

Blueberries and Neuronal Aging

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Key Words

Antioxidants · Cognition · Inflammation · Polyphenols · Signaling

Abstract

As the population of people in the United States over the age of 65 years continues to increase, so too will the incidence of age-related pathologies, including decreases in cognitive and motor function. In cases of severe deficits in memory or motor function, hospitalization and/or custodial care would be a likely outcome. This means that unless some way is found to reduce these age-related decrements in neuronal function, health care costs will continue to rise exponentially. Evidence is accumulating that consumption of blueberries may be one strategy to forestall or even reverse age-related neuronal deficits, as well as their subsequent behavioral manifestations, in order to increase healthy aging. Research suggests that the polyphenolic compounds found in blueberries exert their beneficial effects either through their ability to lower oxidative stress and inflammation or directly by altering the signaling involved in neuronal communication. These interventions, in turn, may protect against age-related deficits in cognitive and motor function. Appropriately, the US Department of Agriculture has figured prominently in these discoveries, through the efforts of two USDA researchers who worked for the department 100 years apart.

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Introduction

The United States Department of Agriculture (USDA) is the federal executive department responsible for developing and executing policies on farming, agriculture, food, and nutrition. According to their mission statement they provide leadership on food, agriculture, natural resources, and related issues based on sound public policy, the best available science, and efficient management. The US federal government is not always commended for its actions, but people may be surprised by the far-reaching impacts of the USDA's Agricultural Research Service (ARS).

The History of Blueberries

Anyone who has heard of the health benefits of blueberries might have come across the name of Dr. James Joseph. However, what people may not realize is that Jim investigated the potential role of blueberries in helping protect against aging when he worked as a USDA scientist at the Jean Mayer, USDA-ARS, Human Nutrition Research Center on Aging (HNRCA) at Tufts University from 1993 until his death in 2010. What is even more remarkable is that Jim's blueberry studies would not have been possible but for the ground-breaking work 100 years

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previously by another USDA scientist, Frederick Coville. In fact, it is safe to say that blueberries would not be the agricultural commodity they are today without the efforts of these two USDA researchers.

Blueberries are one of the only native US crops whose path to domestication is well documented, and they are also among the most recently domesticated crops [1]. Until 1911, blueberries were picked from the wild or from wild bushes that were replanted elsewhere. It was USDA botanist Frederick Coville who led the way to blueberry cultivation when he discovered that plants must be grown in moist, acidic soil [1]. He subsequently made the first successful crosses of several blueberry varieties still popular today for their important traits such as size and flavor [1]. The USDA research program that Coville founded in New Jersey still carries out critical research to protect and expand the US blueberry crop [1]. This research is critical given that consumer demand for blueberries is increasing rapidly, in part due to recognition of blueberry's health benefits [2].

Not only do blueberries taste good, but they fit in well with the advice to eat five servings of fruits/vegetables a day, are low in calories, and high in nutrients. Consequently, in the United States, demand for blueberries has grown, with both fresh and frozen per capita consumption increasing over the last three decades [2]. Since 2002, per capita fresh blueberry consumption has nearly tripled to over 1.1 pounds/person, compared to an annual per capita estimate of 0.25 pounds in the 1990s [2]. As stated above, this increased consumption is due, in part, to consumer awareness of the health benefits derived from eating blueberries, particularly the work of Dr. Joseph, myself, and other members of his lab, showing the positive effects they have on age-related behavioral deficits.

Age-Related Behavioral Declines

In the last century, the lifespan of humans has almost doubled, due to advances in healthcare, nutrition, and sanitation. Consequently, the percent of the population that is over the age of 65 years has and continues to increase, making age-related pathologies a growing concern. Of all the diseases that are associated with increasing age, such as arthritis, cardiovascular disease, cancer, cataracts, hypertension, metabolic disorder, and osteoporosis, dementia might be one of the most insidious and costly. Dementia robs older adults of their independence, control, and identity.

Two of the most dreaded consequences of dementia with aging are problems moving around and difficulty remembering things. Research has demonstrated that as both animals and humans age, psychomotor and cognitive functioning decreases, even in the absence of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. The brain is responsible for conscious control of movement, and alterations in motor function may include decreases in balance, muscle strength and coordination, while memory deficits are seen on cognitive tasks that require the use of spatial learning and memory [3]. These changes may be the result of losses of neurotransmitter receptor sensitivity and associated second messengers, as well as alterations in age- and calcium-sensitive signaling agents (e.g. mitogen-activated protein kinases) associated with memory, especially the conversion of short- to long-term memory. These deficits in neuronal communication are expressed, ultimately, as declines in motor and cognitive behavior.

While the mechanisms that might precipitate these losses of neuronal signaling and subsequent behavioral deficits during aging remain to be discerned, increased susceptibility to the long-term effects of oxidative stress and inflammation are involved [4, 5]. When we are young, our brains are well protected against the ravages of oxidative stress and inflammation. However, as we age both protection and repair from damage are reduced and sensitivity to these insults increases. The brain appears to be particularly vulnerable to oxidative stress because the brain utilizes 10% of the body's oxygen at rest and up to 50% during mental activity; these high rates of oxygen consumption may result in excessive reactive oxygen species being produced. If this is the case, then one approach to improving neuronal functioning might be to alter the neuronal environment and reduce the impact of the oxidative and inflammatory stressors [6].

Antioxidant Potential of Blueberries

We are told that eating more fruits and vegetables will reduce the risk of encountering such ubiquitous killers as cardiovascular diseases and cancer. But questions arise as to the nature of their beneficial properties in relation to diseases of the brain such as Alzheimer and Parkinson's diseases. All plants, including blueberries, synthesize a vast array of chemical compounds that are not necessarily involved in the plant's metabolism. These 'secondary compounds' instead serve a variety of functions that enhance the plant's survivability, including combating oxi-

dative stress and inflammation. Research suggests that phytochemical compounds contained in colorful fruits and vegetables exhibit potent antioxidant and anti-inflammatory activities that can reduce the age-related sensitivity to oxidative stress or inflammation [7]. These effects could arise due to the types, quantities, and combinations of dietary antioxidants/anti-inflammatories found in them. Therefore, the key to reducing the incidence of age-related deficits in behavior might be to alter the neuronal environment with polyphenolic-rich foods such as blueberries, such that neuroinflammation and oxidative stress, and the vulnerability to them, are reduced. Moreover, recent work also suggests that the polyphenolic compounds found in fruits and vegetables may actually directly influence neuronal communication and signaling, which may also contribute to their beneficial effects with respect to cognitive and motor behaviors.

Polyphenolics are one class of phytochemicals of which over 4000 different structures have been identified [8], and these occur ubiquitously in foods of plant origin (e.g. fruits, vegetables, nuts, seeds, grains, tea, and wine). Examples of polyphenolic families include phenolic acids, stilbenes, coumarins, tannins, and flavonoids. Fruits and vegetables are known to contain numerous polyphenols and, until Dr. Joseph and others began studying them in earnest almost 15 years ago, their beneficial health effects on brain function had not been scientifically studied. The question arises, however, as to which of the nutritional polyphenols may be the most effective antioxidants/anti-inflammatories.

It appears that some of the most beneficial brain health effects result from consumption of a large class of polyphenols known as flavonoids. A subset of the flavonoids known as anthocyanins is particularly abundant in brightly colored fruit such as berry fruits, Concord grapes, and acai fruits. Anthocyanins are responsible for the colors in the fruits and they have been shown to possess potent antioxidant and anti-inflammatory activities [9]. The antioxidant properties of fruits and vegetables were extensively studied by Dr. Ronald L. Prior, another USDA Scientist who worked in the laboratory next to Jim Joseph at the HNRCA in the mid- to late 1990s. Dr. Prior and his colleagues developed an automated assessment of antioxidant capacity called the oxygen radical absorbance capacity (ORAC) assay [10]. Using this procedure they determined the total antioxidant activity of various fruits and vegetables. The ORAC rankings showed that berry fruits such as blueberries, cranberries, blackberries, and strawberries were among the highest foods on this scale. Based on this information, we started to wonder

what would happen if we fed these high antioxidant foods to animals at different ages to investigate their beneficial effects.

Berries and Age-Related Behavioral Declines: The Initial Studies

We hypothesized that if increases in oxidative stress and declines in antioxidant defense mechanisms are causative factors in age-related decrements in behavior, then feeding fruits and vegetables with the highest antioxidant properties to animals would forestall or reverse age-related changes in performance. Therefore, in our first study, Fischer 344 (F344) rats underwent long-term feeding from adulthood (6 months) to middle age (15 months) with a control diet (AIN-93), a diet supplemented with a traditional antioxidant, vitamin E (500 IU/kg diet), or diets with extracts of strawberry or spinach that contained identical antioxidant content (based upon ORAC), to determine if the feeding would prevent age-related decrements in motor and cognitive behavior as well as brain function [11]. A number of different parameters known to be sensitive to oxidative stress were prevented by the antioxidant diets including: (1) receptor sensitivity, as measured by oxotremorine-enhanced dopamine (DA) release in isolated striatal slices and cerebellar Purkinje cell activity; (2) calcium buffering capacity, i.e. the ability of striatal synaptosomes to extrude calcium following depolarization, deficits of which ultimately result in reduced cellular signaling and eventually cell death; (3) changes in signal transduction assessed by carbachol-stimulated GTPase coupling/uncoupling in striatal membranes, and (4) cognition (spatial learning and memory) as measured by Morris water maze performance [11]. Spinach-fed rats demonstrated the greatest retardation of age-effects on all parameters except GTPase activity, where strawberry had the greatest effect; strawberry and vitamin E showed significant but equal protection against these age-induced deficits on the other parameters.

However, given that people may only begin to eat healthier when they start seeing declines in old age, our second study was directed towards older rats, who already have motor and cognitive deficits. Therefore, our subsequent experiment found that dietary supplementations (for 8 weeks) with spinach, strawberry, or blueberry extracts in a control diet (AIN-93) were also effective in reversing age-related deficits in brain and behavioral function in aged (19 months) Fischer 344 rats. We added

blueberries to this study because they were found by Dr. Prior in the interim between our two studies to have the highest ORAC of all the fruits and vegetables he had tested up to that point. While all of the supplemented diets showed positive effects on cognitive behavior, rats on the blueberry diet showed the greatest improvements in motor performance, carbachol-stimulated GTPase activity, and oxotremorine-enhanced DA release [12]. Additionally, the blueberry-fed group showed no decrements in calcium buffering following exposure to an oxidative stressor [12]. Interestingly, even though these diets were supplemented based on equal ORAC capacity, they were not equally effective in preventing/reversing age-related changes. Therefore, it seemed that antioxidant activity alone was not predictive of the potency of these compounds. In fact, oxidative stress markers (as measured by DCF fluorescence and glutathione levels in the brain) were only modestly reduced by the diets [12], suggesting that these fruit and vegetable polyphenolics possess a multiplicity of actions, aside from antioxidant potential, and that differences in the polyphenolic composition of these extracts could account for the positive effects observed. Therefore, subsequent investigations in our laboratory and others attempted to determine how the blueberries were working to produce their beneficial effects.

Blueberries and Age-Related Behavioral Declines: Subsequent Studies

After these initial studies, several questions arose in both the scientific and lay communities, which we attempted to answer in subsequent studies. One of the first questions we wanted to answer was whether or not the protective effects of the berries would be observed if superimposed on a natural ingredient, cereal-based diet (NIH-31), or did they just work with a less nutrient-dense, casein-based purified diet (AIN-93). Therefore, in our next study, we found that the beneficial effects of blueberries, and other foods, were seen even when superimposed on an already well-balanced, healthy rodent diet ('chow') which was more representative of a balanced human diet [13]. This study was also the first in which we standardized the amount of blueberries added to the rodent diet at 2%, which is approximately 4 g/day of blueberry consumption for a rat. This dose is roughly equivalent to 0.5–1 cup/day for humans [14]. Since this study, our laboratory and others have used the modified NIH-31 with blueberry supplemented at 2% of the diet (see papers reviewed in [15] and updated in [16]).

Another concern was whether or not the blueberry compounds were crossing the blood brain barrier, or simply exerting their effects peripherally. We conducted a study that showed that the anthocyanins contained in blueberries did enter the brain, and their concentrations were correlated with cognitive performance [17]. These findings show that, once consumed, dietary polyphenols from berries are both neuroavailable and related to behavioral performance. Interestingly, our cell studies and one preliminary animal study have shown that the whole blueberry is more effective than any single phenolic family, possibly because different polyphenols may exert their effects through different and/or independent mechanisms.

Further studies in Dr. David Malin's lab, another of Jim Joseph's collaborators, explored the time course of dietary blueberry's effects [18]. In aging F344 rats, age-related object memory decline was reversed and prevented by maintenance for 1 month on a 2% blueberry-enriched diet. However, feeding rats a blueberry-enriched diet for a somewhat longer duration (2 months as opposed to 1 month) resulted in a more prolonged benefit after diet termination. Specifically, rats that had consumed the blueberry diet for 2 months showed no decline in performance after two or four weeks on the control diet, whereas rats that only ate the blueberry diet for 1 month showed a decline in performance after two weeks and were not significantly different from the age-matched controls after four weeks on the control diet.

Furthermore, a second experiment in this study showed consuming the blueberry diet for 1 month reversed some pre-existing decline in object memory in F344 rats [18]. Rats displayed near-random object memory performance (no preference for a novel object) at 19 months of age, indicative of an age-related decline. They were then placed on either a blueberry or a control diet and reevaluated for changes in object recognition 1 month later. Object memory significantly improved in the blueberry-fed group, but not the control group. These findings show that deficits in cognition are reversed in aged rats by as little as one month of dietary supplementation with blueberry; however, at least two months of supplementation are required to maintain the benefit long-term.

Possible Mechanisms of Action of Blueberries

In the last 10 years, subsequent studies from our group and others have examined the possible mechanisms of action behind these neuronal and behavioral improve-

ments with berries, such as anti-inflammatory activities or changes in neuronal signaling capability. These studies were reviewed by us in the *Journal of Agriculture and Food Chemistry* in 2008 [15] and recently updated by us in the same journal [16].

In addition to possibly acting through antioxidant and anti-inflammatory effects, other possible mechanisms for the berry fruit's positive effects include: direct effects on signaling to enhance neuronal communication, the ability to buffer against excess calcium, enhancement of neuroprotective stress shock proteins, and reduction of stress signals such as nuclear factor kappaB (NF- κ B). Measures of hippocampal plasticity, including neurogenesis, were also enhanced in rats on a blueberry-enriched diet [19]. Additionally, extracellular signal regulated kinase (ERK) activation and insulin-like growth factor-1 were increased in the supplemented animals, and were positively correlated with improvements in spatial memory [19].

The importance of the signaling properties of the blueberries to functional measures was shown in a study from David Morgan's laboratory in collaboration with ours in which APP/PS1 transgenic mice, given blueberry supplementation for 8 months of age, exhibited increased alternation behavior in a Y-maze, compared to the nonsupplemented transgenic animals, and at a level seen in nontransgenic mice [20]. Interestingly, even though behavioral declines were prevented, there were no differences in the number of plaques found in the brains of blueberry-supplemented and nonsupplemented APP/PS1 mice. However, the blueberry-supplemented APP/PS1 mice exhibited enhancement of GTPase activity and greater levels of hippocampal ERK, as well as striatal and hippocampal protein kinase C α (PKC), than those seen in the transgenic mice maintained on the control diet [20]. Two of the most important functions of PKC may be the regulation of synaptic plasticity and modulation of short- to long-term memory. Therefore, it appears that berry fruits mediate signaling pathways involved in inflammation and cell survival, in addition to enhancing neuroplasticity, neurotransmission, and calcium buffering, all of which lead to attenuation of age- and pathology-related deficits in behavior.

Another interesting possibility for the long-term beneficial effects of blueberries is an effect called hormesis [21]. It may be that short-term exposure to plant substances, such as blueberries, could increase reactive oxygen/reactive nitrogen species which, in turn, would lead to upregulation of antioxidant defense mechanisms to enhance long-term protection. A recent study [21] has

shown that 2-day feeding with a blueberry diet resulted in significant increases in total reactive oxygen species, superoxide, and peroxynitrite production in kidney, brain, and liver tissues compared to control-fed rats. However, after long-term feeding (6–12 weeks), there was evidence of reduced reactive oxygen/reactive nitrogen species. Therefore, the authors propose that a hormesis effect might be responsible for the long-term effects, in that once the initial increase in oxidative stress is produced, the antioxidant defenses are activated, which then leads to long-term protection.

Dietary Blueberry Intervention in Humans

Recent clinical trials have extended these effects on cognition to humans. Dr. Robert Krikorian and colleagues, one of which was Jim Joseph, reported that daily supplementation of between 6 and 9 ml/kg blueberry juice for 12 weeks among older individuals with mild cognitive impairment improved cognitive function, particularly with respect to improved paired associate learning, word list recall, and reduced depressive symptoms [22]. Although one limitation of this study was the small sample size, it points to the need for larger clinical trials. Dr. Krikorian is currently running two different blueberry trials with a much larger sample size. In fact, it was Jim Joseph who inspired Dr. Krikorian to run the initial clinical trial and who advised him along the way.

Conclusions

It is evident that blueberries are able to protect against oxidative stress and inflammation in aging and the consequent changes in cognitive and motor behavioral deficits. Additionally, blueberries may exert their effects directly on the brain via alterations in cellular signaling. Whole blueberries appear to be more effective than individual components, showing that the whole is greater than the sum of its parts. Thus, nutritional interventions containing polyphenolics, such as berry fruits, may prove to be a valuable asset in strengthening the brain against the ravages of time as they could retard or prevent the development of age-related neurodegenerative diseases. Early nutritional interventions may even prevent or delay the onset of diseases such as Alzheimer's disease, because they can reduce oxidative stress and inflammation superimposed upon a stress-vulnerable aging brain. However, more mechanistic studies on blueberries and other foods

are needed, as are studies concentrating on other neurological and vascular conditions. There is also a need for more human studies to build on the behavioral and mechanistic findings established so far in the basic science studies. An initial clinical study showed some promise, demonstrating cognitive enhancement after as little as three months of regular berry consumption among

older adults who already exhibited age-related cognitive decline. Therefore, nutritional intervention with blueberries may be effective in forestalling or even reversing the neurological changes associated with aging. If this is the case, and given that blueberries taste good and do not have the side effects of many drugs, we owe something to two USDA Scientists that helped make this possible.

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