*ANAND ANJALI [1], EKA LOMINADZE [1], GURAM MATITASHVILI [1], NIKA KAPANADZE [1], TEKLE DARTSIMELIA [1], TINATIN TKEMALADZE [2]*

# OXIDATIVE STRESS AND ANTIOXIDANTS WITH EMPHASIS ON AGEING

**(Review Article)**

[1]USMD program, Tbilisi State Medical University, Tbilisi, Georgia
[2]Department of Molecular and Medical Genetics, Tbilisi State Medical University, Tbilisi, Georgia

https://doi.org/10.52340/jecm.2022.02.14

## Abstract

*Numerous theories have been postulated to understand the process of aging. One of them links degenerative senescence to reactive oxygen species (ROS), which are natural byproducts of oxygen metabolism. Understanding and supporting this theory may be of great importance due to number of reasons. Firstly, there are well-established risk factors that can increase the production of ROS in our body. Secondly, there are different types of antioxidants that can neutralize toxic effects of ROS. Examples of antioxidants include natural enzymes produced in our body, as well as certain exogenous supplements. Moreover, antioxidant supplementation is already offered by some facilities as a hope to decrease age-related complications. Therefore, it is crucial to understand, how ROS is involved in the process of aging and if there is any evidence how alteration in ROS and antioxidants levels influence the process of ageing.*

## Introduction

Aging is a natural inevitable process. It starts after a person reaches the maximum level of growth in his or her 20s. After certain age the functioning of the human body declines gradually, involving physical and cognitive aspects of health. Aging is accompanied by many chronic illnesses that are associated with the daily wear and tear, which makes us vulnerable to different cardiovascular, nervous system, reproductive, urinary, metabolic, musculoskeletal and digestive problems, as well as the decrease in cognitive functions. There are many factors, which determine the human body's response to this transitioning process, lifestyle choices and genetics are said to be two of the main contributors.

### *1. Different theories of aging*

Over many years researchers have tried to decode the exact mechanism behind aging and as a result we were presented with seven main theories, which could be discussed as individual reasons or consequences of aging, or be considered as small components of a very complicated process. The theories mentioned below describe the physiological as well as psychological and social contributors.

The disengagement theory by Elaine Cumming and William Earle Henry [14] gives 9 postulates with the assumptions that disengagement is an inevitable process of cutting the social ties by an older person realizing that he or she does not have much time to live. This is a process, which is caused by losing the previous abilities and skills and can be considered as a defense mechanism for the elderlies to avoid humiliation and damage to reputation. However, at the same time, it can be seen as a balancing necessity as the younger generation is replacing the older one. With the complete disengagement from the society the elderly find a new role in life in order to avoid the crisis of identity and still feel that they are worthy.

The activity theory, by Robert J. Havighurst [17], can be considered an answer to the above-mentioned postulate. It denies the pessimism of the disengagement theory and suggests that there is no withdrawal, as the elderly are feeling happiest when they engage in the social activities, which is reflected in the increased longevity.

While talking about the physiological triggers of aging, the neuroendocrine theory should be discussed. This theory states that with age the functional responsiveness is lost, which is directly translated to the slower current transmission in the neuroendocrine axis. This means slower response of the muscles and delay in obtaining the homeostasis, which is crucial for the person's physical wellbeing [15].

Professor Imre Zs-Nagy from Debrecen University, Hungary described the theory of membrane [57]. According to the Nagy's research the cell membranes solidify more and more and become less lipid with ageing, which subsequently decreases the efficiency of normal function conduction, which further causes the toxic accumulation of lipofuscin, a cellular toxin. According to the theory, as we age, lipofuscin deposits are seen in the brain, heart, liver and skin. There are studies, finding high lipofuscin levels in the

patients with myocardial diseases, associated with sudden cardiac death, and its increased levels in the different parts of the brain leading to brain damage [28]. This theory states that this pigment is highly disruptive and has a major effect on the normal organ function [39]. This theory also suggests that as the cell membranes become more solid, the sodium and potassium transport and electrical and heat transfer are impaired, which results in the decreased cell-to-cell communication and is apparently attributed to aging.

The cross-linking theory by Johan Bjorksten [56] is one of the oldest theories of aging and refers to the oxygen-dependent accumulation of the cross-linked glycosylated proteins. With age there is increased possibility for the oxygen to associate with glucose and protein. Thus, glycosylated proteins are damaging to the cells and tissues, which in turn decreases the speed of the physiological bodily processes causing aging.

The mitochondrial theory is a variant of the free radical theory of aging [49]. Its key point is that the accumulation of excessive damage to the mitochondria and the mitochondrial DNA, which happen over time leads to aging. It has been observed that functional deterioration of mitochondria and the mitochondrial DNA mutations, caused by the ROS, which themselves are the result of the age-related respiratory enzyme impairment, are increased in the cells in an age-dependent manner [2,25]. Human mitochondrial DNA, which is not protected by histones and is exposed to high levels of ROS and free radicals in the matrix of mitochondria, is susceptible to oxidative damage and somatic mutations. Multiple different mitochondrial DNA mutations have been found in patients with mitochondrial diseases, and some of them are also observed in aging human cells. The incidence and abundance of these mutant mitochondrial DNAs increase with age, particularly in tissues with great demand for energy. On the contrary, recent studies [31] have demonstrated that the ability of the human cell to deal with oxidative stress is compromised in aging. This theory is supported by the observation that intracellular levels of H2O2 and the oxidative damage to DNA and lipids are significantly increased with age. Moreover, the mitochondrial pool of reduced glutathione declines and the DNA damage is enhanced in aging tissues. Taken together, these observations and our previous findings [8] that mitochondrial DNA mutations and oxidative damage are increased in aging human tissues, suggest that mitochondrial theory of aging is mature.

The last one is the free radical theory of aging, which is the most discussed and well-known out of these seven. It was proposed by Denham Harman and states, that the accumulation of free radicals produced during aerobic metabolism causes oxidative damage to the cells and subsequent aging [25]. This theory can be linked to the mitochondrial theory, as it states that the ROS primarily damage the mitochondrial DNA and the degree of damage determines the lifespan of an individual.

Majority of the above-mentioned theories support association between ROS and aging. Therefore, further detailed exploration of this topic could bring better understanding of the aging process.

### *2. What are reactive oxygen species and how they might be involved in ageing process*

ROS are natural byproducts of oxygen metabolism. They are mainly produced in mitochondria during oxidative phosphorylation. Normally, most of the oxygen inhaled from air is reduced to produce water, however, 0.1-2% can be incompletely reduced and result in formation of ROS. Examples of ROS include superoxide anions and hydrogen peroxide. In small quantities, they are needed for the maintenance of human physiological processes and are thought to be involved in cellular growth, apoptosis, signaling pathways and generation of the inflammatory response against pathogens [18]. However, in high amounts it can induce damage by oxidizing certain cellular components. This is called oxidative stress.

Researchers have proven that ROS directly damage crucial elements of the cell, specifically lipids, nucleic acids and proteins [2]. Lipid peroxidation leads to the formation of cyclic endoperoxides and unsaturated aldehydes that are toxic for cellular membrane and enzymes. ROS also damages all four bases, resulting in formation of double-strand breaks and cross-links in nucleic acids, they further lead to peptide fragmentation and oxidation of amino acid residues [3].

These findings have served as the foundation for several ageing theories, aiming to prove, that through these processes ROS accelerate degenerative senescence. Free radical theory of aging dates back

as 1956 [4] and since then it has been a topic for many researches. However, the exact impact that ROS might have on aging is still controversial. Oxidative stress is known to be a contributing factor for developing certain degenerative and age-related diseases such as Alzheimer's disease, Parkinson's disease, and virtually all cardiovascular diseases [1,4]. However, whether these toxic effects are also the cause for cellular aging is largely debated.

One of the widely accepted theories states that toxic effects on nucleic acids lead to accumulation of somatic DNA mutations that affect cellular longevity [32]. However, presence of DNA repair mechanisms largely questions this theory. Studies have shown that prokaryotic and mammalian cells are capable of successfully repairing mutations induced by oxidative stress [6,8]. But there is no data about whether or not these repair mechanisms decline throughout the years.

In the late 20th century, another theory had been introduced, which linked oxidative stress on mitochondrial DNA to senescence. According to this hypothesis, mtDNA damage can lead to mutations that directly block the replication capacity of the cells [5]. Some research also state that oxidative stress depletes the biochemical pool necessary for cellular division [6].

Experimental studies have also been conducted on birds with different lifespan to identify if there is significant correlation between levels of ROS and life expectancy. One of the studies performed in 2018 demonstrated positive association. According to it, long-lived birds tended to have higher levels of antioxidants [52]. However, another study that linked oxidative status to telomere length, suggested no apparent impact of antioxidants on the senescence of long-lived birds [7,23,41].

Despite the presence of numerous theories, none of them have been sufficient enough to confirm if free radicals produce significant impact on aging. However, they provide a basis for further research and exploration of the topic that might be beneficial for preventing a number of age-related complications. Prevention can be achieved through different types of antioxidants that can neutralize ROS and alleviate its toxic effects or through modifying risk factors that increase oxidative stress.

## *3. Risk factors that increase ROS*

### Exogenous factors

There are several exogenous causes that induce oxidative stress, which include environmental pollutants, cigarette smoke, e-cigarettes, ionizing and non-ionizing radiation, drugs, and foods. Chemical substances such as pesticides and heavy metals like mercury, lead, arsenic, cadmium, chromium and organic solvents, are also known contributors of oxidative stress.

### 3.1 Environmental Pollutants

Environmental pollution significantly affects human health and is among the leading contributors of mortality and morbidity in people [12]. It poses a global threat especially in rapidly developing countries with increasing population and metropolitan development resulting in the decline of air quality, which conversely affects developing countries [12]. Air pollutants contain a mixture of gases, particulate matter, and chemicals whose source and constituents are difficult to determine. Particulate matter with an aerodynamic diameter ≤ of 2.5 μm (PM2.5), originating from combustion processes are considered most harmful and[54], they mostly consist of carbon particles, with other organic molecules like sulfates, nitrates, and polycyclic aromatic hydrocarbons [50]. The physical and chemical properties of the particles i.e., size, structure, composition suggest their significance on health [48].

Experimental and epidemiologic studies have recognized ROS as essential mediators of particle toxicity, with a specific association to respiratory and cardiovascular diseases [1, 19]. Increased mortality from ischemic heart disease, heart failure, and lung diseases, like asthma, chronic obstructive pulmonary disease (COPD) [11, 48].

### 3.2 Cigarette smoke

Cigarette smoke and smoking is an important risk factor in the generation of ROS. Cigarette smoke is an aerosol that consists of toxic substances, chemicals, and carcinogenic agents [26]. More than 4000 harmful compounds have been identified in cigarette smoke such as carbon material, quinones, organic solutions, heavy metals, polycyclic aromatic hydrocarbons, and N-nitrosamines [44]. There are more than 50 carcinogens in cigarette smoke that have been recognized by the International Agency for Research on Cancer (IARC) with “sufficient evidence for carcinogenicity” [26, 44]. Cigarette smoke can be divided into

mainstream smoke (inhaled by the smoker) and side stream smoke (the smoke that goes into the air from a burning cigarette and is the main part of second-hand smoke). Cigarette smoke has two phases: the tar phase and the gas phase. The tar phase contains stable polycyclic aromatic hydrocarbons and nitrosamines, and the gas-phase contains nitric oxide, carbon particles, and peroxyl radicals [51]. In the presence of iron, tar can produce hydroxyl radicals and hydrogen peroxide.

In an experimental study conducted to demonstrate the relationship between cigarette smoking and oxidative stress in patients with coronary artery disease, the results concluded that cigarette smokers have increased oxidative damage and reduced antioxidants than non-smokers and are at an increased risk of developing coronary artery disease [29]/

### 3.3 E-cigarette

The practice of using e-cigarettes has become widely popular, ever since they have been marketed as a healthier substitute to traditional cigarettes [21, 40]. There are also reports that state that e-cigarettes aid in smoking cessation [24]. As a result, the consumption of e-cigarettes has increased, especially among the youth population. Since 2014, the most commonly used tobacco product in the United States among the youth are e-cigarettes. This raises a concern, as there is a substantial lack of awareness of the detrimental health effects of e-cigarettes in the overall population. There is minimal evidence about the observation that e-cigarettes are safer than tobacco cigarettes and the risks associated with the long-term use of e-cigarettes. Additionally, recent epidemiological studies have shown several adverse health effects associated with inhalation of e-cigarette aerosols regardless of the nicotine levels [9, 38, 42]. E-cigarette aerosols contain ultrafine particles derived from environmental pollution and have shown to cause equal health effects of environmental pollutants by stimulating inflammation, oxidative stress and starting the process of endothelial dysfunction which eventually leads to cardiovascular and lung disease (20, 21, 30, 37].

### 3.4 Radiation and chemotherapy

Radiation therapy is one of the major treatment modalities for various types of cancers, however it is often presenting with toxic side effects. Types of ionizing radiation include alpha particles, beta particles, gamma rays, and X-rays. These rays can all lead to increased oxidative stress. Alpha particles have less penetrative energy to the outer layer of the skin and is it not a major concern. All the other types of ionizing radiation are indeed penetrative. Gamma rays and X-rays are the most commonly used types of ionizing radiation in a medical setting. Ionizing radiation increases oxidative stress by inducing ROS due to the radiolysis of water molecules.

Chemotherapeutic agents that generate high levels of ROS include anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin), which generate the highest levels of oxidative stress, platinum-containing complexes (e.g., cisplatin, carboplatin), alkylating agents, epipodophyllotoxins (etoposide and teniposide), and the camptothecins (topotecan and irinotecan) [13].

### 3.5 Drugs

Aspirin, antipyretic agents, antipsychotics, antiretrovirals, and analgesic non-steroidal anti-inflammatory drugs (NSAIDS) generate ROS. The mechanisms of drug-induced oxidative stress differ [16].

### 3.6 Foods

There are several types of nutritional sources that can lead to an increase in oxidative stress. Evidence suggests that high quantities of macronutrients (carbohydrates, fat and proteins) induce oxidative stress [46]. Dietary carbohydrates are essential to mention as they contain high glycemic load and contribute to the long-term effects of nutritionally mediated inflammation and aid in the development of cardiovascular diseases, diabetes, obesity, and cancer [36,47]. Processed foods such as snack foods and cereals contain trans-fatty acids, which help in the generation of ROS as they have acrylamide which gives rise to oxidative stress [55]. When vegetable or animal lipids are heated in the microwave, free radicals are generated. Meat is considered as part of the normal diet in many developed countries even though meat is high in proteins it can be a source of toxins due to the presence of N-nitroso compounds, heterocyclic amines, and polycyclic aromatic hydrocarbons associated with cooking and grilling the meat in high temperatures [33]. High concentrations of ethanol can be damaging to the body as it generates ROS. Dietary intake of iron and copper can also increase oxidative stress and its buildup in bodily tissues can increase the risk of cancer [22].

**Endogenous factors**

Dysfunction in the mitochondrial respiratory chain and other enzymes like xanthine oxidase, lipoxygenases, glucose oxidase, myeloperoxidase, cyclooxygenase and nitric oxide synthase are generators of ROS endogenously.

### *4. Types of antioxidants*

Antioxidants are substances that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate. The role of antioxidants, as the definition suggests, is to prevent the damage to cellular components arising as a consequence of chemical reactions involving free radicals. Antioxidants delay or prevent damage mainly by using their free radical scavenging property [34]. There are several groups of antioxidants which include antioxidant enzymes, chain breaking and transition metal binding proteins.

First example of antioxidant enzymes is catalase, which has 2 stage conversion of hydrogen peroxide to water and oxygen. It is mainly located within the cells in peroxisomes and demonstrates the greatest activity in liver and erythrocytes. Another example is superoxide dismutase, which catalyzes the dismutation of superoxide to hydrogen peroxide. Later, hydrogen peroxide is removed by catalase or glutathione peroxidase, which is also another antioxidant enzyme. Glutathione peroxidase catalyzes the oxidation of glutathione using hydroperoxide [53]. Plasma form of this enzyme is mainly synthesized in the kidney and has the highest concentration in the liver. These enzymes are produced in the body, however there are certain exogenous factors that can function as antioxidants. For example, physical exercise leads to increase in antioxidant levels in heart, muscle and liver tissues and reduces free radical production and subsequent damage [53].

Chain breaking antioxidants are small molecules which receive an electron from a radical or donate an electron to radical and form stable byproducts. These are divided into aqueous phase and lipid phase antioxidants. Lipid phase antioxidants react with radicals in lipoprotein particles and in membranes. One of the most notable ones is vitamin E, which has 8 different forms and 2 classes, tocopherols and tocotrienols, both of which have antioxidant function [10, 53]. Another interesting property of Vitamin E is that it helps to structurally stabilize membranes. Vitamin A also exhibits antioxidant property, however it does not show any dependency of oxygen saturation.

Flavonoids are polyphenolic antioxidants found in fruits, vegetables, tea and wine. However, not much is known about their absorption and metabolism. Some studies showed that intake of flavonoids decreases the incidence of coronary heart disease [53].

Aqueous phase chain breaking antioxidants scavenge radicals in aqueous compartment. One of the most important members of this group is Vitamin C. Ascorbate scavenges radicals such as superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid, aqueous peroxyl radicals, and singlet oxygen. Vitamin C undergoes 2 electron reduction to semdehydroascrobyl radical and subsequently to dehydroascorbate, which later on hydrolyses to diketogulonic acid which is broken down to oxalic acid [43].

Another important example is uric acid, which is converted to allantoin. It provides protection against ozone. Albumin bound bilirubin also plays a major role in the protection of a neonate from oxidative damage. Melatonin is unique in the way that it does not undergo redox cycling and cannot be reduced to its former state and it is called a terminal antioxidant [53].

In conclusion, all of these antioxidants can reduce oxidative stress and theoretically can be used as risk reduction agents. It is still debatable and there is even evidence that synthetic antioxidants are dangerous to health. Overall, it is not proven entirely that antioxidants can have an important impact in prevention of free radical damage and more research is required on this matter.

### *5. Role of antioxidants in preventing age-related complications.*

Aging is a very complex topic, thus various studies have arranged for understanding its mechanism and association with oxidative stress. Studies find that the amount of oxidants and antioxidants are balanced in healthy humans. However, there are different genetics, environmental or lifestyle factors that misbalance equilibrium and organisms begin the journey of damage until balance equilibrates. Such factors

are cigarette smoking, obesity, air pollutants, ultraviolet B, G6PD deficiency and many more. Studies show that ceasing cigarette smoking, exercising and avoiding obesity are major life extensions.

Increased oxidative stress leads to atherosclerosis, Alzheimer's, dementia, cancer and various diseases that alter quality of life and lifespan. In order to solve or reduce the prevalence of latter disorders, dozens of trials preceded for finding correlation between antioxidant use and decreasing complications of oxidative damage. One of the studies was done from 1993 to 1999 for finding whether long-term supplementation with vitamin E decreases the risk of cancer, cancer death, and major cardiovascular events [27,41]. From the total cancer patients, the control group had 156 (3.3%) deaths compared to placebo group 178 (3.7%). From cardiovascular patients 1022 (21.5%) deaths was in control patients and placebo 985 (20.6%) respectively. As a result, there was no major association between vitamin E and preventing major cancer or cardiovascular events.

In 2003, 980 elderly "free of dementia" subjects showed no association between intake of carotenes, vitamin C, vitamin E antioxidants and decreased rate of Alzheimer [27,35]. Neither higher intake of vitamin C, E and beta carotene showed promising results in 1999 for pulmonary middle aged male patients living in Finland (n = 1248), Italy (n = 1386), and the Netherlands (n = 691) [45]. All three antioxidants had positive results on the pulmonary function before adjustment for energy intake. Cerebral ischemia showed disappointing results for 26593 male smokers, aged 50–69 years taking lycopene, lutein zeaxanthin, vitamin C, flavanols, flavones, vitamin E. However, intake of beta carotene was inversely associated with cerebral ischemic patients [27].

One of the few associations between the antioxidant vitamins and the delayed aging was vitamin C and its inverse relationship with diastolic pressure for >20 years old males and females, and comparison of vitamin A and E levels with higher risk of hypertension for the same cluster of patients.

Solely increased antioxidant intake does not reduce the aging and the complications of oxidative stress. Reducing oxidative stress such as ceasing cigarette smoking and following healthy diet and following lifestyle extends the lifespan and the quality of life in the most effective way.

## Conclusion

Numerous studies have found an association between aging and ROS, however, there is some data that contradict this theory. For this reason, it can be concluded that damages caused by ROS can serve as causal factors, but clearly, they do not account for all the consequences that come with aging.

Experiments conducted in order to test a free radical theory of aging gave us an opportunity to expand our understanding of this complex process. It has been proven that there is an increase in oxidative stress with aging which alters cellular longevity and causes cell damage, at the same time antioxidant enzymes have shown some protective effects. However, these processes cannot fully explain the root cause of aging.

Modifying risk factors that can increase oxidative stress is thought to be an important intervention in order to improve the quality of life and increase lifespan to some point. Important risk factors include air pollution, cigarette smoke, processed or reheated food, a diet rich with high quantities of macronutrients (carbohydrates, fat, and proteins), high concentrations of ethanol, certain drugs, chemotherapy, and radiation. These are the risk factors that can be easily modified and if decreased might result in significantly improved health in an aging population.

However, it is still unclear, whether increased antioxidant supplementation can prevent age-related complications. Supplementation of antioxidants such as lycopene, lutein zeaxanthin, vitamin C, flavanols, flavones, and vitamin E have not shown promising results in preventing major cancer, cardiovascular events, or hypertension. All these outlines the fact that aging is a multifactorial process and cannot be reduced to any single cause.

## References:

1. Abrams JY, Weber RJ, Klein M, et al. Associations between Ambient Fine Particulate Oxidative Potential and Cardiorespiratory Emergency Department Visits. Environ Health Perspect 2017 Oct 26;125(10):107008.
2. Adelman R, Saul RL, Ames BN. Oxidative damage to DNA: relation to species metabolic rate and life span. ProcNatlAcadSci (USA) 1988 Apr;85(8):2706-8.

3. Agarwal S, Sohal RS. Relationship between susceptibility to protein oxidation, aging, and maximum life span potential of different species. ExpGerontol1996 May-Jun;31(3):365-72.
4. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. ProcNatlAcadSci (USA) 1993 Sep 1;90(17):7915-22.
5. Arnheim N, Cortopassi G. Deleterious mitochondrial DNA mutations accumulate in aging human tissues. Mutat Res 1992 Sep;275(3-6):157-67.
6. Barnett YA, King CM. An investigation of antioxidant status, DNA repair capacity and mutation as a function of age in humans. Mutat Res 1995 Oct;338(1-6):115-28.
7. Beaulieu, Michaël, et al. "Oxidative Status and Telomere Length in a Long-Lived Bird Facing a Costly Reproductive Event." Functional Ecology, British Ecological Society, 2011, 25(3):577–85.
8. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free RadicBiol Med 2000 Aug;29(3-4):222-30.
9. Chatterjee S, Tao JQ, Johncola A, Guo W, Caporale A, Langham MC, Wehrli FW. Acute exposure to e-cigarettes causes inflammation and pulmonary endothelial oxidative stress in nonsmoking, healthy young subjects. Am J Physiol Lung Cell MolPhysiol 2019 Aug 1;317(2): L155-L166.
10. Chen J, He J, Hamm L, Batuman V, Whelton PK. Serum antioxidant vitamins and blood pressure in the United States population. Hypertension. 2002 Dec;40(6):810-6.
11. Choi J, Oh JY, Lee YS, et al. Harmful impact of air pollution on severe acute exacerbation of chronic obstructive pulmonary disease: particulate matter is hazardous. Int J Chron Obstruct Pulmon Dis 2018 Mar 28; 13:1053-1059.
12. Cohen AJ, Brauer M, Burnett R, et al. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet. 2017 May 13;389(10082):1907-1918.
13. Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr Cancer Ther 2004 Dec;3(4):294-300.
14. Cumming and Henry, Growing Old. Cambridge University Press, 1961.
15. Dean W. Neuroendocrine Theory of Aging, Chapter 2 Adaptive Homeostat Dysfunction, 2018.
16. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012; 2012:645460.
17. Diggs J. Activity Theory of Aging. In: Loue S.J., Sajatovic M. (eds) Encyclopedia of Aging and Public Health. Springer, Boston, 2008.
18. Dean Dröge W. Free radicals in the physiological control of cell function. PhysiolRev 2002 Jan;82(1):47-95.
19. Falcon-Rodriguez CI, Osornio-Vargas AR, Sada-Ovalle I, Segura-Medina P. Aeroparticles, Composition, and Lung Diseases. Front Immunol 2016 Jan;20(7):3.
20. Farsalinos KE, PolosaR. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: a systematic review. TherAdv Drug Saf 2014 Apr;5(2):67-86.
21. Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Voudris V. Characteristics, perceived side effects and benefits of electronic cigarette use: a worldwide survey of more than 19,000 consumers. Int J Environ Res Public Health 2014 Apr 22;11(4):4356-73.
22. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. Cancer Epidemiol Biomarkers Prev 2014 Jan;23(1):12-31.
23. Fusco D, Colloca G, Lo Monaco MR, Cesari M. Effects of antioxidant supplementation on the aging process. ClinInterv Aging 2007;2(3):377-87.
24. Hajek P, Phillips-Waller A, Przulj D, Pesola F, Myers Smith K, Bisal N, Li J, Parrott S, Sasieni P, Dawkins L, Ross L, Goniewicz M, Wu Q, McRobbie HJ. A Randomized Trial of E-Cigarettes versus Nicotine-Replacement Therapy. N Engl J Med 2019 Feb 14;380(7):629-37.
25. Harman D. Aging: a theory based on free radical and radiation chemistry. JGerontol 1956 Jul;11(3):298-300.
26. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999 Jul 21;91(14):1194-210.
27. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. Stroke. 2000 Oct;31(10):2301-6.
28. Kakimoto Y, Okada C, Kawabe N, Sasaki A, Tsukamoto H, Nagao R, Osawa M. Myocardial lipofuscin accumulation in ageing and sudden cardiac death. Sci Rep 2019 Mar 1;9(1):3304.

29. Kamceva G, Arsova-Sarafinovska Z, Ruskovska T, Zdravkovska M, Kamceva-Panova L, Stikova E. Cigarette Smoking and Oxidative Stress in Patients with Coronary Artery Disease. Open Access Maced J Med Sci 2016 Dec 15;4(4):636-40.
30. Kerr DMI, Brooksbank KJM, Taylor RG, Pinel K, Rios FJ, Touyz RM, Delles C. Acute effects of electronic and tobacco cigarettes on vascular and respiratory function in healthy volunteers: a cross-over study. JHypertens 2019 Jan;37(1):154-66.
31. Kirkwood TB, Kowald A. The free-radical theory of ageing--older, wiser and still alive: modelling positional effects of the primary targets of ROS reveals new support. Bioessays. 2012 Aug;34(8):692-700.
32. Krause KH. Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases. ExpGerontol 2007 Apr;42(4):256-62.
33. Ledesma E, Rendueles M, Díaz M. Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and prevention, Food Control, 2016;60:64-87.
34. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010 Jul;4(8):118-26.
35. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003 Feb;60(2):203-8.
36. Ma XY, Liu JP, Song ZY. Glycemic load, glycemic index and risk of cardiovascular diseases: meta-analyses of prospective studies. Atherosclerosis. 2012 Aug;223(2):491-96.
37. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, Fry RC, Jaspers I. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. Am J Physiol Lung Cell MolPhysiol 2016 Jul 1;311(1): L135-44.
38. Muthumalage T, Prinz M, Ansah KO, Gerloff J, Sundar IK, Rahman I. Inflammatory and Oxidative Responses Induced by Exposure to Commonly Used e-CigaretteFlavoring Chemicals and Flavored e-Liquids without Nicotine. Front Physiol 2018 Jan 11; 8:1130.
39. Nakano M, Oenzil F, Mizuno T, Gotoh S. Age-related changes in the lipofuscin accumulation of brain and heart. Gerontol1995;41Suppl 2:69-79.
40. Polosa R, Cibella F, Caponnetto P, Maglia M, Prosperini U, Russo C, Tashkin D. Health impact of E-cigarettes: a prospective 3.5-year study of regular daily users who have never smoked. Sci Rep 2017 Nov 17;7(1):13825.
41. Sadowska-Bartosz I, Bartosz G. Effect of antioxidants supplementation on aging and longevity. Biomed Res Int 2014; 2014:404680.
42. Schweitzer KS, Chen SX, Law S, et al. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. Am J Physiol Lung Cell MolPhysiol 2015 Jul 15;309(2): L175-87.
43. Simioni C, Zauli G, Martelli AM, et al. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget 2018 Mar 30;9(24):17181-17198.
44. Smith CJ, Livingston SD, Doolittle DJ. An international literature survey of "IARC Group I carcinogens" reported in mainstream cigarette smoke. Food ChemToxicol 1997 Oct-Nov;35(10-11):1107-30.
45. Tabak C, Smit HA, Räsänen L, Fidanza F, Menotti A, Nissinen A, Feskens EJ, Heederik D, Kromhout D. Dietary factors and pulmonary function: a cross sectional study in middle aged men from three European countries. Thorax. 1999 Nov;54(11):1021-6.
46. Tan BL, Norhaizan ME, Liew WP. Nutrients and Oxidative Stress: Friend or Foe? Oxid Med Cell Longev 2018 Jan 31; 2018:9719584.
47. Turati F, Galeone C, Gandini S, Augustin LS, Jenkins DJ, Pelucchi C, La Vecchia C. High glycemic index and glycemic load are associated with moderately increased cancer risk. MolNutr Food Res 2015 Jul;59(7):1384-94.
48. Weber S, Uzu G, Calas A, Chevrier F, Besombes J. L, Charron A, Salameh D, Ježek I, Močnik G, Jaffrezo J.L: An apportionment method for the oxidative potential of atmospheric particulate matter sources: application to a one-year study in Chamonix, France, AtmosChemPhys2018;18: 9617–29.
49. Wei YH, Ma YS, Lee HC, Lee CF, Lu CY. Mitochondrial theory of aging matures--roles of mtDNA mutation and oxidative stress in human aging. Zhonghua Yi XueZaZhi (Taipei). 2001 May;64(5):259-70.
50. Weinstein JR, Asteria-Peñaloza R, Diaz-Artiga A, et al. Exposure to polycyclic aromatic hydrocarbons and volatile organic compounds among recently pregnant rural Guatemalan women cooking and heating with solid fuels. Int J Hyg Environ Health 2017 Jun;220(4):726-735.

51. Witschi H. Carcinogenic activity of cigarette smoke gas phase and its modulation by beta-carotene and N-acetylcysteine. ToxicolSci 2005 Mar;84(1):81-7.
52. Xia, C., Møller, A.P. Long-lived birds suffer less from oxidative stress. Avian Res 2018; 9:41.
53. Young IS, Woodside JV. Antioxidants in health and disease. J ClinPathol 2001 Mar;54(3):176-86.
54. Zaira L, Lisa K, Marianne G, Air pollution causing oxidative stress, Current Opinion in Toxicology, 2020;20–21:1-8.
55. Zamani E, Shokrzadeh M, Fallah M, Shaki F. A review of acrylamide toxicity and its mechanism. Pharm Biomed Res 2017;3(1):1-7.
56. Zs-Nagy I, Nagy K. On the role of cross-linking of cellular proteins in aging. Mech Ageing Dev1980;14(1–2):245-51.
57. Zs-Nagy I. The Membrane Hypothesis of Aging. Florida: CRC Press, 1994.