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Does Caffeine Consumption Modify Cerebrospinal Fluid Amyloid-β Levels in Patients with Alzheimer's Disease?

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Abstract. Caffeine may be protective against Alzheimer's disease (AD) by modulating amyloid-β (Aβ) metabolic pathways. The present work aimed to study a possible association of caffeine consumption with the cerebrospinal fluid (CSF) biomarkers, particularly Aβ. The study included 88 patients with AD or mild cognitive impairment. The consumption of caffeine and theobromine was evaluated using a validated food questionnaire. Quantification of caffeine and main active metabolites was performed with liquid chromatography coupled to tandem mass spectrometry. The levels of $A\beta_{1\text{-}42}$, total tau, and phosphorylated tau in the CSF were determined using sandwich ELISA methods and other Aβ species, $A\beta_{X\text{-}38}$, $A\beta_{X\text{-}40}$, and $A\beta_{X\text{-}42}$, with the MSD Aβ Triplex assay. The concentration of caffeine was 0.79 ± 1.15 μg/mL in the CSF and 1.20 ± 1.88 μg/mL in the plasma. No correlation was found between caffeine consumption and $A\beta_{42}$ in the CSF. However, a significant positive correlation was found between the concentrations of theobromine, both in the CSF and in the plasma, with $A\beta_{42}$ in the CSF. Theobromine in the CSF was positively correlated with the levels of other xanthines in the CSF, but not in the plasma, suggesting that it may be formed by central metabolic pathways. In conclusion, caffeine consumption does not modify the levels of CSF biomarkers, and does not require to be controlled for when measuring CSF biomarkers in a clinical setting. Since theobromine is associated with a favorable Aβ profile in the CSF, the possibility that it might have a protective role in AD should be further investigated.

Keywords: Alzheimer's disease, amyloid-β, biomarkers, caffeine, cerebrospinal fluid, metabolism, mild cognitive impairment, phosphotau, theobromine, total tau

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INTRODUCTION

Caffeine is the most widely consumed psychoactive drug worldwide [1]. Substantial evidence now exists that caffeine, an adenosine A_1 and A_{2A} receptor antagonist, may be protective against neurodegenerative disorders, including Alzheimer's disease (AD). These effects may involve modulation of amyloid-β (Aβ) metabolic pathways [2].

Experimental studies suggest that caffeine can attenuate neuronal damage and learning deficits caused by Aβ, using *in vitro* and *in vivo* rodent models [3, 4]. Chronic ingestion of caffeine may also counteract the cognitive deficits observed in a transgenic mouse model of AD in which the mutant human amyloid-β protein precursor (*AβPP*) gene is overexpressed [5]. Interestingly, in this animal model, caffeine decreased cortical and hippocampal Aβ levels, as well as cerebral interstitial fluid Aβ levels, probably by reducing the expression of both γ-secretase and β-site AβPP cleaving enzyme (BACE1), the enzymes involved in the production of Aβ from AβPP cleavage [5, 6]. Similar effects of caffeine on amyloid metabolism were also observed in the periphery, with reduced plasma Aβ levels after both acute and chronic caffeine administration in AD transgenic mice [6]. Interestingly, chronic caffeine intake may also prevent the development of spatial memory deficits and the build-up of proinflammatory and oxidative stress markers in a murine model of AD-like tau pathology [7].

Regarding human studies, epidemiological evidence suggests that caffeine may be protective against AD, dementia, cognitive impairment, and cognitive decline (see review and meta-analysis in [8]). A case-controlled study also showed that higher blood caffeine levels are associated with a reduced risk of dementia or delayed onset, in patients who already have cognitive impairment [9]. In agreement with animal studies, preliminary data in humans also suggest that acute caffeine administration modifies plasma Aβ levels, both in young adults and in elderly people [10].

Beyond the relevance of the protective effects of caffeine on cognitive decline and AD for prevention strategies [2], the observations that caffeine may influence Aβ levels may have diagnostic implications. Biomarkers in the cerebrospinal fluid (CSF) that reflect AD pathology are available, namely $A\beta_{42}$, total tau (T-tau), and phosphorylated tau (P-tau) [11]. Several Aβ species may be detected in the CSF, but $A\beta_{42}$ has been mostly used in clinical practice, since it reflects Aβ deposition in the brain [12, 13]. Nowadays the use of CSF biomarkers in AD is part of routine clinical diagnosis and clinical trial assessment [14], is becoming generalized particularly at reference centers [15], and has been formally recommended [16]. It is thus important to know more about potential factors that could influence CSF biomarkers. The possibility that caffeine influences Aβ metabolism raises the question whether caffeine consumption could be a confounder that should be taken into account when measuring CSF Aβ levels. In particular, the data reviewed above that caffeine may influence cerebral interstitial fluid levels of Aβ suggest that this may be reflected by CSF Aβ measurements.

The general aim of the present work was to study a possible association of caffeine consumption with the levels of CSF biomarkers, particularly $A\beta_{42}$, in patients with AD or mild cognitive impairment (MCI), a condition that is generally considered an initial clinical phase of AD. The finding of such an association would point out caffeine as a possible important confounder when evaluating CSF biomarkers, and strengthen the evidence that caffeine, by modifying the amyloid processing, could be neuroprotective in AD.

METHODS

Recruitment of participants was performed at 6 participating clinical centers within the Biomarkers for Alzheimer's disease and Parkinson's disease (BIOMARKAPD) consortium that aims at validating CSF biomarkers for clinical use, namely Lisbon, Coimbra, Thessaloniki, Athens, Copenhagen, and Izmir.

Participants

Patients were diagnosed as having MCI according to the European Alzheimer's Disease Consortium (EADC) criteria [17] or AD according to the DSM-IV criteria [18]. The BIOMARKAPD project was approved by the ethical committees of the participating centers.

Caffeine consumption

The consumption of caffeine and theobromine was evaluated from informants/caregivers using a validated food questionnaire [19–21]. This questionnaire assesses dietary habits in the 12 months preceding the baseline interview. In the present study, an abridged version focusing on food items that contain caffeine was used, namely chocolate (either in bar or powder), chocolate snacks, cola drinks, iced tea, coffee (including latte and other beverages containing coffee),

Table 1
Characteristics of the study population

	All patients $n = 88$	MCI $n = 37$	AD $n = 51$	Statistical significance
Age, years, mean ± SD, (range)	66.3 ± 8.6 (48–89)	65.8 ± 8.1 (48–83)	66.8 ± 9.0 (48–89)	NS#
Gender, F/M, *n* (%)	56/32 (64%/36%)	23/14 (62%/38%)	33/18 (65%/35%)	NS*
Caffeine consumption, mg/day, mean ± SD (range)	95.2 ± 60.8 (0.0–286.9)	102.0 ± 61.9 (1.4–286.9)	90.4 ± 60.1 (0.0–225.0)	NS#
Theobromine consumption, mg/day, mean ± SD (range)	37.2 ± 91.4 (0–687.5)	16.9 ± 28.2 (0–123.8)	54.6 ± 119.7 (0–687.5)	NS#
$A\beta_{X-38}$ (CSF) (pg/mL)	1817 ± 814 (521–4324)	1993 ± 879 (657–4324)	1690 ± 747 (521–3812)	NS#
$A\beta_{X-40}$ (CSF) (pg/mL)	4753 ± 1704 (1651–9514)	5108 ± 1757 (2325–9514)	4496 ± 1634 (1651–8523)	NS#
$A\beta_{X-42}$ (CSF) (pg/mL)	286 ± 196 (70–1213)	**358 ± 201** (98–990)	**234 ± 177** (70–1213)	***p* = 0.003**#
$A\beta_{1-42}$ (CSF) (pg/mL)	482 ± 213 (179–1219)	**583 ± 206** (260–980)	**408 ± 188** (179–1219)	***p* < 0.001**#
T-tau (CSF) (pg/mL)	577 ± 398 (125–2209)	487 ± 388 (125–2209)	642 ± 395 (131–1870)	NS#
P-tau (CSF) (pg/mL)	70 ± 40 (19–210)	63 ± 36 (19–210)	75 ± 42 (19–209)	NS#

AD, Alzheimer's disease; MCI, mild cognitive impairment; NS, non-significant; #Student's t test; *χ^2 test; Statistically significant values ($p < 0.05$) are shown in bold.

and tea (black and green). For each food item, the informants/caregivers were enquired about the average consumption frequency, being able to choose one from nine possible answers ranging from "never" to "six or more times per day". The portion size usually consumed was also asked. The information collected from the food frequency questionnaires was then used to estimate the average daily caffeine and theobromine intake. To assess the content in caffeine and theobromine of the food items, a Food and Drug Administration report was used [22].

Biological samples

Collection of CSF was performed at the participating centers according to the recommendations of BIOMARKAPD [23]. CSF was collected in polypropylene tubes and centrifuged at room temperature, 2000 *g* for 10 min, to remove cells and debris. Samples were then aliquoted to biobanking tubes and stored at –80°C. Blood was collected in EDTA tubes, centrifuged according to routine local protocols, and plasma aliquots were also frozen at –80ºC.

Quantification of caffeine and metabolites

Quantification of caffeine and main active metabolites (theobromine, theophylline, paraxanthine) in the CSF (88 samples) and plasma (70 samples, one center did not have plasma available) was performed with liquid chromatography coupled to tandem mass spectrometry.

Samples were analyzed on a CTC PAL/ekspert™ ultraLC 110 (Eksigent) coupled to a hybrid triple quadrupole/linear ion-trap 4000 QTrap mass spectrometer operated by Analyst 1.6.1 (AB Sciex). The chromatographic separation was performed in a 3 μm Gemini C_{18} column (50 × 2.0 mm, 110 Å, Phenomenex) with a 4 × 2.0 mm C_{18} guard-column (Phenomenex) and column temperature was maintained at 40°C. The flow rate was set to 500 μL/min and mobile phase A and B were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The LC program was: 2% of B (0–0.3 min), 2–20% of B (0.3–8.0 min), 20–80% of B (8.0–8.1 min). After each sample analysis, a 4-min run was performed from 80 to 2% of B for column equilibration.

The ionization source (ESI Turbo V) was operated in the positive mode for caffeine, theobromine and paraxanthine (5500 V); and in the negative mode for theophylline (–4500 V). The other source parameters were the same for both ionization modes, 35 psi for nebulizer gas 1 (GS1), 40 psi for the nebulizer gas 2 (GS2), 30 psi for the curtain gas (CUR), and the temperature was 600°C. Each molecule was analyzed by Multiple Reaction Monitoring (MRM) where Q1 and Q3 were at unit resolution and transitions are shown in Supplementary Table 1.

Plasma (50 μL) and CSF (30 μL) samples were spiked with the internal standards 7-beta-hydroxyethyltheophyline (11.2 ng), caffeine-trimethyl-$^{13}C_3$ (3.9 ng), and florfenicol (3.6 ng) followed by a protein precipitation step using methanol and centrifugation at 14,000 × *g* for 20 min. The supernatant was collected and evaporated in a speedvac and samples were resuspended in 100 μL of 2% ACN + 0.1% FA. Absolute quantification of each molecule in plasma and CSF samples was performed

using calibration curves prepared in blank solvent with concentrations ranging between 5–776 ng/mL for caffeine and 4.7–720 ng/mL for theobromine, paraxanthine, and theophylline. The volume of injection for biological samples and calibration curves was 10 μL and peak areas were integrated using MultiQuant software (version 2.1, AB Sciex).

Quantification of CSF biomarkers

The levels of $A\beta_{1\text{-}42}$ in the CSF were determined using a sandwich ELISA (INNOTEST® β-AMYLOID(1-42), Innogenetics, Ghent, Belgium), specifically constructed to measure Aβ containing both the first and 42nd amino acid, as previously described [24]. In this assay, the monoclonal antibody 21F12, which is highly specific for the C-terminus of $A\beta_{42}$ was used for capture, and 3D6, which is specific to the N-terminus was used as detector. Other Aβ species were assessed with the MSD Abeta Triplex assay (MSD, Rockville, MD), using a multiplexed method in which C-terminally specific antibodies are used to selectively capture Aβ forms ending at amino acids 38, 40, and 42, respectively, which are then quantified using the 6E10 detector antibody. This assay is thus not specific to the 1st amino acid of the Aβ peptides (the epitope of 6E10 lies within amino acids 3 to 8 in the Aβ sequence) and the measured Aβ forms are therefore called $A\beta_{X\text{-}38}$, $A\beta_{X\text{-}40}$, and $A\beta_{X\text{-}42}$ in the current paper.

Total tau (T-tau) concentration in the CSF was determined using a sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium) specifically constructed to measure all tau isoforms irrespectively of phosphorylation status, as previously described [25]. Tau phosphorylated at threonine 181 (P-tau) was measured using a sandwich ELISA method (INNOTEST® PHOSPHO-TAU(181P), Innogenetics, Ghent, Belgium), as described previously in detail [26]. Measurements of CSF biomarkers with clinical relevance were performed at the same reference laboratory in Mölndal, where normal values are $A\beta_{1\text{-}42}$ >550 pg/ml, T-tau <400 pg/ml, and P-tau <70 pg/ml.

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 21 for Windows (2010 SPSS Inc., an IBM Company). The primary statistical analysis was the correlation between the average daily consumption of caffeine and $A\beta_{42}$ in the CSF, using the Pearson test. Other secondary correlation analyses also used the Pearson test. Differences between patients with MCI and AD were evaluated with the Student's t test for numerical variables and the χ^2 test for the categorical variable. Values are presented as mean ± SD. Statistical significance was considered for $p < 0.05$.

RESULTS

The study included 88 patients with AD or MCI, whose characteristics are shown in Table 1. The daily consumption of caffeine estimated with the food questionnaire was 95.2 ± 60.8 mg/day. The daily consumption of theobromine was also estimated on the basis of chocolate consumption using the same food questionnaire and was 37.2 ± 91.4 mg/day. No significant differences were found in daily consumption of either caffeine or theobromine between MCI and AD patients. Regarding CSF biomarkers, T-tau was 577 ± 398 pg/mL, P-tau 70 ± 40 pg/mL, and $A\beta_{1\text{-}42}$ 482 ± 213 pg/mL. Patients with AD had lower levels of the 42 amino acid long form of Aβ, $A\beta_{1\text{-}42}$ (408 ± 188 pg/mL) as compared to MCI patients (583 ± 206 pg/mL; $p < 0.05$, Student's t test), as well lower levels of the Aβ forms ending at amino acid 42, $A\beta_{X\text{-}42}$ (234 ± 177 pg/mL) as compared to MCI patients (358 ± 201 pg/mL; $p < 0.05$, Student's t test), while no significant differences were found for $A\beta_{X\text{-}38}$, $A\beta_{X\text{-}40}$, T-tau, and P-tau between the two clinical groups (Table 1).

The values for caffeine and main metabolites in CSF and plasma are shown in Table 2. The concentration of caffeine was 0.79 ± 1.15 μg/mL in the CSF and 1.20 ± 1.88 μg/mL in the plasma, no significant differences being found in any measured xanthine between MCI and AD patients, who were joined together in further analyses. As expected, the consumption of caffeine, evaluated by the food questionnaire, was positively correlated with the levels of caffeine, theophylline and paraxanthine, both in the plasma and in the CSF (Table 3). However, the consumption of caffeine was not correlated with the levels of theobromine, either in the plasma or in the CSF (Table 3). The levels of caffeine in the plasma and in the CSF were strongly correlated ($r = 0.741$, $p < 0.001$).

In the principal statistical analysis planned, no correlation was found between caffeine consumption and $A\beta_{42}$ (either the full form $A\beta_{1\text{-}42}$ or $A\beta_{X\text{-}42}$) in the CSF, as shown in Table 4. On the other hand, a significant positive correlation was found between CSF levels of $A\beta_{42}$ and theobromine, as well as between plasma $A\beta_{42}$ and theobromine levels, while other caffeine main active metabolites did not correlate with CSF levels of $A\beta_{42}$ (Table 4).

Table 2
Caffeine and main metabolites in CSF and plasma

	Caffeine	Theophylline	Theobromine	Paraxanthine
CSF, μg/mL, mean ± SD (range), $n = 88$	0.79 ± 1.15 (0.00–5.99)	0.031 ± 0.038 (0.00–0.18)	0.38 ± 0.49 (0.00–2.37)	0.25 ± 0.30 (0.00–1.57)
Plasma, μg/mL, mean ± SD (range), $n = 70$	1.20 ± 1.88 (0.00–8.77)	0.15 ± 0.14 (0.00–0.61)	0.67 ± 0.86 (0.00–4.04)	0.74 ± 0.78 (0.00–3.60)

Caffeine and main metabolites, in CSF and plasma, were not significantly different ($p > 0.05$) between MCI and AD patients.

Table 3
Correlations between caffeine consumption and xanthine concentrations

	Caffeine (plasma) (μg/mL)	Theophylline (plasma) (μg/mL)	Theobromine (plasma) (μg/mL)	Paraxanthine (plasma) (μg/mL)	Caffeine (CSF) (μg/mL)	Theophylline (CSF) (μg/mL)	Theobromine (CSF) (μg/mL)	Paraxanthine (CSF) (μg/mL)
Caffeine Consumption (mg/day)	**$r = 0.288$** **$p = 0.016$**	**$r = 0.394$** **$p = 0.001$**	$r = -0.10$ $p = 0.932$	**$r = 0.262$** **$p = 0.029$**	**$r = 0.248$** **$p = 0.020$**	**$r = 0.311$** **$p = 0.003$**	$r = 0.024$ $p = 0.821$	**$r = 0.277$** **$p = 0.009$**

r, Pearson's correlation; *Statistically significant values ($p < 0.05$) are shown in bold.

We next ascertained what factors could be associated with the levels of theobromine in plasma and CSF. The levels of theobromine in plasma and CSF were highly inter-correlated (Table 5). Theobromine in the CSF did not correlate with caffeine consumption, theobromine consumption, or the levels of caffeine, theophylline or paraxanthine in the plasma, but was positively correlated with the levels of caffeine, theophylline and paraxanthine in the CSF (Table 5).

DISCUSSION

In the present study, we could not find an association between either caffeine consumption, caffeine concentration in plasma or caffeine concentration in the CSF, and the levels of the core AD CSF biomarkers, in patients with AD or MCI.

The daily consumption of caffeine estimated with the food questionnaire was 95 mg per day. This value, notwithstanding substantial variation in caffeine concentration and actual volume in coffee beverages, may correspond roughly to one cup of coffee and is lower than the estimated daily caffeine consumption in many European countries [1]. Interestingly, the average daily caffeine intake was previously shown to decline after the diagnosis of AD. This could be due to the disability or disinclination of patients with cognitive complaints in going to the coffee-shop or preparing coffee at home, but frequently the caregivers may restrict consumption because they believe coffee could be potentially harmful, particularly to sick people [27].

The possibility that diet can influence Aβ metabolism was previously raised. In a previous study, food intake did not influence plasma $A\beta_{42}$ levels, compared to the fasting condition, and the authors assumed that it would be unlikely that CSF $A\beta_{42}$ levels would be affected when the plasma levels were not [28]. However, manipulating the saturated fat and carbohydrates content in the diet during four weeks modified CSF $A\beta_{42}$ levels both in healthy subjects and patients with MCI [29]. Regarding caffeine, preliminary data suggest that acute caffeine administration rapidly lowers $A\beta_{42}$ levels in plasma, both in young and old individuals [10].

As expected, the caffeine consumption evaluated from the food questionnaire, the plasma and the CSF caffeine levels were highly inter-correlated. However, neither of these caffeine measurements was correlated with the CSF biomarkers. Also as anticipated, abnormal values were found for CSF biomarkers. Lower levels of $A\beta_{42}$ (either the full form $A\beta_{1\text{-}42}$ or $A\beta_{X\text{-}42}$) were found in patients with AD as compared with patients with MCI. Since CSF $A\beta_{42}$ levels are reduced very early in the disease process, and do not appreciably decrease during the clinical course of AD, a likely explanation for the less diminished levels of $A\beta_{42}$ in MCI patients is that some of these may actually not have AD pathology. The present study thus probably recruited MCI patients with as well as without AD pathology. It is recognized that a proportion of MCI cases do not evolve to AD but to other forms of dementia instead, and some cases may even be due to reversible causes [30]. Another explanation is that some patients, for largely unknown reasons, may have Aβ pathology reflected in positive amyloid scans but not in altered CSF $A\beta_{42}$ levels [31].

Caffeine, chemically 1,3,7-trimethylxanthine, is metabolized by the cytochrome P450 enzyme system in the liver. The main metabolites

Table 4
Correlations between CSF biomarkers and consumption and xanthine concentrations

	Caffeine consumption (mg/day)	Theobromine consumption (mg/day)	Caffeine (plasma) (μg/mL)	Theophylline (plasma) (μg/mL)	Theobromine (plasma) (μg/mL)	Paraxanthine (plasma) (μg/mL)	Caffeine (CSF) (μg/mL)	Theophylline (CSF) (μg/mL)	Theobromine (CSF) (μg/mL)	Paraxanthine (CSF) (μg/mL)
$A\beta_{X-38}$ (CSF)	$r = -0.034$	$r = 0.119$	$r = -0.110$	$r = -0.107$	$r = 0.155$	$r = -0.096$	$r = 0.144$	$r = 0.003$	$r = 0.127$	$r = -0.022$
(pg/mL)	$p = 0.750$	$p = 0.301$	$p = 0.363$	$p = 0.377$	$p = 0.200$	$p = 0.431$	$p = 0.180$	$p = 0.979$	$p = 0.238$	$p = 0.838$
$A\beta_{X-40}$ (CSF)	$r = -0.014$	$r = 0.140$	$r = -0.079$	$r = -0.053$	$r = 0.116$	$r = -0.055$	$r = 0.142$	$r = 0.020$	$r = 0.104$	$r = 0.000$
(pg/mL)	$p = 0.898$	$p = 0.222$	$p = 0.518$	$p = 0.664$	$p = 0.341$	$p = 0.651$	$p = 0.187$	$p = 0.852$	$p = 0.336$	$p = 0.998$
$A\beta_{X-42}$ (CSF)	$r = -0.067$	$r = 0.072$	$r = -0.007$	$r = -0.010$	$\mathbf{r = 0.477}$	$r = 0.007$	$r = 0.026$	$r = 0.014$	$\mathbf{r = 0.379}$	$r = -0.025$
(pg/mL	$p = 0.537$	$p = 0.530$	$p = 0.951$	$p = 0.935$	$\mathbf{p < 0.001}$	$p = 0.952$	$p = 0.810$	$p = 0.900$	$\mathbf{p < 0.001}$	$p = 0.820$
$A\beta_{1-42}$ (CSF)	$r = 0.039$	$r = 0.092$	$r = 0.070$	$r = 0.088$	$\mathbf{r = 0.433}$	$r = 0.104$	$r = 0.016$	$r = 0.062$	$\mathbf{r = 0.328}$	$r = 0.022$
(pg/mL)	$p = 0.719$	$p = 0.421$	$p = 0.566$	$p = 0.470$	$\mathbf{p < 0.001}$	$p = 0.392$	$p = 0.884$	$p = 0.569$	$\mathbf{p = 0.002}$	$p = 0.838$
T-tau (CSF)	$r = -0.046$	$r = 0.195$	$r = -0.094$	$r = -0.105$	$r = -0.187$	$p = -0.125$	$r = 0.110$	$r = -0.058$	$r = -0.132$	$r = -0.046$
(pg/mL)	$p = 0.670$	$p = 0.087$	$p = 0.438$	$p = 0.386$	$p = 0.122$	$p = 0.302$	$p = 0.309$	$p = 0.594$	$p = 0.222$	$p = 0.668$
P-tau (CSF)	$r = -0.065$	$r = 0.174$	$r = -0.087$	$r = -0.106$	$r = -0.185$	$p = -0.110$	$r = 0.062$	$r = -0.069$	$r = -0.129$	$r = -0.060$
(pg/mL)	$p = 0.550$	$p = 0.127$	$p = 0.474$	$p = 0.383$	$p = 0.126$	$r = 0.366$	$p = 0.567$	$p = 0.525$	$p = 0.233$	$p = 0.579$

r, Pearson's correlation; *Statistically significant values ($p < 0.05$) are shown in bold.

Table 5
Correlations between theobromine concentration and consumption and xanthine concentrations

	Caffeine consumption (mg/day)	Theobromine consumption (mg/day)	Caffeine (plasma) (μg/mL)	Theophylline (plasma) (μg/mL)	Theobromine (plasma) (μg/mL)	Paraxanthine (plasma) (μg/mL)	Caffeine (CSF) (μg/mL)	Theophylline (CSF) (μg/mL)	Theobromine (CSF) (μg/mL)	Paraxanthine (CSF) (μg/mL)
Theobromine (plasma) (μg/mL)	$r = –0.010$ $p = 0.932$	$r = 0.066$ $p = 0.616$	**$r = 0.248$** **$p = 0.039$**	**$r = 0.253$** **$p = 0.035$**		**$r = 0.267$** **$p = 0.026$**	$r = 0.221$ $p = 0.066$	**$r = 0.273$** **$p = 0.022$**	**$r = 0.924$** **$p < 0.001$**	$r = 0.224$ $p = 0.062$
Theobromine (CSF) (μg/mL)	$r = 0.024$ $p = 0.821$	$r = 0.000$ $p = 0.999$	$r = 0.128$ $p = 0.291$	$r = 0.214$ $p = 0.076$	**$r = 0.924$** **$p < 0.001$**	$p = 0.162$ $p = 0.179$	**$r = 0.218$** **$p = 0.041$**	**$r = 0.326$** **$p = 0.002$**		**$r = 0.308$** **$p = 0.004$**

r = Pearson's correlation; *Statistically significant values ($p < 0.05$) are shown in bold.

of caffeine with pharmacological activity, paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine) [32, 33] were quantified. The most interesting, as well as unexpected, finding of the present work is that the concentration of theobromine in the CSF was highly and positively correlated with CSF levels of $A\beta_{42}$ (either the full form $A\beta_{1\text{-}42}$ or $A\beta_{X\text{-}42}$). A similar correlation was found between CSF $A\beta_{42}$ and plasma theobromine levels. We estimate that the levels of theobromine in the CSF explain about 10% of the total CSF Aβ42 variation. It would relevant to know whether the correlation between the concentrations of theobromine and CSF levels of $A\beta_{42}$, observed in patients with MCI or AD, also holds true in healthy subjects, or in patients with other types of dementia. This correlation could reflect a general pharmacological effect of theobromine on Aβ processing, and be detected in all subjects, or instead be specific of patients with Aβ pathology. At this point, since healthy controls were not included in the study, the observed correlation between the concentrations of theobromine and CSF levels of $A\beta_{42}$ is pertinent to the clinical population.

Theobromine is one of the main metabolites of caffeine and easily penetrates the blood-brain barrier [34], but seems to be devoid of the mood and vigilance effects typical for caffeine [35]. Chocolate foods and beverages constitute the major source of dietary theobromine [36]. Consumption of chocolate has been associated to better cognitive performance in the elderly [37], and this effect has generally been attributed to the content of flavonoids and not xanthines in chocolate [38]. However, in the present study, the levels of theobromine in the CSF and plasma were not correlated with the estimated intake of theobromine from chocolate, which suggests that most theobromine in plasma and CSF does not directly come from dietary sources. Interestingly, theobromine in the CSF does not correlate with caffeine consumption or the levels of caffeine, theophylline, or paraxanthine in the plasma, but instead correlates with levels of caffeine, theophylline, and paraxanthine in the CSF. The possibility that caffeine may be metabolized in the brain by specific, local enzymatic pathways has been previously advanced [1]. Taken together, these findings suggest that some individuals may demethylate caffeine predominantly to theobromine centrally due to differences in local caffeine metabolism. These individual differences might be related to genetic polymorphisms in the complex enzymatic pathways involved in the metabolism of caffeine, or to environmental factors, for instance the administration of drugs that share the same enzymatic pathways [39]. In these individuals, higher concentrations of theobromine appear to be associated to a favorable Aβ profile in the CSF.

In conclusion, caffeine consumption does not modify the levels of CSF biomarkers in patients with MCI or AD, and does not require to be controlled for when measuring CSF biomarkers in a clinical setting. Since theobromine is associated with a favorable Aβ profile in the CSF, the possibility that this xanthine, formed upon caffeine metabolism and also directly ingested from chocolate products, might have a protective role in AD should be further investigated.

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SUPPLEMENTARY MATERIAL

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