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Role of Walnuts in Maintaining Brain Health with Age[1–3]

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Abstract

Because of the combination of population growth and population aging, increases in the incidence of chronic neurodegenerative disorders have become a societal concern, both in terms of decreased quality of life and increased financial burden. Clinical manifestation of many of these disorders takes years, with the initiation of mild cognitive symptoms leading to behavioral problems, dementia and loss of motor functions, the need for assisted living, and eventual death. Lifestyle factors greatly affect the progression of cognitive decline, with high-risk behaviors including unhealthy diet, lack of exercise, smoking, and exposure to environmental toxins leading to enhanced oxidative stress and inflammation. Although there exists an urgent need to develop effective treatments for age-related cognitive decline and neurodegenerative disease, prevention strategies have been underdeveloped. Primary prevention in many of these neurodegenerative diseases could be achieved earlier in life by consuming a healthy diet, rich in antioxidant and anti-inflammatory phytochemicals, which offers one of the most effective and least expensive ways to address the crisis. English walnuts (*Juglans regia* L.) are rich in numerous phytochemicals, including high amounts of polyunsaturated fatty acids, and offer potential benefits to brain health. Polyphenolic compounds found in walnuts not only reduce the oxidant and inflammatory load on brain cells but also improve interneuronal signaling, increase neurogenesis, and enhance sequestration of insoluble toxic protein aggregates. Evidence for the beneficial effects of consuming a walnut-rich diet is reviewed in this article. J. Nutr. 144: 561S–566S, 2014.

Introduction

In many countries, growing populations and increased mean life spans are leading to a large aging population. With this demographic shift comes an increased incidence of neurodegenerative diseases. Worldwide, >35.6 million people are living with dementia, and in the United States, nearly 7 million people have been diagnosed with dementia and other neurologic disorders (1). Neurodegenerative diseases are highly debilitating, severely affecting quality of life and imposing an economic burden on both individuals and society. Cures for most neurodegenerative disorders have yet to be discovered, in part due to irreversible loss of brain cells during the covert pathogenesis of these debilitating diseases, which often take 10–15 y to manifest clinically, and the poorly understood familial-genetic linkage (2).

Neurodegenerative diseases are associated with chronic exposure to oxidative stress and inflammation, loss of protective signaling, and the accumulation of toxic proteins. During aging, these factors trigger a cascade of altered molecular events, which ultimately disrupt or destroy cells within the brain. Damage to individual cells modifies interneuronal communications, causing deficits in memory, cognition, and motor function. These cellular modifications ultimately lead to the pathogenesis of

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neurodegenerative diseases, such as Alzheimer disease (AD),[4] Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, prion disease, and dementia (2,3). Although the central nervous system is particularly vulnerable, the mutually perpetuating effects of oxidative stress and inflammation also affect other organ systems, increasing older adults' risk of developing other diseases such as heart disease, cancer, arthritis, diabetes, and other age-related disorders. Therefore, systemic protection from oxidative stress and inflammation could not only protect the brain from their direct effects but also from a variety of related pathologies.

Dietary interventions may be able to prevent or forestall neurodegeneration. Epidemiologic investigation of the Mediterranean diet, which consists of high amounts of fruits, vegetables, cereals, and fish and minimal amounts of alcohol, red meat, and dairy products revealed substantial reductions in the risk of AD in a large community-based, case-control cohort study in 194 AD patients and 1790 non-AD patients (4). A similar investigation reported a decreased risk of dementia with increased flavonoid-rich-diet consumption in a cohort study in 1367 participants > 65 y of age (5). A few other studies also indicated the benefits of the Mediterranean diet in reducing the risk of dementia, as well as mortality, in AD patients, indicating the vital role of fruit and nut bioactive compounds on cognitive health (6,7).

English walnuts (*Juglans regia* L.) are rich in α-linolenic acid (ALA; 18:3n−3) and linoleic acid (LA; 18:2n−6) as well as other polyphenolics, phytosterols, and micronutrients. Feeding studies from our laboratory have shown that dietary supplementation with walnuts can improve memory, cognition, and motor function in aged animals (8–10). Although most of these studies have linked walnuts' effects to their high PUFA content, walnuts' notable polyphenol content plays an important role in reducing the inflammation and oxidative stress in the aging brain. This review addresses contemporary research into the effects of dietary walnuts on cognition, motor function, and brain health.

Aging and Metabolic Effects on Brain Health

The central nervous system is metabolically demanding, consuming nearly one-fourth of total oxygen intake and accounting for >20% of the metabolic rate at rest (11,12). The brain's high metabolic rate results in the generation of disproportionate amounts of reactive oxygen and nitrogen species (13). The damaging effects of these free radicals are usually countered by endogenous oxidoreductase enzymes and intrinsic proteins, which act as molecular quenchers at the cellular level. However, aging, as well as other factors, alters the homeostasis between the generation and quenching of these highly reactive elemental species, leading to increased oxidation at the cellular level. Increased oxidative stress and lipid peroxidation initiate a cascade of proinflammatory signals, leading to the dystrophy and death of brain cells. Altered homeostasis of oxidation, inflammation, and protein aggregation has been attributed to the death of neurons, which is directly related to impairment in various cognitive domains, such as learning, decision making, judgment, problem solving, and memory (14–17).

The coordination and execution of cognitive processes depends on the appropriate detection and propagation of signals from both the environment and surrounding cells in the brain. The responsivity of each cell depends on the composition of the cell membrane, through which all signals must pass. FAs are abundant within neuronal membranes, where they play a role in maintaining structural integrity, modulating enzyme activity, and generating secondary messengers and other signaling molecules (9). The PUFA composition of neuronal membranes decreases during aging and contributes to the decline of neuronal function observed in aging. This alteration is prevalent in the aged brain, particularly the cortex, hippocampus, striatum, and cerebellum, where reduced PUFA concentrations contribute to changes in neuronal morphology and a decrease in membrane fluidity and synaptic plasticity (18–20). A number of neuronal functions are affected by deficits in membrane FA composition, all of which reduce the cells' ability to propagate and transmit signals within the brain. Therefore, increased availability of PUFAs may counteract PUFA depletion in neuronal membranes.

Walnut Phytochemicals and In Vitro Studies

Walnuts are a rich source of nutrients and bioactive phytochemicals. Walnuts contain large amounts of PUFAs such as ALA and LA, which have been shown to boost brain health and function even with an increase in age (8,21). Every 100 g of walnuts (*Juglans regia*) contain 38 g of LA and 9 g of ALA, as well as 4.4 g of saturated (palmitic acid, 16:0) and 8.7 g of monounsaturated (oleic acid, 18:1n–9) FAs. In humans, ALA from walnuts is then converted through a series of sequential desaturation and elongation reactions into essential PUFAs such as EPA (20:5n–3) and DHA (22:6n–3) in the liver. Both EPA and DHA play an important role in brain health not only by reducing oxidative stress and altering the immune function but also in maintaining synaptic plasticity, neuronal membrane stability, gene expression, and neurogenesis (8,22).

Even though PUFAs play an important role in brain health, the presence of other phytochemical components contributes to healthy neuronal processes. Other important nutrients in walnuts include, but are not limited to, polyphenols, vitamin E, folate, ellagitannins, ellagic acid monomers, polymeric tannins, melatonin, pectin, flavonoids, carotenoids, alkaloids, nitrogen-containing or organosulfur compounds, and a variety of minerals. Along with PUFAs, other phytonutrients provide direct neuroprotection (8,9,23–26) as well as indirect protection through improved lipid profiles and endothelial function and increased plasma antioxidant capacity. Polyphenols present in walnuts include, but are not limited to, hydroxycinnamic acids such as chlorigenic acid, caffeic acid, P-coumaric acid, ferrulic acid, and sinapic acid; hydrobenzoic acids such as syringic acid and ellagic acid; and compounds such as gallic acid, glansrin, juglone, and syringaldehyde (27). Polyphenols promote neuronal calcium homeostasis in the striatum and hippocampus, regions of the brain crucial for primary and secondary memory functions (28,29).

Melatonin is another bioactive compound found in walnuts. Endogenous melatonin, which is primarily synthesized by the pineal gland, plays a critical role in regulating circadian rhythms (30). Melatonin deficiency has been linked to degeneration of

[4] Abbreviations used: Aβ, amyloid β; AD, Alzheimer disease; ALA, α-linolenic acid; *APOE4*, apoliproprotein ε4; BDNF, brain-derived neurotrophic factor; CREBP, cAMP response element-binding protein; DA, 6-hydroxy dopamine, LA, linoleic acid; NPD1, neuroprotection D1; TLR-4, Toll-like receptor 4.

cholinergic neurons in the basal forebrain and the deposition of aggregated proteins, such as amyloid β (Aβ) peptides, leading to cognitive impairment and dementia (31). Reiter et al. (30) reported that consumption of walnuts increased blood melatonin concentrations, which correlated with an increase in "total antioxidant capacity" of the serum with "total antioxidant capacity," indicating the ability of the blood to detoxify free radicals.

Walnut extract is neuroprotective against a variety of stressors. Previously, we showed that treating cells with walnut oil protected the cells from increases in inflammation and oxidative stress by inhibiting LPS-induced activation of microglial cells (32). When BV-2 mouse microglial cells were treated with walnut extract prior to LPS stimulation, production of NO and expression of inducible NO synthase were substantially reduced. In the same study, walnut extract also reduced the production of TNF-α, a proinflammatory mediator. We have also shown that calcium buffering in hippocampal cells was substantially altered by LPS and 6-hydroxy dopamine (DA) stressors (33). Walnut extract protected against LPS-induced, but not DA-induced, loss of calcium recovery (34). Another study showed that walnut extract counteracted Aβ-induced oxidative stress and cytotoxicity in PC12 cells of rat adrenal medulla (35). In a recent study from our laboratory, we examined the cellular mechanisms underlying walnuts' protective effects on neuronal health and functioning in aging brain (34). Primary hippocampal neurons were pretreated with walnut extract or with the PUFAs found in walnuts. The cells were then exposed to DA and LPS, and cell death and calcium buffering dysregulation were measured (34). Results indicated that walnut oil extract, ALA, and DHA provided substantial protection against cell death and calcium dysregulation; the effects were pretreatment concentration-dependent and stressor-dependent. Conversely, LA and EPA were not as effective at protecting hippocampal cells from these insults. The whole-walnut extract was most beneficial because it does not contribute the cellular toxicity effects. We have also reported that PUFAs found in walnuts attenuate neuroinflammation by modulating microglial reactivity. In a study using mouse microglial cells, walnut extract altered the response stimuli to the chemically induced inflammatory stress through phospholipase D2–mediated internalization of Toll-like receptor 4 (TLR-4) (32).

Mechanistic Animal Studies Supporting Walnut Effects on Cognitive Function

In an effort to determine the bioavailability of FAs in the diet, at the site of action, rats fed diets containing 15% LA and 3% ALA were examined for whole-body distribution of deuterated LA and ALA after a single-dose oral administration (36). The brain concentration of LA peaked at 8 h post-administration. Although ALA was not detected in brain tissue, ALA metabolites including EPA, DPA, and DHA remained elevated up to 25 d post-administration (36). Studies have also shown a decline in FA enzyme activity in the liver of aged animals (37), potentially allowing the presence of ALA and LA in the blood, where it could be taken up in the brain. Additionally, Davis et al. (38) found that mice fed a whole-walnut diet comprising 155 g of whole walnuts/kg diet, which is the equivalent of 80 g (~3 ounces) of walnuts/d in humans, to provide 20% of energy from fat, had changes in plasma markers, which were echoed in the liver metabolomics results. It was concluded that the walnut diet's beneficial effects probably represent the effects of whole walnuts' multiple constituents and not a specific FA or tocopherol (38).

In isolation, dietary DHA can improve learning and memory in rodent models of aging. Aged mice (9 mo) whose diet contained 20% DHA in the form of the green algae *Chlorella vulgaris* for 8 wk made fewer working memory errors in an 8-arm radial water maze (39). Dietary walnuts can also improve cognition. In 1 study, rats that ingested 80 mg/d of walnuts, in addition to their standard diet, for 28 d had enhanced learning and memory in the radial arm maze and reduced anxiety on the elevated plus maze (40). In a recent study from our laboratory (9), 19-mo-old Fischer (F344) rats were fed diets containing 0, 2, 6, or 9% (wt:wt) ground walnuts with skin for 8 wk. Rats fed a diet containing 2 or 6% walnuts had improved balance, coordination, and strength; however, rats fed the 9% diet had impaired motor performance relative to controls. Working memory in the water maze was also enhanced in rats fed diets containing walnuts; however, rats fed the 9% walnut diet had impairments in reference memory. The 6% walnut diet produced the best overall results among the aged rats. The 6% diet contained 5.4 g of ALA and 22.9 g of LA/kg, which, interestingly, is equivalent to the recommended dietary intake of 1 ounce/d (~28 g) of walnuts for humans (8,22,25). We subsequently found that walnut consumption was associated with substantially lower acetylcholinesterase activity in the striatum brain region of aged animals (22).

Older adults are at a higher risk of seizure disorders, the incidence and prevalence of which increase after 60 y of age (41). Acute symptomatic seizures among older adults are often the result of acute neural insults or metabolic disturbances. Asadi-Shekaari et al. (42) also showed that the addition of walnut kernels to the diet of male rats was preventive against experimentally induced epilepsy. In one of our recent studies on aged rats that were supplemented with 6–9% walnuts, walnuts substantially inhibited the activation/phosphorylation of P38 MAPK and the transcription factor NF-κB (43). The results also showed that 6% walnut supplementation activated cAMP response element-binding protein (CREBP), a constitutively expressed nuclear transcription factor that plays a critical role in neuronal survival in the hippocampus and striatum (43). n–3 FAs that are rich in walnuts are converted to EPA and DHA, which act as precursors for anti-inflammatory eicosanoids and neuroprotection D1 (NPD1), respectively. NPD1 has been shown to attenuate the activation of inflammatory signaling mediators such as prostaglandins synthesized from arachidonic acid (long-chain n–6 PUFAs) by cyclooxygenase-2 (44). Therefore, walnut phytochemicals, which can effectively inhibit prooxidant and proinflammatory mediators, may be 1 method of reducing the risk of dementia, seizure, and other neurologic disorders among older adults.

The loss of protein homeostasis in the brain, whereby brain cells accumulate insoluble, misfolded, or damaged protein or organellar structures, is the hallmark of many age-related neurodegenerative diseases. In a separate study, which used the brains of rats supplemented with 6 and 9% walnut diets, our laboratory showed substantially reduced aggregation of polyubiquitinated proteins and activated the neuronal housekeeping function, known as autophagy, in the striatum and hippocampus (43). The clearance of polyubiquitinated protein aggregates such as p62/sequestosome 1 was more pronounced in the hippocampus, a critical region in the brain involved in memory function (43). Importantly, the clearance of toxic protein aggregates was in conjunction with reductions in oxidative stress and inflammation.

Human Studies Linked to Walnut Consumption

Most human studies associate walnuts and walnut PUFAs with their cardiovascular benefits (45–48). Reductions in cardiovascular disease risk factors may be associated with better brain health because cardiovascular disease is also associated with the development of cerebrovascular disease, stroke, and mild cognitive impairment (49,50). A walnut- and walnut-oil–rich diet reduced inflammatory and cardiovascular risk factors among hypercholesterolemic men and women (51). A walnut-enriched diet can also improve endothelium-dependent vasodilatation in type 2 diabetic individuals (24) and overweight adults with visceral adiposity (52). Increased vasculature and improved endothelial function have direct bearing on cerebral health and cognitive function.

Generally, increased nut intake, and walnut intake in particular, has been shown to improve cognition among older adults. Recently, the Doetinchem Cohort Study reported that higher nut intake at baseline was associated with improved processing speed, cognitive flexibility, memory, and global cognitive function (7). Another large, parallel-group, multicenter randomized, controlled clinical trial (PREDIMED, Prevención con Dieta Mediterránea) recently provided compelling evidence for the ability of nuts to counteract depression and age-related cognitive decline. This study, spanning 7 y with a minimum follow-up of 5 y, enrolled 7447 persons (55–80 y of age), with 3 randomized intervention arms (53). The first group received the Mediterranean diet supplemented with virgin olive oil (1 L/wk), the second group received the Mediterranean diet supplemented with 30 g/d of mixed nuts (15 g walnuts, 7.5 g almonds, 7.5 g hazelnuts), and the third group received a low-fat control diet. After 3 y, participants who were on the Mediterranean diet supplemented with nuts had substantially improved concentrations of plasma brain-derived neurotrophic factor (BDNF), particularly among the individuals with a history of depression (53). BDNF, a member of the neurotrophin family, is highly expressed in cortical and hippocampal neurons and promotes the induction of long-term potentiation, synaptic plasticity, neuronal survival and differentiation, axonal elongation, and neurotransmitter release (54). BDNF can cross the blood-brain barrier, and lower plasma and brain BDNF protein concentrations have been implicated in enhanced aggressiveness, hyperactivity, and hyperphagia, as well as in an array of brain disorders, such as epilepsy, AD, Huntington disease, autism, schizophrenia, and major depression (55). A subset of PREDIMED participants were assessed for neuropsychological testing, which revealed that higher intakes of both total olive oil and virgin olive oil, coffee, walnuts, and wine improved both memory and overall cognitive functions (56). Furthermore, the study found that walnuts, among all other nuts included in the study, were associated with substantial improvements in working memory (56). The study also reported that the Mediterranean diet with nuts reduced the risk of stroke by 46%, which indirectly establishes the benefits of nuts on age-associated cognitive decline caused by vascular deterioration.

Further supporting the benefits of walnuts on cognitive function, Pribis et al. (57) reported that daily supplementation with 60 g of walnuts for 8 wk substantially improved inferential verbal reasoning in a double-blind, randomized, placebo-controlled cross-over (6-wk washout) study in young college students. The study was conducted in healthy, cognitively intact young adults, which may explain why no differences in memory, mood, or nonverbal reasoning were detected between the control and diet group.

Walnuts may also play a role in preventing AD. Dai et al. (58) reported that supplementation with fruit and vegetable juice, rich in polyphenols, at least 3 times/wk was attributed to a slower onset of AD, particularly in patients who are apolipoprotein ε4 (*APOE4*) carriers (58). *APOE4* plays a critical role in promoting amyloid accumulation, neurotoxicity, oxidative stress, and neurofibrillary tangles (59). In 2 longitudinal cohort studies, the Washington Heights and Inwood Columbia Aging Project (WHICAP; population-based) and the Predictors Study (clinic-based), which followed people for an average of 4 y, researchers showed that the presence of at least 1 *APOE4* allele was associated with faster cognitive decline in the earliest stages of AD (60). In another study, Yasuno et al. (61) supplemented 41 participants aged ≥65 y with n–3 PUFAs from fish, lycopene, and Ginkgo biloba extracts daily for 3 y and compared them with 622 participants of a similar age group without supplementation. They showed that the combination of antioxidants improved cognitive function in aged persons after 3 y, and the improvement in cognitive function with supplementation was observed both in *APOE4* noncarrier (E4−) and *APOE4* carrier (E4+) groups (61). Although n–3 PUFAs from animal sources differ from n–3 PUFAs from walnuts, because humans convert ALA from walnuts into EPA and DHA, it can be inferred that walnuts rich in n–3 PUFAs, in combination with other high-antioxidant compounds, may delay the cognitive decline associated with AD.

In conclusion, age-related increases in oxidative stress and inflammation, especially when coupled with metabolic and cardiovascular dysfunction, lead to neurodegeneration and cognitive decline. This process of brain aging occurs even in the absence of specific neurodegenerative diseases. Although most chronic neurodegenerative diseases cannot currently be cured, preventive measures earlier in life can protect cognitive function in old age and may prevent or delay the onset of debilitating neurodegenerative diseases. Dietary interventions provide a safe and palatable means of modifying the body's internal environment and, importantly, the neuronal environment within the brain. Walnut polyphenols and tocopherols can reduce oxidative stress and inflammation; furthermore, PUFAs help maintain neuronal membrane integrity and attenuate protein aggregation involved in AD. In rodent studies, the addition of dietary walnuts, equivalent to a single serving of walnuts for humans, was sufficient to improve both motor and cognitive behavior in aged animals. In humans, the inclusion of walnuts in the diet improved cardiovascular health, which is itself a risk factor for neurodegenerative diseases and age-related cognitive decline. Taken together, this evidence suggests that the integration of walnuts into a healthy diet could be an effective means of prolonging health spans, slowing the processes of brain aging, and reducing the risk of chronic neurodegenerative disease.

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