Aging: Is Oxidative Stress a Marker or Is It Causal? (44454)

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Abstract. **Rapid developments in free radical biology and molecular technology have permitted exploration of the free radical theory of aging. Oxidative stress has also been implicated in the pathogenesis of a number of diseases. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, protein), and data in transgenic *Drosophila melanogaster* support the hypothesis that oxidative injury might directly cause the aging process. Additional links between oxidative stress and aging focus on mitochondria, leading to development of the mitochondrial theory of aging. However, despite the number of studies describing the association of markers of oxidative damage with advancing age, few, if any definitively link oxidative injury to altered energy production or cellular function. Although a causal role for oxidative stress in the aging process has not been clearly established, this does not preclude attempts to reduce oxidative injury as a means to reduce morbidity and perhaps increase the healthy, useful life span of an individual. This review highlights studies demonstrating enhanced oxidative stress with advancing age and stresses the importance of the balance between oxidants as mediators of disease and important components of signal transduction pathways.** [P.S.E.B.M. 1999, Vol 222]

In 1956 Harman proposed the free radical theory of aging that attributes the aging process to nonspecific damage to macromolecules (DNA, lipids, protein) by free radicals (1). Since that time, rapid developments in free radical biology and molecular technology have permitted the acquisition of data in support of the role of oxidative stress or injury as a major contributor to the aging process and to the pathogenesis of a number of diseases (2–6). This theory purports that oxidative stress develops when the well-regulated balance between pro-oxidants and antioxidants gets out of control in favor of the pro-oxidant. Oxidants are produced as byproducts of normal metabolism (e.g., mitochondrial electron transport, various oxygen-utilizing enzyme systems, peroxisome function), by phagocytic cells, and from lipid peroxidation. They induce oxidative damage to nucleic acids, lipids, and protein and lead to cellular damage and some of the characteristics of aging (2, 4, 7). Numerous studies have been conducted in the last decade to elucidate the biochemical and molecular mechanisms of aging (5, 8). The general consensus appears to be that the basic mechanisms underlying the aging process are multifactorial and that reactive oxygen species (ROS) are a contributing factor. However, the extent of their contribution remains uncertain (5). Nevertheless, great interest remains in searching for factors that, if modulated, could reduce the level of oxidative stress and injury and thereby alter the course of the aging process.

Aging is an inevitable biological process and characterized by a general decline in physiological function. Aging may be defined as the increased probability of death as the age of the organism increases with the accumulation of diverse adverse changes (9). This is counterbalanced by repair and maintenance factors that contribute to the longevity of the organism. In human beings, it is difficult to distinguish between the phenotypic expression of aging due to the aging process *per se* and the diseases known to occur with a higher prevalence in the aged population, such as cardiovascular disease, arthritis, osteoporosis, and cancer. Advances in medicine and public health have resulted in a large increase in the average life span of humans in developed countries, but many of these advances have not altered aging itself and hence there has been no substantial change in the maximum human life span (9, 10). Slowing the aging process has important implications for society and the

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world. If slowing the aging process increases vitality and the quality of life over the entire life span of an individual, there may be a direct benefit. However, in an increasingly overpopulated world there is danger if the morbidity associated with aging is not reduced and longevity increased. In trying to achieve this goal of minimal morbidity and increased longevity, exploration of the various hypotheses for the aging process will undoubtedly reveal ways to increase the healthy, useful span of an individual's life. This review will focus on the evidence for a role of oxidative stress in the aging process and whether there might be benefit in reducing oxidative injury in the older population through nutrition or dietary supplementation.

Evidence for Oxidative Damage

Oxidative stress is associated with a disturbance in the balance between pro-oxidants (ROS and reactive nitrogen species) and antioxidants, in favor of the pro-oxidant (11). Oxidative damage to DNA, proteins, and lipids accumulates with age (Table I) and contributes to degenerative diseases

Table I. Evidence of Oxidative Injury with Advancing Age: Selected Studies

Species	Tissue	Macromolecule	Index/Observation	Reference
Rats	Whole body	Lipid	↑ Ethane, ethylene, butane, and pentane excretion (lipid peroxidation products) with age	(55)
Rat	Brain, liver, heart	Lipid	↑ Superoxide radicals and lipid peroxides	(56)
Human	Erythrocytes, fibroblasts	Protein	↑ Protein carbonyl content or altered enzyme activity with ↑ age	(57)
Human	Heart, liver, brain, kidney (autopsy)	mtDNA	↑ 5 kb deletion with age (newborn–87 yrs) (PCR)	(22, 58–60)
Human	Heart (autopsy)	mtDNA	↑ 8-OH-dg with age in patients without heart dysfunction (24–97 yrs)	(61, 62)
Human	Heart (autopsy)	mtDNA	↑ mtDNA deletions with age and ischemia (PCR) (30–81 yrs)	(63)
Human	Brain (autopsy)	mtDNA/nDNA	↑ 8-OH-dg in both mtDNA and nuclear DNA with advancing age (42–97 yrs); mtDNA > nDNA	(58)
Mice	Skeletal muscle, brain, heart, liver, kidney	DNA	↑ 8-OH-dg content	(64)
Mouse	Brain, heart, kidney	Protein, DNA	↑ Protein carbonyl content and mitochondrial oxidant generation with age	(65)
Human	Skeletal muscle (biopsy)	mtDNA	↑ mtDNA rearrangements in elderly subjects (54–78 yrs) *via* LX PCR	(66)
House flies	Wing	Mitochondrial protein	↑ Protein carbonyl content	(67)
Gerbil	Brain, heart, kidney, testis	Protein/DNA	↑ Protein carbonyl content and 8-OH-dg with age	(68)
Bovine	Submitochondrial particles from beef heart	Lipid/protein	↑ Thiobarbituric reactive substances, ↑ protein carbonyl content	(42)
Rat	Liver	Lipid	↑ 4-hydroxynonenal, MDA correlated with mitochondrial dysfunction and membrane rigidity	(69)
Rat/human	Tail collagen/skin collagen	Collagen	All ↑ with age: N^{ε}-(1-deoxy-D-fructosyl) lysine N^{ε}-(carboxy-methyl) lysine Pentosidine o-tyrosine	(70, 71)
Mice	Cardiac and skeletal muscle	Protein	↑ o-tyrosine, o-o′-dityrosine content	(72)
Human	Skeletal muscle (biopsy)	DNA, lipid, protein	↑ 8-OH-dg, MDA, protein carbonyl content (25–93 yrs)	(73)

Abbreviations: 8-OH-dg, 8-hydroxy-deoxyguanosine; MDA, malondialdehyde.

and the aging phenomenon by disrupting cellular homeostasis (2, 4, 5, 7, 12).

In addition to finding evidence of damage to macromolecules, supporting evidence that oxidative damage might cause aging directly is provided by studies using transgenic *Drosophila melanogaster* overexpressing genes encoding for the antioxidants Cu/Zn superoxide dismutase (SOD) and catalase. Orr and Sohal (13) demonstrated that these transgenic Drosophila live 34% longer than controls. An important observation made was that this effect was seen only with an optimal balance between SOD and catalase. In their previous studies, overexpression of Cu-Zn SOD alone or catalase alone had only a minor incremental effect on the average life span and none on the maximum life span. In *Caenorhabditis elegans*, the *age*-1 mutant lives twice as long as the wild type and also has increased levels of SOD and catalase (14, 15). In contrast to the Drosophila and *C. elegans* mutants, knock-out mice for *GPX1*, *SOD1*, *SOD2* or *SOD3*, which encode for glutathione peroxidase and superoxide dismutase, do not display a phenotype of rapid aging (16, 17). Of interest is that the genetic link between antioxidant capacity and longevity is found in animals whose adult cells are postmitotic. In mammals, the organs shown to be most vulnerable to the accumulation of oxidative damage are also postmitotic and include the brain, heart and skeletal muscle. However, whether the accumulation of oxidative damage in postmitotic cells actually causes aging is not known. The link between ROS and aging in organs consisting of dividing cells is less clear.

Another link between oxidative stress and aging has focused on mitochondria, which consume $\approx$ 85% of the oxygen used by the cell *in vivo* and are the greatest source of oxidants. Mitochondria supply most of the ATP necessary for cell function and contain the only DNA outside the nucleus in mammalian cells. Mitochondrial DNA (mtDNA) has a high mutation rate for several reasons, including limited repair mechanisms, the absence of histones, and its proximity to the free radical-generating inner mitochondrial membrane (18–20).

The high rate of mtDNA mutations led to the mitochondrial theory of aging, a refinement of the free radical theory proposing that accumulation of detrimental mtDNA mutations during life is a cause of human aging (21–25). Several recent reviews have focussed on age-associated oxidative damage and mtDNA mutations (26,27). Support for this hypothesis comes from several sources: 1) the existence of numerous, potentially pathogenic mtDNA deletions in a variety of aged tissues (26, 27); 2) aging-dependent functional alterations of mtDNA from human fibroblasts transferred into mtDNA-less cells (28); and 3) the decline in the activity of several critical mitochondrial enzymes in human muscle with advancing age (29–32). However, the dilemma regarding causality remains, leading investigators to examine the phenotypic and functional consequences of oxidative injury, and specifically mitochondrial abnormalities, in which to delineate the intermediate steps leading to cellular aging.

Phenotypic and Functional Consequences of Oxidative Injury

Despite the number of studies describing the association of markers of oxidative damage to DNA, proteins, and lipids with advancing age, few, if any studies definitively link oxidative injury to altered energy production or cellular function. Markesbery recently reviewed the evidence supporting increased oxidative stress in the pathogenesis of neuron degeneration and death in Alzheimer's disease (AD) (33, 34). He concluded that despite the number of studies demonstrating oxidative alterations in AD (such as increased brain iron, aluminum, and mercury content that stimulates free radical generation; increased markers of lipid peroxidation (e.g., 4-hydroxynonenal and isoprostanes) in CSF; increased lipid, protein, and DNA oxidation in AD brain; decreased energy metabolism and decreased cytochrome-*c* oxidase activity in the brain in AD; advanced glycation end products, malondialdehyde, protein carbonyls, peroxynitrite, heme-oxygenase-1 and SOD-1 in neurofibrillary tangles), it is not known whether ROS generation is a primary or secondary event (33). *In vitro* studies show that free radicals are capable of mediating neuron degeneration and death, but tissue injury itself can induce ROS production. Regardless of whether free radicals are generated secondarily to other causes, they are indeed deleterious and part of a cascade of events leading to neuron death, suggesting that therapeutic regimens aimed at reducing ROS or preventing their formation may be beneficial. Parkinson's disease was one of the first neurodegenerative diseases associated with a defect in mitochondrial function but not necessarily as a consequence of oxidative injury (35, 36). Recently, Urano *et al.* (37) examined the biochemical and biophysical changes caused by oxidative stress and concluded that they were similar to those observed in aging and could be attenuated by vitamin E.

In intact rat hepatocytes, Hagen *et al.* (38) attempted to link the age-related common 4977 base pair deletion in mtDNA to age-related alterations in mitochondrial membrane potential and oxidant production, but they were unable to correlate the mtDNA deletions with these functional changes. In healthy humans, Brierley *et al.* (39) recently reported that muscle fibers from elderly subjects with very low activity of cytochrome-*c* oxidase, part of which is encoded by mtDNA, also have high levels of mutant mtDNA. This was the first report on normal human tissue demonstrating a direct age-related connection between a biochemical and a genetic defect and suggesting that mtDNA abnormalities may indeed be involved in the aging phenotype in human muscle. Several months later, Kopsidas *et al.* (40) reported that cytochrome-*c* oxidase-deficient fibers, regardless of age, were accompanied by extensive mtDNA rearrangements and reduced levels of full-length mtDNA. They concluded that this link between mtDNA mutations and cytochrome-*c* oxidase activity had important implications for the understanding of the molecular basis of the aging

process. However, the dilemma is the leap from mtDNA injury to specific enzyme activity, which ignores the transcriptional, translational, post-transcriptional or post-translational modifications, that could also influence enzyme activity. Elliott *et al.* (41) very recently described a mitochondrially encoded transcript in a human lymphocyte cell line that represented incomplete or alternative post-transcriptional processing. Of interest is that these transcripts were encoded in the region of the common deletion. This finding implies that the link between the mitochondrial gene and the phenotype is also a complex integration of multiple steps and raises the level of complexity in relating mtDNA injury to phenotypic expression.

Damage by oxidative stress to mitochondrial components also includes lipid peroxidation and protein oxidation. Lipid peroxidation can be harmful in mitochondria that contain cardiolipin as a major component of the inner mitochondrial membrane (42). Cardiolipin is required for the activity of cytochrome-*c* oxidase (43) and other mitochondrial proteins (44). Protein oxidation (45) has been described to affect respiratory chain enzymes (46), ATPase (46), the ADP/ATP transporter (42), and the opening of the permeability transition pore (47). In 1989, Linnane *et al.* (22) proposed that the critical factor in the progressive decline in the performance of individual tissues and organs leading to age-related disease and senescence was the loss of bioenergetic capacity of the cells of aging tissue. Their early work in deltoid muscles was consistent with the recent findings described above (39, 40). Furthermore, they suggested that circumventing the bioenergy depletion of cells from aging tissue by boosting the ability of cells to produce ATP would be beneficial in restoring cell function. To this end, they attempted to use redox therapy to reverse the lower levels of respiratory chain components with coenzyme Q_{10} (48). Cells can survive in the absence of a functional mitochondrial respiratory chain provided they have a means to reoxidize NADH generated by glycolysis. The plasma membrane oxidoreductase system that enables the reoxidation of cytosolic NADH to NAD^+ with molecular oxygen acting as the electron sink provides one mechanism to achieve this. However, a source of redox supplementation is necessary (e.g., coenzyme Q_{10} or potassium ferricyanide). Linnane and his co-workers have demonstrated an amelioration of mitochondrial dysfunction in rats with coenzyme Q_{10}. Using other compounds to enhance the redox status, Hagen *et al.* (49) reversed age-related mitochondrial decay with supplementation by acetyl-L-carnitine, N-*tert*-butyl-α-phenyl-nitrones (50) and most recently, lipoic acid (51).

Finally, one cannot ignore caloric restriction as a means to retard aging and attenuate the oxidative stress associated with advancing age. Table II summarizes recent animal studies examining the efficacy of caloric restriction on reducing indices of oxidative injury. Masoro recently published a thoughtful review of the effect of dietary restriction (DR) on aging and the response to stressors (52). He concluded that studies to date show that the antiaging activity of

Table II. Recent Studies on the Effect of Caloric Restriction on Oxidative Injury

Reference	Species	Outcome
(72)	Mouse	60% restriction prevented the increase in o-o'-dityrosine levels in cardiac and skeletal muscle but not o-tyrosine.
(74)	Rhesus monkey	30% restriction ameliorated the age-associated increase in certain cytokines, probably induced by oxidative stress.
(75)	Rat	40% restriction prevented the age-related increase of mtDNA deletions in liver but not brain.
(76)	Mouse	40% restriction attenuated age-associated decline in skeletal muscle mass and function and reduced protein and lipid oxidative damage in skeletal muscle mitochondria. Damage not reversible by 6 weeks of caloric restriction begun at 18–22 months.

DR is not caused by a reduction in body fat nor by the reduction in metabolic rate. It appears that DR-induced changes in carbohydrate metabolism and in oxidative metabolism could, in part, underlie the antiaging activity. An important component of the response to DR is the enhancement of processes such as antioxidant capacity, repair or immune responses that protect the organism from damaging action of long-term, low-intensity stressors (ROS, high and low temperatures, radiation and infectious agents, and immune or inflammatory processes).

With respect to DNA injury as a cause of the aging process, it is uncertain which is the primary signal: 1) oxidant injury to DNA leading to impaired respiration leading to cell dysfunction and aging; or 2) aging inducing cell dysfunction by yet another mechanism leading to impaired oxidation and excess oxidants leading to DNA or other macromolecular injury. Therefore, despite the number of studies demonstrating a significant relationship between aging and markers of oxidative injury, causality has not been clearly established. However, this does not preclude attempts to reduce oxidative stress as a means to reduce morbidity and perhaps increase the healthy, useful life span of an individual.

Implications for Nutrient Supplementation

Recognizing that oxidative stress may be an adverse factor in a large number of chronic diseases and aging, the question arises as to whether supplemental antioxidants are beneficial. The most convincing evidence for the role of oxidative stress and protection by antioxidants in the disease process is for heart disease (53, 54). However, many of the epidemiological and clinical studies have addressed the ef-

ficacy of antioxidant therapy on disease outcome, not the mechanisms. Suitable markers of oxidative injury *in vivo* have been difficult to identify and validate, making definitive studies nearly impossible to execute. Nevertheless, as recently reviewed by McCall and Frei (53), the current evidence is insufficient to conclude that antioxidant vitamin supplementation materially reduces oxidative damage in humans. Future studies will need to address targeting therapy to specific tissues and finding robust markers for functional consequences of the attenuation of oxidative stress. It will be important to balance the role of oxidants as mediators of disease and as important components of signal transduction pathways (77).

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