The neuroprotective effects of cocoa flavanol and its influence on cognitive performance

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Cocoa powder and chocolate contain numerous substances among which there is a quite large percentage of antioxidant molecules, mainly flavonoids, most abundantly found in the form of epicatechin. These substances display several beneficial actions on the brain. They enter the brain and induce widespread stimulation of brain perfusion. They also provoke angiogenesis, neurogenesis and changes in neuron morphology, mainly in regions involved in learning and memory. Epicatechin improves various aspects of cognition in animals and humans. Chocolate also induces positive effects on mood and is often consumed under emotional stress. In addition, flavonoids preserve cognitive abilities during ageing in rats, lower the risk for developing Alzheimer's disease and decrease the risk of stroke in humans. In addition to their beneficial effects on the vascular system and on cerebral blood flow, flavonoids interact with signalization cascades involving protein and lipid kinases that lead to the inhibition of neuronal death by apoptosis induced by neurotoxicants such as oxygen radicals, and promote neuronal survival and synaptic plasticity. The present review intends to review the data available on the effects of cocoa and chocolate on brain health and cognitive abilities.

Introduction

The cocoa bean, as any bean, is rich in fat that represents 50% or even more of the total weight. The next most important ingredients are proteins or nitrogenous elements, including theobromine (1.0–2.5%) and caffeine (0.06–0.4%). Starches and sugars together form 20–25% of the weight of the bean. Most importantly, cocoa beans are a concentrated source of anti-oxidants, in particular flavonoids, with the flavan-3-ols and their derivatives being present in high concentrations [1]. The flavan-3-ol compounds are mostly present in the cocoa bean in the form of epicatechin and catechin [2], which can also serve as building blocks for the polymeric procyanidin type B-2 [3]. However, during processing of the bean to cocoa powder and chocolate, the concentration of anti-oxidants can be affected by a variety of biological processes and treatments such as fermentation, roasting and ditching [4]. Genetic variability can also generate a 1–4-fold difference in the anti-oxidant content of fresh cocoa beans [5] and the content of epicatechin has also been reported to vary from 2.66 mg g^{-1} in Jamaican beans to 16.52 mg g^{-1} in Costa Rican beans [6].

Cocoa beans contain low variable amounts of caffeine (0.06–0.4%), a well-known psychostimulant. Cocoa powder contains the highest amount of caffeine followed by unsweetened baking chocolate. Dark chocolate will vary considerably in the amount of caffeine (35–200 mg 50 g^{-1}) while milk chocolate contains relatively low amounts of caffeine (14 mg 50 g^{-1}). The cocoa bean is also the most concentrated source of theobromine, another methylxanthine. Unlike caffeine, theobromine, also present in cocoa beans, has only a mild stimulatory effect on the central nervous system. The amount of theobromine varies with the finished product. Dark chocolate, unsweetened baking chocolate and cocoa powder contain more theobromine than milk chocolate and chocolate syrups. For example, 50 g milk chocolate contains about 75 mg theobromine while the same weight of very dark chocolate can contain up to 220 mg theobromine. The effects of the methylxanthines, and mainly those of caffeine, have been extensively reviewed elsewhere both concerning cognition and mental performance [7, 8] and the preventive effects of this methylxanthine on age-related cognitive decline and neurodegenerative diseases [9, 10] and will not be detailed here.

Cocoa also contains some other compounds with potential biological activity. These are biogenic amines such as serotonin, tryptophan, phenylethylamine, tyrosine, tryptamine and tyramine. The concentration of these compounds increases during fermentation and decreases during roasting and alkalinization. In general, these concentrations are irrelevant in healthy subjects since these compounds are metabolized in the intestinal mucosa, liver and kidneys by the monoamine oxidases (MAO). The effects of biogenic amines are only expressed in people with MAO deficiency and could lead to headaches and increased blood pressure and hence often to chocolate avoidance [11]. These effects will not be discussed here.

In addition, a few other compounds with biological activity can be found in cocoa beans and derived products. These are anandamide, an endogenous ligand for the cannabinoid receptor found in low amounts, $0.5\ \mu g\ g^{-1}$, salsolinol and tetrahydro-β-carbolines (THBCs). The latter compounds are found in milk and dark chocolate, and cocoa (5, 20, $25\ \mu g\ g^{-1}$ for salsolinol and 1.4, 5.5 and $3.3\ \mu g\ g^{-1}$ for THBCs, respectively). However, there is no evidence that the consumption of chocolate increases the concentration of these compounds in circulating blood. Finally, magnesium can also be found in cocoa and chocolate ($90\text{–}100\ mg\ 100\ g^{-1}$ in cocoa $vs.$ $43\text{–}50\ mg\ 100\ g^{-1}$ in dark chocolate [11].

In summary, this review will be devoted mostly to the health effects of cocoa and chocolate resulting from the high level of anti-oxidants present in cocoa and chocolate rather considering them as functional foods. This review will try to analyze whether cocoa and chocolate can be considered as nutraceuticals providing health benefits, including the potential prevention of some diseases. Several review articles have been dealing recently with the potential neuroprotective and cognition enhancing properties of flavonoids from various sources [12–15]. In the present review we will concentrate on the potential effects of flavonoids from cocoa and chocolate with a particular emphasis on brain activity and potential neuroprotective action. In addition, the effects of chocolate on mood will be considered.

Bioavailability and penetration of flavanols into the brain

Epicatechin is rapidly absorbed in humans and is detectable in plasma 30 min after ingestion. Epicatechin concentrations reach a peak 2–3 h after ingestion and return to baseline value by 6–8 h after consumption of flavanol-rich chocolate. The overall effects of a daily regular consumption may potentially accumulate [16], mainly if absorbed in high doses [17].

To exert any effect on the brain, anti-oxidants need to cross the blood–brain barrier (BBB) to enter the brain. Their permeability is proportional to their lipophilicity and inversely proportional to their degree of polarity. Catechin and epicatechin have been shown to cross the BBB in two BBB cell lines, one from rat and one from human origin. The process is time-dependent, stereoselective, epicatechin crossing more efficiently the BBB than catechin [18]. In animals *in vivo*, epicatechin was found to enter the brain after oral ingestion and was detected in the brain [19, 20]. Brain concentrations of epicatechin were even found to increase upon repetitive exposure to a grape seed polyphenol extract [21].

There are not many data available on the precise distribution of flavonoids within brain tissue, and especially no regional data available for epicatechin. After chronic administration, higher concentrations of tangeretin were found in the rat striatum, hypothalamus and hippocampus [22]. In blueberry supplemented rats, anthocyanins were detected in the cortex, hippocampus, striatum and cerebellum [23]. However, the possibility for epicatechin and most likely the other flavonoids as well to cross the blood–brain barrier and accumulate in the brain suggests that they may represent good candidates for a direct positive action on the brain, including cognition and possibly neuroprotection (for review see [15]).

Cerebrovascular and cognitive effects of flavonoids from cocoa and chocolate

For optimal brain functioning, cerebral blood flow (CBF) needs to be well maintained to support constant oxygen and glucose supply to neurons as well as waste excretion. Increase in CBF represents a potential means to improve cerebral function. The principal polyphenols that enhance CBF in humans come mainly from cocoa, wine, grape seeds, berries, tea, tomatoes and soya [24]. At the cardiovascular and peripheral level, polyphenol-rich cocoa induces vasodilatation. In one study that looked at cocoa flavanols and vasodilatation, 27 healthy individuals received daily 920 ml of a flavanol-rich cocoa drink (821 mg of flavanols/dose) over 4 days. Peripheral arterial tonometry showed that there was a 29% increase in amplitude at 12 h after the last dose of cocoa. On the 5th day, an additional dose of cocoa led to a 33% increase after 90 min [25]. The mechanism leading to vasodilatation is nitric oxide (NO)-dependent because a nitric oxide synthase (NOS) inhibitor administered after 4 days of cocoa ingestion completely reversed the increase in vasodilatation [25, 26]. Moreover, this study showed that cocoa enriched with flavanols improved measures of endothelial function to a greater degree in healthy elderly persons than in the younger population. Thus, flavanols may be useful in counteracting decreases in endothelial function associated with ageing [27]. Indeed, during ageing endothelium-dependent vasodilation properties attenuate or can even be lost [28]. The latter function is almost exclusively medi-

ated by NO [29]. There appears to be a causal link between cocoa or chocolate ingestion, flow-mediated vasodilation and the release of NO induced by epicatechin in the circulation [25, 30–32].

The consequences of the ingestion of cocoa or cocoa flavanols on CBF have not been explored in animals. In human studies, it was reported that the ingestion of a single dose or a 1 week treatment with cocoa rich in flavanol (900 mg day^{-1}) increases CBF in grey matter [33] and reverses endothelial dysfunction in a dose-dependent manner [17], which suggests its potential in the treatment of cerebrovascular problems [34]. Arterial spin-labelling magnetic resonance imaging (ASL-MRI) reported increased CBF which reached a maximal level at the first time of measurement, i.e. 2 h after ingestion of the flavanol-rich drink. The peak effect of flavanols might occur earlier since the half-life of elimination of epicatechin in humans was found to be fast, i.e. 1.9 and 2.3 h for 40 and 80 g chocolate, respectively [35]. The use of transcranial Doppler ultrasonography also allowed showing an increase in CBF through the middle cerebral artery after the consumption of flavanol-rich cocoa [27, 36, 37]. Finally, in a double-blind randomized placebo-controlled study, blood oxygenation level dependent (BOLD)-functional magnetic resonance imaging (fMRI) showed increased signal in some brain regions, after the acute consumption of a flavanol-rich cocoa drink. In the response to task switching in the young participants tested, no significant effects of chocolate were found in reaction times, the cost of switching between two sets of rules, or heart rate after the ingestion of flavanol-rich cocoa. The authors considered that the fMRI changes may rather reflect cognitive changes that could not be measured in the tests used possibly because participants were young and likely operating at a high level of cognitive ability [34].

In humans, there is a relative paucity of clinical trials exploring the effects of dark chocolate or cocoa on neuropsychological function in different types of healthy individuals. This is observed despite the indication that the anti-oxidants contained in cocoa and dark chocolate may have beneficial effects on the healthy and possibly less healthy brain. Most of the research on the relation between anti-oxidants, cognition and brain health have rather concentrated on flavonoids in soya, berries, wine, tea, vitamins, curcuma, etc. and much less has been reported on chocolate and cocoa (for review see [38–40]). A recent randomized, single-blind, order counterbalanced, crossover design study reported an acute improvement of visual and cognitive function linked to the consumption of cocoa flavanols. The study was performed on 30 healthy adults given dark chocolate containing 720 mg flavanols or a matched quantity of white chocolate. Cognitive performance was assessed using a visual spatial working memory for location task and a choice reaction time task designed to engage processes of sustained

attention and inhibition. Compared with the control condition, cocoa flavanols improved visual contrast sensitivity and reduced the time required to detect motion direction. Since performance improved in different tests, flavanol-related changes could be indicative of quite general mechanisms producing an increase in motivation or attentiveness on the tasks. These acute effects could result both from increased CBF and from increased blood supply to the retina [41]. Indeed, there is a link between retinal blood flow and function [42] and hence flavonoids may influence the function of retinal neurons. In this respect, anthocyanins have been found to accumulate in the brain and eyes of pigs exposed to anthocyanins extracted and powdered from blueberries. This suggests that these compounds may act directly at the sites where their benefits have been documented such as in cognition and vision [43, 44].

In another study testing sustained mental demand in 30 healthy adults, the consumption of drinks containing 520 mg or 994 mg cocoa flavonoids compared with a matched control improved cognitive performance in serial subtraction tasks. The consumption of both doses improved serial threes performance (task consisting of counting backwards in threes from a given number). The 994 mg cocoa flavonoid containing beverage significantly accelerated rapid visual information processing but resulted in more errors in the serial sevens subtraction. The consumption of the 520 mg flavanol enriched drink also reduced self-rated mental fatigue, possibly reflecting the demanding and fatiguing nature of and the level of stress induced by the tasks. These doses of flavanol also improved mood. The mechanisms underlying these effects are unknown but they are most prominent when the concentration of epicatechin and CBF rates are at their highest level [34], suggesting that they may be related to the known effects of cocoa flavonoids on endothelial function and CBF [45]. Several studies using brain imaging techniques reported a correlation between CBF and cognitive function in humans [27, 34, 46]. A recent randomized, double-blind placebo-controlled trial on 63 middle-aged volunteers (40–65 years) studied steady-state visually evoked potential (SSVEP) topography changes after cocoa flavanol consumption (250 or 500 mg vs. a low cocoa flavanol drink given over a 30 day period). Accuracy and reaction time were not affected by flavanol exposure while SSVEP amplitude and phase difference were affected in several posterior parietal and centro-frontal areas during memory encoding, working memory hold period and retrieval. These data suggest increased neural efficiency in spatial working memory as a result of cocoa flavanol consumption [47]. In contrast with the previous studies, a double-blind, placebo-controlled, fixed dose, parallel group clinical trial looked at the effect of a 37 g dark chocolate bar associated with 8 ounces (237 ml) of an artificially sweetened cocoa beverage or a matched placebo given to a group of healthy subjects (41 men and 60 women over

60 years) for 6 weeks. In this study the treatment did not improve any neuropsychological, haematologic or physiologic variables [48].

The flavonoids are considered to influence cognitive function by influencing the signalling pathways that are involved in normal memory processing but the precise mechanisms of action have not yet been clarified. It is known that cocoa flavanols act on CBF and endothelial function and these features were examined using preclinical models. The treatment with one of the major chocolate flavanols, epicatechin, added to mice chow at the dose of 500 μg g^{-1} (daily supply of 2.5 mg) stimulated angiogenesis while it enhanced retention of spatial memory and dendritic spine density in the dentate gyrus of the hippocampus only when exercise was combined with epicatechin administration. These authors also found that the epicatechin treatment upregulated genes associated with learning in the hippocampus while it did not affect hippocampal adult neurogenesis [20]. The effects of flavonoid-rich foods on cognitive function have been linked to the ability of flavonoids to interact with the cellular and molecular paradigms responsible for memory and learning [49, 50], including those involved in long term potentiation and synaptic plasticity [51]. These effects have been hypothesized to lead to enhanced neuronal connection and communication and hence greater capacity for memory acquisition, storage and retrieval [50]. However, most of the studies mentioned above have been limited to the hippocampus and one cannot exclude parallel effects in other brain regions. In relation to this point, it was reported that cocoa administered orally to rats in large amounts (100 mg 100 g^{-1}) showed anxiolytic properties in the elevated T-maze test [52]. Anxiety levels are largely regulated at the amygdalar level [53] which would imply possible effects of the flavonoids on brain regions outside the hippocampus.

In summary, the flavonoids contained in cocoa and chocolate appear able to improve various types of cognitive and visual tasks, possibly as the result of more efficient perfusion of blood to different neural tissues, clearly both forebrain and more posterior cortex and possibly also influence retinal blood flow and visual function.

Potential neuroprotective properties of cocoa and chocolate flavanoids

Flavonoids exert a multiplicity of neuroprotective actions, including the capacity to protect neurons from damage induced by neurotoxins, reduce neuroinflammation, and promote memory, learning and cognitive function. These effects are related to two common processes. First, as detailed later, flavonoids interact with signalization cascades involving protein and lipid kinases that lead to the inhibition of neuronal death by apoptosis induced by neu-

rotoxicants (such as oxygen radicals) and to the promotion of neuronal survival and synaptic plasticity. Concurrently, they induce beneficial effects on the vascular system and on CBF mainly by improving endothelial function and stimulating angiogenesis. Via these mechanisms, the lifelong consumption of flavonoid-rich nutrients has the potential ability to limit neurodegeneration and prevent or even reverse age-related cognitive decline (for review see [15, 54]).

Age-related cognitive decline

In this respect a recent preclinical study showed an effect of a cocoa flavonoid rich extract (ACTICOA powder; Barry Callebaut) on cognitive decline in aged rats. ACTICOA powder given orally to the rats at the dose of 24 mg kg^{-1} daily between 15 and 27 months of age affected the onset of age-related cognitive deficits that appeared at 21 months. ACTICOA powder improved cognitive performance in two tests. At 17, 21 and 25 months, in the light extinction paradigm, treated rats were more active and discriminated better between the active and inactive lever. In the Morris water maze, the performance of ACTICOA-treated rats remained stable between 21 and 25 months while that of control rats declined. In this spatial task, both short and long term memory were improved by the treatment. The lifespan of treated rats was also prolonged by 11% over the 27 month study. Finally ACTICOA powder maintained high urinary free dopamine concentrations in old Wistar rats which the authors hypothesized to reflect possibly the neuroprotection of the dopaminergic nigrostriatal system. Indeed, urinary dopamine concentrations have been related to the severity of parkinsonian symptoms in humans [55, 56]. The results obtained in this animal model suggest that ACTICOA powder may be beneficial in retarding age-related brain impairments, including cognitive deficits in normal ageing. Whether these data can be extended to age-related cognitive decline in humans and to neurodegenerative diseases is not yet clear and would require further preclinical and clinical exploration [57]. Likewise the same ACTICOA extract or vitamin E, that has powerful anti-oxidant properties, was orally administered to rats for 14 days before heat exposure at 40°C during 2 h. Both treatments significantly reduced free radical production by leucocytes. Moreover, rats treated with ACTICOA or vitamin E had better cognitive performance since they were able to discriminate between the active lever and inactive levers in a light extinction paradigm and their spatial long term memory retrieval was preserved in the Morris water maze. Thus, cocoa flavonoids are able to counteract the overproduction of free radicals and their deleterious consequences on cognition [58].

In humans, three studies assessed the consequences of flavonoid intake on normal age-related cognitive decline. The first study, concerning old men, assessed cognitive decline by using the Mini-Mental State Examination (MMSE). In 1990, the authors found cognitive impairment

(MMSE score ≤25) in 154/473 men (32%) and cognitive decline from 1990 to 1993 (drop >2 points) in 51/342 men (15%). They found no association between the intake of vitamins C or E and the risk of cognitive decline while they reported a tendency to an inverse relation between flavonoid intake and risk of cognitive decline, though this was not statistically significant [59]. In the PAQUID (Personnes Agées Quid) study, the relationship between flavonoid intake and cognitive function and decline was prospectively examined among subjects aged 65 years or more. The study included 1640 subjects free from dementia at baseline in 1990 and with reliable dietary assessment who were tested four times over a 10 year period. Cognitive function was assessed with MMSE, Benton's Visual Retention Test and 'Isaacs' Set Test at each visit. Information on flavonoid intake was collected at baseline. The selected food items included citrus fruits, kiwis, other fruits, dried fruits, cabbage, spinach, French beans, asparagus, sweet pepper, oat flakes, chocolate, tea, coffee, soup and fruit juice. This study showed that after adjustment for age, gender and educational level, flavonoid intake was associated with both better cognitive performance at baseline and better evolution of performance over time. The most positive evolution was found in subjects in the two highest quartiles of flavonoid intake compared with subjects in the lowest quartile. After 10 year follow-up, subjects with the lowest flavonoid intake had lost on average 2.1 points on the MMSE, whereas subjects with the highest quartile had lost 1.2 points. This study raises the possibility that dietary flavonoid intake might be associated with better cognitive evolution [60]. Finally a Norwegian cross-sectional study considered the cognitive influence of the intake of flavonoids from chocolate, wine and tea. The relation between the consumption of these items and cognitive performance was explored in 2031 participants (aged 70–74 years) including 55% women. Participants who consumed the three types of food or beverages performed significantly better in cognitive tests and had a lower prevalence of poor cognitive performance than those who did not. The associations between the intake of these food and drinks, and cognition were dose-dependent. Most cognitive functions tested were influenced by intake of these foods or beverages. The effect was maximum for the consumption of ~10 g day^{-1} for chocolate, 75–100 ml day^{-1} for wine, almost linear for tea, most pronounced for wine and modestly weaker for chocolate intake. In contrast, there was no effect of each food or beverage analyzed separately. Thus, in the elderly, a diet containing large amounts of some flavonoid-rich foods is associated with better performance in several cognitive abilities in a dose-dependent manner [61].

Altogether, the studies cited above agree with the possibility that dietary flavonoids might be associated with age-related cognitive preservation and the effect could be stronger if flavonoids are taken together from different food sources.

Alzheimer's disease

Several studies have looked at the relation between anti-oxidant intake and dementia, most often the risk of Alzheimer's disease. In Alzheimer's disease excessive production and deposition of amyloid beta (Aβ) peptide lead to microglial activation, and the resultant production of inflammatory mediators further boosts Aβ production and induces death and dysfunction of neurons. Aβ production is mediated by β- and γ-secretase activities and prevented by α-secretase. It was shown recently that in cultured human neuroblastoma cells, low concentrations of NO up-regulate the expression of α-secretase, and down-regulate that of β-secretase. These data suggest that cerebrovascular NO might suppress or limit the production of Aβ [12, 62]. This preventive action can be achieved by adopting various nutritional and lifestyle measures including the consumption of cocoa powder or chocolate [32, 62]. Indeed, as developed earlier, the flavanols contained in cocoa powder and mainly epicatechin act directly on the endothelium of brain vessels to stimulate the activity of the constitutive endothelium NOS form (eNOS) to induce vasodilatation and improve cerebrovascular perfusion [13, 27, 32].

Results from prospective observational studies relating intake of anti-oxidants and vitamins with Alzheimer's disease are conflicting (for review see [63]). In the Washington Heights-Inwood Columbia Aging Project, no relation between anti-oxidants and the incidence Alzheimer's disease was found [64]. As mentioned earlier in this review, efficient CBF is critical for optimal brain function, and several studies indicate that there is a decrease in CBF in patients with dementia [46, 65]. It is also known that cerebral vascular atrophy leads to 'Mild Cognitive Impairment' (MCI) syndrome that often evolves towards Alzheimer's disease. The hypothesis would be that the beneficial properties of flavanols on cerebrovascular function could allow delaying the evolution of MCI to Alzheimer's disease [65]. A clinical trial was performed on 1367 subjects aged over 65 years among whom 66 developed dementia. The relative risk of developing dementia adjusted to age for the two highest consumptions of flavonoids was 0.55 (95%CI 0.34, 0.90; $P = 0.02$). After further adjustment for gender, education level, weight and vitamin C intake, the relative risk decreased to 0.49 (95% CI 0.26, 0.92; $P = 0.04$) [66]. Thus, it appears that anti-oxidant flavonoids intake is inversely related to the risk of dementia. However, in this study flavonoids came mainly from fruits, vegetables, wine and tea. Complementary studies concentrating specifically on chocolate and on large population samples remain necessary.

Recent preclinical studies reported that a 5 month treatment with the LMN diet, rich in polyphenols, dry fruits and cocoa, induced neurogenesis in the subventricular zone and hippocampus of adult mice [67] and was able to prevent age-related cognitive impairment and

neuropathology in wild type (WT) and Tg2576 mice, a mouse model of Alzheimer's disease. This improvement correlated with a 70% increase in cell proliferation in the subventricular zone of the brain. These results support the critical role of polyphenols as human dietary supplements in possibly counteracting or slowing down cognitive decline during ageing and neurological diseases such as Alzheimer's disease [68].

Stroke

Some data are also available on the relation between flavonoid intake and neuronal loss and function after stroke. A meta-analysis of three studies concerning a sample of 114 009 participants reported a 29% reduction of the risk of stroke in high chocolate consumers compared with low consumers [69]. In one study, the inverse association between chocolate and stroke was even stronger than for myocardial infarction [70]. A very recent human study examined the relationship between the total anti-oxidant capacity (including fruits, vegetables, tea, coffee, chocolate) and the risk of stroke in women from the Swedish Mammography cohort. This study included 31 035 women free of cardiovascular disease (CVD) history and 5680 women without CVD history at baseline. The authors reported that dietary total anti-oxidant capacity was inversely associated with stroke in CVD-free women (17% risk reduction) and haemorrhagic stroke in women with CVD history (45% risk reduction) [71]. Likewise, mice pretreated orally with 5, 15 or 30 mg kg^{-1} epicatechin 90 min before middle cerebral artery occlusion (MCAO) had significantly smaller lesion volumes and improved neurologic scores compared with the control group. Mice that were post-treated with 30 mg kg^{-1} of epicatechin at 3.5 h after MCAO also had significantly smaller infarct volumes and improved neurologic scores [72].

A recent study also reported that treatment with dark chocolate prevents the inflammation of the vagus nerve resulting from a 16 month exposure of mice to the polluted air of Mexico city. Mice exposed to polluted air had a significant imbalance in genes coding for anti-oxidant defences, apoptosis and neurodegeneration at the level of the dorsal vagal complex and this imbalance was mitigated by chocolate administration [73].

The potential neuroprotective effects of the other constituents of chocolate are not known, with the exception of the neuroprotective effect of caffeine on various neurodegenerative diseases such as age-related cognitive decline, Alzheimer' disease [10] and Parkinson's disease [9] that were the subject of numerous studies and recent meta-analyses. However, compared with coffee, tea and soft drinks that represent the major sources of caffeine supply in our diet, the caffeine content of chocolate is much lower and cannot itself account for the known effects of caffeine on neurodegenerative diseases, but it may contribute.

Mechanisms of action underlying chocolate flavonoid effects on the brain

Flavonoids were first considered to exert anti-oxidant actions via their potential to scavenge free radicals, or their influence on the intracellular redox status. However, this classical hydrogen-donating anti-oxidant activity of flavonoids *in vivo* has been challenged, particularly in the brain, where flavonoids concentrations are usually quite low [49]. The effects of flavonoids in the brain are rather mediated by the ability to protect vulnerable neurons, enhance neuronal function and stimulate regeneration [50] via interaction with neuronal intracellular signalling pathways controlling neuronal survival and differentiation, long term potentiation (LTP) and memory. However at this point most of these mechanisms remain hypothetical and have not been experimentally demonstrated [14, 15, 74, 75]. Flavonoids could act also at different levels of the deleterious cascade of neuronal injury and death. A recent cDNA microarray study on the human colon adenocarcinoma Caco-2 cell line reported a change in the expression of several genes involved in the cellular response to oxidative stress. In addition, the down-regulation of the expression of other genes involved in DNA replication, transcription and recombination, DNA oxidative damage and inflammatory response suggests additional mechanisms for cocoa polyphenol actions [76]. There is a growing body of evidence to suggest that flavonoids and other polyphenols may be able to counteract neuronal injury, thereby delaying the progression of brain pathology [49, 51, 77].

The neuronal loss observed in neurodegenerative diseases and in stroke patients is considered to result from multiple processes, including neuroinflammation, glutamatergic excitotoxicity, increases in iron and/or depletion of endogenous anti-oxidants [78, 79]. The inflammatory cascade is believed to play a critical role in the development of the chronic low grade inflammation diseases such as Alzheimer's and Parkinson's disease [80, 81] and in the injury associated with stroke [82]. The flavanols, catechin and epigallocatechin gallate, are able to attenuate microglia and/or astrocyte mediated inflammation via a whole cascade of mechanisms that compromise neuron survival when not inhibited. These include iNOS and cyclooxygenase (COX-2) expression, NO production, cytokine release and NADPH oxidase activation leading to subsequent reactive oxygen species generation. All these effects are linked to the ability to modulate directly various protein and lipid kinase signalling pathways (for review see [15, 49, 54, 83, 84]). These include, for example, the inhibition of tyrosine kinase, protein kinase C and mitogen-activated protein kinase (MAPK) signaling cascades. The latter cascades involve p38 or ERK1/2 which regulate both iNOS and the expression of the cytokine tumour necrosis factor-alpha (TNF-α) in activated glial cells. Inhibitory or

stimulatory actions of these pathways affect neuronal function by altering the phosphorylation state of target molecules, leading to changes in caspase activity and/or by gene expression (for review see [15, 54, 83, 84]). For example, flavonoids block oxidative-induced neuronal injury by preventing the activation of caspase-3, hence supporting their potent anti-apoptotic action. The flavanols, epicatechin and 3-O-methylepicatechin, also protect neurons against oxidative damage via a mechanism involving the suppression of c-Jun N-terminal kinase and downstream partners, c-jun and pro-caspase-3 (for review see [15, 54, 83, 84]). Likewise the flavanol epicatechin that was shown to prevent stroke damage in mice is also active against excitotoxicity induced by N-methyl-D-aspartate (NMDA). The neuroprotection associated with epicatechin is almost abolished in transgenic mice lacking the neuroprotective enzyme heme oxygenase 1 (HO1) or the transcriptional factor nuclear factor (erythroid-derived 2)-like 2, or Nrf2. Nrf2 induces the expression of various genes including those that encode for several anti-oxidant enzymes, and hence might play a physiological role in the regulation of oxidative stress [72]. Together with ERK1/2, epicatechin induces also CREB activation in cortical neurons and increased expression of CREB regulates gene expression [32]. CREB is a transcription factor that binds to the promoter region of several genes involved in synapse remodelling, synaptic plasticity and memory, such as growth factors (BDNF, NRF), the glutamate NMDA receptor subtype and genes involved in angiogenesis, such as VEGF [85].

Chocolate and mood

Cognition is quite difficult to define simply and is the result of many other functions. It involves the participation of various levels of memory, attention, executive functions, perception, language and psychomotor functions. All these functions are influenced by the arousal and energetic level, physical well-being, motivation and mood. Since the latter function has been shown to be influenced by chocolate consumption, and although the mood effects are not directly associated to epicatechin concentration in chocolate, we will consider this aspect here.

It is a common belief that eating chocolate can improve mood states and make people feel good. Chocolate is often associated with emotional comfort. This effect seems to be linked to the capacity of carbohydrates including chocolate to promote this type of positive feelings through the release of multiple gut and brain peptides [86]. Although chocolate contains two analogues of anandamine that bind to the same brain sites as cannabis, any association with pleasure from chocolate is likely to be indirect since the analogues of anandamine inhibit breakdown of endogenous anandamine [87]. In addition, the increase in cannabinoids in circulating blood or urine cannot be accounted for by chocolate consumption, even in very large quantities [88].

The antidepressant-like effect of a cocoa polyphenolic extract was evaluated in rats. A the doses of 24 and 48 mg kg^{-1} 14 days^{-1}, this extract significantly reduced the duration of immobility in a forced swimming test without having any effect on locomotor activity in the open field, confirming that the antidepressant-like effect of cocoa polyphenolic extract in the forced swimming test model is specific [89].

The most likely basis for the attraction of chocolate would be that it stimulates the release of endorphins [90]. Indeed it was shown that the intake of sweet food is increased by opiate agonists and decreased by opiate antagonists [91, 92]. Chocolate may interact with some neurotransmitter systems such as dopamine (chocolate contains the dopamine precursor tyrosine), serotonin and endorphins (contained in cocoa and chocolate) that contribute to appetite, reward and mood regulation. The contribution of the dopaminergic system to chocolate craving and eating is, however, likely to be general rather than chocolate specific. Concerning serotonin, the situation is complex. After ingestion of carbohydrates, brain serotonin concentrations rise only when the protein component of the meal is less than 2% [86]. Chocolate contains 5% of its calorie content as protein, which would be sufficient to negate any serotonin effect. Furthermore, even extreme dietary manipulations of tryptophan, the precursor of serotonin, result in physiological changes that are too slow to account for mood effects that are described during or soon after eating chocolate [93]. Chocolate could also interact with opioids. The opioid system plays a role in the palatability of preferred foods [94], releasing opioids such as endorphins as food is eaten which could by itself enhance the pleasure of eating [95]. Opioids released in response to ingestion of sweet and other pleasantly palatable foods [96, 97] can increase central opioidergic activity, in turn stimulating the immediate release of beta-endorphin in the hypothalamus and producing an analgesic effect [96].

Poor mood stimulates the eating of comfort foods such as chocolate. The attitudes to chocolate are of two separate types [98]. The first factor is called craving and is associated with a prominent preoccupation with chocolate and eating it compulsively which mostly occurs when under emotional stress, suggesting a link between negative mood and an intense desire to consume chocolate [99]. The association between chocolate craving and consumption under emotional stress was shown in one study. The subjects had to listen to background music inducing a happy or sad mood, and chocolate intake was increased by the sound of the sad music [98].

Another factor to consider is the palatability of food. In rats, many data show that endogenous opiates regulate food intake by modulating the extent to which pleasure is induced by palatable foods. In humans the critical factor to

satisfy chocolate craving is the taste and the feel in the mouth [100]. Chocolate is mostly craved by females and predominantly in the perimenstrual period. Men and women differ in their response to satiation, leading to the hypothesis that the regulation of food intake varies between both sexes [101].

The composite sensory properties of chocolate are more likely to play a prominent role in chocolate liking or craving than more simple explanations of its role in appetite and satiety. For instance, if a caloric deficit motivates chocolate craving, both milk chocolate and white chocolate should appeal equally, but it is not the case. If psychoactive substances or magnesium deficit underlie chocolate craving, then milk chocolate and unsweetened cocoa powder should appeal equally, but again it is not the case. If the appeal is the unique sensory combination of chocolate, then chocolate is the only way to satisfy that craving [102].

When looking at the brain pathways involved in chocolate consumption, it appears that different brain areas are recruited depending on whether subjects eat chocolate under high motivation or when they rate chocolate as unpleasant. Different neural substrates appear to underlie different motivation systems, one controlling positive/appetitive stimuli while the second one is associated with negative/aversive stimuli. Modulation of brain activity was observed in cortical chemosensory areas, such as the insula, prefrontal regions and caudomedial and caudolateral orbitofrontal cortex. In the latter cortices, there were opposite patterns of activity when chocolate was rated as pleasant *vs.* unpleasant [103]. A fMRI study reported also significant taste-related activation in the orbitofrontal and insular cortices [104]. Another study using fMRI reported that individual differences in trait reward sensitivity (as measured by the Behavioral Activation Scale) predict activation to pictures of appetizing foods (i.e., chocolate cake, pizza) involved in food motivation and hedonics in a fronto-striatal-amygdala-midbrain network. This trait reward measures prediction of food craving, overeating and relative body weight (in both healthy and overweight populations). Pharmacological stimulation of this circuit in animals can over-ride satiety and cause overeating of highly palatable foods [105].

The odour of chocolate itself also influences brain activity. Exposure of human subjects to the odour of chocolate was associated with significant reductions in theta activity with a trend towards significance when compared with no-odour control. In a second testing, the EEG response to the odour of real chocolate was compared with no odour or hot water. The odour of chocolate was associated with significantly less theta activity than was any other stimulus. The authors hypothesized that the alterations in theta activity reflect shifts in attention or cognitive load during olfactory perception, with a reduction in theta indicating reduced level of attention and higher level of distraction [106]. Furthermore, the sight of chocolate produced more

activation in chocolate cravers than non-cravers in the medial orbitofrontal cortex and ventral striatum. For cravers *vs.* non-cravers, a combination of a picture of chocolate with chocolate in the mouth produced a greater effect than the sum of the components in the medial orbitofrontal cortex and pregenual cingulate cortex. Furthermore, the pleasantness ratings of the chocolate and chocolate-related stimuli had higher positive correlations with the fMRI BOLD signals in the pregenual cingulate cortex and medial orbitofrontal cortex in the cravers than in the non-cravers [107].

The motivation for chocolate preference seems to be primarily, if not entirely, sensory. Liking the sensory properties could originate in innate or acquired liking based on the sweetness, texture and aroma of chocolate, or it could be based in part on interactions between the post-ingestional effects of chocolate and a person's state (e.g., mood, hormone concentrations). Surprisingly there is little evidence for a relation between addiction to chocolate and liking chocolate [100]. However, chocolate consumption fails to activate the shell of the nucleus accumbens [108], the key structure for dependence to drugs [109, 110].

Conclusions

Cocoa powder and chocolate contain a large percentage of flavonoids that display several beneficial actions on the brain. In addition to their beneficial effects on the vascular system and on cerebral blood flow, flavonoids interact with signalization cascades involving protein and lipid kinases that lead to the inhibition of neuronal death by apoptosis induced by neurotoxicants such as oxygen radicals, and promote neuronal survival and synaptic plasticity. They enter the brain and stimulate brain perfusion provoking angiogenesis and changes in neuron morphology that have been mainly studied in hippocampus. Epicatechin, the main flavonoid present in cocoa and chocolate improves various aspects of cognition in animals and humans. Chocolate also induces positive effects on mood and is often consumed under emotional stress. In addition, flavonoids preserve cognitive abilities during aging in rats, lower the risk for developing Alzheimer's disease and decrease the risk of stroke in humans. All these properties are of great interest but at present it is not clear when the consumption of cocoa and chocolate should be initiated to generate beneficial effects on age-dependent cognitive decline and neurodegenerative diseases and many studies are still necessary to explore the neuroprotective potential of cocoa and chocolate. On the other hand, cocoa is most often consumed in the form of energy-rich chocolate, hence potentially detrimental especially because of the risk of weight gain, mainly in individuals vulnerable to certain eating problems leading to hyperphagic obesity. Nevertheless, on the basis of the present knowledge, it appears that the benefits from moderate cocoa or choco-

late consumption likely outweigh the possible risks [85, 111]. Moreover, a very recent human study reported that frequent chocolate consumption might actually be associated with a lower body mass index [112]. Although these results are intriguing, as quoted by the authors, they are in line with preclinical data from mice given a 2 week treatment with epicatechin from cocoa. The cocoa polyphenol improved mitochondrial function, including increased volume, cristae density and protein content for oxidative phosphorylation [113]. These data warrant further research on potential mechanisms involved.

Competing Interests

There are no competing interests to declare.

REFERENCES

1 Gu LW, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL, USDA ARS. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J Nutr 2004; 134: 613–7.

2 Whiting D. Natural phenolic compounds 1900-2000: a bird's eye view of a centuries chemistry. Nat Prod Rep 2001; 18: 583–606.

3 Clapperton J, Hammerstone JF, Romanczyk R, Yow S, Chau J, Lin D, Lookwood R. Genetic Variation in Cocoa Flavour. In 16th International Conference Groupe Polyphenols; 1992: 112–5.

4 Clapperton J. Contribution of genotype to cocoa (*Theobroma cacao* L.). Tropic Agric (Trinidad) 1994; 71: 303–8.

5 Rusconi M, Conti A. *Theobroma cacao* L., the food of the gods: a scientific approach beyond myths and claims. Pharmacol Res 2010; 61: 5–13.

6 Kim H, Keeney PG. (–)-Epicatechin content in fermented and unfermented cocoa beans. J Food Sci 1984; 49: 1090–2.

7 Lorist MM, Tops M. Caffeine, fatigue, and cognition. Brain Cogn 2003; 53: 82–94.

8 Nehlig A. Is caffeine a cognitive enhancer? J Alzheimers Dis 2010; 20: (Suppl 1): S85–94.

9 Costa J, Lunet N, Santos C, Santos J, Vaz-Carneiro A. Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. J Alzheimers Dis 2010; 20: (Suppl. 1): S221–38.

10 Santos C, Costa J, Santos J, Vaz-Carneiro A, Lunet N. Caffeine intake and dementia: systematic review and meta-analysis. J Alzheimers Dis 2010; 20: (Suppl. 1): S187–204.

11 Smit HJ. Theobromine and the pharmacology of cocoa. Handb Exp Pharmacol 2011; 200: 201–34.

12 McCarty MF. Toward prevention of Alzheimers disease–potential nutraceutical strategies for suppressing the production of amyloid beta peptides. Med Hypotheses 2006; 67: 682–97.

13 Patel AK, Rogers JT, Huang X. Flavanols, mild cognitive impairment, and Alzheimer's dementia. Int J Clin Exp Med 2008; 1: 181–91.

14 Spencer JPE. The impact of flavonoids on memory: physiological and molecular considerations. Chem Soc Rev 2009; 38: 1152–61.

15 Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP. The neuroprotective potential of flavonoids: a multiplicity of effects. Genes Nutr 2008; 3: 115–26.

16 Cooper KA, Donovan JL, Waterhouse AL, Williamson G. Cocoa and health: a decade of research. Br J Nutr 2008; 99: 1–11.

17 Heiss C, Finis D, Kleinbongard P, Hoffmann A, Rassaf T, Kelm M, Sies H. Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. J Cardiovasc Pharmacol 2007; 49: 74–80.

18 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: 39–44.

19 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: 1693–702.

20 van Praag H, Lucero MJ, Yeo GW, Stecker K, Heivand N, Zhao C, Yip E, Afanador M, Schroeter H, Hammerstone J, Gage FH. Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. J Neurosci 2007; 27: 5869–78.

21 Ferruzzi MG, Lobo JK, Janle EM, Cooper B, Simon JE, Wu QL, Welch C, Ho L, Weaver C, Pasinetti GM. Bioavailability of gallic acid and catechins from grape seed polyphenol extract is improved by repeated dosing in rats: implications for treatment in Alzheimer's disease. J Alzheimers Dis 2009; 18: 113–24.

22 Datla KP, Christidou M, Widmer WW, Rooprai HK, Dexter DT. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport 2001; 12: 3871–5.

23 Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM, Joseph JA. Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 2005; 8: 111–20.

24 Ghosh D, Scheepens A. Vascular action of polyphenols. Mol Nutr Food Res 2009; 53: 322–31.

25 Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. J Hypertens 2003; 21: 2281–6.

26 Hollenberg NK, Fisher ND, McCullough ML. Flavanols, the Kuna, cocoa consumption, and nitric oxide. J Am Soc Hypertens 2009; 3: 105–12.

27 Fisher ND, Sorond FA, Hollenberg NK. Cocoa flavanols and brain perfusion. J Cardiovasc Pharmacol 2006; 47: (Suppl. 2): S210–4.

28 Heiss C, Kleinbongard P, Dejam A, Perré S, Schroeter H, Sies H, Kelm M. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. J Am Coll Cardiol 2005; 46: 1276–83.

29 Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Lüscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995; 91: 1314–9.

30 Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. JAMA 2003; 290: 1030–1.

31 Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, Mietus-Snyder ML. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004; 23: 197–204.

32 Schroeter HC, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A 2006; 103: 1024–9.

33 Fisher ND, Sorond FA, Hollenberg NK. Cocoa flavanols and brain perfusion. J Cardiovasc Pharmacol 2006; 47: (Suppl. 2): S210–S214.

34 Francis ST, Head K, Morris PG, Macdonald IA. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. J Cardiovasc Pharmacol 2006; 47: (Suppl. 2): S215–20.

35 Richelle M, Tavazzi I, Enslen M, Offord EA. Plasma kinetics in man of epicatechin from black chocolate. Eur J Clin Nutr 1999; 53: 22–6.

36 Sorond FA, Lipsitz LA, Hollenberg NK, Fisher ND. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. Neuropsychiatr Dis Treat 2008; 4: 433–40.

37 Sorond FA, Hollenberg NK, Panych LP, Fisher ND. Brain blood flow and velocity: correlations between magnetic resonance imaging and transcranial Doppler sonography. J Ultrasound Med 2010; 29: 1017–22.

38 Ancelin ML, Christen Y, Ritchie K. Is antioxidant therapy a viable alternative for mild cognitive impairment? Examination of the evidence. Dement Geriatr Cogn Disord 2007; 24: 1–19.

39 Joseph J, Cole G, Head E, Ingram D. Nutrition, brain aging, and neurodegeneration. J Neurosci 2009; 29: 12795–801.

40 Macready AL, Kennedy OB, Ellis JA, Williams CM, Spencer JP, Butler LT. Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. Genes Nutr 2009; 4: 227–42.

41 Field DT, Williams CM, Butler LT. Consumption of cocoa flavanols results in an acute improvement in visual and cognitive functions. Physiol Behav 2011; 103: 255–60.

42 Huber KK, Adams H, Remky A, Arend KO. Retrobulbar haemodynamics and contrast sensitivity improvements after CO2 breathing. Acta Ophthalmol Scand 2006; 84: 481–7.

43 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: 705–12.

44 Kalt W, Hanneken A, Milbury P, Tremblay F. Recent research on polyphenolics in vision and eye health. J Agric Food Chem 2010; 58: 4001–7.

45 Scholey AB, French SJ, Morris PJ, Kennedy DO, Milne AL, Haskell CF. Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. J Psychopharmacol 2010; 24: 1505–14.

46 Ruitenberg A, den Heijer T, Bakker SL, van Swieten JC, Koudstaal PJ, Hofman A, Breteler MM. Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. Ann Neurol 2005; 57: 789–94.

47 Camfield DA, Scholey A, Pipingas A, Silberstein R, Kras M, Nolidin K, Wesnes K, Pase M, Stough C. Steady state visually evoked potential (SSVEP) topography changes associated with cocoa flavanol consumption. Physiol Behav 2012; 105: 948–57.

48 Crews WD Jr, Harrison DW, Wright JW. A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. Am J Clin Nutr 2008; 87: 872–80.

49 Spencer JPE. Flavonoids: modulators of brain function? Br J Nutr 2008; 99: (E Suppl. 1): ES60–77.

50 Spencer JPE. Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. Proc Nutr Soc 2008; 67: 238–52.

51 Spencer JPE. The interactions of flavonoids within neuronal signalling pathways. Genes Nutr 2007; 2: 257–73.

52 Yamada T, Yamada Y, Okano Y, Terashima T, Yokogoshi H. Anxiolytic effects of short- and long-term administration of cacao mass on rat elevated T-maze test. J Nutr Biochem 2009; 20: 948–55.

53 Davis M. The role of the amygdala in fear and anxiety. Annu Rev Neurosci 1992; 15: 353–75.

54 Williams RJ, Spencer JP. Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med 2012; 52: 35–45.

55 Crowley TJ, Hoehn MM, Rutledge CO, Stallings MA, Heaton RK, Sundell S, Stilson D. Dopamine excretion and vulnerability to drug-induced Parkinsonism in schizophrenic patients. Arch Gen Psychiatry 1978; 35: 97–104.

56 Hoehn MM, Crowley TJ, Rutledge CO. The Parkinsonian syndrome and its dopamine correlates. Adv Exp Med Biol 1977; 90: 243–54.

57 Bisson JF, Nejdi A, Rozan P, Hidalgo S, Lalonde R, Messaoudi M. Effects of long-term administration of a cocoa polyphenolic extract (Acticoa powder) on cognitive performances in aged rats. Br J Nutr 2008; 100: 94–101.

58 Rozan P, Hidalgo S, Nejdi A, Bisson JF, Lalonde R, Messaoudi M. Preventive antioxidant effects of cocoa polyphenolic extract on free radical production and cognitive performances after heat exposure in Wistar rats. J Food Sci 2007; 72: S203–6.

59 Kalmijn S, Feskens EJ, Launer LJ, Kromhout D. Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men. Am J Epidemiol 1997; 145: 33–41.

60 Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. Am J Epidemiol 2007; 165: 1364–71.

61 Nurk E, Refsum H, Drevon CA, Tell GS, Nygaard HA, Engedal K, Smith AD. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. J Nutr 2009; 139: 120–7.

62 Pak T, Cadet P, Mantione KJ, Stefano GB. Morphine via nitric oxide modulates beta-amyloid metabolism: a novel protective mechanism for Alzheimer's disease. Med Sci Monit 2005; 11: BR357–66.

63 Luchsinger J, Mayeux R. Dietary factors and Alzheimer's disease. Lancet Neurol 2004; 3: 579–87.

64 Luchsinger JA, Tang M, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003; 60: 203–8.

65 Nagahama Y, Nabatame H, Okina T, Yamauchi H, Narita M, Fujimoto N, Murakami M, Fukuyama H, Matsuda M. Cerebral correlates of the progression rate of the cognitive decline in probable Alzheimer's disease. Eur Neurol 2003; 50: 1–9.

66 Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. Eur J Epidemiol 2000; 16: 357–63.

67 Valente T, Hidalgo J, Bolea I, Ramirez B, Anglés N, Reguant J, Morelló JR, Gutiérrez C, Boada M, Unzeta M. A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. J Alzheimers Dis 2009; 18: 849–65.

68 Fernández-Fernández L, Comes G, Bolea I, Valente T, Ruiz J, Murtra P, Ramirez B, Anglés N, Reguant J, Morelló JR, Boada M, Hidalgo J, Escorihuela RM, Unzeta M. LMN diet, rich in polyphenols and polyunsaturated fatty acids, improves mouse cognitive decline associated with aging and Alzheimer's disease. Behav Brain Res 2012; 228: 261–71.

69 Buitrago-Lopez A, Sanderson J, Johnson L, Warnakula S, Wood A, Di Angelantonio E, Franco OH. Chocolate consumption and cardiometabolic disorders: systematic review and meta-analysis. BMJ 2011; 343: d4488.

70 Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J 2010; 31: 1616–23.

71 Rautiainen S, Larsson S, Virtamo J, Wolk A. Total antioxidant capacity of diet and risk of stroke: a population-based prospective cohort of women. Stroke 2012; 43: 335–40.

72 Shah ZA, Li RC, Ahmad AS, Kensler TW, Yamamoto M, Biswal S, Doré S. The flavanol (-)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. J Cereb Blood Flow Metab 2010; 30: 1951–61.

73 Villarreal-Calderon R, Torres-Jardón R, Palacios-Moreno J, Osnaya N, Pérez-Guillé B, Maronpot RR, Reed W, Zhu H, Calderón-Garcidueñas L. Urban air pollution targets the dorsal vagal complex and dark chocolate offers neuroprotection. Int J Toxicol 2010; 29: 604–15.

74 Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radic Biol Med 2004; 36: 838–49.

75 Rendeiro C, Spencer JP, Vauzour D, Butler LT, Ellis JA, Williams CM. The impact of flavonoids on spatial memory in rodents: from behaviour to underlying hippocampal mechanisms. Genes Nutr 2009; 4: 251–70.

76 Noé V, Peñuelas S, Lamuela-Raventós RM, Permanyer J, Ciudad CJ, Izquierdo-Pulido M. Epicatechin and a cocoa polyphenolic extract modulate gene expression in human Caco-2 cells. J Nutr 2004; 134: 2509–16.

77 Mandel S, Youdim MB. Catechin polyphenols: neurodegeneration and neuroprotection in neurodegenerative diseases. Free Radic Biol Med 2004; 37: 304–17.

78 Jellinger KA. Cell death mechanisms in neurodegeneration. J Cell Mol Med 2001; 5: 1–17.

79 Spires TL, Hannan AJ. Nature, nurture and neurology: gene-environment interactions in neurodegenerative disease. FEBS Anniversary Prize Lecture delivered on 27 June 2004 at the 29th FEBS Congress in Warsaw. FEBS J 2005; 272: 2347–61.

80 Hirsch EC, Hunot S, Hartmann A. Neuroinflammatory processes in Parkinson's disease. Parkinsonism Relat Disord 2005; 11: (Suppl. 1): S9–15.

81 McGeer EG, McGeer PL. Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27: 741–9.

82 Zheng Z, Lee JE, Yenari MA. Stroke: molecular mechanisms and potential targets for treatment. Curr Mol Med 2003; 3: 361–72.

83 Spencer JP. Flavonoids and brain health: multiple effects underpinned by common mechanisms. Genes Nutr 2009; 4: 243–50.

84 Spencer JP, Vafeiadou K, Williams RJ, Vauzour D. Neuroinflammation: modulation by flavonoids and mechanisms of action. Mol Aspects Med 2012; 33: 83–97.

85 McCarty MF. Vascular nitric oxide may lessen Alzheimer's risk. Med Hypotheses 1998; 51: 465–76.

86 Parker G, Roy K, Mitchell P, Wilhelm K, Malhi G, Hadzi-Pavlovic D. Atypical depression: a reappraisal. Am J Psychiatry 2002; 159: 1470–9.

87 di Tomaso E, Beltramo M, Piomelli D. Brain cannabinoids in chocolate. Nature 1996; 382: 677–8.

88 Tytgat J, Van Boven M, Daenens P. Cannabinoid mimics in chocolate utilized as an argument in court. Int J Legal Med 2000; 113: 137–9.

89 Messaoudi M, Bisson JF, Nejdi A, Rozan P, Javelot H. Antidepressant-like effects of a cocoa polyphenolic extract in Wistar-Unilever rats. Nutr Neurosci 2008; 11: 269–76.

90 Benton D, Donohoe RT. The effects of nutrients on mood. Public Health Nutr 1999; 2: 403–9.

91 Reid LD. Endogenous opioid peptides and regulation of drinking and feeding. Am J Clin Nutr 1985; 42: (5 Suppl): 1099–132.

92 Giraudo SQ, Grace MK, Welch CC, Billington CJ, Levine AS. Naloxone's anorectic effect is dependent upon the relative palatability of food. Pharmacol Biochem Behav 1993; 46: 917–21.

93 Young SN, Smith SE, Pihl RO, Ervin FR. Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology (Berl) 1985; 87: 173–7.

94 Si EC, Bryant HU, Yim GK. Opioid and non-opioid components of insulin-induced feeding. Pharmacol Biochem Behav 1986; 24: 899–903.

95 Ottley C. Food and mood. Nurs Stand 2000; 15: 46–52.

96 Parker G, Parker I, Brotchie H. Mood state effects of chocolate. J Affect Disord 2006; 92: 149–59.

97 Fullerton DT, Getto CJ, Swift WJ, Carlson IH. Sugar, opioids and binge eating. Brain Res Bull 1985; 14: 673–80.

98 Willner P, Benton D, Brown E, Cheeta S, Davies G, Morgan J, Morgan M. 'Depression' increases 'craving' for sweet rewards in animal and human models of depression and craving. Psychopharmacology (Berl) 1998; 136: 272–83.

99 Hetherington MM, MacDiarmid JI. 'Chocolate addiction': a preliminary study of its description and its relationship to problem eating. Appetite 1993; 21: 233–46.

100 Rozin P, Levine E, Stoess C. Chocolate craving and liking. Appetite 1991; 17: 199–212.

101 Smeets PA, de Graaf C, Stafleu A, van Osch MJ, Nievelstein RA, van der Grond J. Effect of satiety on brain activation during chocolate tasting in men and women. Am J Clin Nutr 2006; 83: 1297–305.

102 Michener W, Rozin P. Pharmacological versus sensory factors in the satiation of chocolate craving. Physiol Behav 1994; 56: 419–22.

103 Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M. Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain 2001; 124: 1720–33.

104 Smits M, Peeters RR, van Hecke P, Sunaert S. A 3 T event-related functional magnetic resonance imaging (fMRI) study of primary and secondary gustatory cortex localization using natural tastants. Neuroradiology 2007; 49: 61–71.

105 Beaver JD, Lawrence AD, van Ditzhuijzen J, Davis MH, Woods A, Calder AJ. Individual differences in reward drive predict neural responses to images of food. J Neurosci 2006; 26: 5160–6.

106 Martin GN. Human electroencephalographic (EEG) response to olfactory stimulation: two experiments using the aroma of food. Int J Psychophysiol 1998; 30: 287–302.

107 Rolls ET, McCabe C. Enhanced affective brain representations of chocolate in cravers *vs.* non-cravers. Eur J Neurosci 2007; 26: 1067–76.

108 Schroeder BE, Binzak JM, Kelley AE. A common profile of prefrontal cortical activation following exposure to nicotine- or chocolate-associated contextual cues. Neuroscience 2001; 105: 535–45.

109 Nehlig A. Are we dependent upon coffee and caffeine? A review on human and animal data. Neurosci Biobehav Rev 1999; 23: 563–76.

110 Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 2002; 137: 75–114.

111 Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. Antioxid Redox Signal 2011; 15: 2779–811.

112 Golomb BA, Koperski S, White HL. Association between more frequent chocolate consumption and lower body mass index. Arch Intern Med 2012; 172: 519–21.

113 Nogueira L, Ramirez-Sanchez I, Perkins GA, Murphy A, Taub PR, Ceballos G, Villarreal FJ, Hogan MC, Malek MH. (-)-Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle. J Physiol 2011; 589: 4615–31.