

The Summer Meeting of the Nutrition Society was held at the University of Surrey, Guildford on 30 June–2 July 2009

Conference on ‘Over- and undernutrition: challenges and approaches’

Nutrition Society Silver Medal Lecture Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain

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The consumption of flavonoid-rich foods and beverages has been suggested to limit the neurodegeneration associated with a variety of neurological disorders and to prevent or reverse normal or abnormal deteriorations in cognitive performance. Flavonoids mediate these effects via a number of routes, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation and a potential to promote memory, learning and cognitive function. Originally, it was thought that such actions were mediated by the antioxidant capacity of flavonoids. However, their limited absorption and their low bioavailability in the brain suggest that this explanation is unlikely. Instead, this multiplicity of effects appears to be underpinned by three separate processes: first, through their interactions with important neuronal and glial signalling cascades in the brain, most notably the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways that regulate pro-survival transcription factors and gene expression; second, through an ability to improve peripheral and cerebral blood flow and to trigger angiogenesis and neurogenesis in the hippocampus; third, by their capacity to directly react with and scavenge neurotoxic species and pro-inflammatory agents produced in the brain as a result of both normal and abnormal brain ageing. The present review explores the potential inhibitory or stimulatory actions of flavonoids within these three systems and describes how such interactions are likely to underlie neurological effects.

Flavonoids: Neurological effects: Molecular actions

Representing one of the most important lifestyle factors, diet can strongly influence the incidence and onset of CVD and neurodegenerative disorders. Various phytochemical constituents of foods and beverages, in particular a class of compounds termed flavonoids, have been avidly investigated in recent years. They have been proposed to exert a multiplicity of neuroprotective actions within the brain, including a potential to protect neurons against injury induced by neurotoxins⁽¹⁾, an ability to suppress neuroinflammation⁽²⁾ and the potential to promote memory, learning and cognitive function^(3,4). This multiplicity of

effects appears to be underpinned by three processes. First, the flavonoids interact with important neuronal signalling cascades in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and differentiation⁽¹⁾. These effects include selective actions on a number of protein kinase and lipid kinase signalling cascades, most notably the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) pathways that regulate pro-survival transcription factors and gene expression. Second, the flavonoids induce peripheral and cerebral

Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; ERK, extracellular signal-regulated protein kinase; iNOS, inducible NO synthase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B.

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vascular blood flow in a manner that may lead to the induction of angiogenesis and of new nerve cell growth in the hippocampus⁽²⁾. Third, the flavonoids may react directly with and scavenge neurotoxic species and pro-inflammatory agents produced in the brain as a result of both normal and abnormal brain ageing⁽³⁾. Thus, the consumption of flavonoid-rich fruits, such as berries, throughout life holds a potential to limit the neurodegeneration associated with a variety of neurological disorders and to prevent or reverse normal or abnormal deteriorations in cognitive performance. The present review will highlight the neuroprotective mechanisms of flavonoids through their ability to interact with neuronal signalling pathways, their potential to inhibit neuroinflammation and their impact on the vascular system. It will also attempt to address whether at present there is enough data to support a causal relationship between the consumption of flavonoids and behavioural outcomes such as memory and learning in human subjects. Finally, in light of this current information, potential future areas of research will be highlighted that may help to fully address the impact of flavonoid-rich diets on human cognitive performance.

Flavonoid: sources and structure

Flavonoids are synthesised in plants from the reaction of a chalcone precursor with three molecules of malonyl-CoA. Under the action of the enzymes chalcone synthase and chalcone flavanone isomerase the chalcone precursor is isomerised into a flavanone^(5,6). Although they share a similar 2,3-dihydro-2-phenylchromen-4-one skeleton structure, the hydroxylation of the C-3 position of ring C allows differentiation between flavanonols from flavanones (Fig. 1). From these central intermediates the pathway diverges into several side branches, each resulting in a different class of flavonoids. Flavonoids are found ubiquitously in plants and as such are major constituents of a variety of fruit and vegetables, beverages such as tea and wine and seeds such as cocoa beans and grape seeds. All flavonoids share a common structure consisting of two aromatic rings (A and B), which are bound together by three C atoms, forming an oxygenated heterocycle (ring C; Fig. 1). Based on variations in the saturation of the basic flavan ring system, their alkylation and/or glycosylation and the hydroxylation pattern of the molecules flavonoids may be divided into seven subclasses: flavonols; flavones; flavanones; flavanonols; flavanols; anthocyanidins; iso-flavones (for review, see Manach *et al.*⁽⁷⁾). In addition, flavanols, which are sometimes referred to as flavan-3-ols, exist both as monomers and oligomers also known as condensed tannins or proanthocyanidins. These oligomeric forms differ on the basis of their constitutive units (e.g. catechin and epicatechin), their sequence and the positions of inter-flavanic linkages (C-4–C-6 or C-4–C-8 in the B-type series, with additional C2–O–C7 or C2–O–C5 bonds in A-type structures)⁽⁸⁾.

The flavanols are found predominantly in green and black teas, red wine, apples and cocoa. Variations in their structure reside in the hydroxylation pattern of ring B and the presence of a gallic acid moiety in C-3 position

(Fig. 1). The lack of a double bond at the 2–3 position and the presence of a 3-OH group on ring C creates two centres of asymmetry. Typical dietary flavanols include catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate and proanthocyanidins, which may be found at high concentration in cocoa, tea, red wine and fruits such as apples, grapes and many berry fruits. The sources of anthocyanins such as pelargonidin, cyanidin and malvidin include red wine and berry fruits such as blueberries (*Vaccinium corymbosum*), blackberries (*Rubus fruticosus*), cherries (*Prunus avium*) and strawberries (*Fragaria × ananassa*). These compounds exist as glycosides in plants, are water soluble and appear red or blue according to the pH of their environment. Individual anthocyanins arise from the variation in number and arrangement of the hydroxyl and methoxy groups around the three rings (Fig. 1). Flavones, (e.g. apigenin, luteolin) are found in artichoke (*Cynara cardunculus*), celery (*Apium graveolens* L.) and parsley (*Petroselinum crispum*), chives (*Allium schoenoprasum*) and other herbs. Hydroxylation on position 3 of the flavone structure gives rise to the 3-hydroxyflavones, also termed the flavonols (e.g. kaempferol, quercetin), which are found predominantly in onions (*Allium cepa* L.), leeks (*Allium ampeloprasum* var. *porrum* (L.)) and broccoli (*Brassica oleracea*; Fig. 1). Dietary flavanones (e.g. naringenin, hesperetin, taxifolin) are found predominantly in citrus fruit and tomatoes (Fig. 1). Finally, isoflavones such as daidzein and genistein are a subclass of the flavonoid family found in soyabean and soya products. They have a large structural variability and >600 isoflavones have been identified to date and are classified according to oxidation level of the central pyran ring (Fig. 1).

Absorption, metabolism and distribution of flavonoids

Although flavonoids have been identified as powerful antioxidants *in vitro*^(9–11), their ability to act as antioxidants *in vivo* is limited by the extensive biotransformation and conjugation that occurs during their absorption from the gastrointestinal tract, in the liver and finally in cells (for review, see Williamson & Manach⁽¹²⁾, Manach *et al.*⁽¹³⁾, Scalbert & Williamson⁽¹⁴⁾ and Spencer *et al.*⁽¹⁵⁾). In the small intestine and liver dietary flavonoids (and other polyphenols) are substrates for phase I (hydrolysing and oxidising) and phase II (conjugating and detoxifying) enzymes, i.e. they are de-glucosylated and metabolised into glucuronides, sulfates and *O*-methylated derivatives^(14,16,17). Further metabolism occurs in the colon, in which the enzymes of the gut microflora induce the breakdown of flavonoids to simple phenolic acids that may then undergo absorption and are further metabolised in the liver^(15,18). The extent of metabolism in the large intestine has been largely ignored to date, although there is now intense interest in the generation, absorption and potential bioactivity of these bacterially-derived forms. Moreover, it has recently been suggested that their metabolism by bacteria in the colon may also result in the selective beneficial growth of several bacterial groups and species⁽¹⁹⁾. Post absorption from the gastrointestinal tract

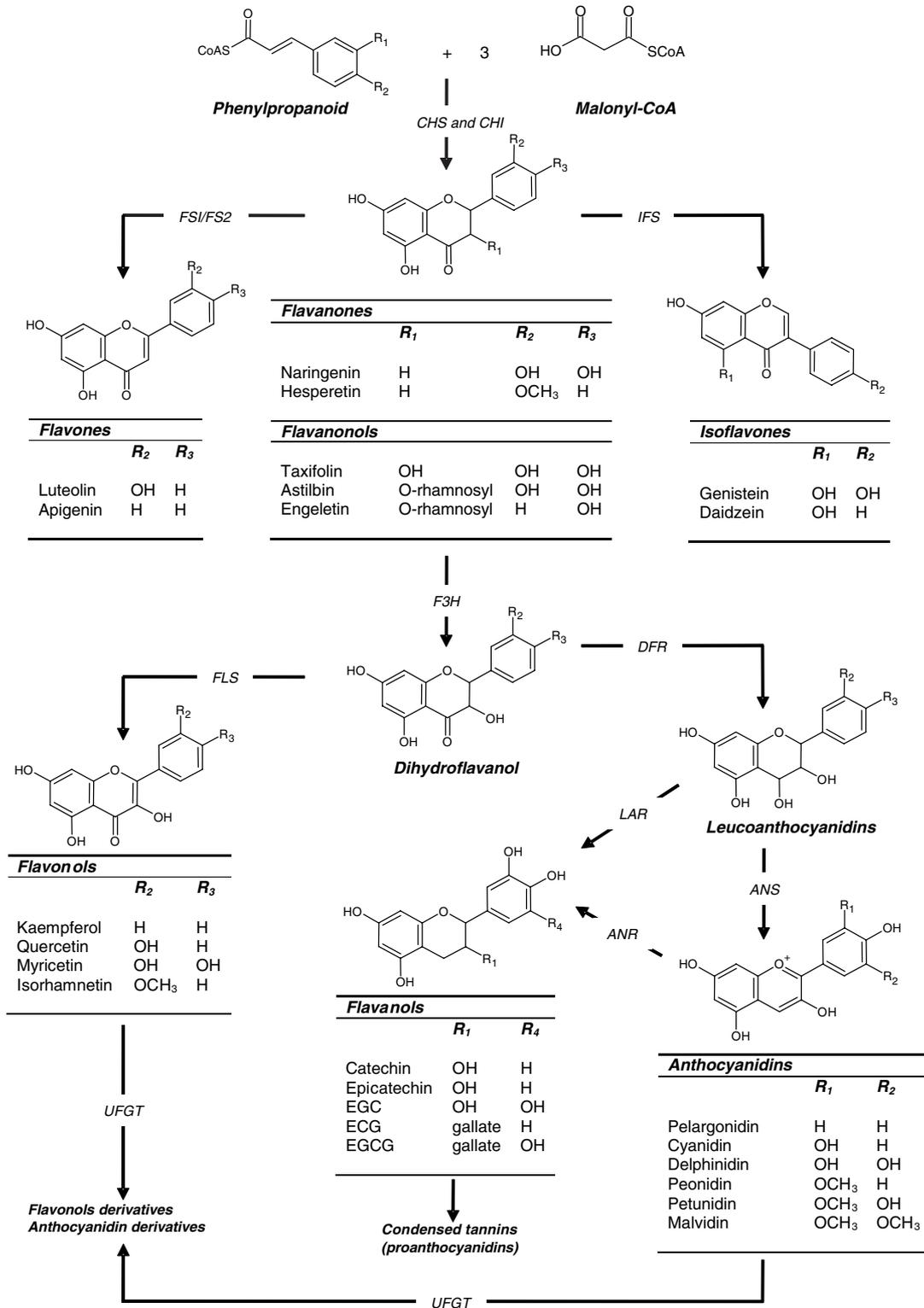


Fig. 1. The structures of the main classes of flavonoids and their biosynthesis. The major differences between the individual groups reside in the hydroxylation pattern of the ring structure, the extent of saturation of ring C and the substitution in the C-3 position. All flavonoids are derived from chalcone precursors that are derived from phenylpropanoid and three molecules of malonyl-CoA and biosynthesised by chalcone synthase (CHS). Various enzymes act to bring about the formation of the various flavonoid classes: chalcone isomerase (CHI), flavone synthase (FSI/FS2), isoflavone synthase (IFS), flavanone 3-hydroxylase (F3H), dihydroflavanol reductase (DFR), anthocyanidin synthase (ANS), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR), UDP glucose-flavonoid 3-O-glucosyl transferase (UFGT), flavonol synthase (FLS). EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate.

flavonoids also undergo at least three types of intracellular metabolism: (1) oxidative metabolism; (2) P450-related metabolism; (3) conjugation with thiols, particularly glutathione⁽²⁰⁾. Many studies have indicated that although glucuronides, sulfates, *O*-methyl derivatives and intracellular metabolites such as flavonoid–glutathione adducts may still participate in antioxidant reactions (in particular, scavenging reactive oxygen and nitrogen species) in the circulation, their effectiveness to do so is greatly reduced relative to their parent aglycones (or indeed those found in plants)^(21–26).

In order for flavonoids to influence brain function directly they must additionally penetrate the blood–brain barrier, which controls entry of xenobiotics into the brain⁽²⁷⁾. Flavanones such as hesperetin, naringenin and their *in vivo* metabolites, along with some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, have been shown to traverse the blood–brain barrier in relevant *in vitro* and *in situ* models⁽²⁸⁾. Their extent of blood–brain barrier penetration is dependent on compound lipophilicity⁽²⁹⁾, i.e. less polar *O*-methylated metabolites may be capable of greater brain uptake than the more polar flavonoid glucuronides. However, evidence exists to suggest that certain drug glucuronides may cross the blood–brain barrier⁽³⁰⁾ and exert pharmacological effects^(31,32), suggesting that there may be a specific uptake mechanism for glucuronides *in vivo*. Their brain entry may also depend on their interactions with specific efflux transporters expressed in the blood–brain barrier such as P-glycoprotein⁽³³⁾, which appears to be responsible for the differences between naringenin and quercetin flux into the brain *in situ*⁽²⁸⁾. In animals flavanones have been found to enter the brain following their intravenous administration⁽³⁴⁾, whilst epigallocatechin gallate⁽³⁵⁾, epicatechin⁽³⁶⁾ and anthocyanins^(37,38) are found in the brain after their oral administration. Furthermore, several anthocyanins have been identified in different regions of the brains of rats⁽³⁹⁾ and blueberry-fed pigs⁽⁴⁰⁾, with eleven intact anthocyanins found in the cortex and cerebellum. Although further work is necessary to establish their bioavailability to the brain, particularly in human subjects, these results suggest that they may localise in the brain and are capable of direct neuroprotective and neuromodulatory actions.

Antioxidants or signalling molecules?

Historically, the biological actions of flavonoids, including those on the brain, have been attributed to their ability to exert antioxidant actions⁽⁹⁾, through their ability to scavenge reactive species or through their possible influences on intracellular redox status⁽⁴¹⁾. However, it is now thought highly unlikely that this classical H-donating antioxidant activity accounts for the bioactivity of flavonoids *in vivo*, particularly in the brain where they are found at only very low concentrations⁽¹⁾. Indeed, it is clear that the concentrations of flavonoids and their metabolite forms accumulated *in vivo*⁽⁴²⁾ are lower (high nM, low μ M) than those recorded for small-molecule antioxidant nutrients such as ascorbic acid and α -tocopherol⁽⁴³⁾. Consequently, the beneficial effects of flavonoid metabolites *in vivo* are unlikely

to result from their ability to out-compete antioxidants such as ascorbate, which are present at higher concentrations (high μ M to mM). However, evidence has accumulated to suggest that the cellular effects of flavonoids may be mediated by their interactions with specific proteins central to intracellular signalling cascades⁽⁴⁴⁾, such as the MAPK signalling pathway and the PI3K/Akt signalling cascade (Fig. 2). For example, flavonoids have been shown to be capable of exerting neuroprotective actions (at low concentration) via their interactions with critical neuronal intracellular signalling pathways pivotal in controlling neuronal survival and differentiation, long-term potentiation and memory^(3,45–47). The present review will examine such actions and how they may impact on the progression of chronic brain disease.

Direct interactions with signalling pathways

Flavonoids have been shown to exert neuronal effects through their interactions with a number of protein kinase and lipid kinase signalling cascades, such as the PI3K/Akt, tyrosine kinase, protein kinase C and MAPK signalling pathways^(48–54) (Fig. 2). Inhibitory or stimulatory actions at these pathways are likely to profoundly affect neuronal function by altering the phosphorylation state of target molecules and/or by modulating gene expression. Although selective inhibitory actions at these kinase cascades may be beneficial in cancer, proliferative diseases, inflammation and neurodegeneration, they could be detrimental during development, particularly in the immature nervous system in which protein kinase and lipid kinase signalling regulates survival, synaptogenesis and neurite outgrowth. In the mature brain post-mitotic neurones utilise MAPK and PI3K cascades in the regulation of key functions such as synaptic plasticity and memory formation^(55,56), thus flavonoid interactions within these pathways could have unpredictable outcomes and will be dependent both on the cell type and disease studied.

MAPK belong to the superfamily of serine/threonine kinases and play a central role in transducing various extracellular signals into intracellular responses^(57,58). The best-characterised MAPK pathways are the mitogenic extracellular signal-regulated protein kinase (ERK) pathway and the stress-activated c-Jun N-terminal kinase (JNK) and p38 cascades (Fig. 2). Once activated ERK, JNK and p38 phosphorylate a number of cytosolic proteins and transcription factors resulting in the enhancement of their transcriptional activities and activation of dependent genes⁽⁵⁹⁾. ERK and JNK are generally considered as having opposing actions, in particular in neuronal apoptosis⁽⁶⁰⁾. ERK1/2 are usually associated with pro-survival signalling^(61–63) through mechanisms that may involve activation of the cAMP response element-binding protein (CREB)^(62,64) (Fig. 2), the up-regulation of the anti-apoptotic protein Bcl-2 and non-transcriptional inhibition of Bcl-xL/Bcl-2-associated death promoter^(62,63). On the other hand, JNK has been strongly linked to transcription-dependent apoptotic signalling^(65,66), possibly through the activation of c-Jun⁽⁶⁷⁾ and other activated protein-1 proteins including JunB, JunD and activating transcription factor 2⁽⁶⁸⁾. Many investigations have indicated that

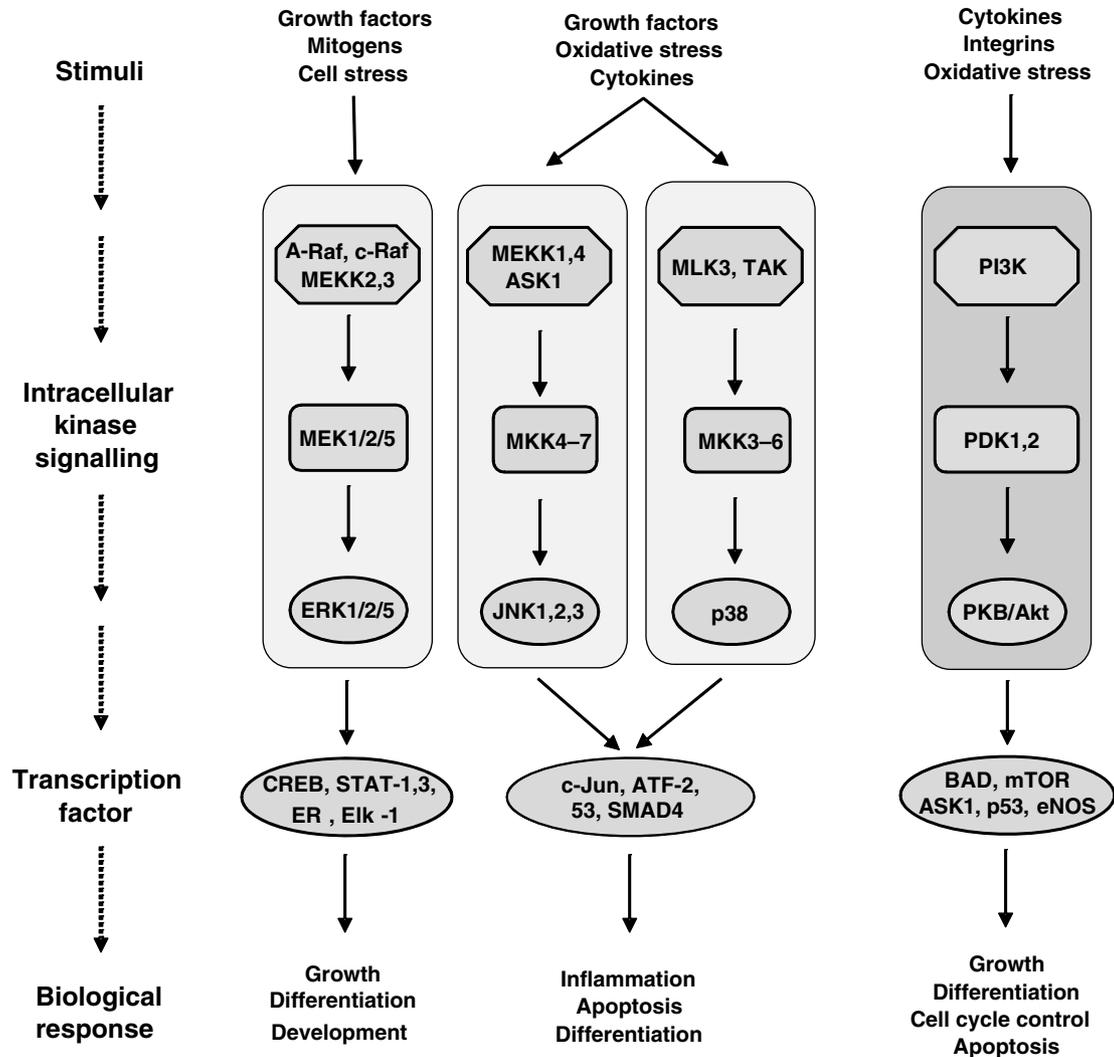


Fig. 2. Potential points of flavonoid action within mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt signalling cascades in neurons and glia. Activation of extracellular signal-regulated protein kinase (ERK) 1/2/5 and Akt are generally pro-survival, whilst inhibitory actions on c-Jun N-terminal kinase (JNK) and p38 pathways are also likely to be neuroprotective. Through their effects on these pathways they may regulate a wide variety of processes, including cell growth, cell proliferation, differentiation, cell cycle entry, cell migration and apoptosis. MEK, MKK, MAPK kinases; MEKK, MEK kinase; CREB, cAMP response element-binding protein; STAT, signal transducer and activator of transcription; ER, oestrogen receptor; ASK1, apoptosis signal-regulating kinase 1; ATF-2, activating transcription factor 2; MLK3, JNK/stress-activated protein kinase activator mixed lineage kinase 3; TAK, transforming growth factor β -activated kinase 1; PDK, phosphoinositide-dependent kinase-1; PKB, protein kinase B; BAD, Bcl-xL/Bcl-2 associated death promoter; mTOR, mammalian target of rapamycin; eNOS, endothelial nitric oxide synthase.

flavonoids and their metabolites may interact selectively within the MAPK signalling pathways^(52,69). The potential modulation of MAPK signalling by flavonoids is important as ERK1/2 and JNK are involved in growth factor-induced mitogenesis, differentiation, apoptosis and various forms of cellular plasticity^(65,66,70-72).

There is much evidence to support the actions of flavonoids on the ERK pathway^(53,73,74), which appear to be mediated by interactions with MAPK kinases MEK1 and MEK2 and potentially membrane receptors^(44,52). Indeed, flavonoids have close structural homology to specific pharmacological modulators of ERK signalling such as PD98059 (2'-amino-3'-methoxyflavone). The flavonol

quercetin and to a lesser extent its *O*-methylated metabolites have been shown to induce neuronal apoptosis via a mechanism involving the inhibition of ERK rather than by induction of pro-apoptotic signalling through JNK⁽⁵⁴⁾. The potent inhibition of ERK activation, and indeed Akt/protein kinase B (PKB) phosphorylation, is also accompanied by downstream activation of Bcl-xL/Bcl-2-associated death promoter and a subsequent strong activation of caspase-3. On the other hand, some flavonoids have been observed to exert a stimulatory effect on ERK1/2. For example, the flavan-3-ol (-)-epicatechin and one of its metabolites, 3'-*O*-methyl(-)-epicatechin, have been shown to stimulate phosphorylation of ERK1/2 and the

downstream transcription factor CREB at physiologically-relevant concentrations⁽⁷⁵⁾. Interestingly, this activation of the ERK pathway is no longer apparent at higher concentrations, suggesting that effects on this pathway are concentration specific. Furthermore, stimulation of ERK1/2 and CREB is not observed with (-)-epicatechin-5-O- β -D-glucuronide, suggesting that effects on the ERK pathway may be dependent on cell or membrane permeability, as has been previously reported⁽⁷⁶⁾. In support of these observations, the protective action of another flavanol, epigallocatechin gallate, against 6-hydroxydopamine toxicity and serum deprivation has been shown to involve the restoration of both protein kinase C and ERK1/2 activities^(77,78).

There is strong evidence linking the activation of JNK to neuronal loss in response to a wide array of pro-apoptotic stimuli in both developmental and degenerative death signalling^(65,68,79). The activation of the JNK pathway and the death of specific neuronal populations are crucial events during early brain development⁽⁸⁰⁾. As with the other MAPK, the core signalling unit is composed of an MAPK kinase kinase, typically MAPK kinase kinases MEK1-4, which phosphorylate and activate MAPK kinases MKK4-7, which then phosphorylate and activate the JNK^(79,81) (Fig. 2). Another MAPK kinase kinase, apoptosis signal-regulating kinase 1, also plays an essential role in stress-induced apoptosis^(82,83). Apoptosis signal-regulating kinase 1 can be activated in response to a variety of stress-related stimuli and activates MKK4, which in turn activates JNK (Fig. 2) and indeed p38⁽⁸⁴⁾. Overexpression of apoptosis signal-regulating kinase 1 has been shown to induce the activation of both JNK and p38 and lead to apoptosis via signals involving the mitochondrial cell death pathway^(80,82). Investigation has indicated that oxidative-induced activation of caspase-3 in neurons is blocked by flavonoids, providing compelling evidence in support of a potent anti-apoptotic action of flavonoids in these cells^(53,73,76,85). The flavanols epicatechin and 3'-O-methyl-epicatechin have been shown to protect neurons against oxidative damage via a mechanism involving the suppression of JNK and downstream partners c-jun and pro-caspase-3^(53,86). Similarly, the flavone baicalein has been shown to inhibit 6-hydroxydopamine-induced JNK activation and neuronal cell death and quercetin may suppress JNK activity and apoptosis induced by H₂O₂^(87,88), 4-hydroxy-2-nonenal⁽⁸⁹⁾ and TNF α ⁽⁶⁹⁾.

In addition to the MAPK pathway flavonoids have been shown to modulate signalling through the serine/threonine kinase Akt/PKB, one of the main downstream effectors of PI3K, a pivotal kinase in neuronal survival⁽⁹⁰⁻⁹³⁾ (Fig. 2). Flavonoids have long been known to modulate PI3K, via direct interactions with its ATP-binding site⁽⁵¹⁾. Indeed, a number of studies have demonstrated that the structure of flavonoids determines whether or not they act as potent inhibitors of PI3K^(50,94). One of the most selective PI3K inhibitors available, LY294002, was modelled on the structure of quercetin^(48,49,95). Quercetin and some of its *in vivo* metabolites have been shown to inhibit pro-survival Akt/PKB signalling pathways by a mechanism of action consistent with quercetin and its metabolites acting at and inhibiting PI3K activity⁽⁵⁴⁾. However, other flavonoids such as the citrus flavanone hesperetin induce the activation

of Akt/PKB and the inhibition of pro-apoptotic proteins such as apoptosis signal-regulating kinase 1, Bcl-xL/Bcl-2-associated death promoter, caspase-9 and caspase-3 in cortical neurons⁽⁷³⁾.

Inhibition of neuroinflammation

Neuroinflammatory processes in the brain are believed to play a crucial role in the development of Alzheimer's disease and Parkinson's disease^(96,97) as well as injury associated with stroke⁽⁹⁸⁾. Activated microglia and/or astrocytes release cytokines and other mediators that have been linked to the apoptotic death of neurons. In particular, increases in cytokine production (IL-1 β , TNF α), inducible NO synthase (iNOS) and NO and increased NADPH oxidase activation⁽⁹⁹⁾ all contribute to glial-induced neuronal death (Fig. 3). The majority of these events are controlled by upstream MAPK signalling, which mediates both the transcriptional and post-transcriptional regulation of iNOS and cytokines in activated microglia and astrocytes^(100,101). Evidence suggests that the non-steroidal anti-inflammatory drug ibuprofen may be effective in delaying the onset of neurodegenerative disorders, particularly Parkinson's disease, by reducing inflammatory injury in specific brain regions⁽¹⁰²⁾. Thus, there is a desire to develop new drugs capable of preventing progressive neuronal loss linked to neuroinflammation⁽²⁾. Recently, the flavanone naringenin, found at high concentrations in citrus fruits, has been found to be highly effective in reducing lipopolysaccharide- and interferon- γ -induced glial cell activation and resulting neuronal injury⁽¹⁰³⁾ via an inhibition of p38 and signal transducer and activator of transcription-1 and a reduction in iNOS expression (Fig. 3). The structurally-related flavanone hesperetin and other flavonoids appear to be incapable of inhibiting pathways leading to NO production, although they have been found to partially alleviate neuroinflammation through the inhibition of TNF α production⁽¹⁰³⁾.

Flavonoids present in blueberry have also been shown to inhibit NO, IL-1 β and TNF α production in activated microglia cells⁽¹⁰⁴⁾, whilst the flavonol quercetin⁽¹⁰⁵⁾, the flavones wogonin and baicalein⁽¹⁰⁶⁾, the flavanols catechin and epigallocatechin gallate⁽¹⁰⁷⁾ and the isoflavone genistein⁽¹⁰⁸⁾ have all been shown to attenuate microglia- and/or astrocyte-mediated neuroinflammation via mechanisms that include inhibition of, in astrocytes and microglia: (1) iNOS and cyclooxygenase 2 expression; (2) NO production; (3) cytokine release; (4) NADPH oxidase activation and subsequent reactive oxygen species generation. All these effects appear to depend on an ability to directly modulate protein kinase and lipid kinase signalling pathways^(45,46). For example, they may act by inhibiting MAPK signalling cascades, such as p38 or ERK1/2, which regulate both iNOS and TNF α expression in activated glial cells^(101,103) (Fig. 3); fisetin inhibits p38 MAPK phosphorylation in lipopolysaccharide-stimulated BV-2 microglial cells⁽¹⁰⁹⁾ and the flavone luteolin inhibits IL-6 production in activated microglia via inhibition of the JNK signalling pathway⁽¹¹⁰⁾. The effects of flavonoids on these kinases may influence downstream pro-inflammatory transcription factors important in iNOS transcription. One of

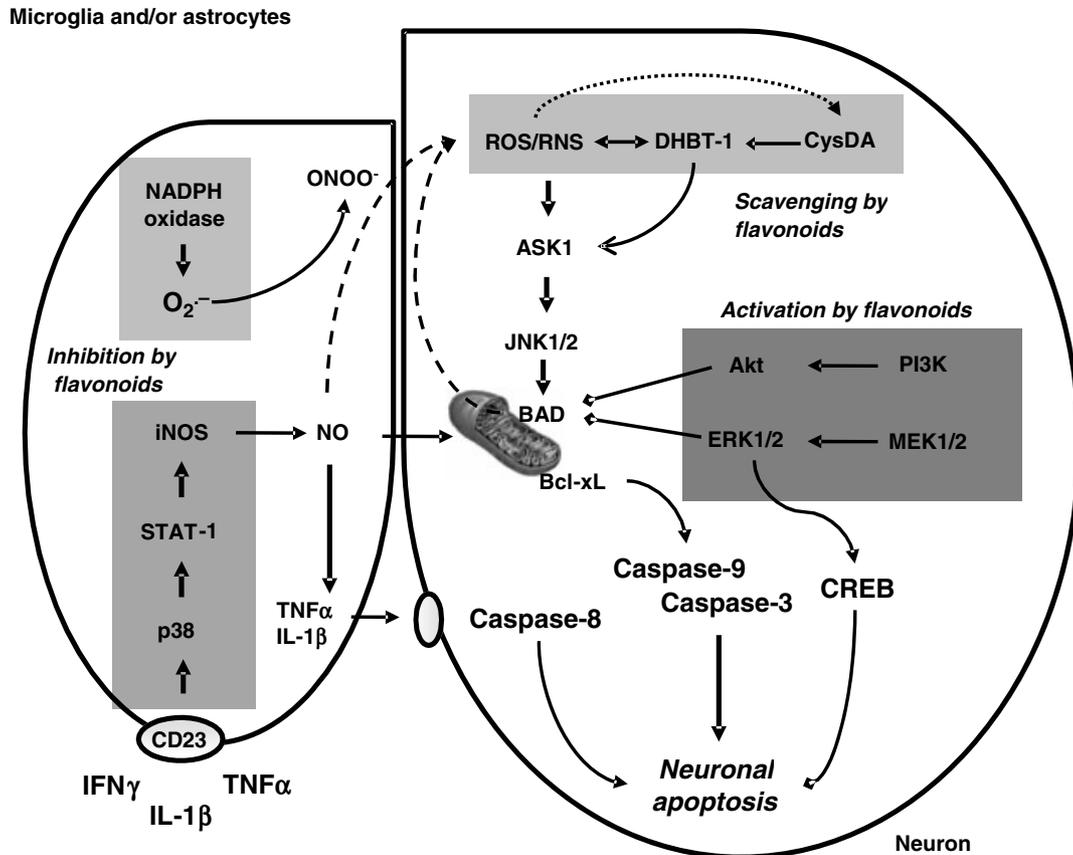


Fig. 3. The cellular mechanisms by which flavonoids and their metabolites protect against neuroinflammation and neuronal injury induced by 5-S-cysteinyldopamine (CysDA), dihydrobenzothiazine (DHBT-1) and related reactive oxygen species (ROS). Flavonoids inhibit the p38 pathway glia cells leading to a reduction in inducible nitric oxide synthase (iNOS) expression and nitric oxide release. In neurons they scavenge neurotoxic species and induce pro-survival signalling pathways, such as extracellular signal-regulated protein kinase (ERK) 1/2 and phosphatidylinositol 3-kinase (PI3K)/Akt, leading to an inhibition of neuronal apoptosis. STAT-1, signal transducer and activator of transcription-1; IFN- γ , interferon- γ ; RNS, reactive nitrogen species; ASK1, apoptosis signal-regulating kinase 1; JNK, c-Jun N-terminal kinase; BAD, Bcl-xL/Bcl-2 associated death promoter; MEK1/2, mitogen-activated protein kinase kinases; CREB, cAMP response element-binding protein. \longrightarrow , Activation; \longleftarrow , inhibition.

these transcription factors, NF- κ B, responds to p38 signalling and is involved in iNOS induction⁽¹¹¹⁾, suggesting that there is interplay between signalling pathways, transcription factors and cytokine production in determining the neuroinflammatory response in the central nervous system. In support of this notion, some flavonoids have been shown to prevent transcription factor activation, with the flavonol quercetin and the flavanone naringenin able to suppress NF- κ B, signal transducer and activator of transcription-1 and activating protein-1 activation in lipopolysaccharide- and interferon- γ -activated microglial cells^(103,105).

Inhibition of toxin-induced neuronal injury

The underlying neurodegeneration observed in Parkinson's disease, Alzheimer's disease, and other neurodegenerative diseases is believed to be triggered by multi-factorial processes, including neuroinflammation, glutamatergic excitotoxicity, increases in Fe and/or depletion of endogenous antioxidants^(112–114). There is a growing body of evidence

to suggest that flavonoids and other polyphenols may be able to counteract this neuronal injury, thereby delaying the progression of these brain pathologies^(1,46,115–119). For example, a *Ginkgo biloba* extract has been shown to protect hippocampal neurons against NO- and β -amyloid-induced neurotoxicity⁽¹²⁰⁾ and studies have demonstrated that the consumption of green tea may have a beneficial effect in reducing the risk of Parkinson's disease^(121–124). In agreement with the latter study, tea extracts and pure (–)-epigallocatechin 3-gallate have been shown to attenuate 6-hydroxydopamine-induced toxicity⁽¹²⁵⁾, to protect against hippocampal injury during transient global ischaemia⁽¹²⁶⁾ and to prevent nigral damage induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine⁽¹²⁷⁾.

The death of nigral neurons in Parkinson's disease is thought to involve the formation of the endogenous neurotoxin 5-S-cysteinyldopamine and its oxidation product dihydrobenzothiazine^(128,129) (Fig. 3). 5-S-cysteinyldopamine conjugates possess strong neurotoxicity and initiate a sustained increase in intracellular reactive oxygen species in neurons leading to DNA oxidation, caspase-3

activation and delayed neuronal death⁽¹²⁸⁾ (Fig. 3). Such adducts may be generated by reactive species⁽¹³⁰⁾ and have been observed post mortem to be elevated in the substantia nigra of patients with Parkinson's disease⁽¹³¹⁾, suggesting that such species may be potential endogenous nigral toxins. However, 5-S-cysteinyldopamine-induced neuronal injury is effectively counteracted by nanomolar concentrations of various flavonoids, including pelargonidin, quercetin, hesperetin, caffeic acid, 4'-O-methyl derivatives of catechin and epicatechin⁽¹³⁰⁾. Furthermore, in the presence of the flavanol (+)-catechin tyrosinase-induced formation of 5-S-cysteinyldopamine is inhibited by a mechanism linked to the capacity of catechin to undergo tyrosinase-induced oxidation to yield cysteinyl-catechin adducts⁽¹³²⁾. In contrast, the inhibition afforded by flavanones, such as hesperetin, is not accompanied by the formation of cysteinyl-hesperetin adducts, indicating that it may inhibit via direct interaction with tyrosinase⁽¹³²⁾.

Reactive oxygen and nitrogen species have also been proposed to play a role in the pathology of many neurodegenerative diseases⁽¹¹³⁾ (Fig. 3). There is abundant evidence that flavonoids are effective in blocking this oxidant-induced neuronal injury, although their potential to do so is thought not to rely on direct radical- or oxidant-scavenging activity^(76,86). Instead, they are believed to act by modulating a number of protein kinase and lipid kinase signalling cascades, such as the PI3K/Akt, tyrosine kinase, protein kinase C and MAPK signalling pathways^(1,46). Inhibitory or stimulatory actions at these pathways are likely to profoundly affect neuronal function by altering the phosphorylation state of target molecules, leading to changes in caspase activity, and/or by gene expression. For example, flavonoids have been observed to block oxidative-induced neuronal damage by modulating the activation of both the MAPK^(53,87–89) and PI3K/Akt⁽⁷³⁾ signalling pathways and the activation of caspase-3^(76,86), providing evidence in support of their potent anti-apoptotic action, and have been found to protect neurons against a variety of neurotoxic insults.

Modulation of synaptic plasticity and neuro-cognitive performance

There is now much evidence to suggest that fruit- and vegetable-derived phytochemicals, in particular flavonoids, are capable of promoting beneficial effects on memory, learning and cognitive performance^(119,133–141). It appears that these low-molecular-weight non-nutrient components are able to impact on memory through their ability to exert effects directly on the brain's innate architecture for memory^(3,47,142,143). The concentrations of flavonoids and their metabolites that reach the brain are thought to be sufficiently high to exert pharmacological activity at receptors, kinases and transcription factors. Although the precise site of their interaction with signalling pathways remains unresolved, evidence indicates that they are capable of acting in a number of ways: (1) by binding to ATP sites on enzymes and receptors; (2) by modulating the activity of kinases directly, i.e. MAPK kinase kinase, MAPK kinase or MAPK; (3) by affecting the function of

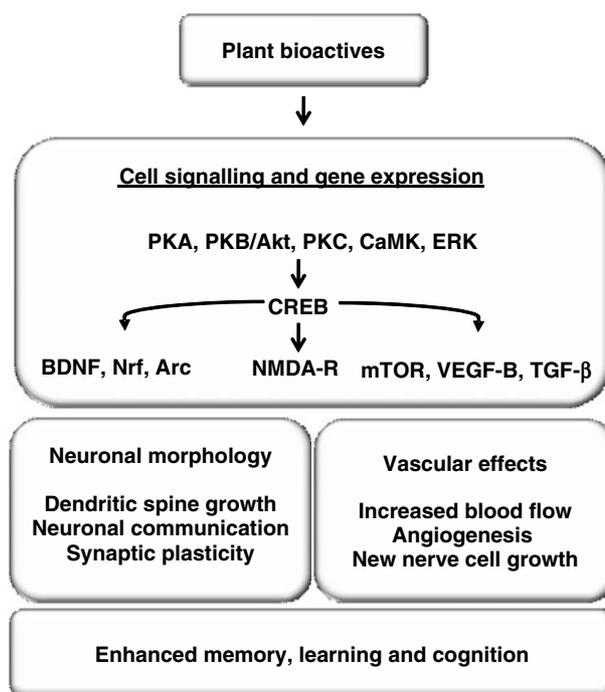


Fig. 4. Flavonoid-induced activation of neuronal signalling and gene expression in the brain. Such processes may lead to changes in synaptic plasticity and neurogenesis in the brain that ultimately influence memory, learning and cognition. PKA, PKB, PKC, protein kinase A, B and C respectively; CaMK, Ca-calmodulin kinase; ERK, extracellular signal-regulated protein kinase; CREB, cAMP response element-binding protein; BDNF, brain-derived neurotrophic factor; Arc, the activity-regulated cytoskeletal-associated protein termed Arc/Arg3.1; NMDA-R, N-methyl D-aspartate receptor; mTOR, mammalian target of rapamycin; VEGF- β , vascular endothelial growth factor β ; TGF- β , transforming growth factor β .

important phosphatases, which act in opposition to kinases; (4) by preserving Ca^{2+} homeostasis, thereby preventing Ca^{2+} -dependent activation of kinases in neurons; (5) by modulating signalling cascades lying downstream of kinases, i.e. transcription factor activation and binding to promoter sequences^(3,4). By affecting such pathways they have the potential to induce new protein synthesis in neurons and thus an ability to induce morphological changes that have a direct influence on memory acquisition, consolidation and storage.

Various individual cascades have been linked with this control of *de novo* protein synthesis in the context of long-term potentiation, synaptic plasticity and memory (Fig. 3): (1) cAMP-dependent protein kinase A; (2) PKB/Akt⁽⁸¹⁾; (3) protein kinase C; (4) Ca-calmodulin kinase⁽⁸³⁾; (5) ERK⁽³⁾. All five pathways converge to signal to CREB, a transcription factor that binds to the promoter regions of many genes associated with synapse re-modelling, synaptic plasticity and memory (Fig. 4). Flavonoids are now well known to modulate neuronal signalling pathways crucial in inducing synaptic plasticity⁽³⁾, and although each of these pathways are known to be involved in increasing the number of, and strength of, connections between neurons, flavonoids appear to interact primarily with the ERK and PKB/Akt pathways^(46,54,75). The activation of these

pathways by blueberry flavonoids, along with the activation of the transcription factor CREB and production of neurotrophins such as brain-derived neurotrophic factor (BDNF), is known to be required during memory acquisition and consolidation. Agents capable of inducing pathways leading to CREB activation will have the potential to enhance both short-term and long-term memory⁽¹³³⁾ by providing a more efficient structure for interpreting afferent nerve or sensory information. One mechanism by which this provision may come about is through flavonoid-induced increases in neuronal spine density and morphology, two factors considered to be vital for learning and memory⁽¹⁴⁴⁾. Changes in spine density, morphology and motility have been shown to occur with paradigms that induce synaptic as well as altered sensory experience and lead to alterations in synaptic connectivity and strength between neuronal partners, affecting the efficacy of synaptic communication (Fig. 4).

Fisetin, a flavonoid found in strawberries, has been shown to improve long-term potentiation and to enhance object recognition in mice by a mechanism dependent on the activation of ERK and CREB^(145,146). Similarly, the flavanol (–)-epicatechin induces both ERK1/2 and CREB activation in cortical neurons and subsequently increases CREB-regulated gene expression⁽⁷⁵⁾, whilst nanomolar concentrations of quercetin are effective at enhancing CREB activation⁽⁵⁴⁾. Blueberry-induced improvements in memory have been shown to be mediated by increases in the phosphorylation state of ERK1/2, rather than that of Ca–calmodulin kinase (II and IV) or protein kinase A⁽¹³³⁾. Other flavonoids have also been found to influence the ERK pathway, with the citrus flavanone hesperetin capable of activating ERK1/2 signalling in cortical neurons⁽⁷³⁾ and flavanols such as (–)-epigallocatechin 3-gallate restoring both protein kinase C and ERK1/2 activities in 6-hydroxydopamine-treated and serum-deprived neurons^(77,78). Furthermore, this ability to activate the ERK pathway is not restricted to neurons and has also been observed in fibroblasts exposed to low concentrations of epicatechin⁽¹⁴⁷⁾.

CREB activation downstream of ERK appears critical in the induction of long-lasting changes in synaptic plasticity and memory^(148–150) and disruption of CREB activity specifically blocks the formation of long-term memory⁽¹⁵¹⁾, whereas agents that increase the amount or activity of CREB accelerate the process⁽¹⁵²⁾. CREB is known to be a critical transcription factor linking the actions of neurotrophins such as BDNF to neuronal survival, differentiation and synaptic function^(153–155). Consequently, the central role of CREB in these processes has led to considerable interest in identifying safe effective agents that may enhance the activity of CREB in specific regions of the brain, as these agents may lead to an improvement in memory⁽¹⁵²⁾. Recent studies have shown that spatial memory performance in rats supplemented with blueberry correlates well with the activation of CREB and with increases in both pro-BDNF and mature BDNF levels in the hippocampus⁽¹³³⁾. Regulation of BDNF is interesting as this neurotrophin is intimately linked to the control of synaptic plasticity and long-term memory⁽¹⁵⁶⁾ (Fig. 5) and decreases in BDNF and pro-BDNF have been reported in

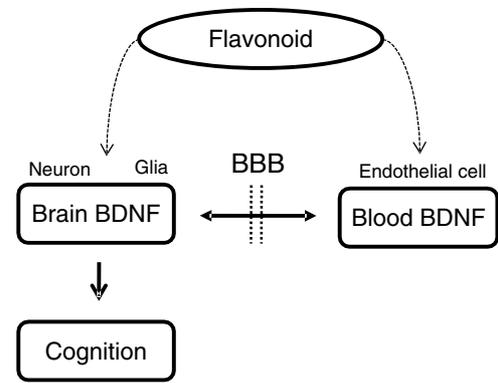


Fig. 5. Generation of brain-derived neurotrophic factor (BDNF) by flavonoids may occur in the brain (neurons and glial cells) and the periphery (endothelial cells). As BDNF may cross the blood–brain barrier (BBB) both sites of generation have the potential to be relevant to changes in cognition. Additionally, plasma measures of BDNF may reflect brain generation in response to flavonoids and general cognitive performance.

Alzheimer’s disease^(157,158). Furthermore, a polymorphism that replaces valine for methionine at position 66 of the pro-domain of BDNF is associated with memory defects and abnormal hippocampal function in human subjects⁽¹⁵⁹⁾.

Flavonoid-induced activation of CREB and BDNF expression has also been shown to lead to the activation of the PI3K/Akt signalling pathway⁽¹³³⁾, presumably via the binding of BDNF to pre- or post-synaptic tropomyosin receptor kinase B receptor. The activation of Akt by flavonoids in the hippocampus triggers the activation of the mTOR pathway and the increased translation of specific mRNA subpopulations⁽¹⁶⁰⁾, including the activity-regulated cytoskeletal-associated protein termed Arc/Arg3.1⁽¹³³⁾, which is known to be important in long-term potentiation and has been proposed to be under regulatory control of both BDNF⁽¹⁶¹⁾ and the ERK signalling⁽¹⁶²⁾ (Fig. 4). Increased Arc/Arg3.1 expression may facilitate changes in synaptic strength and the induction of morphological changes such as that observed when small spines are converted into large mushroom-shaped spines through a mechanism dependent on actin polymerisation⁽¹⁶³⁾. In support of this role, studies have indicated that changes in neuronal morphology occur in response to flavonoid supplementation⁽¹⁶⁴⁾ and that certain flavonoids can influence neuronal dendrite outgrowth *in vitro*⁽⁷⁸⁾. Furthermore, nobiletin (a poly-methoxylated flavone found in citrus peel) also induces neurite outgrowth⁽¹⁶⁵⁾ and synaptic transmission⁽¹⁶⁶⁾ via its ability to interact directly with MAPK and protein kinase A signalling pathways, whilst its metabolite 4’-demethylnobiletin exerts similar effects via the same pathways⁽¹⁶⁷⁾.

Effects on the peripheral and cerebrovascular system

Recent dietary interventions in human subjects using flavanol-containing foods have substantiated epidemiological data for an inverse relationship between flavanol intake and the risk of CVD, indicating various potential

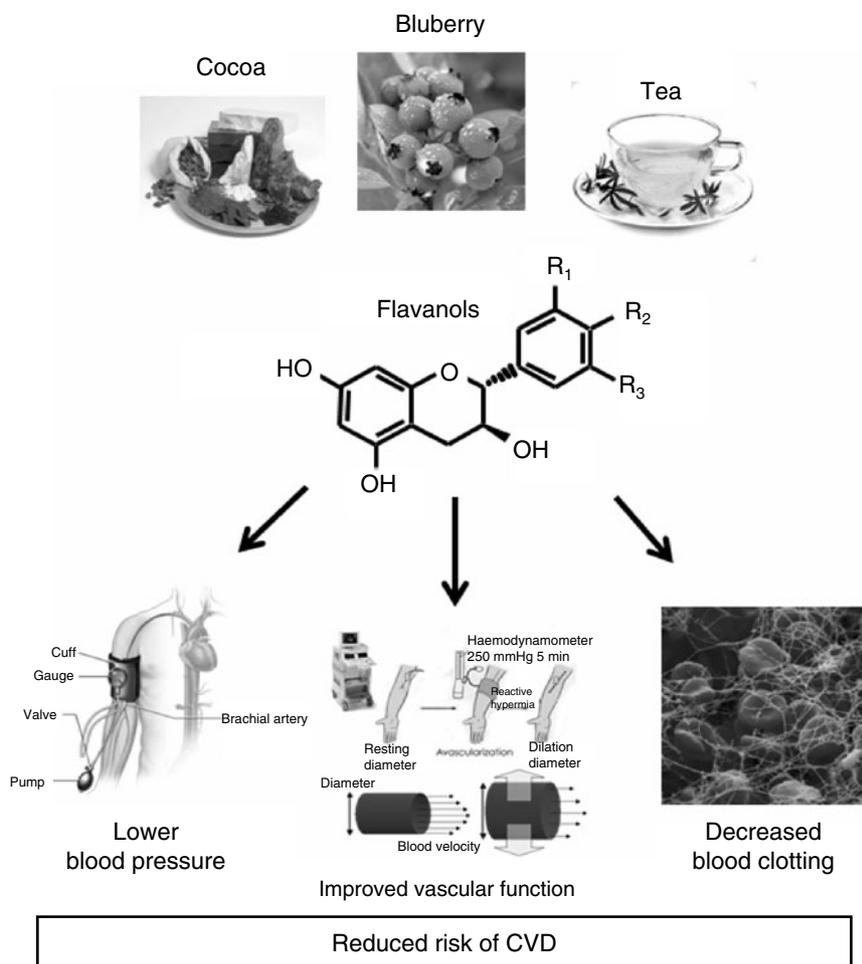


Fig. 6. The peripheral vascular and cardiovascular effects of flavonoid-rich foods. Flavanols in particular have been shown to induce a number of cardiovascular risk factors including blood pressure, vascular function and blood clotting. Such vascular effects are also thought to play a role in determining brain blood flow and changes in cognitive performance. Blueberry, *Vaccinium corymbosum*.

flavanol-mediated bioactivities including the improvement of vasodilatation^(168–172), blood pressure⁽¹⁷³⁾, insulin resistance and glucose tolerance⁽¹⁷⁴⁾, the attenuation of platelet reactivity⁽¹⁷⁵⁾ and the improvement of immune responses and antioxidant defence systems^(176,177) (Fig. 6). The intake of flavanol-rich foods such as cocoa or of pure (–)-epicatechin has been shown to be highly effective in improving peripheral blood flow and surrogate markers of cardiovascular function⁽¹⁷⁸⁾. The intake of flavanols has been shown to result in acute elevations in the level of circulating NO species, an enhanced flow-mediated dilatation response of conduit arteries and an augmented microcirculation^(172,178,179). For example, increases in flow-mediated dilatation and plasma NO species in patients with hypertension, diabetes and coronary artery disease have been observed after consumption of flavanol-rich cocoa or chocolate^(172,174) and flavanol-rich cocoa reverses endothelial dysfunction in smokers⁽¹⁸⁰⁾ and hypercholesterolaemia in post-menopausal women⁽¹⁸¹⁾. Furthermore, increases in flow-mediated dilatation in patients with coronary artery disease have also been observed following

consumption of black tea or grape juice^(182,183). It has also been reported that flavanols might help to lower blood pressure in subjects with hypertension^(173,184,185) and in healthy individuals⁽¹⁷⁴⁾. It has been proposed that these effects are mediated by an ability of flavonoids to increase circulating NO levels, perhaps through actions directly on endothelial NO synthase^(168,186–188). Indeed, *in vitro* experiments have indicated that cocoa flavanols⁽¹⁸⁶⁾ and grape-derived polyphenols^(189,190) have the ability to induce endothelium-dependent dilatation via a direct activation of the endothelial NO synthase and NO production. Although, it is unlikely that oligomeric forms of flavanols may exert such actions *in vivo* (because of their poor absorption), flavanol monomers, specific flavanol metabolites, flavanols and flavones have also been observed to influence NO production and endothelium-dependent relaxation *in vivo*^(178,191–193).

This ability to affect vascular function may also play a role in determining flavonoid effects in the brain, in particular effects on cognition, which are known to also depend on brain blood flow. There is evidence to suggest that

flavonoids are capable of preventing many forms of cerebrovascular disease, including those associated with stroke and dementia^(194,195). Flavonoids, in particular flavanols, have been shown to influence cerebrovascular blood flow^(196,197) and these vascular effects are potentially important as increased cerebrovascular function is known to facilitate adult neurogenesis in the hippocampus^(143,198) (Fig. 4). Indeed, new hippocampal cells are clustered near blood vessels, proliferate in response to vascular growth factors and may influence memory⁽¹⁹⁹⁾. Furthermore, efficient cerebral blood flow is vital for optimal brain function, with several studies indicating that there is a decrease in cerebral blood flow in patients with dementia^(200,201). Brain imaging techniques such as functional MRI and transcranial Doppler ultrasound have shown that there is a correlation between cerebral blood flow and cognitive function in human subjects⁽²⁰¹⁾. For example, cerebral blood flow velocity is lower in patients with Alzheimer's disease and low cerebral blood flow is also associated with incipient markers of dementia. In contrast, subjects without dementia with higher cerebral blood flow are less likely to develop dementia. As mentioned earlier, flavonoids are capable of inducing increased cerebral blood flow in human subjects 1–2 h post intervention^(196,197). After consumption of a flavanol-rich cocoa drink the blood oxygenation level-dependent functional MRI shows an increase in blood flow in certain regions of the brain, along with a modification of the flow oxygenation level-dependent response to task switching. Furthermore, arterial spin-labelling sequence MRI also indicates that cocoa flavanols increase cerebral blood flow for ≤ 2 h post ingestion⁽²⁰²⁾. In support of these findings, an increase in cerebral blood flow through the middle cerebral artery has been reported after the consumption of flavanol-rich cocoa using transcranial Doppler ultrasound⁽¹⁹⁶⁾.

Present and future perspectives

The actions of dietary flavonoids on cognition appear to involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins and neuroinflammation, a potential to activate synaptic signalling and an ability to improve cerebrovascular blood flow. These effects appear to be underpinned by an ability to interact with cell signalling cascades in the brain and the periphery, leading to an inhibition of apoptosis triggered by neurotoxic species, the promotion of neuronal survival and differentiation and an enhancement of peripheral and cerebral blood perfusion. Such effects induce beneficial changes in the cellular architecture required for cognition and consequently provide the brain with a more efficient structure for interpreting afferent nerve or sensory information and for the storage, processing and retrieval of memory. Furthermore, such interactions also protect the brain against neuronal losses associated with ageing, which is particularly relevant as this innate brain structure is known to deteriorate with aging, with neuronal populations or synaptic connections lost over time, leaving the system less efficient in the processing and storage of sensory information.

However, although flavonoid consumption may have the potential to limit or even reverse age-dependent deteriorations in brain function, there are a number of questions still to be resolved. Most notably, at present there are no data in support of a causal relationship between the consumption of flavonoids and behavioural outcomes in human subjects. In order to identify such relationships future intervention studies will be required to utilise better-characterised intervention materials, more appropriate controls and more rigorous clinical outcomes. Whilst cognitive behavioural testing in human subjects and animals provides an appropriate way of assessing function, *in vivo* structural and dynamic quantitative assessments will ultimately be required to provide hard evidence of effects in the brain. For example, it would be highly advantageous to directly link behavioural responses to changes in hippocampal volume and density, changes in neural stem cell and progenitor cells and alterations in brain blood flow using MRI and functional MRI techniques. Functional MRI measures may be used to assess changes in blood flow that underlie improved cognitive functioning as a result of flavonoid supplementation. In addition, such haemodynamic changes may be further compared with changes in grey matter density and to biomarkers of neural stem and progenitor cells using H^1 -NMR spectroscopy. Such an approach will be essential to provide links between flavonoid intake and brain function in a mechanistic, dynamic and quantitative way. Taking such an approach it may also be possible to assess other factors relating to intake such as what time frame is required to gain maximum beneficial effects, which flavonoids are most effective in inducing these changes and in which doses?

Furthermore, the modulation of neurotrophic factors such as BDNF represent useful targets for the prevention of cognitive decline as they are known to be critical in both the protection and repair of neurons in the central nervous system^(203,204). For example, levels of brain BDNF have been shown to correlate with human learning, memory and cognitive function^(205–207). Future efforts should focus on whether the ability of flavonoids to induce improvements in cognition is mediated by their ability to induce BDNF and/or other neurotrophin production in either the brain or the periphery (Fig. 5). Although highly-specific behavioural tests exist to determine cognitive performance in human subjects, presently there is a lack of biochemical markers that can be used as surrogates of human cognitive performance. BDNF may be one such functional biomarkers, as it has been shown to cross the blood–brain barrier and thus levels in the plasma may reflect levels in the brain⁽²⁰⁸⁾ (Fig. 5). As flavonoid consumption has been reported to increase BDNF expression in rat brain and this increase is related to an improvement in spatial working memory⁽¹³³⁾, there is a possibility that the same may occur in human subjects and that increases in brain BDNF may be detected as a biomarker in plasma (Fig. 5). Thus, future studies should investigate the usefulness of BDNF and other brain-derived components as biomarkers of cognitive changes in human subjects and whether they also respond to intervention with flavonoid-rich foods.

Finally, the potential impact of diet on healthcare costs should not be ignored. Dementia costs in the UK alone

have been estimated to be £17 × 10⁹ per year. Development of a treatment that would reduce severe cognitive impairment in older individuals by only 1% per year would cancel out all estimated increases in the long-term care costs for the ageing population⁽²⁰⁹⁾. Also, there is intense interest in the development of drugs capable of enhancing memory and learning, both in adults and children, and there is a strong possibility that in the future specific nutrients, in particular fruit-derived flavonoids, might act as precursors for the development of a new generation of memory-enhancing drugs.

Acknowledgements

The author is funded by the Biotechnology and Biological Sciences Research Council (BB/F008953/1; BB/E023185/1; BB/G005702/1), the Food Standards Agency (FLA-VURS) and the EU (FP7 FLAVIOLA). The author declares no conflict of interest.

References

- Spencer JPE (2008) Flavonoids: modulators of brain function? *Br J Nutr* **99**, E Suppl. 1, ES60–ES77.
- Vafeiadou K, Vauzour D & Spencer JP (2007) Neuroinflammation and its modulation by flavonoids. *Endocr Metab Immune Disord Drug Targets* **7**, 211–224.
- Spencer JPE (2009) The impact of flavonoids on memory: physiological and molecular considerations. *Chem Soc Rev* **38**, 1152–1161.
- Spencer JPE, Vauzour D & Rendeiro C (2009) Flavonoids and cognition: The molecular mechanisms underlying their behavioural effects. *Arch Biochem Biophys* **492**, 1–9.
- Dixon RA & Steele CL (1999) Flavonoids and isoflavonoids – a gold mine for metabolic engineering. *Trends Plant Sci* **4**, 394–400.
- Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol* **126**, 485–493.
- Manach C, Scalbert A, Morand C *et al.* (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–747.
- Cheyrier V (2005) Polyphenols in foods are more complex than often thought. *Am J Clin Nutr* **81**, Suppl., 223S–229S.
- Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–956.
- Rice-Evans C (2001) Flavonoid antioxidants. *Curr Med Chem* **8**, 797–807.
- Rice-Evans C (1995) Plant polyphenols: free radical scavengers or chain-breaking antioxidants? *Biochem Soc Symp* **61**, 103–116.
- Williamson G & Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* **81**, 243S–255S.
- Manach C, Williamson G, Morand C *et al.* (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**, 230S–242S.
- Scalbert A & Williamson G (2000) Dietary intake and bioavailability of polyphenols. *J Nutr* **130**, 2073S–2085S.
- Spencer JPE, Abd El Mohsen MM, Minihane AM *et al.* (2008) Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr* **99**, 12–22; Epublication 1 August 2007.
- Spencer JPE, Chowrimootoo G, Choudhury R *et al.* (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett* **458**, 224–230.
- Spencer JPE (2003) Metabolism of tea flavonoids in the gastrointestinal tract. *J Nutr* **133**, Suppl., 3255S–3261S.
- Scheline RR (1999) Metabolism of oxygen heterocyclic compounds. *CRC Handbook of Mammalian Metabolism of Plant Compounds*, pp. 243–295. Boca Raton, FL: CRC Press, Inc.
- Tzounis X, Vulevic J, Kuhnle GG *et al.* (2008) Flavanol monomer-induced changes to the human faecal microflora. *Br J Nutr* **99**, 782–792.
- Spencer JPE, Kuhnle GG, Williams RJ *et al.* (2003) Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem J* **372**, 173–181.
- Spencer JPE, Schroeter H, Crosssthai AJ *et al.* (2001) Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. *Free Radic Biol Med* **31**, 1139–1146.
- Miyake Y, Shimoi K, Kumazawa S *et al.* (2000) Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats. *J Agric Food Chem* **48**, 3217–3224.
- Terao J, Yamaguchi S, Shirai M *et al.* (2001) Protection by quercetin and quercetin 3-O-beta-D-glucuronide of peroxynitrite-induced antioxidant consumption in human plasma low-density lipoprotein. *Free Radic Res* **35**, 925–931.
- Shirai M, Moon JH, Tsushida T *et al.* (2001) Inhibitory effect of a quercetin metabolite, quercetin 3-O-beta-D-glucuronide, on lipid peroxidation in liposomal membranes. *J Agric Food Chem* **49**, 5602–5608.
- Yamamoto N, Moon JH, Tsushida T *et al.* (1999) Inhibitory effect of quercetin metabolites and their related derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch Biochem Biophys* **372**, 347–354.
- da Silva EL, Piskula MK, Yamamoto N *et al.* (1998) Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. *FEBS Lett* **430**, 405–408.
- Abbott NJ (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat* **200**, 629–638.
- Youdim KA, Qaiser MZ, Begley DJ *et al.* (2004) Flavonoid permeability across an in situ model of the blood-brain barrier. *Free Radic Biol Med* **36**, 592–604.
- Youdim KA, Dobbie MS, Kuhnle G *et al.* (2003) Interaction between flavonoids and the blood-brain barrier: in vitro studies. *J Neurochem* **85**, 180–192.
- Aasmundstad TA, Morland J & Paulsen RE (1995) Distribution of morphine 6-glucuronide and morphine across the blood-brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. *J Pharmacol Exp Ther* **275**, 435–441.
- Sperker B, Backman JT & Kroemer HK (1997) The role of beta-glucuronidase in drug disposition and drug targeting in humans. *Clin Pharmacokinetics* **33**, 18–31.
- Kroemer HK & Klotz U (1992) Glucuronidation of drugs. A re-evaluation of the pharmacological significance of the conjugates and modulating factors. *Clin Pharmacokinetics* **23**, 292–310.
- Lin JH & Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinetics* **42**, 59–98.

34. Peng HW, Cheng FC, Huang YT *et al.* (1998) Determination of naringenin and its glucuronide conjugate in rat plasma and brain tissue by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* **714**, 369–374.
35. Suganuma M, Okabe S, Oniyama M *et al.* (1998) Wide distribution of [H-3](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **19**, 1771–1776.
36. Abd El Mohsen MM, Kuhnle G, Rechner AR *et al.* (2002) Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radic Biol Med* **33**, 1693–1702.
37. Abd El Mohsen MM, Marks J, Kuhnle G *et al.* (2006) Absorption, tissue distribution and excretion of pelargonidin and its metabolites following oral administration to rats. *Br J Nutr* **95**, 51–58.
38. Talavera S, Felgines C, Texier O *et al.* (2005) Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *J Agric Food Chem* **53**, 3902–3908.
39. Passamonti S, Vrhovsek U, Vanzo A *et al.* (2005) Fast access of some grape pigments to the brain. *J Agric Food Chem* **53**, 7029–7034.
40. Kalt W, Blumberg JB, McDonald JE *et al.* (2008) Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. *J Agric Food Chem* **56**, 705–712.
41. Pollard SE, Kuhnle GG, Vauzour D *et al.* (2006) The reaction of flavonoid metabolites with peroxynitrite. *Biochem Biophys Res Commun* **350**, 960–968.
42. Abd El Mohsen MM, Kuhnle G, Rechner AR *et al.* (2002) Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radic Biol Med* **33**, 1693–1702.
43. Halliwell B, Zhao K & Whiteman M (2000) The gastrointestinal tract: a major site of antioxidant action? *Free Radic Res* **33**, 819–830.
44. Schroeter H, Boyd C, Spencer JPE *et al.* (2002) MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. *Neurobiol Aging* **23**, 861–880.
45. Williams RJ, Spencer JPE & Rice-Evans C (2004) Flavonoids: antioxidants or signalling molecules? *Free Radic Biol Med* **36**, 838–849.
46. Spencer JPE (2007) The interactions of flavonoids within neuronal signalling pathways. *Gen Nutr* **2**, 257–273.
47. Spencer JPE (2008) Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proc Nutr Soc* **67**, 238–252.
48. Matter WF, Brown RF & Vlahos CJ (1992) The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem Biophys Res Commun* **186**, 624–631.
49. Vlahos CJ, Matter WF, Hui KY *et al.* (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* **269**, 5241–5248.
50. Agullo G, Gamet-Payrastra L, Manenti S *et al.* (1997) Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. *Biochem Pharmacol* **53**, 1649–1657.
51. Gamet-Payrastra L, Manenti S, Gratacap MP *et al.* (1999) Flavonoids and the inhibition of PKC and PI 3-kinase. *Gen Pharmacol* **32**, 279–286.
52. Kong AN, Yu R, Chen C *et al.* (2000) Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res* **23**, 1–16.
53. Schroeter H, Spencer JPE, Rice-Evans C *et al.* (2001) Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. *Biochem J* **358**, 547–557.
54. Spencer JPE, Rice-Evans C & Williams RJ (2003) Modulation of pro-survival Akt/PKB and ERK1/2 signalling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. *J Biol Chem* **278**, 34783–34793.
55. Lin CH, Yeh SH, Lin CH *et al.* (2001) A role for the PI-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron* **31**, 841–851.
56. Sweatt JD (2001) Memory mechanisms: the yin and yang of protein phosphorylation. *Curr Biol* **11**, R391–R394.
57. Cobb MH & Goldsmith EJ (1995) How MAP kinases are regulated. *J Biol Chem* **270**, 14843–14846.
58. Goldsmith EJ & Cobb MH (1994) Protein kinases. *Curr Opin Struct Biol* **4**, 833–840.
59. Karin M (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* **270**, 16483–16486.
60. Xia Z, Dickens M, Raingeaud J *et al.* (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270**, 1326–1331.
61. Anderson CN & Tolkovsky AM (1999) A role for MAPK/ERK in sympathetic neuron survival: protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. *J Neurosci* **19**, 664–673.
62. Bonni A, Brunet A, West AE *et al.* (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* **286**, 1358–1362.
63. Kaplan DR & Miller FD (2000) Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* **10**, 381–391.
64. Crossstwaite AJ, Hasan S & Williams RJ (2002) Hydrogen peroxide-mediated phosphorylation of ERK1/2, Akt/PKB and JNK in cortical neurones: dependence on Ca(2+) and PI3-kinase. *J Neurochem* **80**, 24–35.
65. Mielke K & Herdegen T (2000) JNK and p38 stresskinases – degenerative effectors of signal-transduction-cascades in the nervous system. *Prog Neurobiol* **61**, 45–60.
66. Yuan J & Yankner BA (2000) Apoptosis in the nervous system. *Nature* **407**, 802–809.
67. Behrens A, Sibilina M & Wagner EF (1999) Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat Genet* **21**, 326–329.
68. Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239–252.
69. Kobuchi H, Roy S, Sen CK *et al.* (1999) Quercetin inhibits inducible ICAM-1 expression in human endothelial cells through the JNK pathway. *Am J Physiol* **277**, C403–C411.
70. Herdegen T, Skene P & Bahr M (1997) The c-Jun transcription factor – bipotential mediator of neuronal death, survival and regeneration. *Trends Neurosci* **20**, 227–231.
71. Castagne V & Clarke PG (1999) Inhibitors of mitogen-activated protein kinases protect axotomized developing neurons. *Brain Res* **842**, 215–219.
72. Castagne V, Gautschi M, Lefevre K *et al.* (1999) Relationships between neuronal death and the cellular redox status. Focus on the developing nervous system. *Prog Neurobiol* **59**, 397–423.
73. Vauzour D, Vafeiadou K, Rice-Evans C *et al.* (2007) Activation of pro-survival Akt and ERK1/2 signaling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. *J Neurochem* **103**, 1355–1367.

74. Llorens F, Garcia L, Itarte E *et al.* (2002) Apigenin and LY294002 prolong EGF-stimulated ERK1/2 activation in PC12 cells but are unable to induce full differentiation. *FEBS Lett* **510**, 149–153.
75. Schroeter H, Bahia P, Spencer JPE *et al.* (2007) (-)-Epicatechin stimulates ERK-dependent cyclic AMP response element activity and upregulates GLUR2 in cortical neurons. *J Neurochem* **101**, 1596–1606.
76. Spencer JPE, Schroeter H, Crossthwaithe AJ *et al.* (2001) Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. *Free Rad Biol Med* **31**, 1139–1146.
77. Levites Y, Amit T, Youdim MB *et al.* (2002) Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. *J Biol Chem* **277**, 30574–30580.
78. Reznichenko L, Amit T, Youdim MB *et al.* (2005) Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *J Neurochem* **93**, 1157–1167.
79. Davis RJ (1999) Signal transduction by the c-Jun N-terminal kinase. *Biochem Soc Symp* **64**, 1–12.
80. Leppa S & Bohmann D (1999) Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* **18**, 6158–6162.
81. Ichijo H (1999) From receptors to stress-activated MAP kinases. *Oncogene* **18**, 6087–6093.
82. Ichijo H, Nishida E, Irie K *et al.* (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* **275**, 90–94.
83. Wang XS, Diener K, Jannuzzi D *et al.* (1996) Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase. *J Biol Chem* **271**, 31607–31611.
84. Matsuzawa A & Ichijo H (2001) Molecular mechanisms of the decision between life and death: regulation of apoptosis by apoptosis signal-regulating kinase 1. *J Biochem (Tokyo)* **130**, 1–8.
85. Schroeter H, Williams RJ, Matin R *et al.* (2000) Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Radic Biol Med* **29**, 1222–1233.
86. Spencer JPE, Schroeter H, Kuhnle G *et al.* (2001) Epicatechin and its in vivo metabolite, 3'-O-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. *Biochem J* **354**, 493–500.
87. Wang L, Matsushita K, Araki I *et al.* (2002) Inhibition of c-Jun N-terminal kinase ameliorates apoptosis induced by hydrogen peroxide in the kidney tubule epithelial cells (NRK-52. E). *Nephron* **91**, 142–147.
88. Ishikawa Y & Kitamura M (2000) Anti-apoptotic effect of quercetin: intervention in the JNK- and ERK-mediated apoptotic pathways. *Kidney Int* **58**, 1078–1087.
89. Uchida K, Shiraishi M, Naito Y *et al.* (1999) Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. *J Biol Chem* **274**, 2234–2242.
90. Kennedy SG, Wagner AJ, Conzen SD *et al.* (1997) The PI 3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev* **11**, 701–713.
91. Coffey PJ, Jin J & Woodgett JR (1998) Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. *Biochem J* **335**, 1–13.
92. Miller FD & Kaplan DR (2001) Neurotrophin signalling pathways regulating neuronal apoptosis. *Cell Mol Life Sci* **58**, 1045–1053.
93. Crowder RJ & Freeman RS (1998) Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J Neurosci* **18**, 2933–2943.
94. Ferriola PC, Cody V & Middleton E Jr (1989) Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem Pharmacol* **38**, 1617–1624.
95. Walker EH, Pacold ME, Perisic O *et al.* (2000) Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* **6**, 909–919.
96. Hirsch EC, Hunot S & Hartmann A (2005) Neuroinflammatory processes in Parkinson's disease. *Parkinsonism Relat Disord* **11**, Suppl. 1, S9–S15.
97. McGeer EG & McGeer PL (2003) Inflammatory processes in Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* **27**, 741–749.
98. Zheng Z, Lee JE & Yenari MA (2003) Stroke: molecular mechanisms and potential targets for treatment. *Curr Mol Med* **3**, 361–372.
99. Kozuka N, Itofusa R, Kudo Y *et al.* (2005) Lipopolysaccharide and proinflammatory cytokines require different astrocyte states to induce nitric oxide production. *J Neurosci Res* **82**, 717–728.
100. Marcus JS, Karackattu SL, Fleegal MA *et al.* (2003) Cytokine-stimulated inducible nitric oxide synthase expression in astroglia: role of Erk mitogen-activated protein kinase and NF-kappaB. *Glia* **41**, 152–160.
101. Bhat NR, Zhang P, Lee JC *et al.* (1998) Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. *J Neurosci* **18**, 1633–1641.
102. Casper D, Yaparalvi U, Rempel N *et al.* (2000) Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro. *Neurosci Lett* **289**, 201–204.
103. Vafeiadou K, Vauzour D, Lee HY *et al.* (2009) The citrus flavanone naringenin inhibits inflammatory signalling in glial cells and protects against neuroinflammatory injury. *Arch Biochem Biophys* **484**, 100–109.
104. Lau FC, Bielinski DF & Joseph JA (2007) Inhibitory effects of blueberry extract on the production of inflammatory mediators in lipopolysaccharide-activated BV2 microglia. *J Neurosci Res* **85**, 1010–1017.
105. Chen JC, Ho FM, Pei-Dawn LC *et al.* (2005) Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IkappaB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia. *Eur J Pharmacol* **521**, 9–20.
106. Lee H, Kim YO, Kim H *et al.* (2003) Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. *FASEB J* **17**, 1943–1944.
107. Li R, Huang YG, Fang D *et al.* (2004) (-)-Epigallocatechin gallate inhibits lipopolysaccharide-induced microglial activation and protects against inflammation-mediated dopaminergic neuronal injury. *J Neurosci Res* **78**, 723–731.
108. Afaq F, Adhami VM, Ahmad N *et al.* (2003) Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea constituent (-)-epigallocatechin-3-gallate. *Oncogene* **22**, 1035–1044.

109. Zheng LT, Ock J, Kwon BM *et al.* (2008) Suppressive effects of flavonoid fisetin on lipopolysaccharide-induced microglial activation and neurotoxicity. *Int Immunopharmacol* **8**, 484–494.
110. Jang S, Kelley KW & Johnson RW (2008) Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. *Proc Natl Acad Sci USA* **105**, 7534–7539.
111. Bhat NR, Feinstein DL, Shen Q *et al.* (2002) p38 MAPK-mediated transcriptional activation of inducible nitric-oxide synthase in glial cells. Roles of nuclear factors, nuclear factor kappa B, cAMP response element-binding protein, CCAAT/enhancer-binding protein-beta, and activating transcription factor-2. *J Biol Chem* **277**, 29584–29592.
112. Barzilai A & Melamed E (2003) Molecular mechanisms of selective dopaminergic neuronal death in Parkinson's disease. *Trends Mol Med* **9**, 126–132.
113. Jellinger KA (2001) Cell death mechanisms in neurodegeneration. *J Cell Mol Med* **5**, 1–17.
114. Spires TL & Hannan AJ (2005) Nature, nurture and neurology: gene-environment interactions in neurodegenerative disease. *FEBS J* **272**, 2347–2361.
115. Joseph J, Cole G, Head E *et al.* (2009) Nutrition, brain aging, and neurodegeneration. *J Neurosci* **29**, 12795–12801.
116. Weinreb O, Amit T, Mandel S *et al.* (2009) Neuroprotective molecular mechanisms of (-)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties. *Genes Nutr* **4**, 283–296.
117. Mandel S & Youdim MB (2004) Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases. *Free Radic Biol Med* **37**, 304–317.
118. Vauzour D, Vafeiadou K, Rodriguez-Mateos A *et al.* (2008) The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* **3**, 115–126.
119. Joseph J, Cole G, Head E *et al.* (2009) Nutrition, brain aging, and neurodegeneration. *J Neurosci* **29**, 12795–12801.
120. Luo Y, Smith JV, Paramasivam V *et al.* (2002) Inhibition of amyloid-beta aggregation and caspase-3 activation by the Ginkgo biloba extract EGb761. *Proc Natl Acad Sci USA* **99**, 12197–12202.
121. Mandel SA, Amit T, Weinreb O *et al.* (2008) Simultaneous manipulation of multiple brain targets by green tea catechins: a potential neuroprotective strategy for Alzheimer and Parkinson diseases. *CNS Neurosci Ther* **14**, 352–365.
122. Mandel SA, Amit T, Kalfon L *et al.* (2008) Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). *J Alzheimers Dis* **15**, 211–222.
123. Kalfon L, Youdim MB & Mandel SA (2007) Green tea polyphenol (-)-epigallocatechin-3-gallate promotes the rapid protein kinase C- and proteasome-mediated degradation of Bad: implications for neuroprotection. *J Neurochem* **100**, 992–1002.
124. Mandel SA, Avramovich-Tirosh Y, Reznichenko L *et al.* (2005) Multifunctional activities of green tea catechins in neuroprotection. Modulation of cell survival genes, iron-dependent oxidative stress and PKC signaling pathway. *Neurosignals* **14**, 46–60.
125. Levites Y, Youdim MB, Maor G *et al.* (2002) Attenuation of 6-hydroxydopamine (6-OHDA)-induced nuclear factor-kappaB (NF-kappaB) activation and cell death by tea extracts in neuronal cultures. *Biochem Pharmacol* **63**, 21–29.
126. Lee S, Suh S & Kim S (2000) Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. *Neurosci Lett* **287**, 191–194.
127. Levites Y, Weinreb O, Maor G *et al.* (2001) Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration. *J Neurochem* **78**, 1073–1082.
128. Spencer JPE, Whiteman M, Jenner P *et al.* (2002) 5-S-Cysteiny-conjugates of catecholamines induce cell damage, extensive DNA base modification and increases in caspase-3 activity in neurons. *J Neurochem* **81**, 122–129.
129. Hastings TG (1995) Enzymatic oxidation of dopamine: the role of prostaglandin H synthase. *J Neurochem* **64**, 919–924.
130. Vauzour D, Ravaioli G, Vafeiadou K *et al.* (2008) Peroxynitrite induced formation of the neurotoxins 5-S-cysteinyldopamine and DHBT-1: Implications for Parkinson's disease and protection by polyphenols. *Arch Biochem Biophys* **476**, 145–151.
131. Spencer JPE, Jenner P, Daniel SE *et al.* (1998) Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. *J Neurochem* **71**, 2112–2122.
132. Vauzour D, Vafeiadou K & Spencer JP (2007) Inhibition of the formation of the neurotoxin 5-S-cysteinyldopamine by polyphenols. *Biochem Biophys Res Commun* **362**, 340–346.
133. Williams CM, El Mohsen MA, Vauzour D *et al.* (2008) Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radic Biol Med* **45**, 295–305.
134. Macready AL, Kennedy OB, Ellia JA *et al.* (2009) Flavonoids and cognitive function: A review of human randomized controlled trial studies and recommendations for future studies. *Genes Nutr* **4**, 227–242.
135. Joseph JA, Shukitt-Hale B, Denisova NA *et al.* (1998) Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. *J Neurosci* **18**, 8047–8055.
136. Joseph JA, Shukitt-Hale B, Denisova NA *et al.* (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* **19**, 8114–8121.
137. Joseph JA, Denisova NA, Arendash G *et al.* (2003) Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci* **6**, 153–162.
138. Joseph JA, Shukitt-Hale B & Casadesus G (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* **81**, Suppl., 313S–316S.
139. Joseph JA, Shukitt-Hale B & Lau FC (2007) Fruit polyphenols and their effects on neuronal signaling and behavior in senescence. *Ann N Y Acad Sci* **1100**, 470–485.
140. Shukitt-Hale B, Lau FC & Joseph JA (2008) Berry fruit supplementation and the aging brain. *J Agric Food Chem* **56**, 636–641.
141. Shukitt-Hale B, Carey A, Simon L *et al.* (2006) Effects of Concord grape juice on cognitive and motor deficits in aging. *Nutrition* **22**, 295–302.
142. Spencer JPE (2009) Flavonoids and brain health: multiple effects underpinned by common mechanisms. *Genes Nutr* **4**, 243–250.
143. Stangl D & Thuret S (2009) Impact of diet on adult hippocampal neurogenesis. *Genes Nutr* **4**, 271–282.
144. Harris KM & Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annu Rev Neurosci* **17**, 341–371.

145. Maher P, Akaishi T & Abe K (2006) Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. *Proc Natl Acad Sci USA* **103**, 16568–16573.
146. Maher P (2009) Modulation of multiple pathways involved in the maintenance of neuronal function during aging by fisetin. *Genes Nutr* **4**, 297–307.
147. Pollard SE, Whiteman M & Spencer JPE (2006) Modulation of peroxynitrite-induced fibroblast injury by hesperetin: a role for intracellular scavenging and modulation of ERK signalling. *Biochem Biophys Res Commun* **347**, 916–923.
148. Pham TA, Impey S, Storm DR *et al.* (1999) CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. *Neuron* **22**, 63–72.
149. Impey S, Smith DM, Obrietan K *et al.* (1998) Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci* **1**, 595–601.
150. Impey S, Mark M, Villacres EC *et al.* (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* **16**, 973–982.
151. Bourtchouladze R, Frenguelli B, Blendy J *et al.* (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79**, 59–68.
152. Tully T, Bourtchouladze R, Scott R *et al.* (2003) Targeting the CREB pathway for memory enhancers. *Nat Rev Drug Discov* **2**, 267–277.
153. Conkright MD, Guzman E, Flechner L *et al.* (2003) Genome-wide analysis of CREB target genes reveals a core promoter requirement for cAMP responsiveness. *Mol Cell* **11**, 1101–1108.
154. Finkbeiner S (2000) CREB couples neurotrophin signals to survival messages. *Neuron* **25**, 11–14.
155. Finkbeiner S, Tavazoie SF, Maloratsky A *et al.* (1997) CREB: a major mediator of neuronal neurotrophin responses. *Neuron* **19**, 1031–1047.
156. Bramham CR & Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol* **76**, 99–125.
157. Peng S, Wu J, Mufson EJ *et al.* (2005) Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* **93**, 1412–1421.
158. Michalski B & Fahnstock M (2003) Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Brain Res Mol Brain Res* **111**, 148–154.
159. Egan MF, Kojima M, Callicott JH *et al.* (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269.
160. Schrott GM, Nigh EA, Chen WG *et al.* (2004) BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. *J Neurosci* **24**, 7366–7377.
161. Yin Y, Edelman GM & Vanderklis PW (2002) The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneuroosomes. *Proc Natl Acad Sci USA* **99**, 2368–2373.
162. Waltereit R, Dammermann B, Wulff P *et al.* (2001) Arg3.1/Arc mRNA induction by Ca²⁺ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. *J Neurosci* **21**, 5484–5493.
163. Lyford GL, Yamagata K, Kaufmann WE *et al.* (1995) Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* **14**, 433–445.
164. van Praag H, Lucero MJ, Yeo GW *et al.* (2007) Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J Neurosci* **27**, 5869–5878.
165. Nagase H, Yamakuni T, Matsuzaki K *et al.* (2005) Mechanism of neurotrophic action of nobiletin in PC12. D cells. *Biochemistry* **44**, 13683–13691.
166. Matsuzaki K, Miyazaki K, Sakai S *et al.* (2008) Nobiletin, a citrus flavonoid with neurotrophic action, augments protein kinase A-mediated phosphorylation of the AMPA receptor subunit, GluR1, and the postsynaptic receptor response to glutamate in murine hippocampus. *Eur J Pharmacol* **578**, 194–200.
167. Al Rahim M., Nakajima A, Saigusa D *et al.* (2009) 4'-Demethylnobiletin, a bioactive metabolite of nobiletin enhancing PKA/ERK/CREB signaling, rescues learning impairment associated with NMDA receptor antagonism via stimulation of the ERK cascade. *Biochemistry* **48**, 7713–7721.
168. Fisher ND, Hughes M, Gerhard-Herman M *et al.* (2003) Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* **21**, 2281–2286.
169. Hirata K, Shimada K, Watanabe H *et al.* (2004) Black tea increases coronary flow velocity reserve in healthy male subjects. *Am J Cardiol* **93**, 1384–1388.
170. Janszky I, Ericson M, Blom M *et al.* (2005) Wine drinking is associated with increased heart rate variability in women with coronary heart disease. *Heart* **91**, 314–318.
171. Stein JH, Keevil JG, Wiebe DA *et al.* (1999) Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **100**, 1050–1055.
172. Heiss C, Dejam A, Kleinbongard P *et al.* (2003) Vascular effects of cocoa rich in flavan-3-ols. *JAMA* **290**, 1030–1031.
173. Taubert D, Berkels R, Roesen R *et al.* (2003) Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA* **290**, 1029–1030.
174. Grassi D, Lippi C, Necozione S *et al.* (2005) Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr* **81**, 611–614.
175. Holt RR, Schramm DD, Keen CL *et al.* (2002) Chocolate consumption and platelet function. *JAMA* **287**, 2212–2213.
176. Sies H, Schewe T, Heiss C *et al.* (2005) Cocoa polyphenols and inflammatory mediators. *Am J Clin Nutr* **81**, Suppl., 304S–312S.
177. Keen CL, Holt RR, Oteiza PI *et al.* (2005) Cocoa antioxidants and cardiovascular health. *Am J Clin Nutr* **81**, Suppl., 298S–303S.
178. Schroeter H, Heiss C, Balzer J *et al.* (2006) (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci USA* **103**, 1024–1029.
179. Fisher ND & Hollenberg NK (2006) Aging and vascular responses to flavanol-rich cocoa. *J Hypertens* **24**, 1575–1580.
180. Heiss C, Kleinbongard P, Dejam A *et al.* (2005) Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J Am Coll Cardiol* **46**, 1276–1283.
181. Wang-Polagruto JF, Villablanca AC, Polagruto JA *et al.* (2006) Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell

- adhesion molecule in hypercholesterolemic postmenopausal women. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S177–S186.
182. Stein JH, Keevil JG, Wiebe DA *et al.* (1999) Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **100**, 1050–1055.
 183. Duffy SJ, Keaney JF Jr, Holbrook M *et al.* (2001) Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation* **104**, 151–156.
 184. Taubert D, Roesen R & Schomig E (2007) Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Arch Intern Med* **167**, 626–634.
 185. Grassi D, Necozione S, Lippi C *et al.* (2005) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* **46**, 398–405.
 186. Karim M, McCormick K & Kappagoda CT (2000) Effects of cocoa extracts on endothelium-dependent relaxation. *J Nutr* **130**, Suppl., 2105S–2108S.
 187. Leikert JF, Rathel TR, Muller C *et al.* (2001) Reliable in vitro measurement of nitric oxide released from endothelial cells using low concentrations of the fluorescent probe 4,5-diaminofluorescein. *FEBS Lett* **506**, 131–134.
 188. Leikert JF, Rathel TR, Wohlfart P *et al.* (2002) Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation* **106**, 1614–1617.
 189. Fitzpatrick DF, Hirschfield SL & Coffey RG (1993) Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am J Physiol* **265**, H774–H778.
 190. Cishek MB, Galloway MT, Karim M *et al.* (1997) Effect of red wine on endothelium-dependent relaxation in rabbits. *Clin Sci (Lond)* **93**, 507–511.
 191. Steffen Y, Jung T, Klotz LO *et al.* (2007) Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cells: protection by (-)-epicatechin. *Free Radic Biol Med* **42**, 955–970.
 192. Ajay M, Achike FI, Mustafa AM *et al.* (2006) Direct effects of quercetin on impaired reactivity of spontaneously hypertensive rat aortae: comparative study with ascorbic acid. *Clin Exp Pharmacol Physiol* **33**, 345–350.
 193. Ajay M, Achike FI & Mustafa MR (2007) Modulation of vascular reactivity in normal, hypertensive and diabetic rat aortae by a non-antioxidant flavonoid. *Pharmacol Res* **55**, 385–391.
 194. Commenges D, Scotet V, Renaud S *et al.* (2000) Intake of flavonoids and risk of dementia. *Eur J Epidemiol* **16**, 357–363.
 195. Dai Q, Borenstein AR, Wu Y *et al.* (2006) Fruit and vegetable juices and Alzheimer's disease: the Kame Project. *Am J Med* **119**, 751–759.
 196. Fisher ND, Sorond FA & Hollenberg NK (2006) Cocoa flavanols and brain perfusion. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S210–S214.
 197. Francis ST, Head K, Morris PG *et al.* (2006) The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S215–S220.
 198. Gage FH (2000) Mammalian neural stem cells. *Science* **287**, 1433–1438.
 199. Palmer TD, Willhoite AR & Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* **425**, 479–494.
 200. Nagahama Y, Nabatame H, Okina T *et al.* (2003) Cerebral correlates of the progression rate of the cognitive decline in probable Alzheimer's disease. *Eur Neurol* **50**, 1–9.
 201. Ruitenberg A, den Heijer T, Bakker SL *et al.* (2005) Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. *Ann Neurol* **57**, 789–794.
 202. Wang Z, Fernandez-Seara M, Alsop DC *et al.* (2008) Assessment of functional development in normal infant brain using arterial spin labeled perfusion MRI. *Neuroimage* **39**, 973–978.
 203. Tapia-Arancibia L, Rage F, Givalois L *et al.* (2004) Physiology of BDNF: focus on hypothalamic function. *Front Neuroendocrinol* **25**, 77–107.
 204. Givalois L, Arancibia S, Alonso G *et al.* (2004) Expression of brain-derived neurotrophic factor and its receptors in the median eminence cells with sensitivity to stress. *Endocrinology* **145**, 4737–4747.
 205. Laske C, Stransky E, Eschweiler GW *et al.* (2007) Increased BDNF serum concentration in fibromyalgia with or without depression or antidepressants. *J Psychiatr Res* **41**, 600–605.
 206. Laske C, Stransky E, Leyhe T *et al.* (2007) BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *J Psychiatr Res* **41**, 387–394.
 207. Laske C, Stransky E, Leyhe T *et al.* (2006) Stage-dependent BDNF serum concentrations in Alzheimer's disease. *J Neural Transm* **113**, 1217–1224.
 208. Shimizu E, Hashimoto K, Okamura N *et al.* (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* **54**, 70–75.
 209. Alzheimer's Research Trust (2009) Dementia statistics. <http://www.alzheimers-research.org.uk/info/statistics/>