# Free Radical Theory: A Review

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## Introduction

Aging can be loosely defined as the accumulation of changes within an organism over time. Aging is a complex, poorly understood, and multi-factorial process. It is generally regarded as progressive, irreversible, deleterious, and endogenous in nature [1]. Over 300 scientific theories seeking to explain the specifics of the process of aging have been posited. Yet, despite the vast amount of research, the plethora of competing theories, and the volumes of related literature, firmly established primary causes of biological aging remain unclear [2,3].

Perhaps, the oldest known attempt to explain the process of aging is the *rate of living theory of aging*. In its ancient incarnation, this theory held that each human is imbued with a finite amount of some undefined, essential, animating substance, and when that substance has been exhausted, death ensues. The early 20th Century version of the rate of living theory suggested that an organism's lifespan is inversely related to its metabolic rate [4]. In 1954, Gershman and Gilbert theorized that the toxic effects of both ionizing radiation and hyperbaric oxygen might result from a common pathway: oxygen free radicals [5]. Two years later, Denham Harman, synthesized these ideas and proposed that free radicals produced during the process of cellular respiration result in cumulative oxidative damage and, in turn, cellular aging and death. In other words, the accumulation of free radical oxidative damage determines lifespan. This theory has come to be known as the *free radical/oxidative stress theory of aging* [3,6,7].

Since its origin in 1956, the oxidative hypothesis of aging has garnered significant evidence and support for the notion that free radicals play an important role in the process of aging, "either as 'damaging' molecules or as signaling molecules" [8]. It has been repeatedly studied, modified, renamed, and propounded [2]. Indeed, oxidative stress mechanisms have been implicated in the pathogenesis of many age-related diseases, including cancer [11,12,3], atherosclerosis [13,14,3], diabetes [15,3], and neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [16]. However, despite the clear role of free radicals and resultant oxidative damage in many age-related diseases, no definitive causal relationship between oxidative stress and the rate of aging and maximum species lifespan has been established [2]. Thus, while the oxidative stress theory does not appear to be the comprehensive explanation of senescence that many have sought to make it, free radical theory remains relevant in that endogenous oxidants are clearly involved in, if not the sole determinant of, biological aging.

**Review of the Relevant Chemistry**

*Definitions*

A *free radical* is defined as any atom or molecule that has one or more unpaired electrons in its outer valence shell. Molecular oxygen (or $O_2$), in its ground state, has two unpaired electrons in its outer shell and is, therefore, a radical species itself.

*Oxidation* of an atom or molecule involves the loss of one or more electrons from that atom or molecule. An *oxidant* forces the oxidation of another atom or molecule by acting as an electron "thief," so to speak.

*Reduction* of an atom or molecule involves that atom or molecule gaining one or more electrons. An *antioxidant*, or reducing agent, acts as a sort of guard, preventing the oxidation of neighboring atoms and molecules by acting as a preferential electron donor.

*Oxygen Basics*

Atoms are most stable when their outer shells are filled with electron pairs (paired electrons spin in opposite directions). Ground state molecular oxygen (diatomic, *triplet oxygen* or $O_2$), while "desiring" to "steal" two additional electrons to fill its outer valence, is relatively unreactive, since its two unpaired electrons occupy degenerate molecular orbitals and are of parallel spin configuration. Therefore, in order for an atom of molecular oxygen to accept two additional electrons and complete its outer valence shell, both of these additional electrons must be of anti-parallel spin configuration relative to the unpaired electrons in the oxygen atom. Electron donor pairs to be offered by most atomic or molecular configurations do not typically fulfill this requirement. As a result, the reduction of molecular oxygen atoms is generally accomplished one electron at a time and the electron donors in these transactions are typically other radicals, transition metals in particular. [3]

One and two electron reduction reactions of molecular oxygen yield the superoxide anion ($O_2^{-}\bullet$) and hydrogen peroxide ($H_2O_2$), respectively. These two radicals, in the presence of transition metals (enzyme-associated iron and copper) in certain valence states, can form the highly reactive and biologically destructive hydroxyl radical ($\bullet OH$). Additionally, there exist high-energy *singlet oxygen* species, which differ from ground state oxygen in that the unpaired outer valence electrons possess opposite (rather than parallel) spin configurations. These excited species of molecular oxygen are much more reactive than ground state, triplet oxygen, since the spin restriction on potential inbound (relative to the oxygen atom) electron pairs (described above) is no longer operative. All of these oxygen species are potentially toxic and can be collectively termed reactive oxygen species, oxygen free radicals, or simply oxidants. [3]

## Free Radical Theory

The free radical/oxidative stress theory of aging proposes that free radicals resulting from cellular respiration cause cumulative oxidative damage over time, leading to senescent degeneration and death. Functionally, oxidative stress results when the homeostasis between the formation of free radical oxidants and the deactivation of these agents and repair of associated damage through endogenous antioxidant defenses and repair mechanisms tilts in favor of the free radicals [9]. This state of oxidative stress basically amounts to an electrochemically *positive* or *electron poor* local environment—a generally toxic state. Although, a variety of free radical species exist, the primary biologically relevant radicals are those resulting from oxygen and nitrogen metabolism [10,3]. For the sake of simplicity, this review will limit discussion to reactive oxygen species only.

## Endogenous Oxidant Formation

There are two main sources of oxygen radical generation *in vivo*: mitochondrial sources and non-mitochondrial sources [8].

In mitochondrial respiration, oxidative phosphorylation is the primary pathway for the generation of metabolic energy. Molecular oxygen is a powerful oxidizing agent and, as such, is an excellent terminal acceptor of electrons in the electron transport chain. "In the textbook scheme of mitochondrial respiration, electron transport involves a coordinated four-electron reduction of $O_2$ to $H_2O$" [3]. However, mitochondrial electron transport is imperfect and, thus, a small percentage of electrons engaged in the electron transport chain react with diatomic oxygen, resulting in the superoxide anion. The superoxide anion can, in turn, be converted into hydrogen peroxide and, subsequently, the extremely reactive hydroxyl radical (3-electron reduction). [16]

Non-mitochondrial sources of oxygen free radicals include hydrogen peroxide degradation via the Fenton Reaction, microsomal cytochrome P450 enzymatic metabolism of xenobiotic compounds, phagocytic cell response to inflammation and infection, and perioxisomal beta-oxidation of fatty acids [3,16]. Additionally, many other enzymes are capable of generating oxygen radicals, both in normal physiologic states and under pathological conditions, frequently in tissue-specific fashion [17,3]. For instance, the neuronal degradation of dopamine by monoamine oxidase can yield hydrogen peroxide and has been implicated as a possible cause of Parkinson's disease [18,3].

As many of the non-mitochondrial sources of oxygen radicals are tissue-specific, these sources are unlikely to play any significant role in the general aging of a given organism. However, they may be involved in the pathogenesis of age-related diseases, which, in turn, may contribute to biological aging in a broader sense. [3]

## Endogenous Antioxidant Defense and Repair Mechanisms

All biological molecules are susceptible to oxidative damage, but the most important classes that are vulnerable to free radical attack are lipids, nucleic acids, and proteins. Lipid peroxidation can lead to changes in membrane permeability and elasticity, as well as deleterious effects on membrane-bound proteins. Oxidation of both nuclear and mitochondrial DNA can result in strand disruptions, abnormal cross-linking, and DNA adducts (covalent bonding of DNA elements to chemical mutagens/carcinogens). Proteins (including vital enzymes) have been shown to undergo oxidative damage at a variety of vulnerable sites and can be rendered biologically inactive, as a result. [8,3]

Endogenous mechanisms for dealing with oxidants and oxidative damage are composed of: (1) antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase); (2) smaller antioxidant molecules resulting from dietary intake of fruits and vegetables (ascorbate, glutathione, tocopherols, carotenoids) and; (3) repair of oxidative damage. While both of these endogenous antioxidant defenses have been studied in depth, the biochemistry of cellular repair of oxidative damage is relatively unexplored. However, it is clear that cells do have the capability to repair oxidized lipids, nucleic acids, and proteins. [3]

## Exogenous Manipulation

### *Caloric restriction*

Caloric restriction has been demonstrated to increase lifespan in some mammals. The rate of living theory would suggest that such an increase in lifespan occurs as a result of a decrease in metabolic rate and, by extension, a decrease in oxygen consumption. However, this mechanism has not been confirmed. Rather, the increase in lifespan appears to result from a combination of a decrease in oxidative stress and an increase in antioxidant defense and repair mechanisms. [9]

### *Exercise*

Over the past three decades, numerous studies have documented that physical exercise, both aerobic and anaerobic, can lead to an increase in the production of free radicals and subsequent oxidative stress in humans and animals. However, whether this increase in oxidative pressure represents a harmful state continues to be a matter of debate. In the past, exercise-induced oxidative stress was generally viewed as a negative condition, one that should be reduced or eliminated in order to maximize performance and health. However, more recent studies have suggested that endogenous free radicals generate a *hormetic* effect. “That is, in response to repeated exposure to toxins and/or stressors the body undergoes favorable adaptations that in turn result in enhanced physiological performance and improved physical health.” [9] For instance, Ristow et al found that insulin sensitivity and molecular mediators of endogenous antioxidant defenses were induced by exercise and that exogenous antioxidant supplementation appeared to block

these effects. These findings, in keeping with the concept of *mitohormesis* (mitochondrial hormesis), suggest that exercise-induced oxidative stress decreases insulin resistance and promotes adaptive increases in endogenous antioxidant defense mechanisms [19].

*Exogenous supplements*

Experimental nutritional antioxidant supplementation has been undertaken in numerous species with a variety of antioxidant compounds. Mean life span has been extended in some cases. However, the results in mammalian studies have been mixed. While no large-scale human trials have studied the relationship between nutritional antioxidant supplementation and aging, several have sought to evaluate the relationship between antioxidant supplementation and the incidence of lung cancer. These large, heavily scrutinized studies involved alpha-tocopherol, beta-carotene, and retinol. No decrease in the incidence of lung cancer was identified with regard to any of the antioxidants involved in the studies. In fact, a slight increase in the incidence in lung cancer and mortality was associated with beta-carotene administration. These results, rather than representing an indictment of free radical theory, highlight the complexity of the human organism and the process of aging. A great deal more study, as well as a more comprehensive knowledge of the underlying biochemistry and molecular biology will likely be required to arrive at a better understanding of the mechanics of senescence and the potential role of exogenous antioxidant supplements with regard to modifying those mechanics. [9]

**Summary**

Studies evaluating oxidative stress phenomena continue to suggest that aging is related to free radical formation and the cumulative effects of oxidative damage over time. Endogenous antioxidant protective mechanisms interact with oxidants in a dynamic fashion, yielding a complex homeostatic environment. These mechanisms become increasingly brittle with age and, thus, the capacity of the organism to maintain this nuanced physiochemical homeostasis decays with age. A variety of diseases have been linked to oxidative stress mechanisms and it is likely that still more pathological states will be found to have roots in cellular oxidative damage. However, free radical oxidative damage does not appear to be the cause of aging *per se*. Rather, it seems that aging results from the interplay of many component processes. Whether the end result of these processes, aging and death, is a sort of inevitable accident or whether it is a precisely programmed event remains to be seen. In any case, given the complexities and subtleties of cellular biology and age-related pathophysiology, a multidisciplinary approach to further investigation into the primary causes of biological aging appears to hold the greatest promise for future research.

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