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REVIEW

Exploring cocoa properties: is theobromine a cognitive modulator?

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Abstract
Nutritional qualities of cocoa have been acknowledged by several authors; a particular focus has been placed on its high content of flavanols, known for their excellent antioxidant properties and subsequent protective effect on cardio- and cerebrovascular systems as well as for neuromodulatory and neuroprotective actions. Other active components of cocoa are methylxanthines (caffeine and theobromine). Whereas the effects of caffeine are extensively researched, the same is not the case for theobromine; this review summarizes evidence on the effect of theobromine on cognitive functions. Considering animal studies, it can be asserted that acute exposition to theobromine has a reduced and delayed nootropic effect with respect to caffeine, whereas both animal and human studies suggested a potential neuroprotective action of long-term assumption of theobromine through a reduction of Aβ amyloid pathology, which is commonly observed in Alzheimer's disease patients' brains. Hence, the conceivable action of theobromine alone and associated with caffeine or other cocoa constituents on cognitive modulation is yet underexplored and future studies are needed to shed light on this promising molecule.

Keywords Cocoa · Theobromine · Cognitive modulator · Cognition

Introduction

Theobroma cacao and its products

Cocoa comes from the processing of seeds of a tropical tree, considered by the Aztecs as a sacred plant. In fact, in the eighteenth century, Linnaeus designated this tree with the Greek genus name of "*Theobroma cacao*" which means "Food of the Gods" (von Linné (Linneaus) 1741; Rusconi and Conti 2010). Each cacao tree provides around 30–40 fruits ("cabosse") every year which are manually harvested; a complex sequence of chemical and physical changes follows according to the product to be reached (cocoa mass, butter, powder, and chocolate) (Romero-Cortes et al. 2013).

Cocoa beans contain about 55% of lipids which represents the main constituent of cocoa butter; it is composed by a predominant fraction of triglyceride molecules species (in particular oleic, stearic, palmitic, and linoleic acid) (Pittenauer and Allmaier 2009); proteins contribute to 10–15% of the dry weight of cocoa seeds and consist mainly of albumin and globulin fractions (Zak and Keeney 1976); the non-protein nitrogen is represented by free aminoacids (0.3%), ammonium formed during fermentation (0.02%), and methylxanthines (theobromine and caffeine); biogenic amines, resulting from microbial decarboxylation of aminoacids of cocoa, consist especially of phenylethylamine (from phenylalanine) and of serotonin (from tryptophan). Carbohydrates are present in the form of mono-, oligo-, and polysaccharides.

Among the polyphenols present in cocoa beans (12–18% of their total weight on dry basis (Lamuela-Raventos 2005)), the main compounds are flavonoids: catechins and flavan-3-ols (about 37%), anthocyanins (4%), and proanthocyanidins (58%). Cocoa flavonoids have a very high antioxidant activity (Maleyki and Ismail 2010).

Finally, cocoa contains low percentage of organic acids, minerals, and vitamins.

Theobromine

Cocoa derivatives are the main sources of theobromine in diet, which may vary in concentration according to different bean

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varieties: Forastero species generally contain the highest amount (del Rosario Brunetto et al. 2007). In cocoa plants, theobromine accumulates in young leaves, and the concentrations of theobromine decreases in parallel with leaves maturation (Koyama et al. 2003). Theobromine (like caffeine which is also contained in cocoa in relatively small amounts) belongs to a class of alkaloid molecules known as methylxanthines (Smit 2011). There is grossly a 1:5 ratio of caffeine to theobromine in chocolate (Bruinsma and Taren 1999); theobromine levels are higher in cocoa beans (approximately 1.2–5 g/100 g) and in dark chocolates (approximately 1 g/100 g) than in milk chocolates (0.1–0.5 g/100 g); higher quality chocolate products as well as African cocoa plants (Fredholm 2011) contain more theobromine (Watson et al. 2013).

Caffeine differs from theobromine only by the presence of one methyl group, which is removed during caffeine metabolism by converting it to theobromine (Wolf 2013) (Fig. 1); other caffeine metabolites are paraxanthine and theophylline. The additional methyl group helps caffeine to better cross the blood-brain barrier (Svenningsson et al. 1999).

Methylxanthines are non-selective adenosine receptor antagonists. Four different adenosine receptors are known (A1, A2A, A2B, and A3); all of them consist in seven transmembrane G protein-coupled receptors linked to different transduction mechanisms (A1 and A3 subtypes' activation results in Gi-mediated decreases in cAMP, whereas activation of A2A and A2B leads to Gs-mediated increases in cAMP). The A1, A2B, and A3 receptors have the highest abundance and are widely distributed centrally (brain). A2A receptors are mostly located peripherally (blood vessels and heart) and have a limited distribution in the brain (striatum, hindbrain) (Dunwiddie and Masino 2001). A2B and A3 are expressed at low levels in neuronal and glial cells and show relatively lower affinity for adenosine receptors (Sheth et al. 2014).

The A1 receptor is coupled to activation of K+ channels and inhibition of Ca2+ channels, thus inhibiting neuronal activity, such as cholinergic transmission; the activation of central nervous system (CNS) A2A receptors inhibits the release

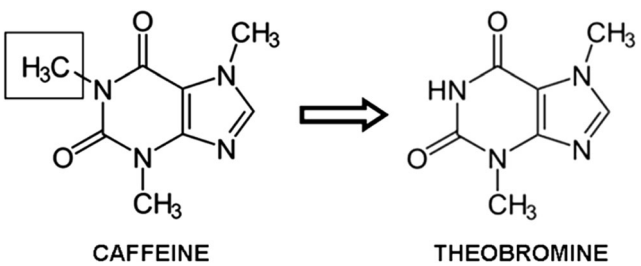

Fig. 1 Metabolism of caffeine. In humans, theobromine does not metabolize into caffeine, although the latter reaction occurs in young leaves of cacao plant. In the liver, theobromine (3,7-dimethylxanthine) is split into 3-methylxanthine and 7-methylxanthine by the enzyme cytochrome P450. 7-Methylxanthine is then metabolized into 7-methyluric acid by xanthine oxidase and into 3,7-dimethyluric acid and 3,7-diaminouracil through less-known reactions

of dopamine and glutamate, probably due to the formation of heteromers with receptors for these neurotransmitters. The A2A receptor is expressed at high levels in only a few regions of the brain and is primarily linked to activation of adenylyl cyclase. Therefore, antagonism of both A1 and A2A receptors seems to be accountable for the stimulant CNS effects of methylxantines (Schindler et al. 2005). Theobromine seems to have equal affinity for A1 with respect to A2A receptors, while caffeine has a slightly lower affinity for A1 receptors (Svenningsson et al. 1999).

Caffeine has weaker stimulant effects than other drugs such as amphetamines: in non-sleep deprived subjects (Marriott, and Institute of Medicine (U.S.), Committee on Military Nutrition Research 1994), it delays sleep and reduce the decline of performance due to fatigue and boredom. A study conducted on sleep-deprived subjects (Penetar et al. 1993) showed that caffeine reversed sleep deprivation without serious side effects: it improves alertness, choice reaction time, sustained attention, and logical reasoning as well as mood, but increases anxiety and nervousness, body temperature, and diastolic blood pressure (neither heart rate nor systolic blood pressure were increased). A possible difference between caffeine and theobromine is that caffeine should have more considerable effect on the CNS due to its extra methyl group, while theobromine most significantly targets smooth muscle, also promoting vasodilatation (Coleman 2004). Even if theobromine demonstrated about one fifth of the stimulant effect of caffeine on CNS, theobromine has a longer half-life (Fredholm 2011).

Theobromine shows also an antitussive and a bronchodilating effect (Simons et al. 1985; Usmani et al. 2005), a diuretic action (Dorfman and Jarvik 1970), and a possible role in reduction of angiogenesis in tumor growth (Smit 2011), all these effects are related to its adenosine receptor antagonistic properties.

Furthermore, methylxanthines are competitive nonselective phosphodiesterase inhibitors (Essayan 2001), thus raising intracellular cyclic adenosine monophosphate (cAMP), activating protein kinase A (PKA), and inhibiting tumor necrosis factor (TNF)-alpha and leukotriene synthesis: all this translates into a reduction in inflammation (Peters-Golden et al. 2005) and tumors development (Sugimoto et al. 2014). To this, it should be added the inhibition of the nuclear enzyme poly (ADP-ribose) polymerase-1, which is associated with the pathophysiology of acute inflammatory diseases and also with chronic inflammation which occurs for example in diabetes and chronic obstructive pulmonary disease (Geraets et al. 2006). A protective effect on enamel has been also attributed to theobromine (Smit 2011), differently from caffeine.

Previous reports of behavior ineffectiveness of theobromine in stimulating CNS (Sprügel et al. 1977; Snyder et al. 1981) have resulted in a reduced number of studies on this topic.

In equine sports, methylxanthines are considered doping agents due to their stimulant effects. Case reports of toxicity in domestic and wild animals exposed to consumption of chocolate were primarily attributed to theobromine, but more recently the importance of a poisonous combination of caffeine and theobromine has been acknowledged (Smit 2011); toxic effects of theobromine have not been described in humans.

In this review, we aimed to assess the literature on the nootropic effect of theobromine and to differentiate theobromine from caffeine in terms of cognitive actions, consequent to their different pharmacodynamics; deepening our knowledge on theobromine effects might help elaborating new prevention and/or treatment strategies in cognitive disorders.

Methods

This study is based on a systematic literature search in three database (PubMed, Web of Science, and Scopus) performed on 15/1/2017 using the following search criteria: (Theobromine OR Cacaos Theobroma OR cacao OR *Theobroma cacao*s OR Theobroma OR Chocolate OR Chocolates OR Cocoa OR Cocoas) AND (neurodegenerative disorders OR cognitive impairment OR cognitive disorders OR cognitive function OR memory OR learning OR dementia OR Alzheimer OR neuroprotection OR clinical trials). No language or time restrictions were applied to the search strategy.

An initial screen of the three databases returned 1841 results from Medline, 47 results from Scopus, and 534 results from Web of Science. Inclusion criteria include (1) papers describing any cognitive effects of theobromine in animals or humans of all ages, (2) no restrictions on publication year, or on language and (3) study designs including cross-sectional, longitudinal, or case-control studies. After excluding duplicates and revision for relevance, 18 studies were retained for the final review and data extraction (Shi and Daly 1999).

Results

Animal studies (Table 1)

After a report of nervousness in hornets subsequent to exposition to methylxanthines including theobromine (Ishay and Paniry 1979), major studies on the effect of theobromine have been carried out on rodents.

Carney in 1982 (Carney 1982) reported that theobromine produced a reduced behavioral effect (scheduled–controlled responding) with respect to caffeine and theophylline in rats. In 1986, the same authors (Carney et al. 1986) did not find any direct effect on spontaneous locomotor activity at different doses of theobromine in mice, as Sprugel et al. already had suggested in 1977 (Sprügel et al. 1977) and attributed this finding to the lack of effect on 5′-N-ethylcarboxamide adenosine. On the other hand, a Japanese study (Kuribara et al. 1992) showed that oral administration of 10 mg/kg of theobromine significantly increased the mouse's ambulatory activity in the subsequent 3 h, while the dosage of 1000 mg/kg of theobromine decreased the activity. Other studies focusing on locomotor activity in mice suggested that theobromine concentrations in chocolate may have behavioral consequences, possibly due to an interaction with other methylxanthines and that can appear delayed with respect to caffeine (Smit 2011).

Ambulatory activity was found to be increased after combined administration of cocoa and green tea, as well as of caffeine and 30 mg/kg of theobromine in the study of He et al. on INR mice (He et al. 2009).

Fernandez-Fernandez et al. (Fernández-Fernández et al. 2015) found a significant decrease of age-related cognitive impairment in mice supplied with a diet rich in theobromine, polyphenols, and polyunsaturated fatty acid (LMN diet); the authors speculated that LMN diet could enhance both cholinergic and adrenergic neurotransmission as shown by a series of in vitro and in vivo experiments and hypothesized that theobromine could be responsible for the effects of this diet, since it was able to increase the level of noradrenaline, dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in PC12 cells derived from rat pheochromocytoma. LMN diet, whose main component is essentially cocoa, has been previously investigated for its antioxidant effect (Valente et al. 2009); moreover, Fernandez et al. in the same study (Fernández-Fernández et al. 2015) showed how theobromine enhanced levels of the antioxidant enzymes Superoxide Dismutase-1 (SOD-1) in human neuroblastoma SH-SY5Y cells and induced the translocation to the nucleus of transcription factor Nrf2, which in turn induced the expression of antioxidant enzymes.

Yoneda et al. (Yoneda et al. 2017) supplemented mice with a diet enriched with 0.05% theobromine for 30 days and demonstrated that theobromine is able to cross the blood-brain barrier since it was measurable during its administration in cerebral cortex (it was detectable on day 30, but not on day 60); moreover, theobromine-nourished mice showed a significant increase of vasodilator-stimulated phosphoprotein (VASP) levels in the brain—thus indicating a concomitant increase the levels of intracerebral cAMP, as well as of phosphorylated cAMP response element-binding protein (CREB) and of protein expression levels of brain-derived neurotrophic factor (BDNF) in both the hippocampus and cerebral cortex with respect to normal nourished mice. This positive modulation of the cAMP/CREB/BDNF pathways observed with theobromine supplementation, which acts as a PDE inhibitor, was hypothesized to enhance cognitive functions; actually, a

Table 1 Animal studies

Study (ref)	Type of study	Animal (available anthropometric features)/cell line	Aim of the study	Theobromine administration			Effects
				Modality	Amount	Duration	
Sprügel et al. 1977	In vivo	NMRI mice (male, 25 ± 1 g)	To assess the influence of caffeine and theobromine on locomotive activity and the brain cGMP/cAMP ratio.	Intraperitoneal injection	45 μg/g caffeine or 180 μg/g sodium theobromine or 180 μg/g sodium theobromin and 45 μg/g caffeine or saline (0.9%)	Acute exposure	Theobromine alone had no effects on locomotive activity nor on brain cGMP/cAMP ratio and antagonized caffeine-stimulating effects on locomotive activity and brain cGMP/cAMP ratio.
Ishay and Paniry 1979	In vivo	Hornets	To ascertain the effect of chemical substances (including theobromine) on hornet behavior	Diet	NS		Theobromine enhanced motility, sensitivity to external stimulus, appetite for proteins, positive geotropism, and phototropism and do not increase mortality.
Snyder et al. 1981	In vivo	ICR mice (male, adult, 25–40 g)	To assess the effects of methylxanthines on locomotor stimulation and on adenosine and benzodiazepine receptor binding; to evaluate behavioral actions of methylxanthines on systems specifically regulated by adenosine.	Intraperitoneal injection	10 μl/g 10 min prior to the 1-h locomotor activity testing period		Theobromine had negligible influence on locomotor activity at all doses (5–10–30-100 μmol/kg). Locomotor stimulation threshold for theobromine was > 100 μmol/kg
Carney 1982	In vivo	Sprague-Dawley rats (male, 250–275 g)	To characterize effects of caffeine, theophylline, and theobromine on scheduled controlled responding.	Intraperitoneal injection	Theobromine 10–320 mg/kg or caffeine 0.32–32 mg/kg or theophylline 1.0–56 mg/kg or saline solution	Acute exposure	A dose-related reduction in responding was found for all the substances tested (caffeine > theophylline > theobromine).
Carney et al. 1986	In vivo	DBA/2J mice (male)	To determinate if theobromine could function in vivo as an adenosin receptor antagonist.	Intraperitoneal injection	Theobromine up to 100 mg/kg or saline solution	_	Theobromine did not have effects on spontaneus locomotor activity. Theobromine blocked adenosin receptor agonists-induced suppression of locomotor activity and hypothermia.
Kuribara et al. 1992	In vivo	dd and ddY strain mice (male, 7 weeks of age, 25–30 g)	To assess behavioral effects of methylxanthines (caffeine, theophylline and theobromine) by ambulatory activity (unconditioned behavior) and discrete lever-press avoidance (conditioned behavior)	Oral	Theobromine 3–1000 mg/kg or caffeine 1–100 mg/kg or theophylline 3–300 mg/kg or controls solutions	Acute exposure	Theobromine 10 mg/kg increased mouse ambulatory activity while theobromine 1000 mg/kg decreased it. Theobromine > 100 mg decreased avoidance behavior. Theobromine 100 mg/kg was toxic.
Shi and Daly 1999	In vivo	NIH Swiss strain mice (white male)	To determinate effect of chronic ingestion of xanthines on receptors and ion channels in brain membranes	Diet	100 mg/kg/day of different xanthines (caffeine, theophylline, theobromine, paraxanthine, IBMX, DMPX, pentoxiphylline)	6–7 days	Caffeine showed a more rapidly and a higher increase of brain level than theobromine. Theobromine ingestion caused an increase in brain levels of A1-adenosine receptors.
He et al. 2009	In vivo	INR mice (male, 7 weeks old)	To investigate the effects of cocoa tea and theobromine compared or associated to green tea and caffeine on ambulatory activity	Diet	200 mg/kg of cocoa tea or green tea or both or water control; caffeine (from 1.25 to 30 mg/kg); coadministration of 10 mg/kg caffeine and theobromine within the 5 to 30 mg/kg dose range.	Acute exposure	Ambulatory activity is increased after combined administration of cocoa and green tea, as well as of caffeine and 30 mg/kg of theobromine.
Fernández-Fernández et al. 2015	In vivo	129S1/SvlmJ mice (male, adult)	To evaluate the effect of LMN diet (cocoa+ hazelnuts+phytosterols+soluble fiber) on both cholinergic and catecholaminergic systems.	Oral	20 g/d LMN diet (NS theobromine amount) or normal diet	10, 20, 30 and 40 days	LMN diet enhanced both cholinergic and catecholaminergic transmissions in 20-day-fed mice
	In vitro	SH-SY5Y cell line	To compare antioxidant effect of theobromine and LMN cream.	Oral	Different doses of theobromine (from 0.001 to 1 μM) or different doses of LMN cream (from 0.1 to 100 μg/mL)	24 h	Theobromine had protective effect at all doses on SH-SY5Y cells damaged with 150 μM H_2O_2 for 24 h, increased the levels of the antioxidant enzymes (Superoxide Dismutase-1 [SOD-1] and Glutathione Peroxidase[GPx]), and

Table 1 (continued)

Study (ref)	Type of study	Animal (available anthropometric features)/cell line	Aim of the study	Theobromine administration			Effects
							induced the translocation of Nrf2 (a transcription factor which activates expression of the gene encoding for SOD-1 and GPx) to the nucleus. Theobromine and LMN cream, showed similar antioxidant effects.
	In vitro	Undifferentiated PC12 cells, derived from rat pheochromocytoma adrenal medulla	To analyze theobromine effect on catecholaminergic metabolism	Oral	Different doses of theobromine (from 1 to 100 μM)	24 h	Theobromine increased the levels of NA, L-DOPA, DA, and DOPAC.
Yoneda et al. 2017	In vivo	C57BL/6NCr mice (male, 21 g)	To investigate whether theobromine could act as a PDE inhibitor in the CNS and affect cAMP/CREB/BDNF pathways and learning behavior	Oral	Normal diet (CN) or diet supplemented with0.05% theobromine (TB)	30 days	Theobromine was detected in the plasma and cerebral cortex. Theobromine did not affect the feeding behavior or glucose metabolism of the mice. Theobromine acted as a PDE inhibitor in the brain, increased cerebral cAMP/CREB/BDNF pathways and motor learning in mice.
Mendiola-Precoma et al. 2017	In vivo	Sprague Dawley rats (male, 6 months old, 500 g)	To study lard-enriched diet (LED) and theobromine effects on cognitive and memory processes.	Oral	Normal diet or LED or 0,5 mg/day or 30 mg/day theobromine supplementation to normal diet or LED	5 months	LED determined cognitive and memory deficits, increased levels of Aβ protein and IL-1β, and was associated with a decrease in gene expression and distribution of A1 purinegic receptor in the hippocampus. Theobromine, at both concentrations tested, restored A1 receptor levels and Aβ levels at 30 mg/day along with cognitive functions.

cAMP, cyclic adenosine monophosphate; *cGMP* cyclic guanosine monophosphate; *DA*, dopamine; *DMPX* dimethyl-1-propargylxanthine; *DOPAC*, 3,4-dihydroxyphenylacetic acid; *IBMX*, 3-isobutyl-1-methylxanthine; *NA*, noradrenalin; *NS*, not specified

significant improvement in motor learning, such as sequence, skill, adaptation, and reversal learning was found in theobromine group compared with normal nourished mice. In addition to a probable cognitive enhancing effect of theobromine, a neuroprotective action has been recently suggested (Mendiola-Precoma et al. 2017): Mendiola-Precoma et al. reproduced a rat model of sporadic Alzheimer's disease (AD) induced by a fat-enriched diet which was specifically able to induce a long-term deterioration in cognitive and memory functions and a decrease in gene expression and distribution in the hippocampus of A1 purinergic receptors (whose main roles are the inhibition of neurotransmitter release and of A2A excitotoxicity). In fact, studies in postmortem brains of AD patients indicated the presence of a lower A1 purinergic receptor density in the hippocampus (Chen and Chern 2011); moreover, A2A receptor antagonists were found to prevent amyloid-β (Aβ) formation (Dall'Igna et al. 2007; Kovács et al. 2011). The authors tested the effect of two different doses of theobromine (0.5 mg and 30 mg/day) and reported, at both concentrations tested, a restoration of A1 receptor levels and, at the higher dose, an improvement of cognitive functions and Aβ levels. Previously, also Shy et al. showed that theobromine ingestion was able to increase brain levels of A1-adenosine receptors.

Human studies (Table 2)

Early publications reported combined and individual effects of caffeine and theobromine in healthy subjects. In the 1970s, Dorfman et al. (Dorfman and Jarvik 1970) showed that the concomitant intake of caffeine and theobromine during evening extended estimated sleep latency and worsened sleep quality with respect to exclusive consumption of theobromine in a group of young volunteers. Few years later, Brunk et al. (Brunk et al. 1973) found a significantly greater subjectively reported stimulation in volunteers receiving caffeine with respect of those receiving theobromine. Conversely, Mumford et al. (Mumford et al. 1994) reported caffeine-like effects on mood and behavior (such as "increase in energy", "motivation to work," and "alert") during an acute exposition to increasing doses of theobromine in seven volunteers. Theobromine showed a very different range of discrimination threshold between subjects, which corresponded to a different individual sensitivity in subjective effects; the basis of different discrimination remained unclear. The same authors reported a slower oral absorption of theobromine with respect to caffeine (peak plasma time 2.5 h vs. 0.5 h). Smit et al. (Smit et al. 2004) investigated how different doses of theobromine (as capsules containing cocoa powder or theobromine plus caffeine or bars of milk or dark chocolate) could influence cognitive performance at tests for examining alertness (Simple Reaction Time task), working memory (Rapid Visual Information Processing task), manual dexterity (Thurstone tapping task); every dose of theobromine was able to improve cognitive performances in at least one of the task of the tests, but in this study, theobromine was always co-administered with a small amount of caffeine; moreover, cocoa powder and its methylxanthine constituents showed identical cognitive effects as well as energetic arousal and hedonic tone so that the authors suggested that methylxanthines represent psycho-pharmacologically active elements of chocolate.

Mitchell et al. (Mitchell et al. 2011) studied behavioral effects of theobromine and caffeine and their association in 24 healthy females in a randomized placebo-controlled and double-blind study. By measuring answers in Bond–Lader Questionnaire (to measure self-reported alertness), Digit Symbol Substitution Test, Workload Questionnaire, and Emotional Reaction Time Test, the authors concluded that an acute exposition to a dose of 700 mg of theobromine did not show stimulating properties. However, after the intake of theobromine, the authors observed a significant increased interest in doing tasks with respect to placebo and a significant delayed effect on the rating of negative words at Emotional Reaction Time Test, which demonstrated changes in emotional valence.

Judelson et al. (Judelson et al. 2013) exposed 24 healthy male volunteers to three experimental doses (100, 200, and 400 mg) of theobromine delivered in a cocoa-based beverage and to three-matched control treatments (0 mg theobromine, 400 mg theobromine, and 100 mg caffeine) delivered in a non-cocoa beverage. At every dose tested, theobromine failed to consistently affect mood state or vigilance.

Baggott et al. (Baggott et al. 2013) conducted a within-subjects placebo-controlled study of three oral theobromine doses (250, 500, and 1000 mg) with a control dose of caffeine (200 mg) in 80 subjects: performances at cognitive measures (Hopkins Verbal Learning Test, Digit Span test, Attention Network Task and Simple Reaction Time) after an acute exposure did not reach a significant improvement, except for alerting performance at Attention Network Task with the highest dose which was responsible for negative mood effects. The authors also assessed the genotype of the A2A receptor gene of their cohort, since previous studies reported that single nucleotide polymorphism affects anxiogenic individual sensitivity to caffeine, but they did not detect any effect on cognitive performances.

Travassos et al. (Travassos et al. 2015) studied the effects of caffeine and theobromine consumption (estimated on the basis of chocolate consumption) on cerebrospinal fluid (CSF) biomarkers, particularly Aβ, in 37 subjects with mild cognitive impairment (MCI) and in 51 patients with AD. A significant positive correlation was detected between plasma as well as CSF levels of Aβ42, a marker of amyloid deposition in the brain and theobromine, while no correlation was found between caffeine consumption or other main active metabolites (other than theobromine) and Aβ42 in the CSF. Curiously, CSF levels of theobromine were positively correlated with the levels

Table 2 Human studies

Study (ref)	Type of study	Sample	Aim of the study	Theobromine administration	Principal results
Dorfman and Jarvik 1970	Randomized controlled trial without placebo	41 healthy volunteers (males, 20–30 years)	To compare cognitive stimulant and diuretic effects of caffeine and theobromine	Increasing doses of theobromine (0 to 375 mg) associated with to 2 different doses of caffeine (125 or 250 mg)	No effect of theobromine on sleep's latency and quality and on urinary sodium excretion.
Brunk et al. 1973	Double-blind randomized controlled trial with placebo	35 healthy volunteers (sex and age N/A)	To compare subjective effects (CNS stimulation), physiological effects (blood pressure, pulse and respiration), biochemical effects (blood glucose and urate).	5 different capsules: theobromine 250 mg or 500 mg; caffeine 250 mg or 500 mg; placebo.	CNS stimulation: caffeine > theobromine, placebo; no differences on physiological and biochemical effects between caffeine and theobromine and placebo
Mumford et al. 1996	Randomized controlled trial	7 healthy volunteers (3 ♂ 4 ♀, 28–46 years)	Discriminative stimulus and subjective effects of theobromine and caffeine	Progressively lower doses of theobromine (max 2 g, min 56 mg) and caffeine (max 178 mg, min 1 mg) 560 mg theobromine, 178 mg caffeine and placebo qd, five times each in mixed sequence	5/7 subjects discriminated 560 mg of theobromine from placebo; 4/7 subjects reported anecdotally caffeine-like effects.
Smit et al. 2004	Double-blind randomized controlled trial with placebo	Study 1) 20 healthy volunteers (3 ♂, 17 females; 18–56 years) Study 2) 22 healthy volunteers (11 males, 11 females; 18–56 years)	To measure the effects on cognitive performance and mood of the amounts of cocoa powder and methylxanthines of 50 g bar of dark chocolate.	Study 1) 2 active treatments (containing identical amounts of methylxanthines representing a 50 g bar of dark chocolate): 11.6 g of encapsulated cocoa powder (CP) 250 mg theobromine+19 mg caffeine (CA + TB) vs. 2 identical placebo. Study 2) 3 different chocolate bars: 0 mg theobromine +0 mg caffeine (zero MX); 100 mg theobromine +8 mg caffeine (low MX); 250 mg theobromine +20 mg caffeine (high MX).	Study 1) Alertness (through SRT task): CP and CA + TB > placeboWorking memory (through RVIP task): CA + TB > placeboEnergetic arousal: CP and CA + TB > placeboHedonic tone: CA + TB > placebo Study 2) Alertness (through SRT task): High MX > placeboWorking memory (through RVIP task): High and Low MX > placebo
Mitchell et al. 2011	Double-blind randomized clinical trial with placebo	24 healthy volunteers (females, 51.1 ± 12.7 years)	To test if caffeine and theobromine have a synergistic effects on cognition, mood, and blood pressure	4 treatments (1 every week): 3 placebo capsules; 2 theobromine capsules (350 mg each) + 1 placebo capsule, 1 caffeine capsule (120 mg) + 2 placebo capsules; 2 theobromine capsules (350 mg each) and 1 caffeine capsule (120 mg).	Alertness (Bond–Lader subscales): increased by caffeine + theobromine and caffeine alone; calmness: increased by theobromine alone; contentment: increased with caffeine alone. Interest in doing tasks: increased by caffeine + theobromine; eagerness to do tasks: increased with caffeine alone; headache symptoms: reduced by caffeine+theobromine; systolic and diastolic blood pressure: decreased by theobromine alone > caffeine+theobromine, increased by caffeine alone.

Table 2 (continued)

Study (ref)	Type of study	Sample	Aim of the study	Theobromine administration	Principal results
Baggott et al. 2013	Randomized double-blind, placebo-controlled within--subjects Williams design	80 healthy volunteers (48 females, 36 males 18–35 years)	To test psychopharmacology of theobromine in healthy volunteers, also in relation to two polymorphisms in the adenosine receptor 2A gene	2 set of capsules (2,5 h interval away from one other): placebo-placebo; theobromine (250 mg)- placebo; theobromine (500 mg)-placebo; theobromine (750 mg)- placebo¸ theobromine (1000 mg)- placebo; placebo-caffeine (200 mg).	Theobromine responses differed according to dose: limited subjective effects at 250 mg and negative mood effects at higher doses, dose-dependently increased heart rate. Polymorphisms in the adenosine receptor 2A gene (rs4822492) appeared to attenuate or prevent theobromine induced increases in systolic blood pressure.
Judelson et al. 2013	Double-blind, Latin Squares crossover design	24 healthy volunteers (males, 23 ± 3 years)	To assess the effect of theobromine doses commonly found in foods on mood and vigilance parameters sensitive to caffeine	100, 200, and 400 mg theobromine	At every dose tested, theobromine failed to significantly affect mood state or vigilance
Travassos et al. 2015	Cross-sectional study	88 patients with cognitive impairment: 37 MCI (23 females, 14 males, 48–83 years) and 51 AD (33 females, 18 males, 48–89 years)	To study association of methylxantines consumption with the CSF biomarkers.	N/A	The consumption of caffeine was not correlated with the plasma nor CSF levels of theobromine (metabolite of caffeine); theobromine is the only metabolite of caffeine whose plasma and CSF level are correlated to plasma and CSF Aβ 42.

of other xanthines in the CSF, but not in the plasma, so it did not result proportional to theobromine-estimated intake from chocolate: this finding suggests that theobromine might be built up through central metabolic processes which promote demethylation of caffeine. On the basis of their findings, the authors hypothesized a possible role of theobromine in neuroprotection from amyloid-mediated neurotoxicity in AD.

Discussion

Our search retrieved relatively few studies investigating the cognitive effects of theobromine alone; a higher number of studies have considered a combination of methylxanthines such as theobromine and caffeine which are both typically contained in cocoa products; theobromine is also one of the metabolites of caffeine.

Major evidence on theobromine effects comes from animal and vitro studies, suggesting a potential cognitive enhancement (through modulation of catecholaminergic and cholinergic systems), an anti-inflammatory and a neuroprotective action.

The few human studies available on healthy volunteers focused on different and insufficiently comparable outcomes after theobromine intake and are prejudiced by limited study duration and size of the sample enrolled. In the range of doses used and along the duration of the studies conducted in humans, theobromine appears to be safe and well tolerated in humans at acute exposure below 1 g, but no studies investigated a long-term use or a chronic intake: it is possible that a longer intake of theobromine may provide long-term cognitive effects which are not revealed in short-term studies.

Limited psychoactive effects of theobromine alone were observed in the study of Baggott et al. (Baggott et al. 2013) who suggested a possible interactive effect of theobromine jointly with caffeine, maybe in the percentage commonly found in cocoa products, as supported by the results of the study of Smit et al. (Smit et al. 2004). Caffeine and theobromine have been previously proposed as the main substances which have a role in the psychopharmacological activity of chocolate (Hetherington 2001), besides biogenic amines and anandamide.

A major role of isolated assumption of caffeine with respect of theobromine in acute CNS stimulation can be postulated. In particular, caffeine showed a most important action on alertness, vigilance, attention, contentment, and eagerness, whereas theobromine exerted a greater effect on motivation and calmness (Brunk et al. 1973; Smit et al. 2004; Mitchell et al. 2011). Combined exposition to caffeine and theobromine demonstrated an acute effect on hedonic tone and working memory (Smit et al. 2004).

A possible role of theobromine in long-term neuroprotection has been recently suggested by both animal and human studies through a dose-dependent reduction of Aβ-amyloid-mediated neurotoxicity, which is known to be a potential underlying pathogenic mechanism of AD (Travassos et al. 2015; Mendiola-Precoma et al. 2017).

The CSF level of theobromine, which does not correspond to blood level, suggests a possible production of this methylxanthine through central demethylation of caffeine; therefore, effects of theobromine may be due to direct consumption as well as to central production from other nootropic methylxanthines such as caffeine.

Smit et Blackburn (Smit and Blackburn 2005) found that caffeine and theobromine could also play a role in our liking for chocolate, especially for dark chocolate. An age and gender dependence of effect of methylxanthines on purinergic receptors could also be postulated (Kovács et al. 2011) and should be taken into account in future studies, as well as a different individual sensitivity (Penetar et al. 1993).

Theobromine may also enhance cognitive performances indirectly through an improvement in cerebral blood flow and a benefit to blood vessels: theobromine is more effective as a blood vessel dilator and heart stimulant and may be the main actor of these effects among cocoa constituents. The relationship between arterial stiffness (due to chronic hypertension) and Aβ-amyloid deposition (Martínez-Pinilla et al. 2015) is well known, thus linking systemic vascular disease to brain amyloid burden. Indeed, differently from caffeine, theobromine showed an effect on both systolic and diastolic blood pressure reduction (van den Bogaard et al. 2010; Mitchell et al. 2011; Baggott et al. 2013; Martínez-Pinilla et al. 2015).

The simultaneous presence of several active substances in cocoa, each with different mechanisms of action and possibly combined effects, could confer to this food a potential neuroprotective effect. Growing evidence is suggestive that flavonoids contained in cocoa products, mainly epicatechin, catechin, and their oligomers, have a role in aiding preservation of cognitive function: laboratory studies have suggested anti-inflammatory and vascular effects, as well as a positive role in synaptic plasticity and in neurogenesis (Vauzour 2014). Microbial species involved in cocoa bean fermentation (cocobiota) may also have a considerable impact among the therapeutical properties of cocoa products through their metabolites such as lovastatin, known for its cholesterol-lowering action (Petyaev and Bashmakov 2016) and subsequent anti-atherogenic properties.

Habitual chocolate intake has been related to better cognitive performances, measured with an extensive battery of neuropsychological tests in a population-based study evaluating 968 community-dwelling participants (Crichton et al. 2016).

With regard to prevention of cognitive decline in the elderly population, more studies are available on caffeine long-term effects: experimental studies on animal and

cell AD models, as well as cross-sectional and longitudinal population-based studies have suggested a protective effect of caffeine for the development of dementia in late-life (Panza et al. 2015); instead, findings on the association of coffee use and progression of mild cognitive impairment to dementia are limited. The study by Travassos et al. (Travassos et al. 2015) on the effects of chocolate consumption (thus on the combined effect of caffeine and theobromine) on CSF in patients with cognitive impairment showed that a higher concentration of theobromine, but not of caffeine, in CSF is associated with a favorable Aβ profile. In some individuals, central caffeine metabolism could privilege demethylation of caffeine to theobromine due to the activation of a preferential enzymatic pathway and/or to environmental factors. This hypothetical variability could share a common biochemical basis of the different discrimination threshold found for theobromine between subjects (Mumford et al. 1996).

It is not known if a positive correlation between theobromine and CSF levels of Aβ42 could be present also in healthy subjects because the study of Travassos et al. has not provided a comparison with healthy controls and no other reports are available in the literature.

Previous studies reported that neural cell cultures with Aβ in the presence of an A2A receptor antagonist completely prevented amyloid-induced neurotoxicity (Rahman 2009); thus, an effect of theobromine on amyloidogenic pathway could be hypothesized. Finally, possible mechanisms of action of methylxanthines, such as theobromine associated with caffeine of cocoa products, comprise direct glial modulation by decreasing release of proinflammatory factors and enhancing astrocyte-mediated glutamate clearance (Sweitzer and De Leo 2011), mechanisms involved in neurodegenerative disorders.

Further studies are needed to investigate the role of theobromine alone, after long-term or chronic exposition (or modified release formulations), in combination with caffeine and other cocoa components for prevention of cognitive impairment and in particular of AD. It is worth noting that a real quantification of spontaneous theobromine consumption through consumption of cocoa derivatives is much more complex than estimation of caffeine intake, even if population studies are the best instrument to determine dementia risk after long-term exposition to a potential protective agent. A way of overcoming this difficulty could be the study of synthetic molecule exposition at different dosage regimens in a RCT: the cohort which may be enrolled in this context could be composed by cognitively normal elderly people and/or subjects with subjective cognitive decline (Rabin et al. 2017) or mild cognitive impairment (Petersen et al. 2014), and the effect of theobromine might be explored combining neuropsychological assessment to neuroimaging and/or functional brain imaging and/or cerebrospinal fluid biomarkers of AD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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