Research article

Antioxidant and neuroprotective properties of blueberry polyphenols: a critical review

Marilù Giacalone, Filippo Di Sacco, Ippolito Traupe, Roberto Topini, Francesco Forfori, Francesco Giunta

Intensive Care Unit, Department of Surgery, University of Pisa, Pisa, Italy

Objective: The aim of this work was to highlight the effects and the possible mechanisms of the action of blueberry polyphenols on the central nervous system (CNS).
Methods: An analysis was carried out, in a temporal order, of the most important literature about this topic and the results have been correlated with the beneficial and protective effects, mainly concerning the CNS.
Discussion: Over the last 10 years an increasing scientific interest has developed about polyphenols, which are very abundant in blueberries, as they have been seen to produce favourable effects related to neuroprotection and linked to a possible decrease of age-related cognitive and motor decline, as shown by the improvement of such functions in animal models with a supplemented diet. Such effects could not only be explained through a purely antioxidant action but also through more complex mechanisms related to inflammation, genic expression, and regulation of cell survival.
Conclusions: Despite the wealth of data from animal studies, there is a relative lack of data concerning human beings, even if some positive results are beginning to emerge. Therefore, blueberry polyphenols could become useful pharmacological agents for various conditions including neurological diseases, but further studies are still necessary to attain this objective.

Key words: Antioxidant, Neuroprotection, Cognitive functions, Inflammation, Ageing

General features of blueberry and its compounds

The blueberry plant is a small shrub common in the mountain brushwood of the Northern hemisphere; however, nowadays there are also plantations in hills and plains. Blueberries belong to the family *Ericaceae* and to the genus *Vaccinium*; several species are to be found in different geographical areas, depending upon the different climatic conditions required for growth. The beneficial effects on human health produced by blueberry polyphenols, as evidenced by many epidemiological studies,[1] have triggered an increasing scientific interest in this subject. Polyphenols are produced by plants for several ecological functions that enhance their ability to survive;[2] they are among the most important vegetable metabolites and they are present in a lot of foods, mainly fruit and vegetables.[3]

Within the family of the polyphenols there are various compounds such as flavonoids, which include anthocyanins; blueberry anthocyanins have received the greatest attention. They have strong antioxidant properties as their chemical structure permits the relocation of the decoupled electron thus forming a stable radical.[4] The mean intake of dietary polyphenols in western countries is thought to be about 1 g/die.[5]

Oxidative stress and ageing

Oxidative stress is a condition of cellular damage derived from the imbalance between antioxidant and pro-oxidant factors in favour of the latter.[6] Pro-oxidants are, in effect, reactive oxygen species and they are referred to as superoxide anions, hydroxyl radicals, hydrogen peroxide, and singlet oxygen. In small amounts they are normal by-products of aerobic metabolism and are promptly neutralized by antioxidant systems; however, a small portion escapes this neutralization to give rise to oxidative stress.[7] In young subjects this does not lead to clinically detectable effects due to the relative abundance of defence systems; however they do occur in aged subjects because of their increased vulnerability[8] and cumulative damage over time.[9]

Correspondence to: Filippo Di Sacco, Intensive Care Unit, Department of Surgery, University of Pisa; c/o Cisanello Hospital, Paradisa Street 2, Pisa, Italy. E-mail: filippo-ds@tiscali.it

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In large quantities, the deleterious effects of oxidative stress are responsible for age-related functional decline,[10] mainly in the CNS because of the very high oxygen exposure and the higher oxygen intake compared with other tissues (20% of total body oxygen).[11]

Therefore, exposure to a high oxygen concentration and cumulative damage give rise to cognitive and motor impairment that is bound to worsen over time in aged subjects, even if they are not affected by any diseases.[12]

In fact, as shown by Joseph *et al.*[13] ageing implies some macroscopic changes (deficits in muscle strength, balance, coordination, and reduced muscle tone as well as cognitive dysfunctions) which are further exacerbated in neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease. Hence, some common elements may be present in varying degrees in both conditions and be responsible for the final damage.

Moreover, microscopic changes can also be observed in ageing and neurodegeneration; they can be related to oxidative stress and include an increase of oxidized/total glutathione ratio, significant lipofuscin accumulation, reduced response to *in vitro* age- and oxidative stress-sensitive tests, that are related to signal transduction functioning.[13] Other changes related to ageing focus on the role of cholesterol: it regulates membrane composition, symmetry, and fluidity: these factors contribute to vulnerability to peroxidation.[14] As indicated, once again, by Joseph, oxidative stress and calcium homeostasis impairment mutually increase cell damage and dysfunction.[13] Finally, ageing also involves a decrease in the sensitivity of several neurochemical systems such as adrenergic, dopaminergic, cholinergic (muscarinic), and opioid systems; these decrements can be attributed to oxidative stress and a decrease in neurotransmitter production, that are highly adverse for signal transduction.[14]

Presentation and discussion of blueberry administration on CNS functions

Therefore, assuming that oxidative stress is the main factor involved in the pathogenesis of physiological brain ageing and neurodegenerative diseases, an increase in antioxidant levels could delay the onset of ageing signs or reverse those already established. One of the first studies in this field of nutritional neuroscience compared the effects of a normal diet (control) and a long-term supplemented one on rats (from 6 to 15 months of age) with strawberry or spinach extracts or with vitamin E; in the supplementation-group, cognitive, motor, and laboratory ageing signs were found to be delayed in comparison with the control group.[15] Therefore, these preliminary results provide evidence that dietary antioxidant supplementation could be involved in the regulation of cognitive functions and hence could affect the performance of animals; so in the next investigation, vitamin E was replaced by blueberries, due to their high antioxidant content. Although the duration of administration (rats from 19 to 21 months of age) was shorter, better performances were detected in supplemented rats than in the control group, with a more significant effect for the blueberry group in comparison with the spinach and strawberry groups.[16] This is the first study which shows an actual reversal of some age-related signs by a dietary supplementation of antioxidants.[16] For both of these studies an oxygen radical absorbance capacity (ORAC) assay was used; it has high specificity for the measure of the antioxidant power of a biological sample or a pure compound due to its response to numerous antioxidants.[17] Performance, in this context, is taken to mean the series of psychomotor, cognitive, and laboratory data obtained through tests to which the rats were submitted; for instance, information about muscle strength, coordination, memory, attention (Morris water maze) cell-signalling pathways, and oxidative stress.[15,16] The Morris water maze is a model widely used in behavioural neuroscience; it represents a spatial learning task that evaluates several cognitive functions typically mediated by the hippocampus; it is also used as an age-sensitive test.[6]

The effects of blueberry antioxidant activity can also be seen in living organisms through an experimental model of the induction of oxidative stress, that is hyperoxia; it represents a strong stimulus in favour of pro-oxidant factors, so far as to provoke changes in the distribution of antioxidants, that are significantly mitigated with long-term blueberry supplementation (8 weeks).[18] Total serum antioxidant capacity (namely ORAC determined in untreated serum) was substantially unchanged in the control group and significantly increased in blueberry-supplemented animals. From this study, blueberry extracts are shown to be particularly rich in anthocyanins (1.28 mg/g fresh weight or 9.11 mg/g dry weight, or 42% of the total contained polyphenols): these data derive from high-performance liquid chromatography analysis.[18] Also in a context of marked oxidative stress, like this one, which is probably different from physiological conditions in living organisms, blueberry antioxidants can display a protective role potentially suitable for future therapies.

In fact, cerebral damage induced by ischaemia – hypoxia in the hippocampus is reduced ($17 \pm 2\%$ of neuronal loss vs. $40 \pm 2\%$ of control group) in rats with a blueberry extract-enriched diet (14.3% of the diet in the treated group vs. 2.5% in the previous study) for 6 weeks.[19] However, due to further studies,

blueberry administration is likely, in several ways, to affect the homeostasis of CNS functioning: one is, therefore, led to hypothesize that the beneficial effects of blueberry may not only depend on purely antioxidant mechanisms but also more complex ones. In fact subsequently, analysing performances of transgenic mice (using the Y-maze) predisposed to the development of AD and supplemented with long-term blueberry extracts, it was demonstrated for the first time that the protective effect may derive from the improvement of neuronal signalling pathways and that it could be possible to counterbalance genetic predisposition to cognitive deficits through dietary changes, since the content of amyloid plaques in treated animals remained unchanged.[20] So, the blueberry role in influencing neuronal plasticity phenomena has been further investigated in subsequent studies. There was found to be an increase in neuronal plasticity, assessed through the relative markers in animals with a blueberry-supplemented diet: an increase in hippocampal neurogenesis, activation of extracellular-tyrosin kinase receptors and correlation of insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 1 receptor (IGF-1R) levels with an improvement in spatial memory in advanced-age rats with short-term blueberry supplementation.[21] Hence, these findings can confirm blueberry correlation to synaptic plasticity. An effect on genic expression has also been shown through the reduction of nuclear factor kappa B (NF-κB); it is a transcription factor that promotes the expression of proinflammatory genes in advanced-age rats to the levels of young animals, with a corresponding memory-improvement correlation.[22] Memory is a cognitive function which is highly sensitive to blueberry influence both for physiological reasons, due to genic expression and synaptic plasticity effects, and topographical ones. Various anthocyanins contained in blueberries indeed have been detected in the cortex, hippocampus, striatum, and cerebellum of long-term blueberry-supplemented rats of advanced age but not in the control group, showing for the first time that polyphenolic compounds are able to cross the blood brain barrier and to localize in several brain regions which are important for learning and memory.[23]

Moreover, a correlation between the Morris water maze performance and the total number of anthocyanin compounds detected in the cortex has been evidenced.[23] Because of this wide distribution, it is important to note that the effects on the CNS regarding memory could imply something more than just retrieval. In fact subsequently, besides classical maze memory tasks with aversive component (that is, the rat's ability to learn to associate a certain route with a noxious stimulus e.g. an electric foot shock and therefore to avoid it), an evaluation of the anxiolytic effect on rats, supplemented for 30 days with lyophilized-blueberry, was carried out.[24]

Improvements in motor functions and long-term memory were found, but the anxiolytic effect did not appear to be significant.[24]

However, in a later study, by using a larger number of rats (30 vs. 20) treated with the same dosage of lyophilized blueberry extract but dissolved in a more concentrated solution (10 vs. 30 ml), for the same time of administration, an anxiolytic effect also emerged,[25] in accordance with the ability of blueberry polyphenols to act in memory neuronal circuits, as well as in associative memory, which, in this context, is related to a pharmacological behavioural component. Nevertheless, their actions are much more complex than foreseen because a genoprotective action was also detected by the electrophoresis of cortical and hippocampal neuron DNA in the supplemented rat group[25] further confirming the multiplicity of targets, and therefore, the variety of the effects of blueberry polyphenols.

Furthermore, the authors note that the effects on the long-term memory of animals could already be detected in the execution of tasks 24 hours after the administration of the highest dose of blueberry extract; nevertheless, a tendency to a visible improvement even though not significant, was also found with a lower dose, concluding that blueberry extract is effective in a wide range of dosages and therefore the composition of the extract and the time of administration are more important than the dosage used.[25]

The effectiveness and the field of action of blueberry supplementation emerges, once again, in another experimental context, a model of ageing: differential cognitive effects of blueberry and strawberry supplementation have been compared through a rodent model of accelerated ageing (high-energy ^{56}Fe particles exposure), showing that strawberry supplementation improves the ability to recall learned information mediated by the hippocampus, while blueberry supplementation improves the ability to learn new information mediated by the striatum.[26] It can be deduced that behavioural deficits and neuronal signalling pathway deficits are related to irradiation with ^{56}Fe particles and can be improved by an antioxidant diet; moreover, the localization of blueberry polyphenols in the CNS determines the final effect on the particular corresponding functions.

In subsequent research, some pharmacokinetic variables such as maximum plasmatic concentration, area under curve plasmatic concentration-time, elimination of half-life, etc. for the 18 main polyphenols have been calculated, and information about the absorption, metabolism, and distribution of such substances have been obtained through data from 97 kinetic studies in human adult subjects.[27] Due to the powerful antioxidant effects of blueberry compounds, their

administration to the astronauts on space missions has been proposed because of the long period of exposure to radiation.[28] Evidence exists that inflammation is an important event in brain ageing; in fact from middle-age onwards an increase of glial fibrillary acidic protein expression occurs[29] and continues even in the absence of inflammatory stimuli.[30] According to this observation, a major production of tumour necrosis factor alpha (TNF-alpha) during cytotoxic reactions has been reported among the elderly[31] and the loss of inhibition of glial cells by neurons may be lacking during ageing.[32] Other studies report an increase of serum concentration of TNF-alpha and interleukin-6 both in elderly mice[33] and in human subjects;[34] in fact it has been proposed that the increased productions of reactive c protein (reactive c protein) found in the elderly may only represent a factor of biological ageing.[35] Moreover, neuronal/glial inflammation induced by central administration of lipopolysaccharide (LPS) has been shown to be able to reproduce a lot of the behavioural, inflammatory, neurochemical, and neuropathological changes seen in the brain of patients with AD in similar regions (e.g. cingulate cortex).[2] Anyway, the correlation between ageing and inflammation had been indicated by an increased expression of NF-κB in aged rats.[22] The LPS model for the induction of inflammation and dysfunction is also used in other experiments; in fact, short-term blueberry nutritional supplement is able to restore the neuroprotective effect, mediated by a heat shock protein (HSP 70) in hippocampal cells of elderly rats, against LPS.[36]

So, these studies highlight the importance of inflammation in age-related phenomena and the possibility of studying them through some experimental *in vivo* and *in vitro* models, that can be also used for blueberry polyphenols. In fact, blueberry extracts also induce a decrease in the production of proinflammatory cytokines and enzymes in murine microglial cells activated by LPS, and such an effect has been seen to occur mainly at the transcriptional level through the reduction of mRNA of several proteins involved in inflammatory processes.[37] Since the enzyme nitric oxide synthetase is virtually inducible in all mammalian cell types by LPS and cytokines, the authors notice that blueberry extracts may have a critical role as anti-inflammatory drugs.[37] Due to another experimental model, microglial cells can also undergo inflammatory activation by beta-amyloid protein, and so blueberry extracts may counteract this action by inducing a significant increase in beta-amyloid clearance, inhibition of its aggregation in the form of fibres and suppression of microglial activation as a response to such a protein in murine cell cultures.[38] Such effects are mediated by the p44/42 mitogen-activated protein kinase pathway.[38] So, not only anti-inflammatory, but also anti-neurodegenerative effects can be detected in these *in vitro* models; this may be relevant in the pathogenesis of certain forms of dementia. Subsequently, on account of its neuroprotective properties and its varied mechanisms, an assessment of the effects of blueberry polyphenols in a whole organism has been proposed.

The nematode *Caenorhabditis elegans* represents a very useful model for this kind of study because of its short lifespan. Basically, using a complex mixture of blueberry polyphenols the mean lifespan of the nematode has been increased by 28% and the ageing-related decline has been attenuated.[39] It represents the first study which demonstrates that the beneficial effects of blueberry polyphenols are distinct from the antioxidant ones in a whole organism.[39] In fact, as reported previously, anti-inflammatory mechanisms could be independent of the other ones; this was seen in another experimental model of inflammation, namely the administration of kainate. Therefore, two later studies considered neuronal inflammation mediated by the excitotoxin kainate; in one of them, blueberry polyphenols were shown to attenuate learning impairment consequent upon neurotoxic insults, and to exert anti-inflammatory effects through changes in genic expression,[40] according to the previous *in vitro* results;[37] in the other study, it was deduced that blueberry supplementation may protect against neurodegeneration and cognitive deterioration dependent on excitotoxicity and oxidative stress.[41] In accordance with the aforementioned considerations, it appears that blueberry polyphenols are able to induce changes in the genic expression of neurons, that is related to synaptic plasticity and the development of cognitive functions.

So, it may be relevant to examine levels of various synaptic plasticity markers, including phosphorylated c-AMP-responsive element binding (f-CREB) and brain-derived neurotrophic factor (BDNF); indeed, significant levels of blueberry flavonoids have been detected in the plasma, the hippocampus and the cortex of rats supplemented for 12 weeks and increased levels of such markers have been found in the hippocampus of such animals.[42] A correlation between f-CREB and BDNF levels and the improvement of spatial memory has also been seen.[42] These data indicate that blueberry polyphenols could induce long-term synaptic changes involving protein synthesis and therefore functional and morphological changes (increase of neuronal communication efficiency, changes in the morphology of dendrites, and other ones), that are expression of synaptic plasticity due to memory improvement.[42] However, according to another study conducted with electrophysiological methods, blueberry flavonoids could not affect the expression of the subunits of the N-methyl-D-aspartate

(NMDA) glutamatergic receptor, which is essential for long-term potentiation (LTP), and synaptic plasticity and undergoes an age-related reduction; however, they could increase NR2B (NMDA receptor subunit 2B) phosphorylation and therefore this could compensate for both the reduction of this receptor and the LTP decline related to ageing.[43] This once again confirms the multiplicity of the targets and the mechanisms of blueberry polyphenols. This wide range of action also affects the main neurochemical systems of the brain, in accordance with its anti-neurodegenerative properties. The central cholinergic system is essential for the regulation of cognitive functions, as evidenced by the extensive loss of cholinergic neurons observed in the forebrain of patients with AD and the learning and memory deficits of anticholinergic drugs, such as scopolamine, in a variety of animal models.[44,45] High doses of blueberry extracts have been shown to improve cognitive performance in mice and this result could be attributed to decreased levels of acetylcholinesterase activity.[46] Reduced levels of lipid peroxidation products have been also found; this represents the first study that correlates the nootropic properties of blueberry polyphenols with the reduction of brain acetylcholinesterase activity, and lipid peroxidation.[46] An increase in low-molecular-weight antioxidants such as glutathione has also been detected.[46] Hence, the increase of antioxidant defences may be relevant not only in neurodegenerative disorders, but also in vascular ones; it is likely to mitigate the effects of brain ischaemia since the administration, for 2 weeks, of blueberry extracts along with other substances (green tea, D3 vitamin, and carnosine), in rats with middle cerebral artery occlusion, induced a reduction of neurological dysfunction consequent upon brain ischaemia and an increase of neurogenesis in the affected areas shown by suitable markers (bromodeoxyuridine, doublecortin).[47] It is interesting to note that significant results were present only after 2 weeks of supplementation, and so it can be concluded that the effects of polyphenols on the brain may depend not only on the dosage used but also on the type of substance administered, the time, and the effect investigated. Also, neurogenesis-related phenomena may be significant and could be useful for the treatment of human pathologies.

Neuroprotective effects were further confirmed in *in vitro* tests such as the incubation of neuronal cultures with biotransformed blueberry juice (with increased antioxidant content) in which the activation of pathways promoting cell survival (p38 and JNK pathways) and the blocking of cell death mediated by extracellular signal-regulated kinase (ERK) 1/2 and MAPK/ERK kinase (MEK) 1/2 induced by hydrogen peroxide were also seen.[48] An increased activity of antioxidant enzymes, that did not emerge from previous studies, was also evident.[48] As may be seen, there are a lot of data concerning tests on animals, but limited information concerning human beings. Nevertheless, in a recent study blueberry juice supplementation for 12 weeks to nine elderly subjects significantly improved the results of memory tests and induced a detectable, albeit not significant, improvement in mood and the normalization of glycemic and insulin levels.[49] In fact, the authors found evidence that hyperglycemia and hyperinsulinemia induce a proinflammatory condition that, apart from ascertained metabolic and cardiovascular diseases, may also promote the onset of neurodegenerative diseases.[49] However, this should be regarded as a preliminary study because of the small number of patients tested and the difficulty of finding a beverage as a placebo with the carbohydrate content and the organoleptic features of blueberry juice.[49] Therefore, other studies should be conducted.

Conclusions

As has been seen, it can certainly be stated that blueberry polyphenols have strong antioxidant properties even though it is less clear as to whether they are able to induce an absolute increase or an increased activity of endogenous antioxidants, and hence this aspect requires further study. The nootropic and neuroprotective properties of blueberry polyphenols are documented; they not only act through purely antioxidant mechanisms, but they also affect various levels of the regulation of cell homeostasis comprising gene expression, as underlined by the improvement of intercellular communication, signalling pathways, synaptic plasticity and its correlation with the improvement of motor and cognitive performance; all these effects could give rise to the anti-ageing, anti-inflammatory anti-excitotoxic effects noted. Blueberry extracts are effective in a wide range of dosages and there is the possibility of a differential action in various brain regions depending upon the different polyphenols involved and their individual pharmacological characteristics. Possible clinical applications could be in the realm of neurological disorders in which oxidative stress, inflammation, and neurodegeneration are associated (e.g. AD, Parkinson's disease, amyotrophic lateral sclerosis, ischemic/infectious/inflammatory diseases). Another possible field of application is anaesthesiology as neuroprotective agents, mainly for operations in which patients have a greater risk of developing intra- and post-operative complications such as bleeding and hypotension; they could also be of use in the intensive care unit for patients with various degrees of impairment in order to allow a more rapid recovery due to the anti-inflammatory and protective effects; for example, blueberry extract could be administrated as parenteral nutritional support. However, despite the preliminary evidence

of their efficacy in human beings, these compounds should not, at the present time, be administered in routine clinical practice, as further human trials are necessary to arrive at appropriate therapeutic strategies.

References

1 Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, *et al.* Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002;113:71–88.
2 Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruits polyphenolic compounds. Am J Clin Nutr 2005;81(Suppl):S313–6.
3 Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significante. Nutr Rev 1998;56:317–33.
4 Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 1996;20:933–56.
5 Pierpoint WS. Flavonoids in the human diet. Prog Clin Biol Res 1986;213:125–40.
6 Galli RL, Shukitt-Hale B, Youdim KA, Joseph JA. Fruit polyphenolics and brain aging. Nutritional interventions targeting age-related neuronal and behavioral deficits. Ann NY Acad Sci 2002;959:128–32.
7 Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. Neurobiol Aging 2005; 26(Suppl 1):S128–32.
8 Cantuti-Castelvetri I, Joseph JA. Differential effect of dopamine catabolism and uptake inhibition of dopamine-induced calcium dysregulation and viability loss. Free Rad Biol Med 1999;27: 1393–404.
9 Harman D. Aging: phenomena and theories. Ann NY Acad Sci 1998;854:1–7.
10 Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 1999;222: 236–45.
11 Halliwell B, Gutteridge JMC. Oxygen radicals and the nervous system. Trends Neurosci 1985;8:22–6.
12 Harman D. Free-radical theory of aging. Increasing the functional life span. Ann NY Acad Sci 1994;717:1–15.
13 Joseph JA, Denisova N, Fisher D, Shukitt-Hale B, Bickford PC, Prior R, *et al.* Membrane and receptors modification of oxidative stress vulnerability in aging. Nutritional considerations. Ann NY Acad Sci 1998;854:268–76.
14 Joseph JA, Denisova N, Bielinski D, Fisher DR, Shukitt-Hale D. Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention. Mech Ageing Devel 2000;116:141–53.
15 Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, *et al.* Long-term dietary strawberry, spinach or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 1998;18:8047–55.
16 Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, *et al.* Reversals of age-related declines in neuronal signal transduction, cognitive and motor behavioral deficits with blueberry, spinach or strawberry dietary supplementation. J Neurosci 1999;19:8114–21.
17 Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 1998;44:1309–15.
18 Cao G, Shukitt-Hale B, Bickford PC, Joseph JA, McEwen J, Prior RL. Hyperoxia-induced changes in antioxidant capacity and the effect of dietary antioxidants. J Appl Physiol 1999;86: 1817–22.
19 Sweeney MI, Kalt W, MacKinnon SL, Ashby J, Gottschall-Pass KT. Feeding rats diets enriched in lowbush blueberries for six weeks decreases ischemia-induced brain damage. Nutr Neurosci 2002;5:427–31.
20 Joseph JA, Denisova N, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, *et al.* Blueberry supplementation enhances signalling and prevents behavioral deficits in an Alzheimer disease model. Nutr Neurosci 2003;6:153–62.
21 Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee HG, Smith MA, *et al.* Modulation of hippocampal plasticity and and cognitive behaviour by short-term blueberry supplementation in aged rats. Nutr Neurosci 2004;7:309–16.
22 Goyarzu P, Malin DH, Lau FC, Taglialatela G, Moon WD, Jennings R, *et al.* Blueberry supplemented diet: effects on object recognition memory and nuclear factor-kappa B levels in aged rats. Nutr Neurosci 2004;7:75–83.
23 Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM, Joseph JA. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 2005;8:111–20.
24 Ramirez MR, Izquierdo I, Bassols Raseira MdC, Zuanazzi JA, Barros D, Henriques AT. Effect of liophylised *Vaccinium* berries on memory, anxiety and locomotion in adult rats. Pharm Res 2005;52:457–62.
25 Barros D, Olavo BA, Izquierdo I, Geracitano L, Bassols Raseira MdC, Henriques AT, *et al.* Behavioral and genoprotective effects of *Vaccinium* berries intake in mice. Pharm Bioc Behav 2006;84: 229–34.
26 Shukitt-Hale B, Carey AN, Jenkins D, Rabin BM, Joseph JA. Beneficial effects of fruit extracts on neuronal function and behaviour in a rodent model of accelerated aging. Neurobiol Aging 2006;28:1187–84.
27 Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr 2005;81:230–42.
28 Rabin BM, Shukitt-Hale B, Joseph JA, Todd P. Diet as a factor in behavioral radiation protection following exposure to heavy particles. Gravitat Space Biol 2005;18:11–7.
29 Rozovsky I, Finch CE, Morgan TE. Age-related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to downregulation. Neurobiol Aging 1998;19:97–103.
30 McGeer PL, McGeer EG. The inflammatory response system of the brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21:195–218.
31 Chang HN, Wang SR, Chiang SC. The relationship of aging to endotoxic shock and to production of TNF-α. J Gerontol 1996; 51:220–2.
32 Chang RC, Chen W, Hudson P, Wilson B, Han DS, Hong JS. Neurons reduce glial responses to lipopolysaccharide (LPS) and prevent injury of microglial cells from over-activaction by LPS. J Neurochem 2001;76:1042–49.
33 Spaulding CC, Walford RL, Effros RB. Caloric restriction inhibits the age-related dysregulation of the cytokines TNF-α and IL-6 in C3B10RF1 mice. Mech Ageing Dev 1997;93:87–94.
34 Volpato S, Guralnik JM, Ferrucci L, Balfour J, Chaves P, Fried LP, *et al.* Cardiovascular disease, interleukin-6, and risk of mortality in older women: the women's health and aging study. Circulation 2001;103:947–53.
35 Kushner I. C-reactive protein elevation can be caused by conditions other than inflammation and may reflect biologic aging. Cleve Clin J Med 2001;68:535–7.
36 Galli RL, Bielinski DF, Szprengiel A, Shukitt-Hale B, Joseph JA. Blueberry supplemented diet reverses age-related decline in hippocampal HSP70 neuroprotection. Neurobiol Aging 2006; 27:344–50.
37 Lau FC, Bielinski DF, Joseph JA. Inhibitory effects of blueberry extract on the the production of inflammatory mediators in lipopolysaccharide-activated BV2 microglia. J Neurosci Res 2007; 85:1010–17.
38 Zhu Y, Bickford PC, Sanberg P, Giunta B, Tan J. Blueberry opposes β-amyloid peptide-induced microglial activation via inhibition of p44/42 mitogen-activation protein kinase. Rejuvenation Res 2008;11:891–901.
39 Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow K. Blueberry polyphenols increase lifespan and thermotolerance in in Caenorhabditis elegans. Aging Cell 2006;5:59–68.
40 Shukitt-Hale B, Lau FC, Carey AN, Galli RL, Spangler EL, Ingram DK, *et al.* Blueberry polyphenols attenuate kainic acid-induced decrements in cognition and alter inflammatory gene expression in rat hippocampus. Nutr Neurosci 2008;11: 172–82.
41 Duffy KB, Spangler EL, Devan BD, Guo Z, Bowker ZL, Janas AM, *et al.* A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. Neurobiol Aging 2008;29:1680–89.
42 Williams CM, Mohsen MAE, Vauzour D, Rendeiro C, Butler LT, Ellis JA, *et al.* Blueberry-induced changes in spatial

working memory correlate in changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. Free Rad Biol Med 2008;45:295–305.
43 Coultrap SJ, Bickford PC, Browning MD. Blueberry-enriched diet can ameliorates age-related declines in NMDA receptor-dependent LTP. Age 2008;30:263–72.
44 Sarter M, Bruno JP. Cognitive functions of cortical acetylcholine: towards a unifying hypothesis. Brain Res Rev 1997;23: 28–46.
45 Silman I, Sussman JL. Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Curr Opin Pharmacol 2005;5:293–302.
46 Papandreou MA, Dimakopoulou A, Linardaki I, Cordopatis P, Klimis-Zakas D, Margarity M, *et al.* Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behav Brain Res 2009;198:352–8.
47 Yasuhara T, Hara K, Maki M, Masuda T, Sanberg CD, Sanberg PR, *et al.* Dietary supplementation exerts neuroprotective effects in ischemic stroke model. Rejuvenation Res 2008;11:201–14.
48 Vuong T, Matar C, Ramassamy C, Haddad PS. Biotransformed blueberry juice protects neurons from hydrogen peroxide-induced oxidative stress and mitogen-activated protein kinase pathway alterations. Br J Nutr 2010;12:1–8.
49 Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, *et al.* Blueberry supplementation improves memory in older adults. J Agric Food Chem 2010; 58:3996–4000.