transgenic models (Hubbard et al. 2012). Generally, CMA and macroautophagy activities decline during ageing (Salminen and Kaarniranta 2009). However, there is a lack of evidence that how microautophagy activity might be affected during the process of ageing (Macian 2019). The dramatic increase in the function of both macroautophagy and CMA has been observed during stress, which facilitates cells to adjust to the environment (Morgunova et al. 2016).

Two well-characterized types of extralysosomal 'waste' are indigestible oxidized protein and senescent mitochondria (Cuervo et al. 2005). The organellar homeostasis is critically regulated by autophagy specifically that of mitochondria (Rubinsztein et al. 2011). Macroautophagy degrades all cellular structures, but mitochondrial

digestion known as mitophagy is of peculiarly great significance because the long-term existence of the cell is dependent on the quality control of such organelles. Macroautophagy is strongly connected with the biogenesis of mitochondria; in few circumstances, the fundamental constituents from the "old" mitochondria are utilized by the cell for the formation of new mitochondria (Yen and Klionsky 2008; Morgunova et al. 2016).

The aged post-mitotic cells contain many mitochondria that are structurally deteriorated and enlarged, exhibiting swelling and disintegration of cristae, usually causing the formation of amorphous material. The mitochondria that are excessively enlarged are frequently named 'giant'. The basic mechanism elaborating age-associated changes in mitochondria is still under discussion. Initial deterioration of mitochondria can be ascribed to the damage by ROS along with an inefficient performance of mitochondrial repair systems such as mtDNA repair as well as Lon and AAA proteases. Defective mitochondria should be degraded and autophagocytosed but the accumulated damaged mitochondria with age either escape macroautophagy or they require replicative advantage over normal mitochondria (Cuervo et al. 2005).

Dysfunctional mitochondrion that has lost membrane potential is more susceptible to release ROS along with toxic apoptotic mediators (Fig. 7.4). These are elim-

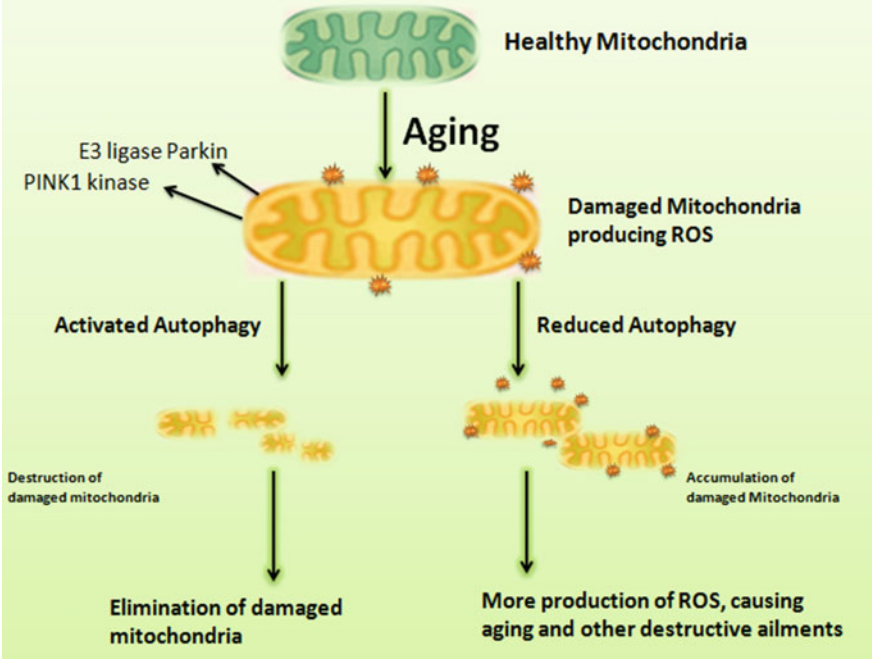

Fig. 7.4 Effect of ageing on autophagy. Activated autophagy causes the elimination of damaged mitochondria by using PINK1 kinase and E3 ligase Parkin markers, while reduced autophagy results in the accumulation of waste product, making the process of ageing more lethal and causing several other destructive ailments. PINK1 kinase: PTEN induced kinase 1

inated selectively by autophagy through ubiquitin Ser65 phosphorylation reactions carried out by PINK1 kinase (PTEN induced kinase 1), following attachment of ubiquitin proteins by the E3 ligase Parkin, compared to "healthy" mitochondrion. The autophagy uses PINK1 kinase and E3 ligase Parkin as markers to distinguish between damaged and healthy mitochondria, promote binding of dysfunctional mitochondria to phagophore by recruiting the mitochondrial autophagy receptors P62/SQSTM1 and NDP52 (Rubinsztein et al. 2011; Gu et al. 2020).

It is strongly suggested that the working of macroautophagy, especially defective mitochondrial breakdown, is impaired due to ageing. Cell organelles undergo a complex and inducible macroautophagy process that includes Beclin1 and other Atg genes activation. The stimulation of macroautophagy occurs in response to hormone treatments and during various stress conditions. Interestingly, both the repression of growth hormone-insulin-like growth factor (GH- IGF-1) axis and caloric restriction can reverse age-associated variations and cause activation of macroautophagy. The increased stress resistance is a special feature of hermetic lifetime extension in *C. elegans* and long-lived mouse models (Cypser et al. 2006; Murakami 2006; Salminen and Kaarniranta 2009).

It has been observed that during ageing, housekeeping processes become compromised as the rate of protein turnover determines the quality of the cellular housekeeping mechanisms. During ageing, the accumulation of waste material in cells after mitosis indicates the inefficiency of physiological housekeep such as the proteolytic system. The proteasomal degradation activity is decreased in ageing (Salminen and Kaarniranta 2009), and the mechanism involves quality control checks, regulation of translation, protein folding, and breakdown by cell machinery. UPS (ubiquitin–proteasome system) and autophagy-lysosome system are involved in it. However, the nature of cross-talk between autophagy and proteasome is still not clear (Sun-Wang et al. 2020).

Indeed, autophagy performs a paramount role in proteostasis regulation, a mechanism which has been recently identified as one of the fundamental age causing mechanism (Macian 2019). Proteins are the components that enable or directly perform several functions of cells, taken together they constitute the "proteome". The term proteostasis or protein stability refers to the capacity of cells to protect protein function and structure against ambient stressors such as changes in oxidative stress, pH, temperature, radiation, and ageing (Ruan et al. 2020; Sabath et al. 2020). Vulnerability in proteostasis correlates with changes in longevity and ageing rates among various species. Different researches identified that long-lived organisms are highly resistant to numerous environmental stressors and protein unfolding, thereby have maintained endogenous enzymatic activities in comparison to the species that are short-lived having less robust/effective proteostasis (Treaster et al. 2014). The proteostasis network present in the cells is well elaborated that involves autophagy, chaperones, protein synthesis, the ubiquitin–proteasome pathway, and the unfolded-protein response. These network constituents altogether are designed for the maintenance of recycling of long-lived products, counteracting protein misfolding, protein turnover and clearing up unfolded proteins. Studies revealed

that proteostasis networks with age can be compromised resulting in the accumulation of protein and the aggregation of damaged and/or unfolded proteins (Carmona and Michan 2016).

One of the main functions performed by autophagy is proteostasis. CMA is engaged in the elimination of potent oxidized proteins by degradation in lysosomes (Barbosa et al. 2019). Cuervo and Dice (2000) demonstrated that during ageing the activity of CMA reduces in rat liver. The impaired transport of defective proteins into lysosomes as well as their binding to the membrane of lysosomes was observed with ageing. Interestingly, the results of various studies concluded that the progressive age-related decreased expression of the receptor protein in CMA uptake and LAMP-2A protein results in the reduced efficiency of CMA. Contrarily, certain targeting protein expression in CMA i.e., HSC70 protein was generally unaffected by ageing. These studies were further extended by the Cuervo laboratory, indicating that in transgenic mice the inhibition of age-associated deterioration of LAMP-2A protein can sustain a functional and an active CMA till older age. Moreover, the CMA conservation was significantly linked with improved liver function and decreased accumulation of defective proteins (Zhang and Cuervo 2008). During ageing, the decline of the LAMP-2A mechanism in lysosomes appears to be post-transcriptional as the LAMP-2A efficiency during transcription is not affected by ageing. This identifies that the function and assembly of the LAMP-2A complex in lysosomal membranes were affected by ageing; hence, impaired CMA properties occur during ageing. Moreover, HSP90 protein levels, a key constituent of LAMP-2A complex assembly, also decrease in the liver of ageing rats (Salminen and Kaarniranta 2009).

7.6 Conclusion

The interplay between redox signalling, autophagy and ageing is although quite dynamic, yet many aspects are still unknown. This highlights the probable challenge to identify specified pathways, the bulk of stimuli and metabolites. It is plausible that ageing process comprises multiple vicious cycles of ROS generation, redox signalling, and autophagial degradation of mitochondria. Defective and effective autophagy may decline or accelerate normal physiological conditions. Much of the data presented here are from those model organisms in which morbidity, mortality, and ageing may vary from what impacts human life and health. Nevertheless, it is expected that the biological progressions allied with the long life of model organisms may defend against ailments that afflict people.

Now the next puzzle is what best should be adopted to improve the healthy ageing process without any side effects. Being aged is a reality that everyone accepts. We want to live a healthy life i.e., healthy ageing. But unfortunately, it is not quite possible because in most cases ageing causes detrimental ailments. There are numerous factors including external as well as internal that collectively contribute to make ageing a detrimental process. Among these, increased oxidative stress and the decreased autophagy process are significant as both are interconnected with each other. During

ageing, if the autophagy activity suppresses due to certain factors, it can neither destroy ROS-producing mitochondria nor maintains the homeostasis process, hence making the process of ageing even worse. Therefore, it is fascinating to speculate, although it is not proven experimentally, that supplements such as inducers that enhance the autophagy activity as well as certain dietary antioxidants may be beneficial to maintain the oxidant-antioxidant balance inside the body, promote health and postpone our inevitable fate.

Compliance with Ethical Standards

Conflict of Interest: All authors declare they have no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 8
Targeting Mitochondria and Redox Dyshomeostasis in Brain Ageing: An Update

Susana Cardoso and Paula I. Moreira

Abstract The increase in life expectancy is one of the highest accomplishments of humankind. Yet, this situation is also one of the most challenging public health issues. Despite the inevitable advancement of biological age, the deterioration of physiological homeostasis is variable and a better knowledge of the molecular mechanisms behind the complexity of the ageing process is a main priority of present societies. As with other organs, ageing induces a significant decrement in the functional capabilities of the brain and the majority of the aged population are confronted with the co-occurrence of diverse brain disorders like neurodegenerative diseases. As substantiated in a vast body of evidence, mitochondria are powerful organelles that not only enable our existence but also the disruption of its function is theorized to have a causative role in physiological and pathological brain ageing. With a focus on mitochondria and associated oxidative stress, we review relevant literature on altered mitochondrial function in physiological and pathological brain ageing, namely in Alzheimer's and Parkinson's diseases. The outcomes of mitochondrial medicine targeted to directly manage mitochondrial and oxidative dyshomeostasis will also be discussed. Also, the efficacy of lifestyle modifications and pharmacological strategies will be reviewed as amenable interventions in brain ageing research.

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Keywords Brain ageing · Mitochondria · Redox imbalance · Mitochondrial medicine · Neurodegenerative disorders

8.1 Introduction

Throughout history, achieving immortality has always been an inherent human instinct among civilizations. And, although life expectancy varies dramatically by country, people are in fact living longer. Due to shrinking fertility rates, improved healthcare infrastructures and early diagnosis of diseases, human lifespan has increased dramatically over the last century (Lunenfeld and Stratton 2013). However, from economics and healthcare perspectives, such ageing demographics have become a great challenge to societies worldwide.

By definition, ageing is a multi-factorial, progressive and normal physiological phenomenon characterized by a lifelong accumulation of cellular and molecular alterations, which frequently results in pathological illnesses (Gonzalez-Freire et al. 2020). As with other organs, normal ageing induces a significant decrement in the functional capabilities of the brain, which often manifests as deterioration of brain sensory, motor, emotive and cognitive processes (Mattson and Arumugam 2018). Whilst a small percentage of old individuals are able to cope with those decrements and have a healthy brain ageing (i.e. with a relative lack of disease and able to perform their everyday activities), the majority of aged population are confronted with disability and fragility and the co-occurrence of diverse brain disorders like dementia and neurodegenerative diseases (Wyss-Coray 2016). In a society with increasing life expectancy and with the rise on the numbers of patients suffering from brain diseases, there has been a substantial interest not only to identify ageing phenotypes but also to investigate the underlying pathways that trigger those phenotypes (Campisi et al. 2019; Vinke et al. 2019).

Among the several theories that have been proposed in an attempt to explain the process of ageing, those that see mitochondria as main actors in the origin and mechanism of ageing occupy a particular place. With origin in the famous "free radical theory of ageing" (Harman 1956), the "mitochondrial free radical theory of ageing" (MFRTA) conceived by Harman (1972) has become an attractive and predominant theory in ageing research. Grounded on observations about the generation, toxicity and the detoxification of mitochondrial-generated reactive oxygen species (ROS), as well as about how these parameters impact the physiological state of cells and organisms with advancing age, the MFRTA predicts a vicious cycle in which, under abnormal conditions, oxidative damage accumulates as a result of an increased production of ROS by mitochondria. This, in turn, leads to an elevated mutation load in mitochondrial DNA (mtDNA), defects in mitochondrial respiration and increased oxidative damage (Hekimi et al. 2011; Sun et al. 2016). Even though MRFTA core premises have been challenged (for review see, Viña et al. 2013), the fact that mitochondria are closely and simultaneously connected to both the energy of the young and the decline of the old cells (Sun et al. 2016), and that defects in

mitochondrial function play a causative role in (brain) ageing pathology cannot be refuted. Without belittling all the other known hallmarks of ageing (Mattson and Arumugam 2018), the purpose of this review is to summarize the importance of mitochondria and redox (dys)homeostasis in both physiological and pathological brain ageing. The latest developments and feasibility of approaches targeting mitochondria to prevent and/or delay brain ageing and age-related pathologies, namely Alzheimer's (AD) and Parkinson's diseases (PD), will also be highlighted.

8.2 Brain Ageing: An Unavoidable Physiological Event

After maturity, when optimal health, strength and appearance are at the peak, the body starts to feel a gradual decline of the normal physiological functions and individuals experience the first signs of age (e.g. the maximum lung, heart and kidney capacities are decreased and arthritic changes, skin wrinkling appear, etc.). In parallel with the ageing of other organs, brain function decline starts to be notorious, with a gradual worsening after middle age (approximately 50 years old) (Singh-Manoux et al. 2012). At this stage, preserving cognitive abilities is an essential part of maintaining a high quality of life. However, the fragility and complexity of the ageing process contributes to the existence of substantial differences in the ability of the older individuals to maintain their cognitive functions in the last years and decades of life. So, understanding the timing and sequence of events in brain ageing has been a major requirement for the identification, prevention, and treatment of any potential age-associated cognitive decline (Carroll 2018).

8.2.1 Mitochondria on the Backstage

It is widely known that during adulthood, the (human) brain undergoes both structural and functional alterations, ultimately causing functional disability in older adults. Among those, changes in prefrontal cortices supporting executive functions have been proposed as a hallmark of healthy ageing (Fjell and Walhovd 2010). As found, there is a prominent thinning of prefrontal cortex by middle age that is widespread across the diverse regions of brain cortex (Salat et al. 2004) whilst alterations in the medial temporal lobe, including the hippocampus, likely reflects the pathological ageing process (Visser et al. 2002). The search for the molecular underpinnings of the observed morphometric effects revealed that there is a significant decline in brain activity during normal ageing (from ~20 to 90 years-old), mainly localized in the medial network including the anterior cingulate/medial prefrontal cortex, dorsomedial thalamus, and subgenual cingulate/basal forebrain (Pardo et al. 2007), directly correlated with a declining brain glucose uptake and metabolism (Pardo et al. 2007). The human brain is known to be a high energy demanding organ that requires about 20% of the body's energy consumption to fulfil its function; in proportion to its size

it consumes more energy than any other tissue (Grimm and Eckert 2017). On the backstage, this requirement is fulfilled by mitochondria, the multifunctional life-sustaining organelles that contribute to neuronal energy and viability through the production of adenosine triphosphate (ATP) via oxidative phosphorylation (Picard et al. 2016). Within the brain, energy generated by mitochondria is used for overall maintenance of cellular processes, buffering of presynaptic Ca^{2+}, neuronal growth, axonal branching and to ensure synaptic transmission (Grimm and Eckert 2017). Using a combination of up-to-date analysis techniques like voxel-based morphometry (VBM) and ^{18}F-fluoro-deoxy-glucose (18FDG)-PET, a previous study have noted a great deterioration, both structurally and functionally (i.e. less glucose metabolism) in the frontal cortex compared with other brain regions like the anterior hippocampus, the thalamus and (functionally) the posterior cingulate cortex during normal ageing (Kalpouzos et al. 2009). More recently, a longitudinal study conducted by Castellano et al. (2019) reported the occurrence of a decreasing brain glucose metabolism in relatively old aged individuals from the baseline to the 4 years of follow-up. Even though the detected alterations were associated with moderately slow decline in cognitive performance, those remained within the normal range for age, prompting authors to suggest that the declining brain metabolism probably reflects a physiological change during the ageing process (Castellano et al. 2019). Likely, ^{13}C/^{1}H magnetic resonance spectroscopic (MRS) studies demonstrated that healthy ageing is associated with abnormal neuronal and glial mitochondrial metabolism (Boumezbeur et al. 2010). Consistent with those studies, in vivo data from aged rats using nuclear magnetic resonance (NMR) spectroscopy revealed that the incorporation of glucose-derived ^{13}C into glutamate, glutamine, aspartate, and gamma-aminobutyric acid (GABA) declined in aged brain, suggesting that normal ageing is associated with a decline in brain glucose metabolism (Miccheli et al. 2003). The above evidence highlights the existence of a metabolic deterioration, involving mitochondria, during the ageing process, which contributes for a general decrease in the overall performance of the brain.

8.2.2 Mitochondria-Redox Status Interplay During Physiological Brain Ageing

Besides its high energy requirements, which makes it strongly dependent on glucose as energy source, compared with other organs the brain is characterized by having a high content in transition metals and low levels of antioxidant defences which makes it a highly vulnerable target to oxidative stress and damage (Moreira et al. 2009). While mitochondria generate the exceptional amount of energy required by neuronal cells, they are also contributing to cellular ROS production. During cellular respiration, an electron that escapes from the mitochondrial electron transport chain (ETC), in particular complexes I and III, binds oxygen (O_2) to form the anionic free-radical superoxide ($O_2^{\bullet -}$) as a physiological by-product of ATP production. The term ROS

is an umbrella word to define a group of reactive free radicals that originate from O_2 including the hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2); each one of them having the capacity to rapidly damage proteins, nucleic acids, and lipids, and affect cell normal functionality and viability (Weidinger and Kozlov 2015). Although evidence discloses ROS as unwanted and toxic by-products of the mitochondrial ETC and a major mediator of age-associated brain cellular damage, at low physiological levels, ROS are also recognized as important signalling molecules regulating physiological gene transcription and protein interactions (Shadel and Horvath 2015). Because of those distinctive roles of ROS, maintaining their balance and regulation is key for cellular normal functions (Martin and Barrett 2002). To achieve this, cells have evolved a strict defence mechanism composed of both enzymatic and non-enzymatic antioxidant defence systems. Among the well-known enzymatic antioxidant defences, mitochondrial copper/zinc superoxide dismutase (Cu/Zn-SOD) and manganese superoxide dismutase (Mn-SOD) takes a crucial role in the conversion of $O_2^{\bullet -}$ into H_2O_2, which is decomposed to innocuous O_2 and H_2O via mitochondrial glutathione peroxidase (GPx) or peroxiredoxins. In turn, many non-enzymatic defences (e.g. glutathione (GSH), vitamins E and C, carotenoids, polyphenols, and flavonoids) are described to decrease the oxidative damage and counteract oxidative stress (Madreiter-Sokolowski et al. 2018).

Using specific mouse models of ageing, several groups have observed that the ageing process is related with an unbalance between oxidants and antioxidants. For instance, SAMP8 mice (accelerated senescence prone 8), an animal model that exhibits an early manifestation of senescence-related phenotypes, present significant age-related changes in the brain levels of the main antioxidant enzymes, Cu/Zn-SOD and Mn-SOD, when compared with age-matched SAMR1 (accelerated senescence-resistant 1) mice (Kurokawa et al. 2001). Consistently, a previous study with SAMP10 mice (a substrain of the senescence accelerated mice) at different ages revealed an age-dependent decrease in SOD activity, and an increase in lipid peroxidation and a decline in cognitive behaviour at the age of 8 months (Wang et al. 2015). Although mechanisms were not evaluated, authors were tempted to suggest that the earlier appearance of oxidative stress could accelerate the pathological process of age-associated neurodegeneration (Wang et al. 2015). More, brains from aged mice (21-months-old) showed a pro-oxidized state due to an imbalance in the glutathione ratio (GSH/GSSG) compared to brains from young mice (3-months-old) (Rebrin et al. 2007). Of note, those alterations were markedly detected in brain areas known to be linked to age-related loss of higher brain functions such as the cortex, hippocampus and striatum (Rebrin et al. 2007). Other studies performed in rodents show a decrement of brain mitochondrial function (i.e. decreased activities of mitochondrial complex I, II, IV and ATP synthesis) with increasing age (Pandya et al. 2016), which is paralleled with a decrease in antioxidant defences (Navarro and Boveris 2004). In close agreement, Zweig et al. (2020) have shown that the loss of nuclear factor erythroid-derived 2 (NRF2), an important antioxidant regulatory transcription factor, induces significant alterations in brain mitochondrial function of aged mice along with disturbances in dendritic complexity and expression of synaptic

plasticity markers culminating in deficits in learning, memory and executive function in NRF2KO animals.

In the same line, a previous study performed in the well-known nonhuman primate model, the Rhesus monkey, brought evidence of a correlation between mitochondrial function impairments in the basal ganglia of aged primates and the motor deficits presented by those animals (Pandya et al. 2015). Concerning human brain tissue studies, post-mortem investigation revealed that the occurrence of brain region-specific differences in the critical balance between oxidant and antioxidant markers and mitochondrial function during physiological ageing render brain cells more susceptible to pathology and selective neuronal degeneration (Venkateshappa et al. 2012). Concurrently, data show the existence of a gradual reduction in the brain levels of the antioxidant GSH in healthy individuals in middle age (~56 years) compared with younger individuals (~26 years) (Mandal et al. 2012). In a likely manner, a previous cross-sectional study reported the occurrence of an age-related increase in oxidative stress markers in the brains of healthy individuals with intact cognitive function (Peskind et al. 2014).

Alongside with an imbalance in oxidative status, aged brains are shown to have higher levels of oxidative damage to both nuclear DNA (nDNA) and mtDNA (Barja 2004; Barja and Herrero 2000). As firstly reported, the amount of damage to DNA in the human brain, determined by the levels of 8-hydroxy-2-deoxyguanosine, increases progressively with normal ageing, although being more pronounced in mtDNA (Mecocci et al. 1993). Mitochondria are the only organelles in the eukaryotic cells that have their own DNA distinct from nDNA. However, its singularities (i.e. close proximity to the source of ROS production, absence of protective histones and less efficient DNA repair machinery) render mtDNA particularly vulnerable to damage (Santos et al. 2013). As organisms grow old, the accumulation of mtDNA oxidative-induced mutations and deletions compromises post-mitotic tissues with high-energy requirements like the brain that strongly rely in a functioning mitochondria (Chomyn and Attardi 2003). In fact, there seems to be a causal link between increased mtDNA mutations and ageing phenotypes; the longer the longevity of a species, the smaller is its mtDNA oxidative damage degree (Barja and Herrero 2000). Other studies showed that neurons in the aged human substantia nigra contain very high levels of mtDNA deletions, likely to be responsible for impaired cellular respiration (Bender et al. 2006; Kraytsberg et al. 2006). A link between mtDNA and brain ageing has further been substantiated by genetic mouse models. Using human mitochondrial transcription factor A (Tfam) transgenic mice, the effects of Tfam overexpression on age-dependent deficits in brain functions were examined (Hayashi et al. 2008). Tfam is a nucleus-encoded protein with a critical role for mitochondrial genome conservation and for mtDNA replication and maintenance. In this particular study, the overexpression of Tfam ameliorated age-dependent declines in motor performance and memory ability alongside with a reduction in lipid peroxidation markers and the restoration of mitochondrial complex enzymatic activities (Hayashi et al. 2008).

Altogether, those evidences demonstrate that the complexity of the (brain) ageing process is accompanied by an intricate connection between oxidative stress and brain mitochondria function alterations and damage that can culminate in an altered brain functioning.

8.2.3 The Importance of Mitochondrial Dynamics in Physiological Brain Ageing

For a homeostatic neuronal function and to meet their metabolic requirements, mitochondria structure, function and localization must be tightly regulated. To achieve this, there is an intermingled relation between the processes of mitochondria dynamics (fusion-fission), biogenesis, degradation, and metabolism, redox signalling, maintenance of mtDNA and cell death (Seo et al. 2010). Many mitochondrial proteins interact closely to meet those challenging functions, and currently the frequently used term "mitochondrial dysfunction" refers to defects not only in mitochondrial bioenergetics but also to any perturbation in mitochondrial number, integrity and morphology. Under physiological conditions, mitochondrial fusion, driven by mitofusins 1 and 2 (Mfn1 and Mfn2) located in outer mitochondrial membrane and by optic dominant atrophy 1 (OPA1) present in the inner mitochondrial membrane, favours the generation of an elongated and interconnected mitochondrial network allowing the spreading of new proteins and mtDNA between the merging organelles. Counterbalancing fusion is the fission process mediated by dynamin-related GTPase protein 1 (Drp1) and mitochondrial fission 1 (Fis1). Unlike fusion, fission allows mitochondrial replication to expand the cellular mitochondrial pool often leading to formation of small, rounded mitochondria. The dynamic coordination between fission–fusion events allows to regulate mitochondria amount and location within the cells and to dispose damaged mitochondria by selective autophagy (Burté et al. 2015). As demonstrated, perturbations in both events by the knock down of Fis 1 protein and by the overexpression of OPA1 leads to a decrease in mitophagy rate and accumulation of damaged mitochondria (Twig et al. 2008). In neurons, mitochondria have to be relocated to the places where more energy is required. This way, mitochondria are assembled in the cell body and need to travel along dendrites and axons to the nerve terminals where the bioenergetics requirements are high and mitochondria are required to fulfil their physiological functions (MacAskill and Kittler 2010). In general, mitochondria movement in the anterograde direction (from cells' body to distal regions) is achieved through kinesin motors whilst its transport in the retrograde direction (towards the cells' body) is ensured by dynein motors. A tight relationship seems to exist between mitochondria morphology and its transport in axons, a process called mitochondrial trafficking (Frederick and Shaw 2007). As shown, Mfn2 by interacting with Miro and Milton (two mitochondria adaptor proteins) can directly regulate mitochondrial transport (Misko et al. 2010). In opposite, genetic manipulations disturbing Drp1 and OPA1

proteins result in an impaired mitochondrial transport and content in axons, with consequent loss of synapses and dendritic spines (Li et al. 2004). Likewise, disruption of fusion machinery due to Drp1 ablation in neurons of adult mouse forebrain was found to cause significant alterations in mitochondrial morphology, transport and content in presynaptic terminals, together with a decrease in oxygen consumption and ATP production (Oettinghaus et al. 2016). A frequently observed characteristic in different models of ageing is an altered and heterogeneous mitochondrial network. Previous data from *Caenorhabditis elegans* revealed that mitochondria size increases during development, followed by a steady maintenance and a progressive decline of size and density during worms' lifespan (Morsci et al. 2016). Using a quantitative proteomics approach, Stauch et al. (2014) investigated the underlying alterations occurring in mice synaptosomal mitochondria during the ageing process. In detail, the authors were able to find age-related alterations in the expression of fission proteins with Drp1 expression increasing between 5 and 12 months of age and decreasing from 12 to 24 months. In turn, fusion machinery, Mfn1/2 and Opa1, decreased from 5 to 12 months and increased from 12 to 24 months, thus showing a shift to a pro-fusion state in aged animals. Concomitantly, proteomics analysis predicted alterations in the mitochondrial biogenesis regulators, nuclear respiratory factor 1 (NRF1) and peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), both being inhibited during aging from 5 to 12 months, but activated from 12 to 24 months. A similar pattern of expression was found in the levels of antioxidant enzymes whereas at the oldest ages, an increase in mtDNA deletions in synaptic mitochondria was also detected (Stauch et al. 2014). Overall, the dynamic proteomic changes occurring with ageing correlated with preservation of synaptic mitochondrial function (Stauch et al. 2014), thereby suggesting the existence of a complex interplay between several cellular pathways towards the preservation of mitochondrial function during healthy ageing. More recently, Reutzel et al. (2020) collected longitudinal data on brain mitochondrial function, cognitive performance, and molecular markers over the entire lifetime of female NMRI mice. In detail, during the brain ageing process, alongside with cognitive impairment, authors detected a significant reduction in mitochondrial respiratory chain activity and reduced mRNA expression of PGC1-α, NRF1, TFAM, cAMP-response element binding protein 1 (CREB1), 5' AMP-activated protein kinase (AMPK), and citrate synthase, key regulators and indicators of mitochondrial biogenesis (Reutzel et al. 2020). Overall, data collected allowed to infer the existence of an intricate association between age-related decline of cognitive performance, brain energy metabolism, and the process of mitochondrial biogenesis during the physiological brain ageing. In order to ensure a functional mitochondrial network and owing to their essential role in energy production, mitochondria must be constantly generated. An earlier study comparing age-related alterations in mitochondrial biogenesis in rat frontal cortex revealed that aged animals (26-month-old) present a 25% loss in mtDNA content versus young rats (6-month-old) animals, alongside with a 35% increase in the mtDNA deletion content and a strong decrease in Tfam bound mtDNA (Picca et al. 2013). Taken together, these findings confirm that mitochondria are critical for maintaining the complex balance of cellular processes that contribute to overall health, specifically a healthy brain

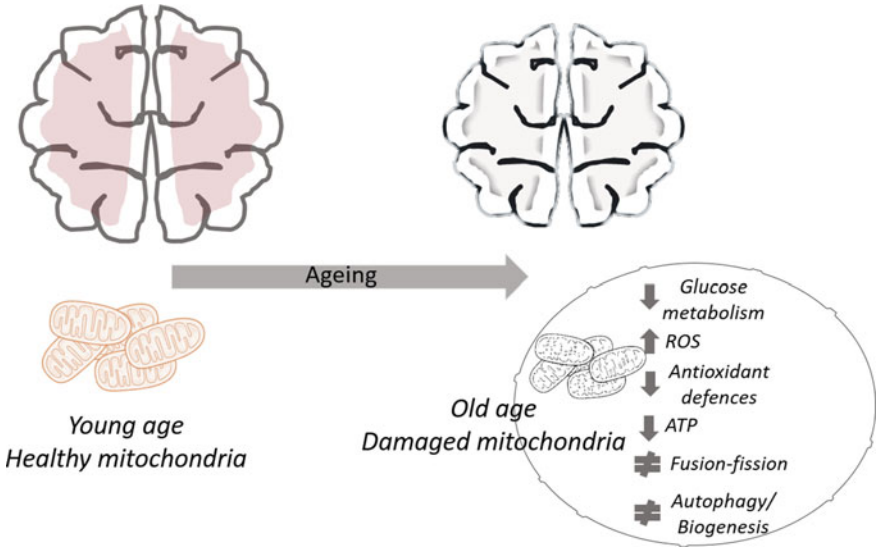

Fig. 8.1 Simplistic representation of mitochondrial function disruption in physiological brain ageing

ageing (Fig. 8.1). Discovering the threshold level that disrupts such balance and bends it to a pathological context remains a challenge.

The inherent need of brain for high energy supply dictates its dependence on functional mitochondria but also renders it sensitive to changes in these organelles. In fact, mitochondria are closely and simultaneously connected to both the energy of the young and the decline of the old cells. During physiological brain ageing, a close connection seems to exist between the age-related declines of cognitive performance and mitochondrial function; the latter characterized by a decline in energy metabolism, an imbalance between reactive oxygen species (ROS) production and antioxidant defences levels, decreased ATP production and disturbances in the machinery responsible for regulating fusion/fission, autophagy and biogenesis processes.

8.3 Pathological Brain Ageing: The Other Side

Despite all the knowledge, it is still quite difficult to draw a line between normal/physiological and pathological brain ageing. Having some clinical differences, most neurodegenerative diseases share several similarities, ageing being a key player in in the onset and progression of these diseases. More, mitochondrial dysfunction and oxidative stress have a relevant role in their etiology. In the next subsections, an overview about mitochondrial deterioration in AD and PD, two major neurodegenerative diseases, will be provided.

8.3.1 Alzheimer's Disease

The relationship between brain ageing and AD is largely known. Representing the most diagnosed type of dementia among the elderly, this illness occurs both in a familial form, which is autosomal dominant (accounting for 1–5% of all cases), and an apparently sporadic type, more common and receiving the diagnosis after age 60. AD is characterized by a progressive decline in cognitive ability, memory loss, and alterations in neuronal physiology that progress through the brain in a non-random manner, with early pathology occurring in the entorhinal cortex and hippocampus (Hampel et al. 2011; Karlawish et al. 2017). AD brains show neurofibrillary tangles (NFTs) mainly composed of hyperphosphorylated tau protein and senile plaques mainly formed by amyloid beta (Aβ) (Cardoso et al. 2016; Tönnies and Trushina 2017). For several years, the amyloid cascade hypothesis, which postulates that Aβ is the culprit of the disease, has dominated the AD field (Mecocci et al. 2018). However, further investigations and the successive failure of Aβ-based therapeutic approaches have challenged the amyloid cascade hypothesis and allowed the consideration of other possibilities for the disease onset and progression. In this context, the "mitochondrial cascade hypothesis" emerged as a baseline, unifying mechanism to explain the neurodegenerative process of AD (Grimm et al. 2016; Swerdlow et al. 2010).

Based on a large amount of evidence it has become clear that a disruption of mitochondria and neuronal metabolism are early events in AD progression (Gibson and Shi 2010; Hauptmann et al. 2009; Tobore 2019). As documented, during AD progression, patients often experience a transitional clinical stage known as mild cognitive impairment (MCI). In this phase, subjects often present an impaired cerebral glucose utilization (Croteau et al. 2018; Friedland et al. 1989; Mosconi et al. 2007), alterations in the expression and/or activities of key mitochondrial energy-related proteins (e.g. pyruvate dehydrogenase (PDH), isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase (α-KGDH), and cytochrome oxidase (COX)) (Bubber et al. 2005; Manczak et al. 2004; Silva et al. 2013). Also, the symptomatic progression of MCI to frank AD as well as the severity of cognitive impairment seems to correlate with disturbances in oxidative status in which the levels of oxidative stress markers surpasses the levels of non-enzymatic antioxidants defences (Aluise et al. 2011; Ansari and Scheff 2010; Nunomura et al. 2012; Pratico et al. 2001). The involvement of oxidative stress in AD is supported by a number of studies which showed the presence of DNA oxidation products, hydroxyl radical adducts of DNA and lipid peroxides like 4-hydroxynonenal (4-HNE) in the brains of AD subjects (Sultana et al. 2013; Swomley and Butterfield 2015). Likely, data from in vivo models of the disease corroborate that the impairment of mitochondrial function and the imbalance of oxidative status towards increased oxidative stress are early events in the pathology of AD (Hauptmann et al. 2009; Yao et al. 2009). Furthermore, Leuner et al. (2012) demonstrated that mitochondria-derived ROS interfere with the amyloidogenic pathway leading to an increased formation of Aβ. Further, the authors could observe that under this scenario, the higher Aβ production can

accelerate mitochondrial dysfunction, increasing ROS levels and consequently Aβ production via enhanced β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) activity (Leuner et al. 2012). As previously proposed, there seems to be an intricate association between the negative behavioral outcomes of AD transgenic mice and the level of synaptic mitochondrial impairment and Aβ content in mitochondrial samples from brain tissue (Dragicevic et al. 2010). Inside mitochondria, Aβ can directly interact with different intracellular proteins like the Aβ-binding alcohol dehydrogenase (ABAD) (Lustbader et al. 2004), and the mitochondrial protein import machinery (the TOM40 complex of the outer membrane and the TIM23 complex of the inner membrane) (Hansson Petersen et al. 2008; Lustbader et al. 2004), compromising mitochondrial function and aggravating the harmful effects of Aβ. In further addition, studies from our laboratory show that isolated brain mitochondria from the triple transgenic mouse model of AD (3xTg-AD) present an impaired energy metabolism culminating in lower ATP levels, increased ROS production and susceptibility to mitochondrial permeability transition pore opening (Carvalho et al. 2012; Resende et al. 2008). Further evidence in support of a primary role of mitochondria in AD was achieved using cybrid cell lines, an in vitro model usually made from SH-SY5Y or NT2 cell lines that are depleted of their own mtDNA and latter fused with platelet cells from AD or age-matched control patients (Swerdlow and Khan 2004). This model, characterized by having mitochondrial genes from AD donors, showed a significant increase in the levels of Aβ and a decrease in complex IV activity, as compared to age-matched control cybrids (Khan et al. 2000; Silva et al. 2013).

It is currently clear that highly interrelated changes in mitochondrial function, morphology, distribution, biogenesis and mito/autophagy contribute significantly to the evolution of the disease (Cai and Tammineni 2016). More specifically, it was found that hippocampal neurons from AD subjects have a reduced mitochondrial content (Baloyannis 2006; Hirai et al. 2001), which can be related to significant reductions in PGC-1α and NRFs protein levels and mtDNA to nDNA ratio (Qin et al. 2009; Sheng et al. 2012). In this line of thoughts, the overexpression of Tfam is found to protect SH-SY5Y cells from Aβ-induced mitochondrial dysfunction (Xu et al. 2009), and PGC-1α overexpression in N2a neuroblastoma cells is able to promote a decrease in secreted Aβ and increase the levels of non-amyloidogenic soluble Aβ (Katsouri et al. 2011). Besides being in a reduced number, mitochondria from AD brains are found to have a reduced size with an altered internal ultrastructure (Baloyannis 2006; Hirai et al. 2001) and reduced levels of mitochondrial fusion machinery (Wang et al. 2009). Concurrently, AD cybrids are found to have significant mitochondrial morphological alterations towards a more fragmented, misshaped, and bleb-like phenotype due to altered expression and distribution of Drp1 and Mfn2 (Gan et al. 2014). Similarly, previous findings reveal that the fragmentation of mitochondrial network in AD can result from the co-localization of Drp1 with Aβ and tau protein; such interaction likely leading to excessive mitochondrial fragmentation (due to an increased fission), and mitochondrial and synaptic deficiencies, ultimately contributing to neuronal damage and cognitive decline (Manczak and Reddy 2012; Manczak et al. 2011). In

close agreement, the partial reduction of Drp1 levels in a Tau transgenic mice alleviates mitochondrial impairment, upholds mitochondria network and enhances mitochondrial biogenesis (Kandimalla et al. 2016). Others demonstrated that tau protein overexpression disrupts mitochondrial dynamics by enhancing mitofusin-associated mitochondrial fusion (Li et al. 2016). Moreover, overexpression of APP or Aβ treatment was found to cause an imbalance in mitochondrial fission/fusion processes promoting an altered distribution of mitochondria, probably triggering Aβ-induced synaptic defects in neuronal cultures (Wang et al. 2008, 2009). Also, in vivo studies using transgenic *Drosophila* show Aβ-induced defects in mitochondrial morphology and transport as critical triggers of neuronal dysfunction (Iijima-Ando et al. 2009; Zhao et al. 2010). Likely, hippocampal neurons treated with Aβ-derived diffusible ligands (ADDLs) present a compromised transport of mitochondria along axons, both in the anterograde and retrograde directions (Wang et al. 2010). Similarly, Aβ overexpression in *Drosophila* promoted a significant depletion of presynaptic mitochondria, probably due to an altered size and shape of mitochondria, and the reduced the velocity of the axonal transport of mitochondria in both directions (Zhao et al. 2010). In another study, primary neurons from Tg2576 APP transgenic mice showed to have a remodeling in mitochondrial dynamics towards increase fission and an impairment in the anterograde mitochondrial movement in comparison with wild-type neurons (Calkins et al. 2011). In this context, the failure of the bidirectional transport of mitochondria during the progression of AD will compromise the supply of functional mitochondria to distal synaptic terminals and delay the backward transport of damaged mitochondria to the cell body for lysosomal degradation leading to inadequate mitophagic removal (Correia et al. 2015). Under normal conditions, autophagy in neuronal cells is a relatively active and well-regulated process to maintain cellular processes and homeostasis. Under these conditions, autophagosomes form around damaged mitochondria followed by the fusion with lysosomes, degradative organelles that allow the degradation of the cargo by hydrolytic enzymes (Rodolfo et al. 2018). However, mounting evidence shows that mitophagy is impaired in the hippocampus of AD patients, in induced pluripotent stem cell-derived human AD neurons, and in AD animal models (Fang et al. 2019). As supposed, defective mitophagy process can occur due to a disturbed induction of autophagic sequestration of mitochondria coupled with a decreased rate of fusion of autophagic vesicles with lysosomes (Correia et al. 2015). In fact, recent data suggests that the first steps of autophagy process, i.e. autophagy induction and autophagosomes formation, are competent while the final stages of the process, namely the fusion of autophagosomes with lysosomes, become impaired during AD development (Bordi et al. 2016). In further agreement, mutant neurons from hAPP transgenic mice and brain tissue from AD patient brains showed markers of autophagy induction, i.e. the presence of autophagic vacuole-like organelles engulfing abnormal mitochondria and a strong induction of parkin-mediated mitophagy due to increased recruitment of parkin to damaged mitochondria, while having a deficit in lysosomal proteolysis (Ye et al. 2015). Parkin is an E3 ligase that translocates to depolarized mitochondria, targeting them for elimination from cells through an autophagy/lysosomal pathway-dependent manner (Tanaka 2010). As reported, parkin overexpression in an AD mouse model

resulted in an enhancement of the autophagic clearance of defective mitochondria, in a decreased intracellular Aβ levels and extracellular plaque deposition, and in the prevention of mitochondrial dysfunction and oxidative stress (Khandelwal et al. 2011; Martin-Maestro et al. 2016). Others showed that the restoration of mitophagy using strong neuronal mitophagy-inducing agents, namely urolithin A (UA) and actinonin (AC), improves memory loss in *Caenorhabditis elegans* and in two mouse models of AD, inhibits Aβ plaques formation and phosphorylation of tau protein and restores mitochondrial homeostasis (Fang et al. 2019).

Clearly there are very strong links between mitochondrial perturbations and AD pathogenesis. Taking into account that the machineries mediating mitochondrial homeostasis are closely interconnected, maintaining a well-functioning mitochondrial network should be the foremost goal.

8.3.2 Parkinson's Disease

PD is the second most common progressive disorder of the central nervous system (CNS) affecting approximately 5% of the population aged over 65 years. Approximately 95% of PD cases are considered of sporadic origin with ageing as its major risk factor (Sarkar et al. 2016). Many studies confirm that PD is caused by a continuous loss of dopaminergic neurons within the substantia nigra pars compacta resulting into a massive reduction of striatal dopamine (DA), and α-synuclein-containing inclusions, called Lewy bodies (Rocha et al. 2018). Clinically, this presently incurable illness is classically characterized by the occurrence of tremors, rigidity, slow movement (bradykinesia), poor balance, and difficulty in walking (Parkinsonian gait). Non-motor symptoms, such as cognitive impairment, hallucinations and other disturbances due to dysfunction of the autonomic nervous system may also occur throughout the course of the disease (Raza et al. 2019).

Throughout the years, several epidemiological, post-mortem analysis, in vitro and in vivo experimental studies have significantly unveiled the underlying molecular mechanisms of PD. Among those, mitochondrial insufficiency has been alleged to be a pivotal event in the pathology of the disease (Bose and Beal 2016). The first clues implicating mitochondrial dysfunction in the pathogenesis of PD emerged with the discovery that chemicals that selectively inhibit mitochondrial complex I (e.g. 1-methyl-4-phenylpyridinium (MPP^+), paraquat, 6-hydroxydopamine, and rotenone) can cause dopaminergic neuron degeneration and the development of parkinsonian phenotypes (Langston 1996). In parallel, several lines of evidence substantiated a link between PD and an impairment in mitochondrial complex I activity, which was found to occur in the substantia nigra (Schapira et al. 1990), frontal cortex (Keeney et al. 2006; Parker et al. 2008), and platelets (Krige et al. 1992) from PD patients. Since then, the understanding of the intricate involvement of mitochondria in PD pathogenesis has considerably expanded, and reports of mutations in multiple PD-associated genes have provided a direct evidence for a primary role of mitochondrial

dysfunction in PD (Parker et al. 2008). Among those, α-synuclein, parkin, DJ-1, phosphatase and tensin homologue deleted on chromosome 10 (PTEN)-induced kinase-1 (PINK1), and leucine-rich repeat kinase 2 (LRRK2)/dardarin, are described to be PD-associated proteins relevant for mitochondrial homeostasis (Grünewald et al. 2019). In addition to its presence in the cytosol and nucleus of neurons, α-synuclein has been shown to localize to mitochondria in a wide range of experimental models disrupting mitochondrial complex I activity and oxidative balance (Devi et al. 2008). Also, compelling data link the function of α-synuclein to the regulation of mitochondrial fission–fusion (towards a fragmented phenotype), transport, and mitophagy (Choubey et al. 2011; Kamp et al. 2010; Pozo Devoto et al. 2017). Parkin, a protein involved in the degradation of oxidatively damaged proteins, was found to physically interact with the outer mitochondrial membrane preventing mitochondrial swelling and cytochrome c release (Darios et al. 2003). Parkin is also associated with both Tfam, enhancing mitochondrial biogenesis (Kuroda et al. 2006), and with mtDNA, protecting it from oxidative damage and stimulating mtDNA repair (Rothfuss et al. 2009). Previously, in in vivo studies made in *Drosophila* transgenic lines, it was also found that the PINK1/parkin pathway inhibits mitochondrial fusion and promotes mitochondrial fission (Deng et al. 2008; Park et al. 2009a, b). DJ-1, a redox-regulated molecular chaperone, was found to preserve mitochondrial integrity under conditions of oxidative stress (Kim et al. 2005; Meulener et al. 2005). Using knockout mice and human carriers of *DJ-1* mutations, it was showed that the loss of DJ-1 significantly disturbs mitochondria homeostasis (Krebiehl et al. 2010). It has been found that DJ-1 depletion impaired mitochondrial respiration, increased mitochondrial ROS generation, reduced mitochondrial membrane potential, alterated mitochondria morphology, and promoted the accumulation of defective mitochondria due to reduced autophagic degradation (Krebiehl et al. 2010).

A primary role of mutations in mtDNA in PD pathogenesis has also been suggested (Esteves et al. 2008). Consistent with an elevated number of mtDNA deletions detected in aged brains and PD patients (Bender et al. 2006; Dölle et al. 2016), variations in the gene encoding polymerase γ (POLG), the only known mtDNA polymerase, which is responsible for replication as well as for repair, have been identified as a risk factor in sporadic PD (Luoma et al. 2007). Further, Grunewald et al. (2016) reported a concomitant deficiency in mitochondrial complex I and low abundances of the mtDNA transcription factor Tfam in neurons from substantia nigra of PD patients. A previous study performed in the "MitoPark" mice known to have a disrupted Tfam gene in dopaminergic neurons showed that these mice have a reduced mtDNA expression alongside with a dysfunctional mitochondria in dopaminergic neurons and manifestation of PD-like behavioural disturbances (Ekstrand et al. 2007). By using the cybrid technique it has been observed that mtDNA from PD patients causes a mitochondrial complex I defect and ATP depletion in healthy recipient cells (Esteves et al. 2008, 2010a). Also, PD cybrids present basal microtubule disruption with a concomitant accumulation of α-synuclein oligomers (Esteves et al. 2010b). As reported, PD cybrids and MPP^+-treated neurons exhibit a disrupted microtubule network due to mitochondrial deficits, which led to the accumulation of α-synuclein that, in turn, impaired mitochondrial function (Esteves et al. 2014). Contrariwise,

authors could observe that the improvement of microtubule-dependent traffic re-establishes autophagic flux, reduces α-synuclein oligomer content, increases mitochondrial membrane potential, and decreases mitochondrial ubiquitination levels, thereby rescuing PD-derived cells (Esteves et al. 2014). This cybrid approach relies on the fusion of NT2 cells previously depleted from their mtDNA (NT2 rho0) with mitochondria isolated from platelets of age-matched healthy individuals or PD patients. This way, data obtained allow to infer that the differences detected in the mitochondrial population between control and PD cybrids are the result of mtDNA variations. Collectively, these studies suggest that mitochondrial dysfunction and associated oxidative stress have a major role in the pathology of PD, which might represent unique targets for therapeutic interventions.

8.4 Mitochondrial Medicine: Targeting Mitochondria and Redox Imbalance on Brain Ageing, Alzheimer's and Parkinson's Diseases

A major goal of modern medicine is to preserve quality of life. Applied to the ageing context, this can be defined as the process of developing and maintaining functional ability that enables wellbeing in older age (Michel and Sadana 2017). Despite being an inevitable process, the recent advances of science in the field of gerontology have showed that it is possible to slow the onset and progression of chronic diseases, preserving functional capacity, and postpone death (Gonzalez-Freire et al. 2020).

The agreement that mitochondrial dysfunction and oxidative stress play a central role in ageing and age-related brain pathologies argues for the considerable interest in developing disease-modifying interventions that strengthen mitochondrial stress resistance and modulate redox homeostasis. As will be detailed in the following subsections, those emerging interventions include lifestyle and pharmacological approaches, both directed towards improving mitochondrial deficits and its consequences (Fig. 8.2).

The agreement that mitochondrial dysfunction and oxidative stress play a central role in ageing and age-related brain pathologies highlights for the need to develop disease-modifying interventions with the ultimate purpose of strengthening mitochondrial stress resistance and modulate redox homeostasis. Presently, those emerging interventions, under the umbrella of mitochondrial medicine, include lifestyle and pharmacological interventions. In the non-pharmacological side physical activity and calorie restriction seem to be the most effective strategies. Considering pharmacological agents, mitochondrial-directed antioxidants, mitochondrial uncoupling and uncoupling compounds as well as mitochondrial transplantation, have been studied as feasible approaches on ageing research.

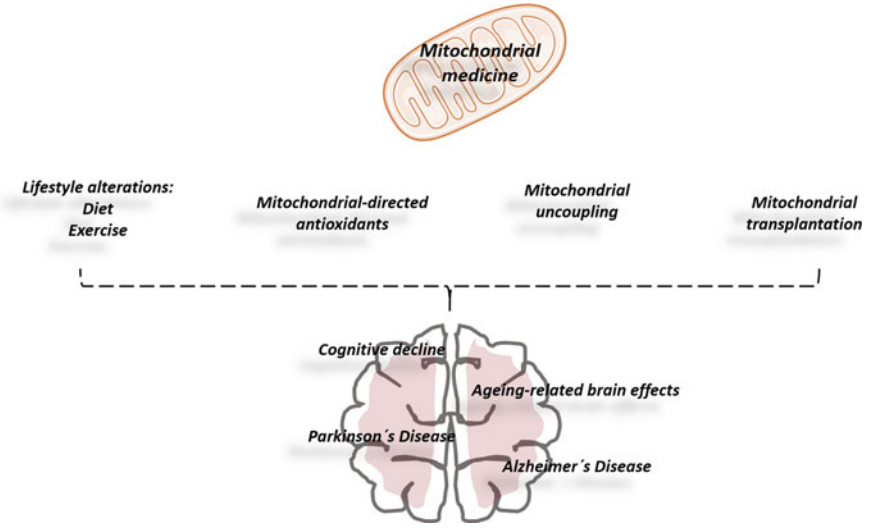

Fig. 8.2 Targeting mitochondria and redox imbalance on brain ageing, Alzheimer's and Parkinson's diseases

8.4.1 Diet and Exercise

A key milestone in the field of ageing research was the observation that restriction of calorie intake prolongs lifespan in a wide range of organisms while improving their health and delaying the appearance of ageing markers. From rodents and short-lived species, including the unicellular yeast, nematodes and invertebrates, to nonhuman primates, several studies support caloric restriction (CR), without malnutrition, as a powerful strategy to extend lifespan and delay the onset of many age-dependent pathologies (Cox et al. 2019; Schafer et al. 2015). Studies in human subjects have also demonstrated that long-term CR could enhance the efficiency of resting energy expenditure, thereby reducing oxidative damage to tissues and organs (Redman et al. 2018). In the context of AD, a prospective epidemiological study provided evidence that individuals with a low calorie intake have a reduced risk of developing AD (Luchsinger et al. 2002). Also, preclinical studies have been shown that CR diminishes AD symptoms and attenuates behavioural deficits (Halagappa et al. 2007; Mouton et al. 2009; Patel et al. 2005). In close agreement, data show that 3xTgAD mice fed with a CR mimetic (2-deoxy-D-glucose, 2-DG) presents an enhancement in brain mitochondrial bioenergetics, a reduction in oxidative stress and the reestablishment of key pathogenic processes in AD (Yao et al. 2011). In a likely manner, original research in PD suggests that CR can lessen the severity of neurochemical deficits and motor dysfunction in a primate model of the disease (Maswood et al. 2004). At the molecular level, CR positive effects seem to rely on the improvement of mitochondrial metabolism, the restoration of oxidative status and resistance to cellular stress, and an increase in mitochondrial biogenesis through activation of the

sirtuin1-PGC1α pathway (Amigo et al. 2017; Lin et al. 2014). As observed, mitochondria adapt to CR conditions by lowering oxygen consumption, ROS generation, and reducing membrane potential without compromising ATP production (López-Lluch et al. 2006). Others also verified that CR or CR mimetics like rapamycin when administered to AD mice models like the 3xTg-AD mice and P301S tau transgenic mice models inhibits mammalian target of rapamycin (mTOR) signalling inducing autophagy and ameliorates tau pathology (Majumder et al. 2011; Caccamo et al. 2010; Ozcelik et al. 2013). Further evidence suggests that CR is capable of upregulating proteolytic systems as well as maintaining autophagy at homeostatic levels in aged α-synuclein-expressing cells (Sampaio-Marques et al. 2018).

Alongside to dietary habits, it is now recognized that regular exercise is an important tool for enhancing both affect and cognitive performance regardless of age (Hogan et al. 2013), whilst physical inactivity and a sedentary lifestyle are considered significant risk factors to develop dementia and neurodegeneration (De la Rosa et al., 2020; Laurin et al. 2001; Radak et al. 2010). In this scenario, epidemiological studies suggest that simple lifestyle alterations may offer an easy approach to enhance brain health in ageing and to slow the onset and progression of AD (Pope et al. 2003). From animals' models of AD, data obtained show that physical exercise is able to inhibit the development of AD-like neuropathology and to improve cognitive behaviour (Garcia-Mesa et al. 2011; Ryan and Kelly, 2016). Concomitantly, mounting evidence from epidemiological data (Chen et al. 2005; Xu et al. 2010), randomized clinical trials (Feng et al. 2019; Kwok et al. 2019), and preclinical studies (Lai et al. 2019; Lau et al. 2011) support a link between different modalities of exercise and alleviation of PD motor symptoms while also improving a range of non-motor symptoms of the disease (Reynolds et al. 2016). Interestingly, it was also found that those benefits are strongly associated with mitochondrial phenotypic changes (Bernardo et al. 2016; Jang et al. 2018) such as improved biogenesis (Jang et al. 2018) and mitochondrial morphology and dynamics (Gusdon et al. 2017; Li et al. 2019), increased activity of mitochondrial respiratory chain complexes (Navarro et al. 2004) and expression of UCP2 (Dietrich et al. 2008), decreased expression/activation of several pro-apoptotic proteins (Cho et al. 2010; Um et al. 2008), and an increased antioxidant capacity (de Sousa et al. 2017).Those mitochondrial adaptations underlie the propitious effects of exercise against brain insults.

From the aforementioned studies, it is suggested that lifestyle interventions, through its ability to modulate and boost mitochondrial resistance, are suitable non-pharmacological approaches to protect or reduce brain ageing and associated diseases.

8.4.2 Antioxidant-Based Therapies

The general assumption that decelerating age-related increases of ROS would be a potent strategy for anti-ageing interventions conducted to an immense research on the antioxidants field and a massive search for dietary supplements, mainly rich in antioxidants, such as vitamin A (retinoids, carotenes), vitamins C and E (tocopherols), lycopene, lutein, ubiquinone, glutathione, polyphenols (flavonoids), resveratrol, and N-acetylcysteine (Sadowska-Bartosz and Bartosz 2014). However, the evolving research advances from preclinical studies to subsequent clinical trials with several of those low-molecular weight antioxidants soon reveal some caveats and did not confirmed the outcomes achieved by preclinical data (Kamat et al. 2008). Numerous variants are now recognized, e.g. the optimal dose; moment of administration, and it has become widely accepted that free radicals have essential physiological roles and are not uniformly harmful (Swerdlow 2009). Under those circumstances, antioxidant-based therapy has evolved with the creation of several mitochondrial-targeted compounds designed to directly reach mitochondria and boost mitochondrial function. In this context, MitoQ, one such mitochondria-targeted antioxidant, has gained prominence with numerous in vitro and in vivo studies demonstrating its protective effects in normal and pathological conditions (McManus et al. 2011; Ng et al. 2014). For instance, MitoQ-treated 3xTg-AD mice showed improved cognitive and neuropathological symptoms compared to untreated 3xTg-AD mice as well as reduced brain oxidative stress, synapse loss, astrogliosis and microglial cell proliferation (Young and Franklin 2019). Also, MitoQ positive outcomes were described in aged rat brain by observing that this compound mitigates peroxynitrite-mediated mitochondrial dysfunction and provides better protection compared to other free radical scavengers and antioxidants (Maiti et al. 2018). Likely, MitoQ was shown to stabilize mitochondrial morphology and function in in vitro and in vivo models of PD (Solesio et al. 2013; Xi et al. 2018). Importantly, a double-blind, placebo-controlled study did not find any positive effects of MitoQ in the progression of PD (Snow et al. 2010). As discussed, antioxidant administration during clinical trials must consider the ideal time-window to begin the treatment, which is often initiated too late in the course of the disease (Persson et al. 2014; Snow et al. 2010). Presently, MitoQ is being clinically assessed in patients suffering from MCI to examine its effects on carotid artery vasodilator function and cerebrovascular blood flow; outcomes will be revealed by the end of 2021 (NCT03514875).

MitoApo, alike to MitoQ, is the conjugated form of the organic compound apocynin with triphenylphosphonium (TPP^+). This newly synthesized mitochondria-targeted compound was found to have strong antioxidant and neuroprotective effects in both cell culture and animal models of PD. MitoApo treatment improved behavioural, mitochondrial and inflammatory processes in MitoPark and MPTP-mice, in addition to protection from MPP^+-induced dopaminergic neurodegeneration in primary mesencephalic neuronal cultures (Langley et al. 2017; Ghosh et al. 2016).

Another example of small mitochondria-targeted antioxidant molecules is the Szeto-Schiller (SS) peptides. SS31, a small water-soluble peptide, is found to cross

the BBB and have the ability to reach neuronal mitochondria conferring neuronal cells protection against several insults. For instance, SS31 has shown positive effects in restoring mitochondrial transport and synaptic viability, and decreasing the percentage of defective mitochondria in primary neurons from Tg2576 mice (Calkins et al. 2012). Similarly, SS31 treatment rescued learning and memory deficits of 10 month-old SAMP8 mice (Jia et al. 2016). Of note, in in vitro models of AD the combined treatment of SS31 and a mitochondrial fission inhibitor (Mdivi1) showed to be more effective than individual treatments to reduce mitochondrial and Aβ-induced toxicities (Reddy et al. 2018). Furthermore, data from an in vivo PD model showed significant neuroprotective effects of SS peptides on dopaminergic neurons of MPTP-treated mice (Yang et al. 2009). More recently, an interesting study reported on the ability of SS31 in reversing the negative neurological consequences of short-term sleep deprivation in ageing mice (Wu et al. 2020). As observed, in sleep deprived mice, treatment with SS31 was able to prevent learning impairments, to preserve hippocampal mitochondrial integrity and synaptic function and to decrease inflammation markers in the hippocampus (Wu et al. 2020).

A different approach using nano-based technologies and diverse nanocarrier delivery systems have demonstrated the successful neuronal delivery of antioxidants like N-acetylcysteine (Mursaleen et al. 2020), curcumin (Mathew et al. 2012), and resveratrol (Neves et al. 2016), which protect against oxidative stress in cellular and animal models of neurodegenerative disorders like AD and PD. As disclosed, nano-engineered platforms represent a promising alternative to circumvent the low bioavailability of bioactive compounds and its poor delivery into CNS due to the blood–brain-barrier, which causes inefficient delivery of bioactive compounds (Babazadeh et al. 2020).

Growing evidence suggests that the targeted delivery of antioxidants and/or compounds with antioxidant properties is a feasible strategy to achieve high concentration within cells and, particularly mitochondria, thereby protecting cells and tissues from oxidative damage through different mechanisms (Jiang et al. 2020). Even though additional studies are needed in order to fully understand the limitations and prospects for using such approaches in ageing and ageing-related brain diseases, the recent positive outcomes from in vitro and in vivo studies may possibly pave the way for further trials design and drug development.

8.4.3 Mitochondrial Uncoupling

Besides natural occurring antioxidants, mitochondria possess endogenously regulated proteins that are closely involved in limiting oxidative injury to cells, the uncoupling proteins (UCPs) (Cardoso et al. 2015). As the name suggests, UCPs are a family of mitochondrial anion-carrier proteins located on the inner mitochondrial membrane, with a primary function of leaking protons from the intermembrane space into the mitochondrial matrix, which uncouples ATP synthesis from electron transport (Ježek et al. 2018). During cellular respiration, the production of $O_2^{\bullet -}$ is

highly dependent on the proton motive force (Δp) created by the efflux of H^+ to the intermembrane space. UCPs, by promoting a mild mitochondrial uncoupling, allow the controlled dissipation of the mitochondrial membrane potential mildly reducing the driving force for ATP synthesis and mitigating ROS production (Kim-Han and Dugan 2005). Although the activation of UCPs can result in modest decreases in the amount of ATP produced by individual mitochondria, strikingly, overall cellular ATP pools may be maintained or even increased (Geisler et al. 2017). In fact, according to the "uncoupling to survive" hypothesis, mild mitochondrial uncoupling, through its effects on metabolic rate and ROS production maintain sufficient ATP production, reduce oxidative damage to DNA, proteins and lipids, being discussed as a potential mechanism to extend lifespan (Brand 2000). Using UCP2 knockout (KO) mice, it was found that UCP2 deficiency shortens mice lifespan compared to wild-type (WT) mice (Andrews and Horvath 2009), probably due to an accelerated ageing process throughout their entire lifespan (Hirose et al. 2016). In further support, previous data reported on the correlation between variants in UCPs genes (UCP2, UCP3 and UCP4) and the longevity rate of humans (Rose et al. 2011).

With five different isoforms, UCPs physiological role seems to depend on their specific location. Within the brain, UCPs (UCP2, UCP4 and UCP5) have recognized roles in neuroprotection and neuromodulation mainly through the modulation of ROS generation (Mehta and Li 2009; Ramsden et al. 2012). For instance, UCP2 protected primary neuronal cultures against Aβ-induced toxicity and oxidative stress (Jun et al. 2015) whereas, in brain biopsies of AD patients all three brain-specific UCPs (UCP2, UCP4 and UCP5) were decreased relative to control brains with the mean levels showing a tendency to be lower in brains with advanced AD (de la Monte and Wands 2006). More recently, an intronic variant of the neuronal UCP4 (UCP4/SLC25A27) gene was identified as affecting the risk of late-onset AD and late-onset sporadic cases of frontotemporal dementia (Montesanto et al. 2016). Also, preclinical studies in animal models of PD revealed that UCP2 overexpression provides protection in MPTP-treated mice by increasing mitochondrial uncoupling and decreasing ROS production in dopaminergic neurons of substantia nigra whilst UCP2 knockout increases dopamine neurons sensitivity to MPTP (Andrews et al. 2005). In the same line, it has been shown that the silencing of UCP5 in SH-SY5Y cells exposed to the mitochondrial toxin MPP^+ exacerbates oxidative stress and cell death without affecting the levels of UCP2 and UCP4 (Ho et al. 2006). Also, posterior studies showed that the in vitro overexpression of UCP5 protects SH-SY5Y cells against MPP +- and dopamine-induced toxicity by preserving mitochondrial function and attenuating ROS production (Kwok et al. 2010). Interestingly enough, UCP4 and UCP5 expression seems to contribute for an improved mitochondrial functionality preservation of female rat brains versus male brains during the ageing process (Guevara et al. 2009, 2011). As disclosed, in comparison to males, aged female rat brains present a better oxidative homeostasis maintenance due to an increased activity of antioxidant enzymes, higher mitochondrial respiratory chain function and increased levels of UCP4 and UCP5 (Guevara et al. 2009, 2011), which ultimately may account for an increased neuroprotection during ageing.

Away from the use of transgenic mice and modified cell lines, fundamental research has looked into artificially uncouplers able to mimic UCPs-mediated brain effects. One well-known example is 2, 4-dinitrophenol (DNP). Formerly used in the clinic as a treatment for obesity, DNP, when used at low doses, induces a mild mitochondrial uncoupling able to reduce oxidative damage in different neuropathological conditions (De Felice and Ferreira 2006; Jin et al. 2004; Wu et al. 2017). It has been reported that DNP can cross the blood–brain barrier (Perry et al. 2013) and, when administered systemically, can act directly on neurons to cause mitochondrial uncoupling and increase cellular resilience, similar to caloric restriction or moderate exercise (Caldeira da Silva et al. 2008). More recently, an in-deep analysis of the underlying neuroprotective mechanisms of DNP revealed that the peripheral administration of low doses of DNP induces several adaptive responses in the main intracellular signalling pathways including inhibition of the mTOR and insulin signalling pathways, and activation of autophagy, class O of forkhead box transcription factors 3a (FoxO3a), Ca^{2+}, CREB and brain-derived neurotrophic factor (BDNF) signalling, ultimately improving mice behaviour performance (Liu et al. 2015). In this line of investigation, recent findings show neuroprotective effects of DNP in the MPTP-induced PD model (Lee et al. 2017). As the authors observed, DNP treatment was able to reduce dopaminergic neuronal cell death and improve functional outcomes; being these effects associated with the modulation of adaptive stress response including NRF2 activation, and with the reestablishment of mitochondrial membrane potential and ROS production levels (Lee et al. 2017). In close agreement, Kishimoto and colleagues (2020) using the 6-OHDA model of PD found that DNP and a novel DNP prodrug (MP201) improve dopaminergic neuronal loss and animals' motor function; outcomes that were associated with reduced microglial and astrocyte reactivity in the substantia nigra and striatum of PD animals. Concomitantly, DNP at low doses ($\leq$20 μM) is able to stimulate neuritogenesis in cultured hippocampal and cortical neurons and to induce neuronal differentiation in mouse neuroblastoma cell line N2A (Wasilewska-Sampaio et al. 2005). Also, co-incubation of nitrophenols like DNP with $A\beta_{1\text{-}42}$ blocked Aβ-toxicity in primary cultures of hippocampal neurons, and when administered in vivo to an AD rat model promoted a reduction in the volume of Aβ deposits in rat brains (De Felice et al. 2001). Although more studies are warranted to give further enlightenments on this, it is certainly interesting to see DNP repurposing potential as a disease-modifying drug in brain pathological contexts.

8.4.4 Mitochondrial Transplantation

Alongside with conventional therapies, research on mitochondria have turned out their potential application in medicine with a novel paradigm of therapeutic intervention. This emerging strategy relates with mitochondrial transplantation. In this process, functional exogenous mitochondria are transferred into mitochondria-defective cells for recovery of the cell viability and, consequently, prevention

and/or reversion of the disease onset and/or progress (Park and Hayakawa 2021; Shanmughapriya et al. 2020). In a pioneering clinical study, mitochondrial transplantation was performed on five paediatric patients undergoing extracorporeal membrane oxygenation support following myocardial ischemia and reperfusion (Emani et al. 2017). In this trial, mitochondrial transplantation procedure involved injection of isolated mitochondria from the abdominal muscles into dysfunctional myocardium. Authors have observed an improvement in myocardial function within 24–48 h following mitochondrial transplantation (Emani et al. 2017). Transposing this approach to the CNS, pivotal studies have showed that brain mitochondria can be transferred from one cell to another cell as a naturally occurring physiological process, also termed mitochondrial exchange. For example, astrocytes are found to transfer healthy mitochondria to stressed neurons promoting neuroprotection and neurorepair (Hayakawa et al. 2016; Huang et al. 2019). Also, the extracellular transfer of endothelial progenitor cells (EPCs)-derived mitochondria to brain endothelial cells after an oxygen–glucose deprivation insult was found to improve receptor cells' health restoring endothelial tightness and angiogenic function (Hayakawa et al. 2018). Considering those proof-of-concept studies, a new paradigm of mitochondrial therapy based on the delivery of exogenous mitochondria was tested for brain injury or disease. In a PD mice model, intravenous supplement of functional mitochondria isolated from human hepatoma cells prevented experimental PD progress by increasing the activity of ETC, decreasing ROS level, and preventing cell apoptosis and necrosis (Shi et al. 2017). Of note, authors observed that after systemic injection, exogenous mitochondria reached different tissue cells, including the brain with high efficiency (Shi et al. 2017). Thus suggesting that this route of administration of mitochondria may be a viable strategy to deliver mitochondria not only to peripheral organs but also to CNS. Another study using Pep-1-conjugated mitochondria (a cell-penetrating peptide) injected into the medial forebrain bundle (MFB) of PD rat models resulted in positive functional outcomes and restoration of mitochondria function of dopaminergic neurons in substantia nigra (Chang et al. 2016). In parallel, mitochondrial transplantation was evaluated in a mouse model of AD achieved with the intracerebroventricular infusion of $A\beta_{1-42}$ (Nitzan et al. 2019). To do so, freshly isolated mitochondria from HeLa cells was delivered in a single intravenous injection to AD-mice; positive outcomes were observed with the improvement of cognitive functions, reduction of neuronal loss and gliosis and reestablishment of mitochondrial functional parameters (Nitzan et al. 2019). Interestingly, imaging and histological staining was able to detect exogenous mitochondria in the mice liver but not in the brain. Further, mitochondrial enzymatic activities were also restored in the liver, prompting authors to suggest the existence of a crosstalk between brain-liver that could underlie the improvement of AD-related deficits (Nitzan et al. 2019). Recently, Zhao et al. (2020) reported on the positive outcomes achieved with the intravenous administration of freshly isolated mitochondria from young mouse liver into aged mice. As observed, mitotherapy significantly improved cognitive and motor performance of aged mice whilst ATP content in mouse brain increased and ROS levels decreased. Besides, heterozygous mtDNA of both aged and young mouse was found to coexist in brain tissue of aged mice after mitochondrial administration (Zhao et al.

2020), suggesting that young mitochondria could contribute to boost mitochondrial function of aged tissues. In a different context, platelet-derived mitochondria was detected in hippocampal neurons 24 h following its intracerebroventricular injection in db/db mice, a model of diabetes (Ma et al. 2020). Concurrently to mitochondrial internalization to hippocampal neurons, diabetes-associated cognitive impairment was alleviated accompanied by restored mitochondrial function as well as decreased accumulation of Aβ (Ma et al. 2020). Using mitochondria isolated from healthy mesenchymal stem cells, Alexander et al. (2021) hypothesized that nasal administration of mitochondria to mice could reverse cisplatin-induced cognitive deficits. Data obtained showed that mitochondria delivered by nasal route is able to reach to brain meninges and enter the brain parenchyma, reversing cisplatin-induced brain mitochondrial abnormalities and restoring both brain structure and function (Alexander et al. 2021). Being the first report of nasally delivered mitochondria, this study highlights this simple and non-invasive route of delivery as a way to facilitate the delivery of mitochondria to the brain thereby reducing the risk for adverse effects and the need to cross the blood–brain and blood-cerebrospinal fluid barriers as well as the amount of mitochondria required to achieve desired outcomes.

As mitochondrial transplantation is being brought to the spotlight with more studies utilizing mitochondrial transplantation for therapeutics in various pathophysiological contexts, it should be noted that several issues still need to be resolved to improve this novel paradigm. For instance, the optimal source of mitochondria and the method of mitochondrial isolation, route of administration, fate tracking within body tissues, the mechanistic aspects of transfer and action, and the safety and effectiveness of therapeutic mitochondria are all important factors needed to be deeply investigated. Future research will certainly provide further enlightenment on this contributing to substantiate this approach.

8.5 Concluding Remarks

Increase in life expectancy is one of the highest accomplishments of humankind. Nevertheless, this situation also presents significant challenges. Economic, social, and healthcare costs are amongst the main consequences of an ageing society. The recognition that the human population is largely heterogeneous with different diseases predisposition, lifespan and response to treatments, difficult the identification and current understanding of factors contributing to the complexity of the ageing process. In this chapter, we have attempted to give an overview on the importance of mitochondria and redox balance in both physiological and pathological brain ageing. The inherent need of brain for high energy supply dictates its dependence on functional mitochondria and also renders it sensitive to changes in these organelles. Many interventions have shown encouraging results in pre-clinical models of ageing and age-related brain diseases characterized by dysfunctional mitochondria and elevated oxidative stress. Nonetheless, most of them still have to fully achieve their promises in ageing humans. With the advent of clinical trials using new

approaches like transplanted isolated mitochondria, future studies should dictate the success of mitochondrial medicine on ageing research.

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Conflict of Interest
All authors declare they have no conflict of interest.

Ethical Approval
This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 9
Importance of CoQ_{10}-dependent Redox Activity in Aging

Guillermo López-Lluch

Abstract Coenzyme Q (CoQ) is a ubiquitous lipidic molecule located in all cell membranes and lipoproteins in blood plasma. Its phenolic head gives it the capacity to accept and donate electrons. For this reason, it is the main lipidic redox compound in cells. This property permits CoQ to take part in the mitochondrial electron transfer chain and in many other activities in mitochondria, and also to act as the major antioxidant in the lipid environment of the organism. During aging, the endogenous synthesis of CoQ decreases. Due to its central function, this decrease can affect many metabolic, signaling, and antioxidant activities, probably severely influencing aging progression. Further, CoQ depletion has been also associated with many aging-associated and metabolic diseases. This chapter revises the importance of this key molecule in cell physiology and aging progression.

Keywords Aging · Longevity · Disease · Coenzyme Q · Ubiquinone · Mitochondria · Antioxidant · Oxidative stress

9.1 Introduction

Coenzyme Q (CoQ) is a ubiquitous molecule with lipid nature made by a quinone-head bound to a polyisoprenoid chain. This chain is variable in length depending on the organism. This isoprene chain has ten units in humans (CoQ_{10}), mainly nine and in minor proportion ten in rodents (CoQ_9 and CoQ_{10}), nine in *Caenorhabditis elegans* worms (CoQ_9), eight in *Escherichia coli* (CoQ_8), and six in *Saccharomyces cerevisiae* (CoQ_6). In this chapter, CoQ_{10} will be used to describe coenzyme Q in human studies, whereas the term CoQ will be used as an indication of all the forms of CoQ. CoQ is mainly located in mitochondria where it is synthesized (Fernandez-Ayala et al. 2005a, b). CoQ is also found in all cell membranes, blood plasma lipoproteins, and seminal fluids.

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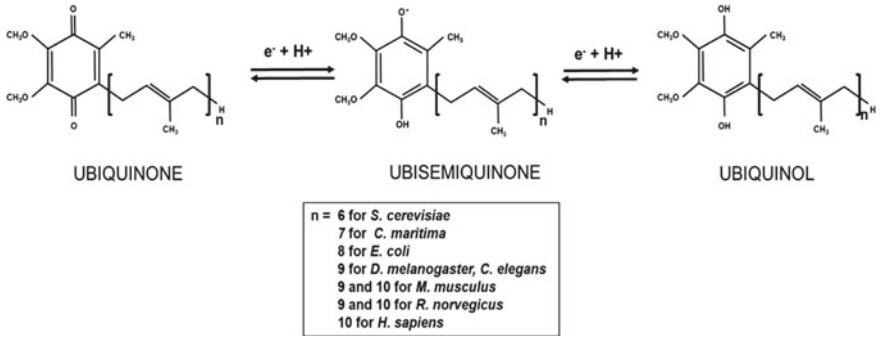

Fig. 9.1 CoQ redox cycle in cell membranes and plasma lipids

In the organism, CoQ is present in oxidized form (CoQ or ubiquinone), in a short-living semi-reduced form (CoQ^{-} or ubisemiquinone) or in a completely reduced form, ($CoQH_2$ or ubiquinol) (Fig. 9.1). To maintain this redox cycle, CoQ is reduced by different reductases. Ubiquinol is oxidized back by transferring its electrons to oxidases or other lipidic compounds such as peroxidized lipids or α-tocopherol located in the membranes.

In recent years, the redox cycle of CoQ has been extensively studied in many cells and organs in relationship with different metabolic diseases associated with mitochondrial deficiency, such as metabolic syndrome, diabetes, hypertension, obesity, and also chronic diseases such as cardiovascular or neurodegenerative diseases among others. An increasing body of evidence indicates that CoQ can play an important role not only in the activity of mitochondria, but also in the avoidance of oxidative damage in cell membranes and plasma lipoproteins and in the regulation of cell survival through inhibiting apoptotic signaling. Further, CoQ levels in mitochondria and in the whole cell can decrease during aging and chronic diseases associated with age and metabolic dysfunction (Lopez-Lluch et al. 2010). For this reason, balanced levels of CoQ and also its redox ratio can be considered biomarkers of mitochondrial function and membrane-linked antioxidant activities in health and disease.

This chapter summarizes some of the most relevant aspects of CoQ redox activity in aging and age-related diseases with special focus on the extramitochondrial activities of CoQ in cell membranes.

9.2 CoQ is Essential for Mitochondrial Activity

CoQ is an indispensable molecule for the physiology of mitochondria. In this organelle, its main function is to transport electrons through the mitochondrial electron transport chain (mETC). Its key role is clear since depletion of CoQ_{10} levels in humans, due to mutations in any of the members of the synthesis machinery, ends in severe mitochondrial diseases with complex clinical manifestations, mainly affecting

children, that include encephalomyopathy, myopathy, cerebellar ataxia, multisystem disease, and nephropathy (Alcazar-Fabra et al. 2021).

In the mETC, CoQ transfers electrons from complexes I and II to complex III (Lopez-Lluch et al. 2018) contributing to the creation of the proton electrochemical gradient needed for the synthesis of adenosine triphosphate (ATP) and many other activities in mitochondria (Turunen et al. 2004).

CoQ is also a structural component of Complexes I and III and it has been demonstrated in *S. cerevisiae* that CoQ is crucial for reaching the right conformation of Complex III (Padilla et al. 2004). Further, recent studies have demonstrated that CoQ is also a critical factor for stabilizing and controlling the assembling of mETC complexes in supercomplexes (Lapuente-Brun et al. 2013; Guaras et al. 2016). In fact, mitochondrial CoQ can be segmented in two pools, one attached and immersed in the supercomplexes I + III, and a free pool available for complex II and other enzymes that use CoQ as an electron acceptor or donor (Hidalgo-Gutiérrez et al. 2021). Interestingly, the assembly of supercomplexes is affected during aging (Milenkovic 2017). Probably, a decrease in CoQ levels in mitochondria during aging could be partially responsible of this effect (Lopez-Lluch et al. 2010).

Mitochondrial CoQ not only receives electrons from mitochondrial electron transport chain complexes, but can also be reduced to ubiquinol by several reductases involved in many other metabolic functions. In any case, reduced CoQ is mainly oxidized back by transferring electrons again to complex III of the mETC. Further, ubiquinol can be also oxidized through interacting with endogenous antioxidants such as α-tocopherol or acting directly in the prevention of lipid peroxidation (Mellors and Tappel 1966).

Apart from the activity in the mETC, mitochondrial CoQ also acts as a redox intermediate in many other mitochondrial activities such as:

- Dihydroorotate dehydrogenase (DHODH): a flavin adenine dinucleotide (FAD)-linked oxidoreductase located on the outer surface of the inner mitochondrial membrane. DHODH catalyzes the synthesis of pyrimidine nucleotides by oxidizing dihydroorotate to orotate using ubiquinone as the final electron acceptor (Evans and Guy 2004).
- Mitochondrial glycerol-3-phosphate dehydrogenase (mGAPDH): is a FAD-linked oxidoreductase located at the outer leaflet of the mitochondrial inner membrane. This enzyme connects glycolysis with oxidative phosphorylation and fatty acid metabolism (Rauchova et al. 1992; Mracek et al. 2013).
- The electron transport flavoprotein ubiquinone oxidoreductase (ETF-Q_0): a membrane iron–sulfur enzyme located at the matrix site of the inner mitochondrial membrane involved in fatty acid β-oxidation and the catabolism of several amino acids. This enzyme transfers electrons from acyl-CoA and N-methyl dehydrogenases to oxidized CoQ (Watmough and Frerman 2010; Henriques et al. 2021).
- Proline dehydrogenases 1 and 2 (PRODH): two oxidoreductases located in the inner leaflet of the inner mitochondrial membrane involved in the proline

and arginine metabolism by reducing ubiquinone through oxidizing proline or hydroxyproline (Summitt et al. 2015).

- Sulfide: quinone oxidoreductase (SQR): a FAD-linked oxidoreductase located at the inner mitochondrial membrane. This enzyme catalyzes the oxidation of H_2S to S_0 reducing ubiquinone as the final acceptor of electrons (Ziosi et al. 2017)
- CDGSH iron–sulfur domain protein 1 (MitoNEET): a recently discovered iron–sulfur protein located at the mitochondrial outer membrane that transfers electrons from reduced flavin mononucleotide to ubiquinone (Tasnim et al. 2020).
- Mitochondrial uncoupling proteins (UCPs) are proton channel proteins located in the inner mitochondrial membrane, essential to dissipate mitochondrial chemiosmotic gradient, an key function to control ROS production in mitochondria. In this function, oxidized CoQ, controls the activity of UCP1 in mitochondria (Echtay et al. 2000, 2001).
- Voltage-dependent anion channel (VDAC1): is located in the mitochondrial outer membrane. Its presence has also been suggested in the plasma membrane (De Pinto et al. 2010). Binding of CoQ through its quinone-head ring can regulate its $Ca2^+$-dependent opening in mitochondria (Murai et al. 2017). In the plasma membrane, its activity as an oxidoreductase has also been suggested (Baker et al. 2004).

It is clear that mitochondrial CoQ is key in the activity of many different enzymatic processes involved in both, catabolism and anabolism. This fact puts CoQ in the center of the control of cell metabolism probably as a metabolic regulator.

As it has been mentioned before, a pool of CoQ can be found attached and engulfed by the supercomplexes I + III. These supercomplexes oxidize the mitochondrial NADH. Another CoQ pool is found free in the membrane interacting with many other enzymes (Lapuente-Brun et al. 2013). Both pools can interact with each other. The increase of the metabolism using the free CoQ pool, for example, β-oxidation of lipids, can generate an increase in the level of ubiquinol free in the membrane that would transfer electrons in a reversed flux to Complex I. This reaction would restore the levels of oxidized CoQ to maintain other metabolic activities. However, it is widely known that in this reverse electron transport, the production of superoxide increases through Complex I (Lapuente-Brun et al. 2013; Scialo et al. 2016). Interestingly, the increase of superoxide could lead to the degradation of Complex I that produces the release of Complex III and the CoQ pool associated with these supercomplexes that would be used in the metabolism of the activities that do not require mitochondrial NADH as an electron source (Hernansanz-Agustín and Enríquez 2021).

The different pools in mitochondria converts the redox CoQ ratio, reduced/oxidised, as a sensor of the metabolic status of the mitochondria (Guaras et al. 2016) and an important factor in the production of reactive oxygen species (ROS) through Complex I. The regulation of ROS production through the redox ratio of CoQ can thus serve as a mechanism of control of the antioxidant response adjusted to the metabolic activity of the cell (Reczek and Chandel 2015; Hernansanz-Agustín and Enríquez 2021). In fact, the ratio of ROS production by this mechanism has been associated with the differentiation of myoblasts to myotubes (Lee et al.

2011); the metabolic shift from carbohydrates to fatty acids (Guaras et al. 2016); the metabolic reprogramming of macrophages to respond to bacterial infection (Mills et al. 2016); the control of physiological levels of oxygen through the carotid body (Fernández-Agüera et al. 2015); the control of the oxidative damage occurring after reperfusion of infarcted heart (Milliken et al. 2020); or in the control of hypoxic signaling (Hernansanz-Agustin et al. 2020).

Further, CoQ is also essential to prevent lipid peroxidation in all cell membranes and in plasma lipoproteins. This antioxidant activity is important also in mitochondrial membranes. In fact, mETC-dependent lipid peroxidation is controlled through the levels of ubiquinol, adding an essential function of CoQ to mitochondrial protection (Takayanagi et al. 1980) even in the absence of α-tocopherol (Mellors and Tappel 1966). Further, a reduced CoQ-dependent antioxidant activity in the mitochondrial membrane has been associated with aging, especially in postmitotic tissues (Ochoa et al. 2003).

All these CoQ-linked activities and functions convert CoQ_{10} levels in mitochondria into a key factor involved in the control of many different mitochondrial activities. Therefore, a decrease of CoQ_{10} levels during human aging can strongly disturb many of these activities increasing importantly the mitochondrial dysfunction associated with aging (Lopez-Lluch et al. 2018).

9.3 Extramitochondrial CoQ_{10}, the Forgotten Key Function

9.3.1 Antioxidant Activity in Cell Membranes

CoQ is not only essential for mitochondrial physiology (Hernandez-Camacho et al. 2018; Lopez-Lluch 2021), but it also acts as the most powerful antioxidant in cell membranes and in plasma lipoproteins (Mellors and Tappel 1966; Takayanagi et al. 1980). A considerable amount of literature demonstrates that CoQ protects all the organic macromolecules from oxidative damage (Maroz et al. 2009; Godic et al. 2014). Ubiquinol prevents both the beginning and the dissemination of lipid peroxidation in cell membranes (Bentinger et al. 2007; Maroz et al. 2009) and also in plasma lipoproteins (Thomas et al. 1997; Sabbatinelli et al. 2020) and in seminal fluid (Alleva et al. 1997) (Fig. 9.2).

In addition to its direct activity as an antioxidant, CoQ in membranes also plays a central antioxidant role by regenerating other main antioxidants such as α-tocopherol or ascorbic acid (Barroso et al. 1997a; Navarro et al. 1998; Lopez-Lluch et al. 2010). This antioxidant activity is maintained through dehydrogenases that transfer electrons from cytosolic NAD(P)H to oxidized CoQ maintaining the CoQ redox cycle. In cells, we can consider two main dehydrogenases: Cytochrome b_5 reductase (CyB5R3) (Villalba et al. 1997) and NAD(P)H quinone dehydrogenase 1 (NQO1) (Takahashi et al. 2008). In the case of plasma lipoproteins it has been recently found a new dehydrogenase, located at the outer surface of hepatocytes' plasma membrane,

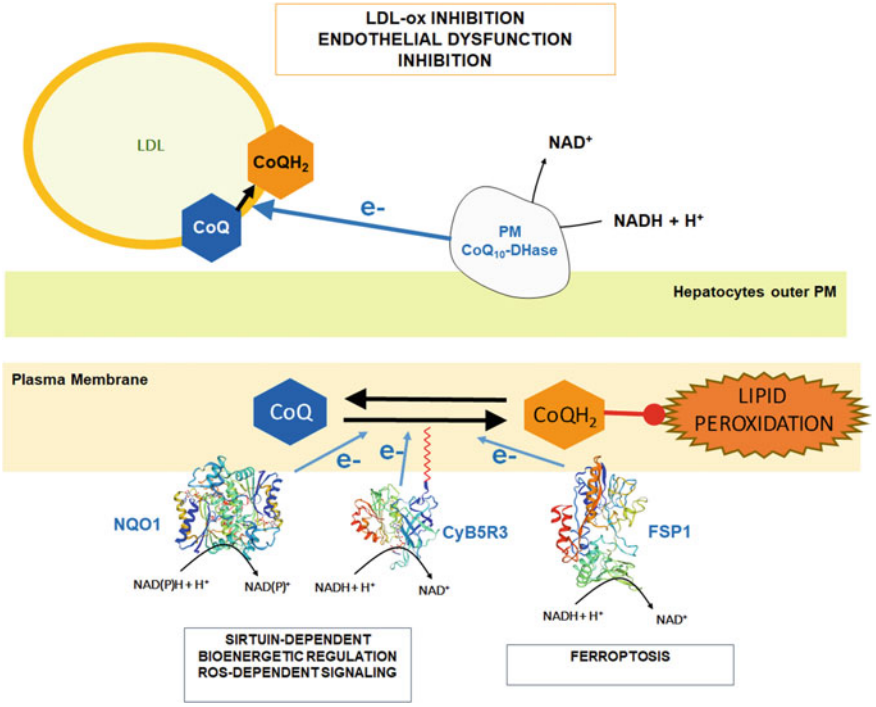

Fig. 9.2 CoQ-dependent antioxidant activities in the plasma membrane. CoQ plays a key role in the protection of membrane lipids against lipid peroxidation and by this mechanism inhibits apoptosis and ferroptosis. Further, CoQ oxidoreductases also maintain a high redox ratio of CoQ in plasma lipoproteins preventing oxidative damage in endothelium and the progression of atherosclerosis. Further, the activity of these enzymes also modulates ROS-dependent signaling and metabolism through sirtuins activation

responsible of the maintenance of the high proportion of reduced/oxidized CoQ_{10} (Takahashi et al. 2019).

9.3.2 Extramitochondrial CoQ Protects Against Apoptosis.

CoQ-dependent oxidoreductases such as CyB5R3 and NQO1 translocate to the plasma membrane after serum deprivation in a response associated with the increase of survival in these stressing conditions (Forthoffer et al. 2002). Other studies demonstrated that NQO1 overexpression protected cells against many other stressors such as energetic stress induced by 2-deoxyglucose or potassium cyanide or proteotoxic stress by incubating with lactacystin, a proteasome inhibitor (Hyun et al. 2012). In this case, no protection against oxidative stress was found. On the contrary, a decrease in the levels of NQO1 by RNA interference increased the vulnerability to death under

energetic or proteotoxic stress indicating a central role of NQO1 in the control or survival of cells under stress conditions.

CoQ_{10} in cell membranes prevents the activation of apoptotic signaling under stress conditions. In this process, CoQ_{10} avoids the release of ceramides and reduces cell death (Barroso et al. 1997b). In this role, CoQ_{10} acts in combination with known antioxidants such as α-tocopherol or ascorbic acid (Barroso et al. 1997a). Moreover, substantial production of superoxide, both intracellularly by mitochondria or through intracellular redox activities or extracellularly by NADPH oxidases found in inflammatory cells, can produce cell damage. Accumulation of cell damage can be eluded by the tandem CoQ/α-tocopherol in cell membranes (Stoyanovsky et al. 1995).

9.3.3 Membrane CoQ Protects Against Ferroptosis.

CoQ-dependent oxidoreductases also play an important role in the regulation of ferroptosis. Ferroptosis is an iron-dependent necrotic cell death caused by lipid peroxidation of polyunsaturated phospholipids of cell membranes (Yan et al. 2021). Recently, ferroptosis suppressor protein 1 (FSP1), a former protein found as apoptosis-inducing factor mitochondrial 2 (AIFM2, AIF2) located in mitochondria, has been shown to be a CoQ_{10}-dependent oxidoreductase that translocates to the plasma membrane. In the plasma membrane, this enzyme plays an important role in the protection against ferroptosis (Bersuker et al. 2019; Doll et al. 2019). Although the exact role of CoQ_{10} in FSP1-dependent protection against ferroptosis remains to be clearly established, it seems that the translocation of FSP1 to the membrane can reinforce the CoQ-dependent protection of plasma membrane against lipid peroxidation under stress conditions (Bersuker et al. 2019; Nehring et al. 2020; Yan et al. 2021). Interestingly, overactivation of CyB5R1 or NADPH-cytochrome P450 reductases could increase ROS production contributing to ferroptosis (Yan et al. 2021), whereas activation of FSP1 and NQO1 blocks it (Bersuker et al. 2019). It seems that the balance in the mechanisms involved in the maintenance of CoQ redox cycle in the membrane is important in the regulation of this cell death process (Stockwell and Jiang 2020; Stockwell et al. 2020).

Ferroptosis has been associated with the progression of neurodegenerative diseases such as Parkinson's disease and the activity of NQO1 or FSP1 would be important in the delay of its progression (Do Van et al. 2016). In fact, plasma membrane redox enzymes have been considered new therapeutic targets for neurodegenerative diseases (Hyun 2019).

9.3.4 CoQ and the Plasma Membrane Redox System.

Extramitochondrial CoQ reductases not only protect cells against lipid peroxidation. These oxidoreductases were discovered as members of an electron transport chain

known as the plasma membrane redox system (PMRS). PMRS activity is involved in the inhibition of sphingomyelinase activity and the release of ceramides (Fernandez-Ayala et al. 2000; Martin et al. 2003), in the control of cell cycle (De Luca et al. 2005), and in the regulation of apoptosis (Barroso et al. 1997a; Li et al. 2019). Further, the activity of these enzymes has also been associated with the regulation of the activity of sirtuins deacetylases by controlling NAD^+/NADH ratio acting as a redox-sensitive molecular switch for metabolic regulation (Ross and Siegel 2017). They can also play a key role in the control of metabolism in mitochondria deficient cells (Gomez-Diaz et al. 1997) and in the regulation of differentiation in neutrophils (Lopez-Lluch et al. 1998, 2001).

CoQ-dependent activities have also been related to the pumping of protons in lysosomes (Gille and Nohl 2000) or with the regulation of the activity of VDAC1 in the plasma membrane (De Pinto et al. 2010). In addition, regulation of VDAC1 activity has also been associated with the control of cell growth and apoptosis.

Due to the central role of CoQ in the activity of these oxidoreductases, levels of CoQ in plasma membrane have been strongly associated with lengthening of survival and longevity. Supplementation with CoQ_{10} enhances the antioxidant protection in the liver plasma membrane of long-lived rats (Bello et al., 2005). Further, supplementation with CoQ_{10} decelerates senescence in a model of accelerated aging in mice, SAMP1 mice (Yan et al. 2006). This supplementation not only protected membranes against oxidative damage, but also protected DNA from damage (Quiles et al. 2005).

All these activities together with the mitochondrial function demonstrate that CoQ is an essential factor for whole cell physiology and its depletion or unbalance during aging or metabolic diseases can be involved in the deterioration of metabolism, cell signaling, survival, and apoptosis in cells.

9.4 Prolongevity Effectors Induce CoQ-Dependent Extramitochondrial Activities

Calorie restriction (CR), is the most known prolongevity intervention successfully affecting many different organisms from yeast to mice and probably humans (Lopez-Lluch and Navas 2016). We determined the effect of CR on the expression and activity of CYB5R3 and NQO1 in plasma membrane from the liver of mice and rats (De Cabo et al. 2004; Lopez-Lluch et al. 2005). Interestingly, in young animals, CR did not affect the activity of these enzymes, but in old animals, an increase in their activity was found. As suspected, this higher activity was accompanied by higher resistance to oxidative damage. Interestingly, the same increase in older animals was found in brain cells (Hyun et al. 2006) in an effect that was later associated with the protection against metabolic and proteotoxic stress (Hyun et al. 2012). In studies performed in the muscle of mice fed with the every-other-day feeding system, mimicking classic CR, the presence of CYB5R3 and NQO1 also increases in old animals, whereas the effect was less clear in young animals (Rodríguez-Bies et al. 2015). Resveratrol,

a polyphenol considered an anti-aging reagent, also regulated the levels of these enzymes in an effect that depended on the organ and the age of mice (Tung et al. 2014, 2015), then, it seems clear that prolongevity effectors regulate CoQ-dependent antioxidant activities.

Accompanying the activity of these enzymes, CR increases the levels of CoQ_{10} in the plasma membrane in the liver of old mice (Lopez-Lluch et al. 2005) and old rats (De Cabo et al. 2004). This increase probably indicates the specific activity of CoQ-dependent antioxidant responses in rodents. Further, only 1 month of CR was able to modify the expression of the enzymes involved in CoQ synthesis and the ratio and levels of CoQ in an organ-dependent mechanism (Parrado-Fernandez et al. 2011) indicating a response of the whole CoQ system to nutritional requirements.

Interestingly, the induction of NQO1 by CR and its mimetic therapies not only affects CoQ-dependent activities, but also many other important cellular functions such as protection against poisonous compounds, modulation of superoxide dismutase activity, stabilization of proteins including p53 and microtubules, control of $NAD(P)^+$ levels, and mRNA translation (For further information, Ross and Siegel 2017, 2021). On the other hand, overexpression of CYB5R3 has been associated with significant improvements in different metabolic parameters associated with lengthening of lifespan in mice (Martin-Montalvo et al. 2016). Transgenic mice overexpressing this CoQ-dependent oxidoreductase not only showed resistance to oxidative damage, but also improved mitochondrial function and a reduced pro-inflammatory profile indicating the importance of this enzyme in the modulation of cell functions.

9.5 CoQ Protects Against Plasma LDL Protection.

In blood plasma, CoQ is located in lipoproteins, mainly in low-density lipoproteins (LDLs), in which it prevents lipid peroxidation with more power than α-tocopherol (Stocker et al. 1991). This fact was demonstrated in experiments of oxidation performed in vitro with lipophilic peroxyl radical generators. CoQ_{10} was the first antioxidant depleted, followed by ascorbate, bilirubin, α-tocopherol, β-carotene, and urate (Neuzil and Stocker 1994). In fact, the ratio, LDL/CoQ_{10} is considered a coronary risk factor (Hanaki 1991).

9.5.1 Treatment with Statins Decrease CoQ_{10} Levels in Plasma

The use of statins to decrease cholesterol synthesis also affects the levels of CoQ_{10} and can be important in the evolution of aging and the increase of oxidation of LDLs in plasma. In fact, the reduction of the amount of CoQ_{10} in LDL has been associated with higher oxidizability of these particles in statin-treated hypercholesterolemic

men (Palomaki 1997). Thus, the use of exogenous CoQ_{10} supplementation has been considered to preserve plasma CoQ_{10} levels in patients treated with these therapeutic compounds (Bargossi et al. 1994). The combination of ubiquinol with statins has been recently proposed to benefit hypercholesterolemic patients with chronic heart failure (Kloer et al. 2020).

9.5.2 CoQ Levels in Plasma Are Affected by Nutrition

Nutrition can also affect the levels of CoQ_{10} in plasma. A study performed with phenylketonuria patients demonstrated that the levels of COQ_{10} in plasma were lower in these patients. This reduction was associated with the strict diet followed by these patients indicating that dietary CoQ_{10} is important in the balance of CoQ_{10} in plasma. Further, hypolipidemic drugs such as gemfibrozil, reduce the levels of CoQ_{10} in plasma (Aberg et al. 1998) probably by decreasing the levels of LDLs that are the particles with higher levels of CoQ_{10}. The use of CoQ_{10} as a dietary supplement in patients taken hypolipidemic drugs has been recently recommended in order to improve different cardiovascular disease (CVD) markers (Zhang et al. 2018). This recommendation confirms the clinical findings that relate disease markers with levels of CoQ_{10} in plasma. In the case of heart failure patients, N-terminal pro-brain natriuretic peptide (NT-proBNP) is considered an indicator of disease severity. A negative relationship between ubiquinol levels and NT-proBNP levels was found in these patients indicating that the severity of this disease correlates with lower levels of CoQ_{10} in plasma (Onur et al. 2015). In this case, oxidation of ubiquinol in plasma was considered a risk factor. The highest CoQ_{10} oxidized in plasma was associated with the highest NT-proBNP levels, indicating a direct correlation of the oxidation of LDLs in plasma with the severity of the disease (Onur et al. 2015). This relationship was also found some years before. In patients with coronary artery disease, the ubiquinol/ubiquinone ratio was significantly lower than that of control patients indicating that the decrease of this ratio must be considered a biomarker of high oxidative stress affecting LDLs associated with the disease (Lagendijk et al. 1997). This decrease in the redox ratio was also found in patients with several other diseases affecting the liver such as hepatitis, cirrhosis, hepatocellular carcinoma (Yamamoto and Yamashita 1997; Yamamoto et al. 1998), indicating that plasma CoQ_{10} redox ratio is a reference of the oxidative stress associated with these diseases (Yamashita and Yamamoto 1997).

9.5.3 Plasma CoQ and the Endothelial Function

CoQ_{10} can also protect the endothelial cells by preventing oxidative damage. For this reason, CoQ_{10} also plays an important role in the positive effect of plasma CoQ_{10} levels in CVD (Gao et al. 2012). Patients with mild to moderate dyslipidemia show

endothelial dysfunction that is improved by ubiquinol supplementation (Sabbatinelli et al. 2020). It has been also demonstrated that CoQ_{10} prevents senescence and dysfunction in vascular endothelial cells caused by oxidative damage (Huo et al. 2018). For this reason, supplementation with CoQ_{10} has been suggested not only to prevent heart failure, but also hypertension and endothelial dysfunction (Yang et al. 2015).

9.5.4 CoQ_{10} Levels in Plasma Are Affected by Aging.

During aging, LDL antioxidant defences tend to decrease and this decline is higher in males under 50 years (Aejmelaeus et al. 1997b). Supplementation with CoQ_{10} was suggested to increase the capacity of LDLs to resist oxidative injury (Aejmelaeus et al. 1997a). This decline starts very early in humans, in comparison with children (0–8 years), adults (29–78 years) already show lower lipid-adjusted CoQ levels in plasma, and the redox ratio was also lower in adults than in children probably indicating a higher consumption of ubiquinol in its antioxidant activity in plasma (Miles et al. 2004).

In our hands, the effect of exercise on CoQ_{10} levels in plasma was different in young than in old subjects. In young people, higher exercise was accompanied by lower CoQ_{10} levels in plasma, even related with cholesterol, but with lower oxidative damage whereas in older individuals, exercise induced an increase in CoQ_{10} in plasma also accompanied by lower oxidative damage (Del Pozo-Cruz et al. 2014b). Interestingly, functional capacity was positively associated with CoQ_{10} in plasma, whereas sedentary people showed lower CoQ_{10} levels and higher oxidative damage including LDL oxidation (Del Pozo-Cruz et al. 2014a).

These and other studies indicate that the maintenance of CoQ_{10} levels in plasma during aging can increase cardiovascular health and that CoQ_{10} is the main indicator of oxidative stress increase associated with aging and age-related diseases. The recent finding of an outer plasma membrane linked oxidoreductase in hepatocytes involved in the reduction of CoQ_{10} in plasma (Takahashi et al. 2019) opens the possibility of studying this reductase as another factor influencing aging progression.

9.6 Importance of CoQ Homeostasis in Aging

The free radical theory of aging situates the unbalance between the creation and elimination of ROS in the epicenter of aging and age-associated degenerative processes. The displacement of this balance to the increase of ROS levels ends in the accumulation of damage in molecules and structures (Miquel 1998). The combination of this unbalance with an inefficient damaged organelle turnover is one of the central causes of physiological decay associated with aging occurring in cells, tissues, and organs. In this sense, healthy aging, in which the deterioration in the functions of

organs and systems is slower, can be reached through the regulation of the production of ROS and the preservation of a well-adjusted antioxidant capacity, reparation, and elimination of damaged macromolecules and cellular structures (Maurya et al. 2016).

9.6.1 Mitochondrial Dysfunction as the Main Cause of ROS Increase During Aging

To produce ATP, mitochondria need molecular oxygen as an acceptor of electrons at the complex IV of the mETC while it is pumping protons from matrix to intermembrane space originating a chemiosmotic gradient. In this process, some electrons leak the mETC. These electrons directly react with molecular oxygen producing superoxide anion as an initial ROS product. At low concentrations, ROS initiate signaling processes that regulate the synthesis and also control the activity of antioxidant enzymes in a hormetic response (Rattan 2008). These antioxidant enzymes are superoxide dismutase (SOD), which transforms superoxide into hydrogen peroxide, and catalase or glutathione peroxidase (GPx) that removes hydrogen peroxide, producing water as the final product.

As above indicated, cells also contain antioxidant molecules such as ascorbic acid, α-tocopherol, glutathione or CoQ_{10} that are used by these enzymes and others such as CYB5R3 or NQO1 to protect macromolecules against oxidative damage. Mitochondrial dysfunction is accompanied by a rise in the production of ROS, surpassing the capacity of the antioxidant systems and causing the increase of damaged components including lipids, proteins, and DNA found in aging (van der Rijt et al. 2020).

Many studies indicate that the accumulation of dysfunctional mitochondrial in cells and tissues is a hallmark of aging (Lopez-Lluch et al. 2018; Haas 2019; Pagano et al. 2020). Mitochondrial dysfunction is also present in many physiological dysfunctions and diseases associated with aging. Among these diseases, we can found sarcopenia, type 2 diabetes, neurodegeneration, cardiovascular disease, liver and kidney dysfunctions, chronic inflammation, stem cell deterioration, vascular damage, and cancer (Hoppel et al. 2017; Waltz et al. 2018; Castelli et al. 2019; Bornstein et al. 2020; Pagano et al. 2020; Patel et al. 2020; Rossman et al. 2020; Sreedhar et al. 2020; Wan and Finkel 2020; Zampino et al. 2020). Dysfunctional mitochondria is responsible for higher ROS production, but it also affects Ca^{2+} homeostasis, synthesis of molecules such as nucleotides, phospholipids and anabolic intermediates, modifications of amino acids, and regulation of apoptosis and the immune response (Lopez-Lluch et al. 2015; Moreno Fernandez-Ayala et al. 2020).

9.6.2 The Decrease of CoQ_{10} in Mitochondria: Cause or Consequence of the Dysfunction of Mitochondria

Because of its essential role in mitochondrial physiology, a decrease in the levels of CoQ_{10} in aged cells and organs can be partially responsible for the unbalanced mitochondrial activity associated with age-related diseases (Lopez-Lluch et al. 2010). However, a direct relationship of the decrease of CoQ_{10} levels with mitochondrial dysfunction has not been clearly demonstrated.

Probably, the disruption of mitochondrial dynamics and turnover can be responsible for the beginning of a vicious cycle in which mitochondrial dysfunction affects CoQ synthesis, while low levels of CoQ contribute to the accumulation of deteriorated mitochondria. In fact, recent transcriptomic and proteomic studies have demonstrated that age-associated mETC dysfunction affects the inner mitochondrial membrane destabilizing the CoQ-synthome ending in a reduction of CoQ synthesis (Kuhl et al. 2017). As the synthesis of CoQ_{10} resides in mitochondria (Fernandez-Ayala et al. 2005a, b), lower levels of CoQ would affect not only mitochondria, but also the antioxidant protection of the rest of the cell membranes and the activities depending on CoQ oxidoreductases.

To clarify this relationship, we can pay attention to sarcopenia associated with chronic statin treatments. Although statins are important in the prevention of cardiovascular disease by lowering LDL levels in plasma, their chronic use in treatments can reduce not only the levels of cholesterol synthesis, but also the synthesis of CoQ_{10} (Campins et al. 2017). Treatment with ubiquinol can rescue the devastating effects of these drugs on mitochondrial levels, metabolism, and rhabdomyolysis indicating that the drop of CoQ_{10} levels in muscle is the main factor in the deterioration of mitochondrial function caused by statin treatment (Vaughan et al. 2013). It is known that the decrease of CoQ_{10} levels activates mitophagy to degrade deteriorated mitochondria (Rodriguez-Hernandez et al. 2009) and increases the dysfunction of mitochondria accompanied by higher oxidative stress and apoptosis in cultured cells (Marcheggiani et al. 2019).

Probably, the relationship of CoQ levels and mitochondrial balanced function works among some limits. The control of the synthesis of CoQ has been associated with the increase of lifespan of mice harboring a mutated central component of the CoQ synthesis (Wang et al. 2015). In these animals, $COQ7^{+/-}$, mitochondria show low activity of mETC, lower ATP synthesis and higher ROS production but accompanied by a higher antioxidant activity probably in response to a hormetic signaling pathway (Lapointe and Hekimi 2008). In other models of animals, reduction in the levels of proteins involved in the synthesis of CoQ is associated with an increase in lifespan without affecting mitohormesis (Rodríguez-Hidalgo et al. 2018). On the other hand, supplementation with CoQ_{10} prevents senescence and dysfunction caused by oxidative stress in vascular endothelial cells (Huo et al. 2018) and decelerates aging progression in SAMP1 mice, a model of accelerated aging (Schmelzer et al. 2010) by a mechanism probably associated with the maintenance of the mitochondrial functions (Tian et al. 2014).

9.6.3 Do CoQ_{10} Levels Decrease During Aging in Humans?

In humans, a very important issue is that it is no clear how CoQ_{10} levels change along time. Most of the studies performed in humans only determine the levels of CoQ_{10} in blood plasma. Nevertheless, these CoQ_{10} levels in plasma may not reflect the right conditions of CoQ_{10} in organs and tissues. However, there is a consensus about a gradual decrease of CoQ_{10} from maturity to old age (Kalen et al. 1989). In the elderly, many organs show a clear decrease in comparison with young individuals (Kalen et al. 1989; Bentinger et al. 2007).

As CoQ_{10} is synthesized in all cells and organs and some tissues and organs such as the central nervous system and muscle did not incorporate CoQ_{10} from plasma, the evolution of these levels during human aging is not clear. However, recent reports have associated the reduction in the levels of CoQ_{10} with the progression of age-related macular degeneration, glaucoma, Alzheimer's disease or Parkinson's disease (Manzar et al. 2020) opening the possibility of using supplements of inducers of CoQ_{10} synthesis in the treatment of these diseases.

As has been indicated before, most of the studies performed in humans are centered on the protection of endothelial cells and the cardiovascular system (Orlando et al. 2020). In fact, CoQ_{10} supplementation has shown therapeutic benefits in aging-related disorders, most of them related to cardiovascular and metabolic diseases (Díaz-Casado et al. 2019) reducing mortality due to cardiovascular dysfunction (Alehagen et al. 2018, 2021). Importantly, deterioration of vasculature is a very important factor in the progression of atherosclerosis and CVD. In this process, inflammation and mitochondrial dysfunction plays an essential role (Salazar 2018). Many clinical studies have demonstrated that CoQ_{10} supplementation reduces the level of cardiovascular fibrosis improving cardiovascular function and reducing cardiovascular-associated mortality in an effect associated with the antioxidant and anti-inflammatory functions (Hargreaves and Mantle 2019). Interestingly, many of these effects can be associated with the prevention of senescence and dysfunction caused by oxidative stress in vascular endothelial cells (Huo et al. 2018).

In resume, the decline of CoQ_{10} has been associated with aging and many age-related diseases apart from CVD or neurodegenerative diseases. CoQ_{10} has been also associated with type 2 diabetes, chronic kidney disease, liver disease, sarcopenia, and importantly with inflammation and immunosenescence (Lopez-Lluch et al. 2010; Hernandez-Camacho et al. 2018; Lopez-Lluch 2021). Its use as a biomarker of oxidative stress in plasma could help in diagnostics in chronic age-related diseases. Further, its use as a nutraceutical in elderly people must increase its homeostasis and help in the protection of cells against oxidative damage and mitochondrial dysfunction during age and age-related loss of capacity.

Fig. 9.3 Essential role of CoQ_{10} homeostasis in aging progression. Maintenance of CoQ_{10} levels and homeostasis can be essential in the progression of aging and age-related diseases by decreasing mitochondrial dysfunction and reducing oxidative damage

9.7 Concluding Remarks

It seems clear that secondary CoQ_{10} deficiency must be considered a key factor in the progression of many age-related diseases. For this reason, strategies focused to maintain or increase CoQ_{10} levels in tissues and organs during aging must help in the maintenance of the physiological functions of these organs. The low capacity of some tissues and organs such as nervous system or muscle to get CoQ_{10} from plasma indicates that the most important strategy must be to induce the endogenous synthesis in these organs. However, in other cases, especially in endothelial cells and immune system, direct supplementation with CoQ_{10} can strongly help in the maintenance of antioxidant capacity, the reduction of oxidative damage, and the prevention of inflammatory responses associated with mitochondrial dysfunction. Only these clear effects put CoQ_{10} as an important nutraceutical for elderly people (Fig. 9.3).

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Chapter 10
Redox Proteostasis in Subcellular Aging

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Abstract Aging is a process which leads to gradual redox status deterioration at the subcellular level. Proteostasis is a dynamic event that regulates protein's redox status within the aging process to maintain redox stability of proteome. Proteostasis also includes the highly complex redox regulatory signaling pathways that affect various functions in the aging cell. At the subcellular level, other cellular organelles besides mitochondria, such as lysosomes, peroxisomes, and endoplasmic reticulum (ER), also produce reactive oxygen species (ROS) that contribute to proteomic aging. The optimum stability and function of proteome may be deteriorated by many aging-related factors such as impaired cellular redoxtasis, nonenzymatic post-translational modifications, and ER stress. Misfolded protein accumulation in the ER lumen interferes signal transduction-related events. Proteasome-autophagy systems possess the removal activity for oxidatively modified proteins and aging organelles. The ubiquitin–proteasome system is major intracellular protein degradation system that controls the garbage recycle process in the aging proteome. Aging-related impaired redoxtasis may cause nonenzymatic post-translational modification- related proteinopathies. The gradual accumulation of oxidatively modified and misfolded protein aggregates is the main characteristics of proteinopathies. Aging-induced interorganellar redox imbalance, impaired oxidative garbage removal, and deposition of modified proteins like amyloid β, tau proteins, α-synuclein, and amyloid polypeptides are all related to age-related protein misfolding diseases. Thus, in the long term, novel antiaging and senolytic strategies to restore proteostasis in aging proteome may provide an effective way to establish promising therapies for Alzheimer's disease and other aging-induced protein misfolding diseases.

Keywords Aging · Redoxtasis · Proteostasis · Protein misfolding · Proteasome autophagy · Ubiquitin–proteasome system

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10.1 Introduction

Aging mainly depends on the optimum efficiency of several interconnected subcellular networks in long-lived postmitotic cells which are also referred to as non-dividing cells (e.g., cardiac myocytes, neurons, and retinal pigment epithelial cells). Interorganellar communication needs to be precisely tuned to prevent subcellular oxidative damage during the aging process. Recent experimental evidence indicates that variations in the subcellular redox status of aging organelles have a pivotal role in regulating their physiological functions with the aging process (Gil-Hernández and Silva-Palacios 2020). Postmitotic cells possess inadequate regenerative activity because of the division and differentiation of stem cells. Therefore, biological waste materials which cannot be removed from the aging cell gradually accumulate and replace normal cellular structures. All these changes lead to interorganellar communication disorders, and eventually cellular death (Çakatay 2010). Cellular functions of the aging organelle mainly depend on communication and proximity with subcellular systems to sustain specific functions (Silva-Palacios et al. 2020).

Available data from previous studies support the presence of subcellular "*redox triangle*" failure in senescence postmitotic cells related to mitochondria, peroxisomes, and endoplasmic reticulum (ER) (Fig. 10.1). Redox triangle detects ROS-mediated oxidative damage signals and redoxtasis imbalances. Redox signaling

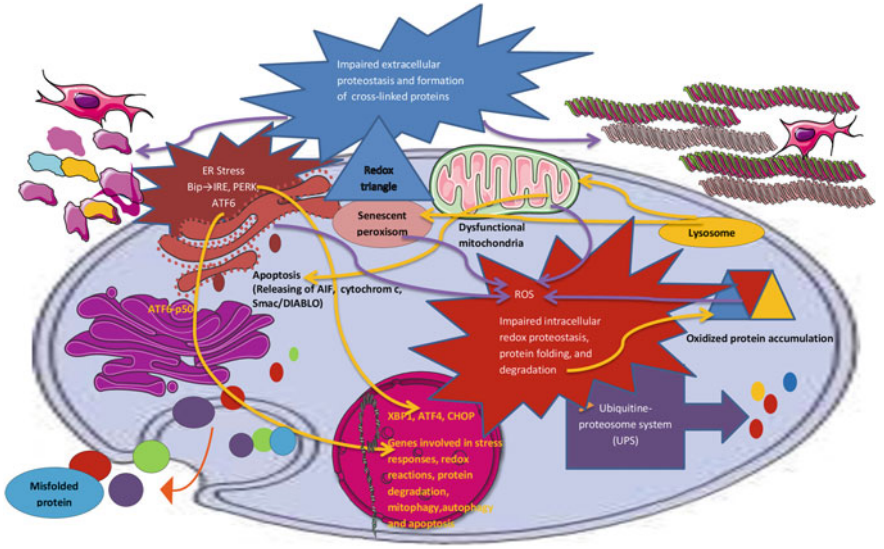

Fig. 10.1 Interorganellar redox regulation and oxidative garbage removal mechanisms of aging cell. Reactive oxygen species (ROS), immunoglobulin binding protein (BiP), ubiquitin–proteasome system, inositol requiring element 1 (IRE-1), serine–threonine ER kinase (PERK), activating transcription factor 6 (ATF6), X-box-binding protein 1 (XBP1), activating transcription factor 4 (ATF4), and homologous protein (CHOP)

process and related enzymes take place in the "redox triangle" created by ER, peroxisomes, and mitochondria (Yoboue et al. 2018). Signaling free radicals can be released by redox triangle via aquaporins. Redox triangle-induced ROS overproduction affects the functioning of ER-mitochondria Ca^{2+} ion transport, ATP synthesis, and oxidative folding activity of proteins within the cisternal lumen of ER. It has been known that redox triangle-controlled relationship occurs during peroxisomal beta-oxidation process. Peroxisomal β-oxidation of very long chain fatty acids (VLCFA) is partially completed and forms NADH that should be moved to mitochondria via outer membrane located porins for its complete oxidation. The optimum redox triangle activity may become inadequate with the advance of the aging process, as determined by decreased activity of peroxisomal catalase in senescent cells (Yoboue et al. 2018) (Fig. 10.1). A list of specific protein oxidation biomarkers in subcellular organelles correlating with aging is given in Table 10.1.

Table 10.1 A list of specific protein oxidation biomarkers in organelles correlating with aging

Organelle	Biomarker	Protein	Reference
Mitochondria	Protein carbonyl groups	Mitochondrial proteins of skeletal muscle	Beltran Valls et al. (2015)
	3-nitrotyrosine	Mitochondrial proteins of skeletal muscle	Murakami et al. (2012)
	3-nitrotyrosine	Mitochondrial proteins of liver: ATP synthase, H(+) transporting mitochondrial F1 complex, β subunit	Marshall et al. (2013)
	3-nitrotyrosine	Subunits of mitochondrial complex I in neurons	Naoi et al. (2005)
	Advanced oxidation protein products	Mitochondrial proteins of heart and brain	Sudheesh et al. (2010)
	Citrate synthase activity	Citrate synthase	Chepelev et al. (2009)
Endoplasmic reticulum	Protein carbonyl groups	Bip/Grp78 PDI, calreticulin	Rabek et al. (2003)
	3-nitrotyrosine	Protein disulfide isomerase (PDI) precursor	Marshall et al. (2013)
	S-nitrosylating PDI	Protein disulfide isomerase	Nakamura and Lipton (2008), Uehara et al. (2006)
Peroxisomes	Protein carbonyl groups	Catalase	Walton and Pizzitelli (2012)
	3-nitrotyrosine	Matrix proteins	Fransen et al. (2012)

The progressive redox failure plays the leading role in the occurrence of degenerative processes with the advancing age. Proteostasis is a highly complicated, subcellularly controlled process that regulates redox stability of proteins within the aging metabolome. Impaired redoxtasis is an inevitable part of subcellular aging which leads to gradual failure of the proteostasis-dependent vital systems, removal of oxidatively damaged proteins, and quality control of related metabolic events in aging (Janikiewicz et al. 2018). At the subcellular level, besides mitochondria, other cellular organelles such as lysosomes, peroxisomes, and ER also produce reactive oxygen species (ROS) that contribute to proteomic aging. Physiologically essential or detrimental properties of ROS depend on their subcellular concentrations. Interorganellar diffusion rate of ROS is likely to be regulated. The place of each organelle on total ROS formation and proteomic aging exhibits a significant variation between cell types and ages (Cecarini et al. 2007).

Giacomello and Pellegrini's terminology (2016) "MAM (mitochondria-associated membrane) fraction" has been used to describe isolated or purified membranes of mitochondria–ER interactions; however, when the subcellular architecture of such contacts has (had) considered, authors refer them as mitochondria–ER contacts "MERCs". Redox status-sensitive proteins are localized to contact sites between the mitochondria and ER, known as MERCs. ER cisternae create membrane contact surfaces with peroxisomes and mitochondria (MAM). Among the advantages of MAM in the aging cell is that it allows the lower rate of ROS formation, the reduced oxidation state of mitochondrial proteins as well as better uptake of Ca^{2+} ions. The close interactions of these contact surfaces increase the ROS and Ca^{2+} ion reuptake which leads to cell death (Gil-Hernández and Silva-Palacios 2020). Unusual organization of MAM can lead to the disruption of ER–mitochondria contact sites with the advance of aging (Cherubini et al. 2020). These contact sites are also implicated in neuronal longevity (De Mario et al. 2017). Hence, MAM dysfunction leads to the development of various neurodegenerative diseases (Xu et al. 2020). Interorganellar matrix and physical interactions permit the transport of various metabolites and ROS that affects the cellular redoxtasis systems.

MAM-localized modulators of ER-mitochondria signal transduction-related crosstalk are known as Ero1-α, calnexin, and selenon/selenoprotein N1 gene (SEPN1). Some of the ROS-generated chaperones and oxidoreductases bind to ER Ca^{2+} ion handling proteins which regulate ER–mitochondria Ca^{2+} ion flux via redox-dependent interactions. ER is an interconnected network of cisternae that fulfills many of the functions related to cellular proteome such as protein biosynthesis, peptide translocation, protein folding, and various enzymatic post-translational modifications such as disulfide formation, glycosylation, and chaperone-related folding. Growing polypeptide chain should remain in unfolded state during its translocation into cisternal lumen. Maturing newly synthesized polypeptides are prone to misfolding as a result of exceeding the critical concentration in cisternal lumen. The accomplishment of protein folding is even more problematic in a massive cisternal network of the ER for secreted proteins, where luminal proteins need to keep their native conformation while being constantly exposed by high-energy collisions with neighboring cisternal proteins (Valastyan and Lindquist 2014). Because of these

unfavorable luminal conditions, many of the ER proteins cannot gain their native conformations, or stably assume the incorrect ones. However, ER blocks improperly folded or incompletely assembled proteins from exiting the ER and destinating to the cytosol or other subcellular components. Unfavorable folding conditions may result in protein folding diseases. Majority of the resident ER proteins such as molecular chaperons and folding enzymes (foldases) collaborate in order to achieve native folding of newly synthesized polypeptide chain and its subsequent release from ER. Chaperones are expressed continuously at the constant level and their expression induces in response to the gradual deposition of unfolded and/or misfolded proteins. ATP-binding and carbohydrate-binding chaperon systems which interact directly to the growing polypeptide chain and indirectly hydrophilic glycosyl groups accomplish together to ensure proper protein passage through the ER and the secretory pathway. Folding enzymes catalyze proline cis–trans isomerization and/or disulfide bond formation, both are necessary for folding to the native conformation (Braakman and Hebert 2013). Incorrectly folded proteins are destined for ER-associated degradation (ERAD) with the help of ubiquitin–proteasome system. The proteasome system is located in the cytoplasm, nucleus, and ER to ensure proper protein folding and prevent aggregation, aforementioned chaperone groups and folding enzymes reduce excessive workload of ERAD (Zeeshan et al. 2016).

The close physical contact of the rough ER to the nucleus ensures the quality control process of protein folding. The rough ER is also able to activate its own signal transduction mechanisms to manage its workload by decreasing the overall rate of translation under ER stress and halting improper folding of proteins. Misfolded protein accumulation in the cisternal lumen triggers a sequence of reactions named unfolded protein response (UPR). Under prolonged and severe ER stress, UPR reduces translation rate, induces luminal protein folding capacity, and ER-related protein degradation rate. It is called the heat shock response in the cytosolic and nuclear compartments. Aging leads to decline in gene expression and folding function of ER-located chaperones. Folding enzymes ensure the fidelity of the protein folding process and UPR (Brown and Naidoo 2012). If the homeostatic response fails, aging cells are directed to undergo apoptosis. UPR also induces an autophagic pathway to eliminate misfolded proteins which cannot be degraded by ERAD. Functions of the UPR components decline with age. During the aging process, the balance between the protective function of UPR and pro-apoptotic signaling was reported; the protective function is significantly diminished and the apoptotic function is getting more robust.

Autophagy displays its protective role against subcellular aging through the removal of intracellular protein aggregates and damaged organelles. It has been suggested that autophagy can ensure neuroprotection by enhancing the removal of these protein aggregates. The growing experimental finding shows that autophagy also decreases with the advance in age; the rate of autophagosome biogenesis and the efficiency of autophagosome/lysosome fusion are getting reduced. Cross-linking is most commonly seen ROS-mediated post-translational modification in long-lived proteins which becomes undegradable by autophagocytosis (Terman and Brunk 2004).

Insoluble protein aggregates known as aggresomes are most commonly seen in senescent neurons. As has been reported in previous papers, prolonged ER stress, interorganellar redox imbalance, protein misfolding-initiated dysregulated ROS cascades, impaired oxidative garbage removal activity, and accumulation of aggresomes have important roles in proteomic aging and the physiopathological mechanism of various age-related proteinopathies such as neurodegenerative diseases, inflammaging, and diabetes mellitus (Terman 2006).

10.2 Mitochondrial Aging and Redox Proteostasis

Mitochondria carry out important physiological tasks such as the production of energy, Ca^{2+} homeostasis, regulation of cell cycle, differentiation, apoptosis, and aging. Insufficient mitochondrial function is generally accepted as one of the important indicators of aging (López-Otín et al. 2013). The role of mitochondria in age-related impaired redox status has been reviewed by the mitochondrial free radical theory of aging (Barja 2014). It is generally assumed that mitochondria contribute approximately 90% of the cellular ROS (Wang et al. 2020). During the cellular aging process, oxidatively damaged mitochondria produces lesser amount of ATP and a higher amount of ROS. Electron transfer chain is embedded in the inner membrane of mitochondria and includes five types of protein complexes: NADH dehydrogenase (Complex I), succinate dehydrogenase (SDH) (Complex II), cytochrome bc1 complex (Complex III), cytochrome c (Cytc) oxidase (Complex IV), and ATP synthase (Complex V). Complexes I, III, and IV pump generated protons across the inner membrane into the intermembrane space, producing electrochemical gradient, which is then utilized by ATP synthase (Figueiredo et al. 2008). Complexes I and III are known as the primary source of superoxide radical anion ($O_2\bullet^-$) production. ROS is passed into the intermembrane space across the complex III. In addition to Complexes I and III-related ROS production, other complexes are also known as producers of ROS, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, cytochrome b5 reductase, flavoprotein– ubiquinone oxidoreductase, and the monoamine oxidase (Balaban et al. 2005).

The flavin mononucleotide and coenzyme Q sites of Complex I, and the quinol oxidase site of Complex III determine the course whether ROS is released to the inside or outside of the mitochondria. Variations in the activity of mitochondrial electron transfer chain with the aging process are likely due to the destabilization of Complexes I and III. If ROS accumulates inside the mitochondria, the mitochondrial DNA coding subunits of electron transfer chain complexes may be oxidatively damaged, resulting in impaired electron flow and insufficient ATP synthesis. If ROS-induced macromolecular damage accumulates outside of the mitochondria with age, downstream cytoplasmic organelles might be oxidatively deteriorated over the cellular life course (Genova and Lenaz 2015). Another characteristic of mitochondrial aging is related to the opening activity of mitochondrial permeability transition (MPT) pore. MPT pore complex is located between mitochondrial membranes which

consist of different macromolecular complexes such as cyclophilin D, the adenine nucleotide translocase, and voltage-dependent ion channel (VDAC) (Bonora and Pinton 2019). MPT increases inner mitochondrial membrane permeability to lower weighted substances (<1.5 kDa) which is triggered by gradual deposition of Ca^{2+} ions in the matrix. The movement of H_2O into the mitochondrial matrix causes mitochondrial swelling, the loss of the inner membrane potential ($\Delta\psi m$), and the uncoupling of oxidative phosphorylation which leads to cellular death (Bonora et al. 2015). Functionally, mitochondria subject to age-related deteriorations include increased ROS formation rate, culminating in oxidative damage to cellular macromolecules as well as impaired bioenergetics. Dysregulated mitochondrial antioxidant enzyme activity and increased ROS formation can influence many of the metabolic pathways such as oxidative phosphorylation, tricarboxylic acid (TCA) cycle, glycolysis, and ATP synthesis. All these seem to be related to diminish mitochondrial dynamics like mitophagy and fusion/fission in the senescent organism that are linked to a higher probability of the occurrence of age-related diseases (Forrester et al. 2018; Gil-Hernández and Silva-Palacios 2020).

Brain mainly includes peroxidizable membrane lipids while featuring a high O_2 consumption rate. Mitochondrial dysfunction has also been known as one of the earliest events in Alzheimer's disease (AD) due to the gradual deposition of β-amyloid peptide (Aβ) in mitochondria, prior to plaque formation process. Even though the exact molecular mechanisms of how Aβ affects mitochondrial redox status are still obscure. The presence of inefficient mitochondrial redox status causes: (i) reduced activities of TCA cycle enzymes, oxidative phosphorylation and ATP production; (ii) a decrease in glucose metabolism and an increase in oxidative damage due to the increased formation rate of ROS (Wang et al. 2020).

Aging mitochondrion seems to be not the only source of ROS formation in the senescent cells in the brain. Microglia cell is considered to be a substantial source of increased ROS production and has been attributed to its own ROS-induced inflammatory activity. In a related context, superoxide forming NADPH oxidase is known as the leading producer of microglial ROS. It has been demonstrated that NADPH oxidase enzyme complex to be activated in the AD brain which can be toxic to neighboring neurons (Desler et al. 2018).

In the cytoplasm of microglia, highly reactive O_2•- is removed by Cu,Zn superoxide dismutase catalyzing the generation of H_2O_2, which in turn is inactivated by reaction with reduced glutathione (GSH) catalyzed by glutathione peroxidase. If the ROS production rate exceeds the antioxidant capacity, O_2•- and H_2O_2 levels will rise. In the presence of redox-active transition metal ions such as Fe^{2+} and/or Cu^{2+}, highly reactive OH· radicals can be formed by Haber–Weiss or Fenton reactions. OH· radical has the highly reactive potential to induce oxidative damage to proteins, lipids, and DNA. Studies related to proteomic approach made the quantitative identification of carbonylated subcellular proteins feasible which are formed by protein oxidation in relation to aging. Considering to these assessments, during organism lifespan, mitochondrial proteins result in the overrepresented ones and also exhibited an enormous increase in carbonylation rate (Cabiscol et al. 2014).

Glial cells are prone to generate an enormous amount of nitric oxide (NO) through the inducible nitric oxide synthase. Activated microglial cells accelerate the formation of more reactive free radicals such as peroxynitrite ($ONOO^-$). $ONOO^-$ initiates lipid peroxidation, protein oxidation, and DNA damage, which lead to neuronal death. There is also extensive evidence support the conclusion that impaired redox homeostasis affects the oxidation status of almost all types of macromolecules in the brain of AD patients (Swomley and Butterfield 2015; Erdoğan et al. 2017; Wang et al. 2020).

10.3 ER Proteome

ER is considered to be a highly active organelle like mitochondria. ER regulates its structural process (e.g., degradation, elongation, fission, and fusion of cisternal membranes) according to its metabolic tasks. The age-related variation in the levels of ER-resident chaperons has clarified to us that aged cell tries to maintain its redox homeostasis in this cellular compartment (Gil-Hernández and Silva-Palacios 2020). ER-resident chaperones such as immunoglobulin binding protein (BiP), thiol-disulfide oxidoreductases, protein disulfide isomerase (PDI), calnexin, glucose-regulated protein 94 (GRP94), and calreticulin are gradually oxidized with the aging process and this detrimental process may also be related to their functional impairment. All these oxidative modifications exhibit a significant correlation with impaired functions of several chaperones and foldases (Brown and Naidoo 2012).

BiP is a member of heat shock 70 protein family and also named glucose-regulated protein 78 (GRP78). It binds transiently to newly synthesized underglycosylated, misfolded, or unfolded proteins transported to the ER lumen. It is also known as heat shock protein 5A(HSP5A) or GRP78. Cisternal unfolded protein accumulation releases BiP. BiP induces transmembrane sensors of ER, serine–threonine ER kinase (PERK), the inositol requiring element 1 (IRE-1), and the activating transcription factor 6 (ATF6), whose signaling recruits several transcription factors such as activating transcription factor 4 (ATF4), X-box-binding protein 1 (XBP1), and homologous protein (CHOP) leading to the activation UPR genetic program. Removal of the BiP from PERK and IRE1 leads to the initiation of UPR through their oligomerization and trans-autophosphorylation. Activation of PERK leads to phosphorylation of the eukaryotic initiation factor (eIF2α) and stimulation of ATF4. Stimulated ATF4 induces its target genes and related redox reactions. ATF4 activation also leads to the induction of CHOP. The activated domain of ATF6 is moved to the nucleus to upregulate ER-related chaperons and protein degradation factors, as well as XBP1 and CHOP expression (Estébanez et al. 2018) (Fig. 10.1).

UPR causes the following processes: (i) upregulation of ER chaperones such as BiP/GRP78 to assist the refolding process of proteins; (ii) inhibition of mRNA translation which is accomplished by PERK which phosphorylates eIF2α thereby reducing the rate of translation; and preventing further synthesis and thus protein

folding (iii) removal of misfolded proteins by the proteasome complex by a process known as ERAD (Brown and Naidoo 2012).

ER-resident transmembrane proteins such as IRE-1, PERK, and ATF6 regulate proteostasis in the cisternal lumen. Transmembrane ER stress transducers (e.g., IRE-1, PERK, and ATF6) have a crucial role to play in mitigating stress and ensuring proteostasis. However, persistent subcellular stress and aging may lead the cell toward apoptosis (Minakshi et al. 2017).

10.4 ER-Related Aging and Redox Proteostasis

The ER is highly sensitive to impaired redox homeostasis and altered redox signaling. All these alterations can influence protein folding, Ca^{2+} ion release, and mitochondrial respiration (Forrester et al. 2018). ER stress and UPR initiate ROS-related cascades and have important roles in the pathogenesis of aging-induced protein misfolding diseases. Redox signaling mediators such as calcium, endoplasmic reticulum oxidoreductin (ERO)-1, GSH/glutathione disulfide (GSSG), glutathione peroxidase 8 (GPX8), NADPH oxidase 4 (NOX4), NADPH-P450 reductase (NPR), and PDI have a strong relationship with ER stress-induced ROS formation (Zeeshan et al. 2016) (Fig. 10.2). GSH/GSSG is considered as the principal thiol redox couple in the ER. Glutathione status of the ER changes during aging process and lower GSH level causes less antioxidant production upon oxidative attack (Rudzińska et al. 2020). GSH is involved in redoxtasis reactions of ER proteins such as the maintenance of protein thiol groups in the reduced form and elimination of H_2O_2. Reducing cytosolic redox environment is known to be unfavorable for the formation of protein disulfide bonds. The luminal molar ratio of [GSH]/[GSSG] in the ER lumen is 1:1 to 3:1 as compared to 30:1 to 100:1 for the ratio of outside of cisternal lumen. In the cisternal lumen, the relative concentration of the GSSG compared to the GSH may contribute to the function of GSSG as the oxidizing peptide during protein folding. Active site of the PDI needs its own oxidized state thiol groups to catalyze the formation of disulfide bonds. A slight shift in the reductive potential of the cisternal lumen transforms PDI to its reduced thiol state (Dixon et al. 2008; Rudzińska et al. 2020). GSH can transform into GSSG by GPX8. GSSG is transformed back to GSH by glutathione reductase with the consumption of NADPH (Fig. 10.2). A list of redoxtasis-related processes in the ER, mitochondria, and peroxisomes is given in Table 10.2.

Knowledge in age-related modulation of ROS formation in the ER is still in the infancy period. However, ER-located proteins like the molecular chaperones BiP/Grp78 and PDI undergo oxidative modification and progressive impairment during aging by some of the studies related to senescent hepatocytes (Rabek et al. 2003; Nuss et al. 2008).

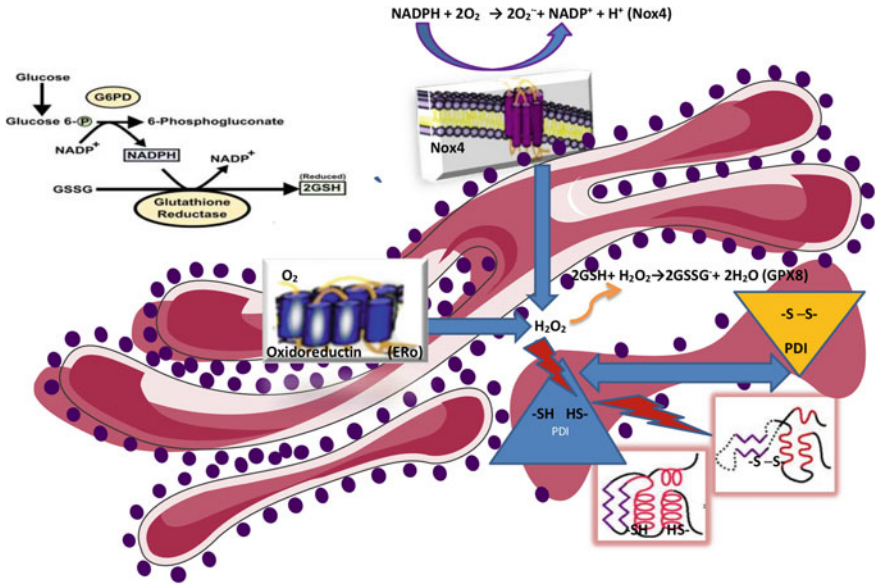

Fig. 10.2 Proteostasis mechanisms during ER stress-related aging. Prolonged ER stress and UPR that induce reactive oxygen species (ROS)-related cascades and are known to play important roles in the pathogenesis of aging-induced protein misfolding diseases. Protein disulfide isomerase (PDI)-endoplasmic reticulum oxidoreductin (ERO)-1, glutathione (GSH)/glutathione disulfide (GSSG), glutathione peroxidase 8 (GPX8), NADPH oxidase 4 (NOX4)

10.5 Mitochondria–ER Signaling-Related Communication

Variations in MAM ultrastructure and aberrant function of ER–mitochondria cooperation cause various age-related diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, metabolic diseases, and inflammation (Gil-Hernández and Silva-Palacios 2020). Cellular functions (e.g., regulation of lipid transfer and Ca^{2+} ion interchange) were the initially clarified functions attributed to MERCs, but additional important physiological roles (e.g., ATP synthesis, the regulation of mitochondrial division, innate immunity, inflammasome assembly, autophagosome formation, processing of the amyloid precursor protein, apoptosis, and redox signaling control) have also been attributed to MERCs, recently (Ray et al. 2014; Moltedo et al. 2019; Garrido-Maraver et al. 2020).

Mitochondria accomplish many vital processes. Increased mitochondrial Ca^{2+} concentration activates matrix enzymes rolled in the TCA cycle, including pyruvate, isocitrate, and α-ketoglutarate dehydrogenases complexes, and stimulates oxidative phosphorylation, which leads to increased production of ATP and MPT pore opening (Janikiewicz et al. 2018; Garrido-Maraver et al. 2020). The gradual decrease in mitochondrial Ca^{2+} ion uptake and the reduced number of MERCs are commonly seen in senescent cells (Janikiewicz et al. 2018). Mitochondria include both crucial regulators

Table 10.2 A list of redoxtasis-related processes in the ER, mitochondria, and peroxisomes

Organelle	ROS producing enzyme	Processes associated ROS production	Processes associated ROS elimination
Mitochondria	NADH: ubiquinone reductase (Complex I)	Superoxide generation related to electron leakage during mitochondrial respiratory chain functioning	Cu, Zn-SOD and Mn-SOD: H_2O_2 production in the mitochondrial matrix with superoxide dismutases
	Succinate dehydrogenase (Complex II)		Glutathione peroxidase 1 (GPX1: mitochondrial isoform) Reduction of H_2O_2 in H_2O using a selenocysteine catalytic residue and reduced glutathione as cofactor
	Cytochrome bc1 complex (Complex III)	Superoxide generation related to electron leakage during mitochondrial respiratory chain functioning	Glutathione peroxidase 4 (GPX4: mitochondrial isoform)
	Pyruvate and α-ketoglutarate dehydrogenases complexes	Undesired superoxide generation during electrons through the flavin co-factor of the DLD (dihydrolipoamide dehydrogenase) subunit	Peroxiredoxin 3 (PRDX3/PRX3) Reduction of H_2O_2 in H_2O using catalytic cysteine residue
	Glycerol-3-phosphate dehydrogenase	Undesired superoxide generation during electrons' fueling of the mitochondrial respiratory chain	Peroxiredoxin 5 (PRDX5/PRX5) PRDX5 is also localized in peroxisomes
Endoplasmic reticulum	ER oxidoreductin(s) (ERo1α and β)	Catalyzed H_2O_2 production using O_2 to initiate disulfide bonds formation during proteins' folding	Glutathione peroxidase 7 (GPX7), Reduction of H_2O_2 in H_2O using catalytic cysteine residue. Contrary to their protein homologs, they do not use GSH but ER reduced proteins as "co-factors"
	Quiescin sulfhydryl oxidase		
	NADPH oxidase 4 (NOX4)	Sequential production of superoxide and H_2O_2 using NADPH and O_2	Glutathione peroxidase 8 (GPX8)
	NADPH-P450 reductase (NPR)	Promotes hydroxyl radical formation	Peroxiredoxin 4 (PRDX4/PRX4) Reduction of H_2O_2 in H_2O using catalytic cysteine residue Glutathione (GSH)/glutathione disulphide (GSSG)

(continued)

Table 10.2 (continued)

Organelle	ROS producing enzyme	Processes associated ROS production	Processes associated ROS elimination
Peroxisomes	Acyl-CoA oxidase(s) (ACOX1, ACOX2 and ACOX3)	(Peroxisomal fatty acids β-oxidation) Flavin dependent H_2O_2 production	Catalase (CAT) Hemoprotein catalyzing the reduction of H_2O_2 in H_2O
	Xanthine oxidase (XO)	H_2O_2 and superoxide production during its catalytic cycle. Involved in purine metabolism	Cu, Zn-SOD: superoxide dismutation into H_2O_2
	D-amino acid oxidase (DAO)	H_2O_2 production during catalyzed oxidation of D isomers of aminoacids	Peroxiredoxin 5(PRDX5/PRX5) Reduction of H_2O_2, ROOH, ONOO, using catalytic cysteine residue (also localized in mitochondria)
	Polyamine oxidase (PAOX)	Involved in the degradation of polyamines. H_2O_2 production during its catalytic process	Epoxide hydrolase 2 Epoxide hydrolase 2 (EPHX2) is a homodimeric enzyme that can bind epoxides and convert them to the corresponding dihydrothiols Glutathione (GSH)/glutathione disulphide (GSSG)

of cell death and potent inductors of apoptosis such as second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein (Smac/DIABLO), apoptosis-inducing factor (AIF), and Cytc. Some of the MAM-resident proteins play a role in ER–mitochondria communication. The participation of glucose-regulated protein 75 (GRP75) is forming a link between Ca^{2+} channels inositol triphosphate receptor (IP3R) and VDAC, while Calnexin (CNX) modulates the activity of sarcoplasmic/ER calcium ATPase 2b (SERCA2b), directly. The ER releases its Ca^{2+} contents through ion channel IP3R and pumps it back through SERCA. Mitochondria imports these Ca^{2+} ions through a series of channels (mCU and VDAC) driven by $\Delta\psi m$ which is also considered to be a significant signaling mechanism, particularly during mitophagy and cell survival decisions. Higher rate Ca^{2+} ions transport to matrix could serve as a homeostatic mechanism to counterbalance the loss of $\Delta\psi m$ in aging cells (Janikiewicz et al. 2018).

Impairment in redox homeostasis needs a synchronic response from ER and mitochondria. ER signaling ensures mitochondrial integrity and mitochondria have crucial components for regulated UPR signaling (Bravo-Sagua et al. 2013).

Mitochondria and ER are both known as ROS generation centers, and therefore the communication at MERCs participates to the detrimental effects of intracellular ROS formation. ER–MT redox crosstalk occurs at MERCSs where it is responsible for ROS formation: oxidative folding activity of the Ero1-α, Ca^{2+} ion movement from ER to MT with the help of IP3R/VADC Ca^{2+} channels, and the electron

transport promoted at mitochondrial electron transfer chain. Ero1-α is known as FAD-dependent oxidase and plays an essential role in protein folding with PDI. Ero1-α activates IP_3Rs to facilitate MPTs as a member of MAM proteome. Ero1-α reforms the oxidized PDI and transfers the electrons from PDI to O_2, leading to H_2O_2 synthesis (Fig. 10.2). During ER stress, Ero1-α oxidizes IP3R1, which potentiates the release of Ca^{2+} ions from the ER. Ca^{2+} homeostatic ion flux from the ER to mitochondria maintains the TCA cycle. High production rate of ROS at MERCs leads to generate redox nanodomains at ER–MT contact sites that modulate ER–MT apposition (Fig. 10.3) (Fan and Simmen 2019; Moltedo et al. 2019).

Nowadays, increasing experimental evidence supports the idea that MERCs-related molecular interactions are closely related to the development of age-related diseases (Moltedo et al. 2019). MERCs are known as the initial occurrence site of Aβ formation (Schreiner et al. 2015) and play a crucial role in the development of AD. The release of Aβ peptide occurs at MERCs throughout the processing of the amyloid precursor protein by the γ-secretase complex, composed of Presenilin 1 and Presenilin 2. Mutated Presenilin 2 proteins affect ER–MT connections and their related biofunctions in genetic types of AD (Zampese et al. 2011).

10.6 Mitochondria–Lysosome Signaling-Related Communication

The proteasome is not merely a subcellular structure able to degrade oxidatively modified proteins. The lysosomal system includes different types of proteases that contribute to protein turnover. Lysosome-related proteolysis targets long-lived proteins and is considered to be non-selective (Cecarini et al. 2007). Correlations between mitochondria and lysosomes in the execution of the apoptosis process are emphasized in the lysosomal-mitochondrial axis theory (Zhao et al. 2003). Lysosomes are the iron-dependent formation sites of OH· radicals and most likely sites for the formation of indigestible substances. Lipofuscin possesses a brown-yellow autofluorescent, electron-dense pigment which includes oxidatively modified protein and lipid residues. Autophagic capacity is insufficient in lipofuscin-loaded cells such as neurons, retinal pigment epithelial cells, and cardiac myocytes. Lysosomal membrane disintegration can be induced in several different ways resulting in apoptosis. Released enzymes can attack various cellular proteins and mitochondria. H_2O_2 diffuses from the mitochondria into lipofuscin-filled lysosomes which are rich in redox-active iron catalyzing transformation H_2O_2 to OH· with Fenton reaction. OH· causes oxidative protein damage to lysosomal membranes that induces leak of lysosomal enzymes and iron into cytosol (Terman and Brunk 2004; Cecarini et al. 2007). Lysosomal enzymes and cytosolic hydrolytic enzymes such as phospholipase A2 permeabilize the outer membranes of mitochondria and lead to releasing of Cytc, AIF, and Smac/DIABLO triggering cell death (Terman and Brunk 2004).

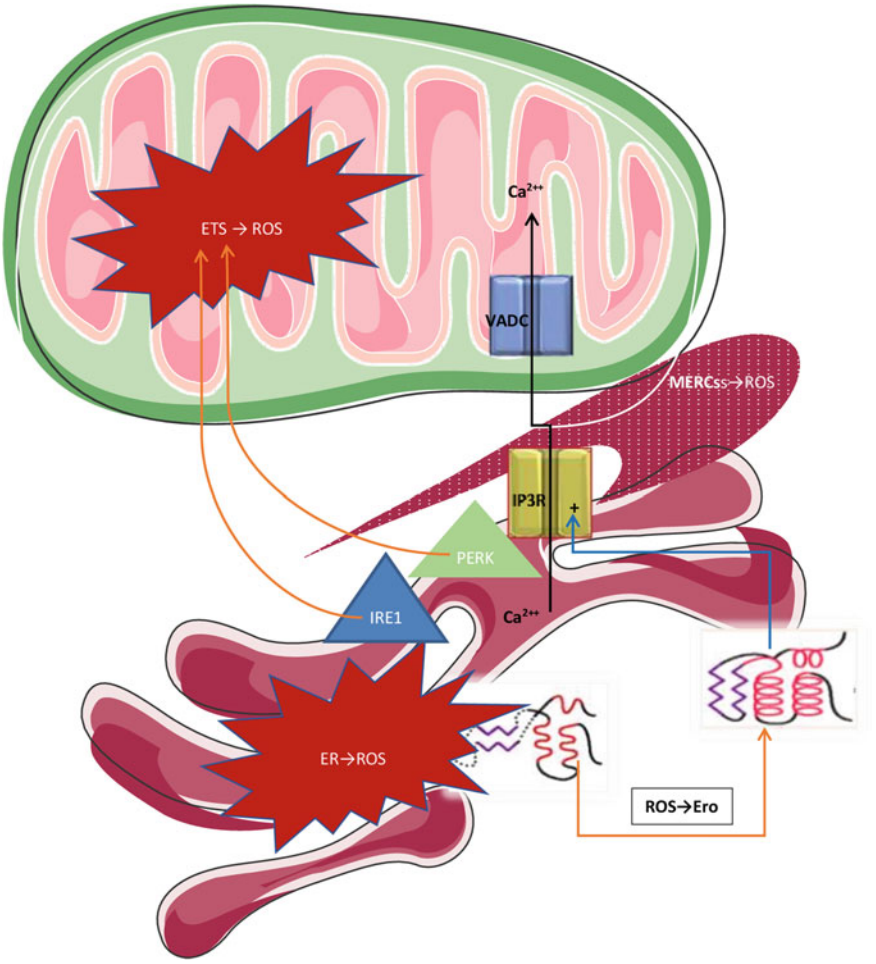

Fig. 10.3 Endoplasmic reticulum–Mitochondria (ER–MT) redox crosstalk occurs at mitochondria–ER contacts (MERCs) where different mechanisms are responsible for reactive oxygen species (ROS) formation: Ca^{2+} ion flux from the ER to MT through the subtype 3 of the 1,4,5- triphosphate receptor/ voltage-dependent anion-selective channel IP3R/VADC Ca^{2+} channels, the oxidative folding activity of the ER chaperone, ER oxidoreductase 1 alpha (Ero1-α), and the electron transport promoted by p66Shc at mitochondrial electron transfer chain. Ero1-α activates IP_3Rs to facilitate mitochondrial permeability transitions as a member of MAM proteome. Ca^{2+} homeostatic ion flux from the ER to mitochondria maintains the TCA cycle. Unfolded protein response (UPR)-unrelated activities of ER transmembrane kinase/ribonuclease 1 (IRE1) and serine–threonine ER kinase (PERK) control mitochondrial ETS activity. Accelerated ROS at MERCs forms redox nanodomains at ER–MT interface that modulates ER–MT apposition

10.7 Peroxisomal Aging and Redox Proteostasis

Peroxisomes are known as multifunctional organelles involved in α-oxidation of branched chain fatty acids such as phytanic acid, β-oxidation of VLCFA, detoxification of glyoxylate, bile acid conjugation, ether lipid synthesis, bile acid conjugation, ROS, and reactive nitrogen species (RNS) formation (Islinger et al. 2018; Walker et al. 2018). Peroxisomal homeostasis needs to be adapted to the metabolic requirements such as peroxisomal proliferation and removal of extensively damaged organelles by autophagy (Walker et al. 2018). Over the last decade, the biological role of peroxisomes has widened well and cellular signaling pathways have also been included. More recent experimental evidences indicate the possible links between peroxisomal aging and impaired cellular redox status.

Peroxisome biogenesis is accomplished by de novo synthesis or division and growth of pre-existing peroxisomes. Peroxisomal biogenesis requires the fusion of two pre-peroxisomal vesicles formed by ER and mitochondria. The growth and division of peroxisomes are accomplished by elongation factors and fission regulators. These processes are strictly regulated by peroxisome biogenesis factors, named peroxins and peroxisomal membrane proteins (Jo et al. 2020). In recent years, the interest increased in nonphysiological roles of peroxisomes (e.g., in cellular stress responses, the combat of pathogens, and antiviral defense as cellular signaling platforms and health aging) (Islinger et al. 2018; Cook et al. 2019). Cytotoxic properties of VLCFA metabolism for inflammatory demyelination and axonopathy are reported. Death of oligodendrocytes and astrocytes, regulation of Ca^{2+} homeostasis, and a marked decrease of the membrane potential of mitochondria in oligodendrocytes are also related to peroxisomal VLCFA metabolism (Islinger et al. 2018). VLCFA triggers oxidative stress characterized by an overproduction of ROS.

It is generally considered that mitochondria are the main ROS formation sites in the aging cell. Expanding knowledge in the last decade showed that the peroxisomes and the ER produce as much or even more ROS than mitochondria. Peroxisome-originated ROS may not only induce aging-related effects, but also function as antiaging signaling effects. Since the peroxisomal matrix contains a high amount of H_2O_2 producing flavoenzymes/oxidoreductases, H_2O_2 is the main product of ROS metabolism in peroxisomes (Table 10.2). Peroxisomes do not only generate H_2O_2, but similar to mitochondria have the ability to form $O_2\bullet^-$ and NO radicals (Cipolla and Lodhi 2017). The superoxide radical anion mainly derives from the enzyme xanthine oxidase. Xanthine oxidase is located in the cytosol as well as in the peroxisomes and is the final enzyme and therefore plays a primary role in purine degradation. H_2O_2 has a comparatively long intracellular half-life with its relatively mild oxidant reactivity and a high diffusion rate. All these properties make H_2O_2 an efficient signaling molecule which has a significant role in cellular differentiation, migration, proliferation, and gene expression. Excess accumulation of H_2O_2 causes impaired proteostasis, which if not balanced will induce cellular dysfunction with aging. The reduction of H_2O_2 produces highly reactive hydroxyl radicals (OH) that can readily react with proteins, lipids, and nucleic acids. OHs may also

alter their macromolecular structures and functions. Oxidation by ROS (like H_2O_2) leads to redox post-translational modifications of cysteine residues. Many of the reactions lead to disulfide bond formation: (i) intramolecular disulfide bonds are often inserted into a reduced protein by disulfide exchange (via formation of mixed disulfides) with GSSG or another oxidized protein molecule (e.g., PDI) and (ii) intermolecular disulfide bonds can be formed with another protein or low molecular weight thiols. Although the peroxisome is generally attributed to be a leading producer of $O_2^{\cdot -}$ and H_2O_2, it also significantly contributes to RNS. NO is formed with the catalytic activity of nitric oxide synthase activity with the transformation of L-arginine to $NO^{\cdot}$ and citrulline. $ONOO^-$ is formed as a consequence of the reaction between $NO^{\cdot}$ and $O_2^{\cdot -}$ (Fransen et al. 2012). Peroxisomal GSH reacts with ONOO- to form S-nitrosoglutathione, known as a signaling molecule. The cellular localization and activities of several peroxisomal matrix proteins are known to be regulated by the cellular redoxtasis system (Wang et al. 2015). ONOO− is also a powerful oxidizing agent and nitrated agent that may inactivate peroxisomal enzymes. Some of the nitrogenous species have structural ability and may trigger direct oxidative and nitrosative modification, often manifested as protein oxidation (Yanar et al. 2020). 3-nitrotyrosine as an important product of tyrosine side chain oxidation reactions is generated due to the reaction with $ONOO^-$ (Yanar et al. 2020). H_2O_2 inside peroxisomes may give rise to reactive OH formation through the Fenton reaction. Carbonylation is the most widely studied nonenzymatic protein modification that takes place as a consequence of aging-related oxidative stress. Metal ion-catalyzed protein carbonylation process is likely to involve the overproduction of OHs (Çakatay et al. 2001). It is very likely that peroxisomes also lead to formation of protein carbonyl group during aging process.

Peroxisomes are sources of ROS and also they protect cells from the oxidative damaging effects of ROS. Peroxisomes include some of the scavenger systems such as catalase (CAT: hemoprotein catalyzing the reduction of H_2O_2 in H_2O), superoxide dismutase (superoxide dismutation into H_2O_2), and peroxiredoxin 5 (reduction of H_2O_2, ROOH, ONOO using catalytic cysteine residue). Epoxide hydrolase 2 (EPHX2) is a homodimeric enzyme which can bind and transforms epoxides into the corresponding dihydrothiols. It has been thought that the primary physiological role of EPHX2 is to detoxify fatty-acid-derived epoxides. GSH is synthesized in the cytosol, from where it is transferred into peroxisomes (Table 10.2). GSSG is thought to be transported to the cytosol with peroxisomal glutathione transporter, wherein it is reduced to GSH by NADPH-dependent cytosolic glutathione reductase (Wang et al. 2015). The diminished CAT targeting to the peroxisome is commonly seen with aging. Senescent cells exhibit a reduced amount of peroxisomal biogenesis factor 5 (PEX5) which leads to diminished recognition affinity. This, in turn, lowers the ability of CAT to be targeted to the peroxisome. It was reported that reduced levels of H_2O_2 cannot be inefficiently degraded by CAT due to its active site which needs the interaction of two molecules of H_2O_2, despite its higher catalytic efficiency. Catalase activity may not actually play a significant role in removing low levels of H_2O_2 from the peroxisomal matrix. CAT is highly prone to its protein oxidation and can be inactivated itself related to higher amounts of peroxisomal H_2O_2. H_2O_2 leakage into

the cytosol induces peroxisomalredox signaling pathway at physiological amounts or causes oxidative damage at excessive amounts (Bonekamp et al. 2009). Gradual accumulation of H_2O_2 causes impaired cellular redox status which if not balanced with redoxtasis systems will promote impaired redoxtasis and cellular dysfunction with aging. As a result, increased H_2O_2 formation, coupled with decreased removal of oxidative damage, promotes impaired redox homeostasis as evidenced by increased lipid peroxidation rate and deposition of lipofuscin granules in hepatocytes. Age-related decline in the peroxisomal import of de novo synthesized CAT, coupled with its already diminished activity, limits its ability to the elimination of H_2O_2 (Walker et al. 2018).

Peroxisomes form a very close physical contact with the ER and with mitochondria (Fig. 10.1). Peroxisomes and mitochondria possess signaling network systems and have crucial roles in regulating redox signaling pathways. Peroxisome-related proteins are also prone to ROS and RNS-mediated oxidative protein damage. Peroxisomal Lon protease homolog-2 possesses the ability to eliminate such oxidatively modified proteins, thus prolonging the useful lifespan of the organelle. Senescent peroxisomes are also removed by autophagy. Autophagic degradation of dysfunctional peroxisomes is named pexophagy. ROS-activated ataxia-telangiectasia mutated kinase targets the peroxisome for degradation in two ways: (i) signaling mammalian target of rapamycin complex 1 to inhibit its suppression of pexophagy and (ii) phosphorylating PEX5 to promote its ubiquitination and its subsequent binding of p62. It was reported that PEX5 may serve as a redox/stress sensor to keep peroxisomal CAT in the cytosol to combat oxidative stress of non-peroxisomal origin (Wanders 2014).

The impaired peroxisomal function can also lead to mitochondrial dysfunction. It is widely assumed that peroxisomal activity is diminished with aging. Decreased expression of peroxisomal matrix proteins involved in ROS and lipid metabolism is commonly seen in aging and age-related disorders (Fransen et al. 2012; Cipolla and Lodhi 2017).

10.8 Concluding Remarks

The regulation of redox status of subcellular proteins has long-term effects on healthy aging and longevity. Recently, the redox-dependent signaling process has been integrated with intracellular ROS production which is no longer considered as just detrimental products of subcellular metabolism but are now highly appreciated for their role in regulating signaling networks in aging. Illumination of the processes related to the regulation of both redox triangle and proteostasis in the subcellular aging would help to identify clinically relevant senolytic and geroprotective therapeutic targets for combating age-related proteinopathies and even increase life expectancy in humans.

Compliance with Ethical Standards

Conflict of Interest: There is no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 11
Modulation of Redox and Aging-Related Signaling Pathways and Biomarkers by Naturally Derived Peptides

Yue Xiao, Qiangqiang Wang, Xinliang Mao, Xiaomin Li, and Zebo Huang

Abstract Nature has provided not only naturally occurring "free" peptides but also cryptic peptides ("cryptides")—bioactive peptide fragments encrypted in the structures of natural proteins. These peptide fragments can be released as functional peptides enzymatically or chemically during gastrointestinal digestion or food/pharmaceutical processing. In the last decade, a large number of naturally derived peptides have been explored for their antioxidant capacity, which are shown to enhance enzymatic as well as non-enzymatic antioxidant systems, including superoxide dismutase, catalase, glutathione peroxidase, and glutathione. Using model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster*, many antioxidant peptides are also found to increase their survival rate under oxidative stress, which can be relatively quantitated as the total or relative survival gain by the change in area under the survival curve (ΔAUC) across the lifespan. As oxidative stress resistance is conveniently used as a prognostic indicator of anti-aging capability, a number of natural peptides are also shown to promote longevity in *C. elegans* and *Drosophila.* The antioxidant and anti-aging activities of peptides are found to involve a range of evolutionarily conserved signal transduction networks, including insulin/insulin-like growth factor-1 signaling (IIS), nuclear factor erythroid-2-related factor 2 (Nrf2), nuclear factor-κB (NF-κB), AMP-activated protein kinase (AMPK), mitogen-activated protein kinases (MAPK), and heat shock response (HSR) pathways.

Keywords Peptide · Cryptide · Redox · Aging · Signaling · Biomarker

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11.1 Introduction

Accumulation of cellular damages caused by intrinsic as well as extrinsic stressors is a universal hallmark of aging, e.g. cumulative impairments by excessive reactive oxygen species (ROS) (Kaushik and Cuervo 2015; Sies et al. 2017). As cells and organisms are constantly facing various stresses throughout their entire lifetime, they have to unremittingly sense deleterious stress signals of both endogenous and exogenous sources and safeguard physiological homeostasis. To survive a persistent stress, they either maintain the current homeostasis in response to a tolerable condition or establish a new homeostatic balance when challenged by a greater stress; otherwise, if such a resilience fails, they may lose integrity even perish (Huang and Tunnacliffe 2006). However, whether aging per se is an adaptation is debatable as the aging process apparently has negative effects on the health of individuals late in life but may arguably be beneficial to the species and population. No matter what it is, aging is widely accepted as a byproduct of natural selection—it happens by a gradual accumulation of coincidental defects in function while evolved to maintain fitness in old age (Stearns et al. 2010; Vijg and Kennedy 2016; Flatt and Partridge 2018; Goldsmith 2019; Singh et al. 2019). Homeostatic level of ROS, for instance, is known to play important roles in normal functions of cells, including regulation of signal transduction and cell communication, but excessive accumulation of ROS may cause detrimental effects (Reczek and Chandel 2015; Shadel and Horvath 2015; Bazopoulou et al. 2019). In aging, oxidative stress occurs when the equilibrium between oxidant and antioxidant is disturbed, tipping the redox balance toward an oxidative status.

Nature has its own wisdom to tackle persistent stress. Under extreme desiccation, for instance, some organisms enter a state of suspended animation known as anhydrobiosis ("life without water"), which is an adaptive strategy characterized by almost no metabolic activity but a reversible homeostasis (Rothschild and Mancinelli 2001; Huang et al. 2010). The mechanisms behind this type of adaptation include, among others, the production of trehalose, extracellular polysaccharides, or late embryogenesis abundant (LEA) proteins through cellular reprogramming (Helm et al. 2000; Browne et al. 2002; Furuki and Sakurai 2018). Interestingly, a number of shared signaling pathways are involved in anhydrobiosis, oxidative stress, and aging, but it is the overall balance of complex signaling network that plays a more pivotal role than signals transmitted through an individual pathway in the signaling outcomes—an engineer's holistic rather than a tinker's partial solution—at organismal level (Huang and Tunnacliffe 2006; Kuzmic et al. 2018; Nesmelov et al. 2018; Ryabova et al. 2020).

Under manageable conditions of oxidative stress, cells and organisms are protected from oxidative damage by activating a set of endogenous antioxidant defense systems, including enzymatic antioxidants (e.g. superoxide dismutase) and non-enzymatic antioxidants (e.g. glutathione). If the intrinsic defense homeostasis is compromised by persistent detrimental stress, exogenous antioxidants may become necessary to help increase the defense function and restore oxidative resilience;

otherwise, cell function may be impaired and disease may occur (Niki 2016; Li et al. 2017a). Given the link between oxidative damage and aging, exogenous antioxidant supplementation may also delay aging and extend lifespan (Finkel and Holbrook 2000). One of such supplements that have attracted increasing attention is antioxidant peptides from natural sources, including both naturally occurring peptides and those derived from natural proteins. These naturally derived peptides are found to exert their regulatory functions through a number of stress and aging-related signaling pathways. Therefore, we examine here recent literature on the antioxidant activity of natural peptides with particular attention being paid to their regulation of redox and aging signaling pathways.

11.2 Antioxidant Activities of Naturally Derived Peptides

For almost all of human history, Nature has provided everything we need to remain healthy, including food and medicine. For example, the oceans cover approximately three-quarters of the surface of the Earth and a variety of marine sourced materials have been widely used for human wellness. Marine peptides, for instance, are shown to have a range of biological and pharmacological functions and have been increasingly used in nutraceutical, cosmeceutical, and pharmaceutical products (Cheung et al. 2015; Pavlicevic et al. 2020). Another example of Nature's wisdom is cryptic peptides ("cryptides"), i.e. bioactive fragments encrypted in the structures of natural proteins (Udenigwe 2014; Iavarone et al. 2018; Zhang et al. 2021). This is of particular interest as these short chains of amino acids may be inactive when they are "hidden" in the sequences of their parent proteins but can be released enzymatically or chemically as functional peptides during gastrointestinal digestion, food processing, or pharmaceutical production. For example, the oyster peptide-rich preparation COP3, which is prepared from *Crassostrea gigas* by digestion with a sequential combination of trypsin and papain, is found to comprise ~2500 peptide sequences with 19 prospective motifs, indicating a repertoire of cryptic peptides ("cryptome") that may contain different subsets of bioactive peptides (Zhang et al. 2021).

In recent years, a large body of evidence has accumulated to support that naturally derived peptides can be used to counteract oxidative stress. These antioxidant peptides are often prepared from natural proteins of animal, plant, and microbial origin. Their antioxidant capability involves almost every aspect of non-enzymatic as well as enzymatic antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) (Table 11.1). Interestingly, ~40% peptides in the antioxidant peptide fractions (containing > 1000 peptides in total) isolated from the protein hydrolysate of the sea cucumber *Apostichopus japonicus* are predicted to have antioxidant potential by the BIOPEP-UWM database and associated program (Guo et al. 2020).

Table 11.1 Antioxidant activities of naturally derived peptides and peptide-rich preparations selected from studies in the last five years

Sources	Peptides	Test models	Antioxidant activities	References
Animal origin				
Sea cucumber *Apostichopus japonicus*	Protein hydrolysate of body walls; peptide fractions; peptides: FETLMPLWGNK, HEPFYGNEGALR, KMYPVPLN	*C. elegans*; PC12 cells; SH-SY5Y cells	Increase of oxidative survival and SOD and CAT activities; decrease of ROS, MDA, and age pigments levels; scavenging of free radicals; lifespan extension; reduction of GSH depletion; alleviation of mitophagy	Guo et al. (2020); Lu et al. (2021)
Sea cucumber *Stichopus variegatus*	Protein hydrolysate; peptide fractions	*Drosophila*; mouse	Increase of SOD and GPx activities and Klotho expression level; decrease of MDA and carbonyl levels; lifespan extension	Lin et al. (2020)
Mussel *Mytilus coruscus*	Protein hydrolysate	HUVEC cells	Increase of SOD, CAT, and GPx activities; decrease of ROS and MDA levels; scavenging of free radicals	Zhang et al. (2020)
Fish	Roe polypeptide	Rat insulinoma cell	Increase of SOD, CAT, and GPx activities and GSH content; decrease of ROS level	Chen et al. (2021)

(continued)

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
	Skin gelatin hydrolysate; peptide: GPA	MODE-K cells; mouse	Decrease of ROS and MDA levels	Deng et al. (2020)
Giant croaker *Nibea japonica*	Protein hydrolysate of swim bladders	HUVEC cells	Increase of SOD, CAT, and GPx activities; decrease of ROS, MDA, and LDH release levels; scavenging of free radicals	Zheng et al. (2020)
Redlip croaker *Pseudosciaena polyactis*	Protein hydrolysate of scales; peptides: DGPEGR, GPEGP MGLE, EGPFGPEG,YGPDG PTG, GFIGPTE, IGPLGA	HepG2 cells	Increase of SOD, CAT, and GPx activities; decrease of ROS and MDA levels; scavenging of free radicals	Wang et al. (2020c)
Soft-shelled turtle *Trionyx sinensis*	Peptide: EDYGA	HepG2 cells	Increase of Nrf2 level; decrease of Keap1 level; scavenging of free radicals	Wang et al. (2020b)
Round scad *Decapterus maruadsi*	Protein hydrolysate; peptide: ILGATIDNSK	*C. elegans*	Increase of SOD and CAT activities and heat shock survival rate; decrease of ROS level; scavenging of free radicals; lifespan extension	Chen et al. (2020)
Red shrimp *Solenocera crassicornis*	Peptide fractions from head	ICR mice	Increase of SOD, CAT, and GPx levels; decrease of MDA content	Jiang et al. (2020)

(continued)

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
Odorous Frog *Odorrana andersonii*	Peptide AOP-P1 from skin secretions: FLPGLECVW	HaCaT cells	Increase of SOD and CAT activities; decrease of MDA and LDH levels	Yin et al. (2020)
Milk	Peptides: ARHPHPHLSFM, AVPYPQR, NPYVPR, KVLPVPEK, VLPVPQK	Caco-2 cells: rat fibroblast cells	Increase of SOD and CAT activities and GSH level; decrease of ROS, NO, and MDA levels; increase of SOD1, TrxR1, Trx1, GR, and NQO1 expression levels; scavenging of free radicals	Tonolo et al. (2020); Kumar et al. (2019)
Locust *Locusta migratoria manilensis*	Peptide fractions	*C. elegans*	Increase of oxidative survival; decrease of ROS and lipofuscin levels; lifespan extension	Cao et al. (2019)
Nile tilapia *Oreochromis niloticus*	Skin gelatin hydrolysate; peptide fractions	IPEC-J2 cells	Decrease of LDH release and ROS levels; prevention of stress-induced death; scavenging of free radicals; increase of GSH levels	Zheng et al. (2018)
Frog *Physalaemus nattereri*	Peptide: TWYFITPYIPDK	Murine fibroblast cells; human microglial cells	Scavenging of free radicals; decrease of GSSG/GSH-eq ratio and ROS level	Barbosa et al. (2018)

(continued)

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
Snake *Bungarus fascia*	Cathelicidin-WA	Mouse; RAW264.7 cells	Decrease of ROS and MDA levels	Wu et al. (2018)
Camel milk	Peptide fractions	Yeast	Increase of oxidative survival; scavenging of free radicals	Ibrahim et al. (2018)
Buffalo ricotta cheese	Peptide: YVEELKPTPEGDL	Intestinal epithelial cells	Increase of HO-1, NQO1, and SOD expression levels; decrease of ROS level	Basilicata et al. (2018)
Hard clam *Meretrix meretrix*	Peptide fractions; peptides: LSDRLEETGGASS, KEGCREPETEKGHR, IVTNWDDMEK	*C. elegans*; mouse	Increase of oxidative survival, SOD and GPx activities, and SOD-3 expression level; decrease of MDA content	Jia et al. (2018); Huang et al. (2018)
Plant origin				
Soybean *Glycine max*	Protein hydrolysate; peptide fractions; peptides: VVFVDR L, VIYVVDLR, IY VVDLR, IYVFVR	Caco-2 cells; HepG2 cells	Increase of mRNA and protein levels of SOD, CAT, and GPx; decrease of ROS level and MDA content; increase of GSH level and GSH/GSSG ratio; scavenging of free radicals	Zhang et al. (2019); Yi et al. (2020)

(continued)

11.2.1 Increase of Survival Under Oxidative Stress by Naturally Derived Peptides

One of the most reliable indicators for in vivo antioxidant competence is the increase of the survival rate of the test organisms under elevated oxidative stress. Interestingly,

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
Wheat germ	Peptides: AREGETVVPG, ADWGGPLPH	Vascular smooth muscle cells; mouse	Increase of SOD activity; decrease of ROS and MDA levels; scavenging of free radicals	Chen et al. (2017); Wang et al. (2020a)
Sorghum *Sorghum bicolor*	Sorghum grain kafirins-derived peptide fraction	Human skin cultures	Increase of SOD, CAT, and GPx activities	Castro-Jácome et al. (2019)
Rice *Oryza sativa*	Peptide-rich extracts of rice and rice bran; peptide: KHNRGDEF	HUVEC cells; HepG2 cells; NIH/3T3 cells; mouse	Increase of SOD, GPx, and GSH levels and GSH/GSSG ratio; decrease of ROS and MDA levels and LDH release; scavenging of free radicals	Liang et al. (2018); Moritani et al. (2020); Wang et al. (2020d)
Angelica sinensis	Protein hydrolysate of roots; peptide fractions	*C. elegans*	Increase of oxidative survival and SOD and CAT activities; decrease of ROS level, MDA content, and age pigment accumulation; lifespan extension	Wang et al. (2016a)

(continued)

a number of peptide preparations are shown to increase the survival of model organisms under oxidative stress, including the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster,* and the yeast *Saccharomyces cerevisiae* (Table 11.1). For example, the protein hydrolysate of the sea cucumber *Apostichopus japonicus* and its identified peptides (Guo et al. 2020; Lu et al. 2021) are shown to increase the survival rate of *C. elegans* treated with paraquat, a widely used superoxide-inducing agent (Wang et al. 2016a; Guo et al. 2020), while the peptide fractions prepared from camel milk are able to enhance the tolerance of yeast cells against H_2O_2-induced oxidative stress (Ibrahim et al. 2018). Several identified peptides (e.g.

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
Sesame *Sesamum indicum*	Peptide-rich preparation	*C. elegans*	Increase of oxidative survival, SOD activity, GSH content, and GSH/GSSG ratio; decrease of ROS and lipofuscin levels; scavenging of free radicals; lifespan extension	Wang et al. (2016b)
Algal and microbial origins				
Arthrospira (Spirulina) platensis	Antioxidant peptide GM15; peptide: LGLDVWEHAYYL	Human blood leucocytes; KB cells	Decrease of ROS level; scavenging of free radicals	Sannasimuthu et al. (2018, 2019)
Chlorella pyrenoidosa and *Yarrowia lipolytica* co-culture	Peptide fractions; peptides: AGYSPIGFVR, VLDELTLAR, LFDPVYLFDQG	HepG2 cells	Increase of SOD, CAT, and GPx activities; decrease of ROS and MDA levels; scavenging of free radicals	Liu et al. (2019)
Bacillus velezensis	Antimicrobial peptide MS15	RAW264.7 cells; HeLa cells	Increase of transcriptional and translational levels of CAT, GPx, SOD1, and HO-1; decrease of ROS and NO levels; scavenging of free radicals	Khan et al. (2020)

(continued)

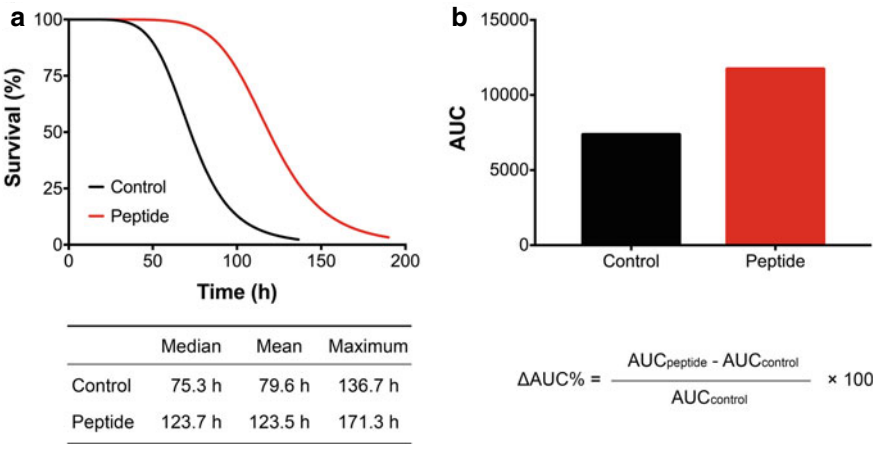
"LSDRLEETGGASS") from the hard clam *Meretrix meretrix* are also shown to improve the survival rate of *C. elegans* under increased oxidative stress induced by paraquat (Jia et al. 2018).

As survival increment under oxidative stress is an unequivocal antioxidant index, we have previously used the change in area under the curve (ΔAUC) to assess

Table 11.1 (continued)

Sources	Peptides	Test models	Antioxidant activities	References
Bacillus amyloliquefaciens	Antioxidant peptide YD1: APKGVQGPNG	RAW264.7 cells	Increase of mRNA and protein levels of SOD1, CAT, and GPx-1; decrease of NO and ROS levels; scavenging of free radicals	Rahman et al. (2018)

the effect of sea cucumber peptides on the total survival gain of *C. elegans* under oxidative stress, in addition to the survival rate per se (Guo et al. 2020). This is particularly valuable to compare the cumulative differences in survival of animal populations across their entire lifespan under persistent stress. As shown in Fig. 11.1a, the survival rate of *C. elegans* population pre-treated with a peptide originated from sea cucumber was much higher than the control where *C. elegans* population was treated with paraquat alone. Quantitatively, the total antioxidant capacity of the peptide, which is expressed as relative total survival gain (ΔAUC%), is ~50%, explicitly indicating its general protective function against increased oxidative stress in *C. elegans* (Fig. 11.1b). Similarly, the survival ΔAUC% of *C. elegans* pre-treated with sea cucumber protein hydrolysate is >20%, demonstrating the in vivo antioxidant activity of the peptide-rich preparation (Guo et al. 2020).



	Median	Mean	Maximum
Control	75.3 h	79.6 h	136.7 h
Peptide	123.7 h	123.5 h	171.3 h

Fig. 11.1 Effect of a sea cucumber peptide on the survival of *C. elegans* under oxidative stress induced by paraquat. **a** Representative Kaplan–Meier survival curves and corresponding survival data. **b** Cumulative survival difference and relative total survival gain or loss (ΔAUC%) of animal populations across the lifespan

11.2.2 Enhancement of Antioxidant Defense Systems by Naturally Derived Peptides

Oxygen is essential for aerobic life on Earth and, due to its high redox potential, is inevitably involved in the production of ROS, which play important roles in many biological processes at the physiological level, including cell signaling and enzyme regulation (Schieber and Chandel 2014; Sies and Jones 2020). Excessive ROS, however, can cause substantial damage to cell function and homeostasis, leading to a state of oxidative stress. Interestingly, almost all the peptides and peptide-rich preparations listed in Table 11.1 are reported to scavenge free radicals and decrease ROS levels and, thus, are capable of reducing such oxidative damage. For example, ROS level is reduced in mammalian cells by fish peptides (Chen et al. 2021) and likewise in *C. elegans* by herbal peptides (Wang et al. 2016a) and in the mouse by snake peptides (Wu et al. 2018).

Enhancing the antioxidant defense system through the regulation of antioxidant enzymes is a well-established feature of the intrinsic ROS clearance machinery. In the last decade, an increasing number of studies indicate that natural peptides can reduce oxidative damage at least partially by upregulating antioxidant enzymes, including SOD, CAT, and GPx. As noted in Table 11.1, most of the peptides are reported to increase the activity of one or more of these antioxidant enzymes. For instance, protein hydrolysates and peptide fractions from sea cucumbers are recently found to increase SOD, CAT, and GPx activities and reduce ROS levels in *C. elegans* and *Drosophila* (Guo et al. 2020; Lin et al. 2020). Various peptides prepared from soybean hydrolysates are also shown to increase both mRNA and protein levels of SOD, CAT, and GPx as well as GSH level and GSH/GSSG ratio in mammalian cells (Zhang et al. 2019; Yi et al. 2020), while peptide fractions from red shrimp head are reported to increase SOD, CAT, and GPx levels in mice (Jiang et al. 2020).

Accumulation of ROS-induced peroxidation products is also recognized as an important biomarker of oxidative stress. For example, the lipid peroxidation product malondialdehyde (MDA) can interact with proteins and DNA to generate covalent adducts with detrimental effects while the protein oxidation product carbonyl groups may cause rapid degradation of proteins, leading to the damage of other cellular components and the onset/exacerbation of cell dysfunction (Ayala et al. 2014; Grune 2020; Sies and Jones 2020). As listed in Table 11.1, a number of peptides are able to reduce the level of peroxidation products in cellular and animal models under oxidative stress. For instance, many peptides are shown to decrease MDA contents in various cellular models, e.g. peptides from milk (Kumar et al. 2019; Tonolo et al. 2020), soybean (Zhang et al. 2019; Yi et al. 2020), wheat germ (Chen et al. 2017; Wang et al. 2020a), and rice (Liang et al. 2018; Moritani et al. 2020; Wang et al. 2020d). Other peptides are found to reduce the level of peroxidation products in animal models, e.g. the peptide preparations from sea cucumbers are able to reduce MDA and carbonyl levels in *C. elegans* and *Drosophila* (Guo et al. 2020; Lin et al. 2020) while the red shrimp peptides can decrease MDA content in mice (Jiang et al. 2020).

11.2.3 Prolongevity Effects of Antioxidant Peptides

ROS-induced damage is one of the major risk factors for aging and age-related diseases (Finkel and Holbrook 2000; Sies and Jones 2020; Xiao et al. 2020). Indeed, the decline in response to oxidative stress is associated with aging, and the status of oxidative stress at the organismal level is often intertwined with the lifespan (Meng et al. 2017; Bazopoulou et al. 2019). Therefore, oxidative stress resistance is commonly used as a prognostic indicator of anti-aging capability, and antioxidant intervention is considered to be a potential strategy to slow aging and promote longevity (Wang et al. 2014; Li et al. 2017a; Luo et al. 2020; Zhong et al. 2021). However, as an intrinsic, progressive, and cumulative phenomenon in the life process, aging per se is difficult to quantitate. In contrast, lifespan or longevity, i.e. the duration from birth to death for an individual, can be quantified with relative accuracy. When this term is applied to populations, nevertheless, the concept is more complicated and needs greater accuracy with subdivided terms to avoid ambiguity, e.g. average lifespan, maximum lifespan, and median lifespan. Regardless, lifespan has been the most acceptable and unequivocal parameter for aging studies in general (Wang et al. 2014).

As summarized in Table 11.1, a number of peptide preparations are reported to have prolongevity effect in the model animal *C. elegans*. For example, the protein hydrolysates and peptide fractions from the medicinal plant *Angelica sinensis* (Wang et al. 2016a), the sesame *Sesamum indicum* (Wang et al. 2016b), the locust *Locusta migratoria manilensis* (Cao et al. 2019), the round scad *Decapterus maruadsi* (Chen et al. 2020), and the sea cucumber *Apostichopus japonicus* (Guo et al. 2020) are shown to extend the lifespan of *C. elegans*. Interestingly, *Angelica* (Wang et al. 2016a), locust (Cao et al. 2019), and sea cucumber (Guo et al. 2020) peptides are also found to decrease the relative levels of lipofuscin and age pigments in *C. elegans*. In addition, the peptide preparation from the sea cucumber *Stichopus variegatus* (Lin et al. 2020) as well as a combination of royal jelly and collagen peptides are shown to extend the lifespan of *Drosophila*, another invertebrate model animal (Lin et al. 2020; Qiu et al. 2020).

11.3 Regulation of Redox and Aging-Related Signaling by Antioxidant Peptides

As an intrinsic feature of life, the highly complex process of aging is known to involve a range of biochemical pathways, including insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS), nuclear factor erythroid-2-related factor 2 (Nrf2), telomere, oxidative stress, and dietary restriction pathways (López-Otín et al. 2013; Chakravarti et al. 2021; López-Otín and Kroemer 2021). Under normal conditions and tolerable stress, these signaling pathways interact with each other and contribute to balancing the signaling network of aging. The molecular homeostasis of the network may,

however, be disrupted by the accumulation of extrinsic as well as intrinsic stresses as aging progresses. Therefore, interventions to balance or rebalance the signaling network are likely to counteract the detrimental stresses and delay aging and age-associated disorders (Li et al. 2014). As shown in Table 11.2, many peptides are able to regulate signaling pathways involved in both the aging process and oxidative stress, including the IIS pathway, which is essential for homeostatic maintenance against internal stress, and the Nrf2 pathway, which plays an important part in the response to external stress (Fig. 11.2).

11.3.1 Regulation of Insulin/IGF-1 Pathway by Antioxidant Peptides

The IIS pathway is evolutionarily conserved from invertebrates to mammals and is well known to play a central role in the determination of lifespan. In *C. elegans*, the critical signaling nodes of this pathway are DAF-2, AGE-1, and DAF-16, which are the homologs of mammalian insulin/IGF-1 receptor, phosphoinositide 3-kinase (PI3K), and FOXO (forkhead box O) transcription factor, respectively. Intriguingly, these key IIS players can be regulated by bioactive supplements such as natural polysaccharides and peptides as demonstrated in an increasing number of studies (e.g. Zhang et al. 2012 and Table 11.2), in addition to their effect on the downstream antioxidant enzymes as summarized in Table 11.1. For instance, the peptides identified from hard clam, purple sea urchin, and locust are shown to unleash their antioxidant power by regulating FOXO/DAF-16 transcription factor (Jia et al. 2018; Zhao et al. 2018; Cao et al. 2019). A number of natural peptides, including those that originated from the wheat germ (Chen et al. 2017; Wang et al. 2020a), snake (Wu et al. 2018), and fish roe (Chen et al. 2021), are also found to modulate the serine/threonine kinase AKT (also known as protein kinase B, PKB), which is itself activated by PI3K and in turn execute diverse downstream functions, in particular regulation of cell survival (Borrie et al. 2017; Hoxhaj and Manning 2020).

11.3.2 Regulation of Nrf2/SKN-1 Pathway by Antioxidant Peptides

In addition to the FOXO/DAF-16 transcription factor, other stress response transcription factors such as Nrf2/SKN-1 also work together with the IIS pathway to regulate oxidative stress and longevity (Tullet et al. 2008; Park et al. 2009). SKN-1 (skinhead-1) is the *C. elegans* ortholog of the mammalian Nrf2, which is an essential stress response transcription factor that is conserved in most eukaryotic models (An and Blackwell 2003). It was originally found to be a master regulator of reduction–oxidation homeostasis but has also been increasingly recognized as a central regulator of

Table 11.2 Major redox and aging-related signaling pathways and key nodes regulated by natural peptides (excluding antioxidant activities shown in Table 11.1)

Pathways	Key nodes	Peptides and references
FOXO/DAF-16	AKT	Fish roe polypeptide (Chen et al. 2021); snake peptide Cathelicidin-WA (Wu et al. 2018); selenoprotein T-derived peptide (Rocca et al. 2018); wheat germ peptides (Chen et al. 2017; Wang et al. 2020a)
	FOXO	Locust peptides (Cao et al. 2019); purple sea urchin gonad peptides (Zhao et al. 2018); hard clam peptides (Jia et al. 2018)
	Antioxidant enzymes	See Table 11.1
Nrf2/SKN-1	Keap1	Mussel peptides (Zhang et al. 2020); red shrimp head peptides (Jiang et al. 2020); soft-shelled turtle peptides (Wang et al. 2020b); soybean peptides (Yi et al. 2020); rice peptides (Wang et al. 2020d); milk peptides (Tonolo et al. 2020)
	Nrf2	Fish roe polypeptide (Chen et al. 2021); mussel peptides (Zhang et al. 2020); red shrimp head peptides (Jiang et al. 2020); soft-shelled turtle peptides (Wang et al. 2020b); soybean peptides (Yi et al. 2020); rice peptides (Moritani et al. 2020; Wang et al. 2020d); milk peptides (Kumar et al. 2019; Tonolo et al. 2020); secretory signal peptide adropin (Chen et al. 2019b); buffalo ricotta cheese peptides (Basilicata et al. 2018); snake peptide Cathelicidin-WA (Wu et al. 2018); bacillus peptides (Rahman et al. 2018; Khan et al. 2020); cocaine- and amphetamine-regulated transcript peptide (Jiao et al. 2018); fish skin gelatin hydrolysate (Zheng et al. 2018)
	ARE	Soft-shelled turtle peptides (Wang et al. 2020b); fish skin gelatin hydrolysate (Zheng et al. 2018); soybean peptides (Yi et al. 2020); milk peptides (Tonolo et al. 2020); fish roe polypeptide (Chen et al. 2021); buffalo ricotta cheese peptides (Basilicata et al. 2018); snake peptide Cathelicidin-WA (Wu et al. 2018); bacillus peptides (Khan et al. 2020)

(continued)

Table 11.2 (continued)

Pathways	Key nodes	Peptides and references
	Antioxidant/detoxifying enzymes	See Table 11.1
NF-κB	IκB	Red shrimp head peptides (Jiang et al. 2020); abalone peptides (Gong et al. 2019)
	NF-κB	Rice peptides (Wang et al. 2020d); selenium-enriched peptides from *Cardamine violifolia* (Yu et al. 2020); red shrimp head peptides (Jiang et al. 2020); Szeto-Schiller peptides (Sun et al. 2020; Hou et al. 2018; Escribano-Lopez et al. 2018); abalone peptide (Chen et al. 2019a); milk peptides (Kumar et al. 2019)
	IκK	NF-κB essential modulator-binding domain peptide (Opazo-Ríos et al. 2020); red shrimp head peptides (Jiang et al. 2020)
	Inflammatory factors: IL-1β, IL-2, IL-6, IL-8, TNF-α	Szeto-Schiller peptides (Escribano-Lopez et al. 2018; Sun et al. 2020); red shrimp head peptides (Jiang et al. 2020); soybean peptide fractions (Zhang et al. 2019); GSE4 (Pintado-Berninches et al. 2019); egg yolk peptides (Young et al. 2010); frog skin peptides (Yang et al. 2009)
AMPK	PKC	Snake peptide Cathelicidin-WA (Wu et al. 2018); wheat germ peptides (Chen et al. 2017; Wang et al. 2020a)
	AMPK	Wheat germ peptides (Wang et al. 2020a); snake peptide Cathelicidin-WA (Wu et al. 2018)
	NOX4	NF-κB essential modulator-binding domain peptide (Opazo-Ríos et al. 2020); Szeto-Schiller peptides (Hou et al. 2018); wheat germ peptides (Chen et al. 2017; Wang et al. 2020a)
MAPK	JNK	Selenium-enriched peptides from *Cardamine violifolia* (Yu et al. 2020); locust peptides (Cao et al. 2019); abalone peptide (Chen et al. 2019a)

(continued)

Table 11.2 (continued)

Pathways	Key nodes	Peptides and references
	ERK	Fish roe polypeptide (Chen et al. 2021); abalone peptides (Gong et al. 2019); selenoprotein T-derived peptide (Rocca et al. 2018); wheat germ peptides (Chen et al. 2017)
	p38	Rice peptides (Wang et al. 2020d); abalone peptides (Chen et al. 2019a; Gong et al. 2019); sesame cake peptides (Ma et al. 2019); milk peptides (Kumar et al. 2019); selenoprotein T-derived peptide (Rocca et al. 2018); snake peptide Cathelicidin-WA (Wu et al. 2018)
HSR	HSF-1	Locust peptides (Cao et al. 2019); recombinant buckwheat glutaredoxin (Li et al. 2018)
	HSP-16.2	Purple sea urchin gonad peptides (Zhao et al. 2018)
	HSP-60	Ile-Leu (Moura et al. 2017)
	HSP-70	Neuroprotective cyclic heptapeptide (Cunningham et al. 2017)
	HSP-90	Leu-Val (Moura et al. 2017)

a range of other cellular functions, including metabolic and proteostatic homeostasis (Dodson et al. 2019; Schmidlin et al. 2019; Lombard et al. 2020). Kelch-like ECH-associated protein 1 (Keap1) is a redox-sensitive protein and a specific repressor of Nrf2 activity in the cytoplasm, but oxidative stress can induce Nrf2 release from the Nrf2-Keap1 complex, causing the nuclear translocation of Nrf2 (Motohashi and Yamamoto 2004; Schmidlin et al. 2019). After translocation into the nucleus, Nrf2 is activated to regulate the expression of target genes, such as antioxidant and detoxifying enzymes, by binding to their antioxidant response elements (ARE) (Schmidlin et al. 2019). Together, the Keap1-Nrf2-ARE pathway is considered as one of the most important defense signaling pathways against oxidative stress (Fig. 11.2).

As summarized in Table 11.2, many peptides and peptide-rich preparations are reported to regulate Nrf2. For example, the peptides from sesame cake are found to upregulate the mRNA levels of *skn-1* and its target gene *gcs-1*, which encode γ-glutamylcysteine synthetase (γ-GCS) (Wang et al. 2016b). Interestingly, the sesame peptides can extend the lifespan and oxidative stress survival of wild-type *C. elegans* but not that of *skn-1* mutant, indicating the modulation of longevity via Nrf-2/SKN-1 (Wang et al. 2016b). Adropin, a secretory signal peptide, is also shown to increase the expression of γ-GCS, which is a rate-limiting enzyme in GSH synthesis (Chen et al. 2019b). Other peptides, e.g. those from fish skin gelatin hydrolysate (Zheng et al. 2018), milk (Kumar et al. 2019; Tonolo et al. 2020), rice (Moritani et al. 2020;

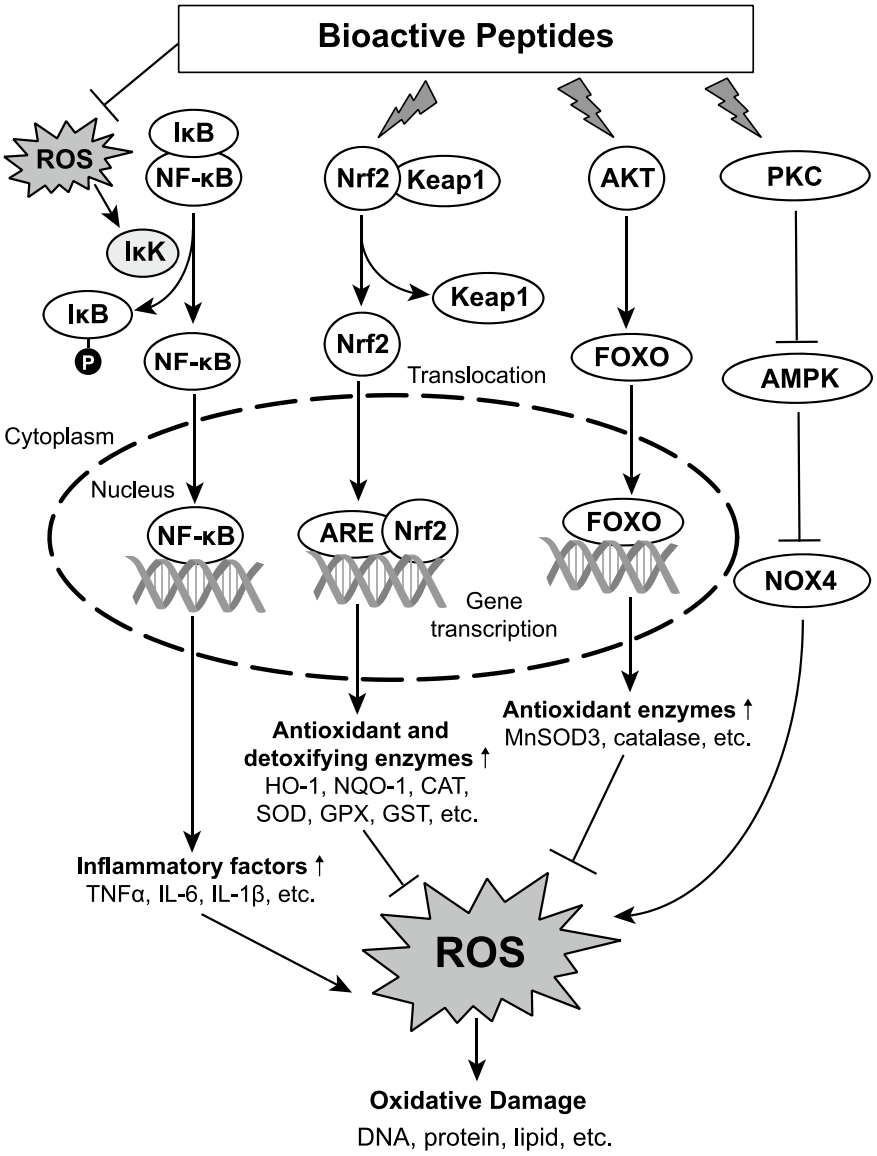


Fig. 11.2 Regulation of redox and aging-related signaling pathways by naturally derived bioactive peptides

Wang et al. 2020d), soybean (Yi et al. 2020), mussels (Zhang et al. 2020), and fish roe (Chen et al. 2021), are also reported to regulate Nrf2 and associated antioxidant enzymes.

A number of natural peptides are found to interact with Keap1, e.g. the peptides identified from mussels (Zhang et al. 2020), soybean (Yi et al. 2020), rice (Wang et al. 2020d), and milk (Tonolo et al. 2020). Some peptides may directly bind with Keap1 and exert their effect on the Nrf2 pathway. For example, the peptide EDYGA obtained from the protein hydrolysate of the soft-shelled turtle is found to modulate the Keap1-Nrf2 pathway by reducing Keap1 expression, leading to an increased nuclear localization and transcriptional activation of Nrf2 (Wang et al. 2020b). Docking analysis suggests that EDYGA may directly bind to Keap1 likely by its glutamate and glycine residues to R415 and R380, respectively, of the Kelch domain receptor pocket, which are the critical points for the Keap1-Nrf2 pathway regulation (Wang et al. 2020b).

The ubiquitin-binding protein p62 (also known as SQSTM1) is reported to interact with the Nrf2-binding site of Keap1, acting as an upstream regulator of Nrf2 which may increase its nuclear localization (Komatsu et al. 2010; Ichimura et al. 2013). As a common component of cellular inclusions, p62 also acts as a selective autophagy receptor for ubiquitinated cargo and, together with ubiquitin, forms phase-separated droplets (Komatsu et al. 2010; Agudo-Canalejo et al. 2021). Interestingly, the peptide-rich fish skin gelatin hydrolysate fractions are found to promote Nrf2 nuclear translocation and increase both mRNA and protein levels of γ-GCS likely through p62-Nrf2 cascade (Zheng et al. 2018). These studies suggest that natural peptides are likely to have other potentials in the Nrf2-mediated cellular pathways, in addition to the oxidative stress response.

11.3.3 Regulation of Other Signaling Pathways by Antioxidant Peptides

Apart from FOXO/DAF-16 and Nrf2/SKN-1 transcription factors, a number of other signaling pathways have also been implicated in the effects of natural peptides on maintaining molecular homeostasis related to aging and oxidative stress (Table 11.2 and Fig. 11.2). For example, the peptides found in abalone (Chen et al. 2019a), milk (Kumar et al. 2019), rice (Wang et al. 2020d), and red shrimp head (Jiang et al. 2020) are found to regulate nuclear factor-κB (NF-κB), which is also an evolutionarily conserved transcription factor with a variety of essential roles in biological processes and disease-related events, including inflammatory and oxidative stress responses (Perkins 2007; Lingappan 2018). A number of peptides have also shown modulatory effects on the upstream regulators and downstream effectors of NF-κB (Fig. 11.2). Red shrimp head peptides, for instance, are able to regulate the inhibitory κB (IκB) and the IκB kinase (IκK) (Jiang et al. 2020). Furthermore, many peptides are found to act on the downstream inflammatory factors such as IL-1β, IL-2, IL-6, IL-8, and

TNF-α, e.g. the peptides from frog skin (Yang et al. 2009), egg yolk (Young et al. 2010), soybean (Zhang et al. 2019), and red shrimp head (Jiang et al. 2020).

The AMP-activated protein kinase (AMPK) is another ubiquitously expressed serine/threonine protein kinase conserved in eukaryotes and plays a central role in several metabolic pathways and metabolic diseases (Day et al. 2017; Herzig and Shaw 2018). As noted in Table 11.2, a number of peptides are capable of regulating key players of this AMPK pathway. For example, the snake peptide Cathelicidin-WA (Wu et al. 2018) and wheat germ peptides (Chen et al. 2017; Wang et al. 2020a) are reported to regulate protein kinase C (PKC), while Szeto-Schiller peptides (Hou et al. 2018) and wheat germ peptides (Chen et al. 2017; Wang et al. 2020a) are found to act on NADPH oxidases (NOX).

The well-characterized, evolutionarily conserved mitogen-activated protein kinases (MAPK) signal transduction networks are widespread in eukaryotes and have important physiological functions, including responses to desiccation (Huang and Tunnacliffe 2004; Huang et al. 2010) and oxidative stress (Li et al. 2017b; Ding et al. 2019). There are three main MAPK families based on phylogenetic, structural, and functional analysis, i.e. c-Jun N-terminal kinases (JNK), extracellular signal-regulated kinases (ERK), and p38 MAPK. As shown in Table 11.2, the peptides from locust (Cao et al. 2019), abalone (Chen et al. 2019a), and *Cardamine violifolia* (Yu et al. 2020) are reported to exhibit antioxidant activity through regulation of JNK. The peptides identified in the wheat germ (Chen et al. 2017), abalone (Gong et al. 2019), and fish roe polypeptide (Chen et al. 2021) as well as selenoprotein T-derived peptides (Rocca et al. 2018) are found to regulate ERK, while the peptides from snake (Wu et al. 2018), milk (Kumar et al. 2019), sesame cake (Ma et al. 2019), abalone (Chen et al. 2019a; Gong et al. 2019), and rice (Wang et al. 2020d) are shown to act on p38 MAPK.

Heat shock transcription factors (HSF) are a family of phylogenetically conserved DNA-binding proteins that were originally discovered as transcriptional activators of genes responsive to thermal stress. The protective mechanism of heat shock response (HSR) has, however, been increasingly recognized as playing pivotal roles to maintain proteostasis (protein homeostasis) under various stress and disease conditions (Gomez-Pastor et al. 2018; Janowska et al. 2019). The HSR pathway primarily involves the expression of heat shock proteins (HSP) to ensure the quality and homeostasis of intracellular proteins and, thus, protect cells from stress damages. As listed in Table 11.2, a number of naturally identified antioxidant peptides are reported to regulate the key members of the HSR pathway, e.g. locust peptides on HSF-1 (Cao et al. 2019), purple sea urchin peptides on HSP-16.2 (Zhao et al. 2018), the dipeptide Ile-Leu on HSP-60 (Moura et al. 2017), a cyclic heptapeptide on HSP-70 (Cunningham et al. 2017), and the dipeptide Leu-Val on HSP-90 (Moura et al. 2017).

11.4 Conclusion

Under normal oxidative stress conditions, cells and organisms can protect themselves from oxidative damage with endogenous antioxidant defense systems. Under persistent detrimental stress, however, exogenous antioxidants become necessary to help their defense against the stress. In the last decade, a large number of naturally derived peptides have been explored for their antioxidant capacity. These antioxidant peptides are found to enhance non-enzymatic as well as enzymatic antioxidant systems, including SOD, CAT, GPx, and GSH. Using model organisms such as *C. elegans* and *Drosophila*, many antioxidant peptides are also shown to increase their survival under increased oxidative stress. As oxidative survival is an unequivocal in vivo antioxidant indicator, the change in area under the survival curve (ΔAUC) is introduced to assess the total survival gain against oxidative stress, which is particularly valuable to compare the cumulative differences of survival across the entire lifespan under persistent stress. On the other hand, as oxidative stress resistance can be conveniently used as a prognostic indicator of anti-aging capability, a number of natural peptides are indeed found to have prolongevity effects in *C. elegans* and *Drosophila*. The antioxidant and anti-aging activities of natural peptides are shown to involve a range of signal transduction pathways, including IIS, Nrf2, NF-κB, AMPK, MAPK, and HSR. The peptides are reported to exert their antioxidant functions by acting on a number of key nodes of these pathways, e.g. AKT/PKB and FOXO/DAF-16 for the IIS pathway and Keap1, Nrf2/SKN-1, and ARE for the Nrf2 pathway.

Compliance with Ethical Standards

Conflict of Interest All authors declare they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 12
Senolytic Phytocompounds in Redox Signaling

Kavitha Thirumurugan

Abstract Senescence is a conserved, quasi-program that continues throughout the development of a living organism. Inviting natural compounds to treat such an ill-planned process requires a hit-and-run approach. Organisms have to protect their safety while opening up for various treatments. The dose, duration, and number of cells/subjects, likely emergence of consequences following treatment have to be considered. The cross-talk of diverse signaling pathways to activate and suppress a particular event precisely at a specific time demands enormous energy. Cells are equipped to execute this complex homeostasis process dynamically. With aging, the overall functional response from the cells declines, so reversing the aging process might help the cells to live healthy for a long time. There are multitudes of clinical trials involving natural compounds as well their synthesized analogs to clear the senescent cells and maintain the youthful metabolic state. This chapter tries to explain the role of senolytics in senescence and the assisting signaling pathways.

Keywords Redox signaling · Senescence · Senolytic phytocompounds · Cell signaling pathways—FoxO · p53 · Nrf · NF-κB · JAK/STAT · mTOR/PI3K/AKT

12.1 Introduction

We all age whether we like it or not. The pertinent question arises how well we age with less age-related complications. As per 2016 data from World Health Organization, the global human life expectancy of both sexes reached 72 years. The aged population (60 and above) are expected to reach 2.1 billion in 2050. Aging is a major risk factor for many diseases like cardiovascular, diabetes, obesity, cancer, arthritis, and neurodegeneration (Kirkland and Tchkonia 2017). Medical advancement increased the lifespan at the cost of quality. These age-related disorders are closely linked with metabolism at the cellular and molecular level affecting senescence. Senescence is a phenomenon of irreversible cell cycle arrest and resistance

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to apoptosis. It is correlated with aging either by the exhaustion of stem cells or the accumulation of senescence cells due to poor immune function and chronic stress (Kang 2019). Senescence is a quasi-program that continues its development albeit loosely. In senescent cells, functional decline occurs due to the constant accumulation of damage that exceeds self-repair mechanisms (Blagosklonny 2006).

Senescence is classified into three types: developmentally programmed, stress-induced premature, and replicative (von Kobbe 2018). Depending upon the intensity of the stress-induced DNA damage, the response manifests at the G1 phase of the cell cycle by p53/p21, and p16/Rb pathways (Kang 2019). Acute senescence is a tightly regulated process targeting specific cells in a tissue (van Deursen 2014). It is beneficial in tissue repair/reprogramming (Mosteiro et al. 2016), fetal development (Storer et al. 2013), and wound healing by PDGF-AA secretion (Demaria et al. 2014). On the other hand, persistent accumulation of senescent cells in aged tissues produce senescence-associated secretory phenotype (SASP) and associated tissue dysfunction (Ellison-Hughes 2020). This chronic senescence is not target-specific and not programmed (van Deursen 2014). Selective clearance of even a small fraction of senescent cells improves age-related functional decline (Baker et al. 2011). Though the senescent cells face a harsh cellular environment that promotes apoptosis, still they tolerate the stress and survive for some time. Pharmacological interventions to remove senescent cells are a better strategy since a study on cyclin-dependent kinase inhibitor p16Ink4a knockout mouse shown retention of a tumor-suppressive function of p19Arf, inducing tumorigenesis (Sharpless et al. 2001).

12.2 Redox Signaling

Redox signaling involves reactive oxygen and nitrogen species (RONS) to regulate mitogen-activated protein kinases (MAPK), protein tyrosine kinases (PTK), and protein tyrosine phosphatases (PTP) (Forman et al. 2002). In mammalian cells, ROS generation is monitored by NADPH oxidase and nitric oxide synthase. NADPH oxidase forms superoxide anion ($O_2^{\bullet-}$) in phagocytes, and nitric oxide synthase produces nitric oxide. The spatial organization of the superoxide anion closer to its target site provides physiological relevance. Otherwise, $O_2^{\bullet-}$ is reduced by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). It is not a radical and uncharged at physiological pH. Due to its limited reactivity, H_2O_2 confers specificity to the reaction (Forman et al. 2002). H_2O_2 reacts with a reduced transition metal to form hydroxyl radicals ($^{\bullet}OH$). These $^{\bullet}OH$ radicals participate in the irreversible oxidation of biological molecules. In cell and molecular signaling, the role of hydrogen peroxide is vital compared to hydroxyl radical. Maintaining a fine balance between oxidation and reduction helps the cell to ward off the pathogen attack and prime during stressful conditions. Tipping the balance in favor of oxidation shifts ROS biology toward pathogenic pathways and harmful effects (Davalli et al. 2016). Low-level ROS induces positive oxidative stress which triggers an adaptive response to a long healthy life. High ROS level stimulates negative oxidative stress that affects

aging adversely (Yan 2014). As ROS creates cellular oxidative stress, their elimination is taken care of by various enzymes. H_2O_2 is removed by catalase in peroxisomes. In other locations, H_2O_2 is reduced by thiol-dependent glutathione peroxidase (Gpx) and thioredoxin peroxidase (Tpx). Gpx requires selenium and Tpx uses reactive cysteines. Oxidation of sulfur present in the cysteines to disulfides and sulfenic acid mediates redox signaling under oxidative stress (Akerboom and Sies 1981). On exposure to hydrogen peroxide, cysteine residues present in the active site of protein tyrosine phosphatase (PTP) oxidized to sulfenic acid, thereby inhibiting phosphatase activity (Denu and Tanner 1998).

12.3 Mitochondrial Dysfunction in the Context of Cellular Senescence

Mitochondrial dysfunction is a warning signal of cellular senescence. The oxidative phosphorylation (OXPHOS) system has five enzymatic complexes: NADH–ubiquinone oxidoreductase (complex I), succinate–ubiquinone reductase (complex II), ubiquinone–cytochrome c oxidoreductase (III), cytochrome c oxidase (IV), and ATP synthase (V) (Signes and Fernandez-Vizarra 2018). Electrons flow from complex I to IV and during this process, leakage of electrons on reaction with oxygen forms superoxide anion, which damages macromolecules. Also, the superoxide anions generate highly reactive secondary ROS that could drive the aging process (Harman 1972). Senescent cells from mitochondria show excess ROS production, and feedback between ROS and checkpoint gene p21 is required for cell senescence (Passos et al. 2010). Mitochondrial ROS is the major player in telomere-induced senescence and maintaining the senescence arrest. Nicotinamide adenine dinucleotide (NAD^+) plays an important role in the TCA cycle and DNA repair. It is an essential coenzyme in redox reactions, and non-redox reactions involving sirtuins, and poly (ADP-ribose)-polymerases (PARP). In human vascular smooth muscle cells, reduced activity of nicotinamide phosphoribosyltransferase (NAMPT) resulted in premature senescence. When the NAMPT gene was added back into aging smooth muscle cells, it delayed the senescence and extended the lifespan along with improved resistance to oxidative stress (van der Veer et al. 2007). Conversion of phosphorylated NAD^+ to its reduced form NADPH protects the cells from oxidative stress. In senescent cells, NAD^+ levels decrease due to its consumption by CD38 and PARP. Low levels of NAD^+ reduce autophagy and mitophagy in senescent cells (Covarrubias et al. 2020). Blocking TNFα or adding NAD^+ reduces inflammation and senescence burden in mice carrying T-cells with mitochondrial dysfunction (Desdín-Micó et al. 2020).

12.4 Senolytic Phytocompounds

Senotherapeutics are pharmacological interventions targeting senescent cells for clearance and relieve associated pathophysiological effects. Senotherapeutics are classified into senolytics, senomorphics, and senoinflammation (Kim and Kim 2019). Senolytics are small-molecule compounds that extend lifespan and defer age-related disorders by the selective killing of senescent cells without affecting nonsenescent cells (Blagosklonny 2018a). These senolytics can be peptides, drugs, antibodies, and natural compounds (Zhu et al. 2015). The first report on senolytics, dasatinib, and quercetin show target pathway protein tyrosine kinase (Zhu et al. 2015). Other reported senolytics are navitoclax, fisetin, luteolin, A1331852, A1155463, and HSP90 inhibitors. Senomorphics are SASP blockers, and they modulate or reverse the phenotype of senescent cells to young cells. Rapamycin, ruxolitinib, metformin, p38 MAPK inhibitors are examples of senomorphics. Senoinflammation is a mediator of immune system clearance of senescent cells (Kim and Kim 2019). A list of compounds targeting senescence is provided in Table 12.1, Figs. 12.1 and 12.2.

12.5 Navitoclax

Navitoclax interacts with the Bcl2 pathway, allows senescent cells to undergo apoptosis by inhibiting Bcl-2. Navitoclax is an anticancer drug and it is toxic to platelets. To improve the potency and reduce the severe thrombocytopenia by ABT-263, taking advantage of the low expression levels of E3 ligase Cereblon (CRBN) in platelets, PZ15227, a PROTAC (proteolysis targeting chimera) that is constructed by linking ABT-263 to a CRBN ligand pomalidomide, has shown improved efficacy and reduced toxicities compared to ABT-263 (He et al. 2020). Navitoclax is effective against HUVEC cells and IMR90 human lung fibroblasts. It is not effective against human primary preadipocytes (Zhu et al. 2016). Silenced GTP-binding RAS-like 3 (DIRAS3) induces senescence in human preadipocytes through activated Akt-mTOR signaling. These premature senescent adipocytes promote the secretion of pro-inflammatory cytokines (IL8, IL6, IL1β, TNFβ), p53, and Cdkn 1a (Ejaz et al. 2017). Pharmacological senolytics reduced the senescent cells and macrophage infiltration (Frasca and Blomberg 2020).

12.6 Dasatinib (D) and Quercetin (Q)

Senescent cells when transplanted to young mice show a plausible ‘hit and run’ effect of D and Q was given the context of the late reappearance of senescent cells following treatment. The presence of senescent cells in young mice causes physical dysfunction and reduced survival. Therefore, selective elimination of senescent cells

Table 12.1 List of compounds targeting senescence

Compound	Property/pathways	Senescent cells	Remarks	Reference
Dasatinib	Tyrosine kinase inhibitor PI3K/Akt inhibitors	HUVEC Preadipocytes MEF BM-MSCs	Induces DNA damage, activates DNA repair pathways Peng et al. (2015)	Kirkland et al. (2017) Justice et al. (2019) Hickson et al. (2019)
Quercetin	Flavonoid PI3K/Akt inhibitors	HUVEC Preadipocytes MEFs BM-MSCs	Reduced ROS via SIRT3 upregulation, reduced SOD2-K68 acetylation Promotes in vitro oocyte maturation in human and aged mice	Kirkland et al. (2017) Justice et al. (2019) Hickson et al. (2019) Cao et al. (2020)
Quercetin surface functionalized magnetite nanoparticles (MNPQ)	Flavonoid Increases AMPK activity	Human fibroblasts	Reduced pro-inflammatory secreted IL-8 and IFN-β in hydrogen peroxide-induced senescent human fibroblasts	Lewinska et al. (2020)

(continued)

Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
Quercetin (Q) + Dasatinib (D) Quercetin (Q) + Dasatinib (D)	Expression of ephrin dependence receptor ligands (EFNB1 or EFNB3), D plus Q reduced p21, PAI-2, and BCL-xL First clinical trial of senolytics: D + Q improved physical function of idiopathic pulmonary fibrosis (IPF) patients	eliminating senescent MEFs; chronologically aged, radiation-exposed, progeroid Ercc1(-/Δ) mice Open label Phase 1 pilot study: 3 days of oral Dasatinib (100 mg) and Quercetin (1000 mg) to subjects with diabetic kidney disease	D more effective to reduce viability of senescent preadipocytes than HUVECs. Q more effective to reduce the viability of senescent HUVECs than preadipocyteD and Q combination reduced the viability of both preadipocytes and HUVECs. Viability of quiescent fat cells not affected by D and Q D + Q-reduced adipose tissue senescent cells in 11 days, reduced $p16^{INK4A}$- and $p21^{CIP1}$-expressing cells, lowered SA β-galactosidase activity	Zhu et al. (2015) Hickson et al. (2019)
Navitoclax (ABT-263)	Bcl-xL inhibitor	HUVEC IMR90 MEFs	Causes trabecular bone loss in aged mice Sharma et al. (2020)	Kirkland et al. (2017) Zhu et al. (2016)
A1331852	Bcl-xL inhibitor, Bcl-2 pro-survival inhibitors	HUVEC	Selective apoptosis in tumor cells	Kirkland et al. (2017)
A1155463	Bcl-2 pro-survival inhibitors	IMR90	Selective apoptosis in tumor cells	Kirkland et al. (2017)

(continued)

Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
Fisetin	Flavonoid PI3K/Akt inhibitors Reduced OS-induced senescence	HUVEC Murine embryonic fibroblasts, Ercc1$^{-/-}$mice showing progeroid syndrome	Reduced oxidative stress Reduced oxidative stress, SA-β-gal, SASP markers Induction of apoptosis, and increased caspase-3,7 activity	Kirkland et al. (2017) Zhu et al. (2017) Yousefzadeh et al. (2018)
17-DMAG	Cell senescence induced with atmospheric oxygen (20%) HSP90 inhibitors Downregulation of anti-apoptotic PI3K/Akt pathway Disruption of the HSP90-AKT interaction, apoptosis of senescent cells	DNA repair deficient Ercc1$^{-/-}$primary murine embryonic fibroblasts, Ercc1 $^{-/\Delta}$ mice, mouse model of a human progeroid syndrome	Reduced p16INK4a expression, extended health span Apoptosis of senescent cells	Fuhrmann-Stroissnigg et al. (2017)
Panobinostat	Histone deacetylase inhibitor Bcl family	Non-Small Cell Lung Cancer (NSCLC), Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines	In senescent cells, decreased histone 3 (H3) acetylation, increased Bcl-xL expression In cancer cells, increased acetylated H3, decreased expression of Bcl-xL. Potential post-chemotherapy senolytic	Samaraweera et al. (2017)
FoxO4-DRI (D-Retro-Inverso peptide)	FoxO4-p53 interaction	Primary human IMR90 fibroblasts and IMR90	FoxO4 peptide neutralized Doxorubicin-induced chemotoxicity	Baar et al. (2017)
Catechins	Nrf2 activation, PI3K/Akt/mTOR inhibition, Bax/Bcl-2 pathway	Human mesenchymal stem cells (hMSCs)	Reduced ROS production, and oxidative stress	Shin et al. (2016) Kumar et al. (2019)

(continued)

Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
MitoQ	Nrf2/PINK1	Human proximal tubular cell line HK-2, C57BL/6 J mice	Reduced oxidative stress	Xiao et al. (2016)
SS31	NF-κB, increased SIRT1/SIRT3	H9C2 cells, Sprague-Dawley (SD) rats	Reduced ROS production, and oxidative stress	Lee et al. (2018a, b)
Melatonin	Rescues replicative senescent cells, interaction of HSPA1L with cellular prion protein (PrPc) for transport to mitochondria	Mesenchymal stem cells (MSCs)	Reduced oxidative stress. Promotes mitophagy and upregulation of heat shock 70 kDa protein 1L (HSPA1L)	Lee et al. (2020)
Equol	Nrf2/ARE	Human skin	Reduced oxidative stress and ROS	Lephart (2016) Liu et al. (2020)
Ganetespib (GT), ABT263, MK2206	HSP90 inhibitor, Bcl-2 family inhibitor, Akt inhibitor	Prostate cancer PCa LNCaP androgen-sensitive cells	MK2206 potently inhibits Akt phosphorylation GT reduces Akt, phospho-S6 protein levels ABT263 not affecting Akt, p-S6 protein levels	Pungsrinont et al. (2020)
Curcumin, o-Vanillin	Nrf2-activation	Human intervertebral disc (IVD)-monolayer and pellet culture	Reduced protein expression of MMP-3, and MMP-13, reduced pro-inflammatory cytokines, IL-6 and IL-8	Cherif et al. (2020) Cherif et al. (2019)

(continued)

Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
Epigallocatechin gallate (EGCG)	Downregulation of PI3K/Akt/mTOR/AMPK, Bax/Bcl-2 Inhibition of hydrogen peroxide-induced senescence p53 and Rb1 pathways	Hydrogen peroxide (150 μM)-induced 3T3-L1 preadipocytes Rat vascular smooth muscle cells (RVSMCs), human articular chondrocytes (HACs), human dermal fibroblasts (HDFs) WI-38 fibroblasts	Suppression of ROS, iNOS, Cox-2, NF-κB, SASP and p53, reduced anti-apoptotic Bcl-2 Suppression of p53-acetylation, No change in SIRT1 activity Reduced reactive oxygen species (ROS), inflammation factors (TNF-α, IL-6, IL-32), decreased expression Rb and p53, increased p-Rb, E2F2, SOD1, and SOD2	Kumar et al. (2019) Han et al. (2012) Zhang et al. (2019b)
Genistein	AMPK activation mTOR inhibition	HUVEC, human vascular smooth cells	Autophagy induction, increased phosphorylation of LKB1-AMPK	Lee et al. (2016)
Resveratrol	p53-CXCR2-mediated cellular senescence	U2OS and A549 cancer cells, normal human fibroblasts	ROS elevation after S-phase arrest	Li et al. (2017)
Olive phenols—hydroxytyrosol (HT) and oleuropein aglycone (OLE)	Inhibition of mTOR	pre-senescent human lung fibroblasts (MRC5), neonatal human dermal fibroblasts	Reduced oxidative stress, ROS, SA-β-gal, p16, SASP	Menicacci et al. (2017)
Rosemarinic acid	Hormetic doses (0.5 μM and 1.0 μM) enhanced stress tolerance, Reducing the accumulation of 8-OhdG (8-hydroxy-2′-deoxyguanosine)	Human skin fibroblast cell-serially passaged p15 to p57	slowdown of telomere loss, 56% reduction of 5-methyl Cytosine (5-mC), and oxidative DNA damage product	Sodagam et al. (2019)

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Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
Ampelopsin	Hormetic doses (0.5 μM and 1.0 μM) enhanced stress tolerance, Reducing the accumulation of 8-OhdG (8-hydroxy-2′-deoxyguanosine)	Human skin fibroblast cell-serially passaged p15 to p57	slowdown of telomere loss, 75% reduction of 5-methyl Cytosine (5-mC), and oxidative DNA damage product	Sodagam et al. (2019)
Cardiac glycosides-Proscillaridin A, digoxin. Combining senolytic and senogenic compounds.	Digoxin-induced senolysis. Reduction in CDKN2A (coding for p16INK4a) expression levels.	A549-human lung adenocarcinoma cell line SK-MEL-103-melanoma cell line Delivery of gamma-irradiated (g-IR) senescent human fibroblasts IMR90 into lungs of mice	Inhibition of Na+/K+ pump, depolarization and acidification, killing senescent cells by apoptosis	Triana-Martínez et al. (2019)
Rapamycin	mTOR inhibitor, Nrf2 activation–regulation of cell cycle arrest, Autophagy activation, STAT3 suppression Autophagy pathway not activated, STAT3 suppression, Nrf2-independent pathway, Inhibition of MAPKAPK2 pathway to reduce SASP	Mouse fibroblasts Nrf2KO fibroblasts, Nrf2KO mice	Reduced SA-β-gal, SASP, p16, p21 Reduced SA-β-gal, SASP. No reduction in p16, p21	Wang et al. (2017) Wang et al. (2017)

(continued)

Table 12.1 (continued)

Compound	Property/pathways	Senescent cells	Remarks	Reference
α-boswellic acid (1 μM concentration)	Autophagy, oxidative stress response, cell cycle regulation, and inflammatory response	Human skin fibroblasts (PCS)-Early passage (p14), and late passage (p58)	Reduced LC3B, SOD1, cell cycle genes, IL6, p53 in both young (p14), and near-senescent cells (p58). Cell cycle genes expressed more in late passage cells.	Lewinska et al. (2019)
Praeruptorin-A (1 μM concentration)	Autophagy, oxidative stress response, cell cycle regulation, and inflammatory response	Human skin fibroblasts (PCS)-Early passage (p14), and late passage (p58)	Increased SOD1, cell cycle genes, IL6, IL15, p53 in both young (p14), and near-senescent cells (p58). LC3B expressed less in early and late passage cells	Lewinska et al. (2019)
Salvianolic acid (1 μM concentration)	Autophagy, oxidative stress response, cell cycle regulation, and inflammatory response	Human skin fibroblasts (PCS)-Early passage (p14), and late passage (p58)	Reduced LC3B, SOD1, IL6, IL15, p53 in young (p14). Increased LC3B, SOD1, IL6, p53 in near-senescent cells (p58) Cell cycle genes expressed less in early and late passage cells	Lewinska et al. (2019)
Berberine	Effective in reducing doxorubicin-induced senescence, extends lifespan of mice	Human fetal lung diploid fibroblasts (2BS and WI38)	Reduced SA-β-gal, p16 Increased cell cycle genes Upregulated expression of phosphorylated-retinoblastoma protein (pRB) Promoted G1/S transition in aged cells	Dang et al. (2020)

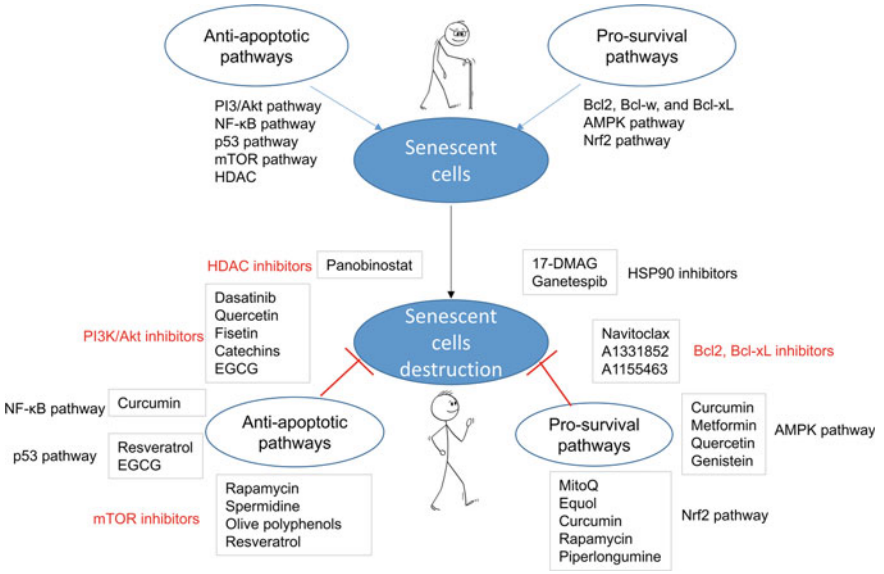

Fig. 12.1 Signaling pathways involved in clearance of senescent cells. Activated anti-apoptotic and pro-survival pathways maintain the senescent cells. Use of senolytics inhibits these pathways and reduces the burden of senescent cells. Involvement of inhibitors of mTOR, PI3K/Akt pathways, along with NFκB, p53 pathways promote apoptosis to destroy senescent cells. Inclusion of Bcl2, Bcl-xL inhibitors, HSP90 inhibitors, and AMPK, Nrf2 pathways, lead to suppression of pro-survival pathways to kill the senescent cells

by the combination of D and Q increased the mice survival by 36% (Xu et al. 2018). Cancer patients going through radiation therapy develop ulcers due to persistent senescent cells in the site and adjacent region. JAK pathway activation is noticed in ulcer patients after radiation therapy. So clearing these cells might reduce the ulcer intensity. Treatment with D (1 mM) and Q (20 mM) cocktail eliminated 40–60% of senescent HOK (human oral keratinocytes) and 10–20% of skin fibroblasts within a day (Wang et al. 2020).

Treating the uterus of 3-month-old and 30-month-old mice with D and Q combination showed an anti-fibrotic effect. The response was observed as p53 upregulation and miR34a downregulation. Aging-related pathway PI3K/Akt1/mTOR, and PTEN not affected in this study as treatment duration is short (Cavalcante et al. 2020).

Intermittent treatment of D (5 mg/kg body weight) and Q (10 mg/kg body weight) to aged mice and hypercholesterolemic mice improved vasomotor function. This is due to increased bioavailability of p-eNOS ^{ser1177}, and p-VASP^{239} in both the media and intima of atherosclerotic vessels. These p-eNOS and p-VASP (vasodilator-stimulated phosphoprotein) are targets of the nitric oxide-activated cGMP-dependent kinase (Roos et al. 2016).

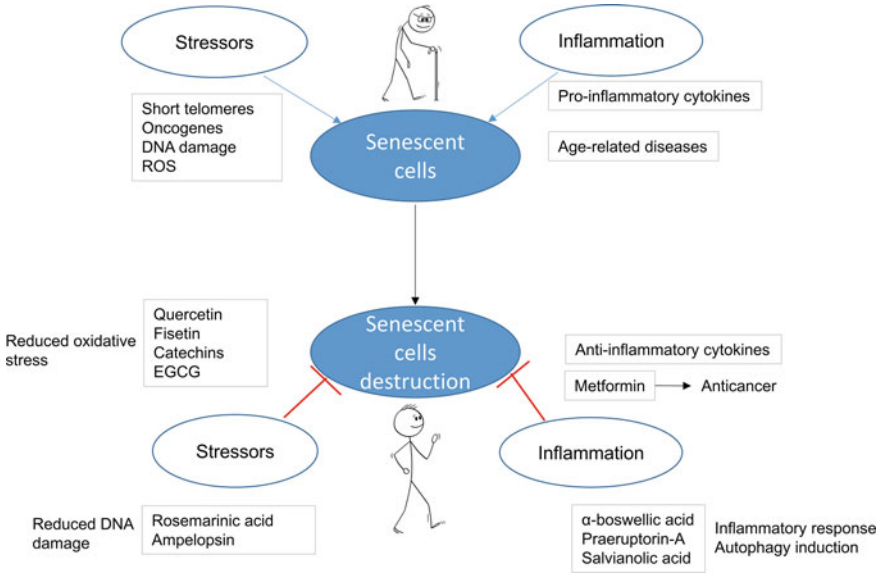

Fig. 12.2 Phytocompounds mediating senescence clearance through regulating inflammation and various stressors. Inflammation and diverse stressors predispose the cells for senescence. Reducing the oxidative stress and DNA damage by administering phytocompounds clear the senescent cells. Natural compounds elicit favorable inflammatory response to destroy the senescent cells through autophagy induction

12.7 Fisetin

Fisetin is a potent flavonoid in clearing senescent cells. Treating primary MEFs from Ercc1-/- progeroid mice with various flavonoids at a dose of 5 μM, fisetin show reduced senescence and SA-β-gal staining (Yousefzadeh et al. 2018). Before fisetin treatment, these MEFs gone through five passages at 20% oxygen for senescence induction. To find the senolytic efficacy in vivo, Ercc-/Δ p16Ink4a luciferase mice fed with fisetin (60 mg/kg body mass/day) intermittently at 6–8 weeks, and 12–14 weeks of age. The low expression of p16Ink4a during a non-treatment period (8–11 weeks) shows the mechanism of senescent cell clearance that does not require continuous exposure to the flavonoid (Yousefzadeh et al. 2018). Treating naturally aged C57/BL6J mice (22–24 months) with fisetin at 100 mg/kg body mass for 5 days by oral gavage show reduced SA-βgal staining (Yousefzadeh et al. 2018). Human omental white adipose tissue explants of an obese female subject cultured in a medium with fisetin at 20 μM for 48 h display reduced SA-β-gal-positive staining, and low expression of SASP factors: IL-6, IL-8, and MCP-1 (Yousefzadeh et al. 2018). Late-life interventions of C57/BL6J mice at 85 weeks of age with fisetin (500 mg/kg body mass) show reduced SA-β-gal-positive staining and low expression of SASP markers (p16, p21, IL-1β, IL-6, IL-10, TNF-α, Cxcl2, MCP-1, and PAI-1) in multiple tissues (fat, spleen, liver, kidney) (Yousefzadeh et al. 2018).

Fisetin acts as a reducing agent. Due to the presence of three hydroxyl groups, it effectively scavenges free radicals (Ishige et al. 2001). Exposing passage 4 human umbilical vein endothelial cells (HUVEC), human lung fibroblasts (IMR90), and human preadipocytes to 10 Gy radiation to induce senescence and then treating them with fisetin at different concentrations (0.5, 5, and 10 μM) provided cell type-specific effects (Zhu et al. 2017). Fisetin induces apoptosis in senescent cells of HUVECs, and not senolytic in IMR90 and human preadipocytes. Choosing an optimal concentration window is essential as lower concentration is not effective to kill the senescent cells and higher concentration might be cytotoxic. Also administering senolytics at intermittent intervals might reduce the chances of side effects and flatten the profile.

12.8 Metformin

The biguanide metformin is an FDA approved antidiabetic drug. In the structure, it has two guanidiniums joined by common nitrogen to inhibit mitochondrial complex 1 and reduce reactive oxygen species. Metformin targets several signaling pathways connected to aging, autophagy, inflammation, stress defense, and survival (Barzilai et al. 2016). Targeting Aging with Metformin (TAME) clinical trial started 6 years ago (2004) with the aim of healthy aging by the American Federation for Aging Research (AFAR) lead by Nir Barzilai. This trial involves 14 institutions and 3000 participants in the age group of 65–79. As aging is associated with dementia, diabetes, cancer, heart disease, and Alzheimer's, the TAME trial will show a paradigm shift of treating age-related diseases in a combo, just by focusing on aging alone.

Treating normal lung human diploid fibroblasts (IMR90), murine macrophage cell line RAW264.7, and AMPKα-activated MEFs with metformin for 6 days show the ability of the drug to block the pro-inflammatory NF-κB, and not affecting the expression of anti-proliferative interferon pathway and p38MAPK (Moiseeva et al. 2013). Independent of AMPK, metformin represses TNFα-dependent IκB expression and reduces the expression of pro-inflammatory cytokines (Moiseeva et al. 2013).

AMPKα-activated MEFs and human mammary epithelial cells (HMEC) treated with metformin (5 mM/L) for 48 h show an increase in ROS levels (Algire et al. 2012). As mitochondrial complex I being the source of ROS, the ability of metformin to interfere with complex I and reduce ATP production to activate AMPK might be the reason for cancer cell death. Also, metformin reduced double-strand breaks after exposure of the cells to paraquat but not after H_2O_2 exposure. In the same study, when oncogenic Ras introduced to primary human fibroblasts, metformin attenuated the increase in ROS production.

12.9 Spermidine

Spermidine is an endogenous natural polyamine that declines with age, particularly the loss of antibody responses in human B lymphocytes. Spermidine post-translationally modifies (hypusinates) eIF5A to maintain cellular basal autophagy. Transcription factor TFEB involved in autophagosome biogenesis requires hypusinated eIF5a for efficient translation. In old B cells, supplementing spermidine levels will boost the immune response via activating the eIF5A–TFEB–autophagy axis. This way, reversing the immune senescence is a promising possibility (Zhang et al. 2019a; Zhang and Simon 2019; Metur and Klionsky 2019).

12.10 Epigallocatechingallate (EGCG)

Superoxide anion has strong nucleophilic properties to influence epigenetic modifications, like DNA methylation and histone modification (Afanas'ev 2014). Epigallocatechin-3-O-gallate (EGCG), an active polyphenol present in green tea is effective in preventing senescence of human dermal fibroblast (HDF) cells. Late passage number cells (PN 30) of RVSMC and HAC treated with SA-β-gal have shown a reduced number of stained senescent cells by EGCG (100 μM) treatment. Similarly, H_2O_2 (150 μM)-treated HDF cells showed reduced protein expression of acetylated p53 following ECGC (100 μM) treatment (Han et al. 2012).

12.11 Rapamycin

A well-known inhibitor of mTOR reduces mitochondrial ROS-induced NFκB activation and decreases SASP while maintaining cell cycle arrest in the aging mouse liver (Correia-Melo et al. 2016). Rapamycin extends overall healthspan and reduces telomere-associated DNA damage foci (TAF) in nfkb1$^{-/-}$ mice (Correia-Melo et al. 2019). Rapamycin extends the lifespan and healthspan of *C. elegans*, *Drosophila melanogaster*, and humans (Lamming et al. 2013; Ehninger et al. 2014; Johnson et al. 2015). Rapamycin suppresses geroconversion so that conversion of reversible cell cycle arrest to irreversible senescence is prevented (Blagosklonny 2014). Rapamycin functions as a senolytic compound to limit hyperfunctional senescent cells thereby protecting the cells/organs from damage and disease progression (Blagosklonny 2018a, b). To study the effect of rapamycin on NAD^+/NADH redox balance in C2C12 myoblast, cells were cultured in rapamycin (100 nM) for 24 h (Zhang et al. 2020). Results show an increase NAD^+/NADH ratio, reduced NADH concentration, and increased ATP concentration in myoblasts cultured longer. When the experiments were conducted with young C57/BL6J mice (8 weeks), there was no significant change in NAD^+/NADH ratio and NADH level. Aged mice (17 months old) treated

with rapamycin (2 mg/kg body mass) display increased NAD^+/NADH ratio and decreased NADH concentration implying a youthful metabolic state (Zhang et al. 2020). In the same paper, optical redox imaging of muscle tissue from 17 months old-aged mice shows reduced NADH. There is a reduced energetic demand by the senescent cells. Senescent cells accumulate more lactate than young cells (McReynolds et al. 2020).

12.12 Senolytics in Cell Signaling Pathways

Senolytics are classified into Bcl family inhibitors, PI3K/Akt inhibitors, and FoxO regulators (Zhu et al. 2020). Bcl family has pro-apoptotic and pro-survival proteins to clear senescent cells. Catechins are Bcl inhibitors. They follow Nrf2, PI3K/AKT/mTOR, and Bax/Bcl-2 pathways. Dasatinib and quercetin, fisetin are PI3K/Akt inhibitors. SASP inhibitors include astaxanthin and equol. Astaxanthin maintains mitochondrial function. Resveratrol is a sirtuin regulator. Rapamycin and spermidine are mTOR inhibitors. Metformin and curcumin are AMPK activators. Curcumin signals AMPK, sirtuin, PI3K/AKT, NF-κB, and Nrf2 pathways (Zhu et al. 2020). Representation of various cell signaling pathways are depicted in Fig. 12.1.

A proteomic atlas of SASP signatures overlaps with aging biomarkers, growth/differentiation factor 15 (GDF15), stanniocalcin1 (STC1), and serine protease inhibitors (SERPINs) in human plasma (Basisty et al. 2020). Primary human lung fibroblasts (IMR-90) and renal cortical epithelial cells exposed to X-irradiation (IR), inducible RAS, atazanavir (ATV) analyzed for secretory (sSASP) and exosome (eSASP) factors. Senescent fibroblasts IMR-90 show expression of DAMPs (damage-associated molecular patterns) high mobility group box 1 protein (HMGB1), and calreticulin (CALR) under IR, RAS, and ATV treatment. Signaling pathways enriched in eSASP are RAS signaling, G-protein signaling, prostaglandin synthesis, and regulation, whereas the sSASP shows ECM-remodeling pathways. This proteomic analysis displays diverse SASP factors specific to cell types and stimuli. Knowing the SASP profiles might help in deducing the efficiency of senotherapeutic senolytics in the future to delay age-related diseases.

12.13 FoxO Pathway

Forkhead homeobox type O family (FoxO) members are FoxO1, FoxO3, FoxO4, and FoxO6 are involved in stress resistance, metabolism, cell cycle arrest, ROS scavenging, apoptosis, tumor suppression, and cell fate determination (Bourgeois and Madl 2018). Cysteine residues present in FoxO act as 'redox sensors' (de Keizer et al. 2011). FoxO activity is inhibited by insulin and IGF-1 signaling pathway (IIS) (Martins et al. 2016). FoxO is phosphorylated by PI3K (phosphoinositide 3-kinase)/Akt and transported to the nucleus for transcriptional inactivation (Dansen

2011). On the contrary, JNK (c-Jun N-terminal kinase) phosphorylates FoxO for nuclear transport and transcriptional activation (Tothova and Gilliland 2007). Loss of FoxO3 results in reduced expression of superoxide dismutase 2 (SOD2) and catalase, and excess accumulation of ROS (Miyamoto et al. 2008). Targeting FoxO4 sequesters p53 in the nucleus. When p53 exits the nucleus, it stimulates apoptotic pathways in the cytoplasm of senescent cells (Baar et al. 2017). FoxO4 links apoptosis and insulin-like growth factor (IGF) signaling. It binds to p53 and mediates apoptosis and cellular senescence. FoxO4-DRI peptide is cell permeable to interfere with the interaction between p53 and FoxO4 and selectively induces apoptosis (Baar et al. 2017). Transferring p53 from the nucleus to mitochondria results in transcription-dependent apoptosis (Mihara et al. 2003).

FoxO is required for the maintenance of stem cell self-renewal. Intracellular redox status regulates stem cell self-renewal and differentiation. Hypoxia stage with low ROS is required for stem cell renewal (Pervaiz et al. 2009), and high ROS is needed for stem cell differentiation (Lee et al. 2018b). Oncogenic stress activates the Ras-Raf signaling cascade. Activated Ras-Raf stimulates PI3K/Akt to phosphorylate and inactivate FoxO, which reduces the activity of detoxifying enzymes MnSOD and catalase. As a result, increased ROS levels trigger Ras-Raf mediated activation of MEK/ERK. JNK and PRAK are stimulated by MEK/ERK activation. Activated JNK phosphorylates FoxO4, and activated PRAK phosphorylates, and activates p53. Interaction between FoxO4 and p53 promotes p21 to induce cellular senescence as a strategy against cell proliferation (Bourgeois and Madl 2018). Unlike FoxO4, the other FoxOs 1 and 3 resist the senescence. In senescent cardiac microvascular endothelial cells (CMEC), FoxO3 phosphorylation by PI3K/Akt translocates FoxO3 from nucleus to cytoplasm and inactivates its activity. This increases the cellular ROS levels due to reduced activity of MnSOD and catalase and reduced p27 (Kip1) activation, whereas in low-passage CMEC cells, FoxO3 overexpression activated MnSOD and catalase, which in turn reduced the ROS levels and p27 (Kip1) activation. FoxO3 helps in suppressing the senescence of CMEC cells through antioxidant/ROS/p27 (Kip1) pathways (Qi et al. 2015).

12.14 p53 Pathway

Activation of the tumor suppressive transcription factor p53 is the most common pathway noticed in cellular senescence. The p53 activates p16, p15, p21, p27—the cyclin-dependent kinase inhibitors (CDKI). These inhibitors prevent retinoblastoma protein (Rb) phosphorylation, and prevent the transition of cell cycle from G1 to S phase (Davan-Wetton et al. 2021), limiting the proliferation potential of the cells while allowing their growth and metabolic activity.

The p53 has pleiotropic functions of apoptosis and tumor suppression via transcription-dependent and transcription-independent processes. DNA binding domain of p53 mediates transactivation and mitochondrial proapoptotic functions. Protective proteins Bcl2 and Bcl-xL interact with p53 at the outer mitochondrial

membrane (OMM) to allow cell survival by inhibiting the release of proapoptotic factors and cytochrome c. In the tumor-rich environment and missense mutations, p53 is not able to interact with Bcl-xL and release cytochrome c (Mihara et al. 2003). Use of RNA interference to develop a mouse model of liver carcinoma deficient in p53 shows stimulation of innate immune response and its co-operative interaction with tumor cell senescence to limit the tumor growth (Xue et al. 2007).

12.15 Nrf Pathway

Normal diploid human skin fibroblast cells (ASF-2), when treated with phenolic diterpenes, carnosic acid (CA), and carnosol (CS) at a hormetic concentration (20 μM) induced GSH, a thiol tripeptide. The level of GSH gradually decreases with aging, leaving the cells vulnerable to various stresses. Also, the treated cells increased the expression of cytoprotective genes like heme oxygenase 1 (HO-1), glutamate-cysteine ligase modulatory subunit (GCLM), NADH (H): quinone oxidoreductase 1 (NQO1), glutathione s-transferase P1 (GST-P1), ferritin heavy chain (FTH1), and thioredoxin reductase 1 (TXNRD1) at protein and mRNA level (Carvalho et al. 2015). In the same study, when CA- and CS-treated cells incubated with N-acetyl cysteine (NAC) showed reduced HO-1 indicating the change in cellular thiol-disulfide redox state. These CA- and CS-treated cells display increased transcriptional activity of Nrf2 (nuclear factor erythroid 2-related factor) in the nuclear fraction. Tipping the balance between oxidation and reduction potential by CA and CS triggers Nrf2 which in turn activated several cytoprotective genes containing antioxidant response element (ARE). Nrf2 activates ARE to maintain cellular redox homeostasis (Sporn and Liby 2012). Nrf2 sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) through proteasomal degradation to combat detrimental ROS (DeNicola et al. 2011). When these cells are incubated with kinase inhibitor LY294002, decreased HO-1 was noticed suggesting involvement of PI3K/Akt pathway. In the investigation of these phenolic diterpenes on senescence, ASF-2 cells continuously cultured in a hormetic concentration of carnosol at 1.5 μM displayed a reduced number of senescence-associated β-galactosidase (SA-β-gal)-positive cells, changes in cell size and morphology (Carvalho et al. 2015). This result suggested carnosol protected the cells against replicative senescence. The same protection offered for CA/CS pre-incubated ASF-2 cells exposed to H_2O_2 (200 μM) against stress-induced premature senescence. Curcumin and rosemarinic acid also showed similar mild stress-induced anti-aging effects through redox state regulation in human skin fibroblast cells (Lima et al. 2011; Rattan et al. 2009).

In cancer cells, activation of the Nrf2 pathway is protective at the early stages and harmful later (Kansanen et al. 2013). Nrf2 plays a dual role as an activator in cancer prevention, and an inhibitor for cancer treatment. Nrf2 activators suppress tumor growth by eliminating carcinogens, and Nrf2 inhibitors promote cell proliferation by metabolic reprogramming. An example of an Nrf2 inducer is Piperlongumine (PL), a natural alkaloid from long pepper. It acts as a senolytic agent at 6.24 μM

(EC_{50}) concentration for replicative induced senescent (RIS) WI38 fibroblasts after 72-h exposure time compared to nonsenescent fibroblasts (EC_{50} value 20.3 μM) (Siepelmeyer et al. 2016). PL wields synergistic senolytic effect at 10 μM concentration, along with a small molecule ABT-263 at 0.08 μM on ionizing radiation-induced senescent (IR-SC) cells via ROS independent, caspase-mediated apoptosis (Wang et al. 2016). The structure of PL has two electrophiles, C2–C3, and C7–C8 olefins, which are essential for its senolytic activity. Senescent cells produce excess ROS and are resistant to oxidative stress and apoptosis (Chandrasekaran et al. 2017). Oxidation resistance 1 (OXR1) is an effective sensor of cellular oxidative stress and a regulator of several antioxidant enzymes that detoxify surplus ROS. Piperlongumine (5 μM) selectively targets OXR1 and reduces its level in senescent cells by proteasomal degradation. OXR1 knockdown kills the senescent cells via reduced antioxidant enzymes thereby driving the cells to oxidative stress. In response, these knockdown cells produce more ROS associated with apoptosis (Zhang et al. 2018).

Osteoarthritis patients show overexpression of gap junction protein connexin43 (Cx43). This leads to an accumulation of senescent cells in articular cartilage and synovial tissue. Treatment of osteoarthritic chondrocytes (OACs) with 10 μM oleuropein (an olive phenolic compound) reduced the number of senescent cells and p53/p21 expression. This decrease is accompanied by reduced transcriptional activity of NF κB and SASP synthesis (Varela-Eirín et al. 2019). Also, the treated cells show Cx43 downregulation, reduced gene expression of proinflammatory cytokines IL6, COX2, IL1β, MMP3, dedifferentiation factors; and enhanced chondrocyte redifferentiation.

APE/Ref-1 (apurinic/apyrimidinic (AP) endonuclease1/redox factor-1) has DNA repair activity, reduces the ROS levels, and bind with HIF-1α (Hypoxia-inducible factor 1α), Nrf2, and p53 in response to oxidative stress (Angkeow et al. 2002).

12.16 NFκB Pathway

NFκB (nuclear factor kappa light chain enhancer of activated B cells) is a master regulator of SASP and the immune system (Flohé et al. 1997; Salminen et al. 2012). Pharmacological inhibition of NFκB extends the lifespan of *Drosophila melanogaster* (Moskalev and Shaposhnikov 2011). NfκB is redox-sensitive, it translocates from the cytoplasm to the nucleus in response to oxidative stress/ROS and a broad range of stimuli (Kabe et al. 2005). In the absence of external stimuli, NF-kB is trapped in the cytoplasm by binding to the inhibitory IkB proteins (Salminen et al. 2008). TNFα is a strong activator of NFκB and H_2O_2 mediates this process in mitochondria of L929 cells (Hennet et al. 1993). NFκB activation by pro-oxidants is inhibited by various thiol-containing compounds, such as NAC, α-lipoic acid, 2-mercaptoethanol, and L-cysteine; sulfur-containing scavengers, and phenolic antioxidants (Meyer et al. 1994). In a study by Rovillain on putative NFκB targets upon senescence, IL-1A and IL-1B genes were highly upregulated, followed by IL-6. These genes contain NF-κB motifs within their promoters (Rovillain et al. 2011). SIRT1 binds to the

p65/RelA protein of NFκB and inhibits its transcriptional activity by deacetylation of p65 (Yeung et al. 2004).

12.17 JAK/STAT Pathway

The burden of senescent cells is high in elderly women with frailty (Justice et al. 2018). Senescent cells are protected by senescent cell anti-apoptotic pathways (SCAPs). IL-10 knockout mice display premature aging (Zhu et al. 2015), and NFκB1 knockout mice show progeroid phenotype along with senescent cell accumulation (Bernal et al. 2014). Human umbilical vein endothelial cells (HUVEC) chronically exposed to TNFα developed senescence and elevated ROS production (Kandhaya-Pillai et al. 2017). Gene expression profiling and ingenuity pathway analysis show strong interferon signature and preferential activation of the JAK/STAT pathway in TNFα-mediated senescent state. The positive feedback mechanism of the STAT pathway shows a dual regulatory role by inducing tumor suppressor genes and cell proliferation. During TNFα-induced senescence, molecular crosstalk occurs among diverse signaling pathways like p38 MAPK, NFkB, p53, and several cytokines-chemokines (Kandhaya-Pillai et al. 2017). Given its crucial role, the JAK/STAT pathway could be a potential therapeutic target to treat senescence-associated inflammatory diseases.

12.18 MTOR/PI3K/Akt Pathway

The mechanistic target of rapamycin (mTOR) is a serine/threonine kinase, present in two forms as mTORC1- complex 1, and mTORC2- complex 2. It is a master growth regulator to sense diverse nutritional and environmental cues in the cell. The nutrient-sensing mTOR pathway promotes the conversion of a quiescent cell to a senescent cell (geroconversion) when the cell is treated with a DNA damaging agent, doxorubicin (Demidenko and Blagosklonny 2008). H_2O_2 produces ROS which stimulates p21 to cause hypertrophy and cell senescence (Chen et al. 2001). PI3K/Akt and Ras/Raf1/ERK pathways activate TOR (Blagosklonny 2006). In human longevity, centenarians display greatly increased insulin sensitivity and preserved glucose tolerance (Paolisso et al. 1996). TOR is activated by insulin and IGF-1. Over-activated TOR accelerates the senescence (Blagosklonny 2006). Rapamycin binds FKBP, this complex interacts with TOR and potently inhibits the yeast cell cycle at the G1 phase (Heitman et al. 1991).

Human vascular smooth muscle cells (VSMCs) show enhanced activation of PI3K/Akt/mTOR signaling in aged cells and not in young cells (Tan et al. 2016). Pre-treating the VSMCs with rapamycin, inhibited replicative senescence by reducing the expression of phosphorylated mTOR at Ser2448, Thr2446 residues. PI3K/Akt is the most common signaling pathway in cancer cells. Akt activation sensitizes the cells

to ROS-mediated apoptosis through increased oxygen consumption and FoxO inhibition. Intracellular increase in ROS predisposes the cells for premature senescence (Nogueira et al. 2008).

12.19 The Current Progress of Senolytics

Recent work by Saccon et al. report that aged mice treated with D + Q specific microbial signatures prominent in the small intestine correlate with reduced senescence and inflammation markers (Saccon et al. 2021). Treated mice show altered microbiota along with a reduced expression of p16, p21 (senescence markers), and Cxcl1, IL1β, IL-6, MCP-1, and TNFα (inflammation markers). This opens the possibility of improving healthspan in elderly subjects through optimized intake of senolytics.

The first human clinical trial conducted on subjects with idiopathic pulmonary fibrosis (senescence driven disease) treated with D + Q for 3 weeks, displayed improved walking endurance, gait speed, and chair rise test performance (Justice et al. 2019; Ellison-Hughes 2020). Diabetic subjects (55–79 yrs) having chronic kidney disease (CKD) treated with D + Q for three days show reduced expression of p16 Ink4a, p21 Cip1, SA-β-gal staining, and CD68 + macrophages in abdominal adipose tissue (Hickson et al. 2019). Also, the level of SASP factors IL-1α, IL-2, IL-6, IL-9 and MMP-2, MMP-9, and MMP-12 reduced in treated subjects.

The human clinical trial was conducted with nine healthy volunteers (51–65 yrs) to study aspects of reverse aging and thymus regeneration, called TRIIM (Thymus Regeneration, Immunorestoration and Insulin Mitigation) (Ray 2019; Fahy et al. 2019). Administering the subjects with recombinant human Growth hormone (0.015 mg/kg) and combining it with dehydroepiandrosterone (DHEA) (50 mg) and metformin (500 mg), and analyzing the DNA from peripheral blood mononuclear cells (PBMC) show regression in epigenetic age by 1.5 yrs. DHEA and metformin did not show thymotrophic effects. The level of C-reactive protein (CRP), CD38+ monocytes, and PD-1 CD8 T-cells reduced after the treatment. There is an increase in lymphocyte to monocyte ratio (LMR) and FGF-21.

12.20 Limitations

Application of senolytics as major therapeutic measure treads caution. Translating in vitro outcomes to in vivo is not ideal as the number of senescent cells is fewer. The absence of specific markers to identify senescent cells is a primary limitation. This is compounded by the heterogeneity in the expression of senescent factors across cell types and stimuli. The reappearance of senescent cells following senolytic therapy may take a longer time and so the treatment regime has to be individual-centric (precision medicine). Care should be taken while fixing the dose and treatment period of senolytics as optimal wound healing requires cell senescence (Partridge et al.

2020). Senescence is a tumor suppressor mechanism and so complete clearance of the senescent cells might endanger the cells with cancer. The presence of senescence effector p16Ink4a promotes insulin secretion from pancreatic beta cells of transgenic mice and so clearing the senescent cells may lead to diabetes (Helman et al. 2016).

12.21 Conclusion

This chapter on the senolytic phytocompounds in redox signaling attempts to bring the salient aspects of individual compounds and their interaction in cell signaling, specifically targeting ROS. Optimal use of senolytics might be therapeutic to delay the progression of age-related diseases. Though there are some limitations, being tissue-specific and selective in clearance, this senolytic research field looks promising.

Compliance with Ethical Standards

Conflict of Interest: There is no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Part III
Redox Biomarkers in Age-Related Disorders

Chapter 13
Impaired Redox Status and Age-Related Neurodegenerative Disorders

Apoorv Sharma, Sandeep Singh, Geetika Garg, and Abhishek Kumar Singh

Abstract Oxidative stress is the major regulatory element during aging and various age-related neurodegenerative disorders. The excess level of reactive oxygen species (ROS) leads to a decline in the antioxidant system which produces an imbalance between oxidants and antioxidants. ROS oxidatively damage and impair biomolecular functions, causing significant neuronal cell degeneration. The brain consumes a large amount of energy for diverse signaling pathways, leading to the overproduction of reactive species and increased oxidative stress (OS). In addition, neuronal membranes, having a high percentage of polyunsaturated fatty acids, are especially vulnerable to ROS, which leads to numerous aging-related neurodegenerative illnesses like Alzheimer's disease, Huntington's disease, Parkinson's disease, and Amyotrophic lateral sclerosis. The relationship between impaired redox status and age-related neurodegenerative disorders needs to be further studied. This chapter strives to increase our comprehension of the key function of OS in neurological illnesses.

Keywords Aging · Oxidative stress · Neurodegenerative disorders · Redox status

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13.1 Introduction

Aging is an inevitable and complex biological process mainly manifested by time-dependent accumulation of cellular damage. An increase in age-associated macromolecular damage leads to a redox imbalance between ROS production and antioxidant defense. The redox impairment is involved in age-related diseases such as cardiovascular disease, cancer, diabetes, and several neurodegenerative disorders including Parkinson's disease and Alzheimer's disease. Despite the hundreds of explored and developed theories, the oxidative stress theory has been extensively studied and received much attention (Harman 1956). According to this theory, advancing age can lead to progressive mitochondrial dysfunction and increased reactive oxygen species (ROS) levels, which leads to mitochondrial deterioration and cellular damage. More recently, cellular and molecular hallmarks proposed by López-Otín et al., contribute to the process of aging (López-Otín et al. 2013).

An imbalance between pro-oxidants and antioxidants generates oxidative stress, resulting in irreversible molecular damage (Sies 2015). Oxidative stress alters the cellular redox homeostasis, associated with many diseases including neurodegenerative disorders, and has been implicated as an important factor in the regulation of growth, senescence, and aging. There are some cellular and molecular hallmarks of aging that include oxidative stress, proteotoxicity, mitophagy, gene alteration, and telomere attrition. Neurotoxicity caused due to unrestricted release of glutamate, excessive calcium ion influx, and hyperactivation of NMDARs leads to ischemic stroke injury (Tripathi et al. 2013; Patnaik et al. 2019). Cellular homeostasis is affected by alteration in intracellular redox status as several cellular signaling pathways involved in the regulation of cell division and stress response systems are sensitive to the redox environment (Chiu and Dawes 2012). Thus, redox homeostasis plays a major role in health and disease.

The brain is highly vulnerable to oxidative damage due to high oxygen and metabolic demands. In addition, moderate antioxidant defenses, high polyunsaturated fatty acids, and highly reactive metals render the brain, particularly neuronal cells and oligodendrocytes, vulnerable to oxidative damage (Bélanger et al. 2011; Cobley et al. 2018). In this chapter, we have focused on different aspects of brain aging regulated by redox biology and the effect of redox imbalance on aging and age-related diseases.

13.2 Oxidative Stress Biomarkers and Redox Status in the Brain

Since the brain is highly vulnerable toward oxidative stress, redox homeostasis is highly important for its normal functioning. According to the free radical theory of aging, an accumulation of oxidative damage to macromolecules occurs with

the progression of age which consequently leads to a loss in neuronal functions, increasing the risk of developing neurodegenerative diseases and cognitive impairment. The redox homeostasis is maintained by two cellular disulfide reductase systems; the thioredoxin system and glutathione system. The thioredoxin system includes thioredoxin (Trx), thioredoxin reductase (TrxR), and NADPH. The glutathione system includes glutathione (GSH), glutathione reductase (GR), and NADPH (Ren et al. 2017). Both of these systems work in parallel and also crosstalk to maintain redox status in the brain. Redox homeostasis is maintained by antioxidant enzymes such as peroxiredoxin, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and some small antioxidants such as GSH, ascorbic acid, vitamin E, and coenzyme Q. As far as the mitochondria is concerned, it is the main site of superoxide and H_2O_2 production, and it also helps in scavenging the exogenous H_2O_2.

It is still necessary to understand the physiopathology of neurodegenerative illnesses like Alzheimer's, Amyotrophic lateral sclerosis, and Parkinson's. Neurodegenerative diseases are marked by impaired redox status as shown by clinical data (Li et al. 2013). Deprivation of GSH in the selective region such as Substantia Nigra (SN) of the brain results in Parkinson's disease (Sian et al. 1994). Whereas, an elevated SOD level in the SN and basal ganglia region of PD patients has been reported, while the other biomarkers such as CAT, GPx, and GR remain unchanged (Marttila et al. 1988). Alzheimer's disease (AD) is also characterized by the alterations in the antioxidant enzymes, such as GPx, CAT, and SOD, in the brain of AD patients, but the results are not consistent (Lovell et al. 1998; Casado et al. 2008). The plasma antioxidant levels are reported to be decreased in AD patients (Foy et al. 1999; Kim et al. 2006), although there are some reports indicating the increase in antioxidants (Giavarotti et al. 2013).

In Amyotrophic lateral sclerosis (ALS), most studies have shown changes in peripheral tissues or in cerebrospinal fluid (CSF), but rarely in the brain (Babu et al. 2008). The GSH level was reduced in erythrocytes, whereas the activity of SOD decreased in red blood cells and the CSF of ALS patients (Boll et al. 2003; Ihara et al. 2005; Nikolić-Kokić et al. 2006). The activity of CAT, another enzymatic antioxidant, was also found to be decreased in red blood cells of ALS patients (Apostolski et al. 1998).

The imbalance between oxidative stress and antioxidant defense mechanism seems to be the universal condition in neurodegeneration.

13.3 Protein Thiol Modification

ROS affects the cell function and interacts with biomolecules resulting in oxidative post-translational modification of proteins. (Davies 2005, 2016; Stadtman 2006; Moldogazieva et al. 2018). Thiol containing cysteine residues are reversibly oxidized to cystine, and thus the post-translational modifications of cysteine are considered to be one of the main drivers of redox signaling (Bindoli et al. 2008; Schöneich

2011; Wani et al. 2014; Go et al. 2015). The irreversible protein modification is the hallmark of pathological neurodegeneration, while the reversible thiol modification involves S-nitrosylation, S-sulfenylation, S-glutathionylation, S-sulfhydration, disulfide formation, sulfinic acid, which are involved in redox signaling.

13.4 Impaired Redox Status and Its Consequences Leading to Aging and Age-Related Neurodegenerative Disorders

Free radical production and oxidative stress play a major role in governing the redox state giving rise to RNS and ROS that are the main crook in the deterioration of neuronal cells (Emerit et al. 2004). Neurodegenerative diseases have some common neuropathological attributes such as (a) abnormal protein dynamics with defective protein degradation and aggregation; (b) oxidative stress and free radical formation; (c) impaired bioenergetics and mitochondrial dysfunctions; (d) neuroinflammatory processes (Jellinger 2010). These common events occurred through different pathways.

Abnormal S-nitrosylation: S-nitrosylation is the post-translational oxidative modification of cysteine residues by nitric oxide (NO) to form S-nitrosothiols (SNOs) (Stamler and Meissner 2001; Hess and Stamler 2012). Increased NO production and abnormal protein S-nitrosylation cause several pathological changes that lead to neurodegenerative sicknesses such as Parkinson's disease, Amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. An abnormal S-nitrosylation causes misfolding of protein disulfide isomerase (PDI), leading to its accumulation and reduced activity. An increased level of nitrosylated PDI is found in patients with sporadic AD and PD (Uehara et al. 2006). Thus, impaired redox status and S-nitrosylation act as risk factors for aging and age-associated neurodegenerative diseases.

S-nitrosylation is also involved in mediating cell death through regulating GAPDH (Hara et al. 2005, 2006). The S-nitrosylation of X-linked inhibitor of apoptosis (XIAP) and GAPDH lead to the progression of cell death which ultimately causes neurodegenerative diseases. The XIAP is an E3 ligase that binds directly to the caspase protein family, causing their degradation and preventing apoptosis, but in PD patients, the nitrosylated XIAP was found making it unable to bind with the caspases. The nitrosative stress contributes to PD pathogenesis through the impairment of pro-survival proteins such as parkin and XIAP through different mechanisms, indicating that abnormal S-nitrosylation plays an important role in the process of neurodegeneration (Tsang et al. 2009). Cys150 of GAPDH on undergoing S-nitrosylation forms a complex with Siah 1, GAPDH-Siah1 complex translocate to the nucleus where it initiates the apoptotic cascade causing uncontrolled neuronal cell death (Hara et al. 2005).

13.5 S-nitrosylation and Mitochondrial Dysfunction

Mitochondria are responsible for the production of energy in the form of ATP which is further used by a cell for its survival and function. Mitochondria also play an important role in fatty acid oxidation, cellular defense, apoptosis, and intermediary metabolism (Ademowo et al. 2017). The mitochondrial DNA, which constitutes around 1% of global cellular DNA, is highly vulnerable toward oxidative stress causing an alteration in mitochondrial DNA, leading to subsequent dysfunction to cause and enhance the illness (Hollensworth et al. 2000). During amyloid toxicity, excessive production of NO mediates the unnecessary nitrosylation of dynamin-related protein 1 (Drp1), results in GTPase hyperactivity and mitochondrial fragmentation, thus impairing bioenergetics and inducing synaptic damage and neuronal loss (Cho et al. 2009; Nakamura et al. 2010). S-nitrosylation causes bioenergetic failures, mitochondrial fragmentation, synaptic deterioration, and ultimately neuronal apoptosis (Nakamura and Lipton 2010). The above-mentioned studies hereby demonstrate that S-nitrosylation acts as a switch signal that regulates the activities of some important protein mediating cell death.

13.6 S-glutathionylation and Neurodegeneration

GSH levels were found to diminish with the progression of age in a variety of senescent taxa, including houseflies, mosquitoes, fruit flies, rats, mice, and humans (Sohal and Weindruch 1996). S-glutathionylation of cysteine residues regulated by glutaredoxin plays an essential role in maintaining cellular homeostasis. S-glutathionylation is involved in redox-activated signal transduction and protects against irreversible protein damage due to oxidative stress (Dalle-Donne et al. 2006). Oxidative stress is an early event in AD (Honda et al. 2004), attributed to excess heavy metal, impaired respiration, and accumulation of amyloid beta protein (Aβ). Aβ accumulation leads to mitochondrial impairment, elevated levels of calcium, and loss of membrane structure and function which exacerbate the oxidative stress on the neuronal cells (Tillement et al. 2011). Redox impairment is considered to promote sulfhydryl oxidation. The development of sporadic Parkinson's disease associated with aging is driven by oxidative stress (Hauser and Hastings 2013; Trist et al. 2019). Genetic mutations in particular proteins such as PARK2 (Parkin), SNCA (α-synuclein), PINK1 (Pink1), and PARK7 (DJ-1) cause familial Parkinson's disease. These proteins control the essential cellular pathways that control respiration and transport, mitochondrial dynamics, calcium homeostasis, ROS generation, autophagy, and apoptosis. The above-mentioned findings suggest that abnormal S-glutathionylation paves the way for various cellular activities like apoptosis, oxidative stress, protein aggregation, mitochondrial dysfunction, and protein degradation that are closely linked to neurodegeneration.

13.7 Sulfhydration and Its Impact on Nervous System

As we know that 'too much of everything is bad'. Too much sulfhydration is also harmful to the nervous system as it leads to chronic inflammation which is one of the hallmarks of neurodegenerative diseases. An important neuronal protein called postsynaptic density 95 (PSD95) is involved in the neuronal maturation process and synaptic density. The protein PSD95 degrades by excessive sulfhydration of GAPDH as sulfhydration of GAPDH by inflammatory cytokines influences its binding to Siah1. The binding increases Siah1 activity. The increased Siah1 activity then mediates the degradation of postsynaptic density 95 (PSD95). This extreme loss of PSD95 is observed in several neurodegenerative diseases like dementia and depression (Manczak et al. 2018). A gene called cystathionine β-synthase (CBS) located on chromosome 21 produces H_2S in the cells. Accordingly, in the patients suffering from Down syndrome or trisomy 21, there will be an unnecessary production of H_2S in the cells leading to hypersulfhydration of its proteins. This hypersulfhydration is considered to be one of the contributing factors in the early onset of AD in adults and retarded brain growth in children (Mir et al. 2014). Several studies suggest that the Trx system globally regulates desulfhydration in cellular systems under different conditions (Ju et al. 2016). The Grx system is also able to reduce protein sulfhydration (Dóka et al. 2016).

13.8 Glutathione Systems in Neurodegenerative Disorders

Antioxidants are considered as a promising approach to slow down the progression of neurodegenerative disorders and limit the extent of neuronal cell loss (Di Matteo and Esposito 2003; Ghosh et al. 2011). The GSH antioxidant system provides cellular defense against ROS in brain cells, directly helps in detoxification of radicals in non-enzymatic reactions, and acts as a substrate for various peroxidases (Dringen 2000). Alterations of GSH metabolism are the major cause of neurodegenerative disorders (Bains and Shaw 1997; Townsend et al. 2003; Ballatori et al. 2009).

GSH System: Glutathione (GSH) is a thiol-containing antioxidant enzyme that plays an important role in neuroprotection. The level of GSH in the brain is abundant about 2–3 mM. Its concentration is highest in the cortex after that cerebellum, hippocampus, striatum, and lowest in substantia nigra. GSH inhibits oxidative stress, it reacts non-enzymatically with oxidants like superoxides, NO, peroxynitrite, and hydroxyl radicals, and reacts enzymatically with enzymes like glutathione peroxidase and GSH-S-transferase (GST). Glutaredoxins (GR) are the enzymes that reduce GSSG and it maintains the ratio of GSH/GSSG in neurons to maintain homeostasis. But under oxidative stress, GSH concentration gets depleted and worsens the neuronal injury in the brain caused by oxidative stress (Madrigal et al. 2001). Aging declines the GSH concentration in the brain and cerebrospinal fluid (Tong et al. 2016).

13.9 Oxidative Stress, Mitochondria, and Age-Related Neurodegenerative Diseases

The mitochondrion contains its own DNA and machinery for synthesizing RNA and proteins. It is highly prone to oxidative stress due to the production of ROS (Douarre et al. 2012) (Fig. 13.1). Mitochondrial DNA damage ultimately leads to mutations in the mitochondrial genome and leads to mitochondrial dysfunction, which induces neurodegenerative diseases (Ott et al. 2007; Cenini et al. 2020).

In oxidative stress condition, the antioxidant defense system cannot cope up with the elevated ROS levels and that causes various age-related pathologies like cardiovascular diseases, neurodegeneration, etc. Oxidative stress disrupts the cellular homeostasis and degrades the biomolecules and causes senescence of diverse cell types like epithelial cells, chondrocytes, neurons, lymphocytes and glial cells, etc. (Wang et al. 2013). Oxidative stress and mitochondrial ROS also cause telomere attrition and dysfunction leading to increased cellular senescence. Degradation of neuronal cells by increased ROS production causes irreversible damage to the brain.

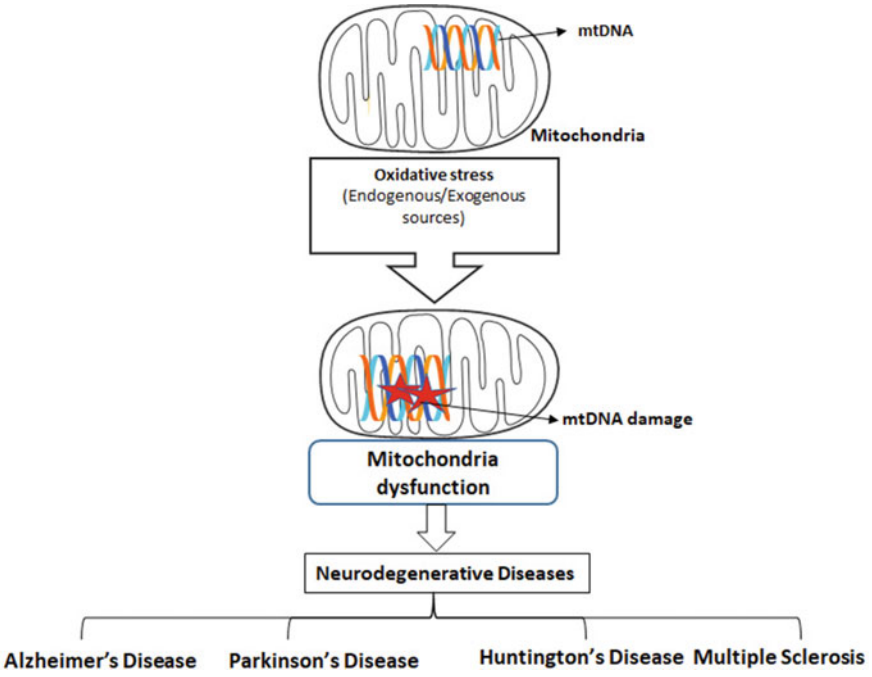

Fig. 13.1 Oxidative stress-mediated mitochondrial dysfunction in neurodegenerative diseases

13.10 Role of Oxidative Stress in Age-Related Neurodegenerative Disorders

13.10.1 Alzheimer's Disease (AD)

Oxidative stress act as a major risk factor in aging and neurodegenerative diseases including AD (Jiang et al. 2016). Enhanced reactive oxygen species (ROS) generation has been linked to age and disease-related loss of mitochondrial function, altered metal homeostasis, and decreased antioxidant defense. Such loss of function activities has a direct impact on neurotransmission leading to cognitive impairment (Tönnies and Trushina 2017). The balance in redox status is essential for maintaining cellular homeostasis. However, in AD, the altered activity of antioxidant enzymes contributes to the accumulation of oxidative damage (Kim et al. 2006). According to mitochondrial cascade theory, in case of sporadic, late-onset of AD, the age-related mitochondrial dysfunction influences the synthesis and processing of amyloid precursor proteins (APP), resulting in Aβ production (Swerdlow et al. 2014). ROS production due to Aβ accumulation causes microglia activation during an inflammatory response leading to the accumulation of amyloid plaques (Nakajima and Kohsaka 2001) (Fig. 13.2). Furthermore, an elevated level of Aβ might speed up the ROS formation by binding directly to mitochondrial membranes, affecting mitochondrial functions, finally resulting in aberrant energy metabolism and synaptic function loss (Beal 2005; Manczak et al. 2006; Gibson et al. 2008; Bose and Beal 2016).

13.10.2 Parkinson's Disease (PD)

Parkinson's disease (PD) is the most common age-associated neurodegenerative disorder. Degradation of dopaminergic neurons in substantia nigra and low dopamine levels is a characteristic feature of PD (Bellucci et al. 2016) (Fig. 13.3). According to some evidence, oxidative stress act as a key factor for dopaminergic neurodegeneration in all forms of PD (Dias et al. 2013; Blesa et al. 2015).

The pathological hallmarks of PD are Lewy bodies in the brain and peripheral nerves. The misfolding and aggregation of alpha-synuclein (α-synuclein) with concomitant cytotoxicity is a hallmark of Lewy body in Parkinson's disease, multiple system atrophy, and dementia due to Lewy bodies (Delenclos et al. 2019). Elevated oxidative stress plays an important role in the progression of early-stage PD occurring prior to the neuronal loss (Ferrer et al. 2011). Increasing mitochondrial ROS production in the substantia nigra pars compacta region due to redox imbalance is associated with severe ETC impairment and oxidative damage (Schapira 1993). A number of environmental toxins and pesticides, including MPTP and rotenone, freely cross lipid membranes and accumulate in mitochondria following inhalation or ingestion (Perier et al. 2005).

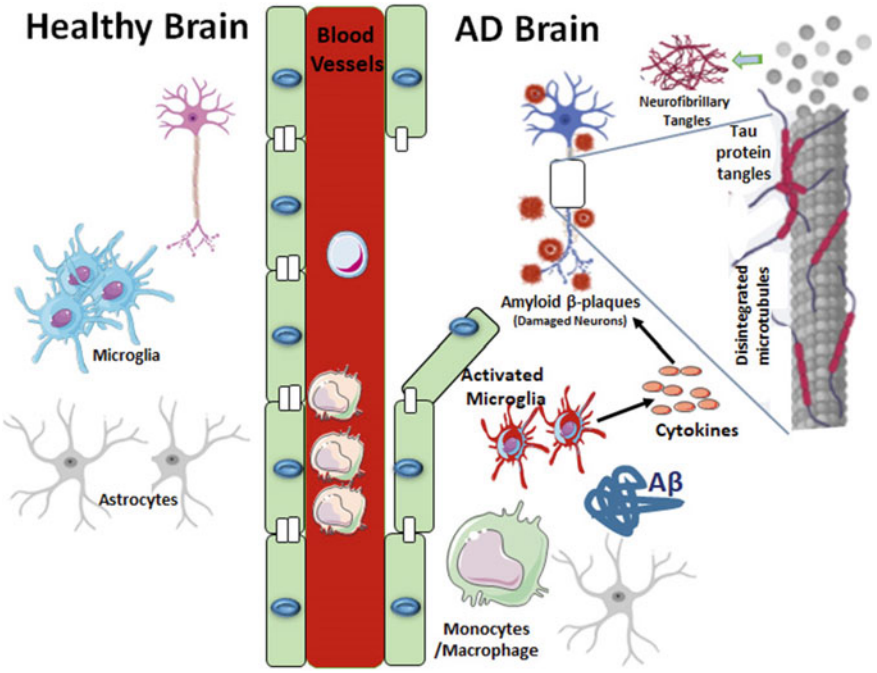

Fig. 13.2 Schematic representation of the pathology of Alzheimer's disease showing its multifactorial interconnected features. The formation of Aβ plaques damages the neurons and neurofibrillary tangles and disintegrate microtubules in the neuron. The blood–brain barrier in the AD brain has been jeopardized

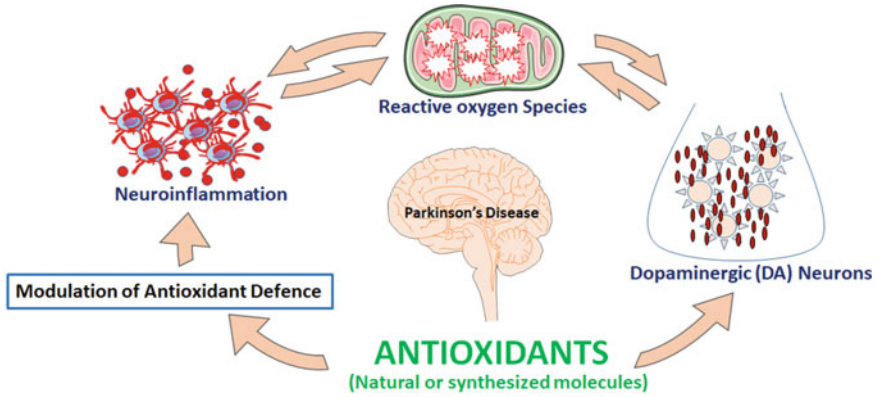

Fig. 13.3 Schematic representation of the pathophysiology of Parkinson's disease. The antioxidants provide protection to dopaminergic neurons and decrease the neuro-inflammation, but ROS on the other hand increases oxidative stress, damages the neurons, and causes neuro-inflammation

13.10.3 Amyotrophic Lateral Sclerosis (ALS)

Oxidative stress is associated with the degeneration of both motor neurons and skeletal muscles in amyotrophic lateral sclerosis (ALS) (Ohta et al. 2019). Before the onset of the disease, the stimulation of the nuclear factor erythroid 2 related factor 2 (Nrf2) antioxidant response element (ARE) was seen in SOD1 mutant mice leading to oxidative stress in distal muscles (Kraft et al. 2007, p. 1). Excitotoxicity, mitochondrial dysfunction, aging, oxidative stress, neuroinflammation, ER stress are the factors that are involved in ALS (Obrador et al. 2020). Mitochondrial dysfunction and oxidative stress are interlinked and enhances the ROS/RNS levels. ROS causes mitochondrial DNA damage, imbalance in Ca^{2+} homeostasis, membrane permeability, lipid peroxidation, protein carbonylation, and enhances oxidative stress, thereby leading to several neurodegenerative disorders including ALS (Guo et al. 2013).

13.10.4 Huntington's Disease (HD)

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting in the deterioration of neurons in the striatum, followed by the cerebral cortex and thalamus deterioration during later stages of the disease (Ross and Tabrizi 2011). It is caused due to mutations in Huntingtin (Htt) gene. Mutation causes the trinucleotide repeats of -CAG- in Htt gene resulting in an abnormal extension of polyglutathione in Htt protein. The role of Htt is unclear, though it has been said that it possesses anti-apoptotic properties, controls synaptic transmission, brain derived neurotropic factor (BDNF) production, and neuronal gene transcription (Ghavami et al. 2014). A number of laboratories have provided evidence supporting the hypothesis that oxidative stress is a primary event in HD neuropathology (Browne et al. 1999; Perluigi et al. 2005; Stack et al. 2008). But the mechanism of this neurodegeneration process is not fully understood, although there is an explanation that involves the cleavage of mutated Htt protein forming aggregates like inclusion bodies damaging the axonal transport. Another explanation involves NMDA receptor excitotoxicity, elevation in caspase activity, autophagy, mitochondrial dysfunction, aspartyl proteases cleavage and proteasome cleavage, and abnormal histone modification (Sadri-Vakili and Cha 2006). Treatments available to date are remacemide, amantadine, tetrabenazine, and levetiracetam. These medications can only help in reducing the symptoms of HD, there are no drugs yet available that could otherwise stop or in some way reverse the adverse effects of HD (Walker 2007).

13.11 Conclusion

From the above data, it can be said that oxidative stress plays a major role in the above-mentioned age-related neurodegenerative disorders. In each of these disorders, the antioxidant defense mechanism is halted due to increased ROS/RNS stress and aging aggravates the situation. The damage is collateral and the result is unwanted and undesirable that we are trying to avoid for a very long time. It is a vicious cycle of OS, mutation, aging, and diseases that right now cannot be avoided but sure can be slowed down. Several antioxidant therapies and vaccines are being made that are helpful to some extent. The calorie restriction mimetics (CRMs) is a new branch of research that has shown some promising results in providing neuroprotection against OS and induction of autophagy. Since there is enormous brain tissues degeneration so stem-cell oriented therapy can be a silver lining. But there is a lot to be done for making some to the point therapeutic targets in controlling these neurodegenerative disorders.

Compliance with Ethical Standards:

Conflict of Interest: All authors declare they have no conflict of interest.

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 14
The Effects of Sirtuin Activators on Cerebral White Matter, Redox Biomarkers, and Imaging Findings in Aging Brain

A. Nedim Kahraman and Hale Z. Toklu

Abstract Aging is usually accompanied by a cognitive decline in quantitative reasoning and perceptual speed, which is associated with decreased white matter volume and integrity in certain brain areas such as the prefrontal cortex. The decline in the antioxidant enzymes has been shown to be associated with the changes in structure and volume of white matter. This chapter focuses on the antioxidant biomarkers and their clinical significance in brain aging in relation to brain white matter.

Keywords SIRT · Brain imaging · Redox · Oxidative · Brain white matter · Aging · Antioxidant · Oxidative stress

14.1 Cerebral White Matter and Aging

The white matter of the brain is primarily composed of myelinated axons and oligodendrocytes. The white color originates from the fatty content and myelin, which helps to speed the conduction of an electric impulse along an axon, allowing the action potential to travel long distances faster. As we understand the pathophysiological changes in neurodegenerative diseases, not only the structural changes but also white matter plasticity has gained attention for its role in neuronal communication (Sampaio-Baptista and Johansen-Berg 2017).

Aging usually accompanied by a cognitive decline in quantitative reasoning and perceptual speed, which is associated with decreased white matter volume and integrity in certain brain areas such as the prefrontal cortex. On the other hand, verbal fluency and semantic memory are less affected (Caserta et al. 2009).

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White matter is vulnerable to decreased blood flow. Hypoperfusion of the white matter due to cerebrovascular aging and small vessel disease is associated with cognitive and sensorimotor decline (Joutel and Chabriat 2017; Yang et al. 2017).

The decreased production of myelin is another factor for age-related changes of the white matter. Degeneration of oligodendrocytes, decreased lactate transport for energy, and decreased levels of fatty acids contribute to the impairment in myelin synthesis. As a result, the conduction of nerve impulses is altered (Liu et al. 2017). In addition to oligodendrocytes, the changes in neuroglia cells such as microglia and astrocytes also influence white matter integrity via increased blood–brain barrier permeability, secretion of proinflammatory cytokines, and increased oxidative stress (Liu et al. 2017). Given the fact that aging is also associated with decreased antioxidant levels, the oxidation–reduction balance is challenged to maintain homeostasis.

Besides senescence, several diseases may lead to early aging of the brain structures. Traumatic brain injury, stroke (both ischemic and hemorrhagic), Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), schizophrenia, infectious diseases lead to such changes in white matter due to enhanced early aging (Griesbach et al. 2018; Joutel and Chabriat 2017; Liu et al. 2017; Marin and Carmichael 2019; Peters and Karlsgodt 2015; Yang et al. 2017).

14.2 Cerebral White Matter Changes and Findings in Diagnostic Imaging

Aging is associated with cognitive decline. Aging-induced cognitive changes were demonstrated with studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), which showed a reduction in the lateralization of brain activity (Guo et al. 2017). The functional changes are often accompanied by structural changes in the brain, which can be seen by imaging techniques- primarily MRI. The T1WI- and T2WI imaging consistently identified the burden of aging and dementia-related decline of structural brain health. Small vessel changes, microhemorrhage, impaired white matter integrity, and atrophy are the most common changes observed in the MRI. Each of these subtle changes can coexist and interact, producing both independent and additive impacts on brain health (Guo et al. 2017). There are controversial reports on the atrophy of whether white matter or gray matter changes are more significant. Nevertheless, the frontal lobe and temporal areas have more prominent changes in comparison to the others (Caserta et al. 2009). Medial temporal lobe atrophy (MTA) is a hallmark change for AD. The shrinking of cortical areas and enlargement of the ventricles are particularly predictive for the progression of dementia. The extend of atrophy, lacunar infarcts, and white matter hyperintensity can be correlated; and the vascular and white matter changes in mid-adulthood can lead to a more severe degeneration of the white matter as the age advances (Gunning-Dixon et al. 2009; Guo et al. 2017). A large-scale investigation demonstrated vascular

changes leading to AD are the very first structural brain changes detected in MRI follow-ups (De Reuck et al. 2015).

MR diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) allow quantification of microscopic water movement. In areas with little or no physical boundaries, such as CSF in the ventricles, water freely diffuses, and therefore it is isotropic. By contrast, the path of a water molecule in white matter is constrained by physical boundaries, such as the myelin sheath, causing the movement to be greater along the axon than across it and typically measured as fractional anisotropy (FA). DTI is therefore sensitive in detecting tightly packed nerve fibers in a locally parallel orientation, characterizing white matter tracts in the brain (Caserta et al. 2009; Sullivan and Pfefferbaum 2007).

T1WI, T2WI, and T2-FLAIR are all suitable for the basal ganglia and surrounding areas, and global atrophy, while T1WI or T2WI is most suitable for malacia, trauma, neoplasm, and malformations. In addition, T2*-weighted gradient recalled echo sequence (T2*GRE) is optimal for detecting microbleeds and calcium deposition, although it is not reliable to score other MRI-based Brain Atrophy and Lesion Index (BALI) categories due to a patchy low signal intensity associated with normal deposition of paramagnets or flow artifacts (Guo et al. 2017). T2-FLAIR is more sensitive in detecting white matter lesions in the subcortical area. On the other hand, the dilated perivascular spaces located in multiple sites are not seen on T2FLAIR (Guo et al. 2017) We also observed white matter changes (seen as hyperintensity in Fig. 14.1) in our patients with dementia (Fig. 14.1).

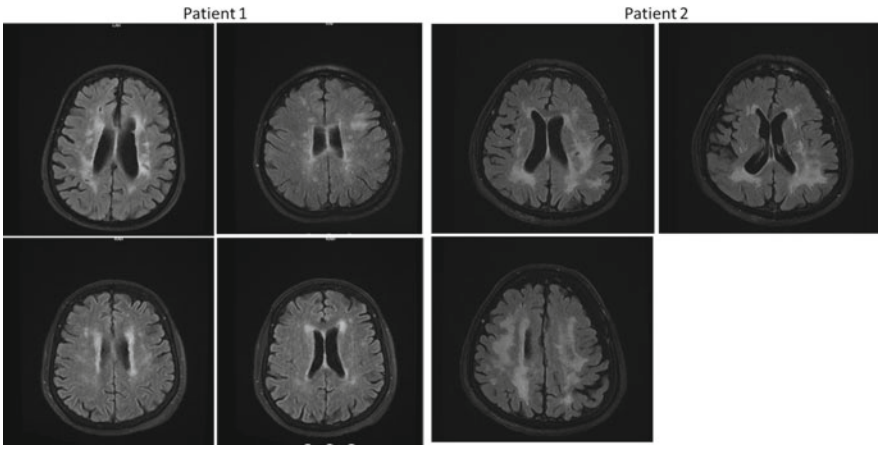


Fig. 14.1 The areas with cerebral white matter lesions show up as areas of increased brightness (leukoaraiosis) due to hyperintensity, when visualized by T2-FLAIR magnetic resonance imaging (MRI)

14.3 Cerebral White Matter Changes and Redox Biomarkers in Brain Tissue

Aging is associated with a systemic decline in activities, which result in physiological changes in structure and function. Oxidative stress is one of the important factors, which affect aging and cognitive function. Genetic polymorphisms that encode antioxidant enzymes are one of the key determinants of healthy aging of the brain (Salminen and Paul 2014).

The brain is particularly vulnerable to oxidative stress because brain cells require a substantial amount of oxygen. The adult brain consumes 20% of the oxygen in the body. While 95% of the oxygen is used for ATP synthesis, approximately 5% forms free oxygen radicals (also known as reactive oxygen species (ROS)) (see Fig. 14.2). The endogenous antioxidant system consists of several enzymes, which serve as free radical scavengers. These enzymes include glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and sirtuins (SIRTs) (Toklu and Ginory 2018). A homeostatic imbalance of ROS and redox biomarkers is associated with long-term potentiation and cognitive function.

The metalloproteins, i.e., GPx, SOD, CAT are the first line of antioxidant defense against ROS (Salminen and Paul 2014). Besides ROS, accumulation of iron, polyunsaturated lipids because of lipid peroxidation, oxidized proteins in large quantities are the challenges for the antioxidant system. The failure in detoxification results in injury in the macromolecules such as DNA and RNA.

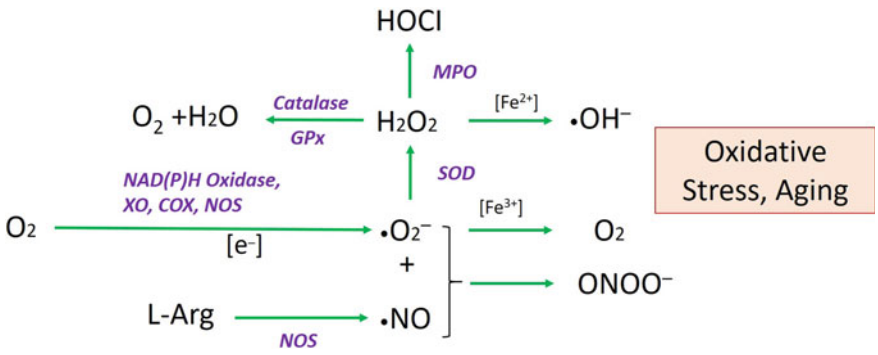


Fig. 14.2 Reactive oxygen species (ROS) have uncoupled electrons, therefore they can bind to macromolecules (DNA, RNA) and/ or cause oxidative damage in protein or lipid structures within the cell. Superoxide ($\bullet O_2^-$) can form a complex with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), or it can be subtracted to superoxide dismutase (SOD) to form hydrogen peroxide (H_2O_2), which may be involved in Fenton reaction with iron and generate hydroxyl radical ($\bullet OH^-$). Both $ONOO^-$ and $\bullet OH^-$ are highly reactive and cytotoxic as they cause DNA damage. However, the endogenous antioxidant system has a number of defensive enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) to neutralize/scavenge these above mentioned reactive metabolites. Abbreviations: COX: cyclooxygenase; GPx: glutathione peroxidase; L-arg: L-arginine; MPO: myeloperoxidase; NOS: nitric oxide synthase; SOD: superoxide dismutase; XO: Xanthine oxidase

Postmortem studies demonstrated a significant decrease in antioxidant enzymes in the hippocampus, frontal cortex, and substantia nigra of aging brains (Venkateshappa et al. 2012).

14.4 Catalase, Glutathione Peroxidase, and Superoxide Dismutase Levels and Brain White Matter Changes

The changes in white matter integrity and antioxidant enzyme levels are crucial for the onset of AD as shown in cadavers (Venkateshappa et al. 2012). Besides the evidence from the brain tissue, lower serum SOD, and GPx levels were shown in the blood of patients with mild cognitive impairment (Kumar et al. 2019). Polymorphisms in CAT and SOD2 genes suggested being the risk factors for enhanced oxidative damage of the white matter and thus cognition among older individuals with these genetic variants (Salminen et al. 2017). Besides aging, CAT, GPx1, and SOD1 and SOD2 levels were significantly lower in oligodendrocytes in the white matter of the brains of the patients with major depression (Szebeni et al. 2014). Decreased white matter integrity was associated with depleted levels of hippocampal glutathione suggesting that this particular disruption may be linked to oxidative stress at the early stages of mood disorders (Hermens et al. 2018) and psychosis (Monin et al. 2015). The disruption in myelin production and white matter maturation in the prefrontal cortex might be the underlying mechanism for advancement to schizophrenia. A randomized controlled study with N-acetyl cysteine seems to be a safe and effective agent to protect white matter integrity in early psychosis (Klauser et al. 2018). Decreased white matter intensities accompanied with decreased antioxidant levels were also observed in migraine patients (Aytac et al. 2014).

14.5 Sirtuins

Sirtuins are transcription-silencing histone deacetylase enzymes, which have a diverse role in cellular function. They modulate physiological and pathological processes such as metabolism, longevity, senescence, cell survival, proliferation, apoptosis, DNA repair, and aging.

SIRTs also have an important role in the pathogenesis of brain injuries such as traumatic brain injuries (closed head trauma, ischemia, stroke), neurodegenerative disorders (Parkinson's, Alzheimer's, Huntington's, Amyotrophic Lateral Sclerosis (ALS)), psychiatric disorders (depression, anxiety, sleep disorders) and aging (Morris 2013; Satoh et al. 2017). The process of normal brain aging is associated with a decrease in white matter volume, neurogenesis, neurotransmitter production, synaptic neurotransmission, and myelination. Sirtuins regulate neurogenesis

and synaptic plasticity. Sirtuin activation was shown to slow down the cognitive decline associated with aging (Toklu and Ginory 2018).

There are seven subtypes of sirtuin enzymes. The brain neurons express all subtypes. SIRT1, SIR5, SIRT6, SIRT7 are expressed in astrocytes, while SIRT2 is detected in myelin-producing cells, i.e., oligodendrocytes. The localization of sirtuins in the brain is summarized in Table 14.1.

Sirtuins (primarily SIRT1) have been shown to contribute to glial progenitor proliferation and regeneration in white matter after neonatal brain injury (Jablonska et al. 2016) and SIRT3 expression in the periventricular white matter was upregulated in hypoxia (Li et al. 2018).

The study cohort comparing SIRT1, 3, and 5 immunoblots and immunohistochemistry in the entorhinal cortex, hippocampus, and white matter of 45 cases demonstrated that: (1) the neuronal subcellular redistribution of SIRT1 is parallel to the decline in its expression, suggesting a loss of neuroprotection which is dependent

Table 14.1 The distribution of subtypes of sirtuins (SIRT) in the brain. Adapted from the work by Toklu and Ginory (2018) with permission from Springer Nature

Enzyme subtype	Intracellular location	Cell type	Region in brain
SIRT1	Nucleus, cytoplasm	Neurons and astrocytes	Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum
SIRT2	Cytoplasm	Neurons and oligodendrocytes	Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum
SIRT3	Mitochondria	Neurons	Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum
SIRT4	Mitochondria	Neurons and astrocytes	Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, preoptic area, striatum
SIRT5	Mitochondria	Neurons and astrocytes	Brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, preoptic area, striatum
SIRT6	Nucleus	Neurons and astrocytes	Amygdala, brain stem, cortex, cerebellum, hypothalamus, hippocampus, olfactory bulb, striatum
SIRT7	Nucleus	Neurons and astrocytes	Amygdala, cortex, hippocampus, striatum, thalamus

to the neuronal population; (2) in contrast to SIRT1 and 3, expression of SIRT5 increases during the progression of AD; (3) which might be related to its appearance in activated microglial cells (Lutz et al. 2014).

Sirtuin-mediated neuroprotection involves several mechanisms such as regulation of DNA repair enzymes, protein kinases, transcription factors, and co-activators (Zhang et al. 2011).

14.6 The Effects of SIRT Activators and Senolytics on White Matter Changes and Redox Biomarkers

Cellular senescence is a phenomenon characterized by the cessation of cell division. In the past, it was thought to be an irreversible cell-cycle arrest mechanism that acts to protect against cancer, but recent discoveries have extended to a role in complex biological processes such as development, tissue repair, aging, and age-related disorders. In humans, senescent cells accumulate in adipose tissue in diabetes and obesity, in the hippocampus and frontal cortex in AD, the substantia nigra in PD, bone and marrow in age-related osteoporosis, lungs in idiopathic pulmonary fibrosis, liver in cirrhosis, retina in macular degeneration, plaques in psoriasis, kidneys in diabetic kidney disease, endothelium in pre-eclampsia, and the heart and major arteries in cardiovascular disease, amongst many other conditions (Kirkland and Tchkonia 2020).

The groundbreaking research on senescent cells and their clearance delaying aging-associated disorders was published less than a decade ago (Baker et al. 2011). Since then, drugs acting on senescent cells are being investigated as senotherapeutics (Kim and Kim 2019; Kirkland and Tchkonia 2020; Wissler Gerdes et al. 2020). Earlier studies focused on the enzymes and signaling pathways such as SIRT, Protein Kinase C (PKC), Protein Kinase A (PKA), Calcium/calmodulin-dependent Protein Kinase (CaMK), Tyrosine Kinase, which are accepted as the key molecules associated with memory and brain senescence (Govoni et al. 2010). Hence, the target of true senolytics is senescent cells, not a single receptor, enzyme, or biochemical pathway (Kirkland and Tchkonia 2020).

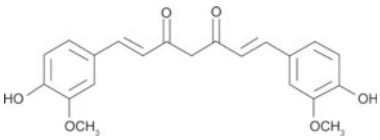
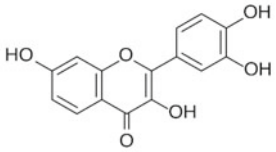
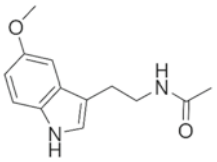
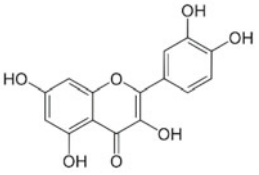
One of the most recent findings in the field is that oxylipin may be a biomarker for tracking the activity of the senescent drugs. Oxylipin is a lipid metabolite, exclusively intracellular in normal conditions, but is released when senescent cells are forced to die. This signaling metabolite is detectible in blood and urine. With a growing list of senolytic drugs in development, detecting this metabolite could verify the performance of senolytic drugs (Wiley et al. 2021).

A publication in 2019 showed that the senolytic combination of *dasatinib and quercetin* selectively cleared senescent cells from the plaque environment, reduced neuroinflammation, lessened Aβ load, and ameliorated cognitive deficits in

Alzheimer's disease in a mice model (Zhang et al. 2019b). Thus, the first human clinical trial (NCT04063124) with senolytic drug treatment with this combination (*dasatinib + quercetin*) is ongoing for Alzheimer's disease. On the other hand, the preliminary results for its efficacy on idiopathic pulmonary fibrosis and diabetic kidney disease are published and looks promising (Hickson et al. 2019; Justice et al. 2019). Recently, fisetin, a flavonoid polyphenol became another candidate as senotherapeutic after showed to extend lifespan (Yousefzadeh et al. 2018). The mechanism of action for fisetin is suggested to be via SIRT1 activation. Prior to fisetin, other SIRT activators also gained attention for their antiaging/senolytic effects. Caloric restriction, rapamycin, melatonin, tempol, vitamin E, and polyphenols, i.e., resveratrol, curcumin, quercetin, and fisetin are some of the most widely studied activators of the sirtuin system, which are further being investigated for their role in modulating cellular senescence (Table 14.2). While SIRT1 activation is associated with senescence and longevity, SIRT1 deficiency results in elevated mTOR (mammalian target of rapamycin) signaling. mTOR is a key kinase enzyme in modulating energy metabolism, nutrient sensing, aging, and longevity. Excessive mTOR activity is inhibited by caloric restriction and several agents like rapamycin. Both activate SIRT 1 and increase life span (Ehninger et al. 2014).

- **Caloric restriction**
  Caloric restriction refers to a dietary regimen that reduces daily calorie intake without incurring malnutrition, i.e. $\geq$ 10% in humans, $\geq$ 20% in animals (Bales and Kraus 2013). Caloric restriction and rapamycin have been shown to increase longevity in mice via SIRT1 activation (Libert and Guarente 2013; Nikolai et al. 2015). Caloric restriction also improves antioxidant status by enhancing SOD, CAT, GPx activities, and increasing GSH concentration in the cerebral cortex and hippocampus (Alugoju et al. 2018; Santin et al. 2011). Caloric restriction preserves white matter integrity, brain energy production, and long-term memory in aging mice and ischemic injury models (Guo et al. 2015; Zhang et al. 2019a).
- **Curcumin**
  Curcumin is the major constituent of turmeric (*Curcuma longa*) root. It has antioxidant, anti-inflammatory, and anticancer activities (Aggarwal et al. 2007; Jurenka 2009). Curcumin also upregulated SIRT1 expression in the brain following stroke (Miao et al. 2016). In a mice model of hypoxic-ischemic brain injury, immediate post-treatment with curcumin was significantly neuroprotective, reducing gray and white matter tissue loss (Rocha-Ferreira et al. 2019).
  The protective effect of curcumin against white matter injury is associated with the protection of oligodendrocytes, inhibition of microglial activation, and suppression of iNOS and NADPH oxidase activation (He et al. 2010). Long-term curcumin supplementation improved white matter integrity in limbic, cerebellar, and brain stem regions in aging primates (Koo et al. 2018). Not only the white matter in brain, but also the white matter of the spinal cord was preserved with nano-formulated curcumin supplementation after spinal cord injury (Krupa et al. 2019).

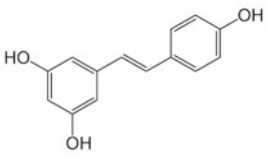
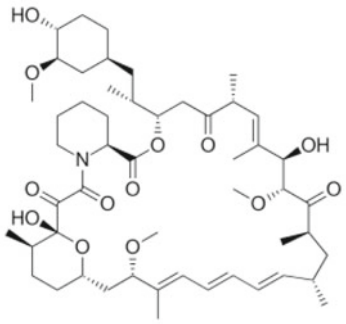
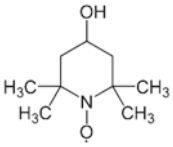
Table 14.2 Sirtuin (SIRT) activators and their sources

Drug	Chemical structure and name	Source (natural/ chemical)
Caloric restriction	–	Intermittent fasting, Fasting diet
Curcumin	 *(1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) - 1,6- heptadiene-3,5-dione*	Turmeric
Fisetin	 *7,3′,4′-flavon-3-ol*	Strawberry, apple, persimmon, grape, onion, kale, kiwi, cucumber
Melatonin	 *N-acetyl-5-methoxy tryptamine*	Eggs, fish, walnuts, peanuts, grains (rice, barley, wheat, oat), sunflower seeds, pistachios, fruits and vegetables (cherries, strawberries, grapes, pomegranate, tomatoes, mushrooms, olives, broccoli, cucumber)
Quercetin	 *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H chromen-4-one*	Grapes, raspberry, nectarine, broccoli, red onion, black tea, red wine

(continued)

- **Fisetin**
  Fisetin is a plant polyphenol found in many fruits and vegetables, such as strawberries, apples, persimmons, onions, and cucumbers. As a polyphenol, fisetin is an antioxidant and immunomodulator which can counteract via AMPK/SIRT1,

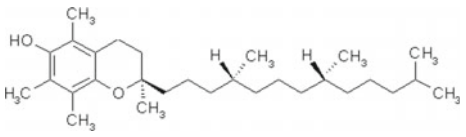
Table 14.2 (continued)

Drug	Chemical structure and name	Source (natural/ chemical)
Resveratrol	 *trans-3,5,4′-Trihydroxystilbene*	Grapes, wine, grape juice, peanuts, cocoa, blueberries, bilberries, and cranberries
Rapamycin (Sirolimus)	 *(1R,15R,16E,18R,19R,21R,23S,24Z,26E,28E,30S, 35R)-1,18-dihydroxy-12-[(2R)-1-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo [30.3.1.04,9] hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone*	A natural antifungal, macrolide antibiotic produced by *Streptomyces hygroscopicus*
Tempol	 *4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl*	Synthetic

(continued)

Nfr2, and PPAR pathways (Iside et al. 2020). Yet no study evaluated the effect of fisetin on cerebral white matter.

Table 14.2 (continued)

Drug	Chemical structure and name	Source (natural/ chemical)
Vitamin E	 *α-tocopherol*	Plant source (Wheat germ oil, Hazelnut oil, Canola/rapeseed oil, Sunflower oil, Almond oil, Safflower oil, Grapeseed oil, Sunflower seed kernels, Almonds, Almond butter, Wheat germ, Canola oil, Palm oil, Peanut oil, Margarine, Hazelnuts, Corn oil, Olive oil, Soybean oil, Pine nuts, Peanut butter, Peanuts) Animal source (Fish, Chicken, Pork)

- **Melatonin**
  Melatonin is a hormone primarily secreted by the pineal gland. Its primary function is to regulate the circadian clock. Melatonin is also a powerful antioxidant whose role goes beyond free radical scavenging. Mostly due to antioxidant activity, neuroprotective effects of melatonin in many central nervous system (CNS) disease conditions such as amyotrophic lateral sclerosis, PD, AD, ischemic injury, neuropsychiatric disorders, subarachnoid hemorrhage and head injury are well documented (Kaur and Ling 2008; Erşahin et al. 2009; Ersahin et al. 2009). Melatonin has been shown to promote myelination in white matter (Villapol et al. 2011). The moderate-to-severe calcification of the pineal gland and white matter hyperintensity was independently associated with elderly individuals (Del Brutto et al. 2020).
- **Quercetin**
  Quercetin is a polyphenolic compound that has antioxidant, anti-inflammatory, immuno-protective, and anti-carcinogenic effects (Andres et al. 2018). Quercetin increased hippocampal SIRT1 levels and improved cognitive function in aged rats (Sarubbo et al. 2018). Other studies showed that quercetin's effect to improve cognitive function was due to the preservation of white matter against hypoxic-ischemic damage (Huang et al. 2012; Takizawa et al. 2003). Furthermore, quercetin improved the age-associated decline in the activities of endogenous antioxidant enzymes such as SOD, CAT, GPx and reduced glutathione content and attenuated elevated levels of protein carbonyl content (PCC), lipid peroxidation, lipofuscin, ROS, and nitric oxide in rat brains (Alugoju et al. 2018).

- **Rapamycin (Sirolimus)**
  Rapamycin, also called sirolimus is an immunosuppressant drug used for preventing the rejection of organ transplants. It is the first pharmacological agent shown to extend lifespan in mammalian species (Carter et al. 2016; Ehninger et al. 2014). As mentioned earlier, caloric restriction has been shown to augment longevity via the activation of SIRT pathway (Zhang et al. 2011), and SIRT1 deficiency results in elevated mammalian mTOR signaling, which has a key role in longevity and energy metabolism (Ghosh et al. 2010). Furthermore, mTOR overactivity has pathological effects on white matter, which can be modified pharmacologically by mTOR inhibitor rapamycin or its derivative everolimus (Lin et al. 2020; Peters et al. 2019; Tillema et al. 2012; Wong 2019; Toklu et al. 2016). Rapamycin was also shown to enhance the differentiation of oligodendrocytes, thus contributes to myelination (Nicaise et al. 2019).
- **Resveratrol**
  Resveratrol is a plant polyphenol with potential therapeutic effects in cancers, cardiovascular, and neurological diseases (Lopez et al. 2015; Toklu et al. 2010). It is a promising agent for brain aging, neurotrauma, epilepsy, Alzheimer's disease, and other neurodegenerative diseases, because of the ability to improve cognitive function and neuronal plasticity (Dias et al. 2016; Lange and Li 2018; Poulose et al. 2015; Sarubbo et al. 2017; Toklu et al. 2010). As a SIRT1 activator and mTOR inhibitor, resveratrol mimics the favorable effects of caloric restriction (Carafa et al. 2016; Dolinsky and Dyck 2011; Ghosh et al. 2010; Villalba and Alcain 2012) but was not proven to prolong life span as caloric restriction does. On the other hand, evidence suggests that resveratrol delays the onset of age-related diseases (McCubrey et al. 2017).
  Preservation of brain white matter, improved cerebral microvascular circulation, improved mitochondrial function, neurogenesis, neuroprotection, and neuronal survival are achieved with resveratrol treatment in experimental studies. Resveratrol treatment also decreased macular degeneration, retinal aging, and aging-induced hearing loss in rats (Karalis et al. 2011; McCubrey et al. 2017; Revuelta et al. 2016).
- **Tempol**
  Tempol is a membrane-permeable free radical scavenger. The protective effect of tempol against neuronal injury and aging is widely being investigated (Dornas et al. 2015; Hamel 2015; Toklu et al. 2017; Wilcox 2010). Tempol has SOD-mimetic effects (Dornas et al. 2015) and was shown to improve cognitive function via white matter protection (Liu et al. 2013).
- **Vitamin E**

  Vitamin E includes a group of lipid-soluble tocopherols and tocotrienols. α-tocopherol is the most plentiful and bioavailable form of vitamin E for humans (La Fata et al. 2014). It is a well-known antioxidant. A recent study has demonstrated that long-term deficiency of vitamin E remarkably decreased the expression of silent mating type information regulation (SIRT)-2 mRNA compared to short-term deficiency (Fukui et al. 2014). Vitamin E is widely studied for its effect on

brain aging and cognitive function (La Fata et al. 2014; Tucker 2016). Frontal lobes exhibited an age-related decline in retinol, total tocopherol, total xanthophyll, and total carotenoid (Craft et al. 2004). Lower carotenoid and vitamin E levels were associated with cerebral deep white matter lesions in aging subjects (Ohshima et al. 2013; Schmidt et al. 1996). Alpha-tocopherol was significantly and inversely related to the presence of beginning confluent and confluent white matter changes after adjustment for the between-group differences in age, arterial hypertension, cardiac disease, and cholesterol (Schmidt et al. 1996). The studies suggest that older adults consuming more polyunsaturated fatty acids and vitamin E rich foods had better white matter integrity and that maintaining white matter microstructural integrity might be a mechanism for the beneficial role of diet on cognition (Gopalan et al. 2014; Gu et al. 2016; Prinelli et al. 2019).

14.7 Clinical Trials with Senolytics/SIRT Activators

At present, there are only a few human clinical trials ongoing with SIRT activators/senolytics. Three of them involve caloric restriction, diet, and aging (NCT01256840, NCT00996229, NCT01219244). Senolytic combination dasatinib and quercetin clinical trials are also ongoing for investigation of their effects in AD (NCT04063124, NCT04685590). See Table 14.3 for the list of other clinical trials.

14.8 Conclusion

Antioxidants and SIRT activators may have beneficial effects in preventing cerebral white matter changes and thus improve neurocognitive function. However, randomized controlled clinical trials are needed to demonstrate the mechanism of action further.

Table 14.3 Human Clinical Trials with SIRT activators, which evaluate brain structure or cognitive function

Drug	Study ID (NCT no) and Title
Caloric restriction/ diet	• NCT01256840 (Long-term Caloric Restriction and Cellular Aging Markers (CRONA)) • NCT00996229 (Effects of Dietary Interventions on the Aging Brain) • NCT01219244 (Effects of Dietary Interventions on the Brain in Mild Cognitive Impairment (MCI))
Dasatinib + Quercetin	• NCT04063124 (Senolytic Therapy to Modulate Progression of Alzheimer's Disease (SToMP-AD)) • NCT04685590 Senolytic Therapy to Modulate the Progression of Alzheimer's Disease (SToMP-AD) Study (SToMP-AD)
Fisetin	• NCT03675724 (Alleviation by Fisetin of Frailty, Inflammation, and Related Measures in Older Adults (AFFIRM-LITE))
Resveratrol	• NCT01504854 (Resveratrol for Alzheimer's Disease) • NCT00678431(Randomized Trial of a Nutritional Supplement in Alzheimer's Disease) • NCT01354977 Effect of Resveratrol on Age-related Insulin Resistance and Inflammation in Humans) • NCT02502253 (BDPP Treatment for Mild Cognitive Impairment (MCI) and Prediabetes or Type 2 Diabetes Mellitus (T2DM))
Melatonin	• NCT03954899 (Disease Modifying Potential of 5 mg of Melatonin on Cognition and Brain Health in Aging) • NCT02395783 Therapeutic Effects of Maternal Melatonin Administration on Brain Injury and White Matter Disease (PREMELIP) • NCT04400266 (Buspirone and Melatonin for Depression Following Traumatic Brain Injury) • NCT04522960 (Melatonin in Alzheimer's disease: Effect on Disease Progression and Epileptiform Activity (MADE)) • NCT03590197 (Effect of Melatonin on Seizure Outcome, Neuronal Damage and Quality of Life in Patients With Generalized Epilepsy) • NCT00940589 (Efficacy of Circadin® 2 mg in Patients With Mild to Moderate Alzheimer Disease Treated With AChE Inhibitor) • NCT04421339 (Melatonin for Huntington's Disease (HD) Gene Carriers With HD Related Sleep Disturbance—a Pilot Study)
Rapamycin	• NCT04629495 (Rapamycin—Effects on Alzheimer's and Cognitive Health (REACH))
Vitamin E	• NCT00040378 (Prevention of Alzheimer's Disease by Vitamin E and Selenium (PREADVISE)) • NCT00753532(Neuroprotective and Cardioprotective Effects Of Palm Vitamin E Tocotrienols) • NCT02263924 (Stroke and Tocotrienol: Unique Role in Neuroprotection (SATURN))

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Chapter 15
Redox Homeostasis in Alzheimer's Disease

Jan Homolak

Abstract Alzheimer's disease (AD) is the most common neurodegenerative disorder with yet unresolved etiopathogenesis that poses a significant socio-economical burden on society. At the molecular level, AD is driven by mutually interdependent pathophysiological processes with the ability to trigger self-sustaining progressive neurodegeneration. Redox homeostasis is maintained by a continuous interaction of molecules regulating the electrophilic and nucleophilic tone of the cell/organism. These messengers also control other processes involved in the proper functioning of the cell. When presented with a redox challenge, the cell either resolves the challenge by fine-tuning the electrophilic and nucleophilic tone to enable resolution without affecting the functioning of other systems or redefines the redox setpoint in a process named heterostasis. Redox heterostasis provides some protection at the expense of the long-term functioning of the cell. The main pathomechanisms of AD (dyshomeostasis of amyloid, tau, and insulin) can all induce redox heterostasis. Inversely, redox heterostasis can trigger all relevant pathomechanisms of AD and mediate their propagation acting as a conjunctive etiopathogenetic amplifier. The mutual interdependence of body redox homeostatic systems could enable the identification of individuals at risk for the development of redox heterostasis, and help prevent the propagation of pathophysiological processes. Redox dyshomeostasis-directed pharmacological interventions could re-establish homeostasis by (i) reducing electrophilic tone by suppressing neuroinflammation (e.g., with NADPH oxidase inhibitors or statins), (ii) providing substrates for the nucleophilic systems (e.g., cysteine), and (iii) stimulating nucleophilic tone by parahormetic activation of the nuclear factor erythroid 2-related factor 2 to reset the redox rheostat.

Keywords Alzheimer's disease · Redox homeostasis · Oxidative stress · Heterostasis · Electrophilic tone · Nucleophilic tone

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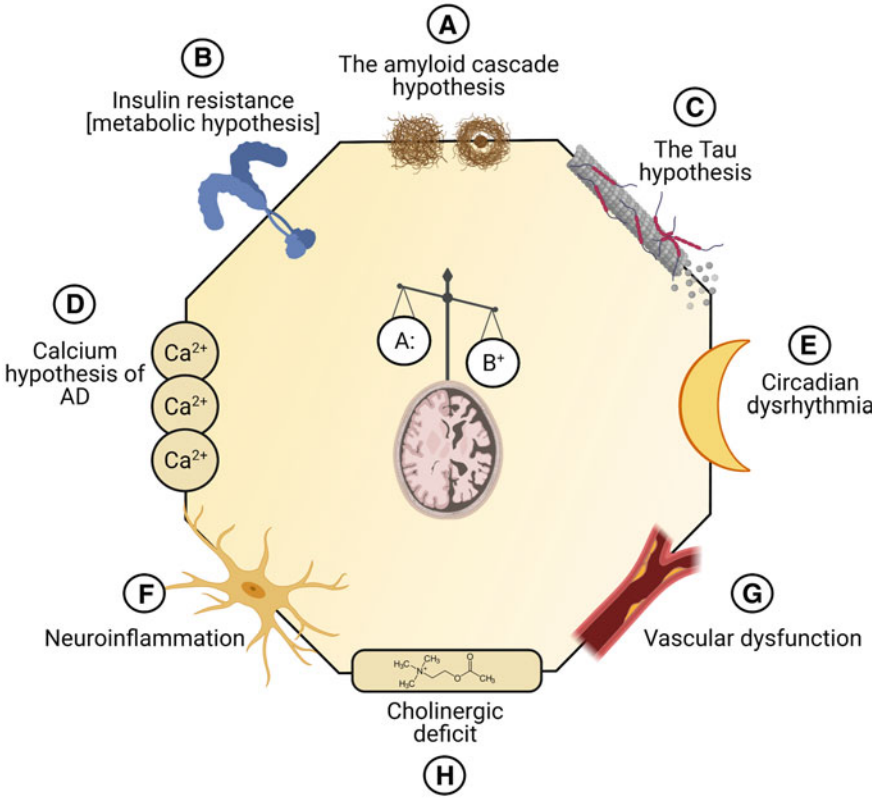
15.1 Alzheimer's Disease

Alzheimer's disease (AD) is the most common type of dementia with yet unresolved etiology. It is characterized by progressive degeneration of neurons accompanied by the development of cognitive impairment and other often neglected neuropsychiatric symptoms such as apathy, depression, confusion, and behavioral changes ("2020 Alzheimer's disease facts and figures" 2020). The prevalence of the disease is on the rise with the increasing median age of the population being one of the most important contributing factors. In 2011, it was estimated that around 24 million people were affected by AD (Ballard et al. 2011), and by the year 2016, the number increased to over 40 million (Brookmeyer et al. 2007; Nichols et al. 2019). Some projections suggest that by the year 2050 the number may increase to around 100 million. In other words, the projections suggest 1 in 85 persons worldwide will be living with the disease (Brookmeyer et al. 2007). The aforementioned predictions are especially worrisome considering a substantial socio-economical burden associated with AD. According to the estimates from 2015, the worldwide cost of dementia was around 818 million USD and was expected to reach 1 trillion USD in 2018 (Wimo et al. 2017).

AD is classified as either early-onset (EOAD) or late-onset (LOAD) depending on whether the disease is diagnosed before the age of 65 (Reitz et al. 2020). Approximately 5–10% belong to the EOAD group. Although it was previously believed that in the majority of patients suffering from EOAD, the disease arises as a consequence of a clear genetic predisposition, recent evidence points toward a more complex underlying biology as positive family history was present in only 60% of patients, and autosomal dominant inheritance was confirmed in just 13% of these families (Bekris et al. 2010). It is currently believed that a fully penetrant autosomal dominant inheritance recognized as a mendelian subtype of EOAD drives the development of the disease in just 0.5–1% of all AD cases (Reitz et al. 2020). In those cases, mutations in amyloid precursor protein, presenilin-1, or presenilin-2 genes are considered to be key etiopathogenetic factors. In contrast, genetic landscapes of the non-mendelian EOAD and LOAD remain much less clear with complex polygenic pathophysiology and inconsistent inheritance patterns. Taken together the etiopathogenetic background of a substantial proportion of cases (~99%) is currently unknown.

Many hypotheses have been proposed over the years providing evidence for the central role of different pathophysiological processes in the context of the development of AD (Fig. 15.1). Nevertheless, attempts of pharmacological exploitation of practically all critical pathobiological processes proposed as key etiopathogenetic events (e.g., amyloid β (Aβ) accumulation or tau aggregation) were unsuccessful so far. On the other hand, mounting evidence suggests extensive interdependence of all processes believed to play the central role in the development of AD-related pathology. Furthermore, redox dyshomeostasis seems to be a critical mechanism by which activation of one AD-related pathophysiological cascade can induce activation of others triggering a self-sustaining vicious cycle driving neurodegenerative processes. Furthermore, a noxious stimulus that introduces a redox disbalance

has the potential to activate any of the proposed main pathophysiological cascades. Conversely, oxidative stress is a downstream consequence of all pathobiological cascades of AD (Fig. 15.1). Consequently, due to its close relationship with the maintenance of cellular equilibrium, redox homeostasis emerges as an important player in the development of AD participating both in early allostatic molecular events as well as in late neurodegeneration-related processes. Understanding the role of redox homeostasis in the development of AD may offer hope for the development of novel strategies to prevent, slow down, and possibly even revert pathobiological processes driving neurodegeneration. This chapter aims to provide an overview of critical mechanisms by which redox homeostasis is involved in the development of the disease from the perspective of currently prominent AD hypotheses, offer an integrative insight into redox homeostasis in the context of neurodegeneration, and discuss diagnostic and therapeutic opportunities that arise as a consequence of understanding redox biology of the disease.



◀**Fig. 15.1** A schematic representation of current hypotheses of Alzheimer's disease (AD). In all hypotheses of AD, a single pathophysiological event is recognized as the main etiopathogenetic factor that activates other pathobiological cascades and initiates a self-sustaining cycle of degeneration of the central nervous system. Redox dyshomeostasis characterized by an excess of electrophiles unopposed by an adequate nucleophilic tone is considered as a downstream pathological process that propagates damage and causes neurodegeneration in all AD hypotheses. (**A**) *The amyloid cascade hypothesis* suggests that the deposition of amyloid-β (Aβ), the main component of the plaques is a primary pathophysiological event in AD. Aβ stimulates mitochondrial production of reactive oxygen species (ROS), generates ROS when coordinated with metal ions, and induces NADPH oxidase (NOX). (**B**) *The insulin-resistant brain state (IRBS)/metabolic hypothesis* proposes that metabolic dysfunction of the brain accompanied by dysregulation of insulin signaling precedes other pathobiological processes and acts as a primary driver of neurodegeneration. IRBS increases electrophilic tone due to downstream metabolic dysfunction, reduces the availability of substrates for the generation of nucleophiles, and downregulates a key regulator of nucleophilic tone nuclear factor erythroid 2-related factor 2 (Nrf2). (**C**) *The tau hypothesis* recognizes hyperphosphorylation of a group of proteins derived by alternative splicing of the microtubule-associated protein tau (MAPT) gene that are involved in the maintenance of the structure and function of microtubules as the main driver of the disease. Dysfunctional tau promotes metabolic dyshomeostasis and ROS production by affecting mitochondrial trafficking, destabilizing mitochondrial membrane, and downregulating factors involved in the maintenance of redox homeostasis (e.g., antioxidant enzymes). (**D**) In *the calcium hypothesis*, compromised handling of cellular Ca^{2+} is considered to be responsible for neurodegeneration. Dyshomeostasis of Ca^{2+} potentiates cellular generation of ROS. (**E**) Circadian rhythm dysfunction occurs early in the course of the disease and exacerbates the deposition of amyloid, inflammatory processes, and IRBS according to the *circadian hypothesis*. Aβ, IRBS, and inflammation promote electrophilic tone, and inadequate activation of reparatory mechanisms that require physiological circadian function downregulates the nucleophilic tone. (**F**) *The neuroinflammation hypothesis* of AD emphasizes the role of central nervous system inflammation as the main pathophysiological self-sustaining process that causes neurodegeneration. Neuroinflammation promotes electrophilic tone by activation of pro-oxidative processes (e.g., NOX). (**G**) In *the vascular hypothesis* of AD, pathophysiological alterations of vascular function are considered a trigger for hypoperfusion and oxidative stress-induced progressive neurodegeneration of sensitive areas of the brain. Vascular redox dyshomeostasis propagates to the neuronal redox system, and the lack of perfusion weakens the nucleophilic reserves of the cell. (**H**) *The cholinergic deficit hypothesis* suggests that the loss of cholinergic signaling acts as a trigger for other pathological alterations observed in AD. Decreased activation of the α7 nicotinic receptor causes a pro-oxidative shift of the redox hypothesis. Finally, dysfunctional redox homeostasis promotes Aβ accumulation, IRBS, tau hyperphosphorylation, calcium dyshomeostasis, circadian dysrhythmia, neuroinflammation, vascular dysfunction, and cholinergic loss

15.2 The Concept of Homeostasis

The concept of homeostasis has been introduced in modern medicine by a Frech physiologist Claude Bernard in the nineteenth century through the idea of *milieu intérieur*. Claude Bernard was working on specific unresolved physiological concepts at the time, such as the role of liver glycogen in the maintenance of plasma glucose, and sympathetic nervous system-mediated regulation of vascular blood flow. Nevertheless, his capacity to provide tremendously insightful generalizations from specific physiological observations led to the postulate that living organisms possess sensors responsible for the regulation of the internal environment and that this ability is

essential for life independent of the external environment (Goldstein and Kopin 2007; Gomes and Engelhardt 2014). Bernard's work on the concept of *milieu intérieur* was later extended by an American physiologist Walter Bradford Cannon who coined the word *homeostasis* to describe a self-regulating process by which biological systems maintain stability while adjusting to changing external conditions. Although homeostasis is a central organizing principle of physiology, the concept is often underappreciated and ignored (Billman 2020), an unfortunate practice leading to the development of non-integrative explanations of biochemical and physiological functions that rarely provide foundations for a better understanding of physiology and pathophysiology of the human body, let alone a successful development of the pharmacological agents for the management of human diseases. One probable contributing factor to the ignorance of homeostatic principles is the fact that homeostasis is regularly described as a set of mechanisms enabling the maintenance of the physiological *steady-state* that is often understood far too literally. Consequently, the importance of acknowledging an interplay of complex dynamic compensatory reactions that orchestrate the response of the organism to a stimulus is neglected. In contrast, Cannon understood the importance of the fact that homeostasis is a rather dynamic process that only maintains stability by change. For example, in his seminal work „*Organization for physiological homeostasis* ", Cannon quotes the words of the Nobel laureate Charles Richet: „ *By an apparent contradiction it* [the living being] *maintains its stability only if it is excitable and capable of modifying itself according to external stimuli and adjusting its response to the stimulation* ". Finally, the importance of the dynamic nature of homeostasis is reflected in the term homeostasis itself as Cannon decided to use the prefix *homeo* (latinized from Greek *homio* meaning similar or alike) instead of *homo* (from Greek *homos* meaning the same or equal) to emphasize homeostasis cannot be accomplished by fixed and rigid constancy (Cannon 1929).

The concept of homeostasis was recognized and adopted by a significant proportion of the scientific community providing strong foundations for further development of physiological thought. The evolution of the concept of homeostasis is beyond the scope of this chapter, however, the contributions of Hans Selye and Sterling and Eyer have to be acknowledged as they provided key ideas upon which redox homeostasis is understood today. Hans Selye, best known for a redefinition of the theory of stress, expanded homeostasis by introducing the term *heterostasis* (*hetero* from Greek *heteros* meaning the other or different). Through the idea of heterostasis, Selye wanted to emphasize that the adaptation of the physiological system to an unusually challenging stimulus can be accompanied by the establishment of a new steady-state by changing the original homeostatic setpoint. Sterling and Eyer indebted the field by introducing the concept of *allostasis*, to further emphasize the importance of variation for maintenance of the homeostasis first mentioned by Cannon. Sterling and Eyer proposed that there is a natural alteration between states of acceptable physiological values that are inherent to the system. Furthermore, they suggested that there is a temporal component to the homeostatic regulation as even a small offset within a

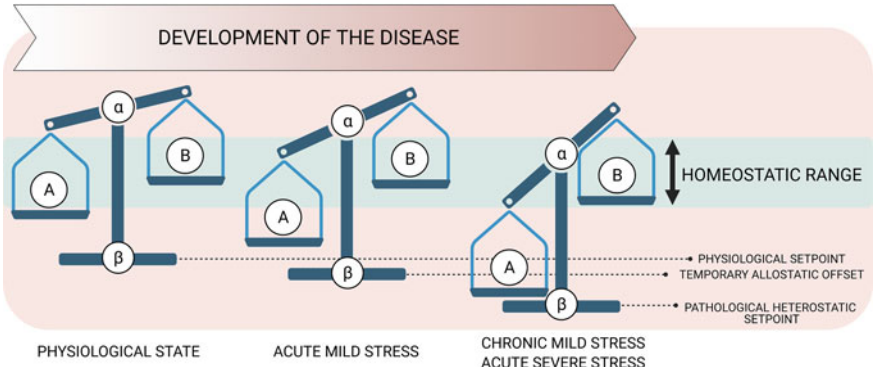


Fig. 15.2 Principles of homeostatic regulation are illustrated using the example of two complex (biological) systems (A and B) interconnected by a set of pre-defined (patho)physiological relationships (α and β). (Left) A shared „static " physiological process/messenger (β) defines the relationship between two complex cellular systems (A and B). α is an adjacent process that enables fine-tuning of A and B maintaining the whole system in the homeostatic range when activation of A or B is required in a response to the stimulus. As long as the challenge is sufficiently small or transient, change in either A or B doesn't require pushing the other system out of the homeostatic range. **(Center)** A more challenging stimulus requires further adaptation of both α and β to maintain both systems in the homeostatic range, however, in order to keep system B functioning, system A is maximally exploited. In the biological system, this would correspond to near-maximal activation or inhibition of the system. For example, B can represent a redox subsystem (e.g., a redox pair), and A can represent a metabolic pathway that has to be sacrificed in order to keep the redox system going in response to a redox challenge. The system is maximally exploited and can work this way only transiently without suffering serious damage due to functional exploitation of one of its subsystems. **(Right)** If the stimulus is too great to be resolved acutely, or if it persists, a novel heterostatic setpoint is required to provide some protection. In this scenario, the redox subsystem (B) is maintained in the homeostatic range, and the metabolic subsystem A is fully sacrificed (e.g., fully inactivated) by rearrangement of the α and β. As all cellular functions are required for its maximal resilience, long-term inhibition of A will cause accumulating damage and lead to the development of the disease. Please note that the redox subsystem and metabolic subsystem were used only for the purpose of demonstration and that the redox system can play the role of A or B and even α or β

physiological range that would benefit the system acutely could overburden it in the long run. The term *allostatic load* has been proposed for the latter concept (Goldstein and Kopin 2007). Key principles of homeostatic regulation and their relationship with health and disease are shown in Fig. 15.2.

15.3 Redox Homeostasis

A general concept of homeostasis also applies to the physiological redox system—a biochemical system comprised of a network of interconnected chemical reactions in which oxidation states of atoms are changed. In chemistry, redox reactions are characterized by a transfer of electrons in which one chemical species—"the reducing

agent"—undergoes oxidation (gives up electrons) to enable the reduction (attainment of electrons) of its redox pair. Although useful for comprehension, there are exceptions to this "general rule" and some reactions go on without an obvious exchange in electrons so it has been proposed that oxidation should simply be defined as an increase in oxidation state. There are additional obstacles in the way of applying the general theoretical concepts of redox chemistry to a biological system with the most obvious one being that the standard conditions, which are generally assumed in theoretical calculations, do not apply to living cells. Furthermore, although individual redox systems are interconnected, the phenomenon of *redox compartmentalization* should not be disregarded as both the living cell and an organism as a whole are structurally defined by hierarchical segmentation that divides the organism/cell into many functionally interdependent but individual compartments. At the organismic level, this is especially evident during the process of embryonic development when a tight regulation of the redox spatio-temporal patterns is involved in the complex orchestration of simultaneous development of many different cell types and tissues. During this period, an interplay of organismic and cellular redox homeostasis provides an important layer of information directing pluripotent cells toward synchronized development of highly differentiated and specialized cells that will provide foundations for the evolution of functional organs in the adult animal. A good example of an organismic redox pattern during the development is an oxidative shift of the glutathione (GSH) redox potential in the zebrafish ontogenesis periods characterized by the greatest degrees of differentiation (gastrulation, organogenesis, pharyngula periods). In this context, the term *redox stress* has been proposed for the deviation of the physiological redox status involved in the regulation of cell proliferation, differentiation, migration, polarity, and regulated death that often results in the development of pathology (Timme-Laragy et al. 2018). Even the infamous drug thalidomide has been proposed to induce limb teratogenesis by a mechanism involving redox misregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (Hansen and Harris 2004).

Functional redox compartmentalization is also evident at the level of individual cells. Some cellular compartments such as the lysosome and the peroxisome are considered more oxidizing, while the others like the nucleus and the mitochondrion exert more reductive properties (Kaludercic et al. 2014). The difference in redox homeostasis at the level of individual subcellular compartments arises as a consequence of different pool sizes of key redox couples and their involvement with distinctive cellular processes that directly or indirectly rely on redox reactions for their proper functioning. For example, cytoplasmic concentrations of the nicotinamide adenine dinucleotide (NAD) range between 200 and 500 μM, while the ratio of the reduced to the oxidized form of NAD is kept between 200 and 800. In contrast, almost twofold higher concentrations of NADs and an approximately 100-fold lower ratio of the reduced to the oxidized form have been observed in the mitochondrion (Wang et al. 2018). The level of redox compartmentalization is functionally important, and redox subsystems are kept relatively separated at the level of individual cells to ensure processes requiring distinct redox environments can all

function simultaneously. For example, although GSH is primarily found in the cytoplasm, a small pool corresponding to approximately 1% of total cellular GSH is found inside the nuclear compartment where it is involved in the maintenance of protein thiols important for the regulation of gene expression and DNA repair mechanisms (Arrigo 1999; Wang et al. 2018). Furthermore, this pool of cellular GSH seems to be differentially regulated as GSH synthesis inhibitor buthionine sulfoximine reduces only the cytoplasmic fraction, while the nuclear remains intact (Jevtović-Todorović and Guenthner 1992).

Several problems stand in the way of truly understanding biochemical redox homeostasis in a living organism. Inability to apply known redox-related theoretical principles to the living cells (because real conditions significantly differ from those theoretically assumed) and conceptual problems related to the comprehension of the spatio-temporal patterns of redox signaling in an extremely complicated biochemical system (a living being) are just "the tip of the iceberg". A commonly used measure, the redox potential of a specific redox couple (e.g., Nernst equation for a reduced/oxidized glutathione redox couple) can be used to illustrate the latter. Although the equation can be used to describe the thermodynamics of the reaction between the oxidized and the reduced fraction of GSH in mathematical terms, the concentration of the specific reactants and products reflect biochemical redox homeostasis much better than a simple ratio of fractions. This is so because absolute concentrations of both the GSH and GSSG are a final result of a set of biochemical processes in which GSH plays a role (e.g., GSSG reduction by nicotinamide adenine dinucleotide phosphate (NADPH), conjugation of GSH to electrophiles, engagement in protein folding, and synthesis, cellular transport of GSH, GSSG, and conjugates, etc.) (Ursini et al. 2016). There are also methodological challenges standing in the way. Although significant steps forward have been made in the field recently, we still have a long way to go to develop the equipment and methods that would enable us to fully appreciate the regulatory role of redox signaling considering many molecules involved in the complex mechanistic interplay are characterized by inherent instability and short half-lives. A single sentence by Buettner et al. illustrates this problem nicely: "*To understand how short-lived, quasi-stable species, such as superoxide, hydrogen peroxide, and nitric oxide, connect to the metabolome, proteome, lipidome, and genome we need absolute quantitative information on all redox active compounds as well as thermodynamic and kinetic information on their reactions, i.e. knowledge of the complete redoxome.*" (Buettner et al. 2013). Considering there are still substantial technical limitations related to methodological tools in practically all of the relevant fields listed (e.g., limitations related to the repeatability of peptide identification and the consistency of quantification in the quantitative proteomics), let alone multiplexing (e.g., distinct sample preparation requirements for different analytical techniques), functionally relevant high-throughput techniques that would capture information related to the *redoxome* in a meaningful pathophysiological context are yet to be developed.

Nevertheless, regardless of the limitations of our tools, the substantial amount of knowledge related to the redox homeostasis we managed to collect over the years clearly indicates that this system is tightly associated with maintenance of function

at the cellular and organismic level and that it is highly relevant for the development of most of the human diseases. Interestingly, this is best evident, not from the recent results generated by novel methods due to technological advances, but rather due to the conceptual leaps that managed to reconcile many apparently paradoxical findings related to the concept of redox homeostasis.

One such successful conceptual leap was done by Ursini et al., who brought back the critical concepts of homeostasis, heterostasis, and allostasis into the context of redox homeostasis of health and disease (Ursini et al. 2016). For a more comprehensive understanding of redox homeostasis as "the golden mean of healthy living", the reader is referred to the informative overview by Ursini et al. (Ursini et al. 2016). Although it was initially believed that electrophiles (molecules that form bonds with their reaction partners (nucleophiles) by accepting both bonding electrons) are exclusively harmful by-products of metabolism, accumulating evidence indicated that they are in fact functional molecules acting as messengers in cellular signaling. Acceptance of this novel idea of electrophiles acting as functional cellular species opened up some important questions that largely contributed to the current understanding of redox homeostasis. For example, if electrophiles are not inherently harmful, are nucleophiles inherently protective? How does the cell controls the production and removal of its electrophile messengers, considering electrophile escape could be harmful to the cell? What are the main signaling mechanisms involved in the regulation of redox homeostasis? If electrophile and nucleophile tone work to maintain the balance of cellular redox homeostasis, when and why does oxidative stress occur? And finally, if electrophiles are not inherently exclusively harmful, what is the mechanism mediating the protective effects of hormesis—a dose-response phenomenon characterized by a low dose stimulation of the protective adaptive response in contrast to inhibition induced by a high dose of the stimulus?

The answers to some of these questions are still being actively sought and the perplexing functioning of the redox homeostasis is an area of active and fruitful research as clarifying the role of redox homeostasis might offer new diagnostic and therapeutic opportunities. Nevertheless, some of the answers have already been proposed.

In contrary to previous beliefs of necessary damage associated with reactive electrophiles, the concept of redox homeostasis neglects the inherent harmful or detrimental nature of both cellular electrophiles and nucleophiles. Instead, both serve important roles in cellular signaling, and either can drive pathophysiological processes in case of its excessive unbalanced production and/or removal. Consequently, a paradigm shift now suggests that just like a stable offset of redox homeostasis (this would be recognized by Selye as *redox heterostasis*) toward an environment characterized by an excess of electrophiles accompanied by oxidative stress is part of a pathological phenotype, a stable offset toward excessive nucleophile accumulation is part of a pathological cascade just as well. Analogously, just like upregulation of the nucleophile tone exerts protective effects in the scenario of excessive electrophile production by balancing the redox environment toward a "golden mean", upregulation of the electrophilic arm can be seen as protective if it drives the cell toward a physiological set point. Furthermore, a temporal component has to be

acknowledged, as even if the cell is able to compensate for an increased electrophilic tone during the exposure to stimuli that directly or indirectly challenge the redox homeostasis (e.g., xenobiotics like nutrients or drugs), a permanent challenge may trigger adaptive processes that establish a new homeostatic setting to provide acute protection at the expense of a permanent functional modification that may be more or less severe (*redox allostatic load*).

Previous theoretical work focused on oxidative stress-related pathophysiology assumed that once the pro-oxidative pathways are activated (e.g., as part of the innate immunity cascade) antioxidant capacity of the cell presents the main defense system protecting from oxidation of cellular components. Nevertheless, this concept fails to acknowledge the dynamic nature of redox homeostasis. In reality, current evidence speaks in favor of simultaneous upregulation of the nucleophilic tone by a fine adjustment of the redox rheostat in order to prevent a stable offset in the redox homeostasis that would encourage the development of a pathophysiological environment by affecting cellular signaling. Furthermore, novel evidence suggests a more precise control of the redox rheostat in contrast to the concept of an on–off switch-like mechanism regulating the cellular redox balance (Ursini et al. 2016).

Initial recognition of the involvement of the "oxidative stress-related" molecules in cellular signaling motivated a search for relevant biochemical mechanisms that might convey redox-related information to classic molecular messengers. The reaction of electrophiles with specific protein thiolates currently stands out as the most well-examined mechanism (Groeger and Freeman 2010). Electrophilic derivatives can be generated either by non-enzymatic free radical-mediated reactions or by enzyme-catalyzed. The enzyme-catalyzed generation of electrophiles often occurs under physiological conditions, while the non-enzymatic pathway is activated under oxidizing conditions upon radical-triggered oxidation of polyunsaturated fatty acids. Under physiological conditions, many electrophiles are continuously produced at low levels to keep the electrophilic tone required for the maintenance of redox homeostasis. Among these electrophilic messengers, α,β-unsaturated aldehydes (e.g., malondialdehyde (MDA), 4-hydroxynonenal (HNE), acrolein) seem to be the most important. Once generated, regulatory electrophiles conjugate with amino acid residues in a process that depends on the nucleophilic reactivity of the target, and the strength of an electrophile's electron-withdrawing group. The thiolate cysteines stand out as highly reactive nucleophilic targets, although other amino acids (e.g., lysine or histidine) are also involved (Doorn and Petersen 2002). Furthermore, thiolate residues of different proteins are characterized by distinct levels of reactivity that depend on a myriad of factors (e.g., acid dissociation constant and steric limitations). On the other side, different electrophiles are characterized by their own properties that affect the reactivity toward biomolecules such as the strength of an electrophile's electron-withdrawing group. For example, a second-order rate constant of HNE for reaction with GSH is ~1.3 M^{-1} s^{-1}, approximately 1000-fold lower than the constant for peroxynitrite indicating a much lower potential reaction rate of GSH and HNE (Groeger and Freeman 2010). It should be emphasized that the abovementioned reaction is illustrative, however, it probably poorly reflects what happens in vivo where the reactivity is also affected by the presence of enzymes (e.g., glutathione S-transferase),

concentration gradients of substrates, pH, etc. A key property of electrophiles that is indispensable for their role in cellular signaling is the fact that they react with thiols reversibly, satisfying the requirement of signaling mechanism temporal resolution (Groeger and Freeman 2010). Once the reaction occurs, the formed disulfide bonds serve as the reversible redox switches affecting the function of cellular proteins with various levels of sensitivity toward changes in the local redox environment. An interesting example of this phenomenon is a metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase that serves the role of a pleiotropic "redox sensor" as electrophile-induced intramolecular disulfide formation at the active site cysteines reversibly inhibits the enzyme in the pro-oxidative environment to divert glycolytic flux into the protective oxidative pentose phosphate pathway (Mullarky and Cantley 2015).

The production and removal of both electrophiles and nucleophiles are tightly regulated processes providing foundations for the maintenance of the redox homeostasis through regulation of the electrophilic and nucleophilic tone. In a dynamic homeostatic balance, rapid fine-tuning of electrophilic and nucleophilic tones enables tight control of the redox environment indispensable for undisturbed cellular functioning. In the case of the disproportionate activation of either the electrophilic or nucleophilic arm, a heterostatic redox offset occurs at the expense of redox environment-sensitive cellular signaling that drives the cell toward the pathological phenotype. In case this imbalance is characterized by an excess in electrophiles unopposed by the nucleophilic tone, a pathophysiological state known as oxidative stress occurs. This phenotypic shift can be induced by a mild continuous harmful pro-oxidative challenge acting as a redox allostatic load or by a substantial oxidative stimulus that overrides the ability of the nucleophilic tone to counteract its action.

Finally, redox homeostasis provides a new context for hormesis, a phenomenon classically defined as a biphasic response to a toxin or stressor that triggers a favorable biological response in the low dose, while the high dose is generally detrimental. In redox biology, oxidative stress has been often discussed in the context of hormesis as a potential hormetic stimulus that might benefit the living organism by stimulating an increase in cellular antioxidant defense and repair mechanisms when presented in a low dose. In contrast, in the context of redox homeostasis, the hormesis seems to provide protection by "resetting" redox heterostatic offset (Ursini et al. 2016). Many health-modifying interventions have been suggested to exploit "oxidative stress-related" hormesis to induce protective effects. For example, physical exercise now recognized as an important intervention for maintenance of health and prevention of chronic non-communicable diseases has been proposed to act as a pro-oxidative hormetin (Radak et al. 2008). Furthermore, some nutritional phytochemicals originally believed to act as chemical antioxidants are now suggested to exert beneficial biological effects due to pro-oxidative triggering via hormesis. One example is 3,5,4′-trihydroxy-trans-stilbene also known as resveratrol, a polyphenol from red grapes and other plants famous for its health-beneficial effects (Plauth et al. 2016). In the context of redox homeostasis primarily defined as the maintenance of the nucleophilic tone, nutritional phytochemicals might address the offset of redox

homeostasis by continuous feedback preservation of the nucleophilic tone via parahormesis (Forman et al. 2014). For a more thorough overview of how nutritional phytochemicals may reset redox heterostasis, please see Ursini et al. and Forman et al. (Forman et al. 2014; Ursini et al. 2016).

15.4 Redox Dyshomeostasis in AD

It is well known that the brain is particularly sensitive to pro-oxidative insults due to the fact that it consumes ~20% of the total oxygen and has a relatively "weak" endogenous antioxidant defense system (e.g., low levels of GSH and catalase). However, other biochemical factors further diminish its ability to combat oxidative challenges. Some examples are the presence of excitotoxic amino acids and auto oxidizable neurotransmitters, high generative capacity for hydrogen peroxide and large content of polyunsaturated fatty acids, neuronal poly (ADP-ribose) polymerase-1 (PARP-1) activity, etc. Many of these factors were discussed as part of the "*13 problems*" that place the brain at risk to succumb to oxidative stress by Barry Halliwell (Halliwell 2006) and were later covered by others (Cobley et al. 2018). Consequently, redox homeostasis of the brain is particularly sensitive to pro-oxidative insults considering its incline toward a relatively oxidized state, and limited ability to upregulate nucleophilic tone in response to the excess of electrophiles. For this reason, redox homeostasis of the brain is also exceptionally important as a relatively mild noxious stimulus can trigger the generation of electrophiles that cannot be counterbalanced by an adequate increase of the nucleophilic tone to prevent the offset of redox homeostasis and redefining of a new pathophysiological heterostatic setpoint. A relatively brittle redox homeostasis could also potentially explain why many different mechanisms can trigger self-sustaining progressive processes driving neurodegeneration at the molecular level. In the context of AD, an acute noxious stimulus such as head trauma or an infection could induce a stable offset of the redox homeostasis laying the foundations for other pathophysiological processes to drive neurodegeneration by induction of oxidative stress. Alternatively, low-grade systemic inflammation that accompanies chronic non-communicable diseases recognized as important risk factors for AD (e.g., diabetes, dyslipidemia, hypertension) could place a considerable allostatic load on the brain redox homeostasis and stimulate a shift toward an excess of electrophiles and relatively oxidized cellular environment. In both cases, the inability to address either acute or chronic electrophilic challenge by adequate upregulation of the nucleophilic tone leads to the establishment of a new pathological redox steady-state that provides some protection, but at the expense of permanent modulation of physiological functions. At the level of neurons, this results in synaptic loss, and at the organismic level, long-term redox heterostasis is reflected by signs and symptoms of neurodegeneration (among other diseased states). Although relatively

limited reserve of the brain redox homeostatic machinery is "bad news" in terms of sensitivity toward the initiation of pathological alterations of the redox environment, it also offers hope as addressing the offset of the redox rheostat of the brain could pose a valuable therapeutic strategy.

15.5 Main Hypotheses of AD from the Perspective of Redox Homeostasis

15.5.1 The Amyloid Cascade Hypothesis of AD and Redox Homeostasis

The amyloid cascade hypothesis has been the most prominent hypothesis of AD guiding the development of models and potential AD therapeutics for the past 30 years. In 1992, Hardy and Higgins proposed that "…the deposition of amyloid β protein (AβP), the main component of the plaques, is the causative agent of Alzheimer's pathology and that the neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition" (Hardy and Higgins 1992). Since its proposal, accumulated evidence from both the preclinical and clinical studies shed a light on the physiological and pathophysiological functions of the molecular cascade involved in the processing of Aβ. Substantial evidence suggests accumulation of Aβ, either due to its increased production or diminished clearance, can trigger microgliosis, astrocytosis, generation of hyperphosphorylated tau, and neurofibrillary tangles, synaptic loss, and memory impairment (Selkoe and Hardy 2016). Nevertheless, the amyloidocentric approach failed to provide a safe and effective drug candidate so far suggesting Aβ might be a part of a more complex puzzle of AD pathophysiology, rather than the sole causative agent and the pathobiological driver of the disease (Karran et al. 2011; Loera-Valencia et al. 2019).

Many reports indicate a bidirectional association of Aβ burden and a pro-oxidative shift of the redox homeostasis (Cheignon et al. 2017; Zhao and Zhao 2013). Aβ seems to be able to induce a pro-oxidative shift of the redox homeostasis when introduced in many different complex biological systems used to model AD. For example, AD-related transgenes that are involved in Aβ production and processing induce oxidative stress in cell models of AD and transgenic animal models of the disease (Zhao and Zhao 2013). Transgenic models sometimes offer limited insight into pathophysiological processes if they fail to model the temporal dimension of the pathological process. Nevertheless, the introduction of exogenous Aβ in either cells or animals is also accompanied by a shift of the redox homeostasis toward a more oxidative state often accompanied by oxidative stress. Furthermore, many nutritional phytochemicals that successfully limit oxidative stress-induced pathology also seem to provide protection in case of altered regulation of Aβ in cell cultures and in animal models.

There are several hypotheses regarding why Aβ promotes a pro-oxidative redox dyshomeostasis. Metal ions such as Zn, Cu, and Fe are abundant in the brain where they are involved in the regulation of many biological functions. For example, high levels of Zn are found in glutamatergic nerve terminals and they are released into the synapse upon neuronal activation achieving millimolar concentrations at which they interact with many postsynaptic proteins such as the N-Methy-D-aspartate receptor (Roberts et al. 2012). Metal ion dyshomeostasis accompanies AD and the concentration of some ions, particularly Zn and Cu can reach levels several times higher than found in the healthy controls (Cheignon et al. 2017). Apart from the pathological concentrations, metal ion dyshomeostasis in AD involves their redistribution to amyloid plaques leaving the cells deficient and disrupting the stability of metalloproteins and metalloenzymes (Roberts et al. 2012). When metal ions are coordinated with Aβ they form catalytically active Aβ-metal complexes that directly contribute to the redox environment by producing H_2O_2 and hydroxyl radicals (Cheignon et al. 2017). Furthermore, Aβ can alter redox homeostasis toward a pro-oxidative state by additional mechanisms. It has been shown that Aβ can increase mitochondrial production of reactive oxygen species (ROS). Aβ induces mitochondrial dysfunction by altering the permeability of the mitochondrial membrane and the activity of mitochondrial enzymes, inducing damage to the respiratory chain that leads to increased production of ROS. Additionally, Aβ can activate NADPH oxidase (NOX), an important regulator of redox balance first discovered in neutrophils where it controls the respiratory burst (Abramov et al. 2004). Subsequently, NOX enzymes were also identified in neurons, glia, and cerebral endothelial cells where they seem to play a role in the maintenance of redox homeostasis of the brain (Tarafdar and Pula 2018). Finally, Aβ oligomers can insert into the membrane bilayer where they promote lipid peroxidation contributing to the enhancement of the electrophilic tone (Butterfield et al. 2013).

15.5.2 The Tau Hypothesis of AD and Redox Homeostasis

The *tau hypothesis* suggests that hyperphosphorylation of a group of proteins derived by alternative splicing of the microtubule-associated protein tau (MAPT) gene that are collectively called *tau* precedes neurodegeneration and triggers pathophysiological mechanisms responsible for the development of AD. The physiological role of tau is believed to be the maintenance of the structure and function of microtubules that are primarily found in neuronal axons. Alterations of tau phosphorylation, often seen as tau hyperphosphorylation, affect its solubility and induce the accumulation of insoluble precipitates. Insoluble aggregates of tau have been reported in different neurodegenerative diseases such as AD and Parkinson's disease where they pile up in histopathological structures recognized as neurofibrillary tangles.

Just as for Aβ, a bidirectional relationship with pro-oxidative redox dyshomeostasis has been observed utilizing different transgenic and non-transgenic cellular and animal models of the disease. Overexpression of mutant forms of human tau

has been associated with increased markers of oxidative stress and enhanced susceptibility to oxidative challenge in both cells and animals (Alavi Naini and Soussi-Yanicostas 2015). Research on animals with human tau transgenes indicates alteration of tau homeostasis is able to affect mitochondrial metabolism and nucleophilic tone by downregulating the expression of proteins involved in mitochondrial respiration as well as those regulating the GSH-mediated protection against excursions of electrophilic messengers (e.g., GSH peroxidases and GSH S-transferase) (Alavi Naini and Soussi-Yanicostas 2015). Interestingly, in some models of tauopathies such as in mice expressing human tau^{P301S}, pathophysiological events targetting redox homeostasis (e.g., decreased expression of the essential mitochondrial antioxidant enzyme manganese superoxide dismutase (MnSOD)) precede the development of neurofibrillary tangles and hyperphosphorylation of tau. Importantly, this early pro-oxidative shift in redox homeostasis is accompanied by behavioral alterations and mitochondrial dysfunctions indicating in some cases tau dyshomeostasis occurs late in the disease progression timeline. Involvement of early disturbance of redox homeostasis in transgenic models of tauopathies led to the suggestions that "…*OS* [oxidative stress] *is a primary and causal player in the neurotoxicity induced by tau mutations, through induction of both apoptosis and dysregulated cell cycle activation*" (Alavi Naini and Soussi-Yanicostas 2015). Dysfunctional tau also promotes metabolic dyshomeostasis and ROS production by affecting mitochondrial trafficking as tau serves the role of a cargo carrier along the microtubule "rail track" (Cheng and Bai 2018). Furthermore, similarly as has been shown for Aβ, fragments of tau possess a copper reduction capacity that enables copper-mediated generation of H_2O_2 that displaces redox homeostasis toward a pro-oxidative state in the presence of metal ion dyshomeostasis (Cheignon et al. 2017; Su et al. 2007).

15.5.3 The Insulin-Resistant Brain State (IRBS)/Metabolic Dysfunction Hypothesis of AD and Redox Homeostasis

Although it was initially believed the brain is not affected by insulin, the discovery of insulin receptors (IR) in the brain (Havrankova et al. 1978; Schulingkamp et al. 2000), as well as their involvement in the modulation of neurometabolic and cognitive processes (Lee et al. 2016) interested neuroscientist in deciphering its cerebral actions in health and disease. Hoyer and colleagues were the first to postulate the action of insulin in the brain might be involved in early metabolic alterations that precede neurodegenerative changes (Hoyer 1997; Hoyer et al. 1994). Early clinical studies soon confirmed the association of insulin resistance and cognitive decline in AD patients and excess body weight, obesity, metabolic syndrome, and diabetes are all recognized as important risk factors for the development of AD today (Cai et al. 2012). IRBS is now recognized as an important etiopathogenetic factor and pharmacological target in AD (Kellar and Craft 2020), and even the term "type 3

diabetes" has been proposed to describe the state of insulin resistance that promotes neurodegeneration in AD (de la Monte and Wands 2008).

Both the physiological and pathophysiological actions of insulin in the brain are still being explored. In the brain, insulin signaling stimulates energy metabolism, neuronal growth, survival, differentiation, protein synthesis, cytoskeletal maintenance and synapse formation, neurotransmitter signaling, and inhibits apoptosis (de la Monte 2012). By modulating many fundamental biochemical pathways, insulin signaling is involved in the regulation of many other processes recognized as important players in the neuropathology of AD such as Aβ turnover and the stability of tau. For an overview of how insulin resistance integrates many other pathophysiological cascades and provides a common link between current AD hypotheses, the reader is directed to a comprehensive review by Alves et al. (2021).

As insulin regulates brain glucose utilization, metabolism, and ATP synthesis, IRBS renders the brain susceptible to oxidative insults by lowering its ability to counteract pro-oxidative shifts of the redox homeostasis. Furthermore, hypoenergosis of the brain promotes the aberrant activity of the mitochondrial transport chain that leads to the formation of excess electrophiles and promotes the activation of pro-inflammatory signaling (de la Monte 2012). Although oxidative stress is considered as one of the key mediators of IRBS-induced neurodegeneration (Dröge and Kinscherf 2008), some studies also reported increased efficiency of the important antioxidant enzymes like catalase, superoxide dismutase, and GSH peroxidase. Although apparently paradoxical, these findings in fact seem to reflect attempts of reactive compensation of the electrophilic excess and suggest an adaptive reaction (Maciejczyk et al. 2019). The compensatory failure is evident from studies that also measured GSH, the most important mediator of the nucleophilic arm of redox homeostasis, as the total concentration of GSH and its ratio to the oxidized form seem to be consistently lower in different animal models of IRBS (Maciejczyk et al. 2019). One potential mechanistic explanation of the observed effects could be depletion of substrates for GSH synthesis as insulin has been recognized as an important regulator of cellular uptake of cysteine—the limiting amino acid for GSH synthesis in humans (Wu et al. 2004). Interestingly, the adaptive response that results in increased efficiency of antioxidant enzymes, particularly GSH peroxidases, may also be one of the causal factors promoting insulin resistance as peroxidases have been recognized as physiologically relevant modulators of IR function via H_2O_2 (Pomytkin et al. 2018). One way by which IRBS could alter redox homeostasis is deficient basal activation on the nuclear factor erythroid 2-related factor 2 (Nrf2), the most important regulator of the nucleophilic tone (Song et al. 2018).

15.5.4 Other Hypotheses of AD and Redox Homeostasis

Many other AD hypotheses have been proposed, and although considered "less prominent" than the amyloid cascade hypothesis, tau hypothesis, and IRBS/metabolic hypothesis by some, substantial evidence exists that each may be

correct and significantly contribute to the understanding of AD and the process of neurodegeneration in general. "Multi-directional" pathobiological processes are an integral part of practically all hypotheses of AD. For example, Aβ accumulation is able to induce IRBS by competing for insulin binding to the insulin receptors (Xie et al. 2002). Conversely, IRBS affects Aβ homeostasis through modulation of serine/threonine protein kinases of the glycogen synthase kinase 3 family (Alves et al. 2021). Furthermore, the mitochondrial hypothesis, closely related to the metabolic hypothesis of AD, but recognized as a separate entity, provides an invaluable mechanistic insight into how different pathophysiological processes (e.g., accumulation of Aβ, tau, or IRBS) work to induce neurodegeneration. Consequently, it is biologically unfounded to consider the hypotheses as individual processes without acknowledging their interdependence. On the other hand, AD could represent an "umbrella term" for many different subtypes of neurodegenerative disorders we currently don't recognize as individual entities, and different pathophysiological cascades could possibly drive the development of the disease in different patients. Other hypotheses of AD are also pathophysiologically related to redox homeostasis. In neuroinflammatory and innate immunity hypotheses of AD upregulation of pro-inflammatory factors is considered as the main initiator event driving prolonged and disproportionate inflammatory processes that promote neurodegeneration via oxidative stress and metabolic dysfunction (Hsieh and Yang 2013). The calcium hypothesis of AD recognizes compromised Ca^{2+} handling as a central pathobiological event that results from upstream metabolic and oxidative damage and perpetuates cellular dysfunction by inducing virtually all major molecular alterations responsible for the development of AD such as Aβ accumulation, hyperphosphorylation of tau, and redox disbalance (Alzheimer's Association Calcium Hypothesis Workgroup 2017). The cholinergic deficit hypothesis proposes that loss of cholinergic signaling is the main molecular event in AD. Interestingly, cholinergic transmission has been considered as an "antioxidative" counterpart to pro-oxidative glutamatergic transmission acting through activation of α7 nicotinic receptor (Guan 2008). Circadian dysrhythmia, often present in the early stage of AD, shifts the redox homeostasis toward an excess of electrophiles by inducing central dyshomeostasis of Aβ and tau, triggering inflammatory processes and metabolic dysfunction (Homolak et al. 2018). Vascular dysfunction, considered an important pathophysiological event in AD, causes a pro-oxidative redox shift as a consequence of brain hypoperfusion, and some suggest endothelial redox homeostasis as a driving factor (Zhu et al. 2007). A more thorough dissection of a bi-direction association of other hypotheses of AD and redox homeostasis is outside of the scope of this chapter, however, an inability to maintain nucleophilic tone upon an electrophile challenge seems to be a common denominator. An integrative approach is thus warranted, and separate acknowledgment of different hypotheses often encountered in the literature (and in this Chapter) primarily serves the purpose of a more structured approach to information rather than to communicate their biological independence.

15.6 Redox Dyshomeostasis: A Conjunctive Etiopathogenetic Amplifier of AD

All relevant pathomechanisms recognized as potential initiators of AD-related pathophysiological processes pose a challenge to the redox homeostasis of the body by either undermining systems involved in the maintenance of the molecular machinery that feeds the nucleophilic tone or stimulating the production and propagation of electrophilic messengers. Conversely, a close relationship of redox homeostatic system with messengers acting as primary drivers of AD (e.g., Aβ, tau, insulin) enables (i) pathological signals to propagate through the biochemical system and trigger other mediators of the disease in a domino effect-like manner (e.g., Aβ-induced IRBS, or neuroinflammation-triggered accumulation of Aβ). Consequently, and (ii) disbalance of the redox homeostasis to trigger pathobiological cascades when acutely challenged and maintain pathophysiological signaling upregulated in case of a stable offset of the redox rheostat (redox heterostasis). From this perspective, redox homeostasis represents an attractive pharmacological target, as (i) its maintenance could increase resilience against acute challenges evolving into a progressive state of neurodegeneration, and (ii) in case of the ongoing pathophysiology its reset could downregulate pathological signal propagation through many signaling pathways simultaneously and restore the capacity of the cell to finally resolve a pathophysiological challenge, rather than delay its resolution at the expense of setting off the homeostatic rheostat.

15.7 Practical Implications and Concluding Remarks

Understanding the role of redox homeostasis in neurodegeneration opens up new opportunities for diagnosing and treating AD patients (Fig. 15.3). Due to its close relationship with many physiological systems, potential new diagnostic strategies that would enable non-invasive and precise assessment of redox homeostasis would probably lack specificity toward pathophysiological alterations related to AD, let alone pathophysiological alterations of specific pathological cascades (e.g., accumulation of Aβ). Nevertheless, in the context of the current understanding of AD, this is not necessarily unfortunate, and a "broad biomarker" would still provide valuable information to a clinician enabling the identification of high-risk patients in the very early stage of the disease. A potential redox biomarker would thus enable a timely intervention with the intention to restore the redox rheostat toward a physiological setpoint before the progression of molecular pathological events. An ideal biomarker would also be able to, again at the expense of its specificity, provide information on redox homeostasis as a whole, rather than the specific alterations of its subsystems. The best approach is still under debate both at the preclinical and clinical level. Measurements of the nucleophilic tone by assessment of GSH-related protective systems would offer important information. Some other techniques are also being examined. For example, we have recently proposed nitrocellulose redox permanganometry (NRP) as a simple

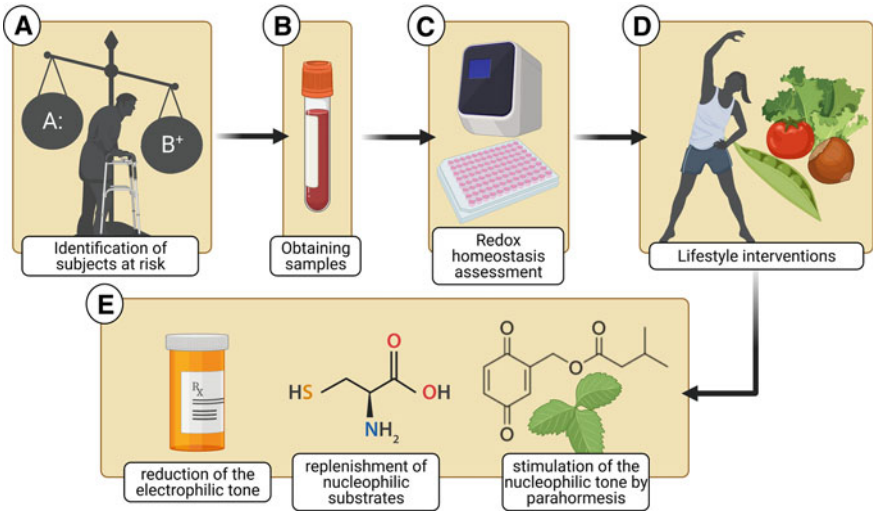


Fig. 15.3 Potential practical implications of the redox homeostasis in the context of prevention and treatment of Alzheimer's disease (AD). **a** Identification of patients at risk of developing neurodegenerative diseases with low reserves of the redox homeostatic system due to allostatic load or inherent biochemical deficiencies. **b** Obtaining appropriate biochemical samples. **c** Analysis of redox homeostasis and the "redoxome". **d** Design and implementation of appropriate lifestyle interventions to reduce the redox allostatic load. **e** Interventions to restore redox homeostasis: reduction of the electrophilic tone by inhibiting neuroinflammation, replenishment of the nucleophilic substrates, and stimulation of the nucleophilic tone by parahormesis

method that would enable estimation of the spatial patterns of reductive capacity in tissue sections following passive diffusion slice print blotting based on quantification of nitrocellulose-trapped MnO_2 precipitate (Homolak et al. 2020). As NRP requires minimal resources and virtually no equipment, and many samples can be processed at once, it could enable novel insights into redox homeostasis at the preclinical level as has been shown in human samples (Homolak 2021) and animal samples from different tissues (Homolak et al. 2021a; c). Measurement of the oxidation–reduction potential (ORP) is also interesting, as assessment of tissue could provide valuable information regarding the "redox balance" in the form of a single value in mV. For example, Cao et al. utilized this principle to predict the antioxidant capacity of urine in diabetic and healthy patients and observed a stable pro-oxidative offset in diabetic subjects (Cao et al. 2016).

Appreciation of the redox homeostasis at the level of the whole organism, rather than at the level of its isolated subunits, is yet another redox homeostasis aspect that should be acknowledged in the context of redox-directed diagnostic and therapeutic strategies. Redox homeostasis relies on the interdependence of its subsystems (e.g., via GSH transport) that are, although compartmentalized (in cellular organelles at the level of the cell, and in organs at the level of an organism) mutually interdependent for their proper functioning. While this may also seem unfortunate as it enables

propagation of oxidative challenge through systems (e.g., propagation of systemic inflammation-induced peripheral pro-oxidative shift onto redox homeostasis of the brain), the mutual interdependence of redox networks also enables potential diagnostic biomarker of one biosystem to inform of the other. For example, a pro-oxidative shift in the urine of diabetic patients reported by Cao et al. (2016) probably also reflects a pro-oxidative redox disbalance of peripheral organs, and maybe even the brain (Muriach et al. 2014). Analogously, an attempt to restore peripheral redox homeostasis (e.g., by fueling the nucleophilic tone of the peripheral systems), could also alleviate some of the electrophilic allostatic load from the central nervous system, and slow down the progression of neurodegenerative processes. In this context, interorgan transport of GSH may be exploited for stabilization of central redox homeostasis by fueling peripheral nucleophilic reserves. (Wu et al. 2004). For example, the gut is recognized as an important potential etiopathogenetic contributor to AD as dysfunctional intestinal barrier enables luminal inflammatory mediators and Aβ to breach into the systemic circulation and trigger peripheral inflammatory processes that drive neuroinflammation (Liu et al. 2020). In a rat model of sporadic AD, morphometric changes indicative of dysfunctional gastrointestinal barrier are accompanied by a pro-oxidative shift of the intestinal redox homeostasis with decreased availability of protein thiols and GSH (Homolak et al. 2021b; c). As the intestine is a major consumer of liver-derived GSH (Wu et al. 2004), failure to maintain gut redox homeostasis can stimulate a pro-oxidative shift of the cerebral redox environment not only by permeability-induced neuroinflammation, but also by limiting the availability of GSH and its precursors for the brain. Conversely, correction of the intestinal redox system would alleviate both allostatic central nervous system redox stressors (neuroinflammation and GSH deficiency). Importantly, as different physiological routes are utilized by different organs to secure adequate delivery of metabolic precursors for GSH synthesis (e.g., small intestine utilizes dietary sources, while the kidney consumes GSH precursors from the arterial blood), different routes should be exploited for replenishment of nucleophilic substrates (Wu et al. 2004). In the particular case of gastrointestinal depletion, an oral route would probably be optimal. The concept of replenishment of cellular nucleophilic stores is supported by findings of successful restoration of GSH synthesis and prevention of GSH depletion by oral or intravenous cysteine and its precursors (e.g., N-acetyl-cysteine, L-2-oxothiazolidine-4-carboxylate) in both humans and animals under physiological and pathological conditions like acute respiratory distress syndrome, or acquired immunodeficiency syndrome (Wu et al. 2004).

Considering pathophysiological cascades involved in the development of AD primarily induce a redox shift toward an excess of electrophiles, other therapeutic strategies that rebalance the redox homeostasis and potentiate nucleophilic tone should also be considered. In this regard, apart from replenishment of important precursors that serve as mediators of the nucleophilic tone (e.g., GSH), inhibition of known potentiators of electrophilic tone (e.g., enzymes involved in activation of inflammatory cascades such as NOX) and stimulation of the nucleophilic system are attractive options. Inhibitors of electrophilic propagators have already been discussed as a potential therapy for neurodegeneration, and have shown some promising results.

For example, direct inhibitors of NOX such as diphenyleneiodonium, or commonly used drugs that inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (statins) that inhibit NOX indirectly have been discussed as a potential therapy for neurodegeneration (Barua et al. 2019). Stimulation of nucleophilic tone is another approach that could disburden the redox allostatic load assuming sufficient substrates are available. For example, many nutritional phytochemicals that have standardly been considered to exert beneficial health effects by direct scavenging of electrophiles, are now considered to act by parahormetic preservation of the nucleophilic tone (Forman et al. 2014). These chemicals have been shown to activate the main regulator of the nucleophilic tone Nrf2 by modulation of cysteines of Kelch-like ECH-associated protein 1 (Keap1) upon oxidation to electrophilic hydroquinones and quinones by endogenous radicals (Forman et al. 2014). Stimulation of the nucleophilic tone by parahormesis is an interesting approach as unlike hormetins (e.g., exercise) that require cellular stress as a mediator, parahormetins provide the same beneficial effects. However, by mimicking electrophiles instead of generating tolerable amounts of those produced endogenously in response to a physiological challenge. This way, the protective nucleophilic tone can be maintained by non-toxic compounds, instead of stimulating the production of endogenous toxic electrophiles in low tolerable doses (Forman et al. 2014).

15.8 Conclusion

To conclude, AD is the most common neurodegenerative disorder with yet unresolved etiopathogenesis that poses a significant socio-economical burden on society. Many hypotheses have been proposed over the years, however, none provided strong foundations for the development of safe and effective therapies for AD. At the molecular level, AD seems to be driven by pathophysiological processes characterized by significant mutual interdependence and the potential to trigger self-sustaining pathology that presents as chronic progressive neurodegeneration. Redox homeostasis is an important physiological system involved in the regulation of ubiquitous cellular functions. It is defined by a continuous interaction of molecules involved in the regulation of the electrophilic and nucleophilic tone at the level of individual cells and the level of an organism. Messengers involved in the maintenance of the electrophilic and nucleophilic tone also play a role in cellular function by interacting with other functional systems. Consequently, many molecules involved in the maintenance of redox homeostasis also modulate metabolism, cellular differentiation, adaptation, and survival. When presented with a redox challenge the cell can resolve the challenge by fine-tuning the electrophilic and nucleophilic tone to enable resolution without affecting the functioning of other systems. Nevertheless, if the stimulus is either too challenging to be acutely resolved, or if it is chronically present (redox allostatic load), the cell adapts by defining a new redox homeostatic setpoint (redox heterostasis) at the expense of proper function of functional systems that rely on redox rheostat revolving around a physiological set point. In

AD, redox homeostasis is shifted toward a heterostatic setpoint characterized by an excess of electrophiles unopposed by increased nucleophilic tone. Many hypotheses of AD have been proposed, however, all proposed pathomechanisms (e.g., Aβ accumulation, tau hyperphosphorylation, IRBS, etc.) have been associated with a pro-oxidative shift in redox homeostasis, and individual pathophysiological events seem to be able to induce one another by affecting redox homeostasis. Conversely, a pro-oxidative drift of the redox homeostasis that results in oxidative stress can trigger all relevant pathobiological initiators and mediators of AD-related neurodegeneration (e.g., Aβ accumulation, tau hyperphosphorylation, IRBS, etc.). Consequently, redox homeostasis can be considered a conjunctive etiopathogenetic amplifier of AD and this important biological role makes it interesting from the perspective of the development of novel diagnostic and therapeutic strategies. Diagnostic opportunities related to redox homeostasis in the context of AD are mostly related to screening that could identify individuals with insufficiently reactive redox homeostatic systems and/or chronically challenged redox homeostasis (allostatic load). In such individuals, correction of redox homeostasis could prevent the propagation of pathophysiological processes in the periphery and in the brain. If redox dyshomeostasis is already present, addressing the offset of the redox setpoint (heterostasis) could slow down or alleviate processes driving neurodegeneration. The main therapeutic strategies targeting redox dyshomeostasis involve reducing the heightened electrophilic tone by addressing neuroinflammation, circadian dysrhythmia, metabolic function nutritional deficiencies, and redox dyshomeostasis of the peripheral systems such as the gut. Additionally, restoration of substrates required for the maintenance of the nucleophilic tone (e.g., GSH) and parahormetic activation of the Keap1-Nrf2 axis could enable potentiation of the nucleophilic tone and contribute to the reset of redox rheostat to the homeostatic setpoint.

Compliance with Ethical Standards

Conflict of Interest There is no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by the author.

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Chapter 16
Aging and Redox Pathways in Diabetes

Carlos S. Botero Suarez, Hilda Merino-Chavez, Kanya Rajagopalan, Chris Triggle, and Mustafa Kinaan

Abstract Diabetes is an increasingly prevalent metabolic disorder associated with hyperglycemia due to insulin resistance or deficiency. Several factors contribute to the development of diabetes including genetic and environmental factors. Diabetes is a significant risk factor for vascular disease, accelerated atherosclerosis, and aging of small and large vessels in the heart, brain, kidneys, nerves, and other tissues. These complications are the result of several detrimental cellular cascades induced by elevated glucose concentrations. Hyperglycemia induces the production of free radical products of oxidative stress, known as Reactive Oxygen Species (ROS). These compounds damage cellular membranes, DNA, proteins, lipids, and other cellular content. The understanding of ROS and its relationship to the pathogenesis of diabetes and its complications can be the key to the development of better treatment options. This book chapter will summarize these hyperglycemia-induced mechanisms and how diabetes medications can alleviate their harmful effects.

Keywords Diabetes · Hyperglycemia · Glucose metabolism · Insulin resistance · Prediabetes

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16.1 Introduction

Diabetes is a disease characterized by a relative or absolute lack of insulin action, insulin resistance, hyperglycemia, and the development of detrimental vascular changes in several organs including the retina, renal glomerulus, and nerves (Beckman et al. 2002). It is also associated with accelerated atherosclerotic disease affecting the heart, brain, and lower extremities (Benjamin et al. 2018). Diabetes is a very common metabolic disorder with increasing prevalence both in the United States, as well as worldwide. Approximately 5.2 million deaths globally are attributed to diabetes, and it carries a mortality rate of 82.4 per 100,000 (Glovaci et al. 2019). Diabetes not only causes significant morbidity and mortality for affected patients, but it also poses a growing healthcare burden with nearly an estimated $245 billion spent annually for diabetes care in the USA (Peter and Lipska 2016).

Reactive Oxidative Species (ROS) are free radical products of oxidative stress, a process where antioxidants fail to buffer and eliminate harmful byproducts of metabolism. ROS can damage cellular membranes, DNA, proteins, lipids, and other cellular content (Kayama et al. 2015; Speakman and Selman 2011; Wu and Cederbaum 2003). Consequently, oxidative stress can lead to the development of overt diabetes mellitus through compromised insulin production and secretion and impaired glucose utilization by the cells by different pathways (Evans et al. 2003; Ferrannini et al. 2016; Sena et al. 2011; Rehman and Akash 2017). Additionally, oxidative stress has been linked to accelerated vascular aging and diabetic complications (Domingueti et al. 2016). The understanding of ROS,their relationship to the pathogenesis of diabetes and its complications can be the key to the development of better treatment options.

16.2 Hyperglycemia and Oxidative Stress

ROS are a group of short-lived oxygenated molecules generated by normal metabolic pathways and include superoxide anion ($\cdot O2-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH-$), and peroxynitrite ($ONOO-$) (Kayama et al. 2015). When ROS are produced in appropriate amounts, they can act as signal transduction molecules providing cell protection. However, elevated levels ofthese unstable molecules can inflict damage to cells through degeneration of DNA, lipids, and proteins, thus inducing cell dysfunction or death (Kayama et al. 2015). DM and hyperglycemia exacerbate oxidative stress state through two main effects: (1) inhibition of antioxidant mechanisms and (2) upregulation of pathways producing ROS (Giacco and Brownlee 2010).

Antioxidants can be enzymatic, such as superoxide dismutase (SOD) and glutathione peroxidase and glutathione reductase, or nonenzymatic, such as Vitamin A, C, and E (Kayama et al. 2015). Each antioxidant works synergistically with other antioxidants and against different types of free radicals. Superoxide dismutase

is found in the cytoplasm and nucleus, while manganese superoxide dismutase is confined to mitochondria, and will convert the superoxide anion radicals to hydrogen peroxide (Maritim et al. 2003). Additionally, superoxide dismutase indirectly inhibits activation of protein kinase C, sorbitol overproduction, and formation of advanced glycation products (AGEs), three pro-oxidative mechanisms (Nishikawa et al. 2000) that will be discussed later in this chapter.

Catalase, an enzyme that breaks down hydrogen peroxide to water and oxygen, acts as an antioxidant on its own. Its level is also reduced in diabetes and is found to be normalized by treatment with captopril, aminoguanidine, melatonin, DHEA, probucol (Maritim et al. 2003). Glutathione is one of the most abundant thiol compounds present in mammalian tissues, which is important for DNA formation, regulation of enzymes, and also protection of cells against reactive oxygen species (Gul et al. 2000). Therefore, glutathione metabolism has a central role in the antioxidant defense system with decreased levels been reported in diabetes (Gul et al. 2000). Intracellular glutathione is oxidized by glutathione peroxidase, an antioxidant enzyme, to glutathione disulfide when neutralizing harmful peroxides. Glutathione peroxidase utilizes selenium in this process. Hence, selenium is generally considered protective against oxidative stress (Gul et al. 2000). The regeneration of glutathione from glutathione disulfide is possible through the activity of another antioxidant enzyme, glutathione reductase. This enzyme utilizes NADPH to catalyze this reaction. Glutathione peroxidase and reductase have been shown to be altered in diabetes (Maritim et al. 2003). Levels of glutathione are reported to be normalized by antioxidants such as vanadyl, DHEA, nicotinamide, melatonin (Maritim et al. 2003).

As for the overproduction of ROS, hyperglycemia mainly causes mitochondrial overproduction of superoxide in the electron transport chain through several metabolic pathways (Nishikawa et al. 2000). These major pathways includeglycolysis (glucose oxidation), Advanced Glycation End-products (AGEs), Protein Kinase C (PKC) activation, Hexosamine, polyol, impaired insulin signaling, and lipid peroxidation pathways. These pathways will be discussed below (Fig. 16.1).

16.2.1 Glycolysis Pathway

Glycolysis, also known as glucose oxidation, is a multistep enzyme-mediated pathway involving both the Krebs cycle as well as the electron transport chain and its function is ATP generation using glucose as its substrate. During this process, glucose is initially phosphorylated by glucokinase or hexokinase to glucose-6-phosphate (G-6-P), then to fructose-6-phosphate (F-6-P),mediated by phosphoglucoisomerase. G-6-P can then be routed into the pentose phosphate pathway to create NADPH, or alternatively can continue along the glycolytic pathway to yield Glyceraldehyde-3-Phosphate (GAP), which is later phosphorylated by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequential reactions of this pathway generate the end product pyruvate, which after decarboxylation can enter the Krebs cycle. Several intermediates are produced in the Krebs cycle including reduced nicotinamide

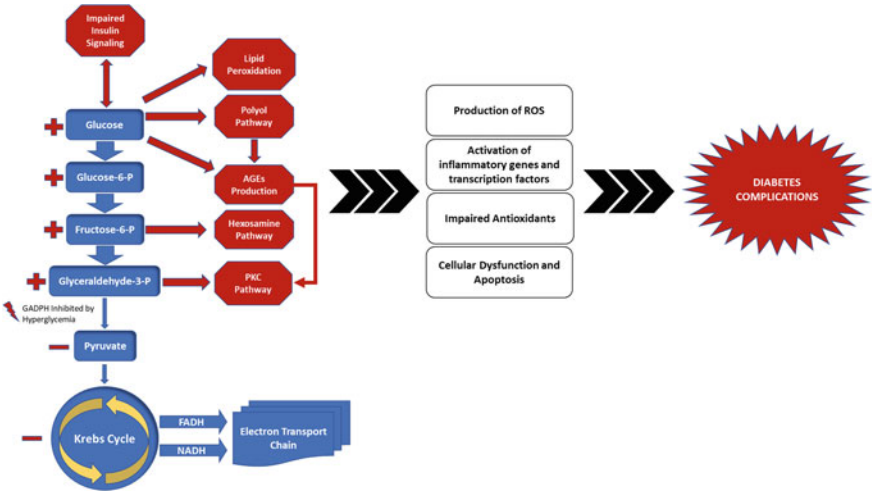

Fig. 16.1 Hyperglycemia results in oxidative stress through several mechanisms. One of the important mechanisms is the disruption of the GADPH enzyme (bolt sign) in the glycolysis pathway. This leads to downregulation of pyruvate production and Krebs cycle (negative sign). Consequently, this leads to accumulation of glycolysis intermediate products (plus sign) which are diverted into oxidative-stress-promoting pathways including polyol, AGEs production, lipid peroxidation, hexosamine, PKC, and impaired insulin signaling pathways. The complex interplay between these altered mechanisms leads to harmful cellular effects causing disease progression, cellular aging, and development of diabetes complications

adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH2). The oxidation of NADH and FADH2 causes a proton concentration gradient required for the electron transport chain (Nishikawa et al. 2000).

Many physiologicalcellular processes, including glucose oxidation, results in the synthesis of a manageable amount of mitochondrial superoxide anion radical (O2•); however, in early hyperglycemic conditions, there is an increase in the glucose oxidation cycle, increasing the activity of the Krebs cycle, which in term leads to increased production of superoxide(Ighodaro 2018). During later stages of diabetes, there is an increase in insulin resistance which lowers the amount of glucose that enters the target cells (Gaster et al. 2001).

The effects of increased superoxide levels can lead to damage of biomolecules, as well as nuclear DNA, which in turn results in DNA repair enzymes, such as poly-ADP-ribose polymerase-1 (PARP-1), to activate. A concurrent effect of PARP-1 activation is inhibition of GADPH and in turn halting the Krebs cycle and pyruvate production. This disruption leads tothe accumulation of glycolytic intermediates such as G-6-P and F-6-P and as well as glucose. Eventually, these intermediates are diverted into pro-oxidative pathways, hence exacerbating the oxidative stress condition. The accumulation of GAP increases the AGE and PKC pathways, while the increase of F-6-P increases the hexosamine pathway, and finally the glucose accumulation leads to the polyol pathways (Ighodaro 2018).

16.2.2 Advanced Glycation End-Products Pathway

Advanced Glycation End-products (AGEs) are lipids and proteins that underwent structural alterations due to exposure and interaction with sugar compounds such as methylglyoxal, glyoxal, and deoxyglucosone (Thornalley et al. 1999). Once AGEs are formed, they can bind to extracellular matrix components orto AGE receptors (AGE-R1, AGE-R 2, AGE-R 3, and RAGE) resulting in ROS generation and promoting oxidative stress (Scivittaro et al. 2000). By activating Receptor for Advanced Glycation End-products (RAGE), AGEs induce a proinflammatory state led by NFkB pathways (Mohamed et al. 1999). AGEs also foment other pro-oxidative stress pathways including the PKC pathways (Boyer et al. 2015). They interact with free amino groups in cellular proteins and cause cross-linking, oligomerization, and aggregation which in turn can impair cellular functions and induce apoptosis (Thornalley et al. 1999).

Hyperglycemia causes autooxidation and the formation of glyoxal, which as stated previously is an AGE precursor. Another mechanism linking hyperglycemia with overproduction of AGEs is the formation of AGEs precursor 3-deoxyglucosone from the disintegration of glucose-derived 1-amino-1deoxyfructose lysine adduct, also known as Amadori product.

16.2.3 Protein Kinase C Activation Pathway

Protein Kinase C (PKC) modulates the activity of target proteins through phosphorylation and a series of reactions. During hyperglycemia, there is an accumulation of dihydroxyacetone-3-Phosphate (DHA-3-P) as a result of inhibition of glyceraldehyde-3-Phosphate dehydrogenase. This increase in DHA-3-P, when combined with fatty acids, leads to de novo synthesis of DAG through the action of 1-acyglycerol-3-P-acyltransferase and phosphatidate phosphohydrolase (Giacco and Brownlee 2010). Diacylglycerol (DAG) plays a key role in this signaling pathway through the upregulation of PKC.

PKC pathway can also be augmented by the binding of AGEs with their respective receptors (RAGE). Upregulation of the PKC pathway has been shown to upregulate ROS-generating enzymes such as lipoxygenases and NADPH-oxidases (Inoguchi et al. 2000).

16.2.4 Hexosamine Pathway

This pathway plays a role in the metabolism of fructose-6-phosphate (F-6-P), a byproduct of glycolysis. Under normal euglycemic levels, a small portion of F-6-P is routedinto the hexosamine pathway where it is converted to glucosamine-6-phosphate, and later into UDP-N-Acetyl glucosamine (UDP-GIcNAc) (Ighodaro 2018).

Hyperglycemia causes an accumulation of F-6-P and other glycolysis intermediates due to downstream glycolysis halt caused by GADPH production inhibition (see Sect. 16.2.1). The diversion of larger amounts of F-6-P into the hexosamine pathway consequently leads to the overproduction of UDP-GlcNAc. Accumulation of UDP-GlcNAc activates O-Glucosamine-N-Acetyl transferase, which leads to increased expression of genes and production of transcription factors including TGF-ß and TGF-α (Figueroa-Romero et al. 2008). These transcription factors inhibit mesangial cell mitogenesis and stimulate the proliferation of collagen matrix as well asthickening of the basement membrane. The end result is a pro-oxidative and toxic role, associated with diabetic complications, especially nephropathy (Schleicher and Weigert 2000).

16.2.5 Polyol (Sorbitol) Pathway

Glucose is metabolized into sorbitol by aldose reductase, using reduced NADPH as its cofactor, and later the sorbitol is transformed to fructose by sorbitol dehydrogenase (SDH). Under normoglycemic conditions, glucose takes its default pathway of glycolysis; however, during hyperglycemic conditions, large amounts of glucose are channeled into the polyol pathway.

The net result of this pathway is the consumption of NADPH and increased sorbitol levels (Srivastava et al. 2005; Gleissner et al. 2008). NADPH plays a crucial role in maintaining redox balance as it is a cofactor of the glutathione antioxidant defense mechanism driven by glutathione reductase (GRx) and glutathione peroxidase (GPx). Thus, the consumption of NADPH, which occurs during a hyperglycemic augmented polyol pathway, leads to decreased levels of these key scavengers of free radicals (Acharya et al. 2010).

Additionally, hyperglycemia also induces an increase in the SDH activity, which utilizes NAD+ as a cofactor to convert sorbitol into fructose (Figueroa-Romero et al. 2008). This increase in fructose can lead to activation of other oxidative stress pathways like protein Kinase C, hexosamine, and AGEs pathways through phosphorylation of glyceraldehyde-3-phosphates and DHA-3-P, de novo synthesis of DAG, and production of methylglyoxal (see Sects. 16.2.3 and 16.2.4) (Ighodaro 2018). These detrimental effects result in a pro-inflammatory and pro-coagulable state in the blood vessels, endothelial dysfunction, reduction of the vasodilator nitric oxide, increase in growth factors, and remodeling of blood vessels. The culmination of these effects

leads to atherosclerosis and lipid deposition, the driving process for cardiovascular and microvascular disease in diabetes (Domingueti et al. 2016; Ahmad et al. 2017).

16.2.6 Insulin Signaling Pathway

Failure of both insulin action as well as insulin secretion in diabetes has also been associated with oxidative stress as seen in the insulin signaling cascade pathway (Rains and Jain 2011). Under physiologic conditions, insulin is secreted in the pancreas by the islet beta cells. As glucose concentrations increase, the glucose-sensing enzyme glucokinase initiates the insulin secretion process by the islet beta cells (Schuit et al. 2001). Glucokinase has a high affinity or low Michaelis constant (Km) for glucose at elevated concentrations, and thus it readily phosphorylates glucose to G-6-P, channeling it to ATP-generating pathways including glycolysis, Kreb cycle, and electron transport chain. The increase in ATP closes ATP-sensitive K+ channel, as well as increases sodium influx, causing a depolarization of the membrane. Subsequently, there is an aperture of voltage-dependent T-type calcium and sodium channels, leading to further increase of sodium and calcium and depolarization of the membrane. Elevated intracellular calcium stimulates the fusion of insulin-containing secretory granules to the plasma membrane, thusreleasing insulin (Rorsman and Renstrom 2003).

Oxidative stress induced by hyperglycemia has been shown to interfere in several pathways contributing to the pathogenesis of insulin resistance and DM (Yaribeygi et al. 2020). Some of the components of the insulin signaling pathway that have been shown to be altered in DM include IRS-1, and IRS-2, PI3K enzyme, and Akt signaling pathways (Paz et al. 1997; Yaribeygi et al. 2020). Hyperglycemia-induced oxidative stress has been also noted to stimulate uncoupling protein-2 (UCP-2). This causes a drop in ATP/ADP ratio resulting in the inhibition of ATP-dependent mechanisms leading up to the secretion of insulin (Robertson et al. 2003; Holley et al. 2015).

Additionally, the quality and quantity of insulin secretedby pancreatic beta cells is reduced due to hyperglycemia-generated ROS and the oxidative damage of the pancreas. Moreover, hyperglycemia-mediated ROS production can alter the shape and function of the mitochondria, which results in the uncoupling of ATP-dependent K + channels and impaired glucose-stimulated insulin secretion (Prattichizzo et al. 2018). Oxidative-stress-mediated loss of beta-cell secretory function, as well as increased insulin resistance, plays an important role in the development of both diabetes type 1 and type 2 (Prattichizzo et al. 2018).

Furthermore, GLUT-4 receptors, which are protein complexes that allow the entrance of glucose into insulin-dependent cells, have reduced expression in Type 2 diabetes mellitus (T2DM) (Gaster et al. 2001). This causes increased insulin resistance and a lowering of glucose entry into the target cells. Oxidative stress can lower the GLUT-4 content by negatively affecting its gene expression as well as it has been shown to reduce the translocation of GLUT-4 into the cell membrane (Pessler et al. 2001; Fazakerley et al. 2018).

16.2.7 Lipid Peroxidation

The process of lipid peroxidation results from the oxidative conversion of polyunsaturated fatty acids to products such as malondialdehyde. These products are referred to as either lipid peroxides or thiobarbituric reactive substances (TBARS) (Memisoğullari et al. 2003). When the correlation between lipid peroxidation and diabetic control has been studied, it has been found that there is a significant malondialdehyde concentration in poorly controlled diabetics, and there is a strong correlation with the degree of peroxidation and glycemic control (Altomare et al. 1992).

These lipid peroxides have deleterious effects on cells both directly and through conversion to hydroxyl radicals, which interact with metals such as iron and copper to form aldehydes that can damage cell membranes. A major contributor in the antioxidant system is ceruloplasmin, as it inhibits iron- and copper-dependent lipid peroxidation (Memisoğullari et al. 2003). Ceruloplasmin level can also be used as a biomarker of oxidative stress, as its increase is associated with persistently high blood glucose over 1 year (Daimon et al. 1998).

TBARS are an indirect measure of intensified free radical production in diabetic complications. Serum levels of TBARS have been found to be significantly increased in all patients with diabetes, more in type II diabetes. They are even more elevated in patients with poor metabolic control and angiopathy suggesting a causation effect (Griesmacher et al. 1995). A decrease in TBARS is therefore an effective method of reducing complications in diabetes. TBARS elevation can be curbed by substances such as nicotinamide, boldine, melatonin, aspirin, enalapril, or may even be reversed with vitamins C, E, beta carotene, melatonin, and gemfibrozil (Maritim et al. 2003). The effect of TBARS in oxidative stress and multi-organ damage will be detailed in further sections.

16.3 Diabetes Progression, Complications, and Oxidative Stress

Oxidative stress contributes in the pathogenesis of diabetes and its complications in many mechanisms as described above. Insulin resistance can precede the clinical diagnosis of type II diabetes mellitus by many years (Freeman and Pennings 2020). This raises the question of whether oxidative stress is present in the earliest stages of insulin resistance and diabetes development. A series of case-controlled studies have previously shown an increase in oxidative stress biomarkers and damage of lipid, protein, and nucleic acid cellular components in patients with prediabetes (Bigagli and Lodovici 2019). Moreover, it has been suggested that the hyperglycemia-driven production of superoxide in the mitochondria is an inciting mechanism in the pathogenesis of diabetes and diabetes-related complications (Ceriello 2006). Additionally, markers of oxidative stress such as MDA (malondialdehyde), GSH (glutathione)

were significantly elevated in prediabetic patients compared with controls in a cross-sectional study that evaluated the risk of cardiovascular disease in these patients (Mahat et al. 2019).

Diabetes is known to cause several debilitating complications which are in part mediated by oxidative stress. The vascular complications of diabetes can be categorized into microvascular and macrovascular, in which oxidative stress is important in the development of both categories (Memisoğullari et al. 2003). Hyperglycemia inflicts damage on the endothelial tissue through shunting of glycolytic intermediates into pathways that promote the production of ROS and AGEs and through increased mitochondrial-driven oxidative stress (Kinaan et al. 2015). In the diabetic microvasculature, the increase in ROS is mainly due to intracellular hyperglycemia (Giacco and Brownlee 2010). In contrast, the ROS production in the macrovasculature of the heart is the result of elevated oxidation of fatty acids, secondary to insulin-resistance pathways (Ndrepepa and Kastrati 2016). Superoxide production also inactivates two critical anti-atherosclerotic enzymes; prostacyclin synthase and endothelial nitric oxide synthase (Giacco and Brownlee 2010). Animal experimental trials have shown a reduction in diabetic nephropathy, retinopathy, and cardiomyopathy by inducing overexpression of superoxide dismutase and thus lowering the amount of superoxide (Shen et al. 2006; Zhang et al. 2006).

Oxidative stress is linked to endothelial dysfunction. In its quiescent physiologic condition, endothelial cells produce nitric oxide (NO) using the endothelial nitric oxide synthase (eNOS) (Alexander et al. 2021). NO is an important gaseous mediator that provides atheroprotective effects against oxidative stress, as well as platelet activation, aggregation, and inflammation (Tousoulis et al. 2012). During this quiescent form, NO mainly functions by targeting transcription factors such as NFκB, cell cycle controlling proteins, and proteins involved in generation of tissue factors, as well as reducing oxidative phosphorylation in the mitochondria (Ghosh and Karin 2002; Moncada and Erusalimsky 2002).

During endothelial activation caused by diabetes or cardiovascular risk factors, there is a switch from a quiescent phenotype toward one that comprises a host defense response. This switch causes the signaling from a NO-mediated silencing of the cellular processes, to activation of redox signaling, and leading to generation of hydrogen peroxide. The process by which eNOS switches from maintaining a quiescent state to switch to endothelial activation and generate ROS is termed *eNOS uncoupling* (Deanfield et al. 2007). The net result of prolonged and repeated exposure to eNOS activation is a dysfunctional endothelium, an alteration of its barrier integrity, progression to senescence, and detachment of endothelium cells into the circulation (Woywodt et al. 2002).

As explained above (Sect. 16.2.6), different metabolic pathways of diabetes can increase mitochondrial superoxide in the endothelium and the myocardium (Domingueti et al. 2016). The hyperglycemia-induced increase in mitochondrial superoxide can cause defective angiogenesis in response to ischemia and can lead to long-lasting epigenetic changes that cause persistent expression of pro-inflammatory genes long after glycemia is normalized, a term called (“hyperglycemic memory”)

(Giacco and Brownlee 2010). Hyperglycemic memory may have clinical implications: First, that early tight control is important to avoid it. Second, the cure of diabetes may not prevent subsequent development of complications, and lastly, novel therapies that alter hyperglycemic memory may be required (Giacco and Brownlee 2010).

With regard to vascular disease, lipid peroxides formed by free radicals is crucial in the formation of atherosclerosis. Type 2 diabetic patients with coronary heart disease were found to have higher lipid peroxide concentrations and TBARS concentrations compared to diabetics without coronary heart disease (Kesavulu et al. 2001). However, some of the antioxidant and lipid metabolic pathways are comparable between diabetic and nondiabetic patients. For example, a study demonstrated increased catalase (CAT) activity and no change in superoxide dismutase (SOD) activity in both diabetic and nondiabetic groups with congestive heart disease (CHD) (Kesavulu et al. 2001). Free vitamin E concentrations in diabetic and nondiabetic subjects were not found to differ either (Kesavulu et al. 2001). Studies suggest that oxidative damage which predisposes to atherosclerosis is reduced by consumption of fruits and vegetables rich in antioxidant vitamins (Nuttall et al. 1999). There is also evidence that probucol, which is an antihyperlipidemic drug and myocardial antioxidant, causes an increase in myocardial SOD, glutathione reductase, and catalase activities as well as a decrease in TBARS (Maritim et al. 2003).

Diabetic kidney disease (DKD) is also involved with renal oxidative stress. Many pathways have been described in the development of diabetic nephropathy, including activation of protein kinase C, AGEs production, and hexosamine pathway (Kumawat et al. 2013). Hyperglycemia also promotes lipid peroxidation, nuclear factor kappa B, and TGF B leading to tissue fibrosis and extracellular matrix synthesis (Turkmen 2017). Studies demonstrated increased oxidative stress in diabetic patients with nephropathy compared to those without (Kedziora-Kornatowska et al. 1998). This has been attributed to decreased SOD and catalase, increased NADH/NAD ratio, and activation of free radical production, leading to diabetic angiopathy and dysfunction of endothelial cells (Kedziora-Kornatowska et al. 1998). There was a positive correlation of HbA1c with malondialdehyde levels and a negative correlation with reduced glutathione, further suggesting that the intensity of oxidative stress in T2DM patients with nephropathy is greater than those without (Kumawat et al. 2013).

Pancreatic β-cells are of great importance in the development of diabetes and are affected by the mitochondrial generation of NADPH-oxidase-dependent ROS generation and oxidative stress pathways. These pathways contribute to insulin resistance, impaired insulin secretion, and late diabetic complications (Newsholme et al. 2019). Antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase are the main enzymatic antioxidants described in the pancreas (Newsholme et al. 2019). The mechanism of insulin secretion modulation and insulin resistance is complex and involves complex intracellular signaling. Some studies have shown how H_2O_2 modulates NFkB and thus alters gene expression (de Oliveira-Marques et al. 2007). Other studies have shown that H2O2 decreases insulin gene expression by altering transcription factors such as PDX1 and V-MafA (Cnop et al. 2014). Antioxidants can also play an important function in the pancreas, as supplementation with β-carotene,

Vitamins C and E has shown to generally improve insulin sensitivity outcomes in humans (Manning et al. 2004; Dakhale et al. 2011; Canas et al. 2012).

16.4 Aging and Diabetes

Aging is a complex process that ultimately leads to the decline or loss of all physiological functions in the body. Amongst the theories that have been proposed to explain this complex process is the free radical theory of aging. The free radical theory of aging was described in 1956 and states that continuous unrepaired oxidative damage is the basis of aging (Harman 1956).

16.4.1 Mitochondrial Free Radical Theory of Aging

The main producer of reactive oxygen species (ROS) in the cell is the mitochondrial electron transport chain. Over 60 years ago, Dr. Harman D proposed the free radical theory in which he states that aging can be attributed to the cumulative damage inflicted by the free radicals on the cellular components, specifically mitochondria. Moreover, it proposed that these free radicals arise from reactions by the oxidative enzymes in the cells (Harman 1956). This process leads to dysfunction of mitochondrial DNA (mtDNA) and other components of the mitochondrial respiratory chain, thuscreating a vicious cycle of more ROS generation and more mitochondrial damage (Lagouge and Larsson 2013).

Recently, this theory has been challenged by researchers suggesting that ROS can be beneficial and may even prolong the lifespan as noted in yeast and Caenorhabditis elegans (Doonan et al. 2008). Furthermore, mice genetically modified to increase mitochondrial ROS and oxidative damage did not show an acceleration in aging (Lopez-Otin et al. 2013). ROS are required for various physiological processes such as cell proliferation, metabolic pathways, gene expression, and cellular signaling culminating in enhanced cellular functions and homeostatic mechanisms. More studies are needed to establish if there is a threshold above which ROS lose their physiological and protective function thus accelerating senescence and aging (Diamanti-Kandarakis et al. 2017).

16.4.2 Aging Beta Cells

Aging is associated with the decline of most of the human physiological functions. Throughout the human life span, the prevalence of diabetes mellitus increases significantly. In 2019, one in five people above 65 years of age had diabetes mellitus worldwide according to the 9th edition of the International Diabetes Federation (IDF).

Glucose intolerance with aging has been attributed to many metabolic causes like lack of physical activity, decreased insulin secretion, peripheral insulin resistance, poor diet, among others (Scheen 2005). Aging is related with an increase in body weight and fat mass along with alterations in the insulin signaling pathway (Ryan 2000). Mechanisms that contribute to defective insulin secretion in the elderly population include increased pancreatic islet amylin deposition, decreased age-related b-cell mass, decreased b-cell sensitivity to glucose as well as gut incretin hormones (Castillo et al. 1995; Scheen 2005).

16.5 Diabetes Medication and Oxidative Stress

Endothelial dysfunction is closely related to oxidative stress, inflammation, and mitochondrial dysfunction and endothelial dysfunction is an early indicator of the development of micro- and macrovascular complications. The effect of antidiabetic agents on oxidative stress and how it correlates with the protection of the endothelium and vascular disease risk reduction has been an area of great interest. There is an accumulation of evidence that several drugs that are used for the treatment of type 2 diabetes in addition to their primary sites of action that contribute to normalizing glycemia also have direct or indirect protective effects on the endothelium and confer vascular protective actions (Ding et al. 2019; Triggle et al. 2020).

The antihyperglycemic drug metformin, first introduced in the late 1950s, remains the first-line pharmacological treatment option in type 2 diabetes that reduces insulin resistance via its effects in the liver to reduce gluconeogenesis and lipogenesis (Foretz et al. 2019). Metformin decreases the rate of glucose production by inhibiting hepatic gluconeogenesis via activation of AMP-activated protein kinase (AMPK) and improves insulin sensitivity by enhancing insulin-induced glucose uptake (Kinaan et al. 2015; Triggle et al. 2020; Teodoro et al. 2018). The therapeutic effects of metformin has been attributed to the activation of AMP-activated protein kinase (AMPK) that results from the inhibition of mitochondria complex 1 and the lowering of the ATP/AMP ratio (Owen et al. 2000; El-Mir et al. 2000). However, the "mitochondria poison" effects of metformin require much higher concentrations of the drug than are needed therapeutically thus raising the likelihood of alternative pathways whereby metformin activates AMPK (Kinaan et al. 2015; Triggle et al. 2020; Fontaine 2018). Activation of AMPK will also enhance the activity of endothelial nitric oxide synthase (eNOS) and improve blood flow (Kinaan et al. 2015; Triggle et al. 2020; Sena et al. 2011). There is extensive literature from both clinical and pre-clinical studies that indicates that metformin has direct effects to protect the endothelium from hyperglycemia-induced oxidative stress via both AMPK-dependent and independent actions (Salvatore et al. 2020). Thus, 12 weeks of treatment with metformin has been demonstrated to improve endothelium-dependent vasodilation in diabetic patients as determined by measuring changes in forearm blood flow after intra-brachial artery administration of the endothelium-dependent vasodilator acetylcholine (Mather et al. 2001). Similarly, a study by Sena et al. where hyperlipidemic rats treated with

metformin for 4 weeks showed improvement in vascular and systemic oxidative stress compared to impaired endothelial-dependent vasodilatation observed in non-treated rats (Sena et al. 2011). Exposure of mouse microvascular endothelial cells to metformin has also been shown to protect against hyperglycemia-induced increases in ROS and endothelial senescence via a cellular mechanism requiring the expression of the deacetylase, sirtuin-1; the protein product of the so-called anti-aging gene, SIRT1 (Arunachalam et al. 2014).

Other antidiabetic medications that have also been reported from in vivo and in vitro studies to modify oxidative function are the thiazolidinediones (glitazones or TZDs) such as rosiglitazone and pioglitazone that mediate their antihyperglycemic actions via binding to the nuclear receptor, PPARγ. TZDs also reduce oxidative stress and inflammation via the inhibition of the expression of inducible NOS (iNOS), reducing the activity of NF-kB via the activation of PPARγ and for pioglitazone also the PPARα receptor (Orasanu et al. 2008; Da Ros et al. 2004). Glucagon-like peptide-1 (GLP-1) enhances the release of insulin via its action in pancreatic beta cells, but GLP-1 receptors are also expressed in other cell types including endothelial cells. GLP-1 receptor agonists have been shown to improve cardiovascular outcomes in diabetic patients via mechanisms that are at least in part independent of their anti-hyperglycemic actions (Honigberg et al. 2020). GLP-1 receptor agonists have also been shown in vitro to protect endothelial cells and have antioxidant effects; however, clinical supportive data for a direct action of the endothelium is controversial, but could be mediated via the activation of the cAMP/protein kinase A signaling pathway (Lim et al. 2017; Nomoto et al. 2015). Thus, the glucagon-like peptide-1 (GLP-1) agonist liraglutide has been shown to enhance the production of NO and antioxidant enzymes including superoxide dismutase (SOD) and catalase (Shiraki et al. 2012). Dipeptidyl peptidase-IV (CD26/DPP-IV) is a ubiquitous, membrane-bound peptidase that catalyzes the breakdown of N-terminal dipeptides including GLP-1. Studies have also demonstrated that DPP-IV inhibitors have antioxidant properties. Thus, data from an in vitro study of mice and human endothelial cells suggest that DPP-IV inhibitors alleviate effects of vascular aging by suppressing inflammation and oxidative stress (Xin et al. 2019).

Finally, selective sodium/glucose co-transporter 2 (SGLT-2) inhibitors have also been shown to have antioxidative effects, which could be a key mechanism that contributes to their cardiac and renal protective benefits (McGuire et al. 2020). It is believed that this action is, in part, due to the suppression of the AGE-RAGE mediated oxidative stress generation (Teodoro et al. 2018). Although the primary site of action of the selective sodium/glucose co-transporter 2 (SGLT-2) inhibitors, the gliflozins, is in the proximal tubule of the kidney, a direct action in the endothelium to reduce hyperglycemia-induced oxidative stress and an endothelial protective action has also been demonstrated in vitro, and in vivo dapagliflozin improves flow-mediated vasodilation in diabetic patients (El-Daly et al. 2018; Shigiyama et al. 2017) (Fig. 16.2).

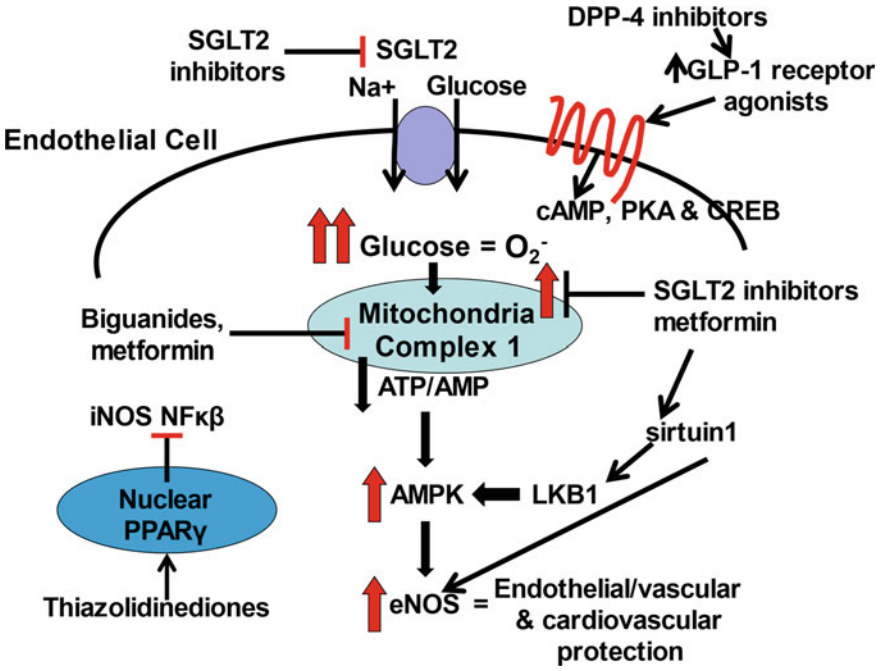

Fig. 16.2 Mechanisms of diabetes medications with Reactive Oxidant Species. Several groups of drugs used as treatment for type 2 diabetes have also been associated with reducing the risk of cardiovascular risk in diabetic patients, at least in part, via the reduction of oxidative stress and protecting endothelial function. Best documented is the biguanide, metformin, with data from both pre-clinical and clinical studies indicating that in addition to its inhibitory effects on liver gluconeogenesis, metformin, via both (AMPK)-dependent and independent actions, enhances endothelial nitric oxide synthase (eNOS) activity. At appropriate concentrations, metformin inhibits mitochondrial complex 1 thereby reducing the ATP/AMP ratio and increasing AMPK. Metformin also, via the deacetylase, sirtuin1, positively regulates the serine/threonine kinase liver kinase B1 (LKB1 or SKT11), which, in turn, enhances the activity of AMPK. In addition, sirtuin1, via the deacetylase of two lysine residues, 496 and 506, enhances the activity of eNOS. The thiazolidinedioes (glitazones, or TZDs), such as pioglitazone, mediate their antihyperglycemic actions via activation of the nuclear receptor PPARγ, but also inhibits activation of the pro-inflammatory transcription factor, NF-κB, and inducible NOS (iNOS) and thus reduces oxidative stress. Glucagon-like peptide-1 (GLP-1) agonists have cardiovascular protective actions, and receptors for GLP-1 are expressed in endothelial cells inferring that via signaling through the cAMP/PKA/CREB (cyclic AMP/protein kinase A/cAMP response element binding protein) axis also have direct protective actions on endothelial function via eNOS and independent of their actions to enhance insulin release. Dipeptidyl peptidase-IV (DPP-4; CD26) inhibitors, via inhibition of the ubiquitous peptidase, DPP-IV, enhance the effects of GLP-1 agonists. Finally, selective sodium/glucose cotransport 2, (SGLT2) inhibitors, the "gliflozins," lower plasma glucose level as a result of the inhibition of SGLT2 in the proximal tubules of the kidney, but SGLT2 are also expressed in murine endothelial cells and SGLT2 inhibitors directly reduce oxidative stress and protect endothelial function in endothelial cells

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Chapter 17
Redox Processes in the Etiopathogenesis of Cerebrovascular Diseases

Karlo Toljan

Abstract The etiology of cerebrovascular diseases is multifactorial, but lifestyle risk factors predominate in most cases. Pathophysiology has features of metabolic and immunological alterations that lead to worsening cerebrovascular health. The process is marked by cerebral atherosclerosis and neuroinflammation, in conjunction with systemic chronic low-grade inflammation. Such processes are also associated with advanced biological aging, and cerebrovascular diseases may be considered a continuum of atherosclerosis, neuroinflammation, and neurodegeneration. On a cellular level, macrophages and microglia play key roles in chronic processes. On a subcellular level, redox status is abnormal in conditions of decompensating cerebrovascular disease, of which stroke is the most severe and acute presentation. Following such an abrupt inflammatory insult, organelles such as mitochondria functionally change, and despite potential reperfusion, a pro-oxidative cellular state is maintained. The neurovascular unit, comprised of the endothelium, accompanying cells of basal lamina, astrocytes, and neurons, represents the crossroads between systemic vascular health and the environment of central nervous system. In chronic cerebrovascular insufficiency, the neurovascular unit is affected by pathophysiological changes, and such a state is marked by inflammatory profile, mostly due to predominant inflammasome activity. The immunometabolic perspective opens the door for wider consideration of pathophysiological, but also therapeutic factors. Most recently, microbiota has emerged as an important component of vascular health, and abnormal levels of microbiota metabolites, such as trimethylamine-N-oxide, have been linked with cardiovascular disease and stroke. By understanding the systemic and local immunometabolic interactions, new diagnostic tools and personalized therapies should become available.

Keywords Immunology · Microbiota · Neurology · Neurodegeneration · Neuroinflammation · Pathophysiology · Reduction–oxidation · Stroke

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17.1 Introduction

Cerebrovascular diseases have considerable epidemiological importance given that strokes, as the acute presentation and the final common pathway of the disease group, globally represent the second largest cause of death and a leading cause of disability (Krishnamurthi et al. 2020; World Stroke Organization 2019). Most strokes are ischemic, 65% globally and 87% in the United States, whereas absolute mortality of ischemic and hemorrhagic stroke is similar (Krishnamurthi et al. 2020). Global stroke incidence rate is around 13 million cases yearly, and 25% of people older than age 25 will experience a stroke during their lifetime. Worldwide, there are around 80 million stroke survivors who are currently alive, and 51% of them are female (World Stroke Organization 2019). Following overt stroke, vascular dementia risk is doubled (Wolters and Ikram 2019). The latter represents 15–30% of all dementia cases, making it the most common type after Alzheimer disease (Wolters and Ikram 2019). Both dementia subtypes can co-exist, and there are mutually shared risk factors. With growing research of pathophysiological aspects, it is becoming apparent that besides the epidemiological connection, cerebrovascular diseases with their sequelae such as vascular dementia, and Alzheimer disease, share some underlying neuroinflammatory and neuropathological components. For example, a distinct clinical-pathological entity such as cerebral amyloid angiopathy, which is associated with a higher risk of hemorrhagic stroke, shares the amyloid-β accumulation with Alzheimer disease as a prominent pathological hallmark (Greenberg et al. 2020). Age, as a non-modifiable risk factor, and classically recognized, largely modifiable, vascular risk factors are associated with certain inflammatory pathways that support a joint immuno-metabolic pathophysiology (Ketelhuth et al. 2019; Wang et al. 2019). A broader approach includes the circadian rhythm and the microbiome as exemplary additional factors to be considered when investigating (neuro)inflammatory pathomechanisms and potential treatments (Bishehsari et al. 2020; Ma et al. 2019). On a subcellular level, overt inflammation reflects an imbalance in molecular redox processes (Hsieh and Yang 2013; Lei et al. 2015). Such processes encompass the exchange of electrons between atoms during which their oxidative states change. Although redox dynamics are an inherent feature of all living organisms, the net exchange should ideally be constrained within limits set by physiologic homeostasis (Ursini et al. 2016). Overburdening the compensatory homeostatic drives would initially set a new state of pathological homeostasis, also termed allostasis (McEwen 2000). The latter represents maintained homeostasis, however, with increased energy demands, altered regulation set points, and overall decreased reactivity, i.e., compensatory mechanisms. Prolonged allostasis leads to latent disease onset, e.g., incipient atherosclerosis or prediabetes. With an increase in allostatic load to uncontrollable levels, a breakdown ensues, and a latent disease becomes unmasked and recognized as a clinical-pathological syndrome, e.g., stroke or dementia (Booth et al. 2015; Guidi et al. 2021). With limited homeostatic strain by certain stimuli or reactants (e.g., preconditioning), occasional favorable effects are observed. Such phenomenon is termed hormesis, and hormetic dose–effect curves are usually represented by U- or

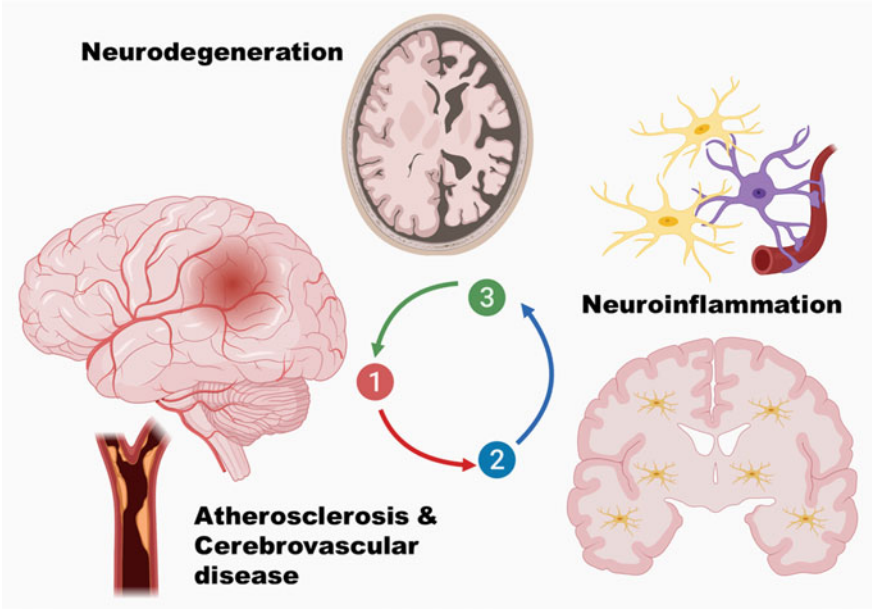

Fig. 17.1 Overview of systemic inflammation, neuroinflammation, and neurodegeneration as a continuum. Cerebrovascular disease as a manifestation of systemic atherosclerosis is pathophysiologically linked to neuroinflammation (yellow cells—activated microglia, purple cell-astrocyte), and both are linked with neurodegenerative processes

inverted U-curves, J-curves, and even mirror-J curves (Calabrese and Mattson 2017; Sedlic and Kovac 2017). A comprehensive understanding of cerebrovascular diseases would unify systemic inflammation, neuroinflammation, and neurodegeneration as a continuum of concurrently present pathophysiology (Fig. 17.1).

17.2 Risk Factors and Etiology

The etiology of cerebrovascular diseases is multifactorial (Fig. 17.2). For stroke in specific, certain risk factors have been well established, and associations remain strong across various populations studied. Hypertension, low intake of fruits and vegetables, high intake of dietary sodium, higher body mass index, smoking, and poor ambient air quality have been demonstrated as globally uniform stroke risk factors with the highest associations, respectively (Feigin et al. 2014). Most of the cerebrovascular risk factors are environmental or lifestyle related. Low physical activity, diabetes, hypercholesterolemia, alcohol use, second-hand smoking, chronic kidney disease, and exposure to environmental toxins such as lead or pollution from solid fuels have all been mentioned as epidemiologically prominent stroke risk factors (Feigin et al. 2014; World Stroke Organization 2019). Looking at an individual level,

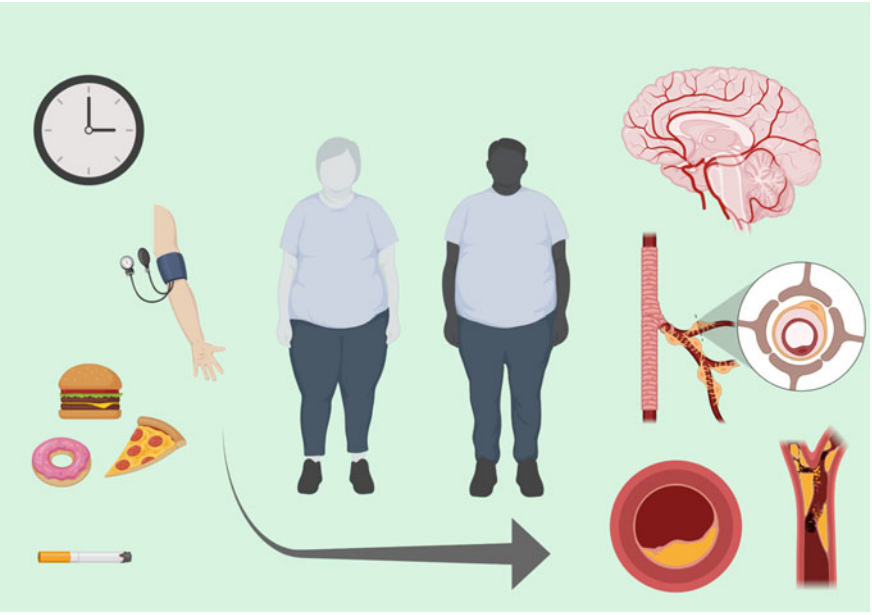

Fig. 17.2 Prominent risk factors for cerebrovascular disease. Hypertension, obesity, poor diet, smoking, and sleep problems including obstructive sleep apnea and circadian rhythm alterations induce immunometabolic changes that lead to systemic atherosclerosis, dysfunction of the neurovascular unit, and ultimately cerebrovascular insufficiency

family history is another contributor associated with stroke occurrence (Chung et al. 2016). Furthermore, genetic studies have determined respective mutations that affect collagen formation, coagulation pathways, hemoglobin synthesis, or cardiac ion channels, and which have been associated with higher incidence of stroke (Boehme et al. 2017). Age as a non-modifiable risk factor is associated with stroke incidence, though comorbidities and overall biological age, rather than chronological age per se seem to be the detrimental factors in cerebrovascular pathophysiology (Kelly-Hayes 2010). Regarding age- and sex-related differences, it has been shown that men have higher rates of stroke than women, but the cumulative incidence switches sometime in the eighth decade, primarily due to overall longer life expectancy of women (Kelly-Hayes 2010). Nevertheless, there are also clear sex-associated different stroke risk factors throughout lifetime, the mechanisms primarily thought to be hormone related. Pregnancy and medications containing hormones have been linked to increased stroke incidence, though protective role of estrogens for vascular health has also been determined (Roy-O'Reilly and McCullough 2018). Complexities are expanded even more with clear sex differences in post-stroke pathophysiological changes as observed in animal models. Female animals experience less oxidative stress, less microglial activation, increased astrocyte activation, and increased reactive vasodilation as compared with male animals, while most of the differences become less evident with castration or aging (Roy-O'Reilly and McCullough 2018).

Our understanding of metabolic and immune changes taking place while the aforementioned risk factors are present is growing, and cerebrovascular disease pathophysiology can be described from population-based, individual-based, or cellular and subcellular levels. Regarding the latter, it is becoming evident that metabolic changes and immune changes are interlinked, and as such favor an immunometabolic perspective (Wang et al. 2019). Inflammatory changes associated with metabolic syndrome, as well as chronic low-grade inflammation associated with metabolic changes, have already been studied in cardiovascular medicine, though more specifically from the clinical perspective of cardiology (Ketelhuth et al. 2019). However, such interactions are being also investigated in cerebrovascular pathophysiology (Arenillas et al. 2007). Additionally, neuroinflammation and neurodegeneration are specific components of chronic cerebrovascular insufficiency (Naveed et al. 2019), which expand the cardiovascular pathophysiological approach of studying systemic immunometabolic alterations. Redox dynamics, as key cellular processes, are affected by involved pathomechanisms (Paspalj et al. 2015; Ye et al. 2016). On a grander scale, such redox imbalances in cerebrovascular milieu are associated with ischemic or hemorrhagic changes (Komsiiska 2019). Clinically, this may become apparent as stroke-related deficits, vascular neurocognitive symptoms, or neuroimaging detected microvascular disease and microhemorrhages.

17.3 Pathophysiological Mediators

17.3.1 Neurovascular Unit

Contemporary view of cerebrovascular (patho)physiology places the neurovascular unit as the key cellular nexus in which the changes are observed and described. The neurovascular unit is comprised of neurons, astrocytes, pericytes, endothelium, vascular smooth muscle cells, and extracellular matrix (Muoio et al. 2014). An insult at any of the components indirectly affects the entire unit. Cerebrovascular endothelial cells maintain central nervous system milieu by tight junctions limiting paracellular pathway, expression of P-glycoprotein enabling clearance of possible toxic substance, as well as controlled transcellular transport via caveolae and exosomes (Garry et al. 2015; Wang et al. 2018). Endothelial cells contain endothelial nitric oxide synthase (eNOS), which is physiologically triggered by shear stress or vascular endothelial growth factor presence. The produced nitric oxide (NO) is salient for maintaining appropriate blood flow and well as a signaling factor for synaptic neuroplastic changes in the brain. Vice-versa, the neuronal produced NO via neuronal NOS (nNOS) also triggers relaxation of the vascular smooth muscle cells and contributes to regulation of local blood flow. However, in conditions of endothelial or neuronal inflammatory changes, such as vasculopathy, acute stroke, or active brain disease, including neurodegenerative ones, excess NO produced by inducible NOS (iNOS) can be neurotoxic (Garry et al. 2015; Wang et al. 2018). Additionally,

on top of the endothelium lies a thick layer of glycocalyx (Haeren et al. 2018). It serves as an appended protectant of the blood–brain-barrier (BBB) integrity and its decreased thickness has been associated with traditional vascular risk factors. The destruction of endothelial glycocalyx exposes the endothelial surface to aggregating platelets, activated leukocytes, and any present systemic inflammatory factors that could then compromise the integrity of the BBB (Zhao et al. 2021). Under local or systemic inflammatory condition, matrix metalloproteinases are induced and cleave the surrounding extracellular matrix, while tight junctions become more permeable (Rempe et al. 2016). Endothelial cells start expressing more leukocyte adhesion and Toll-like receptors, through which activity downstream inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) cascade is promoted (Rempe et al. 2016; Wang et al. 2018; Zhao et al. 2021). Chronic endothelial inflammation, including such related to chronic infections (Lindsberg and Grau 2003), neuroinflammation, and neurodegeneration represent a vicious cycle that is usually evident as cerebral hypoperfusion, ischemia, or (micro)hemorrhages, and loss of brain tissue (Quick et al. 2021; Stanimirovic and Satoh 2006). Recent insights into astrocyte function in neurovascular coupling provide evidence of their specific role as 'intracranial baroreceptors', i.e., governing the sympathetic output in regard to cerebral perfusion (Marina et al. 2020).

17.3.2 Immune Cells

Immune cells present in the blood circulation, as well as in the central nervous system itself, partake in the intercellular communication within the neurovascular unit. Additionally, with the findings suggestive of actual meningeal lymphatics (Papadopoulos et al. 2020) and the dynamic 'glymphatic' system (Jessen et al. 2015), systemic and central nervous system immune interactions appear much more integrated than previously imagined. From the immune cell subtypes, macrophages and microglia get much attention as crucial subsets involved in chronic inflammatory processes. The former is for vessel pathology such as atherosclerosis (Peng et al. 2020), whereas the latter for neurodegenerative processes, including vascular dementia and Alzheimer disease (Park et al. 2017). Interestingly, besides parenchymal microglia, there is also a fraction of perivascular macrophages in close proximity to the central nervous system and with prominent phagocytic capacities, including directed at amyloid β, but also possibly being involved as contributors to inflammatory changes related to hypertension and BBB dysfunction (Koizumi et al. 2019; Park et al. 2017). Neutrophils have been studied as part of non-specific immunity response in acute stroke. Their numbers in infarcted and penumbral area peak early, and there are prominent accompanying pro-inflammatory changes, with consequential BBB disruption and impaired revascularization. Activated neutrophils also release neutrophil extracellular traps (NETs), which are made of DNA parts and granule proteins, and which have been associated with non-specific inflammation including of autoimmune nature (Kang et al. 2020). While neutrophils appear within the first hour of stroke, peak by day 3, and

macrophages by day 4, T-cells can persist longer and may have protective effects if Treg subpopulations function appropriately and are preventing autoimmune reactions during healing phase (Planas 2018). The subclass of natural-killer cells is present very early in acute stroke, as part of non-specific immune response, but the higher early activity may actually be associated with higher level of subsequent post-stroke immune depression (Chen et al. 2019).

Immune cell subtype ratios appear to be remarkably robust markers in various clinical stroke scenarios. Lower lymphocyte to monocyte ratio was associated with hemorrhagic transformation risk in acute ischemic stroke patients (Song et al. 2020) as well as worse outcomes in a different patient population (Ren et al. 2017). The same ratio was associated with poorer prognosis in cases of cerebral venous thrombosis (Li et al. 2019). In a meta-analysis including more than 3700 patients, a higher neutrophil to lymphocyte ratio was associated with hemorrhagic transformation and poorer outcome in ischemic stroke patients (Zhang et al. 2019). Even in case of acute stroke treatment with thrombolysis, higher neutrophil to lymphocyte ratio showed an association with poorer outcome (Pektezel et al. 2019). Another marker is platelet to lymphocyte ratio, and which has been shown to be associated with an unfavorable ischemic stroke outcome in case of a higher ratio value (Xu et al. 2019). These ratios remain to be validated in larger cohorts, but due to apparent persistent trends with certain ratio values and clinical features, an underlying mechanistic explanation may exist. As of now, it is definitely evident that post-stroke period is marked by a highly dynamic (neuro)immunological environment with probable impact on short-term and long-term events (Kömürcü et al. 2020).

17.3.3 Subcellular Mechanisms

In optimal conditions, the pro-inflammatory and anti-inflammatory cascades are within physiological limits, otherwise also seen through net balanced redox processes with a baseline favoring of a cellular nucleophilic tone (Ursini et al. 2016). In pathological conditions associated with pro-inflammatory and ultimately oxidative conditions, the net redox balance is turned to electrophilic. Reactive oxygen species (ROS) arising from activity of mitochondria, NADPH oxidase, xanthine oxidase, lipoxygenase, or cytochrome P450 enzymes (Ma 2010), as well as reactive nitrogen species (RNS) produced by iNOS (Hess et al. 2005), have physiological roles in cellular signaling, e.g., for post-translational modifications or S-nitrosylation. However, in allostatic overload or manifest metabolic disease associated with chronic inflammation, net excess of ROS and RNS causes further pathological changes by production of highly damaging lipid peroxides, notably 4-hydroxy-2-nonenal (HNE) (Csala et al. 2015). As a response to imbalance ratio, a feedback response triggering erythroid 2–related factor 2 (Nrf2) pathway provides extra nucleophilic support by binding to nuclear antioxidant response element and inducing transcription of anti-inflammatory and antioxidant enzymes, e.g., heme-oxygenase-1, as well as limiting the activation of pro-inflammatory NF-κB, COX-2, and iNOS pathways (Ahmed et al. 2017).

The latter is also implicated in downregulation of inflammasome, a salient protein complex implicating in the generation of a powerful pro-inflammatory signal, interleukin 1 (Ma 2013). However, under circumstances when Nrf2 is activated by cholesterol or monosodium urate, it seems that the predominant net effect is activation rather than inhibition. Experiments with Nrf2-knockout animals also show diminished activation of inflammasome, and it appears to have at least a permissive role in its proper functioning (Jhang and Yen 2017). Otherwise, in case of acute inflammatory insult such as a stroke or intensifying chronic inflammation such as progressive vasculopathy, cellular plasma membrane pattern recognition receptors respond to disease-associated molecular patterns by inducing downstream signaling via NFκB and mitogen-associated protein kinase. These pathways induce the transcription of inflammasome components and following the adaptor protein recruitment of NLRP3 in the cytoplasm, the inflammasome complex forms. A pro-inflammatory cellular environment with the formed inflammasome creates a vicious cycle of intensified damaging pathways (Ma et al. 2018). By sustaining uncontainable oxidative damage, mitochondria also become a source of a vicious cycle, especially in case of reperfusion following ischemic stroke. Following the greater flow of oxygen and other circulating metabolites, due to interim sustained damage and abnormal functional status of enzymes typically involved in redox reactions, the mitochondria turn into free radical producing sources (Yang et al. 2018). Less dramatically, but still recognized, abnormal endothelial mitochondria are characteristic for a neurodegenerative process such as Alzheimer disease, though such findings were also described in cerebral amyloid angiopathy (Parodi-Rullán et al. 2019).

17.4 Future Clinical Implications

17.4.1 Diagnostics

With the expanding understanding of the immune aspects related to cerebrovascular diseases, especially acute stroke, a broader set of targeted diagnostics could be considered in each clinical scenario (Fig. 17.3). Starting from basic blood counts and the mentioned ratios, namely lymphocyte to monocyte, neutrophil to lymphocyte, and platelet to lymphocyte, one could possibly obtain additional information with prognostic value. Furthermore, a biomarker such as high-sensitivity C-reactive protein may also be investigated as a tool to predict recurrent stroke and poor functional outcome as some analyses point to such possible utility (Li et al. 2016). Besides other acute phase reactants such as D-dimers and von-Willebrand factor, central nervous system-derived proteins such as S100B are also outcome predicting biomarker candidates (Miao and Liao 2014). The redox status biomarkers at this point do not provide any additional clinical value, as patients usually present with a manifesting disease or a syndrome and getting a snapshot baseline value in a specific moment would not change acute management. However, from pathophysiological perspective, there

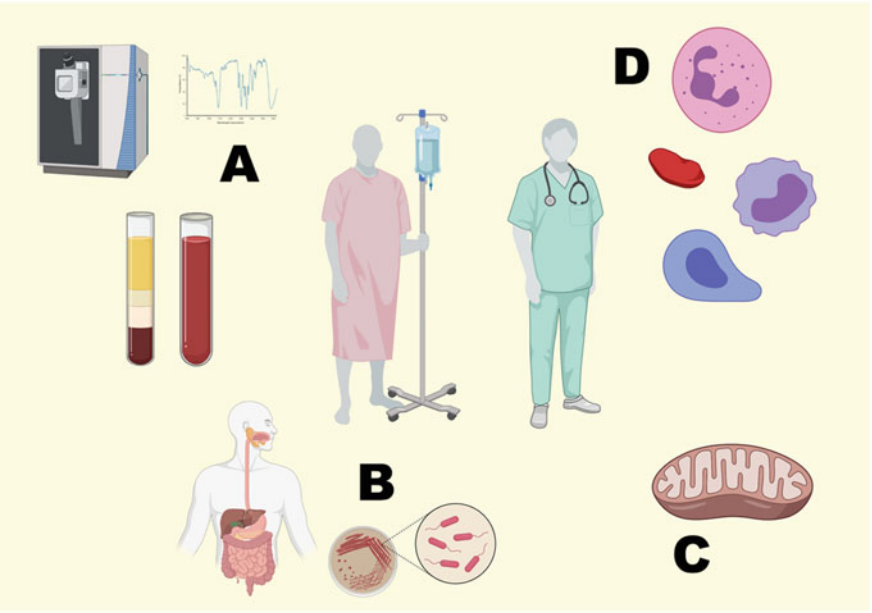

Fig. 17.3 Opportunities for future diagnostics and treatment of cerebrovascular diseases. A. Analysis of blood and serum on multiple metabolic and immunologic biomarkers, including genetic analysis and epigenetic markers to maximize personalized approach. B. Analysis of microbiota and microbial metabolites that seem to be implicated in systemic atherosclerosis and may provide opportunities for risk stratification, as well as new treatment modalities, i.e., microbiota modulation. C. Mitochondrial health seems crucial for immunometabolic processes on a subcellular level and for balanced redox status. Mitochondria could present treatment targets once more specific mitotherapies will be available. D. Various ratios of blood cells appear as potentially useful biomarkers and may have predictive value in cerebrovascular diseases, especially acute stroke

is value in studying the dynamics of redox or inflammatory status markers, as it would likely improve preventative and possibly curative attempts, as well as help with risk stratification (Miao and Liao 2014; Seet et al. 2011). A newer perspective when assessing cardiovascular health and considering preventative steps has been the focus on the gut microbiota and metabolites from commensal microbials. Initially, a higher ratio of *Firmicutes* to *Bacteroidetes* bacteria has been found in patients with atherosclerosis and associated vascular risk factors (Tang et al. 2017). Among the microbial metabolites which are present in the blood, trimethylamine N-oxide (TMAO) has been detected as a potential proatherogenic and prothrombotic metabolite, one that possibly correlates with cardiovascular risk even more than classic biomarkers such as lipoproteins (Dong et al. 2020). Trimethylamine is synthesized from dietary choline and L-carnitine by microbiota metabolism, then absorbed through the gut and metabolized to TMAO in the liver, ultimately being excreted via urine. Therefore, foods such as eggs and red meat, which have been considered harmful for cardiovascular health due to contained cholesterol, are now

being looked at from a different angle. Gut dysbiosis seems an important step in the pathophysiology of TMAO and presumably in the final process of atherogenesis. Animal studies are suggestive of TMAO-enhancing prothrombotic effects such as increasing the size of the atherosclerotic plaque and increasing platelet reactivity (Liu and Dai 2020). There are also negative animal studies with similar experimental designs failing to replicate such findings, though being substantially less common than experiments confirming results consistently. The mechanistic studies seem to point to mitochondrial ROS production and inflammasome activation as a common pathway, following inhibition of sirtuins in the liver (Boini et al. 2017; Chen et al. 2017; Liu and Dai 2020). Prospective human trials, some lasting 3–10 years, have shown a definite association between TMAO levels and the presence of cardiovascular disease, though again, in some experiments with overall much smaller patient populations the association was not confirmed (Liu and Dai 2020). A meta-analysis and a systematic review including 923 patients concluded that TMAO is definitely a new cardiovascular risk factor with potential use for personalized risk stratification (Guasti et al. 2021). Larger studies assessing prospective cerebrovascular risks are still needed, but two nested case–control studies demonstrated clear association of higher TMAO levels and occurrence of stroke (Gencer et al. 2020; Nie et al. 2018). A meta-analysis of available patient data from 6150 participants showed an inverted U-curve trend for TMAO and risk of stroke, with curve peak at 10 μmol/L being associated with the highest risk of stroke (Farhangi et al. 2020). Microbiota-based translational approaches offer new diagnostic tools, primarily blood tests detecting microbial metabolites such as TMAO, as well as microbiota composition analysis assessing for a presence of dysbiosis.

17.4.2 Therapeutic Targets

Based on epidemiological data, lifestyle modifications offer much in terms of prevention alongside conventional pharmacotherapies aimed at minimizing vascular risk factors, ideally when applied in allostatic loading phase or latent disease. On top of recommendations to quit smoking, control body weight, maintain normotension and normoglycemia, limit alcohol drinking, and increase relative dietary intake of vegetables and fruits, future advice may include minimizing consumption of dietary choline and carnitine. Microbiota manipulation with antibiotics and pro- and prebiotics could also be considered as an opportunity to address certain aspects of pathophysiology (Ahmad et al. 2019).

Regarding future pharmacotherapies or interventions, it seems there are several targets. In case of acute inflammatory burden with a huge electrophilic load, such as acute stroke, a greater number of therapeutic nucleophiles seem reasonable, though exact timing and how long one should maintain such an intervention remains to be investigated. The redox dynamics may be pharmacologically manipulated on a systemic level, but ideally only to a degree that homeostasis on a cellular level is maintained. Targeted delivery of chosen therapeutics to exact target is challenging,

and some additional opportunities are opening with further investigation of nanotherapies as ready for use modalities. Nanoparticles containing antioxidants or being made of materials with favorable redox properties, either organic or metal, are being investigated in animal models, and following appropriate safety profiles achieving clinical translation should be possible (Li et al. 2020).

In case of chronic (neuro)inflammatory processes, immunometabolic perspective offers opportunities to target the metabolic signaling to affect the immunological, and vice versa. Medications such as metformin and rapamycin are being investigated as drugs that not only improve insulin sensitivity but also seem to offer an overall rejuvenating or 'anti-aging' effect on the level of the entire organism. Without specific neurorestorative medications, such non-specific pharmacological approaches may offer a potential benefit for conditions such as chronic cerebrovascular disease, or as a preventative in the still latent phase (Blagosklonny 2019). From the side of immunomodulation and vascular health, the positive trial of colchicine for secondary cardiovascular benefits opens a perspective that would enable a broader consideration of using immunotherapies for improvement of (cerebro)vascular health (Nidorf et al. 2020).

17.5 Conclusion

Cerebrovascular health is a reflection of neurovascular unit integrity. Once compromised by acute or chronic noxious stimuli, a coupling cascade of neuroinflammation and neurodegeneration ensues. A common link of both processes is an associated pro-inflammatory status and a redox imbalance favoring net oxidative conditions. Stroke as an acute event and vascular dementia as a form of chronic cerebrovascular insufficiency are clinical entities with considerable epidemiological importance. With the expanding focus on non-traditional vascular risk factors and by exploring pathophysiological relationships, additional diagnostic and therapeutic targets may have been identified in gut microbiota and systemic immune–metabolic interactions. Further understanding of redox changes in the context of time is warranted since the subcellular environment changes differently in response to acute or chronic noxious stimuli. Such alterations have profound effects on cellular (patho)physiology, which is ultimately reflected in overall clinical status.

Disclosures None.

Ethical Statement This article does not contain any studies with human participants performed by any of the authors.

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Chapter 18
Redox Status in Age-Related Acute Mesenteric Ischemia

Suleyman Demiryas and Anıl Orhan

Abstract Acute mesenteric ischemia (AMI) is defined as the ischemia of the gastrointestinal organs secondary to occlusive or non-occlusive circulatory pathologies. Without doubt, it is one of the most lethal gastrointestinal pathologies for elderly patients. Although new treatment modalities and surgical options are introduced for modern clinical use, the mortality rates of AMI still remain significantly high in elderly population. Like all ischemic conditions, ischemic alterations and reperfusion damage have the utmost importance in AMI during the whole process. Previously, it was believed that the ischemia was responsible for the final damage alone and restoration of recirculation to an ischemic bowel was indispensable for the treatment. However, today we know re-introduction of oxygen supply to ischemic tissue also creates further oxidative damage due to the additional formation of reactive oxygen species (ROS). Reactive oxygen species exhibit serious detrimental effects on intestinal tissues, not only causing direct damage on the tissue, but also act as chemo-attractants as well as contributing to the various systemic inflammatory reactions. The interval between ischemia–reperfusion, chronological age, and the length of the affected segment also play an important role for the ROS generation rate. All these factors contribute to overall survival rates of elderly individuals.

Keywords Mesenteric ischemia · Aging · Ischemia–reperfusion injury · Reactive oxygen spices · Oxygen radicals

Co-Author's Dedication As the co-author, I would like to dedicate my efforts on this chapter to my beloved father who unexpectedly passed away a short while ago. He was not only a father to me but also an idol, a hero, a friend, a confidant, and a mentor.—Anıl Orhan, MD

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18.1 Introduction

The most basic definition of mesenteric ischemia is the ischemic condition of gastrointestinal segments due to the partial or complete impairment of the gastrointestinal system blood flow and oxygen supply. It was first described in the fifteenth century by Antonio Beniviene in Italy and mesenteric ischemic pathologies remained as an attractive topic ever since (Boley et al. 1997). Interruption of the blood flow might be acute or chronic, various etiological factors may contribute to severity of this circulatory failure. The terminal damage may differ from local ischemia of a single segment to widespread necrosis of the gastrointestinal tract (Bala et al. 2017). Since the intestines cover a lengthy portion of the gastrointestinal system, majority of the clinical cases have mainly intestinal involvements, and the term "mesenteric ischemia" is almost synonymously used for "intestinal ischemia". The incidence of AMI is roughly around 0.1–0.2%, which is relatively low compared to other benign gastrointestinal disorders; but it does have a relatively high mortality rate in general population (ranging from 27.9% to ~80% according to literature) (Schoots et al. 2004; Gupta et al. 2011; Aliosmanoğlu et al. 2013; Adaba et al. 2015; Bala et al. 2017). Although incidence rate of AMI in elderly population is roughly around 0.07%, it remains significantly higher compared to most of the common gastrointestinal emergencies such as acute appendicitis and it has higher mortality rates (1.8 times mortal compared to acute cholecystitis) in patients above 75 years of age (Kärkkäinen et al. 2015). Probably, the most significant factors determining the overall outcome are the delay of the hospital admission and the length of affected segments. Age, gender, chronic comorbidities such as hypertension, heart and kidney failures, hypercoagulability are several of the other risk factors that might have an impact on the prognosis (Crawford et al. 2016). All those predisposing risk factors directly or indirectly influence the intensity of ischemia–reperfusion damage which basically is the preparatory factor for the tissue damage throughout the process.

Unfortunately, elderly patients suffering from mesenteric ischemia disregard their complaints until the very end, and their negligence may result in an increased risk of intestinal necrosis. On the other hand, acute incidents might have more catastrophic and life-threatening consequences since most of the elderly patients suffering from chronic ischemia develop vascular collaterals to compensate regional blood flow. Overall, mesenteric ischemic pathologies are particularly challenging both for the sufferers and the clinicians; not only because of the pathology itself but also for the difficulties while choosing and manipulating an appropriate treatment for each case. In this chapter, the basics of the AMI clinic and the impact of ROS-induced ischemia–reperfusion response on AMI will be revealed.

18.2 Anatomical Aspects of the Intestinal Blood Flow

Since mesenteric ischemia is a circulatory pathology, a better understanding of the gastrointestinal vascular anatomy is essential for clinical practices.

18.2.1 Arterial Circulation

The gastrointestinal tract maintains its arterial blood flow with the help of three arteries. These three arteries are Coeliac Trunk, Superior Mesenteric, and Inferior Mesenteric Arteries (Fig. 18.1). The coeliac trunk is the first major branch of the abdominal aorta. It arises from the anterior wall of the abdominal aorta approximately around the twelfth thoracic—first lumbar vertebral levels (Feller and Woodburne 1961; Geboes et al. 2001). Shortly after originating from the abdominal aorta, the coeliac trunk divides into three separate arteries: Left Gastric Artery, Splenic Artery, and Common Hepatic Artery. Those arteries are mainly responsible for the arterial blood flow of several supramesocolic organs such as the stomach, duodenum, liver, gallbladder, and spleen (Geboes et al. 2001).

Superior Mesenteric Artery (SMA) arises from the abdominal aorta around the inferior edge of the first lumbar vertebra level (Geboes et al. 2001; Ashley and Menard 2019; Da Silva et al. 2020). SMA descends posteriorly to pancreatic corpus and splits into jejunal and ileal branches, ileocecal, right colic, and middle colic arteries which are responsible for maintaining the arterial circulation of jejunal segments,

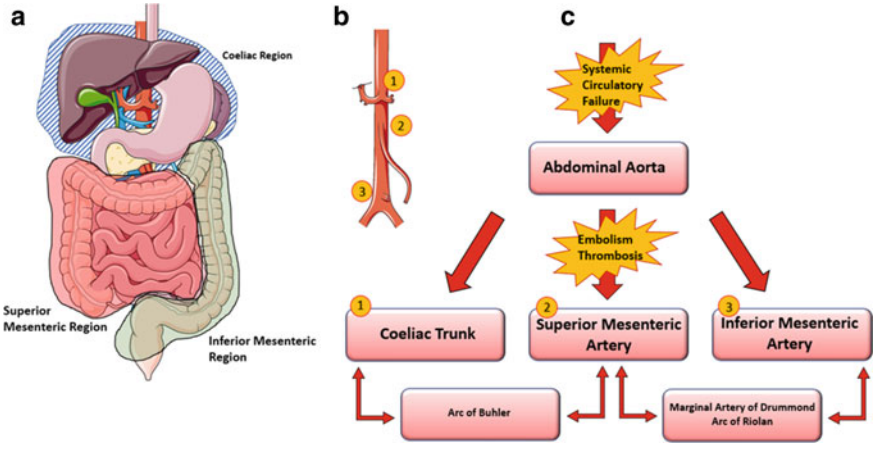

Fig. 18.1 Three major arteries responsible for gastrointestinal circulation. **a** areas of coeliac, superior mesenteric, and inferior mesenteric blood flow. **b** Origin of each artery from abdominal aorta (1—coeliac trunk, 2—superior mesenteric artery, 3—inferior mesenteric artery). **c** Possible pathologies for occlusive/non-occlusive acute mesenteric ischemia clinic and circulatory collaterals for maintaining the continuity of the gastrointestinal blood flow

ileal segments, ileocecal region, ascending colon, and proximal two-thirds of the transverse colon (Kolkman and Geelkerken 2017; Da Silva et al. 2020; White et al. 2020). Inferior Mesenteric Artery (IMA) is the last major branch of the abdominal aorta before it descends and divides into two Common Iliac Arteries. IMA separates from the abdominal aorta around the third lumbar vertebra levels; delivers left colic, sigmoidal, and superior rectal arteries which complete the arterial circulation of distal one-thirds of the transverse colon, descending colon, sigmoid colon, and the rectum (Geboes et al. 2001; Lawson 2018; Netz et al. 2019).

The coeliac trunk, SMA, and IMA have diameter of 6, 7, and 1 mm respectively, and occlusion of these arteries decrease the mesenteric vessel surface 70, 87, and 4% (Memet et al. 2019). So, it is safe to say that SMA is the most important of the three in case of a vascular occlusion (Memet et al. 2019).

18.2.2 Arterial Anastomoses and Their Clinical Significance

What makes this circulatory structure more robust and resistant to probable injuries is the formation of some connections between major arteries. "The marginal artery of Drummond" is an arterial arc formed between SMA and IMA along the mesenteric margin of the colon, and if one of the arteries is occluded, it can be compensated with the help of the marginal artery (Netz et al. 2019). "Arc of Riolan" is another interconnection between the SMA and IMA, and just like the marginal artery, it can be useful to sustain the blood flow of the intestines in case of an occlusion (Netz et al. 2019). The "Arc of Bühler" (an anastomosis between the coeliac trunk and SMA), "Arcs of Barkow" (anastomoses between epiploic arteries of the splenic artery and SMA), and "Kirk's Arcade" (an anastomosis between right gastroepiploic artery and dorsal pancreatic artery) are other interconnections, which might be beneficial to preserve the circulation, but they are rarely seen among the population (Sise 2014; Olewnik et al. 2017). These networks help to maintain the durability of the visceral circulation and they can avoid an extensive malperfusion in case of a vascular occlusion (Clair and Beach 2016).

18.2.3 Venous Circulation

Majority of the venous drainage of the gastrointestinal system is provided with the help of the Portal System. Most of the veins follow their arterial counterparts and finally combine into three major veins: Splenic Vein, Superior Mesenteric Vein (SMV), and Inferior Mesenteric Vein (IMV). Generally, IMV drains into Splenic Vein, just before Splenic Vein and SMV merge together to form the Portal Vein (PV) (Carneiro et al. 2019; Netz et al. 2019) (Fig. 18.2). However, the anatomy of the PV shows significant variations like IMV draining into SMV or into spleno-mesenteric confluence, and additional veins draining into PV such as gastric veins (Lee et al.

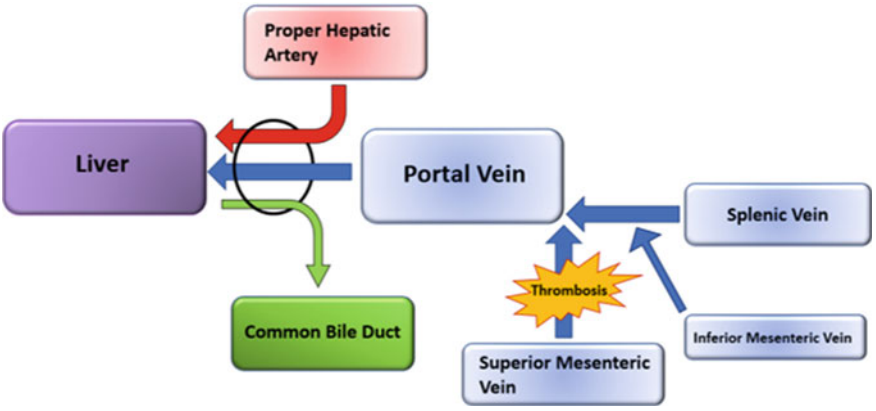

Fig. 18.2 Simplified outline of the portal circulation and possible pathologies for acute venous mesenteric ischemia

2011). Overall, the portal system delivers approximately 70–75% of the liver blood flow with the rest being supplied by the Proper Hepatic Artery (Lautt and Greenway 1987).

18.3 Etiopathogenesis

Although there are different reasons for mesenteric circulatory failures, four major causes of age-related mesenteric ischemia: an embolism to SMA, an acute thrombosis of SMA, mesenteric venous thrombosis, and non-occlusive mesenteric ischemia (Figs. 18.1 and 18.2; Table 18.1) (Savlania and Triphaki 2017). Age is an important risk factor for the mesenteric ischemia etiology, people above 80 years of age have a significantly increased risk of mesenteric ischemia, compared to people who are 60 years of age (Kärkkäinen and Acosta 2017). Moreover, a cohort study showed that while SMA embolism and SMA thrombosis have similar incident rates under 75 years, SMA thrombosis has a twofold increase compared to SMA embolism above 75 years, and incidence of SMV thrombosis is higher in patients under 75 years. (Kärkkäinen and Acosta 2017).

18.3.1 Occlusive Mesenteric Ischemia

18.3.1.1 SMA Embolism

Almost 50% of mesenteric ischemia cases occur due to an embolism to SMA (Lock 2001; Bala et al. 2017; Savlania and Triphaki 2017). Heart is the main source of these

Table 18.1 Possible etiological factors for mesenteric ischemia

Arterial embolism	Arterial thrombosis	Venous thrombosis	Non-occlusive mesenteric ischemia
• Cardiac diseases • Dysrhythmias • Myocardial dysfunction & infarction • Valve pathologies • Prior embolism	• Atherosclerosis • Aortic diseases (e.g., dissection) • Hypercoagulability • Increased blood viscosity	• Prothrombotic States • Protein C & S deficiencies • Antithrombin III deficiency • Factor V Leiden mutation • Antiphospholipid syndrome • Hyperhomocysteinemia • Pregnancy & oral contraceptive use • Inflammatory processes (e.g., Pancreatitis, peritonitis, etc.) • Neoplasms • Liver pathologies	• Hypovolemia & shock • Major surgery & insufficient preoperative preparation • Heart & renal failures • Critically ill patients & intensive care unit patients

embolies; cardiac dysrhythmias, myocardial dysfunction, anatomical deformation of cardiac structures such as valve pathologies or endocarditis may end up with an embolism formation (Bala et al. 2017). Since SMA is a sizeable artery with an oblique pattern, embolies occlude SMA more frequently than any other abdominal artery branches (Bala et al. 2017; Savlania and Triphaki 2017). Ottinger (1978) divided SMA into 4 separate segments (Region 1—the Origin, Region 2—the main trunk including the origin of the middle colic artery, Region 3—the main trunk beyond the origin of the middle colic artery, and Region 4—the more peripheral portion of the SMA), occlusion of each segment creates different clinical manifestations (Carver et al. 2016, Kärkkäinen and Acosta 2017) (Fig. 18.3).

18.3.1.2 SMA Thrombosis

Mesenteric arterial thrombosis represents 25–30% of all cases (Bala et al. 2017; Savlania and Triphaki 2017). The primary factor for thrombosis is the presence of an atherosclerotic formation and several factors such as hyperlipidemia, hypertension, diabetes, hypercoagulability, vasculitis, aortic pathologies, (f.e: aortic dissection) and infections may worsen the situation (Bala et al. 2017; Savlania and Triphaki 2017; Lawson 2018; Orihashi 2018). Even though SMA embolism is still considered as the major cause for AMI, SMA thrombosis is becoming the primary reason for age-related AMI due to several factors, such as routine utilization of anticoagulant drugs

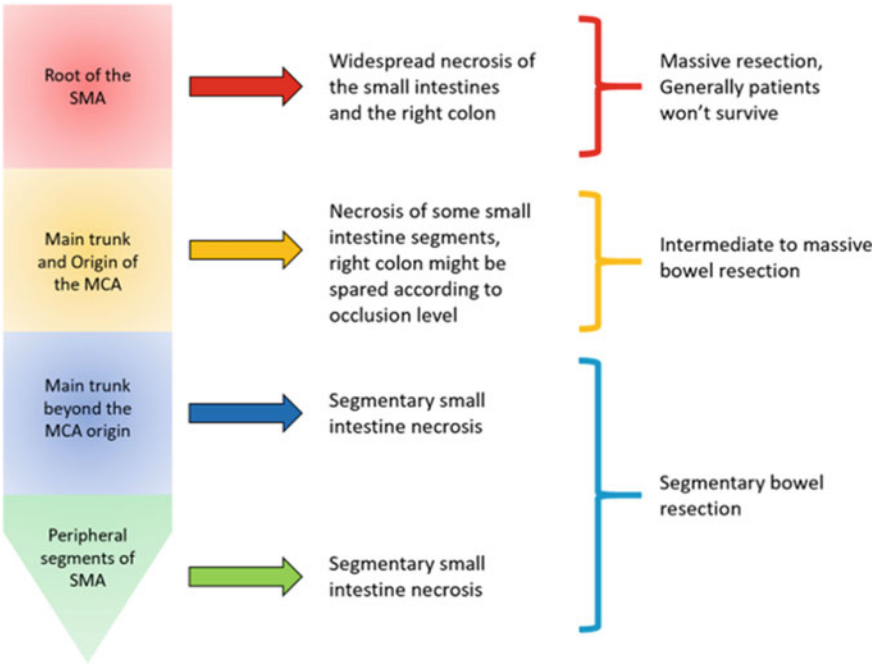

Fig. 18.3 Classification of SMA subsegments and their clinical significance. A proximal occlusion is more likely to cause severe conditions compared to peripheral portions (SMA: Superior Mesenteric Artery, MCA: Middle Colic Artery)

which prohibits embolism formation, and increased risk of atherosclerosis in the elderly population (Kärkkäinen and Acosta 2017).

The initial onset of mesenteric thrombosis has several differences compared to arterial embolism. Patients suffering from partial stenosis of an artery may develop collaterals in the time being, and this may prevent the development of apparent symptoms (Carver et al. 2016; Savlania and Triphaki 2017). In addition to that, the majority of predisposing lesions are generally located at the SMA origin, thrombosis at this level may end up with extensive necrosis if no collaterals are present or collaterals are occluded, and mortality rates of these patients remain to be the highest (around 90%) (Cangemi and Picco 2009; Wyers 2010).

18.3.1.3 SMV Thrombosis

Mesenteric venous thrombosis is not common as arterial embolism or arterial thrombosis, but the mechanism leading to venous ischemia damage is fairly different. There are lots of unique etiological factors which may contribute to venous thrombosis, such as prothrombotic states and coagulation diseases (Protein C and S deficiencies, Factor V Leiden mutation, Antithrombin III deficiency, antiphospholipid syndrome,

hyperhomocysteinemia), inflammatory processes (pancreatitis, peritonitis), liver cirrhosis, portal hypertension, abdominal surgical procedures, neoplastic processes and chemotherapy (Olgun et al. 2014; Savlania and Triphaki 2017). Once a thrombus is formed, it triggers venous congestion, which slowly deteriorates the arteriolar perfusion (Harnik and Brandt 2010; Carver et al. 2016). Disruption of the arteriolar perfusion leads to the inability to provide oxygen into the tissue, and ischemia starts to damage the enterocytes (Russell et al. 2015). Necrosis, bacterial translocation, and systemic sepsis are the terminal results of this chain of events, and they rapidly evolve into a status of hemodynamic instability and multiorgan failure (Russell et al. 2015).

18.3.2 Non-occlusive Mesenteric Ischemia

Non-occlusive mesenteric ischemia (NOMI) is the ischemia of the gastrointestinal segments without any arterial or venous circulatory obstruction (Versyck et al. 2018). Non-occlusive mesenteric ischemia roughly takes about 5–20% of all ischemic cases (Versyck et al. 2018; Stahl et al. 2020). Since the vascular pathway is not undermined with an obstructive lesion and gastrointestinal segments receive extensive ischemic damage due to the systemic nature of this pathology, patients suffering from NOMI have high mortality rates (above 70%) (Schoots et al. 2004). Critically ill patients suffering from abdominal complaints requiring vasopressor support should be suspected of having NOMI (Bala et al. 2017). Hypovolemic shock, major abdominal or cardiac surgeries, heart, and renal failures are the most common causes of NOMI, and the primary treatment remains conservative unless bowel necrosis is not detected (Kärkkäinen and Acosta 2017).

18.3.3 Age-Related Arterial Alterations, Oxidative Damage, and Mesenteric Ischemia

As predicted, advancing age has a remarkable impact on the mesenteric vascular abnormalities leading to mesenteric ischemia. Since age is a non-modifiable risk factor, it has inevitable and progressive effects on the circulatory anatomy and physiology. The density and the synthesis rate of elastic fibers gradually reduce throughout aging, and collagen fibers replace elastin mesh (Collins et al. 2014). The decrease in tropoelastin expression and smooth muscle cell quantity, matrix metalloproteinase interactions, plasmin, thrombin, and ROS-related oxidative protein modifications are commonly seen causes of elastin disappearance (Collins et al. 2014). Age-related loss of elastin network results in increased arterial wall stiffness and intima-media thickness (Collins et al. 2014). Other age-related arterial alterations include calcification of the luminal walls and dimensional variations (Collins et al. 2014). All

these modifications may lead to several vascular pathologies such as stenosis, aortic aneurysms, and aortic dissection, and enable atherosclerotic plaques to form much easier.

Aging also has detrimental effects on ischemia/reperfusion progression, and antioxidant activities which neutralize ROS damage (Shah et al. 1999). Elderly patients are much more resistant to ischemia compared to younger ones, possibly due to their collateral formations. However, they are much more susceptible to reperfusion injury (Shah et al. 1999). A decrease of antioxidant defense capacity due to advancing age may be a relevant explanation. Moreover, production of anti-inflammatory and protective mediators, such as endothelial nitric oxide, decreases over the time (Miyashiro et al. 1997; Sheridan et al. 2007). As expected, ischemia–reperfusion related redox reactions and inflammatory processes tend to be much more aggressive without the presence of all these protective mediators. Aging-dependent impaired intravascular redox homeostasis is another factor that may contribute to the vulnerability of elderly patients to vascular damage. Presence of oxidized proteins, lipids, and DNA in the plasma increases parallel to aging and it accelerates both the structural and functional intravascular alterations (Çakatay et al. 2008, 2010; Cebe et al. 2014).

18.3.4 Sex, Oxidative Damage and Mesenteric Ischemia

Sex has always been an important predisposing factor for almost every disease, and it is not different for age-related AMI. According to the experimental studies, the increased risk of cardiovascular diseases for males, detrimental effects of testosterone, and protective effects of estrogen are possible reasons for gender-related differences caused by ischemia–reperfusion injuries (Mester et al. 2018; Hundscheid et al. 2020). A recent study (Hundscheid et al. 2020) has shown that females display less epithelial damage, less inflammatory response, and less release of enterocyte-specific biomarkers compared to males after suffering similar mesenteric ischemia–reperfusion damage. Since estrogens are strong antioxidants, which have anti-apoptotic activities, and anti-inflammatory properties, females remain much more resistant to oxidative stress and ROS damage (Hundscheid et al. 2020). These immunological differences increase the survival rates of females compared to males, decrease the intensive care unit (ICU) admissions, and decrease the possibility of severe sepsis and septic shock (Hundscheid et al. 2020). According to a retrospective study (Peterson et al. 2011), women who are between 18 and 40 years of age had 33% and women who are over 50 years of age had 17% lower mortality compared to men after gastrointestinal surgery. Same study concluded that effects of estrogens may remain potent beyond the reproductive age, but further assessment is needed for confirmation (Peterson et al. 2011).

18.4 Reperfusion Injury and Acute Mesenteric Ischemia

18.4.1 Effects of Ischemia on Cellular Metabolism

Probably the most critical phenomenon determining the severity of ischemic conditions is the ischemia–reperfusion injury. Whatever the reason is, hypoxemia triggers mitochondrial dysfunction, and it evolves to the disruption of the aerobic cellular respiration if the hypoxemia prolongs (Cerqueira et al. 2005; Wu et al. 2018). The absence of aerobic respiration decreases ATP production which is indispensable for keeping basic cellular mechanisms ongoing. These mechanisms include the functioning of several ATP-dependent ion channels and intracellular organelles such as ribosomes and endoplasmic reticulum (Wu et al. 2018). If the duration of hypoxemia increases, cells promote anaerobic cellular respiration in order to regenerate ATP to maintain energy. However, anaerobic cellular respiration is not effective as its aerobic counterpart, and moreover, anaerobic respiration produces several side products such as lactic acid (Wu et al. 2018). All of these intracellular interactions create a hyperosmolar acidotic state in the cell which initiates organelle disintegration, cell swelling, and eventually cellular death (Wu et al. 2018) (Fig. 18.4).

18.4.2 ROS Formation and Reperfusion

Reperfusion is the crucial stage for post-ischemic events. During a standard ischemia/reperfusion process, when oxygen is redistributed to the tissues, it is possible to restore the energy supply back, and toxic materials can be removed if the cellular damage is reversible (Grace 1994; Cerqueira et al. 2005). However, if the ischemia is severe and extensive, a stronger reperfusion answer might be initiated, which can lead to apoptosis and cell death (Cerqueira et al. 2005; Kalogeris et al. 2012). This manifestation is also called as the “oxygen paradox”, first described by Hearse, Humphrey and Chain back in 1973 (Hearse et al. 1973), which can be defined as an increase of damage instead of improvement in ischemic tissues when reoxygenation starts (Hess and Manson 1984; Reginelli et al. 2013). At this point, the presence of ROS is one of the key factors accelerating the ischemia/reperfusion damage. Under physiological circumstances, ROS are generated during normal cellular reactions and antioxidant activity eliminates these side products (Shah et al. 1999). However, ischemia followed by reperfusion increases the amount of ROS to a level which antioxidant activity cannot manage to neutralize all (Fig. 18.4). The electron transport chain is considered to be the major ROS source inside the cell (Bhattacharyya et al. 2014). Other endogenous ROS sources include the xanthine oxidoreductase system, nitric oxide synthase activity, the respiratory burst initiated inside the activated neutrophils, myeloperoxidase activity, and arachidonic acid metabolism (lipoxygenase and cyclooxygenase pathways) (Cerqueira et al. 2005; Kalogeris et al. 2012; Bhattacharyya et al. 2014; Wu et al. 2018).

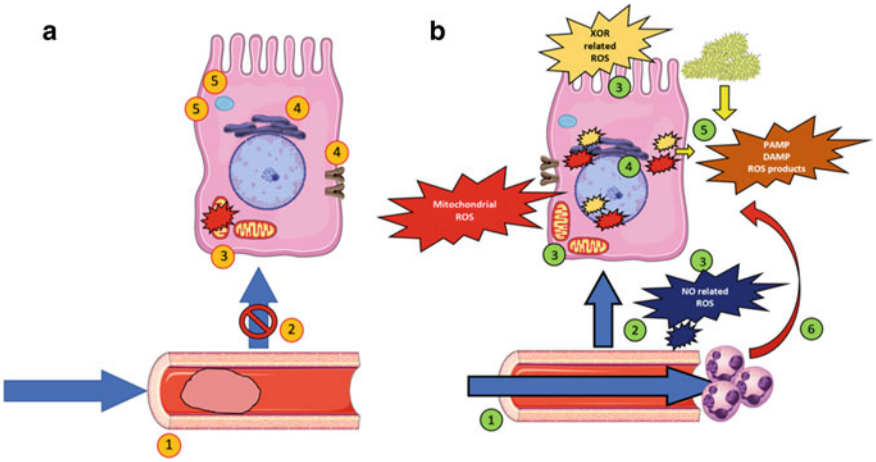

Fig. 18.4 Ischemia/reperfusion reactions in enterocytes. **a** Occlusion of arteries (1) results in hypoxemia (2). Mitochondria switches to anaerobic respiration and ATP levels decrease (3). ATP-dependent functions of organelles gradually reduce (4) which triggers intracellular electrolyte imbalances, swelling, and organelle disintegration. Lysosomal disintegration and apoptosis eventually lead to cellular death (5). **b** When vascular continuity is established (1), oxygen is reintroduced into the ischemic region (2). Oxygen initiates the generation of reactive oxygen species (ROS) in intracellular and intercellular compartments (3). ROS further damages organelles, cell membrane, and intracellular structures (4). Increased concentrations of ROS, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) (in case of a bacterial contamination/translocation) attract neutrophils and macrophages inside the ischemia/reperfusion region which causes further inflammatory reactions (5 and 6). [XOR—Xanthine Oxidoreductase, NO—Nitric Oxide]

18.4.2.1 Mitochondrial ROS

Under normal circumstances, oxygen is converted to carbon dioxide and water after a chain of reactions inside the mitochondria during the aerobic respiration (Osellame et al. 2012). These chains of reactions are conducted by two different cycles integrated into each other: The citric cycle (the Krebs cycle) and the electron transport chain. The citric acid cycle is a series of biochemical reactions in which a two-carbon acetyl group of acetyl-CoA and four-carbon oxaloacetate form a six-carbon citrate (Osellame et al. 2012). The citric cycle also transfers electrons released during the reactions onto the cofactors nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). These cofactors carry electrons to the electron transport chain located on the inner-mitochondrial membrane (Osellame et al. 2012). The electron transport chain is a group of protein, which generate a proton efflux using the electrons released from the citric acid cycle (Osellame et al. 2012). This gradient builds a potential difference, and it is used for the synthesis of ATP (Osellame et al. 2012). During these reactions, a fairly small amount of oxygen molecules (around 1–2%) escape the normal pathway, and they convert to oxygen-derived free radicals (Cerqueira et al. 2005; Bhattacharyya et al. 2014). Even though the amount of

ROS produced through the mitochondrial reactions is relatively small under normal circumstances, it has an essential impact on the cellular aging process (Fusco et al. 2007).

18.4.2.2 Xanthine Oxidase and ROS

The liver and intestines accommodate considerable amounts of xanthine oxidoreductase (XOR) compared to the other organs; and therefore, these organs are much more susceptible to ROS damage induced by XOR reactions after an ischemia–reperfusion related event (George and Struthers 2009). XOR is a flavoenzyme that takes part in purine metabolism. It catalyzes the oxidation of hypoxanthine to xanthine, and further to uric acid. Xanthine oxidoreductase can be active in two forms as xanthine dehydrogenase (XDH) and xanthine oxidase (XO), and it is the only enzyme capable of forming urate in humans (George and Struthers 2009). Under the ischemic conditions, XDH forms XO (Schoenberg and Beger 1993). XO is unable to continue its activity without the presence of molecular oxygen and hypoxanthine accumulates inside the ischemic tissue (Shah et al. 1999; Kalogeris et al. 2012). During the reperfusion stage, XO rapidly converts the accumulated hypoxanthine to uric acid after the oxygen is reintroduced into the tissue; however, this reaction results in an increased concentration of ROS like superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2), which also acts as a signal transducer, since it exceeds the limits of the antioxidant capabilities of the tissue (Shah et al. 1999; Bhattacharyya et al. 2014). Finally, H_2O_2 undergoes a further reaction which forms a very reactive hydroxyl radical (OH^-) (Cerqueira et al. 2005).

18.4.2.3 Nitric Oxide Synthase and ROS

Nitric oxide synthase (NOS) activity is another potential ROS source. NOS is a heme-containing oxidoreductase enzyme that converts L-arginine to L-citrulline and nitric oxide (NO). NOS enzymes have different isoforms based on their gene expressions and locations: neuronal NOS (nNOS—NOS 1 gene located at chromosome 12), inducible NOS (iNOS—NOS 2 gene located at chromosome 17), and endothelial NOS (eNOS—NOS 3 gene located at chromosome 7) (Alderton et al. 2001, Bhattacharyya et al. 2014). NO has various roles in human physiology as it induces vasodilatation, has anti-aggregate effects on thrombocytes, inhibits adhesion capabilities of neutrophils & thrombocytes, inhibits smooth muscle cell proliferation, and such (Roselli et al. 1998). However, when NO combines with O_2^-, they form a potent reactive nitrogen oxide species (RNS): peroxynitrite ($ONOO^-$) which is primarily responsible for so-called “the nitrosative stress” (Cerqueira et al. 2005; Kalogeris et al. 2012).

18.4.2.4 Antioxidant Defense Mechanisms

Antioxidant defense is crucial for countering the harmful effects of ROS. Antioxidant defense systems include endogenous enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase; endogenous non-enzymatic antioxidants such as glutathione, thioredoxin, and melatonin; and exogenous antioxidants such as vitamin C—E and A, zinc, iron, selenium, manganese, and copper (Bhattacharyya et al. 2014). Similar to other body compartments, glutathione is the most abundant antioxidant in the gastrointestinal tract (Brown et al. 2014). The activity of glutathione is regulated by several glutathione dependent enzymes, such as glutathione reductase, glutathione peroxidase (Gpx), glutathione S-transferase, and glutaredoxin (Grx). These enzymes have various isoforms; however, some of these isoforms are highly expressed in the intestines and gastrointestinal system (e.g., Grx 2 and 5, Gpx 1 and 2) (Godoy et al. 2011). Superoxide dismutase, catalase, and peroxiredoxins are other important antioxidant enzymes, which protect the gastrointestinal tract against oxidative stress. All of these antioxidant systems remove ROS by undergoing oxidation themselves and they neutralize the potential threat to other tissues (Bhattacharyya et al. 2014). Capabilities of antioxidant enzymes reduce with age (Kozakiewicz et al. 2019), and elderly patients become more susceptible to ROS-related damage.

18.4.2.5 Reperfusion Related ROS Damage in Acute Mesenteric Ischemia

Ischemia–reperfusion damage is probably the most influencing factor for intestinal viability in case of an AMI event. As expected, an ischemia–reperfusion sequence is much more detrimental compared to ischemia itself (Bhattacharyya et al. 2014). Reperfusion has two major stress factors on the ischemic tissue: the creation of ROS and the accumulation of neutrophils/macrophages inside the reperfusion zone (Bhattacharyya et al. 2014).

ROS created during oxidative and nitrosative stresses trigger cellular alternations, and damage using various mechanisms. They can directly damage cellular macrostructures, such as cell membranes, DNA, cellular proteins, and lipids; or they can affect cellular signal mechanisms and molecular pathways (Kalogeris et al. 2012). ROS are responsible for lipid peroxidation of cell membranes, which deteriorate the cell membrane integrity, and this also leads to the formation of "lipid peroxide" radicals during the process (Horton and Walker 1985; Cerqueira et al. 2005). In addition to ROS activity, these lipid peroxides further damage the other lipid components of all cellular membranes, including lysosomes, and the release of proteolytic enzymes eventually leads to necrosis and cell death (Cerqueira et al. 2005).

Neutrophil/macrophage migration to the ischemia region is the second stress factor inflicting damage on the enterocytes during the ischemia–reperfusion event. As discussed above, ischemia triggers a cascade of events, and these reactions eventually lead to an inflammatory response. This inflammatory response may occur with

the absence of microorganisms, which is called "sterile inflammation", or it may contain pathogens, which adds another dimension to the whole process (Kalogeris et al. 2012). As the inflammation progresses, pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs) concentrations increase gradually (Kvietys and Granger 2012). When the reperfusion starts, in addition to an accumulated production of ROS, PAMPs and DAMPs activate the intravascular (neutrophils) and perivascular (macrophages/mast cells) immune cells (Kvietys and Granger 2012; Bertoni et al. 2018). When the activated neutrophils and macrophages arrive at the ischemic region, they do not only initiate phagocytosis but also release hydrolytic enzymes (e.g., collagenase, elastase, proteases, cathepsins) and inflammatory cytokines, which exuberate the damage potential (Cerqueira et al. 2005; Kalogeris et al. 2012; Kvietys and Granger 2012) (Fig. 18.4).

The accumulation of leukocytes also causes another status which is known as "the no-reflow phenomenon" (Cerqueira et al. 2005; Kalogeris et al. 2012). This phenomenon can be described as the failure of microvascular reperfusion despite the blood flow re-established during an ischemic condition (Kloner et al. 2018). An increase of chemo-tactical factors and ROS production precipitate an exaggerated neutrophil migration to the capillaries. A decrease of NO synthesis and overexpression of endothelial adhesion molecules enhance the adhesion and aggregation capabilities of leukocytes. With the help of the acidic environment in the ischemic tissue, these leukocytes lose their flexibility, and they physically occlude these microvascular structures (Gute et al. 1988; Kalogeris et al. 2012). Moreover, the mucosal barrier disruption initiated with neutrophils results in an edema formation that increases the perivascular pressure, which prevents microvascular perfusion (Gute et al. 1988; Kalogeris et al. 2012). This is probably one of the important reasons why necrosis continues to develop, even if the reperfusion of the ischemic tissues commences.

18.5 Clinical Manifestations of Acute Mesenteric Ischemia

18.5.1 Clinical Presentation

Clinical presentation of AMI differs according to the underlying etiology. If the ischemia is triggered right after an acute embolic event, or when thrombosis finally occludes the lumen completely, symptoms incline to be more apparent and clamorous. Pain is the most common and consistent symptom among the patients, and peritoneal irritation signs (e.g., abdominal guarding, rebound tenderness) are common if the necrosis of an intestinal segment and/or perforation is present (Cudnik et al. 2013; Clair and Beach 2016; Lawson 2018).

On the other hand, partial occlusion of mesenteric arteries provokes a totally different set of symptoms which are similar to chronic mesenteric ischemia. The most common symptom of these patients is postprandial abdominal pain, which is also called as "the mesenteric angina" and duration of the pain may extend up to

four–six hours after the meal (Kolkman and Geelkerken 2017). Patients prefer to eat their meals less than usual to avoid pain and this leads to another symptom, loss of weight (Kolkman and Geelkerken 2017). Abdominal distension, vomiting, diarrhea, and blood in the stool are some of the other symptoms which these patients may suffer (Oldenburg et al. 2004; Carver et al. 2016; Kolkman and Geelkerken 2017).

There are several distinctive physical examination findings which the clinician must be aware of, and if these findings are present, mesenteric ischemia must be included in the differential diagnosis. "Paradoxical abdominal pain" is the first of these findings. Patients complain from an insufferable abdominal pain whereas the physical examination reveals only minimal tenderness in the early mesenteric ischemia (Carver et al. 2016; Bala et al. 2017). The probable explanation for this paradox is the lack of transmural infarct and lack of peritoneal irritation at the early stages. Auscultation of bowel sounds is another helpful examination finding; however, it is only useful at the later stages (Carver et al. 2016). If the ischemia evolves into necrosis, peristaltic activity of necrotic segments will cease and the clinician will not be able to hear any bowel sounds, which can be identified as "the silent abdomen" (Cudnik et al. 2013).

Unfortunately, none of these examination findings are helpful to diagnose AMI on their own and many of these findings can also be seen in other abdominal pathologies like colitis, pancreatitis, irritable bowel syndrome, diverticulitis, gastric diseases and such (Clair and Beach 2016). The diagnosis still remains as a challenge today, and patients suspected of mesenteric ischemic pathologies can be missed easily if they do not undergo further evaluation (Carver et al. 2016).

18.5.2 Diagnostic Tools for Acute Mesenteric Ischemia

18.5.2.1 Laboratory Assessment

Clinical Biomarkers

As we mentioned above, the clinical presentation of mesenteric ischemia can be insignificant at the first glance, and it might require additional confirmatory tests to diagnose AMI before it's too late.

Laboratory routines such as white blood count, serum urea and creatinine levels, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase (LDH), creatinine kinase, amylase are not particularly useful to predict the AMI since their levels tend to increase in other abdominal pathologies and inflammatory processes as well (Powell and Armstrong 2014). Even though all these clinical laboratory biomarkers are not useful on their own in the early stages of mesenteric ischemia, a rapid increase of these biomarkers is beneficial to guess the severity of the inflammatory process and serial laboratory routines are advised to determine the AMI development in the early phases (Powell and Armstrong 2014).

Lactate

Lactate has two separate isomers. L-lactate is an end-product of the glycolysis and it is formed from pyruvic acid by the LDH (Isfordink et al. 2018). L-lactate is produced by all human cells, especially under vigorous activity and restricted oxygen perfusion (Powell and Armstrong 2014). Liver can eliminate large amounts of lactate from the portal system and limits its serum concentrations (van den Heijkant et al. 2013; Powell and Armstrong 2014). The second isomer is D-lactate, which is not synthesized by human cells but produced by bacterial flora (Isfordink et al. 2018). D-lactate can be metabolized by D-LDH, and increased levels of D-lactate might be related with gastric bypass surgery, short gut syndrome, probiotic use, and bacterial overgrowth inside the intestinal lumen (Powell and Armstrong 2014; Isfordink et al. 2018).

Like many other diagnostic modalities, lactate levels do not increase in the early beginning of the ischemia (Kärkkäinen and Acosta 2017). However, when the extent of ischemia grows and lactate level exceeds the clearance capabilities of the liver, serum lactate level gradually increases. Even though the increase of L-lactate and D-lactate levels have high sensitivity (around 80–90%), they have low specificity (around 40–45%) (Cudnik et al. 2013). It is believed that lactate is helpful to determine whether if there is an ischemic event ongoing or not; however, it is not useful for differentiating the underlying pathology. The solitary use of lactate is not recommended to differentiate the early intestinal ischemia or the necrosis, and it is unreliable unless other confirmatory tests support the presence of the clinic (Bala et al. 2017).

D-Dimer

D-dimer is a fibrin degradation product, it is a useful marker to evaluate fibrinolysis and D-dimer levels can increase in a variety of thrombotic events (Powell and Armstrong 2014; Memet et al. 2019). Since the fibrin degradation is a physiological reaction, low levels of D-dimer are detectible in healthy individuals (Linkins and Takach Lapner 2017). The increase of D-dimer levels is age dependent, and it has been shown that D-dimer levels of people over 70 years of age are 2–5 times greater than those who are under 50 years of age (Haase et al. 2013).

D-dimer is a useful biomarker to exclude the presence of occlusive mesenteric ischemia in the early stages and it shows high sensitivity, specificity, and accuracy (60%, 82%, and 79%, respectively] (Block et al. 2008; Bala et al. 2017). However, D-dimer has a high sensitivity (96%) but a low specificity (40%) overall, similar to lactate (Cudnik et al. 2013). In the clinical use, D-dimer is one of the most essential biomarkers to evaluate whether if there is a thrombotic process ongoing or not. On the other hand, D-dimer level can increase in different inflammatory or organ related pathologies as well, which decreases its use in the differential diagnosis.

Fatty Acid Binding Protein (FABP)

Fatty Acid Binding Proteins (FABPs) are intracellular proteins responsible for fatty acid transport in the tissues. FABPs have different tissue specific subtypes, and liver-FABP (L-FABP) and ileal-FABP (I-FABP) are the FABPs mainly expressed in the

gastrointestinal system (Furuhashi and Hotamisligil 2008). Since I-FABP is mainly located at the mucosal villi of the enterocytes, they are fairly specific for intestines (Memet et al. 2019). Experimental studies revealed that aging reduces the lipid absorption and is associated with reduced abundance of I-FABP (Woudstra et al. 2004). I-FABP levels are low in the circulation due to its fairly rapid clearance rate, and I-FABP levels rapidly increase if there is an enterocyte damage, which makes I-FABP a viable marker to determine the intestinal injury (Powell and Armstrong 2014; Memet et al. 2019).

I-FABP is much more promising compared to lactate and D-dimer as a potential biomarker for detecting intestinal injury in clinical use. Combined sensitivity and specificity rates of I-FABP tests for AMI diagnosis are above 70% and 85%, respectively, according to several meta-analysis (Cudnik et al. 2013; Sun et al. 2016). I-FABP levels progressively increase in accordance with the ischemia duration and only return to its baseline values when reperfusion is established (Ho et al. 2020). Probable disadvantages of I-FABP tests arise if the differential diagnosis includes other intestinal diseases such as inflammatory bowel diseases and Coeliac disease, and its unable to predict the extent and the duration of the ischemia (Ho et al. 2020).

Experimental Biomarkers

Serum alpha-glutathione S-transferase (alpha-GST) and cobalt-albumin binding essay (CABA) are potential biomarkers, which might have high diagnostic accuracy, but these tests do not have extensive clinical use (Cudnik et al. 2013; Bala et al. 2017).

Unfortunately, none of these biomarkers, including lactate, d-dimer, and I-FABP, reflect the ROS reactions on their own. However, they are useful for physicians to understand whether if there is a severe ischemia/reperfusion event that is ongoing or not.

18.5.2.2 Radiological Assessment

Radiological assessment of AMI is essential. Since physical exam and laboratory findings do not reveal the underlying pathology for most of the cases, radio-diagnostic evaluation is the main modality for a swift and accurate diagnosis.

Main radiological tests for diagnosing AMI include abdominal X-rays, Doppler ultrasonography, contrast enhanced multidetector imaging (computerized tomography (CT) & magnetic resonance imaging (MRI)), and angiography (McCarthy et al. 2015; Savlania and Triphaki 2017). However, X-ray films are only helpful at the later stages of AMI (only if intestinal distention or perforation develop), Doppler ultrasonography can be challenging due to technical difficulties (e.g., patient's body proportion & weight, presence of intestinal gas), and angiography is an invasive method which clinicians do not prefer unless they wish to perform therapeutic interventions (Jaster et al. 2016; Savlania and Triphaki 2017). On the other hand, contrast enhanced CT is a non-invasive method which has an excellent sensitivity and specificity, it is easily accessible, and widely accepted as the first choice imagining for AMI (McCarthy et al. 2015; Copin et al. 2018; Sinha et al. 2020).

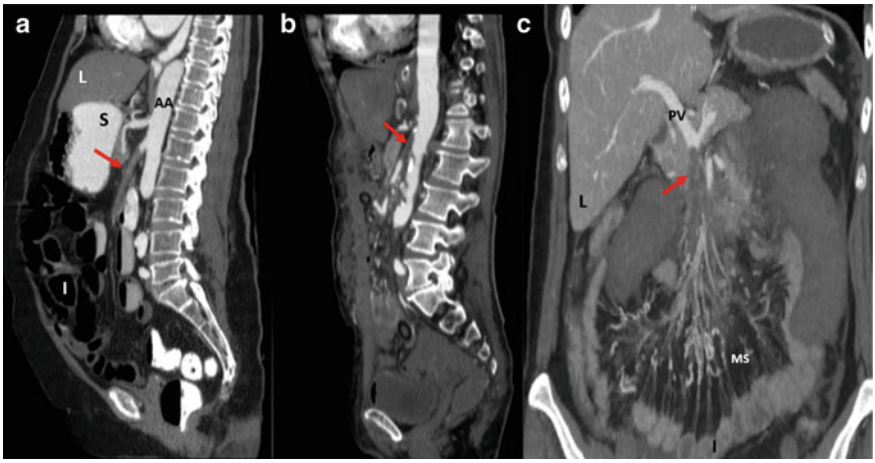

Fig. 18.5 Contrast enhanced abdominal CT images: (**a**) sagittal view of SMA occlusion (**b**) sagittal view of SMA thrombosis (**c**) coronary view of SMV thrombosis (red arrows show emboly/thrombus formations) (L: liver, S: stomach, I: intestines, AA: abdominal aorta, PV: portal vein, MS: mesentery)

Multidetector CT findings include intraluminal filling defects in occlusive mesenteric ischemia (embolus or thrombosis) (Fig. 18.5), vasoconstriction in non-occlusive mesenteric ischemia (reversible if the underlying cause is removed), thickening and edema of the intestinal wall, pneumatosis intestinalis, mesenteric strands, paper-thin bowel wall, and porto-mesenteric venous gas in severe ischemia and necrosis (Copin et al. 2018; Kanasaki et al. 2018; Sinha et al. 2020) (Fig. 18.6). Several other gastrointestinal diseases such as Crohn's disease, infectious colitis, radiation enteritis might also mimic mesenteric ischemia CT findings (Sinha et al. 2020). Even if the bowel findings are similar in these pathologies, occlusive mesenteric pathologies are distinctive due to vascular emboly/thrombus formations.

The correlation between radiological findings and ROS response in mesenteric ischemia is not well established. Since ischemia–reperfusion and ROS-related damage increase with the length of affected segments, CT is useful for clinicians to understand the magnitude of the reperfusion damage ongoing.

The use of nuclear imaging such as scintigraphy is not efficient since AMI is an emergency, and such imaging methods will consume time, which is precious both for the patient and the physician.

18.5.3 Surgical Procedures

Probably the first successful surgery for mesenteric ischemia conducted by Elliot, who resected infarcted intestines, created a pair of stomas, and reanastomosed them

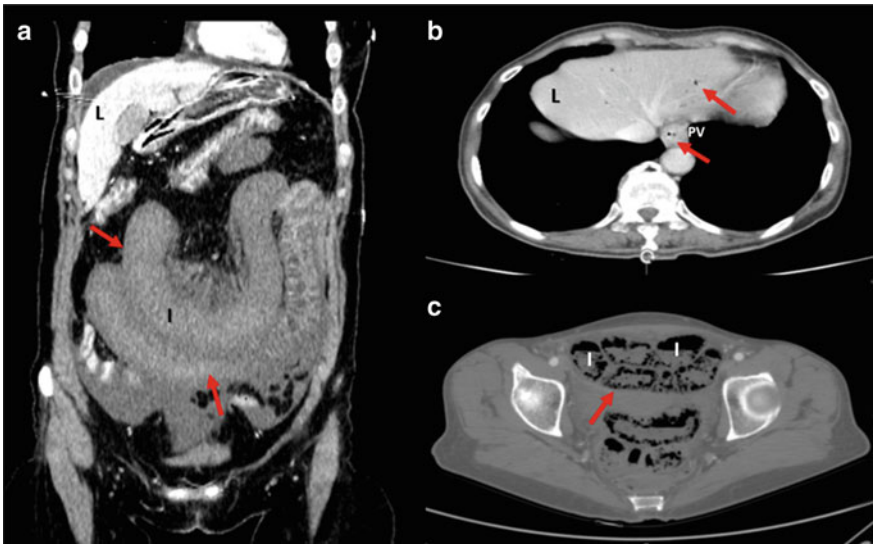

Fig. 18.6 Contrast enhanced abdomen CT images: (**a**) intestinal edema and bowel wall thickening (coronal view) (**b**) portal venous gas (axial view) (**c**) pneumatosis intestinalis (axial view) (red arrows indicate pathological alterations) (L: liver, I: intestines, PV: portal vein)

afterward back in 1895 (Boley et al. 1997). However, treatment modalities of mesenteric ischemia took a huge leap forwards when Klass introduced the concept of re-establishing the blood flow to ischemic but viable bowel (Boley et al. 1997). The modern surgical intervention is based on this concept; resection of necrotic intestines and reintroduce the blood flow to ischemic segments if they are recoverable (Carver et al. 2016; Bala et al. 2017).

Laparotomy is the only option for the surgeon if physical examination indicates obvious peritonitis. Once the laparotomy goes underway, the surgeon must evaluate the intestinal segments as a whole. The presence of necrotic segments leaves no option but excision (Fig. 18.7). However, determining the viability of ischemic segments is the major challenge for the surgeon. Excessive resection of intestines might result in intestinal failure, so the primary goal must be preserving the intestines. Peristalsis of the intestinal segments can be determined by intraoperative examination (hands and eyes) and electromyography, oxygenation can be determined by photoplethysmography, and perfusion can be determined by Doppler ultrasonography, fluorescein, and indocyanine green if technical capabilities are available (Bryski et al. 2020). The basic principle for laparotomies must be based on the "Damage Control Surgery" strategy, the surgeon must consider minimal traumatization (e.g., excision of necrotic segments, evaluation and revascularization of vascular structures, irrigation, and aspiration if the intraabdominal cavity is contaminated) and must plan re-laparotomies if necessary (Bala et al. 2017). Anastomoses should be left for re-laparotomies (second or third look re-laparotomies) if the surgeon suspects the viability of the intestinal segments.

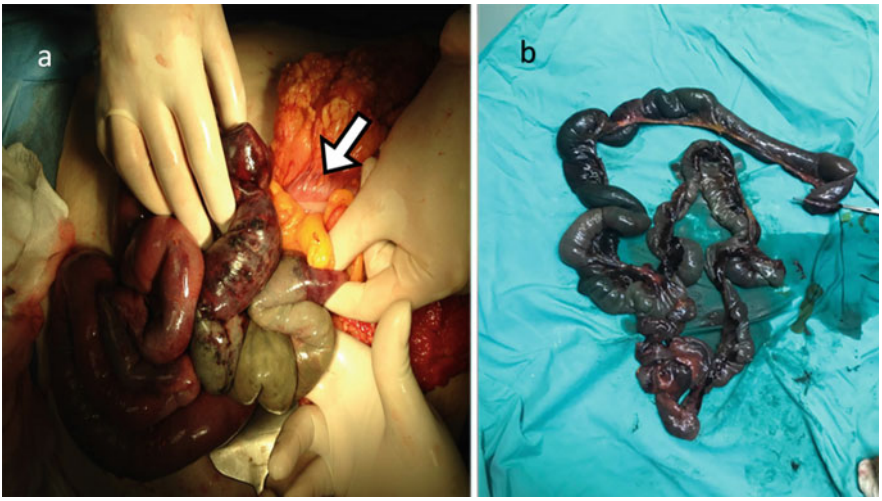

Fig. 18.7 Intraoperative images (**a** Intraoperative examination of necrotic intestinal segments, white arrow shows a normal colonic segment. **b** Excised necrotic intestine)

Revascularization is another essential component of the surgical procedures. Most of the time, since the extend of ischemia and/or necrosis depends on vascular occlusion, surgeon must be aware of vascular continuity. Intraoperative physical examination (e.g., palpating SMA), Doppler ultrasonography, and catheterization are widely used methods to assess the luminal occlusions (Clair and Beach 2016; Kanasaki et al. 2018). Minimally invasive endovascular therapies (EVT) have gained considerable popularity when introduced into routine clinical usage, and performing EVT is a beneficial option if the patient's clinical condition is not deteriorating (Tilsed et al. 2016). EVT is also a plausible choice for elderly cases and patients with poor preoperative condition if there is no evidence of bowel necrosis (Acosta 2015; Zhao et al. 2016). However, the presence of peritonitis leaves no option but the laparotomy, and hybrid procedures which include resection of necrotic segments and intraoperative revascularization (Savlania and Triphaki 2017).

18.5.4 Prognosis of Acute Mesenteric Ischemia

Managing elderly patients suffering from AMI is a challenging task, and unfortunately, the overall prognosis is not favorable in many cases. Mortality rates may increase under the presence of advanced age, colonic involvement, multiorgan failure and sepsis, time delay to surgical interventions, and ICU admission (Acosta-Merida et al. 2006; Carver et al. 2016; Caluwaerts et al. 2019). It also has been shown that high levels of lactate, presence of renal failure, and necessity of vasopressor usage in the postoperative ICU period increase the mortality rates (Murphy et al. 2018). It is

advised to consider palliative care for elderly cases who have late-stage presentation and excessive necrosis, because they are unlikely to benefit from invasive procedures (Tilsed et al. 2016). Comorbidities also increase the mortality rates for patients who have significant comorbidities and suffer from poor long-term survival if they undergo aggressive treatment (Carver et al. 2016).

Our own surgical experience is amenable to the literature as well. A total of 27 patients were operated for AMI in 15 years. Out of 27 patients, 13 died due to the detrimental effects of the surgery or postoperative complications (48.1%). Thromboembolic diseases were the primary cause (21/27), and SMA was the most occluded vessel (22/27) according to our preoperative and intraoperative examinations. Age was a significant factor for mortality; the median age of patients who died and survived from AMI were 72 and 57.3, respectively. Only five patients were below 50 years of age, neoplastic processes were the major cause of AMI in this group (4/5), and only one patient succumbed to death due to preoperative cardiac arrest. Colonic involvement was another factor, which was significantly associated with mortality; only three of eleven patients who were suffering from colonic involvement managed to survive (3/11). Finally, revascularization significantly increased the survival rates (no embolectomy group: 30% vs embolectomy group: 65%).

18.5.5 Potential and Promising Treatment Modalities

In the last two decades, physicians concentrated on preventing and ameliorating the ischemic damage on the tissue. Experimental studies proved that we still have room for improvement.

Interleukin 6 (IL-6) is a well-known mediator which has both proinflammatory and anti-inflammatory effects. An experimental study demonstrated that low doses of IL-6 decreased intestinal injury, improved perfusion, and modulation of interleukin 10 (IL-10) and vascular endothelial growth factor. However, the same study also showed higher levels of IL-6 worsened the inflammatory response (Te Winkel et al. 2019).

Etanercept is a tumor necrosis factor-alfa (TNF-α) inhibitor which is widely used for rheumatological diseases. The increase of TNF-α has been associated with an increased free radical generation and decreased NO production. A study on etanercept and ischemia–reperfusion damage showed that the use of etanercept reduced the effects of inflammation, and restored vascular contractility during the reperfusion stage (Öziş et al. 2018).

Allopurinol is a xanthine oxidase inhibitor, which is widely used for treating diseases with increased levels of uric acid such as gout and paraneoplastic disorders. Since xanthine oxidase metabolites are important components in ROS-related AMI damage, allopurinol decreases the epithelial damage induced by xanthine oxidase related ROS (Frishman et al. 2008; Carver et al. 2016).

Other promising agents include sesamin (Sayhan et al. 2019), β-alanine and aprotinin (Dominowski and Kirsch 2021), glutamine (Xu et al. 2020), and tachykinin

peptides (Umer et al. 2020). It is safe to say that future studies will enhance our capabilities to deal with AMI much more effectively.

18.6 Conclusion

Acute mesenteric ischemia is an important age-related disease, which has high rates of mortality and morbidity. Even though it is a fairly unique disease in gastrointestinal disorders, underlying mechanisms are fairly basic, and ischemia–reperfusion reactions play a crucial role through the process. Elderly patients are more inclined to suffer from the disease due to age-related degenerative alterations of vascular structures and vulnerability against oxidative damage. The interval between the start of the complaints and hospital admission is fairly important because ischemia may evolve to necrosis, which has an immense impact on the course of the treatment and survivability. Mortality rates still remain high, despite notable advancements achieved in surgery and postoperative care. Experimental studies are concentrated on preventing or recovering the ischemia–reperfusion related tissue damage, and even though they are not introduced to extensive clinical use, they show significant potential. A better understanding of ischemia–reperfusion damage in enterocytes will surely allow clinicians to produce better diagnostic methods for detecting AMI in the early stages.

Conflict of Interest All authors declare they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed specifically for this chapter by any of the authors.

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Chapter 19
Redox Signaling and Biomarkers in the Acute Setting

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Abstract The role of redox biomarkers is highly applicable to the diagnosis and management of patients in the acute setting, especially in the context of the aging patient population. Several biomarkers are in active clinical usage; however, many more novel biomarkers are undergoing current research. The process of redox signaling and its associated biomarkers are involved in the potential clinical management of a wide variety of pathologies. Redox biomarkers have been especially noted to be associated with processes characterized by inflammation and cellular damage. As a result, oxidative stress at a cellular level is often implicated in pathologies requiring acute and timely diagnosis, including sepsis, pulmonary and cardiovascular disease, neurologic injury, and trauma. The geriatric population is particularly vulnerable to morbidity and mortality when subject to injury and disease. The discovery of redox signaling biomarkers that would allow for earlier diagnosis of life-threatening conditions, in addition to providing guidance on prognosis, could be an effective method for decreasing the detrimental effects seen in the aging population. Sepsis, as it represents a spectrum of disease that affects multiple organs, has been one of the areas most studied with regard to relevant biomarkers; tests measuring levels of C-reactive protein and procalcitonin are already in common usage. Research into disorders of the respiratory, cardiovascular, and neurologic systems have also yielded specific biomarkers that may reflect underlying inflammation, tissue ischemia, or chronic disease and are influenced by redox signaling. This chapter discusses specific biomarkers and their clinical applications in acute settings, such as emergency departments.

Keywords Redox signaling · Biomarkers · Aging · Sepsis · Acute respiratory distress syndrome · Cerebral ischemia · Acute coronary syndrome · Traumatic brain injury · Oxidative stress

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19.1 Introduction

Redox signaling plays an important role in an innumerable sum of biological processes, thereby producing the clinical manifestation of a vast spectrum of diseases, via mechanisms initiated at a cellular level. Oxidative stress is a common underlying cause in the pathophysiology of many clinical disease processes, triggering the occurrence of redox reactions that may disrupt normal signaling and biologic pathways within the cell. Biomarkers produced by, or as a result of redox signaling, are garnering interest for their potential role in the acute medical setting. Current research indicates their ability to monitor goals of treatment, act as prognostic indicators, and even have utility as potential therapeutic targets for some disease states. Major clinical guidelines have already adopted the use of some biomarkers, such as C-reactive protein in sepsis. In this case, the biomarker is implicated in the downstream reactions created by redox signaling to induce a pro-inflammatory state. Subsequent measurement of its elevation becomes evidence to support critical diagnoses. In order to be relevant to a clinician evaluating disease in real time, a biomarker must be measurable, temporally related to the disease process, and correlate with disease severity or presence. The acute care setting also requires biomarkers to be easily measurable and utilize cost-effective testing prior to widespread dissemination and use (Cournot 2018).

The aging population is uniquely vulnerable to the disruption of normal cellular function, which leads to disease processes and associated morbidity. Ischemic and inflammatory insults often result in irreversible tissue damage, in contrast to younger patients with more physiologic resilience and tissue plasticity. Sepsis, acute pulmonary disease, cardiovascular insults, traumatic brain and spine injury, and stroke account for significant morbidity and mortality among the elderly and have been associated with oxidative stress and production of redox biomarkers. While promising, redox signaling biomarkers must be closely evaluated for specificity to the acute phase of the disease. Biomarkers in the acute setting are of particular interest to clinicians treating the aging population, as correct disease identification and treatment initiation are even more critical to the geriatric patient. The use of redox signaling biomarkers in the elderly patient experiencing an acute disease process may be challenging, however, because of confounding factors due to the underlying co-morbidities which are often present in this cohort. Exploration of potential redox signaling biomarkers must be clearly investigated in all age groups and subsequently applied to the geriatric population to determine their clinical usefulness. The following chapter explores different biomarkers involved in the process of redox signaling and discusses their relationship to the diagnosis and management of the acute disease.

19.2 Redox Biomarkers in Sepsis

Sepsis is an acute condition caused by a dysregulated immune response and triggered by viral, bacterial, or fungal infection. It does not represent a singular disease state; it instead encompasses a spectrum ranging from the presence of the infection itself and the initiation of physiologic disturbances to septic shock and a state of physiologic collapse with resulting end-organ damage. The advent of antibiotics in the modern era has provided for a reliable means of intervention on the condition; however, sepsis continues to represent a significant cause of morbidity and mortality in both the generalized and hospitalized population (Samraj et al. 2013). Statistics indicate mortality from sepsis at 40–70%, despite the advanced treatment methods designed around the condition (Liu et al. 2013). Early identification and diagnosis of sepsis is the mainstay of the Surviving Sepsis Campaign, in addition to the administration of antibiotics within the first hour of recognition and guided fluid resuscitation (Coopersmith et al. 2018). Early identification of patients at risk for sepsis is key to reducing mortality. Biomarkers may serve as an important tool in facilitating recognition of high-risk patient populations, early identification of patients with sepsis, and in monitoring disease progression. These critical steps are essential to the goal of improving patient outcomes (Pierrakos 2021). The Campaign guidelines, which outline methods of sepsis management, recommend the use of biomarkers as a key part of the clinical evaluation when sepsis is suspected. This chapter will discuss several biomarkers which could be beneficial in caring for potentially septic patients.

C-reactive protein (CRP) and procalcitonin (PCT) are biomarkers already used clinically. CRP activates the complement cascade by functioning as an opsonin and binding to soluble or particulate ligands. Via receptor binding, multiple signaling pathways are initiated that induce a pro-inflammatory state (Majzunova et al. 2013). CRP levels have been shown to increase in an age-dependent fashion in the context of normal aging and have been implicated in acute cardiac events and atherosclerosis (Tang 2017). In pathologic states, C-reactive protein gradually increases, beginning from 4–6 h after initial infection and subsequently doubling every 8 h, reaching a peak at 36–50 h post-infection. CRP serum levels increase as a result of signaling from increased inflammatory cytokines, such as IL-6 (Del Giudice and Gangestad 2018), and are typically proportionate to the degree of inflammatory insult. Using CRP levels to correlate with bacterial infections has shown a sensitivity of 68–92% in various studies, with a corresponding specificity of 40–67%. Over the course of treatment, a rapid decline in the level of C-reactive protein is a positive prognostic sign, indicating a strong response to therapy (Nora et al. 2017). In addition to CRP, a newer but analogous biomarker procalcitonin (PCT) has become increasingly used as a marker of bacteremia. Production of PCT is triggered by pro-inflammatory cytokines, but significantly increases as a result of bacterial endotoxin; as a result, it has demonstrated usefulness in distinguishing between bacterial and non-infectious sources of inflammation. Its clinical utility is related to its rapid rise in the context of infection, with peak levels after 6 h and a half-life of 24–36 h. Procalcitonin rise

occurs significantly in advance of positive blood cultures and thus can contribute to the early identification of sepsis (Nora et al. 2017).

The host survival from infection depends on the body's immune system to identify pathogens and mount an effective immune response utilizing the innate and adaptive immune system. The innate immune system recognizes pathogens by different receptors known as pathogen-associated molecular patterns (PAMPs) that are displayed on the cellular surface. Toll-like receptors (TLRs) are found on macrophages, monocytes, and dendritic cells. A cluster of differentiation 14 (CD14) is one such receptor and is able to identify groups of ligands associated with bacteria. Lipopolysaccharide (LPS) is a ligand associated with Gram-negative bacteria. Lipoprotein-Binding Protein presents lipopolysaccharide to CD14, initiating signaling pathways to upregulate cytokine production. The complex consisting of lipopolysaccharide, lipoprotein-binding protein, and CD14 leads to gene expression for both soluble and membrane-bound forms of CD14 (mCD14 and sCD14). Soluble CD14 is cleaved by cathepsin D, and a subtype of sCD14 is formed—sCD14-ST, also known as presepsin. Presepsin is released via exocytosis into the general circulation and can be measured as a marker of infection and innate immune response, triggered by the presence of a pathologic micro-organism. (Henriquez-Camacho and Losa 2014).

Presepsin may represent a method by which to maximize early identification of sepsis, as an increase in the marker is noted within 2–4 h of the infectious stimulus, with a half-life of 4–5 h. In contrast to procalcitonin, presepsin does not become elevated as a consequence of the tissue damage inflicted by burns and also has the capability of rising to high levels in immunocompromised patients. It does present the disadvantage of being expressed at lower levels in patients receiving chemotherapy (Shozushima et al. 2011). As patients' infection severity progressed from sepsis to septic shock, levels of plasma presepsin were found to correlate with the increased infection severity; presepsin was noted to have greater specificity than procalcitonin in the diagnosis of sepsis (Venugopalan et al. 2019). The Acute Physiology and Chronic Evaluation (APACHE II) score, as a validated predictor of adverse outcomes and disease severity, is often used as a comparison for new biomarkers, along with the Sequential Organ Failure Assessment (SOFA) score. A strong positive correlation was found between the levels of presepsin and the APACHE II score, supporting a potential usage of presepsin as a reflector of the severity of sepsis and local infection (Shozushima et al. 2011); however, another multi-center trial with 106 patients demonstrated an average sensitivity and specificity of 71% for both parameters when presepsin was evaluated as a marker to differentiate between infection and non-specific systemic inflammation (Henriquez-Camacho and Losa 2014).

Another recent biomarker developed in order to improve the diagnosis of sepsis is known as soluble triggering receptor expressed by myeloid cells-1 or sTREM-1. As described by the marker's formal designation, sTREM-1 is a glycopeptide receptor on myeloid cells, and its expression has been found to be upregulated in sepsis secondary to pathogenic bacteria or fungi (Li et al. 2014). sTREM-1 was noted to be as accurate as procalcitonin (PCT) in the diagnosis of bacterial sepsis, with a sensitivity of 82% and a specificity of 86%. In admitted septic patients, sTREM-1 levels were found to be independently associated with mortality, and a rapid decrease of sTREM-1 was

associated with improved mortality. Serum measurements of sTREM-1, PCT, and interleukin-6 (IL-6) levels of patients in the non-survival group were shown to be significantly higher than those in the survival group on day 1 ($P < 0.01$). The 28-day mortality prediction was 0.792 for PCT, 0.856 for sTREM-1, 0.953 for SOFA score, and 0.923 for APACHE II score. Serum PCT, sTREM-1, and IL-6 levels showed a decreasing trend over time in the survival group ($P < 0.05$). sTREM-1 has the disadvantage of being a relatively high-cost test, and it is not currently widely available among institutions for clinical use. This study, however, demonstrated that serum sTREM-1 may offer prognostic utility for sepsis mortality, especially in combination with the APACHE II score (Jedynak et al. 2018).

Adrenomedullin (ADM) is a substance produced as a result of physiologic stress and has several varying effects on multiple organ systems throughout the body. It is produced by cells in numerous tissues and acts as a vasodilator, in addition to having anti-microbial and anti-inflammatory effects. Adrenomedullin causes vasodilation by binding to receptors on the smooth muscle of the vasculature and inhibits muscle contraction by the induction of potassium channels. This leads to hyperpolarization and resultant closure of voltage-gated calcium channels, which ultimately causes an overall reduction in the available intracellular calcium necessary for muscle contraction and vasoconstriction. Potential physiologic stressors leading to the increased production of ADM include hypoxia, increased plasma cytokine levels, and higher levels of lipopolysaccharide (LPS) associated with Gram-negative bacteria. Higher adrenomedullin levels were associated with decreased vascular tone and an increase in sepsis severity and mortality. Therapies based on targeting of adrenomedullin have been developed, as the downstream regulation of vascular tone may be the direct cause of the increase in mortality. A monoclonal antibody called Adrecizumab has been developed to further examine the possibility of decreasing adrenomedullin's deleterious effects. In an animal model, the antibody was able to improve 7-day survival in septic mice, an effect which was noted to be more pronounced with repeated administration (Geven et al. 2018). Upcoming human studies may elucidate the potential value of targeted anti-ADM therapy in decreasing mortality from sepsis. In a similar fashion to presepsin, pro-ADM, the prohormone fragment of adrenomedullin, was found to be higher in patients with sepsis than with patients meeting systemic inflammatory response (SIRS) criteria. In febrile neutropenic patients, pro-ADM was found to successfully distinguish sepsis from non-specific SIRS without a source of infection (Angeletti 2013). The advantage of measuring the prohormone protein fragment, compared to measuring adrenomedullin itself, is that the prohormone is a more stable compound that can be measured in even trace amounts in bodily fluids (Henriquez-Camacho and Losa 2014).

Interleukin-27 (IL-27), a simultaneously pro- and anti-inflammatory cytokine, has also been examined as a potential biomarker for the identification of sepsis. IL-27 acts as a T-cell regulator and is manufactured by signal transduction pathways within antigen-presenting cells (APCs) following the introduction of infectious stimuli. Its efficacy may be superior in the pediatric population, as it was shown to have identified critically ill children with bacterial infections and differentiated them from those with sterile inflammation from other causes (Wong et al. 2012). A specificity >90% for

distinguishing bacteremia in both children and immunocompromised patients has been demonstrated in other studies, reflecting its potential clinical utility (Hanna et al. 2015). The accuracy of IL-27 was further enhanced by using it as a diagnostic tool in conjunction with procalcitonin, a technique that improves both the positive and negative predictive value.

Interleukin-6 (IL-6), which belongs to the same family of cytokines as interleukin-27, has indicated a similar potential to act as a marker of immune response and activation. IL-6 is produced and acts on a wide variety of tissues and has been particularly implicated in the viral immune response. Interleukin-6 activates various cellular signaling pathways in target organs to mediate gene expression and upregulate receptors, proteins, and other key substances in the inflammatory cascade (Velasquez-Salinas et al. 2019). Multiple studies were retrospectively reviewed in a meta-analysis involving 2680 critically ill patients, comparing IL-6 to CRP in the clinical management of sepsis. IL-6 was found to have a sensitivity of 0.68 and specificity of 0.73, compared to PCT with a sensitivity of 0.78 and specificity of 0.67. The AUC of IL-6, PCT, and CRP for diagnosis of sepsis was 0.80, 0.83, and 0.71, respectively (Ma et al. 2016). This comprehensive review provides evidence that the IL-6 test has moderate diagnostic performance when compared to more traditionally used inflammatory biomarkers such as PCT and CRP in differentiating sepsis from non-infectious SIRS in adults. IL-6 and PCT have similar diagnostic value, which could be useful in emergency department settings to improve patient care and outcomes in patients who are potentially septic in need of early therapeutic interventions.

In addition to early identification and diagnosis of sepsis, prognostication and assessment of short-term mortality in the acute setting is another essential aspect of patient management. A biomarker referred to as suPAR, or soluble urokinase plasminogen activator receptor, has shown promise in predicting outcomes in septic patients. The uPA receptor is expressed on a number of cell types, most with an immune-based function, such as macrophages and lymphocytes. The receptor itself is a component of other immunologic functions and basic biologic tasks, such as chemotaxis, cell adhesion, migration, tissue remodeling, and signal transduction. Levels of suPAR are increased in a non-specific manner in blood and other tissues in the infectious state and have not shown the same diagnostic specificity of other markers such as C-reactive protein. Although it does not improve the diagnostic capability of currently known biomarkers, suPAR measurements may be analogous to severity scores in predicting the prognosis (Donadello et al. 2012).

A clear link between elevated suPAR levels and increased mortality has been demonstrated for some specific high mortality diseases, such as HIV, tuberculosis, and malaria; but even more applicable to the aging and critically ill patient, an association has been observed even without an obvious diagnosis of infectious disease (Eugen Olsen 2011).

suPAR levels could also be beneficial in emergency department triage, in the risk stratification of patients at low risk versus high risk of mortality. If successful, its usage in the clinical setting could improve patient management and flow in emergency departments, as well as identify patients in need of expedited care and more immediate physician assessment. A study by Schultz et al. (2019) assessed the correlation

of suPAR levels with patients who were categorized based on acuity. Patients were divided into four triage categories for the purposes of research analysis: Red, Orange, Yellow, and Green. Patients were categorized in the emergency department using a standard emergency department triage method, taking into account abnormal vital signs and patient complaints. The goal of the study was to improve 7-day mortality predictions by using both standard triage and suPAR level. The study cohort included greater than 9000 patients in whom a documented triage level was available. The research indicated that utilization of suPAR measurements in concert with standard triage consistently correlated with all-cause mortality within the first seven days of admission, regardless of age and complaint. The ability to predict mortality early in the emergency department course has not yet been shown to decrease the recognized mortality; however, more investigation is required to determine the full potential of this biomarker.

Many biomarkers have been investigated to assist with the risk stratification of patients in the acute setting and to function as early diagnostic tools for septic patients. Early identification of potential sepsis will facilitate earlier treatment with broad-spectrum antibiotics, to potentially decrease mortality and improve patient care (Dupuy 2013). The other advantage of discovering additional biomarkers, in addition to assisting physicians to monitor the effectiveness of therapy, is to guide the prognosis of critically ill patients. A continued need exists for effective diagnostic biomarkers in bacterial sepsis, and several are already available for clinical use; however, the lack of sensitivity and specificity of the known biomarkers represents a challenge in facilitating their widespread clinical use and ability to improve patient management. More investigation and clinical trials are needed to further investigate the use of sepsis biomarkers in the acute setting (Table 19.1).

Table 19.1 Redox signaling biomarkers with potential use in sepsis. Overview of relevant redox biomarkers discussed in the text and their projected clinical uses. Most of these biomarkers are novel and not widely available for testing in the acute setting; however, ongoing research may bring them into increased clinical use

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
C-reactive protein (CRP)	Sepsis, acute cardiac events, atherosclerosis, hypertension, Parkinson's disease, diabetes, chronic kidney disease	Functions as an opsonin and activates the complement cascade; induces a pro-inflammatory state by binding to its receptors and activating signaling pathways	Often correlates with severity of infection; allows monitoring of antibiotic therapy over time. Can also indicate severe atherosclerosis

(continued)

Table 19.1 (continued)

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
Procalcitonin (PCT)	Sepsis	Triggered by bacterial endotoxin and inflammatory cytokines	Differentiating between bacterial and non-infectious causes of inflammation
Presepsin	Sepsis	Formed from exocytosis and proteolysis of immune complex after bacterial recognition	Evaluates immunocompromised patients with severe infection, follows infection severity
sTREM-1	Sepsis	Glycopeptide receptor expressed on the surface of myeloid cells. Expression increased in bacterial and fungal sepsis	In patients admitted for sepsis, rapid decrease was associated with improved mortality
Adrenomedullin (ADM)	Sepsis	Produced during physiologic stress; causes vasodilation by binding with receptors on blood vessels. Also has anti-microbial and anti-inflammatory effects	Correlates with septic shock, relaxation of vascular tone, and increased mortality. ADM-targeted therapies may be beneficial
Interleukin-27 (IL-27)	Sepsis	Heterodimeric cytokine expressed by antigen-presenting cells that activates an inflammatory response	Strong positive predictive value in patients with bloodstream infections, critically ill pediatric patients
Interleukin-6 (IL-6)	Sepsis, cognitive disease, neoplasm	Pro-inflammatory cytokine, anti-inflammatory myokine. Stimulates production of CRP	Differentiates sepsis from non-infectious SIRS, mediates chronic low-level inflammation
Soluble urokinase plasminogen activator receptor (suPAR)	Sepsis	Cleaved fragment of urokinase-type plasminogen activator system, takes part in numerous immunologic processes	Correlates with short-term mortality in Emergency Department patients and disease severity, ED triage applications

19.3 Redox Biomarkers in Pulmonary Disease

Respiratory diseases, independent of systemic infection, are also a common cause of presentation to the acute setting. As our population continues to age, the incidence and complexity of lung diseases continue to increase. As the lung is one of the organs most directly responsible for the processes of oxygenation and ventilation, it plays an

important role in reduction–oxidation physiology (Hecker 2018). There are a wide variety of respiratory diseases that can present in the acute setting, including acute respiratory distress syndrome (ARDS), acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), asthma, idiopathic pulmonary fibrosis (IPF), and infections with bacteria, fungi, and viruses, including those caused by the highly infectious SARS-CoV-2 virus. Oxidative stress and inflammatory responses play important roles in all of these pulmonary diseases. Reactive oxygen species in particular have been implicated in the pathophysiology of these disease processes. They are involved in the regulation of growth factors and growth factor receptors via kinase and phosphatase activity and mediate pulmonary vasoconstriction via endothelin-1 (Berkenbosh et al. 2001; Villegas 2014). NADPH and glutathione (GSH) also play important redox roles in lung disease as enzyme cofactors (Freyhaus et al. 2011; Fitzpatrick et al. 2009).

ARDS and ALI can be thought of as two diseases on the same disease spectrum, with the former being a more severe version. ALI/ARDS can arise from a variety of diseases including pneumonia, sepsis, trauma, aspiration, drowning/submersion injuries, and iatrogenic causes including barotrauma and volutrauma. ARDS remains a clinical diagnosis and no single biomarker has been identified and placed into clinical practice that can reliably diagnose ARDS. The 2012 Berlin definition of ARDS requires that onset take place over no more than one week, along with bilateral opacities consistent with pulmonary edema identified either on plain radiographs or computed tomography, PaO_2/FIO_2 ratio <300 mmHg on a minimum of 5 cm H2O of PEEP, and it "must not be fully explained by cardiac failure or fluid overload." Histologically, ALI/ARDS is identified with diffuse alveolar injury. Ultimately, the diagnosis of ALI/ARDS remains a clinical diagnosis requiring multiple sources of data. An ideal biomarker for ALI/ARDS would be a substance that can reliably and reproducibly be detected and the presence of which has a high degree of specificity and sensitivity for ALI/ARDS (Bhargava and Wendt 2012; Cross and Matthay 2011). While ALI/ARDS is inarguably a complex disease process, there is considerable evidence implicating reactive oxygen species in the pathophysiology. This then makes redox biomarkers a natural potential target for ARDS diagnosis, monitoring, and prognostication.

A large number of potential biomarkers for ALI/ARDS are under investigation at this point. Angiopoietin-1 (ANGPT-1) and angiopoietin-2 (ANGPT-2) are among these potential biomarkers. ANGPT-1 and ANGPT-2 act in antagonism to each other with ANGPT-1 providing a stabilizing effect on pulmonary endothelium, reducing apoptosis, and acting as an anti-inflammatory while ANGPT-2 does the opposite. Both absolute levels of ANGPT-2 and the ANGPT-2:Ang-1 ratio are being studied as potential biomarkers for ALI/ARDS and predictors of mortality. Another potential biomarker in respiratory disease, and in particular in ALI/ARDS, is hydrogen peroxide. Kietzmann et al. found that patients with ARDS have levels of hydrogen peroxide in expired breath condensate—approximately 480% greater than healthy controls (Kietzmann et al. 1993). Patients with ARDS have also been found to have drastically diminished concentrations of glutathione, a naturally occurring antioxidant, when compared to healthy controls (Bunnell and Pacht 1993).

COPD is another disease process frequently encountered in the acute care setting. Traditionally, COPD is diagnosed with spirometry, and this remains the gold standard for COPD diagnosis. However, performing spirometric testing in the acute care setting is often impractical, if not impossible. This motivates the search for a quick and accurate test to diagnose and potentially risk-stratify patients with COPD in the acute setting. Biomarkers are one potential type of such test which has begun to show promise. Like ARDS, COPD is a complex multifactorial disease process in which oxidative stress plays a major role. When compared with healthy patients, those with COPD have also been found to have elevated levels of hydrogen peroxide, and during COPD exacerbations, it has been found these levels are even more increased than baseline (Dekhuijzen et al. 1996).

The significant amount of mucosal endothelium in the respiratory tract is susceptible to repeated oxidative stress, as a function of normal respiration in room air—an atmosphere composed of 21% oxygen. Multiple cells in the lung have been implicated in the production of reactive oxygen species (ROS) including tracheal and alveolar epithelium, Clara cells, and vascular endothelium, as a response to oxidative injury (Comhair et al. 2005). Asthma, as a chronic inflammatory disorder, is intimately related to the production of both reactive nitrogen and oxygen species at the cellular level. Oxidative stress on the respiratory system, which is often a result of exposure to micro-organisms or environmental pollutants, leads to the presence of ROS and RNS. Alveolar macrophages have been shown to be capable of producing high levels of these compounds when subjected to stress via a significant number of metabolic pathways (Kinnula et al. 1991). These types of cells contain endogenous enzymes to combat the generation of free radicals, and hydrogen peroxide is a common by-product of the reduction within the cell. When vast amounts of inflammatory cells are diverted to the endothelium of the lung parenchyma to address an oxidative insult, the production of hydrogen peroxide is increased and subsequently exhaled in the breath condensate of patients (Fig. 19.1). Measurement of these levels could play a major role in the diagnosis and assessment of asthma severity and other inflammatory-mediated lung diseases (Fridovich and Freeman 1986).

Ambade et al. conducted an observational study in which they compared patients with and without COPD as diagnosed by spirometry and compared serum levels of a number of potential biomarkers including superoxide dismutase 3, glutathione peroxidase, catalase, ceruloplasmin ferroxidase activity, C-reactive protein, and surfactant protein D (SPD). Of these, they found SPD to hold the most promise in smoking patients, with ferroxidase activity most promising in non-smokers (Ambade et al. 2015). Surfactant protein D is used by alveolar macrophages to improve recognition of bacteria, thus, promoting phagocytosis. Alveolar macrophages also internalize and degrade SPD (Hartl and Griese 2006).

NADPH oxidase-2 (NOX-2) is an important source of superoxide anion, playing a key role in systemic inflammation and in the pathogenesis of many diseases caused by RNA viruses, including influenza (To et al. 2017; Violi et al. 2017). The virus SARS-CoV-2, commonly known as COVID-19 and the root cause of a global pandemic, has been shown to cause widespread systemic inflammation, most prominently in the respiratory system, and it has been postulated that NOX-2 may play an important

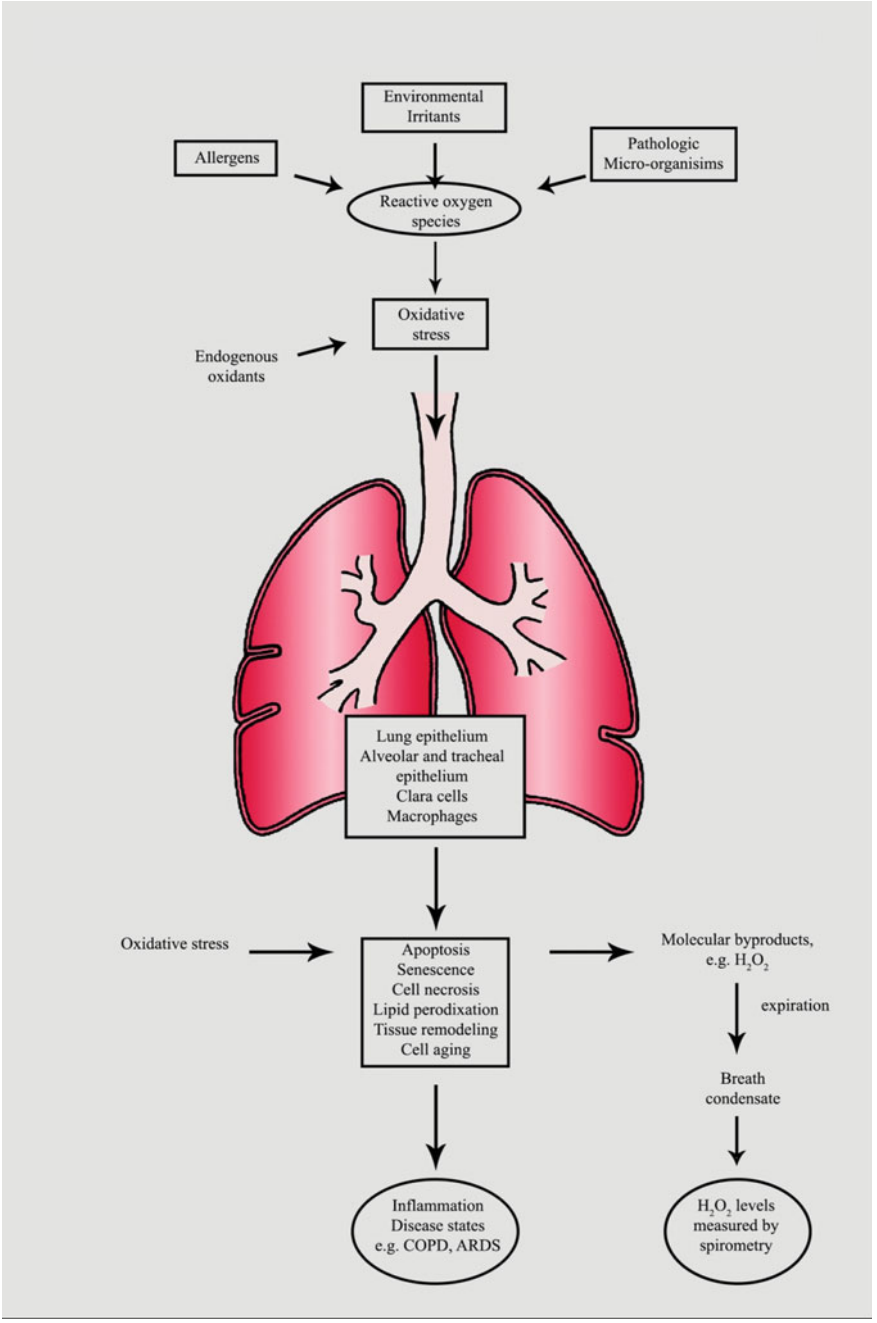

Fig. 19.1 H_2O_2 levels measured in breath condensate can be used as an indirect measurement of increased inflammation and lung pathology caused by oxidative stress

role in the pathophysiology of the virus (Loffredo and Violi 2020). Additionally, it was observed that critically ill patients with COVID-19 have elevated neutrophil to lymphocyte ratios (Fu et al. 2020) and elevated levels of reactive oxygen species (Laforge and Violi 2020). NRF2 is a redox-sensitive transcription factor, which plays an important role in COPD as well as in respiratory viral infections. Under normal circumstances, oxidant species will cause the translocation of NRF2, which in turn has a cascade of antioxidant effects. However, it has been observed in respiratory viral infections that NRF2-mediated pathways become inhibited, thus, causing a pro-inflammatory response with further oxidative damage (Casola 2014).

Due to the prominent role played by oxidative stress in pulmonary disease, researchers have been searching for antioxidant chemicals to be used as potential therapies. One such antioxidant is *N*-acetylcysteine (NAC), which has multiple anti-inflammatory and anti-oxidative effects, including acting as a direct scavenger of free radicals and raising levels of glutathione (Aruoma et al. 1989). For its effect on glutathione levels, NAC is commonly used in clinical practice in treating patients with acetaminophen overdose. It has also been studied in patients with asthma, ALI/ARDS, and sepsis and found to have no mortality benefit. While no mortality benefit for NAC has been demonstrated in these diseases, other clinically important benefits have been observed, including decreased COPD exacerbation frequency, improved oxygenation, and faster recovery in ALI/ARDS (Bernard et al. 1997; Suter et al. 1994; Szakmany et al. 2012). However, NAC has also been found to depress cardiac performance in patients with septic shock (Peake et al. 1996). Thus, the utility of NAC in lung disease remains limited, and no definitive clinical utility in the treatment of pulmonary disease has been established. Glutamine and antioxidant supplementation have also been studied in critically ill, mechanically ventilated patients. The REDOXS study (Heyland et al. 2006) was a large-scale randomized trial, in which 1200 such patients admitted to the intensive care unit received one of three potential interventions: (1) glutamine supplementation, (2) antioxidant supplementation, and (3) glutamine and antioxidant supplementation against a placebo arm. The antioxidant supplementation consisted of co-administration of selenium, zinc, β-carotene, vitamin E, and vitamin C. While they used a novel design, implementing both enteral and parenteral vitamin supplementation, this study showed no improvement with any of the treatment arms compared to placebo. Other potential therapeutics targeting redox pathways have been studied with similarly ineffective results. The nitric oxide synthase inhibitor 546C88 was evaluated in patients with septic shock based on the hypothesis that it would improve mortality due to its antioxidant effects, but was found to do the opposite (Lopez et al. 2004).

The lung plays a vital role in maintaining oxygenation and ventilation and is particularly prone to diseases caused or mediated by reactive oxygen species and oxidative stress. This supports the conclusion that redox biomarkers are, mechanistically and otherwise, an appropriate potential target for the diagnosis, evaluation, and treatment of pulmonary disease (Table 19.2).

Table 19.2 Redox signaling biomarkers associated with respiratory pathology. As the primary center of physiologic respiration, the pulmonary system encompasses a number of biomarkers that can indicate underlying inflammation and physiologic disturbance

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
Angiopoietin-1 (ANGPT-1)	ALI/ARDS	Stabilizes pulmonary endothelium, acts as an anti-inflammatory	Under investigation as a predictor of mortality
Angiopoietin-2 (ANGPT-2)	ALI/ARDS	Antagonist to ANGPT-1	Ratio of ANGPT-1: ANGPT-2 also being researched
Hydrogen peroxide	ARDS, COPD, asthma	Produced by reduction of reactive oxygen species and oxidative stress; increased levels in breath condensate with pulmonary pathology	Diagnosis and risk stratification of COPD and asthma
NADPH oxidase-2 (NOX-2)	Sepsis, respiratory disease	Important source of superoxide anion; causes widespread systemic inflammation	Postulated to play a role in the pathophysiology of COVID-19
NRF2	COPD, respiratory viral infection	Redox-sensitive transcription factor, activated by oxidant species leading to antioxidant effects	NRF2-mediated pathways noted to become inhibited in respiratory viral infections, causing a pro-inflammatory response

19.4 Redox Biomarkers in Cardiovascular Disease

With advanced medicine comes prolonged median life expectancy. Recent studies estimate that by the year 2030, one-fifth of the global population will be over the age of 65 (Kahn et al. 2016). This is expected to cause increased demand on the healthcare system, which will require novel solutions to optimize care for the aging population. Acute settings such as emergency departments will be of particular relevance for the care of the elderly since there is a linear association between age and emergency department visits (Ukkonen et al. 2019). In addition to sheer volume, elderly patients pose a distinct challenge in the acute setting, as they may be more likely to require an extensive evaluation for potentially life-threatening conditions that occur less commonly in younger individuals without co-morbidities. Since cardiovascular disease is the most common cause of mortality in the general population, it remains standard of care to consider this diagnosis in the elderly patient and prioritize efficient diagnostic and treatment modalities. This section will focus on the potential for redox biomarkers to mitigate elderly cardiovascular disease and the current role of other cardiac-related biomarkers in the acute setting.

According to the World Health Organization's 2020 report, cardiovascular disease (CVD) has been the leading cause of death worldwide for more than 20 years. While treatments and tertiary prevention methods have improved, the undeniable and persistent global mortality necessitates improved primary and secondary disease prevention. Despite ample public health programs to increase risk factor awareness and patient education, the burden of CVD continues to prevail across the world. Aging, one of the most significant CVD risk factors, is considered a nonmodifiable risk factor and, therefore, is a noteworthy limitation when pondering the persistence of severe cardiovascular disease despite valid public health efforts. In addition to age being an independent and nonmodifiable risk factor, other modifiable risk factors, such as smoking and obesity, have an accruing effect with time and, therefore, age. Aging has a substantial impact on vasculature morphology. Age-related endothelial cell dysfunction and heightened elastin degradation prompt vascular wall thickening and perivascular fibrosis. Subsequently stiff, thickened, and fibrotic vessels affect impedance, pulsatility, and eventually ejection fraction (Costantino et al. 2016).

Recent research involving oxidative cell signaling suggests redox biomarkers may be the much-anticipated key to advancing CVD prevention strategies by identifying potential targets for intervention, and even prevention, of age-related cardiovascular pathology. Reactive oxygen species (ROS) are natural byproducts of oxidative phosphorylation. While their production and role are vital to normal cell function, in excess, they can also be destructive (Karimi Galougahi et al. 2015). Aging causes significant damage to cellular ROS pathways, manifesting a cascade of cellular reactions due to the dysregulated ROS presence. By understanding the consequences senescence has on molecular pathway signaling and cell homeostasis, oxidative biomarkers can be targeted and incorporated into clinical practice. Silent mating-type information regulation 2 homolog 1 (SIRT1) has a central role in age-related CVD. In a normal functioning cell, SIRT1 is responsible for downregulating the ROS-producing mitochondrial adaptor protein p66Shc*p66Shc* and enhancing endothelial cell function which ultimately reduces calcification of vascular smooth muscle cells (Chen et al. 2013). Aging leads to dysregulation of SIRT1, thus, restricting its cardioprotective attributes. Further investigation may yield innovative therapies for enhancing the function of SIRT1 and improving outcomes in cardiovascular disease. Another important component in age-related CVD is the activated protein-1 transcription factor JunD. Studies have shown an inverse relationship between age and JunD, consequently leading to increased superoxide anions (Paneni et al. 2013). The disproportionation of superoxide anion ($O2\cdot-$) forms two ROS, hydrogen peroxide ($H2O2$) and hydroxyl ($\cdot HO$). Additionally, the unpaired electron of superoxide anion ($O2\cdot-$) leads to its swift binding with the unpaired electron of nitric oxide (NO) thereby depleting bioavailable nitric oxide (NO). Nitric oxide is a fundamental vasodilator; therefore, its depletion leads to vasoconstriction, cell proliferation, platelet aggregation, and thrombus formation (Klabunde 2012).

Uric acid, myeloperoxidase (MPO), Serum NOX2-derived peptide (sNOX2dp), 8-hydroxy-2'-deoxyguanosine (8-OHdG), lipid oxidation, and antioxidant enzymes such as glutathione peroxidase (GPX-1) have all been associated with redox pathways involved in CVD (Karimi Galougahi et al. 2015). Atherosclerosis and plaque

formation lay the foundation for sudden and potentially devastating cardiac events. Lipoproteins accumulate in the intima of the vascular endothelium and, when oxidized, are consumed by macrophages. This leads to foam cell formation and the fatty streaks that are characteristic of early atherosclerosis in the vasculature. Over time, lipid-rich deposits continue to accumulate, forming an atherosclerotic plaque. Foam cell apoptosis occurs due to prolonged stress and is inadequately cleared, creating a necrotic core. A fibrous cap builds on the forming complex, which can destabilize and rupture, leading to vascular occlusion (Khandkar et al. 2021). Clinically, this mechanism underlies the majority of presentations of ST-elevation myocardial infarction in the acute setting.

Chest pain, one of the most common emergency department complaints, can be a symptom of an underlying life-threatening condition which may be difficult to diagnose within a varied patient population. Of the potential etiologies of chest pain, a significant cause is an acute ischemic event resulting in myocardial infarction, but there is an extensive list of other sources such as gastroesophageal reflux disease or costochondritis. The emergence of biomarkers specific for cardiomyocyte necrosis, cardiac troponins, has greatly improved the ability to rapidly diagnose acute myocardial infarction and leads to prompt intervention. In 2017, the American College of Cardiology Federation and the American Heart Association indicated that cardiac troponins and natriuretic peptides are the first-line biomarkers in risk stratification (Passino et al. 2019). Troponin I (cTnI) and troponin T (cTnT) have superseded the previously used creatine kinase-MB and are widely accepted as the standard for assessing myocardial infarctions in the acute setting. The latest, high sensitivity troponin (hs-cTnT) has higher sensitivity but worsened specificity, which delays disposition in the acute setting and is an area for future improvements. Biomarkers based on redox signaling may represent an important adjunct to the diagnosis and treatment of cardiovascular disease (Table 19.3).

Similar to cardiovascular disease, congestive heart failure is a common cause of presentation to both emergent and non-emergent settings, manifesting in a variety of ways, such as chest pain, shortness of breath, and lower extremity edema. Biomarkers specific to cardiomyocyte stretch, natriuretic peptides, have become the standard for diagnosis after studies showed the ability to improve the previously low specificity and sensitivity of clinically diagnosed heart failure (Nadar and Shaikh 2019). Currently, N-terminal-proBNP (NT-proBNP) and B-type natriuretic peptide (BNP) are the two most thoroughly studied and used. Myocardial stretch activates the BNP gene, which triggers the creation of the prohormone proBNP. The prohormone is subsequently cleaved into the biologically active form, BNP, and the biologically inactive but stable form, (NT-proBNP). Both BNP and NT-proBNP involve renal clearance and, therefore, can be unreliable for diagnosing heart failure in chronic kidney disease patients. Research has implied atrial natriuretic peptide (ANP) is not useful for diagnosis because of its swift clearance; however, there is an ongoing discussion about prognosis possibilities of ANP prohormone, mid-regional proANP (MR-proANP) (Nadar and Shaikh 2019).

Table 19.3 Biomarkers implicated in cardiovascular disease. Redox signaling biomarkers are promising as future diagnostic tools; however, most currently used biomarkers are primarily involved in general signaling pathways

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
8-OHdG	Ischemic stroke, cardiovascular disease	Produced by reactive oxygen species; indicates overall oxidative stress	High levels were correlated with increased cognitive impairment 30 days post-CVA
N-terminal-proBNP	Cardiac disease	Myocardial stretch activates the BNP gene, which triggers creation of proBNP and NT-proBNP	Prognostication and diagnosis of congestive heart failure
SIRT1	Cardiovascular disease	Downregulates ROS-producing mitochondrial protein, reduces calcification of vascular smooth muscle, cardioprotective attributes	May be a target for therapy when upregulated
Troponin T (cTnT)	Cardiac disease	Non-redox mechanism	Reflective of myocardial injury and is the standard diagnostic tool for acute coronary syndromes

Although many redox signaling biomarkers have been identified and linked to cardiovascular pathophysiology, the ability to easily obtain and rapidly measure these biomarkers has proven to be a challenge, and continued research is required for clinical validation and application.

19.5 Redox Biomarkers in Trauma

As individuals age, their risk for life-altering traumatic injury increases greatly. In addition, elderly people have a poorer prognosis and a more difficult recovery following significant trauma. Common traumatic injuries in the elderly are often the result of falls leading to traumatic brain injury (TBI), spinal cord injury (SCI), and other orthopedic fractures. There is a great focus currently on changing systems in order to provide more appropriate care to geriatric patients. Redox biomarkers used in trauma are extensive, and research into their utility is rapidly growing and evolving. While many of the markers currently being studied are not used in clinical practice, the efficacy of such markers has been proven in various scenarios. With an aging population, traumatic injuries from falls and motor vehicle collisions demonstrate the value of the further study of prognostic and diagnostic biomarkers in the acute

setting. This section will discuss the most widely researched biomarkers in trauma including those for TBI, SCI, polytrauma, and burns.

Biomarkers currently being studied in patients with traumatic brain injury (TBI) include the following: glial fibrillary acidic protein (GFAP), S100B protein, neuron-specific enolase (NSE), peroxisome proliferator-activated receptor gamma (PPARγ), isoprostanes, malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), matrix metalloproteinases (MMPs), and neuroprostanes. These biomarkers can be analyzed from serum, whole blood, or in some cases cerebrospinal fluid (CSF). Oftentimes, many of these markers are difficult to obtain in an immediate fashion, which leads to low sensitivity in acute injury. Additionally, they are not always specific to the central nervous system, which can make interpretation of their values a challenge. While CSF biomarkers could provide more biomarkers specific to the CNS, the invasive and time-consuming nature of acquiring these samples makes wide-scale adoption less feasible.

Mendes Arent et al. (2014) discussed electrophilic aldehydes such as MDA and 4-HNE. MDA is a product of lipid peroxidation, which can occur following a TBI. This biomarker provides information about oxidative stress, secondary injuries, and neurotransmitter dysfunction (Mendes Arent et al. 2014). There are a number of biomarkers mentioned; however, the ones specific to redox reactions include those seen after oxidative stress to membrane lipids after trauma. MDA and 4-HNE have been shown to have higher levels in the first 30 min after trauma and they maintain their elevation for up to 72 h after brain injury. Animal models have shown these levels correlate with brain edema, cognitive disorders, and the amount of injury. PPARγ has been described as a potential biomarker in TBI and ischemic stroke. It is widely expressed in multiple tissues, especially in the brain, where it has primarily protective effects. There are multiple studies done in rats which prove an increase in PPARγ, in the ipsilateral cerebral cortex, occurs acutely after ischemic stroke for a period spanning from 4 h to up to 14 days post-stroke. However, conflicting results show decreased PPARγ-binding activity in the contralateral side of the brain. Overall, PPARγ has inconsistent evidence in TBI as some experiments have shown increased levels and others have shown decreased. Similarly, PPARγ has shown mixed results in spinal cord trauma (Cai et al. 2018). It is believed that PPARγ may help protect neurons from damage, but the paucity of literature as it pertains to PPARγ and trauma mandates further study before any reliable conclusions can be made.

Anthonymuthu and colleagues (2018) investigated both primary (mechanical forces) and secondary mechanisms (molecular pathways leading causing inflammation, electrolyte imbalances, oxidative stresses) of TBI. Lipid peroxidation biomarkers such as isoprostanes, malondialdehyde (MDA), and hydroxynonenal (HNE) were the preferred subjects of their literature due to their greater stability and greater half-life than other markers. The researchers found that HNE-modified proteins, F2-isoprostanes, and MDA all were significantly increased following TBI. These markers can elucidate multiple helpful factors including the severity of oxidative stress in the brain. MDA was the only biomarker in this paper discovered to have a prognostic role (Anthonymuthu et al. 2018). Furthermore, Yen and colleagues (2015) added to the discourse on F2-isoprostanes and F4-neuroprostanes for TBI.

In moderate and severe TBI, patients showed elevated levels of F2-isoprostanes and F4-neuroprostanes in their CSF. They also found that those with higher levels of both markers ended up having a lower GCS score and worse outcomes overall (Yen et al. 2015). Lorente primarily assessed prognostic biomarkers of TBI in his publication. Of the redox biomarkers mentioned, the elevation of TIMP-1 and MDA is associated with increased mortality in TBI patients. He also mentions MMPs, which appear often in the literature pertaining to biomarkers in brain trauma. This paper specifically notes tissue inhibitor of matrix metalloproteinases-1(TIMP-1) as one marker that has shown statistically significant elevations in TBI patients that did not survive the event. Serum TIMP-1 levels have also been shown to be an adequate indicator of 30-day mortality in this subset of patients. MDA, a product of phospholipid peroxidation, has higher levels in patients with TBI and this was borne out by multiple studies. There is also a positive correlation between MDA levels and mortality. MDA levels > 1.96 nmol/mL have an association with elevated 30-day mortality. TIMP-1 levels greater than 220 ng/mL have the same 30-day mortality association (Lorente 2015).

Redox biomarkers in spinal trauma have been less studied than that of brain trauma. The most prominent markers include Acrolein, apoprotein A-1, and an assortment of biomarkers including S-100B, NSE, B-APP, and GFAP. In polytrauma, biomarkers such as tissue factor pathway inhibitor (TFPI), total bilirubin, and paraononase–arylesterase (PON-AE) are showing promise. Lastly, patients with trauma from a burn can present with changes in interleukins, TND, IL-1, 4-HNE, CD163, NT-CNP, and 8-hydroxy-2'-deoxyguanosine. The novelty of these various markers is astonishing and we can only hope for further research into their use in the acute setting. Yet another lipid peroxidation marker relevant to trauma is Acrolein. It is involved in many CNS processes, but in the area of spinal trauma, Acrolein is an ominous marker proving to be especially toxic and leading to axonal injury with inflammation and demyelination. It is elevated in damaged spinal cord tissue and in the urine of animal models with spinal cord injury. Acrolein's value as a biomarker stems from its congruence with the severity of spinal cord injury. The degree of elevation of acrolein directly relates to the amount of spinal cord damage. Although promising, there is still much work to be done to improve the accessibility of the acrolein quantification methods (Tully et al. 2014).

TBI resulting in diffuse axonal injury (DAI) was studied by Frati and colleagues (2017). They found that oxidative stress is heavily involved in the outcomes of those with DAI. Their paper mentions multiple markers such as SBDPs, pNF-H, and others. However, there is no mention of any biomarkers related to oxidative stress. S-100B, NSE, B-APP, and GFAP represent oxidative biomarkers that show promise in DAI and have also been linked to TBI and other CNS trauma (Frati et al. 2017). Each of the aforementioned has shown to be useful in the acute setting of DAI and also good for prognosis after injury. Not to be overlooked in spinal trauma is the value of apolipoprotein A-1 as a biomarker for spinal cord injury, which was discussed in Zhang et al.'s research. They highlight that apoA-1 is significantly decreased in spinal cord injury and neurodegenerative disorders. While understudied, apoA-1 has

the potential as a biomarker indicative of oxidative stress in the acute and chronic phases of spinal trauma (Zhang et al. 2021).

Sandesc et al. from Austria investigated oxidative stress biomarkers in polytrauma patients in a prospective study. Reactive oxygen species pose additional threats to the complex polytrauma patient, as they often face many other challenges such as prolonged mechanical ventilation, biochemical pathway changes, and possibilities for sepsis. Free radical production in these patients leads to lipid peroxidation and disruption of the phospholipid membranes of cells. Oxidative stress also has effects on coagulation factors, and this study found that patients who received antioxidant therapy showed statistically significant improvement in their APTT, TBCs, and PT and decreased complication rates in their polytrauma patients. Additionally, they showed that total bilirubin (TBIL) levels were increased in the polytrauma patient receiving antioxidant therapy. They believe that the higher levels in the treatment group were due to decreased free radicals and a consequent reduction in the consumption of bilirubin. Previous studies have demonstrated a decrease in plasma levels of paraoxonase–arylesterase (PON-AE) protein activity when oxidation–reduction potential was at its greatest. This means that when they had worsening oxidation status, the PON-AE levels were lower. While further research is needed, this study showed strong evidence that the reduction of oxidative species with targeted therapies can improve outcomes in polytrauma patients (Sandesc et al. 2018).

Burn patients were treated with antioxidants including Vitamin C, E, A, selenium, and others in the study performed by Bratu and colleagues (2016). They continued to research, investigating the biomarkers used to indicate oxidative stress in burn patients. The SIRS response from severe burns leads to a pro-inflammatory cascade and the production of free radicals. Increased free radicals lead these patients to be more immunocompromised, with higher rates of complications and mortality. Biomarkers of oxidative stress in burn patients include multiple interleukins, TNF, and others. IL-1 has shown to be the most specific and has been most extensively studied. As in other forms of trauma, this study mentions 4-HNE as a specific marker for redox reactions. While microRNAs, CD163, and others are specific markers for burn patients, they do not directly relate to redox and oxidative stress. The research concluded that with the abundance of oxidative stress in burns patients, Vitamin C brings many wonderful benefits (Bratu et al. 2016).

19.5.1 Redox Biomarkers in Stroke

Stroke is one of the deadliest ailments that affect the elderly. The aftermath of a stroke can be so detrimental to a geriatric patient that they never may fully recover. There are a wide variety of redox biomarkers being studied currently. A partial list includes the following: MMP, tenascin C, thioredoxin, isoprostanes, neuroprostanes, apolipoprotein B, MDA, 8-ODdG, and S100. We will discuss each of these promising markers.

Zang et al. found biomarkers that could predict poor outcomes in acute stroke patients. They looked at patients who had received an endovascular thrombectomy in the setting of acute ischemic stroke. They found that MMP-9, thioredoxin, and tenascin C were the biomarkers that indicate an unfavorable outcome when elevated. Conversely, decreased levels of gelsolin were associated with worse outcomes. This study was limited in that it only discussed those in which the thrombectomy had been completed successfully (Zang et al. 2020). Thioredoxin is a well-researched biomarker that has informative value in various neurological illnesses. Oraby and Rabie (2019) studied the versatility of thioredoxin as a marker in acute ischemic stroke. Thioredoxin is known to be released from cells during oxidative stress, and this paper found that thioredoxin was higher in the acute ischemic stroke patient. There was a correlation between thioredoxin level and the severity of the prognosis. In this case, both the diagnostic and prognostic value of thioredoxin was displayed (Oraby and Rabie 2019). Seet Raymond et al. (2011) revealed that F2-isoprostanes, HETE's, and F4-neuroprostanes each increase in the 6–12 h after acute stroke. They are primarily signs of increased oxidative stress, especially in lipid membranes. While each of them may have value in the acute phase, F4-neuroprostanes was the only marker found to have the prognostic ability (Seet Raymond et al. 2011). Lorenzano and colleagues investigated biomarkers of oxidative stress which could help predict diffusion–perfusion mismatch in acute ischemic stroke patients. They concluded that mismatch was associated with elevated plasma levels of oxygen radical absorbance capacity (ORAC) and F2-isoprostane. The degree of ORAC elevation was significantly associated with the volume of mismatches in the penumbra which was salvageable (Lorenzano et al. 2019).

Ng et al. (2017) looked into a wide variety of biomarkers in cerebral ischemia in order to enhance clinical decision making. Equipping clinicians with the ability to grade the severity of stroke patients in the acute setting could impact care across the spectrum. They discussed MMP-9 and its ability to be a marker of blood–brain barrier disruption and can even predict greater risk for hemorrhagic transformation in recombinant tissue plasminogen activator (tPA) when the value is >140 ng/ml. Additionally, it can also indicate early neurologic deterioration and the likelihood of malignant infarction. The neuroprostanes, a frequently studied biomarker, have been added to the CHADs-VASc score to help improve its performance in predicting vascular risk (Ng et al. 2017).

Byun et al. discussed oxidized phospholipids and their ability to provide pertinent information about recurrent ischemia in the aftermath of stroke and transient ischemic attack. They found that the oxidized phospholipids on Apolipoprotein B, when elevated, were able to predict recurrent stroke and also predict coronary ischemic events. The levels were elevated in those with recurrent stroke and in those with co-morbidities associated with poor stroke outcome, such as diabetes and cardiovascular risk. While this study used ApolipoB levels to determine statin treatment groups, they found that the oxidized version of the Apolipoprotein (OxPL-apoB) also was predictive in stroke patients that did not have coronary artery disease (Byun et al. 2017).

Zhang and Bi (2020) looked specifically at biomarkers in the post-stroke period. They were primarily concerned with cognitive impairment in the months after an ischemic stroke. Cognitive impairment is markedly increased already in those with stroke. This worsens with the patient's age and can be one of the most detrimental factors for these patients. 8-Hydroxydeoxyguanosine (8-OHdG) is produced by reactive oxygen species and is a biomarker that indicates overall oxidative stress. Oxidative stress is associated with cognitive impairment and likewise, high levels of 8-OHdg were correlated with increased cognitive impairment in a post-stroke period of up to 1 month. Additionally, they spoke on Malondialdehyde (MDA) which has begun to develop a depth of research in recent years. MDA levels indicate oxidative stress from lipid peroxidation. MDA levels are elevated in acute ischemic stroke and also are predictive of the patient having a low cognitive test score. MDA has been linked independently to cognitive impairment in the post-stroke phase (Zhang and Bi 2020). Researchers have also studied multiple biomarkers and supported a 4 marker screening test for stroke which included MMP-9 and S100-B. The panel had sensitivity approaching 90%. S100-B is found in central nervous system astrocytes and its elevation has been associated with increased oxidative stress and inflammation in subarachnoid hemorrhage (Kernagis and Laskowitz 2012). Chong studied only this marker and concluded that S100-B should be studied further as it is promising to predict severity and understand prognosis in subarachnoid hemorrhage (Chong 2016). The use of multiple markers may be an area of further research in order to help improve the accuracy and value of the biomarkers mentioned throughout this section (Table 19.4).

Table 19.4 Redox signaling biomarkers associated with damage to the neurologic system. Markers specific to the neurologic system can be detected in the event of tissue damage and injury and guide patient management

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
Acrolein	Spinal cord injury	Lipid peroxidation marker	Appears to be toxic and leads to axonal injury with inflammation and demyelination. Elevated in damaged spinal cord tissue
Apolipoprotein B	Ischemic stroke, coronary ischemic events	Oxidized phospholipids on structure are associated with stroke outcomes	Levels elevated in recurrent stroke and co-morbidities associated with poor stroke outcomes

(continued)

Table 19.4 (continued)

Biomarker	Relevant disease states	Redox-associated pathophysiology/mechanism	Clinical use
Isoprostanes, neuroprostanes	Ischemic stroke, TBI	Signs of oxidative stress in lipid membranes	Increase 6–12 h after acute stroke, also have elevated levels following moderate to severe TBI
MDA	TBI	Product of lipid peroxidation	Shown to have higher levels in the first 30 min after trauma, stay elevated for 72 h after brain injury; predicts low cognitive test score. Positive correlation with increased levels and increased mortality
MMP-9	Ischemic stroke	Marker of blood–brain barrier disruption	Can predict high risk of hemorrhagic transformation in TPA, early neurologic deterioration, and likelihood of malignant infarction
PPARy	TBI, ischemic stroke	Expressed in multiple tissues with neuroprotective effects	Animal studies show an increase acutely after ischemic stroke from 4 h to 14 days post-stroke, associated with increased mortality
Thioredoxin	Acute ischemic stroke	Released from cells during oxidative stress	Correlation noted between levels and the severity of prognosis

19.6 Conclusion

Redox biomarkers clearly represent an important modality to decipher the complex physiology of acute processes and responses in the aging population. This chapter describes specific biomarkers in the setting of sepsis, acute pulmonary disease, cardiovascular insults, traumatic brain and spine injury, and stroke--disease states frequently encountered in the acute or emergent setting. While some biomarkers have strong diagnostic correlation and potentially promising prognostic value, the field is young. More study is needed to understand the stress reactions of the aging population, the clinical significance and use of biomarker monitoring, and how widespread adoption and utilization of these tools will affect the practice of acute care medicine.

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