Review Article

Increased Cerebrospinal Fluid Production as a Possible Mechanism Underlying Caffeine's Protective Effect against Alzheimer's Disease

Peter Wostyn,[1] Debby Van Dam,[2] Kurt Audenaert,[3] and Peter Paul De Deyn[2, 4]

[1] Department of Psychiatry, PC Sint-Amandus, Reigerlostraat 10, 8730 Beernem, Belgium
[2] Laboratory of Neurochemistry and Behavior, Institute Born-Bunge and, Department of Biomedical Sciences,
  University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium
[3] Department of Psychiatry, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium
[4] Department of Neurology and Memory Clinic, Middelheim General Hospital (ZNA), Lindendreef 1,
  2020 Antwerp, Belgium

Correspondence should be addressed to Peter Wostyn, wostyn.peter@skynet.be

Alzheimer's disease (AD), the most common type of dementia among older people, is characterized by the accumulation of β-amyloid (Aβ) senile plaques and neurofibrillary tangles composed of hyperphosphorylated tau in the brain. Despite major advances in understanding the molecular etiology of the disease, progress in the clinical treatment of AD patients has been extremely limited. Therefore, new and more effective therapeutic approaches are needed. Accumulating evidence from human and animal studies suggests that the long-term consumption of caffeine, the most commonly used psychoactive drug in the world, may be protective against AD. The mechanisms underlying the suggested beneficial effect of caffeine against AD remain to be elucidated. In recent studies, several potential neuroprotective effects of caffeine have been proposed. Interestingly, a recent study in rats showed that the long-term consumption of caffeine increased cerebrospinal fluid (CSF) production, associated with the increased expression of Na$^+$-K$^+$ ATPase and increased cerebral blood flow. Compromised function of the choroid plexus and defective CSF production and turnover, with diminished clearance of Aβ, may be one mechanism implicated in the pathogenesis of late-onset AD. If reduced CSF turnover is a risk factor for AD, then therapeutic strategies to improve CSF flow are reasonable. In this paper, we hypothesize that long-term caffeine consumption could exert protective effects against AD at least in part by facilitating CSF production, turnover, and clearance. Further, we propose a preclinical experimental design allowing evaluation of this hypothesis.

1. Introduction

Alzheimer's disease (AD), the most common type of dementia among older people, is a progressive neurodegenerative disorder characterized clinically by a gradual decline in cognition and daily functioning and behavioural alterations [1]. Principal neuropathological hallmarks of AD include extracellular senile plaques containing β-amyloid (Aβ) derived from β-amyloid precursor protein (APP) after sequential cleavage by β-secretase and γ-secretase, and intracellular neurofibrillary tangles caused by abnormally phosphorylated tau protein [1]. Early-onset familial AD caused by mutations in the genes encoding APP and the γ-secretase-complex components presenilin-1 and presenilin-2 accounts for less than 5% of the total number of AD cases [2]. The discovery of pathogenic mutations in these three genes in rare patients with autosomal dominant, early-onset AD provided incontestable evidence that aberrant APP processing can be sufficient to trigger the pathological cascade leading to AD [3]. The pathological accumulation of Aβ in the far more common late-onset AD is more likely to be the result of defects in the clearance of Aβ [3]. Despite major advances in understanding the molecular etiology of the disease, progress in the clinical treatment of AD patients has been extremely

limited. Therefore, new and more effective therapeutic approaches are needed. Accumulating evidence from human and animal studies suggests that the long-term consumption of caffeine, the most commonly used psychoactive drug in the world, may be protective against AD [4–7]. Epidemiologically, a small case-control study showed that caffeine intake was significantly inversely associated with AD [6]. A prospective study also showed that daily coffee drinking was associated with a reduced risk of AD after 5-year follow-up [7]. There is also substantial evidence for a beneficial role of caffeine in animal models of AD [4, 5]. The mechanisms underlying the suggested protective effect of caffeine against AD remain to be elucidated. In this paper, we present a hypothesis which speculates that long-term caffeine consumption could exert protective effects against AD at least in part by facilitating cerebrospinal fluid (CSF) production, turnover, and clearance. Further, we propose a preclinical experimental design allowing evaluation of this hypothesis.

2. Presentation of the Hypothesis

There is evidence that production and turnover of CSF help to clear toxic molecules such as $A\beta$ from the interstitial-fluid space of the brain to the bloodstream [8]. CSF production and turnover have been shown to be decreased in ageing, normal pressure hydrocephalus (NPH), and AD [8]. Using the Masserman technique, Silverberg et al. [9] measured a 50% decrease in CSF production among AD patients when compared with Parkinson's disease controls. Mean CSF production in AD was 0.20 ± 0.06 mL/min, and in controls was 0.42 ± 0.13 mL/min [8]. The authors calculated a threefold decrease in CSF turnover in AD [8]. Age-associated reduction in CSF production, with diminished clearance of $A\beta$, may be a key factor in the onset and progression of AD [8] and may be a particularly important mechanism of amyloid toxicity in late-onset AD cases in whom overproduction of $A\beta$ may not be operative [10].

There is some scientific rationale for considering AD, at least in part, to be a choroid plexus (CP) disease, in that reduced CSF production and turnover may contribute to the difficulty in clearing $A\beta$ from the aging brain [11]. CSF is produced mainly by the four choroid plexuses that are found one in each ventricle of the brain [12]. The CPs are highly vascularized villous structures covered by a single layer of epithelial cells [13, 14]. CPs have multiple functions of synthesis, secretion, active transport, and selective reabsorption of deleterious substances [13]. In young adults, CSF is completely renewed six times a day [11]. Structural changes in the CP coincide with diminished CSF production in ageing, AD, and NPH [8]. In AD, choroid plexuses present similar, although much more pronounced, abnormalities than those observed in ageing [13, 14]. The CP in AD shows epithelial atrophy, basement membrane thickening, cyst formation, lipid accumulation, fibrosis, calcification, and hyalinization and amyloid deposition in choroidal blood vessels [8].

A review by Brown et al. [12] highlighted the molecular mechanisms of CSF production. The epithelial cells of the CP

secrete CSF, by a process that involves the transport of Na^+, Cl^- and HCO_3^- from the blood to the ventricles of the brain [12]. This creates an osmotic gradient that is accompanied by the secretion of H_2O [12]. The movement of ions across the cellular membrane is mediated by specific transporters and ion channels that are distributed unequally on the basolateral and apical sides of the CP epithelial layer [12]. Na^+-K^+ ATPase, K^+ channels, and Na^+-K^+-$2Cl^-$ cotransporters are expressed in the apical membrane [12]. By contrast the basolateral membrane contains Cl^--HCO_3^- exchangers, a variety of Na^+-coupled HCO_3^- transporters and K^+-Cl^- cotransporters [12]. Aquaporin 1 (AQP1) mediates water transport at the apical membrane, but the route across the basolateral membrane is unknown [12].

Among the numerous proteins involved in choroidal CSF production, it is known that Na^+-K^+ ATPase plays an important role in CSF secretion [15]. The Na^+-K^+ ATPase is a ubiquitous protein which catalyses 1 molecule of ATP to exchange $3 Na^+$ ions for $2 K^+$ ions across the cell membrane [16]. In the choroid plexus, this enzyme is located in the luminal surface and provides the driving force for CSF production [15]. Inhibitors of the Na^+-K^+ ATPase pump, for example, the cardiac glycoside ouabain, have been shown to reduce CSF production and the movement of Na^+ into the CSF [12]. Moreover, it has been shown that ageing affects choroidal proteins involved in CSF production [17]. Masseguin et al. [17] compared choroid plexuses of Sprague-Dawley rats aged 10 or 20 months with those of 3-month-old ones. Progressive and age-related changes in the Na^+-K^+ ATPase, carbonic anhydrase II and AQP1 expressions at the apical and/or cytoplasmic level, as suggested by both the decreases in the intensities of immunocytochemical and in situ hybridization signals, indicated that ageing decreases notably the protein expression of the enzymes and transporters known to regulate the CSF production in choroid plexus [17]. As noted above, with age, CSF production decreases and could increase the risk for development of late-onset AD [8].

In a recent study in young adult Sprague-Dawley rats, Han et al. [15] found that the long-term consumption of caffeine, a nonselective adenosine A_1 and A_{2A} receptor antagonist, increased CSF production, associated with the increased expression of Na^+-K^+ ATPase and increased cerebral blood flow. Caffeine (0.3 or 0.6 g/L) was added to the drinking water for 3 weeks in this study [15]. This low dose of caffeine (0.3 g/L) has been shown to be representative of the standard daily human consumption [15]. The authors found a significant increase in the production of CSF in the caffeine-treated rats compared to the control rats (5.02 ± 0.15 versus $2.95 \pm 0.12 \mu$L/min, $P < .01$) [15]. By contrast, acute treatment with caffeine decreased the production of CSF [15]. The rats treated with caffeine just once, before measurement, showed a significantly reduced production of CSF, by 22.3% compared to the control rats [15]. The "effect inversion" of caffeine was mediated by increased expression of the A_1 adenosine receptor, in the choroid plexus of rats chronically treated with caffeine [15]. In accordance with previous results showing increased expression of Na^+-K^+ ATPase in A_1 adenosine receptor transgenic mice, this study

showed that the A_1 adenosine receptor regulates the expression of Na^+-K^+ ATPase in the choroid plexus [15]. Because caffeine is commonly ingested chronically, it is important to note that long-term exposure to adenosine receptor antagonists like caffeine can have effects that resemble the acute effects of adenosine receptor agonists, due likely to up-regulation of adenosine receptors (A_1 and A_{2A}) and adaptive changes leading to adenosine receptor sensitization [4]. The results of the study by Han et al. [15] showed that the A_1, but not the A_{2A}, adenosine receptor, was increased in the choroid plexus of the caffeine-treated rats compared to the control rats. It is important to note, however, that in another study, the same level of caffeine administration to AD transgenic mice did not affect A_1 or A_{2A} receptor densities or expression in either cerebral cortex or hippocampus [5]. Therefore, the up-regulation of A_1 adenosine receptors reported by Han et al. [15] might be specific to the choroid plexus. Alternatively, this A_1 adenosine receptor up-regulation might be specific to normal young adult rats. Furthermore, in their study, Han et al. [15] noted a significant increase in the cerebral blood flow of the caffeine-treated rats compared to the control rats. This finding is contrary to the well-documented central vasoconstrictive properties of caffeine that lead to a decrease in cerebral blood flow in both animals and humans [18]. Given that cerebral blood flow affects the production of CSF [15], it would be interesting to investigate whether caffeine-induced enhancement of CSF production also occurs in other species or animal models.

Of major interest for the hypothesis presented here, recent epidemiological and experimental studies suggest that long-term caffeine consumption may be protective against AD [4–7]. However, findings from cross-sectional studies have been contradictory [19, 20]. Some studies indicated no association between coffee drinking and AD, while others found an inverse association between caffeine intake and AD [19, 20]. Most of the longitudinal studies have consistently found the cognitive benefits of long-term caffeine consumption among the group of oldest people [19]. Several studies have shown that long-term (years to decades) caffeine consumption may result in improved cognitive function or may protect against memory impairment observed in AD and the ageing process [19]. In a 1989 paper by Lammi et al. [21], low coffee consumption was reported to be associated with mental disability after a 25-year follow-up of 716 Finnish men. In the Canadian Study of Health and Aging (CSHA), daily coffee drinking decreased the risk of AD by 31% during a 5-year follow-up of subjects aged 65 years or older [7]. A case-control study compared the daily intake of caffeine in 54 AD patients with 54 nondemented matched controls [6]. Patients with AD had an average daily caffeine intake of 73.9 ± 97.9 mg during the 20 years that preceded diagnosis of AD, whereas the controls had an average daily caffeine intake of 198.7 ± 135.7 mg during the corresponding 20 years of their lifetimes [6]. The main finding of this study was a significant inverse association between caffeine intake and AD, and this association was independent of other habits eventually related to caffeine consumption, medical disorders that might influence caffeine intake and potential risk factors for AD [6]. The Finland, Italy, and the Netherlands Elderly prospective cohort study (the FINE study) evaluated cognitive functioning and coffee consumption in 676 healthy elderly men over a 10-year period [22]. An inverse and J-shaped association was observed between the number of cups of coffee consumed and cognitive decline, with the least cognitive decline for three cups of coffee per day [22]. This decline was 4.3 times smaller than the decline of non-coffee drinkers [22]. The French Three City Study of 7017 individuals aged 65 years and older without dementia examined the association between caffeine intake, cognitive decline, and incident dementia [23]. While no relation between caffeine intake and cognitive decline was found in men, this prospective study found that women with high rates of caffeine consumption (more than three cups per day) had less decline in verbal retrieval, and to a lesser extent in visuospatial memory over 4 years than women consuming one cup or less [23]. The protective effect of caffeine was more notable in older versus younger women [23]. No relationship was observed between caffeine intake and the risk of dementia during 4 years of follow-up [23]. A Portuguese cohort study recently confirmed a protective effect of caffeine in women [24]. From a cohort of 648 subjects aged 65 and over, 531 participants were selected for this study [24]. 309 participants completed the follow-up evaluation (median follow-up: 48 months) [24]. This study found that caffeine intake was associated with a lower risk of cognitive decline in women, but not significantly in men [24]. Cognitive decline was defined by a decrease of at least 2 points in the score of the Mini-Mental State Examination from baseline to the follow-up visit [24]. The Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study investigated the associations of midlife coffee and/or tea consumption to the development of dementia and AD in late-life [20]. After following 1409 subjects for an average of 21 years, this study found that coffee drinkers at midlife had lower risk of dementia and AD later in life compared with those drinking little or no coffee [20]. The lowest risk (65% decreased) was found in individuals who drank 3–5 cups of coffee per day during their midlife years [20]. Santos et al. [25] recently conducted a systematic review and meta-analysis of published studies quantifying the relation between caffeine intake and cognitive decline or dementia. The authors found a trend towards a protective effect of caffeine, but the large methodological heterogeneity across a still limited number of epidemiological studies precluded robust and definite statements on this topic [25]. It should also be noted that there are inherent limitations of observational epidemiological studies, in that causality cannot be inferred from associations alone. A recent review explored the relation between caffeine intake, diabetes, cognition and dementia, focusing on type 2 diabetes [26]. The conclusion was that, although epidemiological studies indicate that coffee/caffeine consumption is associated with a decreased risk of type 2 diabetes and possibly also with a decreased dementia risk, at present it is not certain that these associations are causal [26].

As noted above, compromised function of the choroid plexus and defective CSF production may be one mechanism implicated in the pathogenesis of late-onset AD [8, 11].

Given that long-term caffeine consumption may augment CSF production [15], and given that increased CSF production may improve the CSF turnover and clearance of potentially toxic metabolites, such as Aβ, it seems reasonable to speculate that long-term caffeine consumption could exert protective effects against AD, at least in part by facilitating CSF secretion. To the best of our knowledge, no previous study has suggested increased CSF production as a possible mechanism underlying the inverse association between caffeine consumption and AD. However, there could be other potential mechanisms of cognitive protection by caffeine. Among them, the antioxidant properties of caffeine, its anti-inflammatory capacities, its ability to block disruptions of the blood-brain barrier, and its well-documented blockade of adenosine A_1 and A_{2A} receptors have been proposed to underlie its ability to protect against AD [5, 27]. Furthermore, in a recent study, Arendash et al. [5] reported that long-term caffeine administration that began in young adulthood protected AD transgenic mice against otherwise certain cognitive impairment in older age, while also limiting their brain production of Aβ due to reduced expression of both β-secretase and presenilin-1/γ secretase. The ability of caffeine to reduce Aβ production was confirmed in neuronal cell cultures from these same transgenic mice, wherein concentration-dependent decreases in both Aβ (1–40) and Aβ (1–42) were observed [5]. In another study, Arendash et al. [28] found that aged, cognitively-impaired AD transgenic mice given a moderate amount of daily caffeine exhibited a restoration of working memory to the level of normal, aged mice. In these same aged AD mice, which had pre-existing and substantial Aβ burden, caffeine treatment reduced both soluble and deposited (insoluble) brain Aβ levels [28]. Cao et al. [27] recently reported that acute caffeine administration to both young adult and aged AD transgenic mice rapidly reduced Aβ levels in both brain interstitial-fluid and plasma without affecting Aβ elimination. A single treatment with caffeine did not affect the half-life of interstitial-fluid Aβ, demonstrating that caffeine had affected brain Aβ production rather than its elimination [27]. The latter, however, is not inconsistent with the idea that long-term caffeine consumption may exert protective effects against AD, at least in part by increasing CSF production and clearance. Indeed, chronic but not acute treatment of rats with caffeine increased CSF production [15]. If our hypothesis were correct, one would expect that enhanced CSF production by chronic caffeine treatment should result in greater clearance of Aβ from the brains of AD transgenic mice, resulting in increased plasma Aβ levels. However, Cao et al. [27] reported that both plasma and brain Aβ levels are reduced by acute or chronic caffeine administration in several AD transgenic lines and ages. Newly produced Aβ enters a dynamic equilibrium between soluble and deposited Aβ in the brain, with continual transport of soluble Aβ out of the brain and into plasma [27]. As noted above, in their study in AD transgenic mice, Arendash et al. [5] found that long-term caffeine administration suppressed brain Aβ production by reducing expression of both β-secretase and presenilin-1/γ secretase. Acutely, such decreased Aβ production would result in lower brain levels of soluble Aβ and consequently lower plasma Aβ levels [27]. Continued caffeine suppression of Aβ production and the resultant lower brain levels of soluble Aβ would induce a flux of deposited (insoluble) Aβ to the soluble form, which is cleared from brain into plasma [27]. Consequently, plasma Aβ levels may be reduced or not changed [27]. Therefore, despite a caffeine-induced increase in CSF production resulting in enhanced clearance of Aβ from the brain, chronic caffeine administration might result in lower plasma Aβ levels due to its ability to decrease Aβ production through suppression of both β-secretase and presenilin-1/γ secretase.

3. Preclinical Evaluation of Hypothesis

The evaluation of preventive or disease-modifying efficacy is not easily accomplished in a clinical setting. Animal models have therefore acquired a strong position in this field of research based on the rapid development of symptoms and/or pathology, availability of potentially large groups of subjects, accessibility to early-stage CNS changes, and the possibility of time-linked observations. The major shortcoming of many preclinical trials scrutinizing disease-modifying efficacy is the lack of a wash-out period to prevent bias from sustained symptomatic treatment effects. Given the acute (i.e., symptomatic) cognition enhancing effects of caffeine, animal model studies assessing preventive or disease-modifying treatment strategies based on caffeine or other adenosine receptor antagonists, should include a wash-out period prior to commencing cognitive and/or behavioural analyses and correlating biochemical parameters. A preclinical treatment schedule to assess disease-modification in transgenic rodent models of dementia based on clinical withdrawal designs has previously been proposed [29]. Treatment starts prior to the first presentation of the symptoms and continues past the time point where symptoms would occur if untreated. Given caffeine has acute cognition enhancing effects that are partially based on its actions on arousal, mood, and concentration, it is essential to include a wash-out period to ensure picking up true disease-modifying effects on cognition (prevention of learning deficits) and not potential acute cognition enhancing effects of caffeine. The primary goal would not be to mimic the physiological conditions of daily caffeine intake in humans in a mouse model, but to have a controlled experiment allowing appraisal of the hypothesis that chronic caffeine intake would alter the progression of AD on increased Aβ clearance via increased CSF production.

CSF production is not easily monitored in rodents, let alone in mice. CSF formation rate can be measured by the ventriculocisternal perfusion method, originally developed in goats by Pappenheimer et al. [30], but downscaled for application in rats and mice over the past decades. Briefly, under stereotactic guidance, one (stainless steel) cannula is introduced into the lateral cerebral ventricle, while another is inserted into the cisterna magna. Artificial CSF containing a (polysaccharide) dye, for example, Blue Dextran 2000 [31] or tetramethylrhodamine isothionate-dextran [32], is infused through the ventricular cannula at a constant rate. CSF samples are collected via the

cisterna magna at 20-min intervals allowing colorimetric (for Blue Dextran 2000) or fluorometric detection (for tetramethylrhodamine isothionate-dextran) of the dye as a measure for CSF production rate (V_f in μL/min) based on the following formula: $V_f = V_i(C_i - C_o)/C_o$ with V_i being the infusion rate of the perfusate, C_i the concentration of the dye in the inflow fluid and C_o the concentration of the dye in the outflow fluid. Application of long-term administration of caffeine in a mouse model for AD with established face, construct, and predictive validity employing a treatment strategy with a wash-out period to prevent bias from known symptomatic treatment effects [29] could allow us to gain further insight in the mode of action underlying the presumed disease-modifying efficacy of caffeine or other xanthine analogs. The measurement of CSF production rate should be preceded by a test battery of behavioural paradigms evaluating various learning and memory levels, as well as BPSD-related alterations, known to be affected in the mouse model. CSF production needs to be linked to Aβ clearance (Aβ CSF levels), as well as CNS plaque load and CNS levels of different (oligomeric) soluble and insoluble Aβ species, preferably in addition to other AD-related pathophysiological pathways including inflammation and oxidative stress.

4. Conclusions

In conclusion, the hypothesis of the present paper is that long-term caffeine consumption could exert protective effects against AD at least in part by facilitating CSF production, turnover, and clearance. This obviously needs to be confirmed by future research. If confirmed, then long-term caffeine consumption could be protective against AD by limiting production of Aβ, due to reduced expression of both β-secretase and presenilin-1/γ secretase, and by facilitating CSF production, with improved clearance of Aβ. It should be stressed, however, that several other mechanisms also might explain caffeine's apparent protective effect against AD.

Abbreviations

Aβ: β-amyloid
AD: Alzheimer's disease
APP: β-amyloid precursor protein
AQP1: Aquaporin 1
CP: Choroid plexus
CSF: Cerebrospinal fluid
NPH: Normal pressure hydrocephalus.

Conflict of Interest

References

[1] P. Wostyn, K. Audenaert, and P. P. De Deyn, "Alzheimer's disease-related changes in diseases characterized by elevation of intracranial or intraocular pressure," *Clinical Neurology and Neurosurgery*, vol. 110, no. 2, pp. 101–109, 2008.

[2] P. Wostyn, K. Audenaert, and P. P. De Deyn, "Alzheimer's disease and glaucoma: Is there a causal relationship?" *British Journal of Ophthalmology*, vol. 93, no. 12, pp. 1557–1559, 2009.

[3] K. Sleegers, J. C. Lambert, L. Bertram, M. Cruts, P. Amouyel, and C. Van Broeckhoven, "The pursuit of susceptibility genes for Alzheimer's disease: progress and prospects," *Trends in Genetics*, vol. 26, no. 2, pp. 84–93, 2010.

[4] X. Chen, O. Ghribi, and J. D. Geiger, "Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer's and Parkinson's diseases," *Journal of Alzheimer's Disease*, vol. 20, supplement 1, pp. 127–141, 2010.

[5] G. W. Arendash, W. Schleif, K. Rezai-Zadeh et al., "Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain β-amyloid production," *Neuroscience*, vol. 142, no. 4, pp. 941–952, 2006.

[6] L. Maia and A. de Mendonça, "Does caffeine intake protect from Alzheimer's disease?" *European Journal of Neurology*, vol. 9, no. 4, pp. 377–382, 2002.

[7] J. Lindsay, D. Laurin, R. Verreault et al., "Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging," *American Journal of Epidemiology*, vol. 156, no. 5, pp. 445–453, 2002.

[8] G. D. Silverberg, M. Mayo, T. Saul, E. Rubenstein, and D. McGuire, "Alzheimer's disease, normal-pressure hydrocephalus, and senescent changes in CSF circulatory physiology: a hypothesis," *Lancet Neurology*, vol. 2, no. 8, pp. 506–511, 2003.

[9] G. D. Silverberg, G. Heit, S. Huhn et al., "The cerebrospinal fluid production rate is reduced in dementia of the Alzheimer's type," *Neurology*, vol. 57, no. 10, pp. 1763–1766, 2001.

[10] B. R. Ott, R. A. Cohen, A. Gongvatana et al., "Brain ventricular volume and cerebrospinal fluid biomarkers of Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 20, no. 2, pp. 647–657, 2010.

[11] J. M. Serot, M. C. Bene, and G. C. Faure, "Normal-pressure hydrocephalus and Alzheimer disease," *Journal of Neurosurgery*, vol. 99, no. 4, pp. 797–798, 2003.

[12] P. D. Brown, S. L. Davies, T. Speake, and I. D. Millar, "Molecular mechanisms of cerebrospinal fluid production," *Neuroscience*, vol. 129, no. 4, pp. 957–970, 2004.

[13] J. M. Serot, M. C. Béné, B. Foliguet, and G. C. Faure, "Morphological alterations of the choroid plexus in late-onset Alzheimer's disease," *Acta Neuropathologica*, vol. 99, no. 2, pp. 105–108, 2000.

[14] J. M. Serot, M. C. Béné, and G. C. Faure, "Choroid plexus, ageing of the brain, and Alzheimer's disease," *Frontiers in Bioscience*, vol. 8, pp. s515–s521, 2003.

[15] M. E. Han, H. J. Kim, Y. S. Lee et al., "Regulation of cerebrospinal fluid production by caffeine consumption," *BMC Neuroscience*, vol. 10, article 110, 12 pages, 2009.

[16] K. S. Richards, K. Bommert, G. Szabo, and R. Miles, "Differential expression of Na/K-ATPase α-subunits in mouse hippocampal interneurones and pyramidal cells," *Journal of Physiology*, vol. 585, no. 2, pp. 491–505, 2007.

[17] C. Masseguin, S. LePanse, B. Corman, J. M. Verbavatz, and J. Gabrion, "Aging affects choroidal proteins involved in CSF production in Sprague-Dawley rats," *Neurobiology of Aging*, vol. 26, no. 6, pp. 917–927, 2005.

[18] B. B. Fredholm, K. Bättig, J. Holmén, A. Nehlig, and E. E. Zvartau, "Actions of caffeine in the brain with special reference to factors that contribute to its widespread use," *Pharmacological Reviews*, vol. 51, no. 1, pp. 83–133, 1999.

[19] A. Rahman, "The role of adenosine in Alzheimer's disease," *Current Neuropharmacology*, vol. 7, no. 3, pp. 207–216, 2009.

[20] M. H. Eskelinen, T. Ngandu, J. Tuomilehto, H. Soininen, and M. Kivipelto, "Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study," *Journal of Alzheimer's Disease*, vol. 16, no. 1, pp. 85–91, 2009.

[21] U. K. Lammi, S. L. Kivelä, A. Nissinen, S. Punsar, P. Puska, and M. Karvonen, "Mental disability among elderly men in Finland: prevalence, predictors and correlates," *Acta Psychiatrica Scandinavica*, vol. 80, no. 5, pp. 459–468, 1989.

[22] B. M. van Gelder, B. Buijsse, M. Tijhuis et al., "Coffee consumption is inversely associated with cognitive decline in elderly European men: the FINE Study," *European Journal of Clinical Nutrition*, vol. 61, no. 2, pp. 226–232, 2007.

[23] K. Ritchie, I. Carrière, A. de Mendonça et al., "The neuro-protective effects of caffeine: a prospective population study (the Three City Study)," *Neurology*, vol. 69, no. 6, pp. 536–545, 2007.

[24] C. Santos, N. Lunet, A. Azevedo, A. de Mendonça, K. Ritchie, and H. Barros, "Caffeine intake is associated with a lower risk of cognitive decline: a cohort study from Portugal," *Journal of Alzheimer's Disease*, vol. 20, supplement 1, pp. 175–185, 2010.

[25] C. Santos, J. Costa, J. Santos, A. Vaz-Carneiro, and N. Lunet, "Caffeine intake and dementia: systematic review and meta-analysis," *Journal of Alzheimer's Disease*, vol. 20, supplement 1, pp. 187–204, 2010.

[26] G. J. Biessels, "Caffeine, diabetes, cognition, and dementia," *Journal of Alzheimer's Disease*, vol. 20, supplement 1, pp. 143–150, 2010.

[27] C. Cao, J. R. Cirrito, X. Lin et al., "Caffeine suppresses amyloid-β levels in plasma and brain of Alzheimer's disease transgenic mice," *Journal of Alzheimer's Disease*, vol. 17, no. 3, pp. 681–697, 2009.

[28] G. W. Arendash, T. Mori, C. Cao et al., "Caffeine reverses cognitive impairment and decreases brain amyloid-β levels in aged Alzheimer's disease mice," *Journal of Alzheimer's Disease*, vol. 17, no. 3, pp. 661–680, 2009.

[29] D. Van Dam and P. P. De Deyn, "Drug discovery in dementia: the role of rodent models," *Nature Reviews Drug Discovery*, vol. 5, no. 11, pp. 956–970, 2006.

[30] J. R. Pappenheimer, S. R. Heisey, E. F. Jordan, and J. C. Downer, "Perfusion of the cerebral ventricular system in un-anesthetized goats," *American Journal of Physiology*, vol. 203, pp. 763–774, 1962.

[31] A. Chodobski, J. Szmydynger-Chodobska, and C. E. Johanson, "Vasopressin mediates the inhibitory effect of central angiotensin II on cerebrospinal fluid formation," *European Journal of Pharmacology*, vol. 347, no. 2-3, pp. 205–209, 1998.

[32] K. Oshio, H. Watanabe, Y. Song, A. S. Verkman, and G. T. Manley, "Reduced cerebrospinal fluid production and intra-cranial pressure in mice lacking choroid plexus water channel Aquaporin-1," *FASEB Journal*, vol. 19, no. 1, pp. 76–78, 2005.