A REVIEW ON THE EFFECT OF FREE RADICAL ON AGING

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ABSTRACT

Aging is such a process through which all human beings go, some people age faster some slower. Aging is an inevitable physiological change occurring in organisms over time. Many factors like lifestyle and genetics can cause aging these are primary factors, some other external factors like environment, stress, improper sleep, and nutrition are also caused by premature aging or unhealthy aging. There are more than 300 theories for aging, and among all Free radical theory is the leading cause of aging. Aging is a process that we can neither stop nor reverse but we can make it healthy or successful aging. Healthy aging is about creating the environments and opportunities that enable people to be and do what they value. Therefore one of the best ways to deal with aging caused by free radicles is to encounter them with various antioxidants. Nutrient antioxidants belonging to exogenous antioxidants are compounds that cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids

Keyword: - *Aging, Free radicals, Antioxidants, Nutrient antioxidants*

1. INTRODUCTION

Aging is such a process through which all human beings go, some people age faster some slower. Aging can be defined as “a gradual deterioration in the ability to perform the physiological activity”. This change is irreversible and unstoppable. Many factors like lifestyle and genetics can cause aging these are primary factors, some other external factors like environment, stress, improper sleep, and nutrition are also caused by premature aging or unhealthy aging. The scientific community has generated over 300 theories to explain the driving forces behind aging [1]. Denham Harman's Free Radical Theory of Ageing, first proposed in 1956, has significantly influenced aging research for the past half-century [2]. Gershman and Gilbert were the first to notice similarities between reactive species produced during cellular respiration and those made by irradiation: the generation of free radicals such as hydroxyl (.OH) and superoxide (O2.-) radicals, as well as the less reactive non-radical oxygen species, hydrogen peroxide (H2O2) [3]. Oxidative stress, cellular senescence, and consequently, SASP factors are involved in several acute and chronic pathological processes, such as CVDs, acute and chronic kidney disease (CKD), neurodegenerative diseases (NDs), macular degeneration (MD), biliary diseases, and cancer. Cardiovascular (CV) risk factors (ie, obesity, diabetes, hypertension, and atherosclerosis) are associated with the inflammatory pathway mediated by IL-1α, IL-6, IL-8, and increased cellular senescence [4].

1. WHAT ARE FREE RADICALS

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an

electron from other molecules, therefore behaving as oxidants or reductants. The most important oxygen-containing free radicals in disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. [5]

1.1 Characteristics of free radicals [5]

- They are highly reactive due to the presence of unpaired electrons in the outermost shell.
- They cause the generation of new radicals.
- They have a very short or ultra-short life span
- Production of free radicals causes damage to the body

1.2 Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (NOS)

In general pro-oxidants/oxidants are termed ROS/RNS. The most important free radicals produced during metabolic reactions are radicals derived from oxygen and ROS. Both the ROS and RNS can be classified into two groups of compounds namely; radicals and non-radicals. Radicals are the species that contain at least one unpaired electron in the shells around the atomic nucleus and are capable of independent existence. The oxygen molecule itself is a radical, and because of the presence of two unpaired electrons, it is referred to as biradical. Examples of the radicals include Superoxide (O_2.-), Oxygen radical (O_2..), Hydroxyl (OH-), Alkoxyradical (RO-), Peroxyl radical (ROO-), Nitric oxide (nitrogen monoxide) (NO) and nitrogen dioxide (NO_2). The high reactivity of these radicals is due to the presence of one unpaired electron which tends to donate it or to obtain another electron to attain stability. The nonradical species include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCI), hypobromous acid (HOBr), ozone(O3), singlet oxygen (10), nitrous acid (HNO_2), nitrosylcati (NO+), nitrosyl anion (NO-), dinitrogen trioxide (N_2O), dinitrogen tetraoxide ((N_2O), nitronium (nitryl)cation (NO_2), organic peroxides (ROOH), aldehydes(HCOR) and peroxynitrite (ONOOH). These nonradical species are not free radicals but can easily lead to free radical reactions in living organisms [6].

Table 1: list of ROS and NOS produced during metabolism. [6]

FREE RADICALS	SYMBOLS	HALF LIFE
Reactive oxygen species (ROS)		
Superoxide	$O_2^{\bullet}$	10-6 s
Hydroxyl	$OH^{\bullet}$	10-10 s
Alkoxyl radical	RO•	10-6
Peroxyl Radical	ROO•	17 s
Non radicals Hydrogen peroxide	H_2O_2	Stable
Singlet oxygen	$1O_2$	10-6s
Ozone	O_3	Stable
Organic peroxide	ROOH	Stable
Hypochlorus acid	HOCl	Stable
Hypobromous acid	HOBr	Stable
Reactive Nitrogen Species (RNS)		
Nitric oxide	$NO^{\bullet}$	Stable
Nitrogrn dioxide	$NO_2^{\bullet}$	Stable
Non radicals		
Peroxynitrite	$ONOO^-$	10-3s
Nitrosyl cation	NO^+	-
Nitrosyl anion	NO^-	-
Dinitrogen trioxide	N_2O_3	-
Dinitogen tetraoxide	N_2O_4	-
Nitrous acid	HNO_2	-
Peroxynitrous acid	ONOOH	Fairly stable
Nitryl chloride	NO_2Cl	-

1.3 Generation of free radicals

The generation of ROS begins with rapid uptake of oxygen, activation of NADPH oxidase, and the production of the superoxide anion radical (O_2), eqn (1),

$$2O_2 + NADPH \quad (oxidase) \quad 2O_2^{\bullet -} + NADP + H^- \ldots\ldots\ldots\ldots (1)$$

The 20 is then rapidly converted to H_2O_2 (eqn (2)) by SOD

$$2O_2^{\bullet-} + 2H^- \xrightarrow{SOD} H_2O_2 + O_2 \text{ …….. (2)}$$

These ROS can act by either of the two oxygen-dependent mechanisms destroying the microorganism or other foreign matter. The reactive species can also be generated by the myeloperoxidase-halide-H2O2 system. The enzyme myeloperoxidase (MPO) is present in the neutrophil cytoplasmic granules. In the presence of the chloride ion, which is ubiquitous, H_2O_2 is converted to hypochlorous (HOCI, eqn (3)), a potent oxidant and antimicrobial agent.

$$CI^{\bullet} + H_2O_2 + H \longrightarrow HOCI + H_2O \text{…….(3)}$$

ROS are also generated from $2O_2^{\bullet-}$ and H_2O_2 via 'respiratory burst' by Fenton (eqn (4) and/or Haber-Weiss (eqn (5) reactions.

$$H_2O_2 + Fe^{2+} \longrightarrow OH + OH^- + Fe^{3+} \text{……. (4)}$$

$$O_2^{\bullet-} + H_2O_2 \longrightarrow OH + OH^{\bullet} + O_2 \text{ ……. (5}$$

The enzyme nitric oxide synthase produce reactive nitrogen species (RNS), such as nitric oxide (NO) from arginine (eqn (6).

$$L\text{-}Arg + O_2 + NADPH \longrightarrow NO^{\bullet} + \text{citrulline..... (6)}$$

An inducible nitric oxide synthase (iNOS) is capable of continuously producing large amount of $NO^{\bullet}$, which act as $aO_2^{\bullet-}$ quencher. The $NO^{\bullet}$ and $O_2^{\bullet-}$ react together to produce peroxynitrite ($ONOO^-$, eqn (7)), a very strong oxidant, hence, each can modulate the effects of other. Although neither $NO^{\bullet}$ nor $O_2^{\bullet-}$ is a strong oxidant, peroxynitrite is a potent and versatile oxidant that can attack a wide range of biological targets.

$$NO^{\bullet} + O_2^{\bullet-} \longrightarrow ONOO^- \text{…… (7)}$$

Peroxynitrite reacts with the aromatic amino acid residues in the enzyme resulting in the nitration of the aromatic amino acids. Such a change in the amino acid residue can result in the enzyme inactivation. However, nitric oxide is an important cytotoxic effector molecule in the defense against tumor cells, various protozoa, fungi, helminths, and mycobacteria The other sources of free radical reactions are cyclo oxygenation, lipooxygenation, lipid peroxidation, metabolism of xenobiotics, and ultraviolet radiations [6].

1.4 Properties of free radical

- **Superoxide Ion Radical ($O_2^{\bullet-}$)** [11,12]

Superoxide anion radical is the most important widespread ROS formed by the enzymatic process, autooxidation reaction, and nonenzymatic electron transfer reactions in which an electron is transferred to molecular oxygen. It is mostly produced within the mitochondria and its reactivity with the biomolecules is low. The enzymes that can produce superoxide include xanthine oxidase, lipooxygenase, cyclooxygenase, and NADPH-dependent oxidase. It can exist in two forms such as O2•- or hydroperoxyl radical (HO2) at low pH. The hydroperoxyl radical is the most important form and can easily enter the phospholipid bilayer than the charged form (O2•-). Under physiological pH the most occurring form is superoxide. It can act as a reducing agent and it reduces iron complexes such as cytochrome-c and ferric-ethylene diamine tetraacetic acid (Fe+-EDTA), in which Fe+3 is reduced to Fe+2. It can also act as an oxidizing agent and oxidize ascorbic acid and tocopherol.

- **Hydroxyl Radical (OH•)** [13,14,15,16]

Hydroxyl radical is the neutral form of hydroxide ion and is a highly reactive free radical. It can strongly react with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates, and cause more severe damage to the cells than any other ROS can do. It is formed in a Fenton reaction, in which H2O2 reacts with metal ions (Fe? 2 or Cu?), often bound in complex with different proteins such as ferritin (an intracellular protein that stores iron) and ceruloplasmin (plasma copper carrying protein) or other molecules. Under stress conditions, an excess of O2•-releases free iron from ferritin, and the released free iron participates in the Fenton reaction to form OH•. It is also formed by the reaction between superoxide radicals and H2O2 in a reaction called Haber– Weiss reaction..

- **Peroxyl Radical (ROO•)** [17,18]

It is derived from oxygen in living systems. The simplest form of peroxyl radical is perhydroxyl radical (HOO•) which is formed by the protonation of superoxide. About 0.3 % of the total O2•- in the cytosol of a typical cell is in the protonated form. It initiates fatty acid peroxidation and also can promote tumor development.

- **Hydrogen Peroxide (H_2O_2)** [19,20,21]

Hydrogen peroxide is formed in vivo in a dismutation reaction catalyzed by the enzyme superoxide dismutase (SOD). It is not a free radical but it can cause damage to the cell at relatively low concentrations (10 lM), but at higher levels, the cellular energy-producing enzymes such as glyceraldehyde-3-phosphate dehydrogenase are inactivated. It can easily penetrate the biological membranes. H2O2 has no direct effect on DNA but can damage

DNA by producing hydroxyl radical (OH-) in the presence of transition metal ions. The major antioxidant enzymes that can eliminate the H2O2 include catalase, glutathione peroxidase, and peroxiredoxins.

Singlet Oxygen ($^1O_2$) [22-29]

It is an electronically high excited, meta-stable state of molecular oxygen and is a highly reactive toxic reactive oxygen species. Upon activation, the molecular oxygen is excited to first state 1Δg and then to the next higher excited singlet state, 1eg. The first excited state, 1Δg, has two electrons with opposite spins in the same π* orbital whereas, the second excited state, 1εg, has one electron in each degenerated π* orbital with opposite spins. The 1Dg state is extremely reactive, and compared to the other electronically excited states] It is produced in vivo by the activation of and eosinophil. It is also formed by some of the enzymatic reactions catalyzed by enzymes such as lipoxygenases, deoxygenates, and lacto peroxidase. It is a highly potent oxidizing agent that can cause DNA damage and tissue damage.

- **Ozone (O_3)** [30-35]

Ozone is a powerful oxidant that may be produced in vivo by antibody antibody-catalyzed water oxidation pathway which plays an important role in inflammation. It can form free radicals and other reactive intermediates by oxidizing the biological molecules. It can cause lipid peroxidation and oxidize different functional groups, for example, amine, alcohol, aldehyde, and sulphydryl, present in proteins and nucleic acids]. It can also cause chromosomal aberrations which may be due to direct attack by O3 or by the free radicals generated by it.

- **Hypochlorous Acid (HOCl)** [36-39]

It is a major oxidant produced by the activated neutrophils at the site of inflammation from hydrogen peroxide and chloride in a reaction catalyzed by the enzyme myeloperoxidase.

HOCl is a strong reactive species involved in oxidation and chlorination reactions. It can oxidize thiols and other biological molecules including, ascorbate, urate, pyridine nucleotides, and tryptophan. HOCl chlorinates several compounds such as amines to give chloramines;

tyrosyl residues to give ring chlorinated products, cholesterol, and unsaturated lipids to give chlorohydrins, and it can also chlorinate DNA..

- **Nitric Oxide or Nitrogen Monoxide (NO•)** [40-45]

It is a small molecule generated in tissues by different nitric oxide synthases (NOS) which convert L-arginine to L-citrulline. In this reaction, one of the terminal guanido nitrogen atoms undergoes oxidation and produces NO. Three types of isoforms of NOS such as neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) are involved in the formation of the NO radical.

It is both aqueous and lipid soluble and therefore it readily diffuses through the cytoplasm and plasma membrane]. The NO• is an important intracellular second messenger that stimulates guanylate cyclase and protein kinases and helps in smooth muscle relaxation in blood vessels. It is identical to endothelium-derived relaxing factor (EDRF) produced by vascular endothelial cells which is an important mediator of vascular responses [42]. It can also act as an important cellular redox regulator] and regulate enzymatic activity by nitrosylating the proteins. Since it is involved in many biological activities like blood pressure regulation, smooth muscle relaxation, neurotransmission, defensive mechanisms, and immune regulation.

- **Peroxynitrite ($OONO^-$) and Other Reactive Nitrogen Species** [46-49]

Peroxynitrite (OONO-) is formed by the reaction between O2□- and NO_. It is highly toxic and can directly react with CO2 to form other highly reactive nitroso peroxo carboxylate (ONOOCO2-) or peroxynitrous acid (ONOOH). The ONOOH further undergoes homolysis to form both OH• and NO2 or rearranges to form NO3. OONO- can oxidize lipids, oxidize methionine and tyrosine residues in proteins, and oxidize DNA to form nitroguanine. The nitrotyrosine residues are considered as a marker of peroxynitrite-induced cellular damage \.

NO reacts with O2 and water to form nitrate and nitrite ions. One electron oxidation of NO• results in nitrosonium cation (NO2□+) while one electron reduction results in nitroxyl anion (NO-). These two ions can react with NO and form N2O and OH_. NO_ can react with a variety of radicals such as H2O2 and HOCl to form N2O3, NO2- andNO3-.

1.5 Sources of Free Radicals

The ROS can be produced from either endogenous or exogenous sources. The endogenous sources of OS include different cellular organs such as mitochondria, peroxisomes and endoplasmic reticulum, where the oxygen consumption is high.

Mitochondria

Most of the intracellular ROS are derived from mitochondria (Fig. 1). The superoxide radicals are produced at two major sites in the electron transport chain, namely complex I (NADH dehydrogenase) and complex III (ubiquinone cytochrome c reductase). The transfer of electrons from complex I or II to coenzyme Q or ubiquinone (Q) results in the formation of a reduced form of coenzyme Q (QH2). The reduced form QH2 regenerates coenzyme Q via an

unstable intermediate semiquinone anion □Q- in the Q-cycle. The formed □Q- immediately transfers electrons to molecular oxygen leading to the formation of superoxide radicals [50].

The superoxide anion is converted to hydrogen peroxide by the action of mitochondrial superoxide dismutase (MnSOD). H2O2 can be detoxified by the Catalase (CAT) and glutathione peroxidase (GPx). The other mitochondrial components which contribute to the formation of ROS include monoamine oxidase, ketoglutarate dehydrogenase, glycerol phosphate dehydrogenase, and p66shc [51].

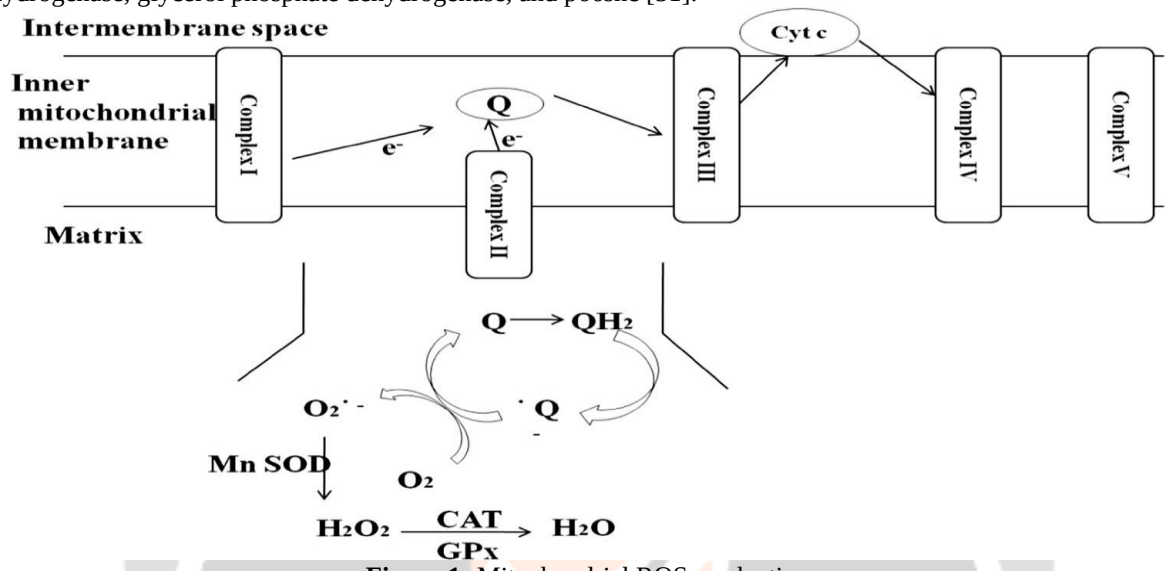

Figure 1: Mitochondrial ROS production

- **Peroxisomes** [52,53]

In peroxisomes, the respiratory pathway involves the transfer of electrons from various metabolites to the oxygen leading to H2O2 formation. But is not coupled to oxidative phosphorylation to produce ATP instead free energy is released in the form of heat. The other free radicals produced in peroxisomes include H2O2, O2•- OH• and NO•. The b-oxidation of fatty acids is the major metabolic process producing H2O2 in the peroxisomes. As reviewed elsewhere, the different peroxisomal enzymes such as acyl CoA oxidases, D-amino acid oxidase, L-a-hydroxy oxidase, urate oxidase, xanthine oxidase, D-aspartate oxidase have been shown to produce different ROS.

- **Endoplasmic Reticulum** [54,55]

The enzymes of endoplasmic reticulum such as cytochrome p-450 and b5 enzymes and diamine oxidase contribute to the formation of ROS. Another important thiol oxidase enzyme, Erop1p catalyses the transfer of electrons from dithiols to molecular oxygen results in the formation of H2O2.

1.6 Molecular Targets of Free Radicals [56]

When there is an imbalance between the free radical production (ROS/RNS) and antioxidant defenses, the former will be produced in higher concentrations leading to oxidative stress and nitrosative stress. Since these free radicals are highly reactive, they can damage all the three important classes of biological molecules including nucleic acids, proteins, and lipids.

- **Deoxyribonucleic Acid (DNA)** [57-59]

Both ROS/RNS can oxidatively damage the nucleic acids. ThemitochondrialDNA ismore vulnerable to the ROS attack than the nuclear DNA, because it is located in close proximity to the ROS generated place. ROS, most importantly, the OH• radical directly reacts with all components of DNA such as purine and pyrimidine bases, deoxyribose sugar backbone and causes a number of alternations including single and double stranded breaks in DNA.

The major free radical induced adducts of the sugar moiety in DNA include glycolic acid, 2-deoxytetrodialdose, erythrose, 2-deoxypentonic acid lactone, 2-deoxypentose-4-ulose. 8-hydroxy deoxyguanosine is considered as the biomarker of oxidativeDNA damage and is involved in mutagenesis, carcinogenesis and ageing.

- **Ribonucleic acid (RNA)** [60-65].

ROS can attack different RNAs produced in the body. The RNA is more prone to oxidative damage than DNA, due to its single stranded nature, lack of an active repair mechanism for oxidized RNA, less protection by proteins than DNA and moreover these cytoplasmic RNAs are located in close proximity to the mitochondria where loads of ROS are produced. Indeed, RNA is subjected to more oxidative damage than DNA in humans. 7, 8-dihydro-8-

oxoguanosine (8-oxoG) is the most extensively studied RNA damage product and its levels are raised in various pathological conditions like Alzheimer's disease, Parkinson's disease, atherosclerosis, hemochromastosis and myopathies.

- **Lipids** [66,67].

The membrane lipids, especially the polyunsaturated fatty acid residues of phospholipids are more susceptible to oxidation by free radicals. The lipid peroxidation is very important in vivo because of its involvement in various pathological conditions. The lipid peroxidation results in the loss of membrane functioning, for example, decreased fluidity, inactivation of membrane bound enzymes and receptors.

- **Proteins** [68,69].

The protein oxidation can be induced by radical species such as O_2 •-, OH•, peroxyl, alkoxyl, hydroperoxyl as well as by the non-radical species such as H_2O_2, O_3, HOCl, singlet oxygen, OONO-. ROS oxidize different amino acids present in the proteins, causing formation of protein– protein cross linkages, results in the denaturing and loss of functioning of proteins, loss of enzyme activity, loss of function of receptors and transport proteins.

2. WHAT IS AGING [70, 71]

Aging is an inevitable physiological change occurring in organisms over time. It ultimately 'leads to death naturally as one gets old along with gradual dysfunctions of all organs in the organisms including unicellular organisms, plants, animals, and humans'. Aging is the direct cause of diseases and death in humans so it is one of the biggest questions among many biological phenomena. Despite the efforts of several researchers, the progression and the mechanisms of aging are not clearly understood yet. Of various theories with regards to the causes of aging, there are two important theories including genetic programming theories of aging, suggesting that aging and lifespan of organ¬isms are genetically determined, and theories of aging related to primary damage, claiming that aging is induced by the accu¬mulation of damages in organisms from multiple harmful fac¬tors. The wear-and-tear theory, error catastrophe theory, the free radical theory, DNA damage hypothesis, loss of adaptive cellular mechanisms, the mitochondrial theory, and the cell membrane theory are known to be included in the theories of aging related to primary damage.

2.1 Factors Associated With Aging

There are many factors which plays very important role in aging. It is very difficult to explain the actual cause of aging but for the understanding the factors which are associated with aging can be categorized into following two types:

i. **Intrinsic factors** [72, 77]

Aging is caused by intrinsic factors and is determined by both, physiological factors and genetic predisposition. Different signs of aging occur as cellular proliferation and hormone levels decrease, or as a result of diverse factors: telomere shortening and accumulation of dysplastic keratinocytes; degradation of the extracellular matrix; mutations in nuclear and mitochondrial genes, Production of reactive oxygen species (ROS) in the mitochondria that results from an oxidative cell metabolism and a decrease in antioxidant activity and multiple lipid or amino acid metabolic aberrations. Such disturbances cause functional and physical changes due to abnormal water distribution or a lack of hygroscopic substances.

Extrinsic factors [78, 80]

Environmental factors such as smoking, automobile contaminants, alcohol consumption, dietary habits, industrial waste, stress and lifestyle options can contribute to aging. These factors do not cause aging directly but they speed up the whole aging process.

2.2 Mechanism of Aging [81, 82]

There are many theories of the biological causes of aging, which suggest that many different mechanisms contribute to the aging process. According to Kirkwood "the rate of accumulation of stress-induced random molecular damage is dependent on the capacity of the antioxidant system and efficiency of repair systems. As these systems are not 100% efficient, cells always contain some unrepaired damage that leads to the activation of a stress response and up-regulation of mechanisms to remove the damage or to prevent cell division. However, these responses also become less efficient with age so that damaged components accumulate leading to cellular defects, which gives rise to tissue dysfunction and aging".

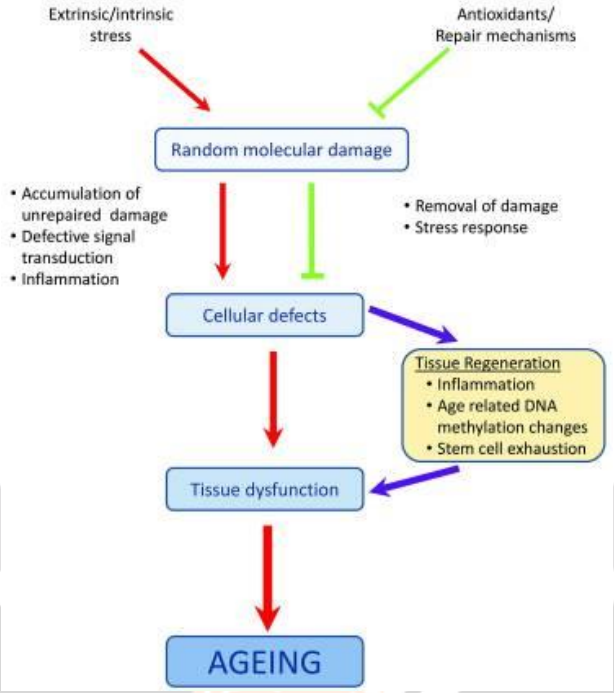

Figure- 2 The Underlying Process of Aging

2.3 Aging Theories [83, 84]

Despite recent advances in molecular biology and genetics, the mysteries that control the human lifespan remain unclear.

Many theories have been proposed to explain the process of aging, but neither of them appears to be fully satisfactory. The traditional aging theories hold that aging is not an adaptation or genetically programmed. Modern biological theories of aging in humans fall into two main categories:

1. Programmed theory
   The programmed theory has three sub-categories:
   a. Programmed Longevity
   b. Endocrine Theory
   c. Immunological Theory
2. Damage or error theories
   The damage or error theory includes:
   a. Wear and tear theory
   b. Rate of living theory
   c. Free radicals theory
   d. Somatic DNA damage theory

3. FREE RADICAL THEORY OF AGEING [85, 92]

The free radical theory of aging (FRTA), and the simultaneous discovery of the important, ubiquitous involvement of free radicals in endogenous metabolic reactions, was proposed by Harman in 1954. The FRTA arose from the application of a broad background in chemistry and biology to a consideration of aging phenomena from the premise that a single common process, modifiable by genetic and environmental factors, was responsible for the aging and death of all living things.

The FRTA postulates that the common aging process is the initiation of free radical reactions (FRRs). These reactions, however, initiated, could be responsible for the progressive deterioration of biological systems over time owing to their innate ability to produce random change due to the high chemical reactivity of the intermediate free radicals.

The FRTA was extended in 1972 with the suggestions that (a) most FRRs were initiated by the mitochondria at an increasing rate with age, and (b) the life span is determined by the rate of free radical damage to the mitochondria. Consequences of mitochondrial aging were discussed in 1983. Collectively, the FRRs initiated by the mitochondria constitute the inherent aging process. Later, it became clear [98, 99] that improvements in general living conditions increased ALE-B by decreasing the FRRs associated with suboptimal living conditions.

The FRTA suggests that measures to decrease (a) the chain lengths of FRRs, e.g., with antioxidants such as vitamin E, and/or (b) their rates of initiation, e.g., by minimizing copper, iron, and other oxidant catalysts, can lower the rate of formation of aging changes, even under optimal living conditions, and in turn decrease the rate of aging and of disease pathogenesis. Many studies now support this possibility..

3.1 Advancement and Corollaries of Free Radical Theory of Aging

a. Oxidants and Evolutionary Theories of Aging
b. Oxidants and the Somatic Mutation Theory of Aging
c. Oxidants and Mitochondrial Theories of Aging

3.2 Age Related Issues and Free Radicals [93-116]

Oxidative stress, cellular senescence, and consequently, SASP factors are involved in several acute and chronic patho¬logical processes, such as CVDs, acute and chronic kidney disease (CKD), neurodegenerative diseases (NDs), macular degeneration (MD), biliary diseases, and cancer. Cardiovas¬cular (CV) risk factors (ie, obesity, diabetes, hypertension, and atherosclerosis) are associated with the inflammatory pathway mediated by IL-1α, IL-6, IL-8, and increased cellular senescence]. Moreover, vascular calcification is linked to SASP-driven osteoblastic transdifferentiation of senescent smooth muscle cells. In many neurodegenerative conditions, including Alzheimer's disease (AD), brain tissue biopsies show increased levels of p16, MMP, and IL-6. Chronic obstructive pulmonary disease, biliary cirrhosis, cholangitis, and osteoarthritis share several damaging SASP profiles including IL-6, IL-8, and MMP. The induction of epithelial to mesenchymal transition mediated by RONS promotes cancer metastasis. In synthesis, given the close relationship between oxidative stress, inflammation, and aging, the oxidation-inflammatory theory of aging or oxi-inflame-aging has been proposed: aging is a loss of homeo¬stasis due to chronic oxidative stress that affects especially the regulatory systems, such as nervous, endocrine, and immune systems. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in which chronic oxidative stress and inflammation feed each other, and consequently, increase the age-related morbidity and mortality.

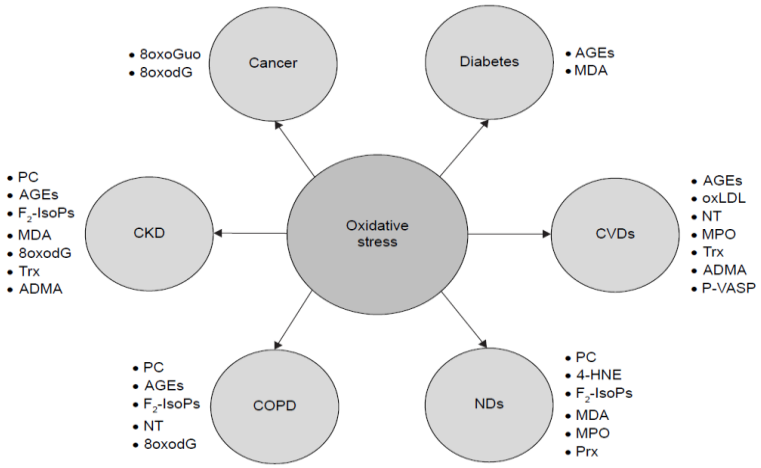

Figure 3 Oxidative stress, age-related diseases, and relative biomarkers**.**

Abbreviations: 4-HNE, *trans*-4-hydroxy-2-nonenal; 8oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine; 8oxoGuo, 7,8-dihydro-8-oxoguanosine; ADMA, asymmetric dimethyl l-arginine; AGEs, advanced glycation end products; CKD,

chronic kidney disease; CVDs, cardiovascular diseases; F2-IsoPs, F2-isoprostanes; MDA, malondialdehyde; MPO, myeloperoxidase; NDs, neurodegenerative diseases; NT, nitrotyrosine; oxLDL, oxidized low-density lipoprotein; PC, protein carbonyl; Prx, peroxiredoxins; P-VASP, phosphorylated vasodilator-stimulated phosphoprotein; Trx, thioredoxin

The connection between oxidative stress and the main age-related diseases are,

Cancer
Cardiovascular disease
Neurological disease
Pulmonary disease
Rheumatoid arthritis
Nephropathy
Ocular disease

3.4 Healthy Aging [117-122]

Aging is a process that we can neither stop nor reverse but we can make it healthy or successful aging. Healthy aging is about creating the environments and opportunities that enable people to be and do what they value. WHO defines healthy aging as "the process of developing and maintaining the functional ability that enables well-being in older age"

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects, and to contribute to disease prevention.

Anti-oxidants can be classified as enzymatic antioxidants and non-enzymatic antioxidants.

The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx).

The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants are produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc. Nutrient antioxidants belonging to exogenous antioxidants are compounds that cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc

4. CONCLUSION

Free radicals, atoms, and molecules containing one or more unpaired electrons in the outer orbit and are capable of independent existence which are highly reactive and cause damage to the body. There are two types of radicals in the body i.e. reactive oxygen species (ROS) for example superoxide, hydroxyl, and alkoxy radicals etc. these radicals form into mitochondria, peroxisome, and endoplasmic reticulum, and the target of these radicals are DNA, RNA, lipids and proteins.

Aging is an unavoidable, irreversible, and unstoppable phenomenon occurring in organisms at one time. Aging can be caused by many factors which are categorized into intrinsic (time and genes) and extrinsic (smoking, alcohol, lifestyle, stress, environment, etc.). This process of aging is defined by many scientists and over three hundred theories were proposed. The most prominent and acceptable theory to date is the Free radical theory of aging (oxidative stress).

Aging can neither be stopped nor avoided but the means of proper diet including functional food and dietary supplements, exercise, adopting a healthy life style, and avoiding such things that cause oxidative stress can make the aging process healthy or successful.

5. REFERENCES

[1] Vina, J., C. Borras, and J. Miquel, *Theories of ageing.* IUBMB life, 2007. **59**(45): p. 249-254.

[2] Harman, D., *Aging: a theory based on free radical and radiation chemistry.* 1955.

[3] Gerschman, R., et al., *Oxygen poisoning and x-irradiation: a mechanism in common.* Science, 1954. **119**(3097): p. 623-626.

[4] Chandrasekaran A, Idelchik MDPS, Melendez JA. Redox control of senescence and age-related disease. *Redox Biol*. 2017;11:91–102.

[5] "New Approach to Dietary Supplement and Nutraceuticals" by Shilpa Mandpe Ambade , Dr. Vishal R. Parate, Dr. Md.Rageeb andDr. Bharat V. Jain, Pee Vee publications , pg no.99

[6] "A Textbook of Dietary Supplement And Nutraceuticals" By Dr. Ajay Kumar Shukla, Dr. Santram Lodhi and Dr. Mohanlal Kori, Pee Vee Publications, pg no. 195 & 19
[7] Michelson AM, McCord JM, Fridovich I. Superoxide and Superoxide Dismutases. London: Academic Press; 1977. p. 320.
[8] Kuppusamy P, Zweier JL. Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation. J Biol Chem. 1989;264(17):9880–4.
[9] Kontos HA, Wei EP, Ellis EF, Jenkins LW, Povlishock JT, Rowe GT, et al. Appearance of superoxide anion radical in cerebral extracellular space during increased prostaglandin synthesis in cats. Circ Res. 1985;57(1):142–51.
[10] McIntyre M, Bohr DF, Dominiczak AF. Endothelial function in hypertension. Hypertension. 1999;34:539–45.
[11] Bielski BHJ, Cabelli DE. Superoxide and hydroxyl radical chemistry in aqueous solution. Active Oxygen in Chemistry. 1996;66–104.
[12] Bielski BHJ, Cabelli BH, Arudi RL, Ross AB. Reactivity of RO2/O2. Radicals in aqueous solution. J Phys Chem Ref Data. 1985;14:1041–100.
[13] Bedwell S, Dean RT, Jessup W. The action of defined oxygen centred free radicals on human low density lipoprotein. Biochem J. 1989;262(3):707–12.
[14] Halliwell B. Oxidants and human disease: some new concepts. FASEB J. 1987;1(5):358–64.
[15] Fenton HJH. Oxidation of tartaric acid in the presence of iron. J Chem Soc Trans. 1894;65:899 910.
[16] Haber F, Weiss J. The catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc London (A). 1934;147:332–51.
[17] De Grey ADNJ. HO2˙: the forgotten radical. DNA Cell Biol. 2002;21:251–7.
[18] Cerruti PA. Pro-oxidant states and tumor activation. Science. 1985;227:375–81.
[19] Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS Lett. 2000;486(1):10–3.
[20] Mates JM, Perez-Gomez C, Nunez de Castro I. Antioxidant enzymes and human diseases. Clin Biochem. 1999;32(8):595–603.
[21] Chae HZ, Kang SW, Rhee SG. Isoforms of mammalian peroxiredoxin that reduce peroxides in presence of thioredoxin. Methods Enzymol. 1999;300:219–26.
[22] Hojo Y, Okado A, kawazoe S, Mizutani T. In vivo singletoxygen generation in blood of chromium (VI)-treated mice an electron spin resonance spin-trapping study. Biol Trace Elem Res. 2000; 76(1):85–93.
[23] Agnez-Lima LF, Melo JT, Silva AE, Oliveira AH, Timoteo AR, Lima-BessaKM, et al. Review DNA damage by singlet oxygen and cellular protective mechanisms. Mutat Res. 2012;751(1):1–14.
[24] Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood. 1998;92(9):3007–17.
[25] Kanovasky JR. Singlet oxygen production by biological systems. Chem Biol Interact. 1989;70(1–2):1–28.
[26] Chan HWS. Singlet oxygen analogs in biological systems: coupled oxygenation of 1, 3-dienes by soybean lipoxidase. J Am Chem Soc. 1971;93(9):2357–8.
[27] Hayaishi O, Nozaki M. Nature and mechanisms of oxygenases. Science. 1969; 164:389–96.
[28] Kanofsky JR. Singlet oxygen production by lactoperoxidase. J Biol Chem. 1983; 258(10):5991–3.
[29] Sies H, Menck CF. Singlet oxygen induced DNA damage. Mutat Res. 1992; 275:367–75.
[30] Lerner RA, Eschenmoser A. Ozone in biology. Proc Natl Acad Sci USA. 2003; 100(6):3013–5.
[31] Goldstein BD, Lodi C, Collinson C, Balchum OJ. Ozone and lipid peroxidation. Arch Environ Heath. 1969; 18:631–5.
[32] Freeman BA, Mudd JB. Reaction of ozone with sulfhydryls of human erythrocytes. Arch BiochemBiophys. 1981; 208(1):212–20.
[33] Mudd JB, Leavitt R, Ongun A, McManus TT. Reaction of ozone with amino acids and proteins. Atmos Environ. 1969; 3:669–81.
[34] Mustafa MG. Biochemical Basis of Ozone Toxicity. Free Radical Biol Med. 1990; 9:245–65.
[35] Fetner RH. Ozone induced chromosome breakage in human cell culture. Nature. 1962; 194:793-4.
[36] Winterbourn CC, Kettle AJ. Biomarkers of myeloperoxidasederived hypochlorous acid. Free Radic BiolMed. 2000; 29(5):403–9.
[37] Albrich JM, McCarthy CA, Hurst JK. Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. Proc Natl Acad Sci USA. 1981;78(1):210–4.
[38] Winterbourn CC. Comparative reactivities of various biological compounds with myeloperoxidase-hydrogen peroxide-chloride, and similarity of the oxidant to hypochlorite. Biochim Biophys Acta. 1985;840(2):204–10.
[39] Prutz WA. Hypochlorous acid interactions with thiols, nucleotides, DNA, and other biological substrates. Arch Biochem Biophys. 1996;332(1):110–20.
[40] Andrew PJ, Mayer B. Enzymatic function of nitric oxide synthases. Cardiovasc Res 1999;43(3):521–31.

[41] Chiueh CC. Neuroprotective properties of nitric oxide. Ann N Y Acad Sci. 1999;890:301–11.
[42] Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chandhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA.1987;84:9265–9.
[43] Wink DA, Mitchell JB. Chemical biology of nitric oxide: insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med. 1998;25(4–5):434–56.
[44] Stamler JS. Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell. 1994;78(6):931–6.
[45] Koshland DE Jr. The molecule of the year. Science. 1992;258(5090):1861.
[46] Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol. 1996; 271:C1424–37.
[47] Douki H, Cadet J. Peroxynitrite mediated oxidation of purine bases of nucleosides and isolated DNA. Free Rad Res. 1996; 24(5):369–80.
[48] Ischiropoulos H, Al-Mehdi AB. Peroxynitrite mediated oxidative protein modifications. FEBS Lett. 1995; 364(3):279–82.
[49] Czapski G, Goldstein S. The role of the reactions of NO with superoxide and oxygen in biological systems: a kinetic approach. Free Radic Biol Med. 1995; 19(6):785–94.
[50] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408:239–47.
[51] Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann NY Acad Sci. 2008; 1147:37–52.
[52] De Duve C, Bauduhuin P. peroxisomes (microbodies and related particles). Physiol Rev. 1966;46:323–57.
[53] Schrader M, Fahimi HD. Review Peroxisomes and oxidative stress. Biochim Biophys Acta. 2006;1763(12):1755–66.
[54] Cheeseman KH, Slater TF. An introduction to free radical biochemistry.Br Med Bull. 1993;49(3):481–93.
[55] Gross E, Sevier CS, Heldman N, Vitu E, Bentzur M, Kaiser CA, et al. Generating disulfides enzymatically: reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1p. Proc Nat Acad Sci USA. 2006;103(2):299–304.
[56] Droge W. Review Free radicals in the physiological control ofcell function. Physiol Rev. 2002;82(1):47–95.
[57] Halliwell B, Gutteridge JM. Free radicals in biology and medicine, vol. Third edition. Midsomer Norton: Oxford University Press; 1999.
[58] Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Radical Biol Med. 2002;32(11):1102–15.
[59] Barja G. The flux of free radical attack through mitochondrial DNA is related to aging rate. Aging (Milano). 2000;12(5):342–55.
[60] Hofer T, Badouard C, Bajak E, Ravanat JL, Mattsson A, Cotgreave IA. Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. Biol Chem. 2005;386(4):333–7.
[61] Abe T, Tohgi H, Isobe C, Murata T, Sato C. Remarkable increase in the concentration of 8-hydroxyguanosine in cerebrospinal fluid from patients with Alzheimer's disease. J Neurosci Res. 2002;70(3):447–50.
[62] Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, Sato N, et al. Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. Neurobiol Dis. 2002;9(2):244–8.
[63] Martinet W, de Meyer GR, Herman AG, Kockx MM. Reactive oxygen species induce RNA damage in human atherosclerosis. Eur J Clin Invest. 2004;34(5):323–7.
[64] Broedbaek K, Poulsen HE, Weimann A, Kom GD, Schwedhelm E, Nielsen P, et al. Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. Free Radical Biol Med. 2009;47(8):1230–3.
[65] Tateyama M, Takeda A, Onodera Y, Matsuzaki M, Hasegawa T, Nunomura A, et al. Oxidative stress and predominant Abeta 42 (43) deposition in myopathies with rimmed vacuoles. Acta Neuropathol. 2003;105(6):581–5.
[66] Siems WG, Grune T, Esterbauer H. 4-Hydroxynonenal formation during ischemia and reperfusion of rat small-intestine. Life Sci. 1995;57(8):785–9.
[67] Bast A. Oxidative stress and calcium homeostasis. In: Halliwell B, Aruoma OI, editors. DNA and free radicals. London: Ellis Horwood; 1993. p. 95–108.
[68] Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 1997;324:1–18.
[69] Butterfield DA, Koppal T, Howard B, Subramaniam R, Hall N, Hensley K, et al. Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants N-tert-butyl-alpha-phenylnitrone and vitamin E. Ann N Y Acad Sci. 1998;854:448–62.
[70] Harman D. The aging process. Proc Natl Acad Sci U S A 1981;78: 7124-8.

[71] Albert MA, Funkenstein HH. The effects of age: normal variation and its relation to disease. In: Asbury AD, McKhann GM, McDonald WI, editors. Diseases of the Nervous System-Clinical Neurobiology. 2nd ed. Philadelphia: WB Saunders; 1992. P.598-611.
[72] Nikolakis G, Makrantonaki E, Zouboulis CC. Skin mirrors human aging. Horm Mol Biol Clin Investig.2013 Dec;16(1):13-28. DOI 10.1515/hmbci-2013-0018.
[73] Poljšak B, Dahmane RG, Godić A. Intrinsic skin aging: the role of oxidative stress. Acta Dermatovenerol Alp Pannonica Adriat. 2012;21(2):33-6.
[74] Farage MA, Miller KW, Elsner P, Maibach HI. Functional and physiological characteristics of the aging skin. Aging Clin Exp Res. 2008 Jun;20(3):195-200.
[75] Hashizume H. Skin aging and dry skin. J Dermatol. 2004 Aug;31(8):603-9.
[76] Kohl E, Steinbauer J, Landthaler M, Szeimies RM. Skin ageing. J Eur Acad Dermatol Venereol. 2011 Aug;25(8):873-84. DOI 10.1111/j.1468-3083.2010.03963.x.
[77] Makrantonaki E, Zouboulis CC. William J. Cunliffe Scientific Awards. Characteristics and pathomechanisms of endogenously aged skin. Dermatology. 2007;214(4):352-60.
[78] Farage MA, Miller KW, Elsner P, Maibach HI. Functional and physiological characteristics of the aging skin. Aging Clin Exp Res. 2008 Jun;20(3):195-200.
[79] Bernhard D, Moser C, Backovic A, Wick G. Cigarette smoke--an aging accelerator? Exp Gerontol. 2007 Mar;42(3):160-5.
[80] Farage MA, Miller KW, Elsner P, Maibach HI. Intrinsic and extrinsic factors in skin ageing: a review. Int J Cosmet Sci. 2008 Apr;30(2):87-95. DOI 10.1111/j.1468-2494.2007.00415.x.
[81] Mc Auley MT, Guimera AM, Hodgson D, et al. Modelling the molecular mechanisms of aging. *Biosci Rep*. 2017;37(1):BSR20160177. Published 2017 Feb 23. doi:10.1042/BSR20160177
[82] Weinert B.T. and Timiras P.S. (2003) Invited review: theories of aging. *J. Appl. Physiol. (1985)* 95, 1706–1716
[83] Jin K. Modern Biological Theories of Aging. *Aging Dis*. 2010;1(2):72-74.
[84] Davidovic M, Sevo G, Svorcan P, Milosevic DP, Despotovic N, Erceg P. Old age as a privilege of the "selfish ones" *Aging and Disease.* 2010;1:139–146.
[85] Harman D. Free radical theory of aging: the "free radical" diseases. *Age* 7: 111–131, 1984.
[86] Harman D. Free radical theory of aging: role of free radicals in the origination and evolution of life, aging, and disease processes. In: *Free Radicals, Aging and Degenerative Diseases*, edited by Johnson JE Jr, Walford R, Harman D, and Miquel J. New York, NY: Alan R. Liss, 1986, pp. 3–49.
[87] Harman D. Lipofuscin and ceroid formation. In: *Lipofuscin and Ceroid Pigments*, edited by Porta, E.A. New York, NY: Plenum Press, 1990, pp. 3–15.
[88] Harman D. Free radical involvement in aging: pathophysiology and therapeutic implications. *Drugs Aging* 3: 60–80, 1993.
[89] Harman D. Aging: prospects for further increases in the functional life span. *Age* 17: 119–146, 1994.
[90] Harman D. Aging and disease: extending the functionallife span. *Ann N Y Acad Sci* 786: 321–336, 1996.
[91] Harman D. Aging: overview. *Ann N Y Acad Sci* 928: 1–21,2001.32.
[92] Harman D. Alzheimer's disease: role of aging in pathogenesis. *Ann N Y Acad Sci* 959: 384–395, 2002.
[93] Beckman, Kenneth B., and Bruce N. Ames. The Free Radical Theory of Aging Matures. *Physiol. Rev.* 78: 547–581, 1998.
[94] KIRKWOOD, T B. Comparative life spans of species, why do spe cies have the life spans they do? Am. J. Clin. Nutr 55, Suppl. 1191S-1195S, 1992
[95] KIRKWOOD, T B., AND M. R. ROSE Evolution of senescence: late. survival sacrificed for reproduction. Philos. Trans. R. Soc Lond B Biol Sci 332 15-24, 1991
[96] KIRKWOOD, T B L Evolution and aging. Nature 270 301-304, 1977
[97] ROSE, M. R., AND C. E. FINCH. Genetics and evolution of aging. In: Contemporary Issues in Genetics and Evolution. Dordrecht, Germany Kluwer, 1994, vol 3.
[98] WILLIAMS, G. C., AND R. M. NESSE. The dawn of Darwinian medi cine. Q. Rev. Biol. 66: 1-22, 1991.
[99] BOHR, V. A., AND R. M. ANSON. DNA damage, mutation and fine structure DNA repair in aging. *Mutat. Res.* 338: 25–34, 1995.
[100] EVANS, D. A., J. P. BURBACH, AND F. W. VAN LEEUWEN. Somatic mutations in the brain: relationship to aging? *Mutat. Res.* 338: 173–182, 1995.
[101] DOLLE, M. E., H. GIESE, C. L. HOPKINS, H. J. MARTUS, J. M. HAUSDORFF, AND J. VIJG. Rapid accumulation of genome rear rangements in liver but not in brain of old mice. Nature Genet. 17: 431-434, 1997
[102] WARNER HR. AND TE JOHNSON Parsing age mutations and time Nature Genet 17 368-370, 1997

[103] HART, R. W., AND R. B. SETLOW. Correlation between deoxyribo nucleic acid excision-repair and life-span in a number of mamma lian species. Proc. Natl. Acad. Sci. USA 71. 2169-2173, 1974.
[104] CORTOPASSI, G., AND E. WANG. There is substantial agreement among interspecies estimates of DNA repair activity Mech. Ageing Dev. 91: 211-218, 1996.
[105] FEIG, D. I., T. M. REID, AND L. A. LOEB. Reactive oxygen species in tumorigenesis. Cancer Res. 54, Suppl. 1890s-1894s, 1994.
[106] GROLLMAN, A P, AND M. MORIYA. Mutagenesis by 8-oxoguanine an enemy within. Tren Genet. 9: 246-249, 1993.
[107] GROLLMAN, A P, AND M. MORIYA. Mutagenesis by 8-oxoguanine an enemy within. Tren Genet. 9: 246-249, 1993.
[108] HARMAN, D The biologic clock: the mitochondria? J. Am. Geriatr Soc. 20: 145-147, 1972.
[109] FLEMING, J. E., J. MIQUEL, S. F. COTTRELL, L. S. YENGOYAN, AND A. C. ECONOMOS. Is cell aging caused by respiration-depen dent injury to the mitochondrial genome? Gerontology 28: 44-53, 1982.
[110] MIQUEL, J. An update on the mitochondrial-DNA mutation hypothesis of cell aging. Mutat. Res. 275: 209-216, 1992.
[111] ARNHEIM, N., AND G. CORTOPASSI. Deleterious mitochondrial DNA mutations accumulate in aging human tissues. Mutat. Res. 275: 157-167, 1992.
[112] BANDY, B., AND A. J. DAVISON. Mitochondrial mutations may in crease oxidative stress: implications for carcinogenesis and aging? Free Radical Biol. Med. 8: 523-539, 1990.
[113] BITTLES, A. H. Evidence for and against the causal involvement of mitochondrial DNA mutation in mammalian ageing. Mutat. Res. 275: 217-225, 1992.
[114] CORTOPASSI, G., AND Y LIU. Genotypic selection of mitochondrial and oncogenic mutations in human tissue suggests mechanisms of age-related pathophysiology Mutat. Res. 338: 151-159, 1995.
[115] Burton DGA, Matsubara H, Ikeda K. Pathophysiology of vascular calcification: pivotal role of cellular senescence in vascular smooth muscle cells. *Exp Gerontol*. 2010;45(11):819–824.
[116] Laberge R-M, Awad P, Campisi J, Desprez P-Y. Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenviron Off J Int Cancer Microenviron Soc*. 2012;5(1):39–44
[117] De la Fuente M, Miquel J. An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. *Curr Pharm Des*. 2009;15(26):3003–3026.
[118] Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018;13:757-772. Published 2018 Apr 26. doi:10.2147/CIA.S158513
[119] Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci*. 2008;4(2):89-96.
[120] World Health Organization, 26 October 2020| Q&A, Healthy ageing and functional ability, Retrieved from https://www.who.int/news-room/questions-and-answers/item/healthy-ageing-and-functional-ability
[121] Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Review. *Crit. Rev. Food. Sci. Nutr.* 2004; 44: 275-295.
[122] Droge W. Free radicals in the physiological control of cell function.Review. *Physiol. Rev*. 2002; 82: 47-95.