Nutrition and Aging 1 (2012) 89–97
DOI 10.3233/NUA-2012-0005
IOS Press

Flavonoid transport across blood-brain barrier: Implication for their direct neuroprotective actions

Ana Faria[a,b,c,*], Nuno Mateus[b] and Conceição Calhau[a]
[a]*Biochemistry Department (U38.FCT), Faculty of Medicine, University of Porto, Porto, Portugal*
[b]*Chemistry Investigation Center (CIQ), Faculty of Sciences, University of Porto, Porto, Portugal*
[c]*Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal*

Abstract. Epidemiological and dietary intervention studies in humans and animals indicate that flavonoid consumption may be capable of promoting brain health. However, the knowledge regarding the brain bioavailability of flavonoids, and thus their potential for direct neuronal and glial actions, remains insufficient and inconsistent. If their direct actions on neurons are to be fully elucidated, knowledge regarding their transport across blood-brain barrier (BBB) and how this is regulated is crucial. Presently, there is sparse, but valuable information regarding the interaction of flavonoids and their metabolites with the BBB and their transfer across it. This review aims to highlight the existing knowledge with regards to these issues by two approaches: firstly by examining data emanating from *in vitro* studies, and secondly by appraisal of the existing *in vivo* data regarding flavonoid bioavailability to the brain.

Keywords: Blood-brain barrier, flavonoid, transport, neuroprotection

1. Flavonoid and neurodegenerative diseases – human and animal studies

Polyphenolic compounds are products of plant secondary metabolism and are found ubiquitously in the plant kingdom and are responsible for the protection of the plant against environmental attack. For example, they possess anti-microbial and anti-fungal properties, exert insect feeding deterrence, protection against UV radiation damage, prevent heavy metal toxicity through chelation and exert anti-oxidant protection against free radicals generated during photosynthesis [1]. Apart from their physiological roles in plants, polyphenols, and in particular flavonoids (a major group of polyphenols) have been postulated to exert beneficial health effects in humans, despite being classified as non-nutrients. With regards to this, several epidemiological studies have provided support for the association between the consumption of polyphenol-rich fruits, vegetables and beverages, such as tea and red wine, and health promotion [2–4].

Numerous studies have indicated that high consumption of fruits and vegetables rich in flavonoids and other polyphenols lead to a reduction in the risk/incidence of age-related neurodegenerative disorders (reviewed in [5–10]). For example, age-related changes in the hippocampus has been shown to be attenuated following intervention with green tea for 7 months, with biochemical, morphological and behavioural assays indicating a reduction in oxidative status and an improvement in spatial learning [11]. Similar effects have been observed following the prolonged consumption of red wine, another rich source of

*Corresponding author: Ana Faria, Department of Biochemistry (U38/FCT), Faculty of Medicine of the University of Porto, Al. Prof. Hernâni Monteiro, Porto 4200-319, Portugal. Tel./Fax: +351 22 551 36 24; E-mail: anafaria@med.up.pt.

ISSN 1879-7717/12/$27.50 © IOS Press and the authors. All rights reserved

polyphenols, here leading to improvements in oxidative status in the cerebellum [12]. There have been many studies that have indicated that berry-enriched diets are effective in preventing age-related object memory declines and improvements in motor and cognitive performance in rodents [13–15].

Human data are also available: elderly adults consuming blueberry juice show neurocognitive benefits [16] and supplementation with concord juice enhances cognitive function in elderly adults with early memory decline [17]. Recent data compiled using databases of polyphenol consumption in a cohort of middle-aged adults indicated a positive relation between long-term flavonoid consumption and cognitive performance, in particular in preserving verbal memory, a vulnerable domain in pathological brain aging [18]. Another population study concluded that a higher consumption of dietary flavonoids, especially flavonols, was associated with lower population rates of dementia in the developed countries studied [19].

Several flavonoids have also been shown to exert positive effects in mice models of Alzheimer's disease. For example, there is data indicating the following functionality: the stilbene resveratrol promotes intracellular degradation of β-amyloid through a mechanism that involves the proteasome [20]; epigallocatechin-3-gallate was found to decrease β-amyloid levels and plaques associated with the promotion of the non-amyloidogenic α-secretase proteolytic pathway [21]; and a polyphenol-rich grape seed extract was shown to prevent β-amyloid deposition and to attenuate brain inflammation in a transgenic mouse model [22]. In agreement with these studies, epidemiology investigations workers have highlighted an inverse association between consumption of the Mediterranean diet and the risk for Alzheimer's disease [23, 24].

With regards to Parkinson's disease, polyphenols have been found to exert beneficial effects on rodent models of the disease. For example, in the unilateral 6-hydroxydopamine (6-OHDA)-treated rat model of Parkinson's disease, several polyphenols have shown putative beneficial effects, with genistein administration protecting against 6-OHDA toxicity [25] and animals pre-treatment with plant extracts rich in polymethoxylated flavones, procyanidins and isoflavones protecting against nigrostriatal dopaminergic lesioning [26]. Green tea polyphenols have also been found to protect dopaminergic neurons, in a dose-dependent manner, against 6-OHDA-induced increases in several oxidative stress parameters [27]. However, to date, there is a lack of epidemiologic studies and clinical trials supporting these animal observations in Parkinson's disease models. Nevertheless, a study from 2007 indicated that there may be a link between dietary patterns high in fruit, vegetables, whole grains, nuts, fish, and poultry intake, and a low intake of saturated fat and a moderate intake of alcohol with a protection against Parkinson's disease [28].

Despite these, and many other, promising studies indicating that polyphenols and flavonoids may protect the brain, there is little evidence regarding their bioavailability to the brain. As such, it is difficult at present to accurately conclude about the precise mechanisms underlying their positive effects. In particular, for their actions to be mediated centrally, i.e. within the brain and CNS, flavonoids and other polyphenols must reach the brain where they may directly interact with neuronal and glial populations. Thus, if one is to conclude on their likely actions in the CNS, it is necessary to first establish whether there are capable of traversing the blood-brain-barrier (BBB). The next sections will review the current information regarding this in order to help assess their potential mechanisms of brain action *in vivo*.

2. Bioavailability of flavonoids

Knowledge about flavonoid biokinetics (the composite actions of their absorption, distribution, biotransformation and elimination) is critical in order to understand the bioactivity of flavonoids in humans. Flavonoids have been detected in the blood after ingestion of beverages or foods rich in these components. All polyphenols and flavonoids are subject to phase I and II metabolism in the small intestine and liver during their absorption. Studies have detected several flavan-3-ols and their metabolites in plasma of human healthy volunteers following consumption of green tea [29–32], with the primary metabolites being O-methylated, sulphated, and glucuronidated conjugates [29, 31]. These metabolites, as well as some aglycone forms have also been found in plasma of humans after ingestion of chocolate and cocoa [33–36].

Despite being incompletely resolved, supplementation with anthocyanin rich foods, e.g. red wine, strawberries, açai or cranberry juice, results in the appearance of metabolites in the plasma 1–3 h later [37–40]. The extent of metabolism of anthocyanins

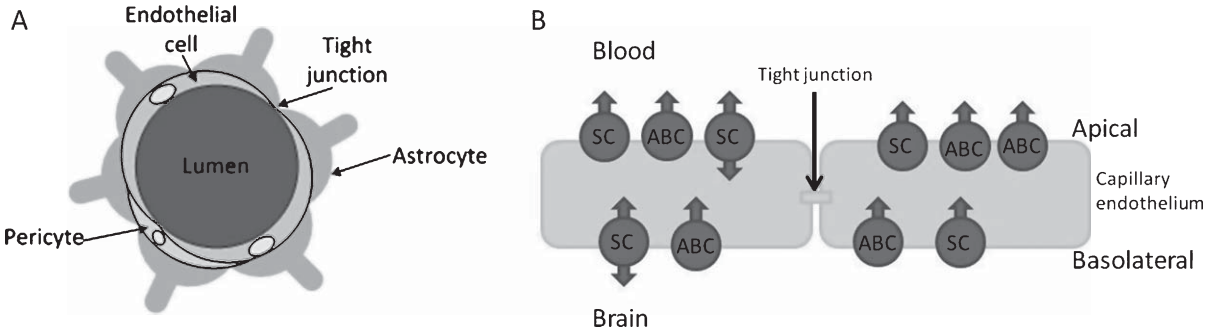

Fig. 1. Schematic representation of the brain capillary (A), illustrating the interaction of capillary endothelial cells, pericytes, and astrocytes that result in tight junctions between endothelial cells, forming the blood–brain barrier (BBB) (adapted from [47]); (B) represents the putative localization of ABC transporters (ABC) as well as solute carriers (SC) associated with the BBB (more detail in Table 1).

remains unclear as information regarding the decomposition of anthocyanins prior to absorption is still emerging. Furthermore, the O-methylation of some anthocyanins yields other anthocyanins, making it difficult to distinguish between the ones that were absorbed and those formed by metabolism. Studies with quercetin (a major flavonol present in the human diet) also indicate that this compound and its metabolites are available in human plasma after food intake [41–45]. The appearance of flavonoids in plasma provides evidence that these compounds are bioavailable to tissues in the periphery, but cannot be used as evidence that they are also present in the brain and CNS. Whether they can transverse the BBB and reach the central nervous system is an issue that will be covered in the next section and is a major focus of this review.

2.1. The blood-brain barrier (BBB)

Free exchange between blood and interstitial fluid occurs in nearly all organs of the body with the exception of the capillaries in the brain, which have evolved to constrain the movement of molecules and cells between the blood and the brain. This important characteristic provides a natural defence for the brain against toxic or infective agents circulating in the blood, and is conferred by cell adhesion molecules allowing endothelial cells to form tight junctions. However, besides fulfilling this important protective role by limiting the movement of substances into the brain [46], the BBB has also evolved to play a crucial role of supplying essential nutrients, hormones and drugs to the brain and in eliminating toxic metabolites from the brain.

The BBB is made up of three cell types: endothelial cells, astrocytes and pericytes (Fig. 1). The endothelial cells that are present in the BBB form a distinct boundary between two different physiological environments. Thus, chemical movement through this barrier implies passage through a biological membrane [47]. There are three major routes by which a compound may cross such a biological membranes: 1) it may pass by a purely passive process; 2) it may involve a carrier (carrier-mediated process) or; 3) it may involve a specialized transport process. Passive processes are only possible if compounds are small, lipid-soluble, non-ionized and if there is a concentration gradient between the two sides of the membrane (principle of Fick's law) [48], and such a passage can occur without energy expenditure. On the other hand, carrier-mediated transport involves one or more transporter(s) and the influx or efflux of substances. This kind of transport is saturable and specific, and can be of the following nature: 1) uniport (transport of a single molecule); 2) symport (transport of two or more different molecules or ions in the same direction) or 3) antiport (transport of two or more different molecules or ions in opposite directions). Finally, specialized transport includes endocytosis, which can occur with or without interaction with membrane receptors and is a form of chemical distribution more likely for larger molecules and proteins [47]. Depending on the nature of the transported compound endocytosis could be phagocytosis, pinocytosis and potocytosis.

Several transporters of different classes have been described in association with the BBB [49–51]. Due to the fact that these cells are relatively impermeable, possessing extensive tight junctions with no fenestrations, transport systems become extremely

important not only to allow nutrients, vitamins and some drugs/xenobiotics transport, but also to actively limit the passage of potential harmful substances into the brain. We detail these in the next sections.

2.1.1. Solute carriers

The endothelial cells forming the BBB express a large number of solute carrier proteins, since most polar molecules are unable to diffuse through the cell membranes of the BBB. These transporters are 'polarized' and are found on the BBB apical and/or basolateral membranes [47, 52]. The precise location and orientation of these transporters either favours the direction of transport from the blood into the brain, or from brain back into the peripheral blood. A wide range of substrates makes use of these carriers, including glucose, several amino acids (charged and neutral), nucleosides, nucleotides and nucleobases, organic cations and organic anions among others (Table 1).

2.1.2. ATP-binding cassette transporters

ATP-binding cassette transporters (ABC transporters) are the main transporters responsible for controlling the passage of lipophilic substances to the brain. One would predict that lipophilic substances may pass the BBB freely and reach the central nervous system (CNS), however, this is not the case since they are actively effluxed from the BBB cells back into the blood. The most abundant ABC transporters in the BBB are P-glycoprotein (P-gp; Multidrug Resistance Protein 1), the Multidrug Resistance-associated Proteins (MRPs family) and the Breast Cancer Resistance Protein (BCRP) (Table 1) [49, 53]. The main function of these efflux transporters is to prevent the entry and accumulation of harmful substances (either endogenous or exogenous) to the brain (at the expense of ATP). P-gp and BCRP are expressed on apical membranes, favouring the transport of substances from the cells back into the blood, whilst MRPs are found in both membranes.

The barrier functions of BBB are regulated during healthy conditions and during pathology, and result from a combination of: 1) the transport barrier or specific transport mechanisms mediating solute flux; ii) physical factors, in particular tight junctions between cells, reducing flux through the intercellular cleft or paracellular pathway; and iii) metabolic competence or the potential of metabolizing molecules [54].

2.2. Accessibility of flavonoids to the brain

One of the main issues regarding the bioavailability of flavonoids to the brain concerns the mechanisms by which flavonoids (conjugated or unconjugated) are transported across the BBB. Furthermore, it is currently unclear as to whether other dietary components may affect the access of flavonoids into the CNS. Several studies have attempted to investigate this question and despite some progresses being made there is still a long way to go before obtaining clear evidences.

2.2.1. In vitro approaches

Various *in vitro* cellular models of BBB have been used to study flavonoid transfer across the BBB. For example, ECV304 (representing the peripheral side of the BBB) co-cultured with C6 glioma cells (representing the CNS side), bEND5 and RBE4 have been the most common cellular models of BBB [55]. Using this approach, various flavonoids of different sub-classes (flavanones and anthocyanins) have been observed to be able to access and transverse the endothelial cells layer. In addition, the major flavonoid metabolites found in the circulation, glucuronides and O-methylated derivatives are also incorporated into these cells. Indeed, in addition to accumulating these flavonoid metabolites, it appeared that these cells were capable of deconjugating glucuronide derivatives liberating aglycone forms which may then be capable of entering glial cells and thus the brain [56]. Using RBE4 cells, flavan-3-ols, flavonols and anthocyanins have been shown to be able to transverse cells in a time-dependent manner, with quercetin the flavonoid showing the least transport efficiency [57].

In another study, the isomers (+)-catechin and (−)-epicatechin were found to be capable of crossing the BBB layer, with, interestingly, a significant difference between the transport of these two isomers reported [58], suggesting the involvement of a stereo-selective process for flavanol passage across BBB probably due to differences in their efflux out of cells. An important observation was that glucuronic acid conjugates of catechin and epicatechin are detected on the basolateral side, confirming the metabolic competence of these cells, which is in agreement with other observations [55] but also that their transport involves, at least in part, a trans-cellular pathway.

More recently, the hCMEC/D3 cell line, an immortalized human cerebral micro-vessel endothelial cell

Table 1
Transporters expressed at the BBB, its substrates (endo and xenobiotics) and identification of localization (adapted from [52, 74]). AP – apical; BL – basolateral; In – influx (blood to endothelium or endothelium to brain); Ef – efflux (brain to endothelium or endothelium to blood); ND – not determined; PAH – polycyclic aromatic hydrocarbon

Transporters	Substrates	Localization	Direction
Solute carriers			
Energy transport system			
GLUT1	Glucose	AP/BL	In
SGLT1	Glucose	BL	Ef
MCT1	Lactate, monocarboxylates	AP/BL	In
CRT	Creatine	AP/BL	In
Amino acid transport system			
CAT1 and 3	Cationic amino acids	AP	In
LAT1 (system L)	Large neutral amino acids	AP/BL	In
SNAT2, 3 and 5	Small neutral amino acids	BL	Ef
ASCT2	L-Asp, L-Glu and others	BL	Ef
EAAT1, 2, 3	Anionic amino acids	BL	Ef
GLYT	Glycine	ND	In
TAUT	Taurine	AP/BL	In, Ef
Nucleoside transport system			
ENT1 and 3	Nucleosides, nucleotides and nucleobases	AP	In
CNT1, 2 and 3	Nucleosides, nucleotides and nucleobases	BL	In
Organic anion/cation transport system			
OAT2	Organic anions	AP	In
OAT3	PAH, organic anions	BL	Ef
OCT2	Organic cations	AP	In
OCT3	Organic cations	ND	ND
OCTN2	Carnitine	AP/BL	In
Other transport systems			
RFC	Folate	ND	ND
PMAT	Amine transporter	BL	Ef
CTL1	Choline	AP/BL	In, Ef
ABC transporters			
P-gp (MDR1)	Vincristine and others	AP	Ef
MRP1	Organic acids, conjugates	AP/BL	Ef
MRP2	Organic acids, conjugates	AP/BL	ND
MRP3	Organic acids, conjugates	AP/BL	ND
MRP4	Nucleosides, topotecan	AP/BL	Ef
MRP5	Nucleosides	AP/BL	ND
BCRP	Mitoxantron, topotecan	AP	Ef

line, has also been utilized as a BBB model. Using this model, the transport of flavan-3-ols and was detected along with their metabolism to glucuronides matching previous observations with RBE4 cells [58]. Furthermore, synthesized O-methylated flavan-3-ols [59] were found to traverse these cells more efficiently compared to the parent compounds in the same model [60]. Since O-methylated forms are observed *in vivo* this may indicate that they may enter the brain to a relatively high degree and thus may play an important role in mediating the effects of flavonoids in the brain.

In addition to cellular models, *in situ* models of the BBB have also been used to assess flavonoid access to the brain. Here, the permeability of radiolabeled quercetin and naringenin was tested in an *in situ* rat model of perfusion, with both quercetin and naringenin found to be localized in all 7 brain regions studied to different degrees following perfusion of the carotid artery [61]. Quercetin showed a lower permeability that naringenin, something that was suggested to be correlated with its lower lipophilicity and/or because naringenin did not seem to be a

substrate for P-gp unlike quercetin. These results were in agreement with cellular models where *in vitro* BBB transport model with quercetin again showing lower permeability than naringenin and, rutin, the rutinoside of quercetin (not found in the circulation *in vivo*) found not to cross the BBB [62].

2.2.2. Animal studies

Studies in both rats [63–65] and pigs [66, 67] have indicated that anthocyanins are able to reach the brain. In addition, another study has also provided information on the localisation of anthocyanins various brain regions and how this is related to cognitive performance [63]. Intact anthocyanins were detected in various brain regions that mediate cognitive behaviour, although no anthocyanin metabolites were detected, only anthocyanins in their glycosylated form. Whilst these findings are interesting and indicate that anthocyanins may reach the brain intact, it remains uncertain as to the exact extent of flavonoid transfer across the BBB.

Other studies have made use of ^{14}C-labeled polyphenols in order to more accurately investigate the tissue distribution and kinetics of these compounds in rodents after oral gavage. Here, anthocyanin glycosides and their metabolites were again shown to accumulate in the brain over time, along with some flavanol and metabolites at higher concentration [68]. The brain bioavailability of flavan-3-ols has previously been reported in other animal studies, where epicatechin, its O-methylated form and its glucuronide metabolites were found in rat brain after oral ingestion via gavage [69]. Furthermore, epigallocatechin-3-gallate, the main flavanol component of green tea, has also been found in the brain of conscious and freely moving rats after intravenous administration [70]. Recently, it has been demonstrated that oral administration of quercetin leads to brain uptake, with the data suggesting that quercetin metabolites may accumulate in different brain regions [56].

3. General remarks and future avenues of study

Weighing the evidence to date, it appears that flavonoids and some of their metabolites are able to traverse the BBB and enter the brain. However, more data are required using cell and animal investigations to precisely define the nature and extent of this uptake. In this respect, cell models have some advantages over *in vivo* studies as they also provide options to study the transporters and the mechanisms involved in flavonoid transfer and thus are able to deepen the knowledge about the mechanisms of bioactivity. One of the main limitations in studying flavonoid bioavailability in either model is the problem associated with detecting the very low levels entering the brain or crossing the cell layer. Predominately detection relies on HPLC coupled with to UV-visible spectroscopy, fluorescence detection and/or MS. However, the detection limits with these techniques may be insufficient to measure accurately the levels in the various tissue samples. As such, future work requires a greater use of radiolabeled flavonoids that may greatly improve detection limits. Although many of these are not commercial available, researchers in this area must strive to have such compounds synthesized in order to avoid the necessity of needing to use high, non-dietary levels of flavonoid intervention in order to allow detection in the brain.

Furthermore, the strategies used for flavonoid ingestion/administration are different between studies with some studies supplementing the animals' diet, whilst others make a continuous perfusion of a solution containing flavonoids and others use gavage to administrate the compounds to the animals. The route of administration may influence the amount ingested by the animals, and will influence absorption and metabolism due to food matrix effects. Such factors will affect flavonoid concentrations reaching the circulation and tissues and may also influence the accumulation pattern of flavonoids in tissues.

Invariably, the models used to assess the bioavailability of flavonoids in brain, for both *in vivo* and *in vitro* studies, are of animal origin (bovine, rat or mouse). However, species differences in flavonoid absorption and metabolism and differences in the structure and function of the BBB make the extrapolation of animal results to humans an impossible task. Indeed, there are considerable differences in the expression and activity of both influx and efflux transporters in the BBB between species that may influence flavonoid transfer and thus brain bioavailability [50]. In this respect, the development of a human cellular model of the BBB [51, 58] was a step forward in evaluating and validating existing results with models from different animal species.

An enduring question in flavonoid research is whether conjugated or unconjugated forms mediate the observed biological effects following supplementation with flavonoid-rich foods. Both have been detected in brain tissue following ingestion of such foods, although the concentrations required *in vivo* for biological responses are unclear. It is generally accepted that hydrophilic flavonoid-glucuronide metabolites cannot cross the BBB, meaning that those glucuronides detected in the brain are formed there. Despite this, there are possibilities that these metabolites attach the cell surface membranes thus facilitating their transfer [56], or are transported in a similar manner to other glucuronides, such as morphine glucuronide. Furthermore, it is also plausible that flavonoid glucuronides are cleaved by β-glucuronidase activity into the resulting hydrophobic aglycone, suggesting the possibility of a transient de-conjugation reaction and subsequent re-conjugation taking place around the BBB [56, 69, 71]. As previously described, the *in vitro* conjugation of flavan-3-ols with glucuronic acid after incubation with a hBBB cell model supports this theory [58]. Anthocyanins, a particular class of flavonoids, are especially intriguing. Several studies have shown they appear in plasma and in brain in their intact form [39, 40, 64, 67], i.e. glycosylated. However, it is generally accepted that, as they are charged, diffusion is not a potential mechanism of their crossing the BBB, and that the precise mechanism of transfer remains to be clarified. It has been hypothesised that that they could use the glucose transporters or bilitranslocase to enter the cell [72, 73].

Overall, there is still a gap regarding our knowledge regarding the mechanisms of flavonoid transport across the BBB. Further, multidisciplinary approaches are required, combining knowledge and techniques of different fields such as nutrition, organic chemistry, food science, pharmacology and biochemistry, in order to precisely determine this crucial issue in flavonoid bioavailability and bioactivity, and to allow the identification of factors (diet or metabolic) that increase or decrease the ability of flavonoids to enter the CNS.

Acknowledgments

Financial support from FCT (PTDC/QUI/65501/2006, SFRH/BPD/75294/2010) is gratefully acknowledged.

References

[1] Cooper-Driver GA, Bhattacharya M. Role of phenolics in plant evolution. Phytochemistry. 1998;49(5):1165-74.

[2] Hollman PC, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem Toxicol. 1999;37(9-10):937-42.

[3] Kaur C, Kapoor HC. Antioxidants in fruits and vegetables - the millennium's health. International Journal of Food Science and Technology. 2001;36(7):703-25.

[4] Van Duyn MA, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. J Am Diet Assoc. 2000;100(12):1511-21.

[5] Aquilano K, Baldelli S, Rotilio G, Ciriolo MR. Role of nitric oxide synthases in Parkinson's disease: A review on the antioxidant and anti-inflammatory activity of polyphenols. Neurochem Res. 2008;33(12):2416-26.

[6] Singh M, Arseneault M, Sanderson T, Murthy V, Ramassamy C. Challenges for research on polyphenols from foods in Alzheimer's disease: Bioavailability, metabolism, and cellular and molecular mechanisms. J Agric Food Chem. 2008;56(13):4855-73.

[7] Lau FC, Shukitt-Hale B, Joseph JA. Age-related neuronal and behavioral deficits are improved by polyphenol-rich blueberry supplementation Oxidative Stress and Age-Related Neurodegeneration. Boca Raton: Crc Press-Taylor & Francis Group. 2006;373-93.

[8] Spencer JP. Nutrients, brain health: An overview. Genes Nutr. 2009;4:225-6.

[9] Spencer JP. The impact of flavonoids on memory: Physiological and molecular considerations. Chem Soc Rev. 2009;38(4):1152-61.

[10] Spencer JP, Vauzour D, Rendeiro C. Flavonoids and cognition: The molecular mechanisms underlying their behavioural effects. Arch Biochem Biophys. 2009;492(1-2):1-9.

[11] Assuncao M, Santos-Marques MJ, Carvalho F, Lukoyanov NV, Andrade JP. Chronic green tea consumption prevents age-related changes in rat hippocampal formation. Neurobiol Aging. 2011;32(4):707-17.

[12] Assuncao M, Santos-Marques MJ, de Freitas V, Carvalho F, Andrade JP, Lukoyanov NV, Paula-Barbosa MM. Red wine antioxidants protect hippocampal neurons against ethanol-induced damage: A biochemical, morphological and behavioral study. Neuroscience. 2007;146(4):1581-92.

[13] 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(2):344-50.

[14] Malin DH, Lee DR, Goyarzu P, Chang YH, Ennis LJ, Beckett E, Shukitt-Hale B, Joseph JA. Short-term blueberry-enriched diet prevents and reverses object recognition memory loss in aging rats. Nutrition. 2011;27(3):338-42.

[15] Shukitt-Hale B, Cheng V, Joseph JA. Effects of blackberries on motor and cognitive function in aged rats. Nutr Neurosci. 2009;12(3):135-40.

[16] Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, Joseph JA. Blueberry supplementation improves memory in older adults. J Agric Food Chem. 2010;58(7):3996-4000.

[17] Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B, Joseph JA. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. Br J Nutr. 2010;103(5):730-4.
[18] Kesse-Guyot E, Fezeu L, Andreeva VA, Touvier M, Scalbert A, Hercberg S, Galan P. Total and specific polyphenol intakes in midlife are associated with cognitive function measured 13 years later. J Nutr. 2012;142(1):76-83.
[19] Beking K, Vieira A. Flavonoid intake and disability-adjusted life years due to Alzheimer's and related dementias: A population-based study involving twenty-three developed countries. Public Health Nutr. 2010;13(9):1403-9.
[20] Marambaud P, Zhao H, Davies P. Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem. 2005;280(45):37377-82.
[21] Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T, Tan J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci. 2005;25(38):8807-14.
[22] Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, Pollard A, Fenech M, Zhou XF. Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse. Neurotox Res. 2009;15(1):3-14.
[23] Gu Y, Luchsinger JA, Stern Y, Scarmeas N. Mediterranean diet, inflammatory and metabolic biomarkers, and risk of Alzheimer's disease. J Alzheimers Dis. 2010;22(2):483-92.
[24] Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. Ann Neurol. 2006;59(6):912-21.
[25] Baluchnejadmojarad T, Roghani M, Nadoushan MR, Bagheri M. Neuroprotective effect of genistein in 6-hydroxydopamine hemi-parkinsonian rat model. Phytother Res. 2009;23(1): 132-5.
[26] Datla KP, Zbarsky V, Rai D, Parkar S, Osakabe N, Aruoma OI and Dexter DT. Short-term supplementation with plant extracts rich in flavonoids protect nigrostriatal dopaminergic neurons in a rat model of Parkinson's disease. J Am Coll Nutr. 2007;26(4):341-9.
[27] Guo S, Yan J, Yang T, Yang X, Bezard E, Zhao B. Protective effects of green tea polyphenols in the 6-OHDA rat model of Parkinson's disease through inhibition of ROS-NO pathway. Biol Psychiatry. 2007;62(12):1353-62.
[28] Gao X, Chen H, Fung TT, Logroscino G, Schwarzschild MA, Hu FB, Ascherio A. Prospective study of dietary pattern and risk of Parkinson disease. Am J Clin Nutr. 2007;86(5):1486-94.
[29] Del Rio D, Calani L, Cordero C, Salvatore S, Pellegrini N, Brighenti F. Bioavailability and catabolism of green tea flavan-3-ols in humans. Nutrition. 2010;26(11-12):1110-6.
[30] Renouf M, Redeuil K, Longet K, Marmet C, Dionisi F, Kussmann M, Williamson G, Nagy K. Plasma pharmacokinetics of catechin metabolite 4′-O-Me-EGC in healthy humans. Eur J Nutr. 2011;50(7):575-80.
[31] Stalmach A, Mullen W, Steiling H, Williamson G, Lean ME, Crozier A. Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. Mol Nutr Food Res. 2010;54(3):323-34.
[32] Stalmach A, Troufflard S, Serafini M, Crozier A. Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. Mol Nutr Food Res. 2009;53 Suppl 1:S44-53.
[33] Baba S, Osakabe N, Yasuda A, Natsume M, Takizawa T, Nakamura T, Terao J. Bioavailability of (−)-epicatechin upon intake of chocolate and cocoa in human volunteers. Free Radic Res. 2000;33(5):635-41.
[34] Natsume M, Osakabe N, Oyama M, Sasaki M, Baba S, Nakamura Y, Osawa T, Terao J. Structures of (−)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (−)-epicatechin: Differences between human and rat. Free Radic Biol Med. 2003;34(7):840-9.
[35] Ritter C, Zimmermann BF, Galensa R. Chiral separation of (+)/(−)-catechin from sulfated and glucuronidated metabolites in human plasma after cocoa consumption. Anal Bioanal Chem. 2010;397(2):723-30.
[36] Mullen W, Borges G, Donovan JL, Edwards CA, Serafini M, Lean ME, Crozier A. Milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans. Am J Clin Nutr. 2009;89(6):1784-91.
[37] Garcia-Alonso M, Minihane AM, Rimbach G, Rivas-Gonzalo JC, de Pascual-Teresa S. Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and antioxidant capacity of plasma. J Nutr Biochem. 2009;20(7):521-9.
[38] Mertens-Talcott SU, Rios J, Jilma-Stohlawetz P, Pacheco-Palencia LA, Meibohm B, Talcott ST, Derendorf H. Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (Euterpe oleracea Mart.) in human healthy volunteers. J Agric Food Chem. 2008;56(17):7796-802.
[39] Milbury PE, Vita JA, Blumberg JB. Anthocyanins are bioavailable in humans following an acute dose of cranberry juice. J Nutr. 2010;140(6):1099-104.
[40] Mullen W, Edwards CA, Serafini M, Crozier A. Bioavailability of pelargonidin-3-O-glucoside and its metabolites in humans following the ingestion of strawberries with and without cream. J Agric Food Chem. 2008;56(3):713-9.
[41] Mullen W, Boitier A, Stewart AJ, Crozier A. Flavonoid metabolites in human plasma and urine after the consumption of red onions: Analysis by liquid chromatography with photodiode array and full scan tandem mass spectrometric detection. J Chromatogr A. 2004;1058(1-2):163-8.
[42] Mullen W, Edwards CA, Crozier A. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Br J Nutr. 2006;96(1):107-16.
[43] Wiczkowski W, Romaszko J, Bucinski A, Szawara-Nowak D, Honke J, Zielinski H, Piskula MK. Quercetin from shallots (Allium cepa L. var. aggregatum) is more bioavailable than its glucosides. J Nutr. 2008;138(5):885-8.
[44] Erlund I, Silaste ML, Alfthan G, Rantala M, Kesaniemi YA, Aro A. Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables. Eur J Clin Nutr. 2002;56(9):891-8.
[45] Mauri PL, Iemoli L, Gardana C, Riso P, Simonetti P, Porrini M, Pietta PG. Liquid chromatography electrospray ionization mass spectrometric characterization of flavonol glycosides in

tomato extracts and human plasma. Rapid Commun Mass Spectrom. 1999;13(10):924-31.
[46] Palmer AM. The blood-brain barrier. Neurobiology of Disease. 2010;37(1):1-2.
[47] McNamara PJ, Leggas M, Drug distribution, In: Hacker M, Bachmann K, Messer W. Pharmacology: Principles and practice: Academic Press; 2009;113-31.
[48] Lehman-McKeeman L. Absorption, distribution and excretion of toxicants. In: Klaassen CD Casarett and Doull's - Toxicology, the basic science of poisons: McGraw-Hill; 2008;131-59.
[49] Taft DR. Drug excretion. In: Hacker M, Bachmann K, Messer W, Pharmacology: Principles and practice: Academic Press; 2009, p. 175-99.
[50] Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, Terasaki T. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. J Neurochem. 2011;117(2):333-45.
[51] Weksler BB, Subileau EA, Perriere N, Charneau P, Holloway K, Leveque M, Tricoire-Leignel H, Nicotra A, Bourdoulous S, Turowski P, Male DK, Roux F, Greenwood J, Romero IA and Couraud PO. Blood-brain barrier-specific properties of a human adult brain endothelial cell line. FASEB J. 2005;19(13):1872-4.
[52] Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis. 2010;37(1):13-25.
[53] Cardoso FL, Brites D, Brito MA. Looking at the blood-brain barrier: Molecular anatomy and possible investigation approaches. Brain Res Rev. 64(2):328-63.
[54] Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):41-53.
[55] Youdim KA, Dobbie MS, Kuhnle G, Proteggente AR, Abbott NJ, Rice-Evans C. Interaction between flavonoids and the blood-brain barrier: *In vitro* studies. J Neurochem. 2003;85(1):180-92.
[56] Ishisaka A, Ichikawa S, Sakakibara H, Piskula MK, Nakamura T, Kato Y, Ito M, Miyamoto K, Tsuji A, Kawai Y, Terao J. Accumulation of orally administered quercetin in brain tissue and its antioxidative effects in rats. Free Radic Biol Med. 2011;51(7):1329-36.
[57] Faria A, Pestana D, Teixeira D, Azevedo J, De Freitas V, Mateus N, Calhau C. Flavonoid transport across RBE4 cells: A blood-brain barrier model. Cell Mol Biol Lett. 2010;15(2):234-41.
[58] Faria A, Pestana D, Teixeira D, Couraud PO, Romero I, Weksler B, de Freitas V, Mateus N, Calhau C. Insights into the putative catechin and epicatechin transport across blood-brain barrier. Food Funct. 2011;2(1):39-44.
[59] Gonzalez-Manzano S, Gonzalez-Paramas A, Santos-Buelga C, Duenas M. Preparation and characterization of catechin sulfates, glucuronides, and methylethers with metabolic interest. J Agric Food Chem. 2009;57(4):1231-8.
[60] Faria A, Meireles M, Pestana D, Monteiro R, Santos-Buelga C, Gonzalez-Manzano S, Duenas M, de Freitas V, Mateus N, Calhau C. Flavonoid metabolites transport across BBB. 5th International Conference on Polyphenols, Sitges, Barcelona. 2011.
[61] Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an *in situ* model of the blood-brain barrier. Free Radic Biol Med. 2004;36(5):592-604.
[62] Yang ZH, Sun X, Mei C, Sun XB, Liu XD, Chang Q. An *in vitro* transport model for rapid screening and predicting the permeability of candidate compounds at blood-brain barrier. J Asian Nat Prod Res. 2011;13(12):1087-97.
[63] 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(2):111-20.
[64] Passamonti S, Vrhovsek U, Vanzo A, Mattivi F. Fast access of some grape pigments to the brain. J Agric Food Chem. 2005;53(18):7029-34.
[65] Talavera S, Felgines C, Texier O, Besson C, Gil-Izquierdo A, Lamaison JL, Remesy C. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. J Agric Food Chem. 2005;53(10):3902-8.
[66] Kalt W, Blumberg JB, McDonald JE, Vinqvist-Tymchuk MR, Fillmore SA, Graf BA, O'Leary JM, Milbury PE. Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. J Agric Food Chem. 2008;56(3):705-12.
[67] Milbury PE, Kalt W. Xenobiotic metabolism and berry flavonoid transport across the blood-brain barrier. J Agric Food Chem. 2010;58(7):3950-6.
[68] Janle EM, Lila MA, Grannan M, Wood L, Higgins A, Yousef GG, Rogers RB, Kim H, Jackson GS, Ho L, Weaver CM. Pharmacokinetics and tissue distribution of 14C-labeled grape polyphenols in the periphery and the central nervous system following oral administration. J Med Food. 2010;13(4):926-33.
[69] Abd El Mohsen MM, Kuhnle G, Rechner AR, Schroeter H, Rose S, Jenner P, Rice-Evans CA. Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. Free Radic Biol Med. 2002;33(12):1693-702.
[70] Lin LC, Wang MN, Tseng TY, Sung JS, Tsai TH. Pharmacokinetics of (−)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain regional distribution. J Agric Food Chem. 2007;55(4):1517-24.
[71] Gutierrez-Merino C, Lopez-Sanchez C, Lagoa R, Samhan-Arias AK, Bueno C, Garcia-Martinez V. Neuroprotective actions of flavonoids. Curr Med Chem. 2011;18(8):1195-212.
[72] Faria A, Pestana D, Azevedo J, Martel F, Freitas Vd, Azevedo I, Mateus N, Calhau C. Absorption of anthocyanins through intestinal epithelial cells - Putative involvement of GLUT2. Molecular Nutrition & Food Research. 2009;53(11):1430-7.
[73] Passamonti S, Vrhovsek U, Mattivi F. The interaction of anthocyanins with bilitranslocase. Biochemical and Biophysical Research Communications. 2002;296(3):631-6.
[74] Ohtsuki S, Terasaki T. Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. Pharm Res. 2007;24(9):1745-58.