

# The beneficial effects of fruit polyphenols on brain aging

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## Abstract

Brain aging is characterized by the continual concession to battle against insults accumulated over the years. One of the major insults is oxidative stress, which is the inability to balance and to defend against the cellular generation of reactive oxygen species (ROS). These ROS cause oxidative damage to nucleic acid, carbohydrate, protein, and lipids. Oxidative damage is particularly detrimental to the brain, where the neuronal cells are largely post-mitotic. Therefore, damaged neurons cannot be replaced readily via mitosis. During normal aging, the brain undergoes morphological and functional modifications resulting in the observed behavioral declines such as decrements in motor and cognitive performance. These declines are augmented by neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and Parkinson's disease (PD). Research from our laboratory has shown that nutritional antioxidants, such as the polyphenols found in blueberries, can reverse age-related declines in neuronal signal transduction as well as cognitive and motor deficits. Furthermore, we have shown that short-term blueberry (BB) supplementation increases hippocampal plasticity. These findings are briefly reviewed in this paper. © 2005 Published by Elsevier Inc.

**Keywords:** Aging; Brain; Oxidative stress; Inflammation; Dietary antioxidants; Polyphenols; Blueberry supplementation; Behavioral deficits; Hippocampal plasticity; Signaling

## 1. Introduction

Normal aging is accompanied by declines in motor and cognitive performance [43]. These declines are amplified in age-related neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and Parkinson's disease (PD). As the elderly population increases, so will the prevalence of these age-related disorders [19,20,62]. In order to improve the quality of life for the elderly and to alleviate the social and economic burdens imposed by the prolongation of life expectancy, it is crucial to devise strategies to impede or reverse age-related neuronal declines. There is substantial evidence that oxidative stress plays an important role in the aging process [33–35]. According to Dr. Denham Harman's free radical theory, aging is the accumulation of oxidative damage to cells and tissues over time. It has been suggested that the behavioral and neuronal deficits seen in the elderly popula-

tion are the result of an increasing vulnerability to damage by free radicals [22,54]. This theory has fostered the field of nutraceutical research, which investigates the effects of dietary antioxidants as a means to reverse or slow the aging process [5,30,40–42,45,88]. Our research has shown that supplementation with fruits and vegetables are beneficial in both forestalling and reversing the deleterious effects of aging on neuronal communication and behavior (reviewed in [43]). The observed protection may be the result of the antioxidant and anti-inflammatory properties of the polyphenolic compounds found in these fruits and vegetables [66].

## 2. Oxidative stress and inflammation in brain aging

### 2.1. Oxidative stress

Reactive oxygen species (ROS) collectively refer to oxygen radicals and non-radicals that are readily converted to radicals [8,10,21]. ROS are the by-products of normal aerobic metabolism [3,14]. It is estimated that approximately

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2–5% of the oxygen consumed by a cell is subsequently converted to free radicals [23,83]. The production of ROS is normally counterbalanced by cellular defense systems [24,32]. However, about 1% of the ROS escape daily elimination to give rise to oxidative cellular damage [4]. The process, by which the production of ROS is not effectively neutralized, leading to cellular damage, is known as oxidative stress [32]. The extent of oxidative stress can be experimentally determined by the quantification of end-products of nucleic acid damage, lipid peroxidation, and protein oxidation [46].

The brain is especially susceptible to oxidative stress [23,32] for the following reasons. Weighing at about 2% of the body mass, the brain utilizes 20% of the total oxygen consumption. Besides, it is enriched with readily peroxidizable polyunsaturated fatty acids. Moreover, the brain is not particularly endowed with antioxidant defenses: it has a very low level of catalase activity and only moderate amounts of the endogenous antioxidant enzymes, superoxide dismutase and glutathione peroxidase. Additionally, the brain has high levels of iron and ascorbate, which are the key catalysts for lipid peroxidation. Also, many neurotransmitters themselves are autoxidized to generate ROS [26,53,64,71]. Except for those in some restricted regions of the brain, neuronal cells are post-mitotic and tend to accumulate oxidative damage [3,79,80]. The notion of increased oxidative stress in the aging brain is supported by numerous studies [6,19,27,47,78]. There is evidence that increased oxidative stress plays an important role in the pathogenesis of neurodegenerative diseases such as AD, PD, and ALS [9,15,77].

## 2.2. Inflammation

Normal brain aging is also associated with elevated levels of neuroinflammation [16,28,63,70]. Inflammation is intimately linked to enhanced ROS production [17,29,52,56]. Local unbridled over-production of ROS has been reported in several inflammatory diseases [11,31,38]. Oxidative stress-mediated inflammation has also been attributed to neurodegenerative disorders including AD and PD [18,36,37,57,61,67]. Neuroinflammation has been connected to enhanced signal transduction leading to the activation genes such as the inducible nitric oxide synthase (iNOS), interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and nuclear factor-kappa B (NF- $\kappa$ B) [1,49,60,65,69,72,81,86]. The neural-protecting microglial cells have been found to be activated by inflammation to produce high levels of ROS and cytokines [11,12,58,68,84].

## 3. Neuroprotective effects of fruit polyphenols

Since the endogenous antioxidant defense systems are not 100% effective, it is plausible to suggest that nutritional antioxidants be exploited to combat the accumulation of oxidative stress over the ever-prolonging human lifespan.

In fact, there is an increased interest in the study of the beneficial effects of nutritional antioxidants on health via the delay of aging and age-related diseases [25,43,49,82,87,89].

### 3.1. Polyphenols in fruits and vegetables

Fruits and vegetables rich in polyphenols have been found to be beneficial to brain function (reviewed in [76]). Some of the fruits and vegetables used in our research include blueberries, cranberries, strawberries, and spinach, all of which are high in antioxidant capacities as measured by the modified oxygen radical absorbance capacity (ORAC) assay [85]. This may account for the positive results observed with blueberry (BB) as well as other berry supplementation in rodent studies conducted in our laboratory as discussed in the following sections.

### 3.2. Cognitive and motor behaviors

Normal aging is accompanied by behavioral deficits, including cognitive and motor performances [2,39,50]. These deficits are probably induced by oxidative stress and inflammation [43]. To investigate whether nutritional antioxidants would be effective in preventing these deficits, we fed Fischer 344 (F344) rats from adulthood (6-month-old) to middle age (15-month-old) with a control diet (ATN-93) or the diet supplemented with either vitamin E (500 IU/kg diet) or extracts of strawberry or spinach containing the same millimolar Trolox equivalents of antioxidant capacity. We showed that long-term feeding of these animals with the supplemented diets prevented a variety of age-related deficits including cognitive performance [45].

In a later study, we found that feeding aged (19-month-old) F344 rats for 8 weeks with diets supplemented with spinach, strawberry or blueberry extracts effectively reversed age-related deficits in neuronal and cognitive function [44]. However, only the BB-supplemented diets improved balance and coordination [44].

Amyloid precursor protein/presenilin-1 (APP/PS1) transgenic mice have been used as a murine model for AD, since these mutations facilitate the production of beta amyloid and consequently Alzheimer-like plaques in several regions of the brain, followed by behavioral deficits [41]. To test if blueberry supplementation would prevent the behavioral deficits, a group of these mice was given blueberry supplementation beginning at 4 months of age and continuing for 8 months. These 12-month-old mice were then tested in a Y-maze to assess cognitive performance. The data showed that the BB-supplemented transgenic mice performed similarly to the non-transgenic mice, but significantly better than the non-supplemented transgenic mice [41]. However, no difference in the number of plaques was observed between the BB-supplemented and non-supplemented APP/PS1 mice, even though behavioral deficits were prevented in the BB-supplemented animals [41].

### 3.3. Signaling and neurogenesis

The discrepancy between plaque formation and improved Y-maze performance seen in the BB-supplemented APP/PS1 mice versus the non-supplemented APP/PS1 mice might be due to alterations in signaling pathways induced by blueberry supplementation [41]. The levels of extracellular signal regulated kinase (ERK) and protein kinase C (PKC) were elevated in the BB-supplemented APP/PS1 mice as compared to those found in the non-supplemented mice [41]. Both ERK and PKC kinases are important in mediating cognitive function, especially in conversion of short-term to long-term memory [55,59,73,74]. Our findings suggested that BB supplementation might enhance neuronal signaling to offset the putative deleterious effect of plaque deposition on behavioral deficits seen in the BB-supplemented transgenic mice [41].

To correlate behaviors with blueberry supplementation-induced alterations in signaling events, young (6-month-old) and old (19-month-old) F344 rats were fed a control or BB-supplemented diet for 8 weeks. These rats were then given a battery of tests to evaluate their motor and cognitive performances. After these tests the hippocampal expression of signaling markers including ERK1 and ERK2, as well as PKC- $\alpha$  and PKC- $\gamma$ , were analyzed by immunoblotting assays. BB supplementation significantly increased the expression of PKC- $\alpha$  in the old rats [75]. The expression of ERK1 and ERK2 positively correlated with inclined screen latency (measurement of muscle tone, strength, and stamina) in the BB-supplemented young and old rats [75] while the expression of PKC- $\gamma$  positively correlated with small plank latency (measurement of balance and coordination) in BB-supplemented old rats [75]. These results suggested that BB-supplementation enhanced motor performance via increasing neuronal signaling [75].

The hippocampus is one of the regions that has the capacity to generate neurons (neurogenesis), but this ability is diminished during aging [48,51] and accompanied by cognitive decline [7,13]. To dissect another possible mechanism underlying the beneficial effect of blueberries on cognition in aged rats, 19-month-old F344 rats were fed either a control or a BB-supplemented diet for 8 weeks. The rats were then tested for their reference and working memory [7]. After behavioral tests, rats were injected with bromodeoxyuridine (BrdU) for 4 consecutive days before they were processed for immunohistochemistry. Neurogenesis in the rat brains was quantified by BrdU incorporation into the genome of dividing cells via immunohistochemistry [7]. The localized expression of ERK and insulin-like growth factor 1 (IGF-1) and its receptor (IGF-1R) was also assayed by immunohistochemistry. The behavioral data revealed an enhanced cognitive performance (fewer errors in reference and working memory) with BB supplementation [7]. The number of BrdU-positive neuronal cells in the dentate gyrus of the aged rats was significantly increased by the short-term BB supplementation [7]. There was an inverse correlation between the number of BrdU-positive cells and the number of total

memory errors [7]. In addition, BB-supplemented aged rats showed significant increases in the protein levels of IGF-1, IGF-1R and ERK, and these increases were also inversely correlated with the number of total memory errors [7]. The data indicated that BB supplementation increased hippocampal plasticity and cognitive performance via concerted mechanisms involving neurogenesis, neurotrophic factor IGF-1 and its receptor, and MAP kinase signal transduction cascades. Taken together, these findings suggest that multiple mechanisms may be involved in the beneficial effects of high antioxidant fruits on aging.

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