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Chapter 15 Oxidative Stress and the Aging Brain: From Theory to Prevention

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I INTRODUCTION: THE FREE RADICAL THEORY OF AGING

Aging is characterized by a progressive decline in the efficiency of physiological function and by the increased susceptibility to disease and death. Currently, one of the most plausible and acceptable explanations for the mechanistic basis of aging is the "free radical theory of aging." This theory postulates that aging and its related diseases are the consequence of free radical-induced damage to cellular macromolecules and the inability to counterbalance these changes by endogenous anti-oxidant defenses. The origin of this explanation has a foundation in the "rate of living theory" [1], according to which the lifespan of an individual depends on its rate of energy utilization (metabolic rate) and on a genetically determined amount of energy consumed during adult life. Pearl [1] proposed that the longevity of an organism is inversely correlated to its mass-specific metabolic rate: increasing an organism's metabolic rate will decrease longevity, whereas factors that decrease the metabolic rate will increase longevity. The correlation between metabolic rate and longevity has been questioned due to the exception posed by birds, which have a high metabolic rate yet live much longer than mammals [2]. However, despite their high rate of oxygen consumption, it has been shown that birds have a low rate of free radical production in brain and in other tissues; their mitochondria produce up to 10-fold fewer reactive oxygen species (ROS) *in vitro* [3, 4]. This observation suggests that the mitochondrial rate of free radical production may be more important than the metabolic rate in terms of longevity. Indeed, the mitochondrial rate of free radical production seems to have a much stronger correlation with maximum longevity.

Harman [5] originally proposed the "free-radical theory" of aging in the mid-1950s. He suggested that free radicals produced during aerobic respiration have deleterious effects on cell components and connective tissues, causing cumulative damage over time that ultimately results in aging and death. He initially speculated that free radicals were most likely produced through reactions involving molecular oxygen catalyzed in the cells by the oxidative enzymes and enhanced by trace metals such as iron, cobalt, and manganese. The skepticism first spread around this theory was weakened by the discovery in 1969 of the enzyme superoxide dismutase (SOD) [6]. The existence of an intracellular enzyme whose sole function is to remove superoxide anions ($O_2^{-\bullet}$) has provided strong biological evidence that free radicals are involved in the aging process. In 1972, Harman expanded his original studies to include the involvement of mitochondria in the physiological processes of aging [7]. Harman proposed that mitochondria generate a significant amount of cellular energy and, through consumption of most of the intracellular oxygen, set the limit on the lifespan. Approximately 90% of cellular oxygen is consumed within the mitochondria, mainly in the inner membrane, where oxidative phosphorylation occurs. Since the early 1970s, several studies have emerged to give support to this theory, and the free radical theory of aging has been expanded to the mitochondrial free radical theory of aging. The premise of the mitochondrial free radical theory of aging is that mitochondria are both producers and targets of reactive oxidative species. According to the theory, oxidative stress attacks mitochondria, leading to increased oxidative damage. As a consequence, damaged mitochondria progressively become less efficient, losing their functional integrity and releasing more oxygen molecules, increasing oxidative damage to the mitochondria, and culminating in an accumulation of dysfunctional mitochondria with age.

Although the deleterious effects of free radicals in the aging process have been demonstrated, ROS also are important in maintaining homeostasis. Recent studies have shown that ROS act as an additional class of small molecules that function as cellular messengers. For example, oxidants (nitrous oxide [NO]) act as signaling molecules to promote long-term potentiation (LTP) [8]. Moreover, it has been shown that stimulation of growth factors induces the production of free radicals that are subsequently involved in regulating the proliferative response [9]. The human organism is equipped with very efficient antioxidative defense mechanisms that, among others, include antioxidative enzymes such as SOD, catalase, glutathione peroxidase, and glutathione reductase [10]. When the production of ROS is prolonged, the endogenous reserves of antioxidants become insufficient, leading to cell damage. Similarly, the production of ROS below physiological levels induces a decreased proliferative response.

II FREE RADICAL GENERATION

Free radicals are chemical species with a single unpaired electron. The unpaired electron is highly reactive as it seeks to pair with another free electron; this results in the production of another free radical. The newly produced free radical is unstable in most cases and, as a result, it can also react with another molecule to produce yet another free radical. Thus, a chain reaction of free radicals can occur, leading to more and more damaging reactions. The majority of free radicals that damage biological systems are oxygen radicals and other reactive oxygen species, which are by-products formed in the cells of aerobic organisms. There are several sites of ROS production: mitochondrial electron transport, peroxisomal fatty acid, cytochrome P-450, and phagocytic cells.

A ROS Generation in the Mitochondria

Mitochondria are the main source of ROS [11, 12]. The generation of mitochondrial ROS is a consequence of oxidative phosphorylation, a process that occurs in the inner mitochondrial membrane and involves the oxidation of NADH to produce energy. This energy is then used to phosphorylate ADP. Mitochondrial electron transport involves four-electron reduction of O_2 to H_2O: NADH and succinate donate electrons respectively to complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) of the mitochondrial electron transport chain. Coenzyme Q accepts electrons from Complexes I and II, and next donates electrons to cytochrome b in complex III (ubiquinone-cytochrome c reductase). In complex III, the electrons are donated to cytochrome c1, and so to cytochrome c, to complex IV (cytochrome c oxidase), which finally reduces O_2 to H_2O. However, during mitochondrial electron transport, a one-electron reduction of O_2 results in $O_2^{-\bullet}$ Studies on isolated mitochondria in the presence of a high, nonphysiological concentration of oxygen estimated that mitochondria convert 1 to 2% of the oxygen molecules consumed into $O_2^{-\bullet}$ [13], but subsequent investigations under more physiological conditions reduced this value to 0.2% [14, 15]. Superoxide anion is detoxified by the mitochondrial mangansese (Mn) superoxide dismutase (MnSOD) to yield hydrogen peroxide (H_2O_2), and the H_2O_2 is then converted to H_2O by catalase. H_2O_2 in the presence of

reduced transition metals can also be converted to hydroxyl radical (OH^-). Each of these by-products is a potential source of oxidative damage to the mitochondria, cellular proteins, lipids, and nucleic acids.

B Nonmitochondrial Generation of ROS

A second source of oxygen radicals is peroxisomal β-oxidation of fatty acids, which generates H_2O_2 as a by-product. Peroxisomes are organelles responsible for degrading fatty acids as well as other molecules [12]. Peroxisomes possess a high concentration of catalase, so whether or not leakage of H_2O_2 from peroxisomes contributes significantly to cytosolic oxidative stress under normal circumstances is still unclear. Phagocytic cells are another important source of oxidants; these cells defend the central nervous system against invading microorganisms and clear the debris from damaged cells by an oxidative burst of nitric oxide, H_2O_2, and O_2^-. Finally, cytochrome P450 enzymes in animals are one of the first defenses against natural toxic chemicals from plants.

III OXIDATIVE STRESS IN THE AGING BRAIN

A Mitochondrial Changes

In the aging brain, as well as in the case of several neurodegenerative diseases, there is a decline in the normal antioxidant defense mechanisms, which increases the vulnerability of the brain to the deleterious effects of oxidative damage [16]. The antioxidant enzymes SOD, catalase, glutathione peroxidase and glutathione reductase, for example, display reduced activities in the brains of patients with Alzheimer's disease [17, 18]. It is believed that free radicals of mitochondrial origin are among the primary causes of mitochondrial DNA (mtDNA) damage. Several studies have found increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, in mtDNA in the aged brain [19, 20]. High levels of 8-OHdG have been found in both nuclear DNA (nDNA) and in mtDNA of the post mortem brains of aged subjects [21]. Other studies have shown that the age-related increase in oxidative damage to mitochondrial DNA is greater than the oxidative damage that occurs to nuclear DNA in rodents [20, 22]. For example, oxidative DNA damage has been detected in human brain mitochondrial DNA and in rat liver at levels more than 10 times higher than in nuclear DNA from the same tissue [19]. This higher susceptibility of mtDNA to oxidative damage may be due to a lack of mtDNA repair mechanisms, a lack of protection by histone proteins, as well as the fact that mtDNA is located close to the inner mitochondrial membrane where reactive oxygen species are generated [21, 23]. In agreement with the mitochondrial free radical theory of aging, an inverse correlation has been shown between the levels of oxidative damage to mtDNA and maximum longevity [24] in both the heart and the brain: slowly aging mammals exhibit lower mtDNA damage than those who age faster. In contrast, this correlation is not associated with nuclear DNA. Mitochondrial DNA has a very high mutation rate; and when a mutation occurs, cells initially contain a combination of wild-type and mutant mtDNAs. During cell division, both types of mtDNA are randomly distributed into the offspring cells. Over many generations, the mtDNA genotype of a cellular lineage can move toward predominantly mutant or wild-type mtDNAs. As the percentage of mutant mtDNA increases, the cellular energy capacity decreases until it falls below the bioenergetic threshold — the minimum energy output necessary for a cell or tissue to function normally. Damage to mtDNA is often accompanied by an increased level of DNA mutations and deletions [25, 26]. Several studies have shown that oxidative-induced mutations in mtDNA accumulate with age in postmitotic tissues such as the brain [27–29]. Several age-related disorders have been shown to be associated with high levels of mtDNA mutations. For example, elevated levels of cortical mtDNA deletions have been found in patients with Alzheimer's disease [30, 31]. Ikebe et al. [32] found 17 times the level of mtDNA deletion in the striatum of Parkinson's disease patients when compared with control subjects. Increasing evidence indicates that accumulation of oxidation of DNA, lipid, and protein by free radicals is responsible for the functional decrease in the aged brain.

B Membrane Composition and Lipid Damage

Aging also is accompanied by changes in membrane fatty acid composition, including a decrease in the levels of polyunsaturated fatty acids (PUFAs) and an increase in monosaturated fatty acids. PUFAs, such as arachidonic acid (AA), are abundant in the aging brain and are highly susceptible to free radical attack. A correlation between the concentration of AA and long-term potentiation has been shown [33, 34], suggesting that oxidative depletion of AA levels may relate to a cognitive deficit in rats. For example, levels of AA are decreased in the hippocampus of aged rats with impaired ability to sustain long-term potentiation. Oxidative damage to lipids can also occur indirectly through the production of highly reactive aldehydes. Peroxidation of AA forms malondialdehyde (MDA), which induces DNA damage by reacting with amino acids in protein to form adducts that disrupt DNA base-pairing. Increased levels of MDA have been found in the aged canine brain [35]. In the aged human brain, increased levels of MDA have been found in inferior temporal cortex and in the cytoplasm of neurons and astrocytes [36], as well as in the hippocampus and cerebellum of aged rodents [37, 38]. Peroxidation of linoleic acid forms 4-hydroxy-2-nonenal (HNE). HNE is more stable than free radicals and it is able to migrate to sites that are distant from its formation, resulting in greater damage. The most damaging effect of HNE is its ability to form covalent adducts with histidine, lysine, and cysteine residues in proteins, enabling a modification in their activity [39]. It has been shown that the HNE-modified proteins, along with neurofibrillary tangles, are present in the senile plaques in aged dogs [40]. Increased levels of HNE have also been found in Alzheimer's and Parkinson's disease [41, 42]. These findings support the hypothesis that lipid peroxidation contributes to the deterioration of central nervous system (CNS) function.

C Protein Oxidation

Most of the studies conducted to assess the role of protein oxidation in aging brains conclude that there is an increase in oxidized proteins. An increase in the oxidation of mitochondrial proteins with age has been demonstrated by measuring the levels of protein carbonyl groups in the human cerebral cortex along with age [43]. Carbonyl formation can occur through a variety of mechanisms, including direct oxidation of amino acid side chains and oxidation-induced peptide cleavage. Increasing evidence suggests that protein oxidation may be responsible for the gradual decline in physiological functioning that accompanies aging. Elevated protein carbonyls have been shown to be present in the hippocampus of aged rats with memory impairment [44]. Increased protein carbonyl levels were found in the frontal and occipital cortex of aged humans [43] and rats [45, 46]. Measuring protein 3-nitro-tyrosine (3-NT) levels is another way to assess the oxidative modification of proteins. Increased 3-NT levels have been identified in the hippocampus and the cerebral cortex of aged animals as well as in the CSF of aged human and in the white matter of aging monkeys [47–49]. 3-NT immunoreactivity has been observed in the cerebellum in the Purkinje cell layer, the molecular layer, and in the cerebellar nuclei of aged rats [50]. However, contradictory findings of decreased protein 3-NT levels of brain homogenate were reported in aged Wistar rats. Recently, proteomics studies enabled the identification of specific proteins that undergo oxidative stress in AD patients [51, 52].

D Oxidative Stress and Chronic Inflammation

Increasing evidence associates aging and age-related diseases with inflammation [53–55]. The key cellular event signaling ongoing inflammation in the brain is the accumulation of reactive microglia in the degenerative areas [56, 57]. Microglia are the resident immune cells of the central nervous system; they constitutively express surface receptors that trigger or amplify the innate immune response. These include complement receptors, cytokine receptors, chemokine receptors, major histocompatibility complex II, and others [58]. In the case of cellular damage, they respond promptly by inducing a protective immune response, which consists of a transient upregulation of inflammatory molecules as well as neurotrophic factors [59]. This innate immune response usually resolves potential pathogenic conditions. However, when chronic inflammation occurs, prolonged activation of microglia triggers a release of a wide array of neurotoxic products [60] and proinflammatory cytokines such as interleukin-1 (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and many others. Elevated protein levels of IL-1β, TNFα and IL-6 have been found in the brains of aged animals [37, 61, 62]. Animal studies have shown that increased levels of IL-6 in the hippocampus and cerebral cortex are primarily from microglia [55]. It has been proposed that the increase in brain microglial activation may be one of the early events that leads to oxidative damage. Activated microglia are indeed the most abundant source of free radicals in the brain and release radicals such as superoxide and nitric oxide [63]. Microglia-derived radicals, as well as their reaction products hydrogen peroxide and peroxynitrite, can harm cells and these products have been shown to be involved in oxidative damage and neuronal cell death in neurological diseases [64]. It also should be noted that microglial cells have efficient antioxidative defense mechanisms. These cells contain high concentrations of glutathione, the antioxidative SOD enzymes, catalase, glutathione peroxidase, and glutathione reductase, as well as NADPH-regenerating enzymes [64]. When the production of ROS is prolonged, the endogenous reserves of antioxidants become exhausted and result in cell damage (Figure 15.1).

E Antioxidant Defense Mechanisms

Several antioxidant defense mechanisms have evolved to protect cell components from the attack of oxidative stress and associated oxidative damage. These mechanisms include antioxidant enzymes, such as SOD, superoxide reductases, catalase, glutathione peroxidases (Gpx), and many heat-shock proteins. The enzymes catalase and SOD are the major defenses against ROS. SOD converts superoxide anions into H_2O_2, and catalase converts H_2O_2 to molecular oxygen and water. SOD exists in two forms: Cu/ZnSOD is present primarily in the cytoplasm while MnSOD is present primarily in the mitochondria.

The hypothesis that lifespan can be enhanced by increasing antioxidant defenses has been controversial because of conflicting results in several aging models. For example, many studies have shown that endogenous levels of antioxidant enzymes in the brain and other tissues do not decrease during aging [65, 66]. Moreover, studies in mammals in which levels of antioxidants are experimentally increased have shown that maximum longevity is not affected [67, 68]. Experiments with *Drosophila melanogaster* have shown that overexpression of MnSOD increased lifespan [69], while overexpression of CuZn-SOD had only minor incremental effects on lifespan [70]. Similarly, enhanced levels of catalase (up to 80%) did not prolong the lifespan of flies, nor did it provide improved protection against oxidative stress induced by hyperoxia or paraquat treatment [71]. In contrast, the simultaneous overexpression of CuZn-SOD and catalase was found to extend and slow down various age-related biochemical and functional alterations in *Drosophila* [72]. Similarly, *Drosophila* selected for longevity and *Caenorhabditis elegans* with the *age-1* mutation (a mutation associated with increased lifespan), were found to have increased activity of CuZn-SOD and catalase [73–75]. These conflicting results suggest that an optimal balance between SOD and catalase is important for lowering the levels of oxidative stress and increasing lifespan. The effect of overexpression of SOD on lifespan has also been observed in mice [76]. Although several studies have shown that elevated CuZN-SOD induced protection against oxidative stress, other reports did not find a correlation between such protection and increased lifespan. For example, homozygous transgenic mice with a two- to fivefold increase of CuZN-SOD showed only a small increase in lifespan [77].

IV EXPERIMENTAL CONTROL OF OXIDATIVE STRESS PATHWAYS

A Genetic Manipulations

Work with *Drosophila*, yeast cells, *Neurospora* (a type of fungus), and the nematode *Caenorhabditis elegans* has established a genetic link between stress responsiveness and lifespan. For example, when exposed to a low-energy environment, *C. elegans* converts to its dauer state in which reproductive function is arrested. During this state, this organism is more resistant to stress. *C. elegans* mutations have been reported to extend life expectancy by 40% to more than 100%. The first of these mutations to be discovered involved the *age-1* gene; mutations in this gene have been shown to increase longevity by about 100% but do not affect reproduction or movement. Biochemical studies have revealed that strains carrying *age-1* alleles have enhanced oxidative defenses. For example, when the wild-type and *age-1* strains were examined for resistance to H_20_2 exposure, the 50% effective lethal dose (LD_{50}) of the wild type remained constant over the lifespan whereas the LD_{50} of the *age-1* strain increased with aging [75]. Moreover, the increased resistance to oxidative stress was associated with elevated antioxidant activity, as shown by an increase in the activity of SOD and catalase [78]. A variety of other life-extending mutations that are correlated with enhanced stress tolerance have been described.

There are a number of mutated genes that regulate the insulin/insulin growth factor 1 (IGF-1) signaling pathway. *Age-1*, *daf-2,* and *daf-16* genes in *C. elegans* are associated with an insulin-like signaling pathway. *Age-1* and *daf-2* suppress the activity of the downstream target *daf-16*, a transcription factor that belongs to the Forkhead family of proteases [79]. Hence, loss of function of either of these upstream regulators enhances *daf-16* function and leads to increased lifespan. Importantly, loss-of-function mutations in *daf-16* not only prevents longevity conferred by the *age-1* and *daf-2 mutations*, but also abolishes stress resistance [80], thereby strengthening the intimate link between longevity and the stress responsiveness associated pathway. These animals are smaller in size, and have a decreased body temperature and a modest increase in antioxidant capacity. Recent studies have shown that knockout mice for the IGF receptor live longer and display greater resistance to oxidative stress [81, 82].

One additional long-lived mutant strain of *C. elegans,* which provides an important link between metabolism, oxidants, and aging, is *clk-1*. *Clk-1* lacks an enzyme required for the synthesis of ubiquinone, or coenzyme Q [83]. Coenzyme Q is an important electron acceptor for both complex I- and complex II-dependent respiration. Overexpression of *clk-1* leads to a reduction in lifespan [84], probably by increasing the rate of metabolism, which in turn might lead to a faster accumulation of damage resulting from metabolic by-products such as ROS. Another mutant with reduced longevity is *mev-1*. *Mev-1* encodes a subunit of the enzyme succinate dehydrogenase cytochrome b, a component of complex II of the mitochondrial electron transport chain. These animals show hypersensitivity to hyperoxia, and have compromised mitochondrial function and increased ROS generation [85]. SOD activity is about half that found in the wild type, and the average lifespan is reduced by approximately 35% [86]. These worms also were shown to exhibit increased levels of nuclear DNA

damage [87]. Similarly, mice heterozygous for SOD2 have an increased incidence of nDNA as well as a significant increase in tumor formation. Another interesting mutation affecting longevity involves the p66shc gene. The p66shc protein belongs to a family of adaptor proteins that regulate protein-protein interaction for several cell surface receptors. These mice live 30% longer than control mice and also have an increased resistance to oxidative stress [88].

Links between longevity and stress resistance, similar to those demonstrated in *C. elegans,* also exist in *Drosophila melanogaster*. Various strains of flies selected for extended lifespan display increased resistance to oxidative stress that in some cases is correlated with enhanced activity of antioxidant enzymes. Methuselah (*mth*), a long-lived mutant, encodes a G protein-coupled receptor that is thought to play a role in signal transduction [89]. This mutant not only enhances longevity, but also increases resistance to heat stress and paraquat (an intracellular ROS generator). *Mth* exhibits a 35% increase in average lifespan and is resistant to several stressors, such as oxidants, starvation, and heat [89]. Another *Drosophila* mutant, *Indy,* belongs to a family of proteins involved in the Krebs cycle. This mutant shows a 50% increase in lifespan [90].

Several groups have been developing animal models with mitochondria deficiencies [91–93]. These models include the adenine nucleotide translocator (ANT-1), mitochondria superoxide dismutase- (SOD2-) deficient mice, Tfam-deficient mice, and the PolgA. ANT-1 - deficient mice are a model for chronic ATP deficiency. These mice have increased production of ROS and hydrogen peroxide and a parallel increase in mtDNA mutations consistent with levels seen in much older mice [94]. SOD2-deficient mice die in the neonatal period from dilated cardiomyopathy or neonatal degeneration in the brain stem [92, 93]. The Tfam-deficient mice exhibit cytochrome *c* oxidase deficiency and die at around 3 weeks of age [95]. PolgA is a more recent mouse model, independently developed by two groups [29, 96], that expresses a deficient version of the nucleus-encoded catalytic subunit of mtDNA polymerase. These mice develop a mtDNA mutator phenotype with a three- to fivefold increase in levels of mtDNA point mutations, as well as an increase in the amount of deleted DNA. This increase in mtDNA is associated with a premature onset of aging-related phenotypes, including osteoporosis, alopecia, kuphosis, and a median survival of 48 weeks of age and a maximum survival of 61 weeks. Interestingly, these mice do not show an increase in oxidative damage markers [96, 97]. One possible explanation for the decreased longevity in these mice is an increase in markers of apoptosis, suggesting a possible decline in regenerative capacity of tissues in these mice [96, 97].

B Caloric Restriction

Caloric restriction is the only reproducible experimental manipulation for extending lifespan in many species. Restriction of food intake by 30 to 50% below *ad libitum* levels during the early growth phase of life has been shown to produce significant increases in the mean lifespan of several species, including insects, mice, fish, and rats [98]. Moreover, calorically restricted rhesus monkeys showed physiological changes similar to those observed in rodents on a calorie-restricted diet. Both calorically restricted monkeys and rats are smaller, mature later, have lower blood glucose and insulin levels, lower body temperature, and increased daytime activity [99]. Although several theories have been proposed to explain the anti-aging effects of caloric restriction, one hypothesis proposes that it acts by decreasing oxidative stress. In support of this hypothesis, it has been shown that caloric restriction can stabilize mitochondrial function and reduce oxidative stress in brain cells [100]. In the same study, the authors showed that the reduction in ROS production is not due to reduced mitochondria oxygen consumption, but rather to a lower percentage release of ROS per total flow in the respiratory chain [100]. It has been shown that caloric restriction decreases H_2O_2 in rat heart mitochondria. On the other hand, contradictory results have been obtained on the effect of caloric restriction on the expression of the antioxidant enzymes SOD, catalase, and GSH-peroxidase. In particular, some reports have shown that caloric restriction did not increase antioxidant defenses, while other studies demonstrated that the activity of SOD, catalase, and GSH was increased in older ages [101, 102]. Caloric restriction also prevents many of the changes in gene expression and transcription-factor activity that normally occur with aging, including basal elevations in expression of heat-shock proteins. Caloric restriction can also induce the expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF). Increased levels of BDNF have been found in neurons in the hippocampus and other brain regions of rodents maintained on caloric restriction [103]. Given all the positive effects of caloric restriction, one could assume that this translates to an improvement in cognition in animals with caloric restriction; however, the data are mixed on this aspect of behavior; although some reports have found positive benefits [104–106], others have found either only small benefits or no benefits at all [107, 108]. Mixed benefits have also been reported on motor behaviors; improvements were noted on some motor learning tasks and complex locomotor behaviors [107, 109] but negative effects of caloric restriction were observed when testing some aspects of drug-induced rotational behavior and stereotopy [110].

Although caloric restriction is a reproducible way to increase the functional and maximal lifespan, it is questionable whether humans will choose to adopt this lifestyle change and it is still controversial whether caloric restriction will increase lifespan in nonhuman and human primates [111]. There is now accumulating evidence that selection of appropriate whole foods or the addition of antioxidants into the diet is beneficial to increasing the functional lifespan, if not the maximal lifespan (for a review, see [112]). One could then argue that caloric selection may be as important as caloric restriction.

V SUPPLEMENTARY ANTIOXIDANTS AS APPROACHES TO IMPROVE BRAIN HEALTH

A Vitamin E

Vitamin E (Vit E) is the most studied antioxidant supplement and has been shown to increase longevity in short-lived species such as *C. elegans* and the rotifer *Asplanchna brightwelli* [113, 114]. There is little evidence that it increases maximum lifespan in rodents [115, 116] but evidence for an improvement in functional lifespan (i.e., improvements in aspects of physiology and brain health) is more compelling. For example, Vit E administered to rodents can improve age-related impairments in LTP [117] and improve cognitive behaviors [118, 119]. Vit E has also been shown to have actions that can prevent neurodegenerative disease in animal models and in *in vitro* studies. For example, Vit E is neuroprotective in apoE-deficient mice [120] and modifies Aβ toxicity in cultured hippocampal neurons [121]. The prevention of Aβ toxicity *in vivo* has also been observed [122]. In that study, Vit E prevented the onset of behavioral deficits induced by infusions of Aβ into the cerebroventricles. However, the data in humans are less convincing; clinical prevention trials using Vit E did not demonstrate improvements in cognition in AD patients, although some improvement in living performance was noted [123, 124]. Epidemiological studies have not always shown a positive link between increased intake of Vit E and decreased incidence of AD, Parkinson's disease or cerebrovascular disease [125,126]. Some studies have noted a correlation between dietary intake of foods high in Vitamin E, such as nuts, or diets high in fruits and vegetables and a decreased incidence of these diseases [127], suggesting that perhaps whole food sources of antioxidants and other phytochemicals may be of benefit.

B Phytochemicals and Polyphenolic Compounds

Fruits, vegetables, nuts, and other whole foods, such as blue-green algae, contain thousands of phytochemicals, including polyphenolic compounds that express anti-oxidant activity and anti-inflammatory compounds [106, 128–130]. Some of these foods, such as the blue-green algae, also contain omega fatty acids, including gamma-linolenic acid (GLA), that can act to reduce lipid peroxidation and also reduce inflammation. Nature has packaged a wide array of phytochemicals that likely act in synergy to promote health. The area of nutritional neuroscience is growing quickly and there are many studies on the effects of whole foods and herbs as neuroprotective agents.

Green tea has been widely studied, with particular interest in one of the polyphenolic components, (−)-epigallocatechin-3 gallate (EGCG). EGCG has been extensively researched for its anticarcinogenic effects [131, 132]; however, it has also been shown to have actions that inhibit pro-inflammatory cytokines [133, 134]. The green tea catechin EGCG has been examined for activity in Alzheimer's disease. EGCG has been shown to reduce amyloid precursor protein cleavage that produces Aβ in primary neurons derived from Swedish mutant APP-overexpressing mice. When EGCG is given to these Tg APP_{sw}2576 mice, there is a reduction in amyloid load in the brain [133]. EGCG also protects against Aβ toxicity in cell cultures, an action that is likely related to its antioxidant activity [136]. Other phytochemicals that have been studied for potential activity in Alzheimer's disease include curcumin and other related flavones, as well as flavonoids such as quercitin [137–139]. Further, blueberries have also been shown to reduce amyloid load in an animal model of AD [140].

Foods such as blueberries, spinach, and spirulina, a blue-green algae with high oxygen radical absorbance capacity (ORAC = 320; [141]), have also been studied extensively for neuroprotective actions. For example, in the cerebellum there is a correlation between the loss of function of β-adrenergic receptors in the aged brain and a loss in the ability to learn complex motor skills [142]. Feeding aged F344 rats a diet rich in spinach improves cerebellar β-adrenergic receptor function and improves motor learning that is associated with a decrease in oxidized glutathione and the pro-inflammatory cytokine TNFα [143, 144]. Further studies have attempted to correlate the ability to improve cerebellar β-adrenergic receptor function with the *in vitro* antioxidant capacity of the foods added into the diet. For example, cucumber is very low in polyphenolic compounds and, when added to the diets of aged rats, produced no improvement in cerebellar β-adrenergic receptor function and no reduction in malondialdehyde or TNFα [141]. On the other hand, the addition of spirulina to the normal rat diet (0.1% w/w) significantly improved β-adrenergic receptor function, decreased malondialdehyde, and reduced pro-inflammatory cytokine levels [141]. Although correlations between reduced markers of oxidative stress and inflammation do not guarantee a causative relationship, there is increasing evidence from many studies supporting this hypothesis.

Blueberries and spirulina have also been shown to be neuroprotective in animal models of Parkinson's disease. In one study by Strömberg et al. [145], blueberries and spirulina both improved the recovery from a neurotoxic 6-hydroxydopamine (6-OHDA) lesion of dopamine projections to the striatum. In this study, there was a reduced lesion area 1 month following 6-OHDA administration; however, the lesion was similar in size to controls at 1 week following the neurotoxic insult, suggesting that what the dietary supplementation was doing was to improve the recovery and regeneration of the dopamine terminals in the striatum following the lesion, rather than preventing the initial damage. One mechanism by which this might occur is through an interaction with microglial activation in response to the injury. This study reports that 1 week following the injury, there was an increased microglial response to the injury. In this early period, the microglia are phagocytotic and aid in repairing the damage caused by the neurotoxic insult; the increased microglial response at this time may help to prepare the brain for regeneration of the dopamine fibers into the lesioned area (Figure 15.2). A second finding of this study was that microglial reaction to the injury continued to increase over time in the lesioned rats fed a control diet but was reduced at during later time points in rats fed the supplemented diet. This chronic phase of the microglial response may actually be detrimental; reducing this later phase of inflammation correlated with the smaller lesion size in the rats that consumed the supplemented diet. Green tea catechins have also been studied for their potential actions in Parkinson's disease. For example, EGCG attenuates 6-hydroxydopamine-induced cell death in neuronal cultures [146, 147].

Neuroprotection from ischemic brain damage is another area where nutritional and herbal approaches have been examined. Multiple epidemiological studies have shown a correlation between diets high in fruits and vegetables, and specifically, the Mediterranean diet, and a decreased risk of cardiovascular disease and stroke [148, 149]. This has also been examined in animal models. For example, a study by Wang and colleagues [150] reported that dietary supplementation with either blueberries, spinach, or spirulina for 1 month prior to a middle cerebral artery inclusion reduced the size of the damage. The most effective supplement in this study was spirulina, which reduced the infarction size by 70% when incorporated into the diet at 0.1% w/w. Blueberries and spinach reduced the infarct size by 50% when incorporated into the rat diet at 2% w/w (Figure 15.3). A study by Sweeney et al. [151] had shown that a much higher dose of blueberries prevented cell loss in the hippocampus following an ischemic event. It must be recognized, however, that all of these studies, when converted to human equivalents, are on the high side if converted to a nutraceutical dosage for optimal health. Perhaps a more interesting approach is to examine the synergy of nutrients to determine if there may be combinations of whole foods that will synergize to produce even more potent effects at maintaining optimal brain health and preventing neurodegenerative disease.

VI SUMMARY AND CONCLUSIONS

In closing, this chapter has summarized some of the literature that supports a role for oxidative stress as one aspect of age and disease that contributes to declines in function of the central nervous system. The free radical theory as proposed by Harmon in the mid-1950s was the catalyst for many studies into aspects of free radical metabolism and mitochondrial function that have shown links between oxidative stress, brain aging, and neurodegenerative disease. Interestingly, in recent years, the oxidative stress theory of aging has also come to include a role for inflammation. These two processes are interlinked and have many feedback loops such that separating the two processes is difficult. Many studies are now defining the critical mitochondrial and inflammatory pathway changes that underlie this increase in oxidative stress and inflammation with age. Genetic manipulations of many species have assisted in delineating the critical aspects of free radical metabolism that either induce premature aging or increase longevity. These genetic manipulations are not, however, applicable as interventions to improve brain health with age. There are two interventional strategies discussed in this chapter: (1) caloric restriction and (2) nutritional intervention. Caloric restriction is the most reproducible way to increase lifespan among many species and reduces many diseases associated with aging. But how many humans will comply with this rigorous lifestyle change? Antioxidants and polyphenolic compounds from fruits, vegetables, nuts, and grains decrease markers of oxidative damage, such as malondialdehyde and protein carbonyls, and decrease levels of pro-inflammatory cytokines either directly or indirectly by reducing oxidative damage. Epidemiological evidence supports that a diet high in fruits and vegetables may help prevent certain diseases of aging, including stroke, diabetes, Parkinson's disease, and Alzheimer's disease. So, your mother was right when she told you to eat your fruits and vegetables!

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Figures

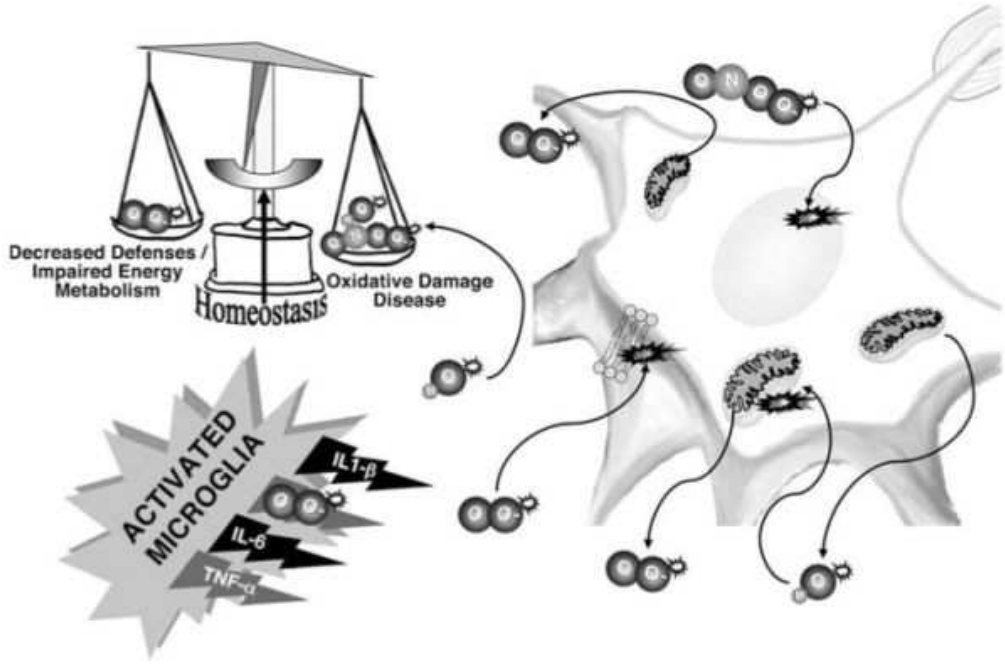

FIGURE 15.1

Oxidative stress and inflammation are two processes that go hand in hand during the aging process and in neurodegeneration and disease. There are at least two major sources for reactive oxygen species (ROS) in the CNS: the mitochondrion and the microglia. ROS are produced as a result of normal production of energy by the mitochondrion and are part of the natural defense system of the microglia to kill invading microorganisms. Under normal conditions, there is a balance of production of ROS, and homeostasis exists where energy metabolism is at the appropriate level in the mitochondrion and the microglia are either at rest, because there is no infection, or are responding normally to an injury or infection. ROS are essential for the normal function of the cell, and underproduction of ROS would lead to a decrease in energy production and a decrease in the ability of microglia to mount a defense against invading organisms. The other end of the spectrum appears to be the situation in the aging brain where increased ROS produced from the mitochondrion and the microglia can lead to damage to lipids, DNA, and proteins. This leads to a functional decline in neurons, such as changes in neurotransmitter receptor signal transduction. The microglia also produce pro-inflammatory cytokines, such as Il-1β TNFα, and IL-6, that provide positive feedback to keep the inflammation cycle active. The rise in ROS may also act as a stressor that activates redox-sensitive signaling pathways. Thus, once activated, these diverse signaling pathways can continue to have damaging effects, leading the neurodegeneration and disease.

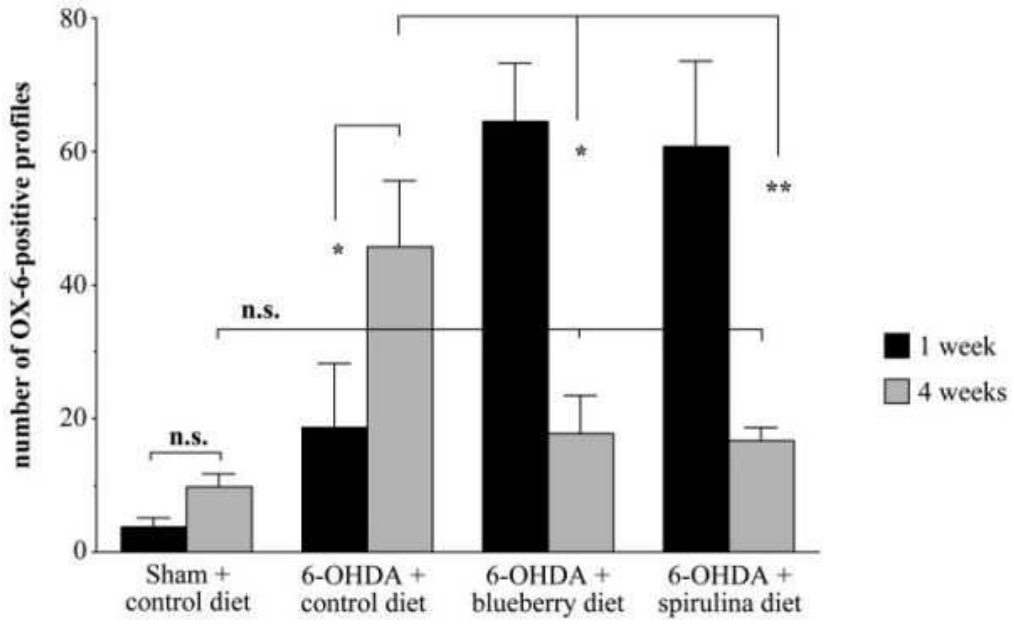

FIGURE 15.2

Bar graph depicting the number of OX-6 positive microglia in the striatum of rats following either sham of 6-hydroxydopamine (6-OHDA) lesions into the corpus striatum. This quantification of MHC class II receptors on microglia demonstrates that in rats treated with 6-OHDA and fed the control diet with normal levels of vitamins and minerals the inflammatory process continues to increase over the 4 weeks following the 6-OHDA insult. In rats that were fed the blueberry or spirulina diet prior to the insult, there was initially a much larger response to the 6-OHDA at 1 week; however, at 1 month following the insult, there was a significant reduction in the numbers of OX-6 positive microglia observed in the striatum. * = $p < 0.05$, $p < 0.01$, one-way ANOVA (see [143] for a full description).

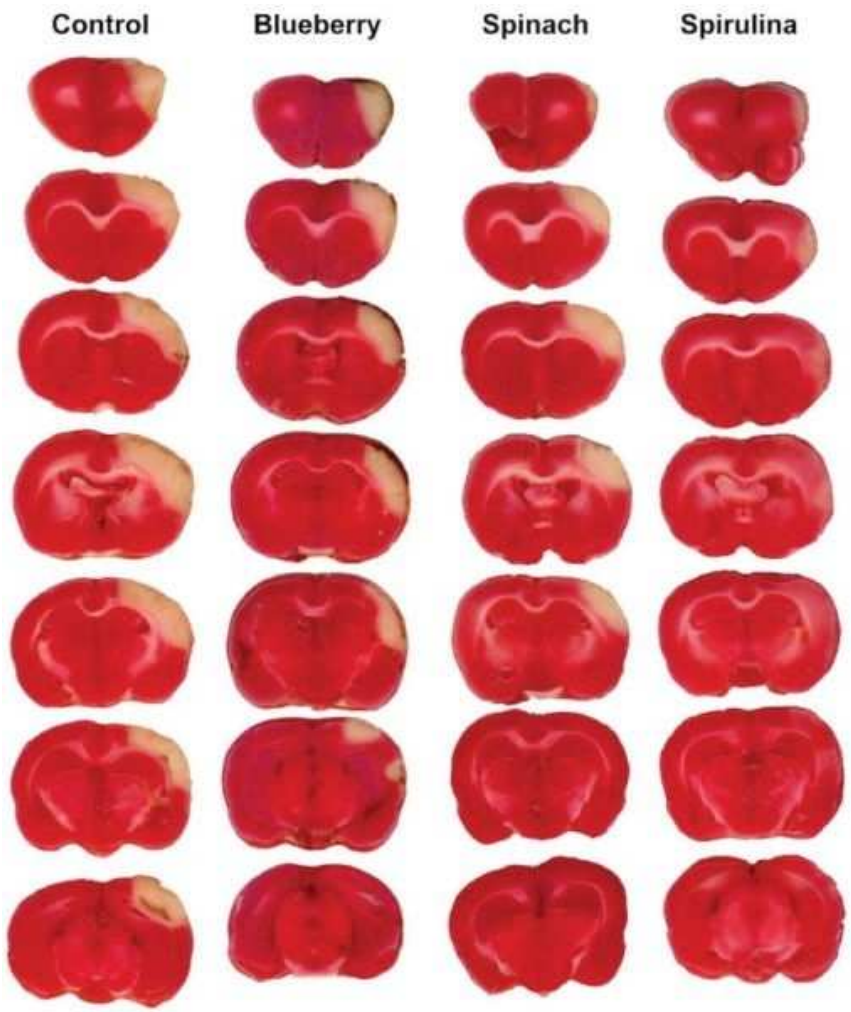

FIGURE 15.3

(SEE COLOR INSERT FOLLOWING PAGE 204) Pretreatment with either blueberry, spinach, or spirulina enriched diets significantly reduced the cortical infarction induced by middle cerebral artery occlusion/reperfusion. The right middle cerebral artery was ligated for 60 minutes. Animals were euthanized for TTC staining 48 hours after ischemia/reperfusion. Marked infarction (white areas) in the right cerebral cortex was found in animals receiving the control diet. Pretreatment with either blueberry, spinach, or spirulina significantly reduced the amount of infarction.