Published in final edited form as:

Neurochem Res. 2009 April ; 34(4): 670–678. doi:10.1007/s11064-008-9808-4.

Oxidative Damage and Cognitive Dysfunction: Antioxidant Treatments to Promote Healthy Brain Aging

Elizabeth Head, Ph.D.¹

¹Institute for Brain Aging & Dementia, Department of Neurology, University of California, Irvine, CA, 92697

Abstract

Oxidative damage in the brain may lead to cognitive impairments in aged humans. Further, in age-associated neurodegenerative disease, oxidative damage may be exacerbated and associated with additional neuropathology. Epidemiological studies in humans show both positive and negative effects of the use of antioxidant supplements on healthy cognitive aging and on the risk of developing Alzheimer disease (AD). This contrasts with consistent behavioral improvements in aged rodent models. In a higher mammalian model system that naturally accumulates human-type pathology and cognitive decline (aged dogs), an antioxidant enriched diet leads to rapid learning improvements, memory improvements after prolonged treatment and cognitive maintenance. Cognitive benefits can be further enhanced by the addition of behavioral enrichment. In the brains of aged treated dogs, oxidative damage is reduced and there is some evidence of reduced AD-like neuropathology. In combination, antioxidants may be beneficial for promoting healthy brain aging and reducing the risk of neurodegenerative disease.

Keywords

acetyl-l-carnitine; Alzheimer disease; beagle; beta-amyloid; dog; canine; lipoic acid; oxidative damage

Introduction

Aging in most species studied to date is accompanied by the progressive accumulation of oxidative damage in many tissues [1–5]. The brain, in particular, is highly vulnerable to oxidative damage as it consumes approximately 20% of the body's total oxygen, has a high content of polyunsaturated fatty acids, and lower levels of endogenous antioxidant activity relative to other tissue [6–8]. Normal metabolic processes result in the release of reactive oxygen species (ROS), which in turn can lead to oxidative damage to proteins, lipids, DNA and RNA [1]. ROS are produced primarily from mitochondria [9], intracellular organelles that are themselves vulnerable to oxidative damage [10].

Mitochondrial dysfunction and production of ROS may be a key contributor to the deleterious effects of aging on the brain. For example, research by Dr. Mori and others provides a significant amount of support for a role of mitochondrial dysfunction leading to oxidative damage in the brain [5, 11–18]. In the senescence-accelerated mouse (SAMP8), which exhibits a shortened life-span and learning impairments, Dr. Mori has also shown significant mitochondrial dysfunction is also observed in liver [5] and in heart [11]. Further, co-factors that specifically target the mitochondria to improve efficiency and reduce ROS such as acetyl-L-carnitine (ALCAR) and lipoic acid (LA) also lead to behavioral improvements and reduced oxidative damage in the brains of aged rodents [10, 18, 19]. Thus, studies in rodents support the hypothesis that oxidative damage leads to behavioral dysfunction [20] and that improving mitochondrial function and reducing oxidative damage may promote healthy brain aging [21]. In combination, based on studies in animals, oxidative damage may lead to neuronal and thus cognitive dysfunction in aging humans [22].

In this brief review, a brief summary of evidence from the human literature will be presented suggesting that oxidative damage is critically involved with both normal and pathological aging. The results of a longitudinal intervention study in a canine model of aging that naturally develops cognitive decline and human-type neuropathology also provide support for the hypothesis that oxidative damage causes neuronal dysfunction. In combination, administration of antioxidants (either as supplements or within food) may be beneficial for healthy brain aging.

Oxidative damage and mitochondrial dysfunction in human brain aging and Alzheimer Disease

Studies in both normal and pathological human brain aging provide evidence in support of a role of oxidative damage in age-associated cognitive losses. Studies of human autopsy tissue show higher levels of oxidative damage to DNA/RNA [23–25], proteins [24, 26–29], lipids [25, 29, 30] in aged as compared to young human brain. Oxidative damage may also play a role in age-associated neurodegenerative diseases such as Alzheimer disease (AD) [31–33]. AD is characterized by the accumulation of senile plaques and neurofibrillary tangles [34]. Senile plaque contain the toxic peptide, beta-amyloid (A β), which is cleaved from a longer precursor protein (APP)[35]. A β is thought to play a causative role in the development of AD [36]. In addition to plaques and tangles accumulating in the AD brain, there is a large body of evidence showing increased protein [24, 27, 28, 37–44], lipid [25, 30, 45–48], DNA [49–51] and RNA oxidative damage [12, 52, 53]. Further, antioxidant activity in the AD brain is reduced relative to age-matched controls [24, 29, 54]. Additional work by Dr. Mori and colleagues also suggest that the cerebrospinal fluid of patients with AD exhibits reduced antioxidant capacity [55]. Proteins particularly vulnerable to oxidative damage have been identified by proteomics and a subset of these proteins may be directly or indirectly involved in the production and accumulation of AD neuropathology [56]. There are many downstream consequences of oxidative damage to proteins that may lead to aberrant signaling that may compromise neuron function [57].

Mitochondrial function also appears compromised with age and disease in the human brain [3, 4, 14, 58]. In normal aging, mitochondrial respiratory chain activity declines [59], mitochondrial metabolism-associated enzymes such as aconitase decrease [60] and the rate of somatic mitochondrial DNA mutations increases [17, 61]. In AD, similar types of losses in respiratory chain activity [15, 62, 63] and increases in mitochondrial DNA mutations [64] are observed but are higher when compared to age-matched controls. Further, in AD, decreased cytochrome oxidase activity in the posterior cingulate cortex is correlated with hypometabolism seen by positron emission studies [65]. A new gene array study in the cingulate cortex shows that energy-metabolism related genes, and specifically a 65% reduction in expression of mitochondrial electron transport chain genes, occurs in AD [66]. Thus, mitochondrial dysfunction and the production of ROS, combined with lower endogenous antioxidant activity may lead to increasing oxidative damage to molecules critically important to neuronal function.

A β , Oxidative Damage and Mitochondrial Dysfunction

Why is oxidative damage more extensive in the AD brain relative to nondemented aged brains? There is evidence of a link between A β and oxidative damage that is based upon work with mouse models of AD and studies of AD brains [67, 68]. It is unclear yet whether oxidative damage may lead to A β deposition or whether A β leads to oxidative damage. In studies of transgenic mice that overexpresses mutant human beta-amyloid precursor protein (Tg2576), lipid peroxidation precedes A β deposition in plaques [69]. Further, APP is increasingly oxidized with age and oxidative modification may in turn favor amyloidogenic cleavage to produce more A β [70]. Beta-secretase protein levels are increased by the lipid peroxidation product, hydroynonenal, which can lead to enhanced production of A β from full length APP [71]. In turn, there is good evidence that A β itself can cause oxidative damage [72–74]. There is also growing evidence of A β causing mitochondrial dysfunction [67]. A β can impair mitochondrial function through several mechanisms [75, 76] including through direct interaction with A β -binding alcohol dehydrogenase (ABAD) located within mitochondria [77]. Another key enzyme involved with the cleavage of APP to A β , the gamma-secretase complex, also appears to be associated with mitochondria [78]. Thus, in the diseased brain, there may be a feedforward loop involving oxidative damage and A β . Oxidative damage and mitochondrial dysfunction may lead to the production of A β , which in turn causes more oxidative damage and mitochondrial dysfunction [79].

Antioxidants in Human Aging and Disease

Based on neuropathology studies, antioxidants may be predicted to be associated with healthy aging and might serve as an approach to reduce the risk of developing AD. However, in contrast to robustly positive outcomes in studies using rodent models, observational studies in humans have shown either a positive effect of antioxidant supplementation on cognition and risk reduction for developing AD [80–82], or no significant effects [83–86]. Difficulties in interpreting human observational studies stem from inconsistencies in the amount of supplements taken, their form and source, their duration and regularity of use, and from the challenges of determining the exact dietary intake of antioxidants. For example, in one study, combinations of antioxidants were

superior to single supplementation [87] and dietary intake of antioxidants has been shown to be superior to supplements in human studies on cognition and risk of developing AD [81, 88]. There have been few systematic clinical trial studies evaluating the effects of antioxidants on cognition in aged individuals or patients with AD. Vitamin E, however, has been shown to delay institutionalization in AD patients, suggesting some beneficial effects [89]. However, vitamin E alone did not improve cognition in patients with mild cognitive impairment, which is thought to be a precursor to AD [90]. In nondemented elderly women, vitamin E alone has little benefit on cognition [85], however, one study showed that supplementation with a combination of vitamins E and C led to improved memory [91].

There are several studies of the effects of mitochondrial co-factors specifically on cognition in patients with AD. In studies where ALCAR was administered to patients with moderate to severe AD, either improved cognition and/or slower deterioration has been observed [92–95]. In early-onset AD patients (less than 65 years of age), only small cognitive improvements were noted [96] but in other studies of younger patients with AD (less than 61 years) there was evidence for slowed disease progression [97, 98]. When the results of all these studies are combined in a meta-analysis, there were clear benefits of ALCAR administration in patients with AD, particularly with respect to slowing cognitive decline [99]. Further, combining ALCAR with acetylcholinesterase therapy in AD may provide additional benefits [100].

Similar evidence of maintenance of function was observed in a study of 9 patients with AD or related dementias receiving 600 mg/day of LA for an average of 337 days [101]. In a follow up study of 48 patients for a longer 48 month treatment period, maintenance of function similar to the smaller study was observed [102]. Interestingly, a recent study of transgenic mice (tg2576) also showed improved learning and memory in LA treated animals with little effect on A β pathology [103]. Thus, LA treatment may also be of benefit for AD through neurobiological mechanisms independent of A β reduction.

There have been no human clinical trials that have combined different cellular antioxidants with mitochondrial co-factors. Thus, we tested the hypothesis that a combination antioxidant diet approach, which included cellular antioxidants, fruits and vegetables and mitochondrial co-factors, can lead to improved cognition and reduced neuropathology in brain aging. To explore these hypotheses we have been working with a canine model of aging. Work from our and other laboratories suggest that this natural model of aging provides unique advantages for the study of oxidative damage and development of AD pathology that complements currently used model systems such as transgenic mice and nonhuman primates. Unique features of the canine model include long life spans, allowing for longitudinal studies and the natural deposition of human-type A β that does not require overexpression of mutant human genes.

The Canine Model of Human Aging

Over the past 15 years we have been working with a dog model of human brain aging. For our studies we use primarily beagles, which have a median life span of 13.5 years (equivalent for males and females). For these studies we consider animals 5 years and

younger to be young, 6–10 years to be middle aged and over 10 years to be old. Additional categorization can be used depending on the criteria – for example, the age at which cognition decline or when brain pathology begins to accumulate. However, beagles over the age of 15 years may also be considered to be successful agers. The maximal lifespan of beagles is approximately 18 years.

The brains of aged dogs progressively accumulate oxidative damage to lipids, proteins, DNA and RNA [104–110]. In addition, dogs naturally develop beta-amyloid pathology [111–115], which is a toxic protein implicated in Alzheimer disease [116]. Neuron loss also occurs in the hilus of the hippocampus [117, 118] in aged dogs along with a reduced ability to generate new neurons [119]. Less neurogenesis is correlated with poorer cognitive performance [119]. By magnetic resonance imaging, cortical atrophy and ventricular widening can be observed with age [120] [121] and is linked to both A β and cognition [122].

In parallel to these neuropathological events in the aged canine brain, there is a progressive loss of a subset of cognitive functions [123–129]. Further, not all aged individuals are equally affected; some aged dogs can learn and remember information as well as younger dogs. Other aged dogs can show severe cognitive impairments [124, 130]. Some types of learning and memory are more vulnerable to aging than others. For example, simple visual discrimination learning and procedural learning remains relatively intact with age in dogs [124]. In contrast, visuospatial function [129] and complex learning ability [131] are compromised with age. In a sensitive measure of spatial learning and memory, subtle age impairments can be detected as young as 6 years of age [129].

In canines, although A β is primarily deposited as diffuse plaques in the canine brain, there is an association between the extent of A β and cognitive decline [132–136]. Oxidative damage may also be associated with behavioral decline. Rofina and collaborators found that increased oxidative end products in aged canine brain [106, 108, 134] correlates with severity of behavior changes due to cognitive dysfunction. For example a significant correlation was found between behavior changes and several measures of oxidative damage [106]. Does oxidative damage lead to A β accumulation and, either in combination with A β or independently of A β , cause cognitive decline? We tested this hypothesis in a longitudinal study of aging in old beagles that we provided with a diet rich in a broad spectrum of antioxidants. In addition, we tested the hypothesis that combining an antioxidant diet with behavioral enrichment, to stimulate neurogenesis and neuronal growth factor release, would provide added benefits.

A Longitudinal Study of Antioxidants in Aged Dogs

We completed a longitudinal investigation of the effects of dietary antioxidant intervention on cognitive function of aged beagle dogs. The experimental subjects were a group of 48 aged beagles (10 to 13 years of age) and 17 young dogs (3 to 5 years old). Each animal was assigned into one of two food groups using a counter balanced design based on extensive baseline cognitive testing. No differences existed between cognitive ability of groups prior to dietary intervention [137]. The two foods were formulated to meet the nutrient profile for the American Association of Feed Control Officials recommendations for adult dogs

(AAFCO 1999). Control and test diets were identical in composition, other than inclusion of a broad-based antioxidant and mitochondrial cofactor supplementation to the test diet. The control and enriched foods had the following differences in formulation on an as fed basis respectively: dl-alpha-tocopherol acetate, (120 ppm vs 1050 ppm), l-carnitine (< 20 ppm vs 260 ppm), dl-alpha-lipoic acid (<20 ppm vs 128 ppm), ascorbic acid as Stay-C (< 30 ppm vs 80 ppm), and 1% inclusions of each of the following (1 to 1 exchange for corn): spinach flakes, tomato pomace, grape pomace, carrot granules and citrus pulp. An additional treatment was also included in the study, behavioral enrichment. The behavioral enrichment protocol consisted of housing animals in pairs (social enrichment), providing two 20 minute outdoor walks per week (physical exercise) and continuous cognitive testing (cognitive enrichment). The cognitive enrichment consisted of a landmark discrimination task [138], an oddity discrimination task [131], and a size discrimination learning and reversal task [133, 139]. Each of these tests measured distinct cognitive domains to minimize practice effects and served as a behavioral enrichment test battery (i.e. control animals did not receive this training). On an annual basis, a second battery of tests was used and all animals were given cognitive testing on measures of discrimination learning, reversal and spatial memory, which were different tasks than those used for the behavioral enrichment treatment. To minimize practice effects we used different stimulus objects for each successive discrimination and reversal problems and in addition, we selected objects that were more difficult for animals to distinguish. This prevented possible floor effects. For the spatial memory task, identical testing procedures were used each year and we predicted that all animals may improve with repeated exposure but that treated animals would benefit more.

After one month of treatment, we tested animals for spatial attention ability using a landmark discrimination task [123]. We observed rapid improvements in function on this measure of learning [138]. Approximately six months after starting the dietary intervention, the dogs were tested on a series of oddity discrimination learning tasks [131]. We observed both age and diet effects with old animals performing significantly worse than young dogs on all phases of the test. With respect to diet, old dogs fed the test food made significantly fewer errors on the more difficult tasks than old controls. Additional improvements in cognition in response to the antioxidant-enriched diet were observed on repeated measures of visual discrimination and reversal learning [137]. Spatial memory was also improved in aged dogs provided with the antioxidant enriched diet after long term treatment (>2.0 years) [140]. Interestingly, young dogs fed the antioxidant diet showed no cognitive improvements on the oddity task as well as other learning problems [137, 141] suggesting the antioxidant diet selectively corrected an oxidative defect present in the aged brain. In all of the cognitive outcome measures we also observed that the antioxidant diet effects were further enhanced in animals that also received behavioral enrichment [137].

Our next experiments were intended to identify the neurobiological mechanisms underlying improved cognition in response to the antioxidant diet. In the brain, we observed decreased protein oxidation and increased endogenous antioxidant enzyme activity, but interestingly, no changes in the extent of lipid peroxidation [109]. These effects were more robust in animals receiving both the antioxidant diet and behavioral enrichment. The extent of diffuse A β in the brains of treated animals was also reduced [142]. However, in analyzing which brain regions were affected by antioxidant treatment we found that the prefrontal cortex

remained unaffected where as more posterior and ventral cortical regions (parietal cortex and entorhinal cortex) showed less A β . Based upon previous work [112, 113] we have learned that A β deposition occurs at different ages in distinct cortical regions in the canine brain. The prefrontal cortex shows A β accumulation between 8 and 9 years of age whereas parietal and entorhinal cortex accumulates A β at later ages (>10 years). Thus, given the age at which the treatment was started in the study animals, the antioxidant treatment was not able to reduce pre-existing A β (prefrontal cortex) but prevented further A β accumulation in the parietal and entorhinal cortex. Antioxidant benefits on the production of A β and deposition into diffuse plaques in the canine study is consistent with a previous report of increased alpha-secretase activity in transgenic mice overexpressing mutant human beta-amyloid precursor protein and thus lowering the production of A β in response to treatment with the antioxidant, green tea epigallocatechin-3-gallate[143].

The mechanisms by which individual components in the diet may contribute to improved neuron function may be complex and involve multiple molecular cascades [109]. For example, primary cortical neurons pretreated with both acetyl-L-carnitine and lipoic acid prior to exposure to oxidative stress, show increased endogenous antioxidant levels [144], which was similar to observations in treated dogs [109]. Importantly, the PI3K, PKG and ERK1/2 cell survival pathways are activated in response to acetyl-L-carnitine and lipoic acid treatment [144]. Acetyl-L-carnitine alone can up-regulate heat shock proteins and protect oxidative damage in cortical neurons exposed to A β [145]. However, the antioxidant enriched diet may have many other beneficial effects including possible anti-inflammatory properties or other direct effects on brain function that have yet to be identified.

In contrast, we have found that behavioral enrichment does not modify A β levels but may improve or enhance the effects of the antioxidant diet by maintaining neuron number in the hippocampus, a region critically involved with learning and memory [117]. The results of these studies strongly suggest that antioxidants and behavioral enrichment improve brain function through independent molecular pathways. Thus, the use of antioxidant supplements in addition to other lifestyle modifications (increased social activity and cognitive engagement) may work additively.

Summary

In humans, oxidative damage has been observed with age in the brain and epidemiology studies also suggest that antioxidant use is beneficial for cognitive function. Further, in the age-associated neurodegenerative disorder, Alzheimer disease (AD), more extensive oxidative damage has been reported relative to normal aging and this may be due to the accumulation of additional A β neuropathology. Targeting mitochondrial function, specifically, may provide benefits to patients with AD by slowing disease progression. Overall, recent reviews of the human epidemiological literature have emphasized that antioxidants may be one of the most promising treatments for preventing AD [146]. Thus, continuing on the theme of recent work of Dr. Akitane Mori's exploring the link between aging, oxidative damage and neurodegeneration, researchers have been making significant strides to find ways in which to promote successful brain aging. Given recent studies by Dr.

Mori describing several natural compounds that may provide brain aging benefits [147], this is an area that will continue to evolve over time.

Acknowledgments

The canine study was supported by funding from the NIH/NIA AG12694.

References

1. Poon HF, Calabrese V, Scapagnini G, Butterfield DA. Free radicals and brain aging. *Clin Geriatr Med*. 2004; 20:329–359. [PubMed: 15182885]
2. Liu J, Mori A. Stress, aging, and brain oxidative damage. *Neurochem Res*. 1999; 24:1479–1497. [PubMed: 10555789]
3. Ames BN, Shigenaga MK. Oxidants are a major contributor to aging. *Annals of the New York Academy of Sciences*. 1992; 663:85–96. [PubMed: 1482105]
4. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA*. 1993; 90:7915–7922. [PubMed: 8367443]
5. Mori A, Utsumi K, Liu J, Hosokawa M. Oxidative damage in the senescence-accelerated mouse. *Ann N Y Acad Sci*. 1998; 854:239–250. [PubMed: 9928434]
6. Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: implications for neuroimaging. *Trends Neurosci*. 2004; 27:489–495. [PubMed: 15271497]
7. Halliwell B, Gutteridge JMC. Oxygen radicals in the nervous system. *Trends in Neuroscience*. 1985; 8:22–26.
8. Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol Aging*. 2002; 23:795–807. [PubMed: 12392783]
9. Perez-Campo R, Lopez-Torres M, Cadenas S, Rojas C, Barja G. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J Comp Physiol [B]*. 1998; 168:149–158.
10. Liu J, Atamna H, Kuratsune H, Ames BN. Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites. *Ann N Y Acad Sci*. 2002; 959:133–166. [PubMed: 11976193]
11. Nakahara H, Kanno T, Inai Y, et al. Mitochondrial dysfunction in the senescence accelerated mouse (SAM). *Free Radic Biol Med*. 1998; 24:85–92. [PubMed: 9436617]
12. Mecocci P, MacGarvey U, et al. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Annals Neurology*. 1994; 36:747–751.
13. Wei YH. Oxidative stress and mitochondrial DNA mutations in human aging. *Proceedings of the Society for Experimental Biology and Medicine*. 1998; 217:53–63. [PubMed: 9421207]
14. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci USA*. 1994; 91:10771–10778. [PubMed: 7971961]
15. Hirai K, Aliev G, Nunomura A, et al. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci*. 2001; 21:3017–3023. [PubMed: 11312286]
16. Miquel J, Economos AC, Fleming J, Johnson JE Jr. Mitochondrial role in cell aging. *Experimental Gerontology*. 1980; 15:575–591. [PubMed: 7009178]
17. Wallace DC. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science*. 1992; 256:628–632. [PubMed: 1533953]
18. Haripriya D, Devi MA, Kokilavani V, Sangeetha P, Panneerselvam C. Age-dependent alterations in mitochondrial enzymes in cortex, striatum and hippocampus of rat brain -- potential role of L-Carnitine. *Biogerontology*. 2004; 5:355–364. [PubMed: 15547323]
19. Crouch PJ, Cimdins K, Duce JA, Bush AI, Trounce IA. Mitochondria in aging and Alzheimer's disease. *Rejuvenation Res*. 2007; 10:349–357. [PubMed: 17708691]
20. Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc Natl Acad Sci U S A*. 1996; 93:4765–4769. [PubMed: 8643477]

21. Liu J. The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: an overview. *Neurochem Res.* 2008; 33:194–203. [PubMed: 17605107]
22. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998; 78:547–581. [PubMed: 9562038]
23. Mecocci P, MacGarvey U, Kaufman AE, et al. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann Neurol.* 1993; 34:609–616. [PubMed: 8215249]
24. Smith CD, Carney JM, Starke-Reed PE, et al. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci U S A.* 1991; 88:10540–10543. [PubMed: 1683703]
25. Montine TJ, Neely MD, Quinn JF, Beal MF, Markesbery WR, Roberts LJ, Morrow JD. Lipid peroxidation in aging brain and Alzheimer's disease. *Free Radical Biology & Medicine.* 2002; 33:620–626. [PubMed: 12208348]
26. Stadtman ER. Protein oxidation and aging. *Science.* 1992; 257:1220–1224. [PubMed: 1355616]
27. Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. *Chem Res Toxicol.* 1997; 10:485–494. [PubMed: 9168245]
28. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. *JBC.* 1997; 272:20313–20316.
29. Cini M, Moretti A. Studies on lipid peroxidation and protein oxidation in the aging brain. *Neurobiology of Aging.* 1995; 16:53–57. [PubMed: 7723936]
30. Markesbery WR, Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging.* 1998; 19:33–36. [PubMed: 9562500]
31. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med.* 1997; 23:134–147. [PubMed: 9165306]
32. Pratico D, Delanty N. Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease. *Am J Med.* 2000; 109:577–585. [PubMed: 11063960]
33. Behl C. Alzheimer's disease and oxidative stress: Implications for novel therapeutic approaches. *Progress in Neurobiology.* 1999; 57:301–323. [PubMed: 10096843]
34. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology.* 1991; 41:479–486. [PubMed: 2011243]
35. Selkoe DJ. Normal and abnormal biology of the beta-amyloid precursor protein. *Annu Rev Neurosci.* 1994; 17:489–517. [PubMed: 8210185]
36. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science.* 1992; 256:184–185. [PubMed: 1566067]
37. Butterfield DA, Kanski J. Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. *Mech Ageing Dev.* 2001; 122:945–962. [PubMed: 11348660]
38. Smith MA, Richey Harris PL, Sayre LM, Beckman JS, Perry G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J Neurosci.* 1997; 17:2653–2657. [PubMed: 9092586]
39. Su JH, Deng G, Cotman CW. Neuronal DNA damage precedes tangle formation and is associated with up-regulation of nitrotyrosine in Alzheimer's Disease brain. *Brain Research.* 1997; 774:193–199. [PubMed: 9452208]
40. Hensley K, Hall N, Subramaniam R, et al. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J Neurochem.* 1995; 65:2146–2156. [PubMed: 7595501]
41. Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B. An assessment of oxidative damage to proteins, lipids and DNA in brain from patients with Alzheimer's disease. *Journal of Neurochemistry.* 1997; 68:2061–2069. [PubMed: 9109533]
42. Aksenov MY, Aksenova MV, Butterfield DA, Geddes JW, Markesbery WR. Protein oxidation in the brain in Alzheimer's disease. *Neuroscience.* 2001; 103:373–383. [PubMed: 11246152]

43. Castegna A, Aksenov M, Aksenova M, et al. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic Biol Med.* 2002; 33:562–571. [PubMed: 12160938]
44. Castegna A, Aksenov M, Thongboonkerd V, et al. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J Neurochem.* 2002; 82:1524–1532. [PubMed: 12354300]
45. Palmer AM, Burns MA. Selective increase in lipid peroxidation in the inferior temporal cortex in Alzheimer's disease. *Brain Research.* 1994; 645:338–342. [PubMed: 8062096]
46. Sayre LM, Zelasko DA, Harris PLR, Perry G, Salomon RG, Smith MA. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. *Journal of Neurochemistry.* 1997; 68:2092–2097. [PubMed: 9109537]
47. Pratico D, Lee MY, Trojanowski JQ, Rokach J, Fitzgerald GA. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *Faseb Journal.* 1998; 12:1777–1783. [PubMed: 9837868]
48. Pratico D, Clark CM, Lee VM, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann Neurol.* 2000; 48:809–812. [PubMed: 11079549]
49. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. *Nucleic Acids Res.* 2007; 35:7497–7504. [PubMed: 17947327]
50. Lovell MA, Gabbita SP, Markesbery WR. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. *Journal of Neurochemistry.* 1999; 72:771–776. [PubMed: 9930752]
51. Gabbita SP, Lovell MA, Markesbery WR. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *Journal of Neurochemistry.* 1998; 71:2034–2040. [PubMed: 9798928]
52. Lovell MA, Markesbery WR. Oxidatively modified RNA in mild cognitive impairment. *Neurobiol Dis.* 2008; 29:169–175. [PubMed: 17920285]
53. Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, Smith MA. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *The Journal of Neuroscience.* 1999; 19:1959–1964. [PubMed: 10066249]
54. Pappolla MA, Omar RA, Kim KS, Robakis NK. Immunohistochemical evidence of oxidative. [corrected] stress in Alzheimer's disease. *Am J Pathol.* 1992; 140:621–628. [PubMed: 1372157]
55. De Deyn PP, Hiramatsu M, Borggreve F, et al. Superoxide dismutase activity in cerebrospinal fluid of patients with dementia and some other neurological disorders. *Alzheimer Dis Assoc Disord.* 1998; 12:26–32. [PubMed: 9539407]
56. Butterfield DA. Proteomics: a new approach to investigate oxidative stress in Alzheimer's disease brain. *Brain Res.* 2004; 1000:1–7. [PubMed: 15053946]
57. Polidori MC, Griffiths HR, Mariani E, Mecocci P. Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease. *Amino Acids.* 2007; 32:553–559. [PubMed: 17273806]
58. Cassarino DS, Bennett JP Jr. An evaluation of the role of mitochondria in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. *Brain Res Brain Res Rev.* 1999; 29:1–25. [PubMed: 9974149]
59. Ojaimi J, Masters CL, Opeskin K, McKelvie P, Byrne E. Mitochondrial respiratory chain activity in the human brain as a function of age. *Mech Ageing Dev.* 1999; 111:39–47. [PubMed: 10576606]
60. Yan L-J, Levine RL, Sohal RS. Oxidative damage during aging targets mitochondrial aconitase. *Proc Natl Acad Sci, USA.* 1997; 94:11168–11172. [PubMed: 9326580]
61. Michikawa Y, Mazzucchelli F, Bresolin N, Scarlato G, Attardi G. Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science.* 1999; 286:774–779. [PubMed: 10531063]
62. Gibson GE, Sheu KF, Blass JP. Abnormalities of mitochondrial enzymes in Alzheimer disease. *J Neural Transm.* 1998; 105:855–870. [PubMed: 9869323]

63. Bosetti F, Brizzi F, Barogi S, et al. Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease. *Neurobiol Aging*. 2002; 23:371–376. [PubMed: 11959398]
64. Coskun PE, Beal MF, Wallace DC. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc Natl Acad Sci U S A*. 2004; 101:10726–10731. [PubMed: 15247418]
65. Valla J, Berndt JD, Gonzalez-Lima F. Energy hypometabolism in posterior cingulate cortex of Alzheimer's patients: superficial laminar cytochrome oxidase associated with disease duration. *J Neurosci*. 2001; 21:4923–4930. [PubMed: 11425920]
66. Liang WS, Reiman EM, Valla J, et al. Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. *Proc Natl Acad Sci U S A*. 2008; 105:4441–4446. [PubMed: 18332434]
67. Reddy PH. Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. *J Neurochem*. 2006; 96:1–13. [PubMed: 16305625]
68. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radic Biol Med*. 2007; 43:658–677. [PubMed: 17664130]
69. Pratico D, Uryu K, Leight S, Trojanowski JQ, Lee VM. Increased Lipid Peroxidation Precedes Amyloid Plaque Formation in an Animal Model of Alzheimer Amyloidosis. *J Neurosci*. 2001; 21:4183–4187. [PubMed: 11404403]
70. Frederikse PH, Garland D, Zigler JS Jr, Piatigorsky J. Oxidative stress increases production of beta-amyloid precursor protein and beta-amyloid (A β) in mammalian lenses, and A β has toxic effects on lens epithelial cells. *JBC*. 1996; 271:10169–10174.
71. Tamagno E, Bardini P, Obbili A, et al. Oxidative stress increases expression and activity of BACE in NT2 neurons. *Neurobiol Dis*. 2002; 10:279–288. [PubMed: 12270690]
72. Butterfield DA. beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Chem Res Toxicol*. 1997; 10:495–506. [PubMed: 9168246]
73. McLellan ME, Kajdasz ST, Hyman BT, Bacskai BJ. In vivo imaging of reactive oxygen species specifically associated with thioflavine S-positive amyloid plaques by multiphoton microscopy. *J Neurosci*. 2003; 23:2212–2217. [PubMed: 12657680]
74. Hensley K, Carney JM, Mattson MP, et al. A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. *Proc Natl Acad Sci U S A*. 1994; 91:3270–3274. [PubMed: 8159737]
75. Kim HS, Lee JH, Lee JP, et al. Amyloid beta peptide induces cytochrome C release from isolated mitochondria. *Neuroreport*. 2002; 13:1989–1993. [PubMed: 12395106]
76. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J Neurochem*. 2002; 80:91–100. [PubMed: 11796747]
77. Lustbader JW, Cirilli M, Lin C, et al. ABAD directly links A β to mitochondrial toxicity in Alzheimer's disease. *Science*. 2004; 304:448–452. [PubMed: 15087549]
78. Hansson CA, Frykman S, Farmery MR, et al. Nicastrin, presenilin, APH-1, and PEN-2 form active gamma-secretase complexes in mitochondria. *J Biol Chem*. 2004; 279:51654–51660. [PubMed: 15456764]
79. Yan SD, Yan SF, Chen X, et al. Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid beta-peptide. *Nat Med*. 1995; 1:693–699. [PubMed: 7585153]
80. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*. 2002; 287:3223–3229. [PubMed: 12076218]
81. Morris MC, Evans DA, Bienias JL, Tangney CC, Wilson RS. Vitamin E and cognitive decline in older persons. *Arch Neurol*. 2002; 59:1125–1132. [PubMed: 12117360]

82. Maxwell CJ, Hicks MS, Hogan DB, Basran J, Ebly EM. Supplemental use of antioxidant vitamins and subsequent risk of cognitive decline and dementia. *Dement Geriatr Cogn Disord*. 2005; 20:45–51. [PubMed: 15832036]
83. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol*. 2003; 60:203–208. [PubMed: 12580704]
84. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, Havlik R, White LR. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology*. 2000; 54:1265–1272. [PubMed: 10746596]
85. Kang JH, Cook N, Manson J, Buring JE, Grodstein F. A randomized trial of vitamin E supplementation and cognitive function in women. *Arch Intern Med*. 2006; 166:2462–2468. [PubMed: 17159011]
86. Fillenbaum GG, Kuchibhatla MN, Hanlon JT, et al. Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. *Ann Pharmacother*. 2005; 39:2009–2014. [PubMed: 16227448]
87. Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, Norton C, Welsh-Bohmer KA, Breitner JC. CCS Group. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. *Arch Neurol*. 2004; 61:82–88. [PubMed: 14732624]
88. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. *Neurology*. 2007; 69:1921–1930. [PubMed: 17998483]
89. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. *The New England Journal of Medicine*. 1997; 336:1216–1222. [PubMed: 9110909]
90. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med*. 2005; 352:2379–2388. [PubMed: 15829527]
91. Chandra RK. Effect of vitamin and trace-element supplementation on cognitive function in elderly subjects. *Nutrition*. 2001; 17:709–712. [PubMed: 11527656]
92. Rai G, Wright G, Scott L, Beston B, Rest J, Exton-Smith AN. Double-blind, placebo controlled study of acetyl-L-carnitine in patients with Alzheimer's dementia. *Curr Med Res Opin*. 1990; 11:638–647. [PubMed: 2178869]
93. Pettegrew JW, Klunk WE, Panchalingam K, Kanfer JN, McClure RJ. Clinical and neurochemical effects of acetyl-L-carnitine in Alzheimer's disease. *Neurobiol Aging*. 1995; 16:1–4. [PubMed: 7723928]
94. Spagnoli A, Lucca U, Menasce G, et al. Long-term acetyl-L-carnitine treatment in Alzheimer's disease. *Neurology*. 1991; 41:1726–1732. [PubMed: 1944900]
95. Bonavita E. Study of the efficacy and tolerability of L-acetylcarnitine therapy in the senile brain. *Int J Clin Pharm, Ther & Toxicol*. 1986; 24:511–516.
96. Thal LJ, Calvani M, Amato A, Carta A. A 1-year controlled trial of acetyl-L-carnitine in early-onset AD. *Neurology*. 2000; 55:805–810. [PubMed: 10994000]
97. Brooks JO 3rd, Yesavage JA, Carta A, Bravi D. Acetyl L-carnitine slows decline in younger patients with Alzheimer's disease: a reanalysis of a double-blind, placebo-controlled study using the trilinear approach. *Int Psychogeriatr*. 1998; 10:193–203. [PubMed: 9677506]
98. Thal LJ, Carta A, Clarke WR, et al. A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer's disease. *Neurology*. 1996; 47:705–711. [PubMed: 8797468]
99. Montgomery SA, Thal LJ, Amrein R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease. *Int Clin Psychopharmacol*. 2003; 18:61–71. [PubMed: 12598816]
100. Bianchetti A, Rozzini R, Trabucchi M. Effects of acetyl-L-carnitine in Alzheimer's disease patients unresponsive to acetylcholinesterase inhibitors. *Curr Med Res Opin*. 2003; 19:350–353. [PubMed: 12841930]
101. Hager K, Marahrens A, Kenkies M, Riederer P, Munch G. Alpha-lipoic acid as a new treatment option for Alzheimer type dementia. *Arch Gerontol Geriatr*. 2001; 32:275–282. [PubMed: 11395173]

102. Hager K, Kenklies M, McAfoose J, Engel J, Munch G. Alpha-lipoic acid as a new treatment option for Alzheimer's disease--a 48 months follow-up analysis. *J Neural Transm.* 2007; (Suppl): 189–193.
103. Quinn JF, Bussiere JR, Hammond RS, et al. Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice. *Neurobiol Aging.* 2007; 28:213–225. [PubMed: 16448723]
104. Head E, Liu J, Hagen TM, et al. Oxidative Damage Increases with Age in a Canine Model of Human Brain Aging. *Journal of Neurochemistry.* 2002; 82:375–381. [PubMed: 12124438]
105. Kiatipattanasakul W, Nakamura S, Kuroki K, Nakayama H, Doi K. Immunohistochemical detection of anti-oxidative stress enzymes in the dog brain. *Neuropathology.* 1997; 17:307–312.
106. Skoumalova A, Rofina J, Schwippelova Z, Gruys E, Wilhelm J. The role of free radicals in canine counterpart of senile dementia of the Alzheimer type. *Exp Gerontol.* 2003; 38:711–719. [PubMed: 12814808]
107. Papaioannou N, Tooten PCJ, van Ederen AM, Bohl JRE, Rofina J, Tsangaris T, Gruys E. Immunohistochemical investigation of the brain of aged dogs. I. Detection of neurofibrillary tangles and of 4-hydroxynonenal protein, an oxidative damage product, in senile plaques. *Amyloid: J Protein Folding Disord.* 2001; 8:11–21.
108. Rofina JE, Singh K, Skoumalova-Vesela A, et al. Histochemical accumulation of oxidative damage products is associated with Alzheimer-like pathology in the canine. *Amyloid.* 2004; 11:90–100. [PubMed: 15478464]
109. Opii W, Joshi G, Head E, et al. Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: Relevance to Alzheimer's disease. *Neurobiol Aging Epub.* 2006
110. Hwang IK, Yoon YS, Yoo KY, et al. Differences in Lipid Peroxidation and Cu,Zn-Superoxide Dismutase in the Hippocampal CA1 Region Between Adult and Aged Dogs. *J Vet Med Sci.* 2008; 70:273–277. [PubMed: 18388427]
111. Cummings BJ, Su JH, Cotman CW, White R, Russell MJ. Beta-amyloid accumulation in aged canine brain: a model of plaque formation in Alzheimer's disease. *Neurobiology of Aging.* 1993; 14:547–560. [PubMed: 8295657]
112. Head E, McCleary R, Hahn FF, Milgram NW, Cotman CW. Region-specific age at onset of beta-amyloid in dogs. *Neurobiol Aging.* 2000; 21:89–96. [PubMed: 10794853]
113. Hou Y, White RG, Bobik M, Marks JS, Russell MJ. Distribution of beta-amyloid in the canine brain. *NeuroReport.* 1997; 8:1009–1012. [PubMed: 9141082]
114. Wisniewski HM, Johnson AB, Raine CS, Kay WJ, Terry RD. Senile plaques and cerebral amyloidosis in aged dogs. *Laboratory Investigations.* 1970; 23:287–296.
115. Wisniewski, HM.; Wegiel, J.; Morys, J.; Bancher, C.; Soltysiak, Z.; Kim, KS. Aged dogs: an animal model to study beta-protein amyloidogenesis. In: Maurer, KPR.; Beckman, H., editors. *Alzheimer's disease Epidemiology, Neuropathology, Neurochemistry and Clinics.* Springer-Verlag; New York: 1990. p. 151-167.
116. Selkoe DJ, Schenk D. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol.* 2003; 43:545–584. [PubMed: 12415125]
117. Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW. Region specific neuron loss in the aged canine hippocampus is reduced by enrichment. *Neurobiol Aging.* 2008; 29:521–528.
118. Hwang IK, Lee CH, Li H, et al. Comparison of Ionized Calcium-binding Adapter Molecule 1 Immunoreactivity of the Hippocampal Dentate Gyrus and CA1 Region in Adult and Aged Dogs. *Neurochem Res.* 2008
119. Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW. Neurogenesis decreases with age in the canine hippocampus and correlates with cognitive function. *Neurobiol Learn Mem.* 2007; 88:249–259. [PubMed: 17587610]
120. Su M-Y, Head E, Brooks WM, Wang Z, Muggenberg BA, Adam GE, Sutherland RJ, Cotman CW, Nalcioğlu O. MR Imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiology of Aging.* 1998; 19:479–485. [PubMed: 9880050]

121. Kimotsuki T, Nagaoka T, Yasuda M, Tamahara S, Matsuki N, Ono K. Changes of magnetic resonance imaging on the brain in beagle dogs with aging. *J Vet Med Sci.* 2005; 67:961–967. [PubMed: 16276050]
122. Tapp PD, Siwak CT, Gao FQ, et al. Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J Neurosci.* 2004; 24:8205–8213. [PubMed: 15385603]
123. Milgram NW, Adams B, Callahan H, Head E, Mackay W, Thirlwell C, Cotman CW. Landmark discrimination learning in the dog. *Learning & Memory.* 1999; 6:54–61. [PubMed: 10355524]
124. Milgram NW, Head E, Weiner E, Thomas E. Cognitive functions and aging in the dog: Acquisition of nonspatial visual tasks. *Behav Neurosci.* 1994; 108:57–68. [PubMed: 8192851]
125. Chan AD, Nippak PM, Murphey H, et al. Visuospatial impairments in aged canines (*Canis familiaris*): the role of cognitive-behavioral flexibility. *Behav Neurosci.* 2002; 116:443–454. [PubMed: 12049325]
126. Head E, Mehta R, Hartley J, et al. Spatial learning and memory as a function of age in the dog. *Behav Neurosci.* 1995; 109:851–858. [PubMed: 8554710]
127. Tapp PD, Siwak CT, Estrada J, Holowachuk D, Milgram NW. Effects of age on measures of complex working memory span in the beagle dog (*Canis familiaris*) using two versions of a spatial list learning paradigm. *Learn Mem.* 2003; 10:148–160. [PubMed: 12663753]
128. Tapp PD, Siwak CT, Estrada J, et al. Size and Reversal Learning in the Beagle Dog as a Measure of Executive Function and Inhibitory Control in Aging. *Learning and Memory.* 2003; 10:64–73. [PubMed: 12551965]
129. Studzinski CM, Christie LA, Araujo JA, et al. Visuospatial function in the beagle dog: an early marker of cognitive decline in a model of human aging and dementia. *Neurobiol Learn Mem.* 2006; 86:197–204. [PubMed: 16616528]
130. Head, E.; Milgram, NW.; Cotman, CW. Neurobiological Models of Aging in the Dog and Other Vertebrate Species. In: Hof, P.; Mobbs, C., editors. *Functional Neurobiology of Aging.* Academic Press; San Diego: 2001. p. 457–468.
131. Cotman CW, Head E, Muggenburg BA, Zicker S, Milgram NW. Brain Aging in the Canine: A Diet Enriched in Antioxidants Reduces Cognitive Dysfunction. *Neurobiology of Aging.* 2002; 23:809–818. [PubMed: 12392784]
132. Cummings BJ, Head E, Ruehl WW, Milgram NW, Cotman CW. Beta-amyloid accumulation correlates with cognitive dysfunction in the aged canine. *Neurobiology of Learning & Memory.* 1996; 66:11–23. [PubMed: 8661247]
133. Head E, Callahan H, Muggenburg BA, Cotman CW, Milgram NW. Visual-discrimination learning ability and beta-amyloid accumulation in the dog. *Neurobiol Aging.* 1998; 19:415–425. [PubMed: 9880044]
134. Rofina JE, van Ederen AM, Toussaint MJ, et al. Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease. *Brain Res.* 2006; 1069:216–226. [PubMed: 16423332]
135. Pugliese M, Geloso MC, Carrasco JL, Mascort J, Michetti F, Mahy N. Canine cognitive deficit correlates with diffuse plaque maturation and S100beta (-) astrocytosis but not with insulin cerebrospinal fluid level. *Acta Neuropathol.* 2006; 111:519–528. [PubMed: 16718348]
136. Colle M-A, Hauw J-J, Crespeau F, Uchiara T, Akiyama H, Checler F, Pageat P, Duykaerts C. Vascular and parenchymal A β deposition in the aging dog: correlation with behavior. *Neurobiology of Aging.* 2000; 21:695–704. [PubMed: 11016539]
137. Milgram NW, Head E, Zicker SC, Ikeda-Douglas CJ, Murphey H, Muggenburg B, Siwak C, Tapp D, Cotman CW. Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: A two-year longitudinal study. *Neurobiol Aging.* 2005; 26:77–90. [PubMed: 15585348]
138. Milgram NW, Head E, Muggenburg BA, et al. Landmark discrimination learning in the dog: effects of age, an antioxidant fortified diet, and cognitive strategy. *Neuroscience and Biobehavioral Reviews.* 2002; 26:679–695. [PubMed: 12479842]
139. Milgram NW, Head E, Zicker SC, et al. Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: a two-year longitudinal study. *Neurobiol Aging.* 2005; 26:77–90. [PubMed: 15585348]

140. Nippak PM, Mendelson J, Muggenburg B, Milgram NW. Enhanced spatial ability in aged dogs following dietary and behavioural enrichment. *Neurobiol Learn Mem.* 2007; 87:610–623. [PubMed: 17303448]
141. Siwak CT, Tapp PD, Head E, et al. Chronic antioxidant and mitochondrial cofactor administration improves discrimination learning in aged but not young dogs. *Prog Neuropsychopharmacol Biol Psychiatry.* 2005; 29:461–469. [PubMed: 15795055]
142. Pop, V.; Head, E.; Nistor, M.; Milgram, NW.; Muggenburg, BA.; Cotman, CW. Reduced A β deposition with long-term antioxidant diet treatment in aged canines. Society for Neuroscience, Abstract Viewer/Itinerary Planner; Washington, DC: 2003.
143. Rezai-Zadeh K, Shytle D, Sun N, et al. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J Neurosci.* 2005; 25:8807–8814. [PubMed: 16177050]
144. Abdul HM, Butterfield DA. Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Free Radic Biol Med.* 2007; 42:371–384. [PubMed: 17210450]
145. Abdul HM, Calabrese V, Calvani M, Butterfield DA. Acetyl-L-carnitine-induced up-regulation of heat shock proteins protects cortical neurons against amyloid-beta peptide 1–42-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease. *J Neurosci Res.* 2006; 84:398–408. [PubMed: 16634066]
146. Rutten BP, Steinbusch HW, Korr H, Schmitz C. Antioxidants and Alzheimer's disease: from bench to bedside (and back again). *Curr Opin Clin Nutr Metab Care.* 2002; 5:645–651. [PubMed: 12394639]
147. Noda Y, Mori A. Antioxidant activities of uyaku (*Lindera strychnifolia*) leaf extract: a natural extract used in traditional medicine. *J Clin Biochem Nutr.* 2007; 41:139–145. [PubMed: 18193108]