# Caffeine and Coffee as Therapeutics Against Alzheimer's Disease

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**Abstract**. Epidemiologic studies have increasingly suggested that caffeine/coffee could be an effective therapeutic against Alzheimer's disease (AD). We have utilized a transgenic mouse model for AD in well-controlled studies to determine if caffeine and/or coffee have beneficial actions to protect against or reverse AD-like cognitive impairment and AD pathology. AD mice given caffeine in their drinking water from young adulthood into older age showed protection against memory impairment and lower brain levels of the abnormal protein (amyloid-$\beta$; A$\beta$) thought to be central to AD pathogenesis. Moreover, "aged" cognitively-impaired AD mice exhibited memory restoration and lower brain A$\beta$ levels following only 1–2 months of caffeine treatment. We believe that the cognitive benefits of chronic caffeine administration in AD mice are due to caffeine itself, and not metabolites of caffeine; this, because our long-term administration of theophylline to AD mice provided no cognitive benefits. In acute studies involving AD mice, one oral caffeine treatment quickly reduced both brain and plasma A$\beta$ levels – similarly rapid alterations in plasma A$\beta$ levels were seen in humans following acute caffeine administration. "Caffeinated" coffee provided to AD mice also quickly decreased plasma A$\beta$ levels, but not "decaffeinated" coffee, suggesting that caffeine is critical to decreasing blood A$\beta$ levels. Caffeine appears to provide its disease-modifying effects through multiple mechanisms, including a direct reduction of A$\beta$ production through suppression of both $\beta$- and $\gamma$-secretase levels. These results indicate a surprising ability of moderate caffeine intake (the human equivalent of 500 mg caffeine or 5 cups of coffee per day) to protect against or treat AD in a mouse model for the disease and a therapeutic potential for caffeine against AD in humans.

Keywords: Alzheimer's disease, amyloid-$\beta$, caffeine, coffee, memory

## INTRODUCTION

The premise that caffeine/coffee may provide a safe, effective, readily available, and inexpensive therapeutic against Alzheimer's disease (AD) is underscored by a growing body of epidemiological literature. Several cross-sectional human studies have reported that caffeine/coffee consumption in both young and aged normal adults is associated with better cognitive performance [1,2]. Caffeine/coffee has been shown to improve information processing in the elderly, as well as prove information processing in the elderly, as well as

in young adults [3]. In a 10-year prospective study involving aging men, Van Gelder et al. [4] found that coffee consumption was linked to slower cognitive decline, particularly with modest coffee consumption of 3 cups per day (approximately 300 mg caffeine). More recently, women with higher coffee consumption over a four year period showed less cognitive decline than those consuming little or no coffee [5] and mid-life coffee consumption has been linked to a 65% decreased risk of AD [6]. Most convincing of the epidemiologic studies in establishing an association between caffeine and AD has been Maia and de Mendonca [7], which reported that AD patients consumed markedly less caffeine during the 20 years preceding diagnosis of AD, compared with age-matched individuals without AD. Although these epidemiologic studies collectively suggest that chronic caffeine/coffee intake delays or reduces the

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risk of AD, the extent to which caffeine/coffee protects against AD is problematic to assess in humans because: 1) retrospective studies are based on recall and cannot unequivocally isolate caffeine/coffee intake from other factors affecting cognition over years, and 2) controlled longitudinal studies involving caffeine/coffee administration over decades are impractical. Moreover, the potential for caffeine/coffee to *treat* established cases of AD has not been reported to date in the scientific literature.

The aforementioned issues concerning epidemiologic, longitudinal, and controlled studies in humans to evaluate the therapeutic potential of caffeine/coffee against AD are at least partially addressed by human amyloid-$\beta$ protein precursor (A$\beta$PP) transgenic mouse models for AD. These AD mice bear one or more human genetic mutations that cause the familial form of AD. One such mutation is the "Swedish" mutation, resulting in a double amino acid substitution at the $\beta$-secretase cleavage site of A$\beta$PP, making such cleavage much more likely and thus greatly increasing production of human A$\beta$ in brains of these transgenic mice. The human A$\beta$ that is produced then aggregates into the A$\beta$ cores of neuritic plaques, as in human brains. During the process of A$\beta$ aggregation, these "A$\beta$PPsw" mice become cognitively impaired in a variety of cognitive domains [8–10], similar to human AD patients. Although A$\beta$PPsw mice are only a model for the A$\beta$ deposition of AD (and do not mimic the full pathologic complexity of the human disease), they do model what is thought to be the primary disease mechanism – A$\beta$ production and aggregation in the brain. As such, highly controlled studies can be done by utilizing these mice to develop insight into what therapeutics could protect against or treat AD in humans. This paper provides a brief overview of the studies we have performed with AD transgenic mice to evaluate the potential of caffeine/coffee for protection and treatment against AD in humans. As well, we mention initial results from our on-going clinical studies involving caffeine administration.

## MATERIALS AND METHODS

The Materials and Methods for the studies/data involving caffeine administration have been described in detail in our previous work [11–13], which is referred to specifically when a particular study is presented and discussed. For our new behavioral study involving long-term theophylline administration to AD mice, appropriate methods are included in the section detailing results of that study.

## RESULTS

### Caffeine to protect against Alzheimer's disease: transgenic mouse studies

Our initial studies were designed to determine whether caffeine could protect against the development of A$\beta$ neuropathology and cognitive impairment in A$\beta$PPsw transgenic mice. In those studies, which are detailed in Arendash et al. [11], we put caffeine into the drinking water of A$\beta$PPsw transgenic (Tg) mice between 4 and 9 months of age (300 mg/L). The resultant amount of caffeine intake for each mouse (approximately 1.5 mg/day) was the human equivalent of 500 mg or 5 cups of coffee per day. Cognitive testing in a comprehensive battery during the final 6 weeks of treatment revealed surprisingly better performance of Tg mice being given caffeine compared to control Tg mice given non-caffeinated water. In caffeine-treated Tg mice, cognitive domains of spatial learning/reference memory, working memory, and recognition/identification were in fact at or near the performance level of non-transgenic control mice, which were being given water. Even when "overall" cognitive performance was assessed by evaluating many cognitive measures collectively in discriminant function analysis (DFA), the superior overall performance of caffeine-treated Tg mice could easily be distinguished from the overall performance of Tg controls. Thus, long-term caffeine treatment protected AD mice from cognitive impairment overall and in multiple cognitive domains.

There are several reasons why caffeine-induced cognitive protection in AD Tg mice is unlikely to involve generalized mechanisms (e.g., increased glucose utilization) and/or the well-known stimulant effects of caffeine to increase alertness/focus. First, long-term caffeine administration had no effect on sensorimotor or anxiety function in such Tg mice [11]. Second, we have found that life-long administration of caffeine in drinking water to normal (non-transgenic) mice does not improve their cognitive performance when tested in older age [12].

Accompanying the cognitive protection provided by long-term caffeine treatment to Tg mice, there were significant 32–37% reductions in their hippocampal (brain) levels of A$\beta$ compared to untreated Tg mice [11]. Importantly, the mechanism for this caffeine-induced reduction in brain A$\beta$ involves suppression of A$\beta$ production because both $\beta$- and $\gamma$-secretase levels were reduced in the hippocampus of caffeine-treated
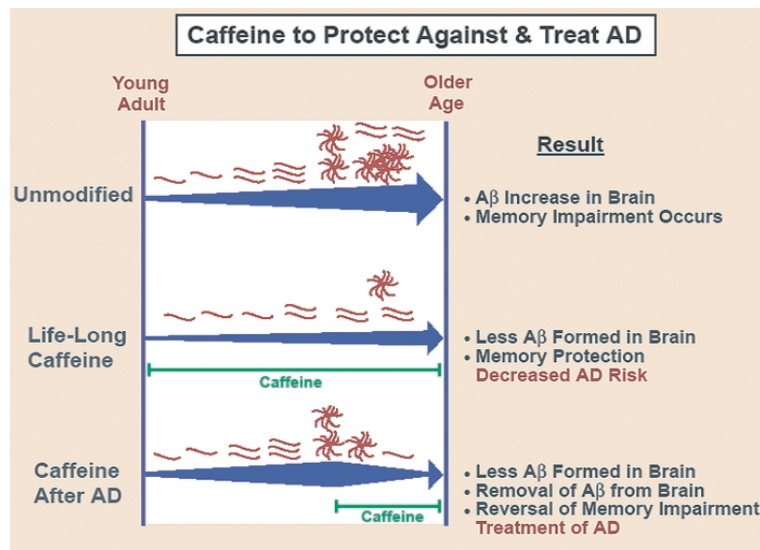
Fig. 1. Protective/treatment effects of caffeine intake on A$\beta$ production/aggregation and memory in AD transgenic (Tg) mice when caffeine consumption is started during young adulthood (life-long caffeine) or after development of AD pathology/memory impairment.

Tg mice [11]. Exactly how caffeine suppresses both of these secretases was explored in our follow-up studies (see next section). Our initial study [11] provided the first evidence that caffeine can directly affect AD pathogenesis by suppressing levels of both enzymes needed for A$\beta$ production. Moreover, we have shown that this suppression of A$\beta$ production by caffeine occurs at physiologic concentrations (1–10 $\mu$M) – caffeine concentrations that are present in the plasma and brain following only 1–2 cups of coffee. *Parenthetically, we are not aware of any AD drug under development by the pharmaceutical industry that has caffeine's profound ability to reduce* <u>*both*</u> A$\beta$ − *producing enzymes*. As depicted in Fig. 1, "unmodified" brain A$\beta$ production and aggregation during aging in Tg mice inevitably results in memory impairment. By contrast, long-term ("life-long") caffeine intake to Tg mice reduces brain A$\beta$ production/aggregation, thus protecting against development of A$\beta$-induced memory impairment (Fig. 1).

*Caffeine to treat Alzheimer's disease: transgenic mouse studies*

To determine if caffeine treatment has the ability to reduce pre-existing memory impairment and A$\beta$ neuropathology, caffeine treatment in drinking water (300 mg/L) was administered for 2 months to "aged" Tg mice already exhibiting both of these AD characteristics [12]. This concentration of caffeine in drinking water, which is identical to that utilized in our prior

protection-based study, results in mice receiving a daily caffeine intake of approximately 1.5 mg/day (e.g., the human equivalent of 500 mg caffeine or 5 cups of coffee per day). Prior to caffeine treatment, aged Tg mice were substantially impaired in a working memory task, the radial arm water maze. Following caffeine treatment, however, these same mice exhibited a restoration of working memory to the level of normal, aged mice [12]. In these same aged Tg mice, which had substantial brain A$\beta$ deposition prior to caffeine treatment, reductions of 40% and above for both soluble and deposited (insoluble) brain A$\beta$ were evident following caffeine treatment [12].

The profound cognitive restoration by caffeine in aged AD mice is likely due to caffeine's aforementioned ability to reduce A$\beta$ production by reducing both $\beta$- and $\gamma$-secretase protein expression [11]. We have further extended these mechanistic findings by showing that 1) caffeine administration to Tg mice was able to reduce the Raf-1/NF$\kappa$B inflammatory pathway, which stimulates brain $\beta$-secretase, and 2) caffeine suppresses both $\alpha$ and $\beta$ isoforms of GSK-3 in neuronal cell cultures, which are linked to PS1/$\gamma$-secretase activity and tau hyperphosphorylation, respectively. Regarding suppression of the Raf-1 inflammatory pathway, we have determined that caffeine stimulates PKA activity in the hippocampus of treated AD Tg mice [12]. This enhanced PKA activity then inhibits (inactivate) cRaf-1 by phosphorylation at serine259, decreasing NF$\kappa$B activity and the expression of NF$\kappa$B-controlled genes such as BACE1, thus resulting in decreased A$\beta$ pro-

duction. Regarding the suppression of GSK-3, caffeine affects both active and holoprotein (total) GSK-3 levels [12], which suggests that caffeine does not diminish GSK-3 activity through direct substrate-level phosphorylation, but rather through alternations in protein production or degradation The extent to which either or both of these caffeine mechanisms involve adenosine receptor antagonism is unknown and currently being investigated.

Our measurement of plasma caffeine levels of aged, behaviorally-evaluated A$\beta$PPsw mice given two months of caffeine treatment underscores the human relevance and physiologic significance of the caffeine treatment in this long-term study. At the caffeine concentration in drinking water that was employed, plasma caffeine levels averaged 26 $\mu$M [13], equivalent to the plasma caffeine concentration expected in humans following intake of several cups of coffee. This caffeine concentration is very close to the 20 $\mu$M concentration that we found to be optimal in our cell culture studies for suppression of A$\beta_{1-40}$ and A$\beta_{1-42}$ production, although caffeine concentrations in the much lower nM range (250–500 nM caffeine) were also effective [11]. This very low nM range of caffeine effectiveness to reduce A$\beta$ production *in vitro* is below the level of any known molecular target, including adenosine receptor antagonism. As such, caffeine's reduction of A$\beta$ production appears to be novel in being independent of adenosine receptors actions. It is important to note that theophylline, one of several active metabolites of caffeine, may be responsible for at least some of the cognitive, neuropathologic, and neurochemical benefits of caffeine. This premise is underscored by the fact that caffeine metabolites such as theophylline have a considerably longer half-life than caffeine. We have explored the contribution of theophylline to the benefits currently being attributed to caffeine in our AD mice, and report our findings in the next section.

Also in this long-term caffeine treatment study in aged AD mice, we found a clear reduction in hippocampal inflammatory cytokines in caffeine-treated mice with higher plasma caffeine levels compared to those with lower caffeine levels. The ability of caffeine to provide beneficial anti-inflammatory effects in the brains of AD mice is consistent with prior studies supportive of its anti-inflammatory capacities [14] and, as such, could represent a potent "non-amyloidogenic" mechanism of caffeine action that may contribute to its ability to protect against/reverse cognitive impairment. Other beneficial mechanisms of caffeine action in AD mice (and perhaps in human AD) include

the antioxidant actions of caffeine [15], its ability to block disruptions of the blood-brain barrier [16], and its well-established antagonism of brain A1 and/or A2A adenosine receptors [1,2,17,18]. Regarding the latter, adenosine receptor antagonism may be central to most or all of the beneficial actions of caffeine against AD. Any combination of such mechanisms of caffeine action may collectively provide the cognitive benefits we have documented in AD mice.

The effects of caffeine on established AD characteristics are summarized in Fig. 1 (Caffeine after AD), which shows caffeine-induced suppression of A$\beta$ production leading to removal of A$\beta$ from the brain, resulting in reversal of cognitive impairment. This study [12], performed in aged AD mice, provides the first evidence that caffeine treatment can reverse cognitive impairment and AD neuropathology in a model for the disease.

*Theophylline and cognitive function in AD transgenic mice*

As a major active metabolite of caffeine, theophylline may be responsible for a significant part of the cognitive benefits we found through long-term caffeine administration. In the aged Tg mouse study just presented [12,13], we found plasma theophylline levels to be strongly correlated with plasma caffeine levels. Moreover, theophylline has a longer half-life than caffeine, with brain concentrations of theophylline usually being higher than those for caffeine following long-term caffeine treatment [19]. Thus, it is important to determine the extent to which theophylline, contributes to the cognitive benefit we are attributing to caffeine administration in our AD mice. We therefore administered theophylline in drinking water (200 mg/L) to A$\beta$PPsw+PS1 double transgenic mice (Jackson Laboratories, Bar Harbor, Maine) or non-transgenic littermates beginning at 5.5 months of age. The resulting dose of theophylline (approximately 1 mg/day) is just slightly less than the 1.5 mg/day dose of caffeine we routinely administer long-term to AD mice. This theophylline dosage presumably resulted in plasma theophylline levels at least as high as those resulting from chronic caffeine administration because AD mice chronically treated with caffeine have a plasma caffeine-to-theophylline ratio of approximately 8-to-1 [13].

At 6 months into theophylline treatment, mice were evaluated in our standard 6-week behavioral battery of sensorimotor, anxiety, and cognitive-based tasks, as
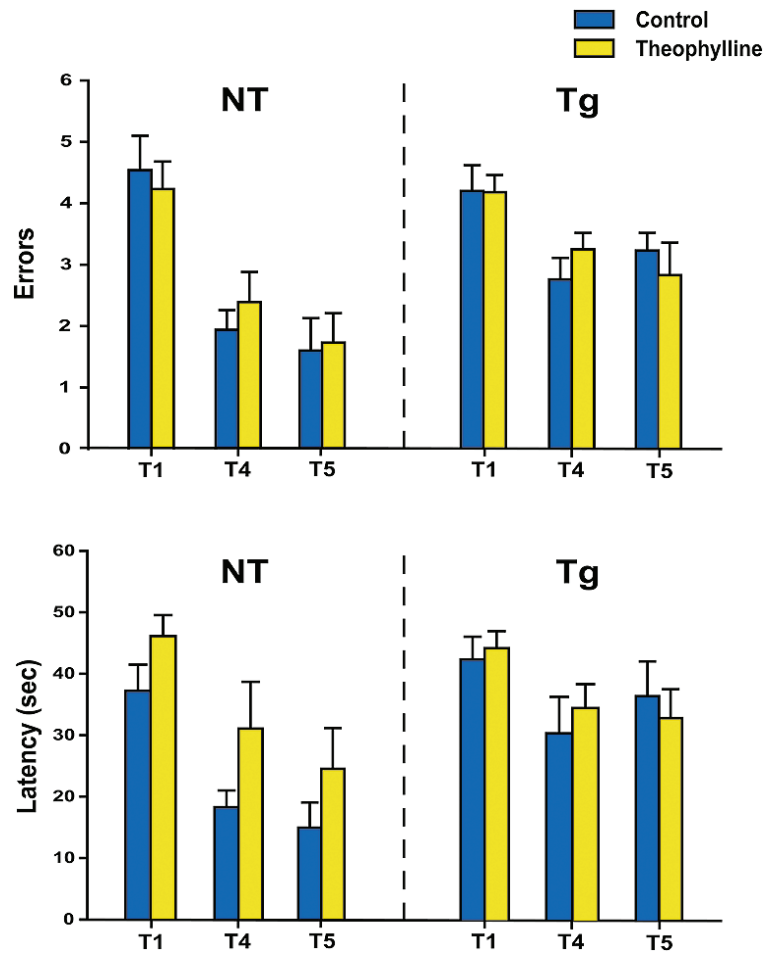
Fig. 2. Inability of long-term treatment with the active caffeine metabolite theophylline to provide cognitive benefit to AD transgenic mice or non-transgenic littermates, as demonstrated by the radial arm water maze task of working memory. The data presented are for Trial 1 (naïve first trial), and Trials 4 and 5 (working memory trials) overall across 12 days of testing at 6 months into theophylline treatment. The number of animals in each group were: NT controls ($n = 7$), NT+theophylline ($n = 8$), Tg controls ($n = 7$), and Tg+theophylline ($n = 9$).

done in our initial caffeine study [11]. Across multiple cognitive-based tasks [Y-maze spontaneous alternation, Morris maze acquisition/retention, circular platform, platform recognition, and radial arm water maze], there were no effects of theophylline treatment in either Tg or non-transgenic mice compared to controls. An example of this surprising lack of cognitive benefit with long-term theophylline treatment is shown in Fig. 2 for the radial arm water maze task of working memory. For both errors and latency during working memory trials T4 and T5, theophylline-treated mice performed no different from controls across 12 days of testing. In addition, there were no effects of theophylline treatment on sensorimotor tasks (open field activity, balance beam, string agility) or on the elevated plus-maze task of anxiety (data not shown). Thus, we conclude that

the cognitive benefits of caffeine administration in our prior protection-based [11] and treatment-based [12] studies were likely due to caffeine itself, and not theophylline. Whether or not the other major caffeine metabolite (paraxanthine) may be responsible for some of caffeine's cognitive benefits in Tg mice remains to be determined.

It is important to put our long-term theophylline treatment study ($> 7$ months of treatment) in proper context of prior work investigating theophylline's effects on cognitive function. Our search of the scientific literature has, firstly, revealed a wealth of studies showing no effect of theophylline administration on the cognitive performance of humans (both adults and children). This is consistent with our present finding of long-term theophylline treatment having no cognitive
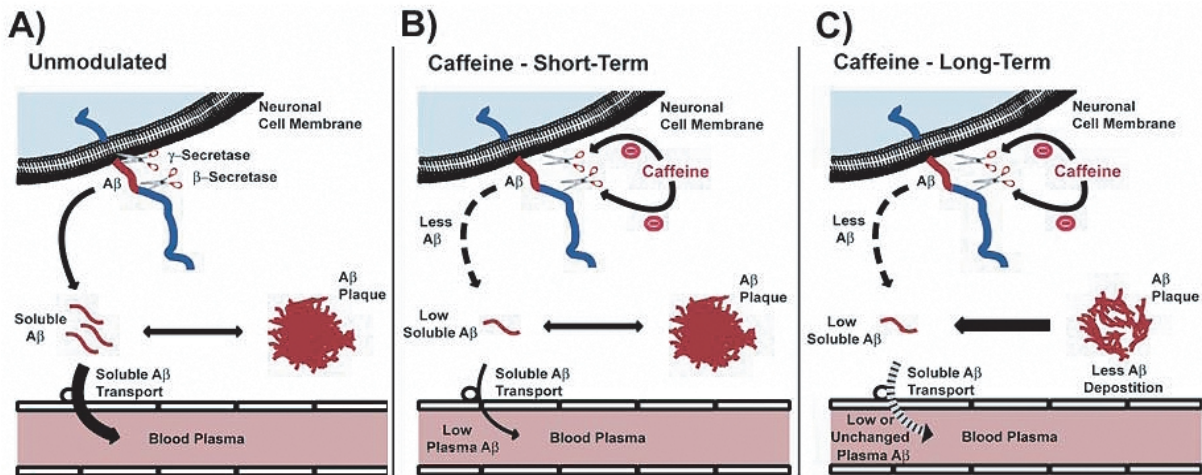
Fig. 3. Brain A$\beta$ production/clearance, the suppressive actions of caffeine on A$\beta$ production, and resultant effects on brain and plasma A$\beta$ levels. (**A**) *Unmodulated*: A$\beta$ is primarily produced in neurons, secreted into the brain extracellular space in soluble form, then enters a dynamic equilibrium between soluble and deposited (insoluble) A$\beta$. Continual transport of soluble A$\beta$ occurs into plasma. (**B**) *Caffeine: Short-Term*: Caffeine suppression of both $\beta$- and $\gamma$-secretase activities reduces A$\beta$ production, resulting in lower soluble A$\beta$ in brain and plasma. The equilibrium between soluble and deposited A$\beta$ is not impacted by this short-term reduction in brain soluble A$\beta$ levels. (**C**) *Caffeine: Long-Term*: Continuous caffeine suppression of A$\beta$ production and resultant chronically lower levels of brain soluble A$\beta$ induce a flux of deposited (insoluble) A$\beta$ to the soluble form, which is cleared from brain into plasma via soluble A$\beta$ transport. Plasma A$\beta$ levels may be reduced or not changed, depending on degree of caffeine-induced suppression of A$\beta$ production. Reproduced with permission from IOS Pres.

effects in normal mice across multiple tasks and cognitive domains. Secondly, no studies had investigated the effects of "long-term" theophylline on cognitive function prior to our present work. Earlier studies only involved "acute/single treatment" theophylline administration to normal mice or as a pre-treatment to mice exposed to a noxious stimulus (e.g., ischemia, microwave irradiation). Particularly since acute administration of caffeine (and presumably theophylline) may have cognitive effects that are different from long-term administration, the behaviorally-based theophylline study that we present in the current manuscript appears to be the first long-term theophylline treatment study investigating cognitive performance in any rodent, either normal, genetically-manipulated, or CNS-injured.

### Acute effects of caffeine/coffee on blood A$\beta$ levels in AD mice and humans

Following formation of monomeric A$\beta$ in the brain, newly produced A$\beta$ enters into a dynamic equilibrium between soluble and deposited A$\beta$ in the brain, with continual transport of soluble A$\beta$ out of the brain and into plasma down a concentration gradient (Fig. 3A). Therefore, blood levels of A$\beta$ may be indicative of caffeine's therapeutic actions on brain levels of A$\beta$. In view of our findings that caffeine decreases A$\beta$ production in AD transgenic mice through suppression of

both $\beta$- and $\gamma$-secretase [11,12], we have hypothesized that ensuing lower brain levels of soluble A$\beta$ will result in lower plasma A$\beta$ levels acutely (Fig. 3B). This hypothesis is supported by the finding that A$\beta$ is rapidly produced and cleared from the brain [20]. Moreover, following almost 2 months of caffeine administration to "aged" Tg transgenic mice (see preceding section), we have found that higher plasma caffeine levels were strongly associated with lower plasma A$\beta$ levels in individual Tg mice ($r = -0.761$; $p = 0.011$).

Considering the above findings, we determined the effects of acute caffeine [13] or coffee administration on plasma and brain A$\beta$ levels in AD transgenic mice. Acute (single treatment) administration of caffeine (1.5 mg/0.2 cc) or saline vehicle was given by i.p. injection or gavage to A$\beta$PPsw transgenic (Tg) mice in young adulthood (3 months old) or in older age (14 months old). At 3 hours following treatment, plasma A$\beta_{1-40}$ levels in 3-month old Tg mice were substantially reduced by 41%. Even the older 14-month old Tg mice showed significant reductions in plasma A$\beta$ following acute caffeine treatment. We have since repeated these studies in additional Tg mice, acutely administering caffeinated or de-caffeinated coffee (i.p.) instead of caffeine (Caffeinated coffee contained the same 1.5 mg caffeine/0.2 cc as in pure caffeine administration). At 3 hours following an initial treatment and 3 hours after another treatment 3 days later, caffeinated
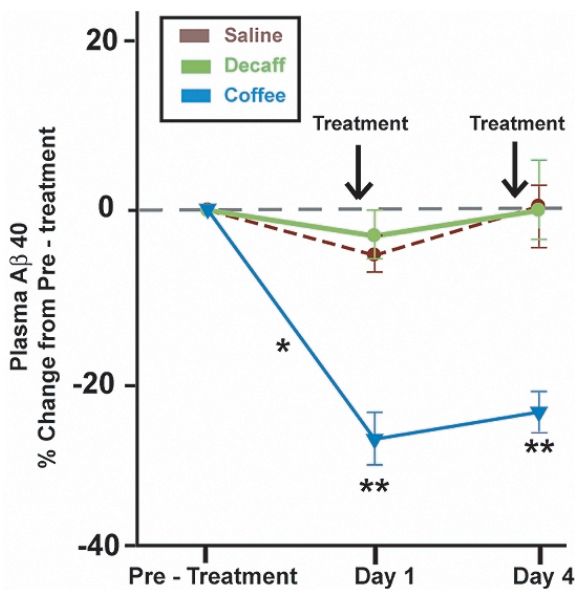
Fig. 4. Ability of (caffeinated) coffee to acutely suppress plasma A$\beta$ levels, but not de-caffeinated coffee or saline control. Treatments (i.p.) were administered on Days 1 and 4, 3 hours prior to taking a blood sample on both days. Percent change from pre-treatment A$\beta$ levels are graphed. Each group consisted of 6–7 A$\beta$PPsw mice. $^*p < 0.02$ for Pre vs. Day 1, coffee group only; $^{**}p < 0.02$ for coffee group vs. other two groups on that day. Absolute values (in pg/ml) for pre – versus post-treatment (Day 1) plasma A$\beta_{1-40}$ levels and associated paired $t$-test statistics were: Saline treatment (6055 $\pm$ 1180 vs. 5400 $\pm$ 381, N.S.), Decaffeinated coffee (6036 $\pm$ 1029 vs. 5662 $\pm$ 809, N.S.), Caffeinated coffee (6851 $\pm$ 1446 vs. 5090 $\pm$ 637, $p < 0.02$).

coffee provided the same beneficial reduction in plasma A$\beta$-40 levels as we had previously reported for pure caffeine (Fig. 4). By contrast, decaffeinated coffee and saline treatments had no effect on plasma A$\beta_{1-40}$ levels. Thus, caffeine is the likely coffee constituent that is critical for decreasing brain and plasma A$\beta$ levels. Although this acute study did not involve measurement of "brain" A$\beta$ levels as in our prior acute and long-term studies [11–13], the fact that acute caffeine administration in our prior work resulted in reductions in both plasma and brain A$\beta$ levels within the same 2–3 hour time course [13] strongly suggests that a similar reduction in brain A$\beta$ occurred with coffee administration, but not with decaffeinated coffee administration.

In a separate group of 3-month old Tg mice, measurement of brain interstitial fluid (ISF) levels of A$\beta$ through *in vivo* microdialysis was performed both before and following caffeine administration (1 mg, i.p.). This single caffeine treatment significantly lowered brain ISF A$\beta$ levels by 32% at 2–3 hours post-treatment, compared to the basal ISF A$\beta$ levels in each

mouse [13]. Follow-up studies showed that this rapid suppression of brain A$\beta$ levels by caffeine was not due to increased A$\beta$ elimination from the brain, but rather was due to decreased A$\beta$ production. These acute studies collectively indicate that plasma A$\beta$ can be a viable biomarker for the efficacy of anti-A$\beta$ therapeutics such as caffeine. It is nonetheless important to underscore that our acute caffeine administration studies involved AD transgenic mice "naïve" to caffeine, whereas any clinical trials in humans would involve many occasional or habitual caffeine users.

As a clinical extension of our studies in AD mice and in view of the aforementioned clinical caveat, we have embarked on acute caffeine and coffee administration studies in humans. Similar to our AD mouse studies, we find that acute oral caffeine administration (400 mg) to aged humans induces an immediate decrease in blood A$\beta$ levels in approximately half of subjects (data not shown). In both young adult and aged humans, we find that blood A$\beta$ levels continue to be modified many hours following caffeine or coffee administration. These initial clinical studies are consistent with caffeine/coffee having an immediate and direct effect on what could be the primary pathogenic mechanism of AD. Inasmuch as the protocols and results of these acute caffeine administration studies will be presented in detail within upcoming manuscripts, they should currently be considered as preliminary observations that suggest a similar response of human and mouse subjects to acute caffeine administration. Additional clinical studies are underway to determine more long-term effects of caffeine and coffee administration to aged humans and AD patients.

## DISCUSSION

The results of our studies indicate a surprising ability of moderate caffeine intake to protect against or reverse AD-like cognitive impairment and A$\beta$ neuropathology in established mouse models for AD. The equivalent amount of daily caffeine intake that our studies suggest is required for human therapeutic benefit against AD is approximately 500 mg. The average US intake of caffeine is only around 150 mg/day and decreases during aging – thus, average caffeine intake appears to be significantly below levels necessary for benefits against AD. An 8 oz. cup of coffee contains 100 mg caffeine, while a similar serving of tea or soft drinks contain only 35 and 25 mg caffeine, respectively. As such, only five cups of coffee per day (a moderate con-

sumption level) are needed to reach the 500 mg level, while many more servings of tea or soft drinks would be required. Moreover, given coffee's significant content of antioxidants and other beneficial phytochemicals, this source of caffeine is clearly preferable over essentially any other source.

Caffeine's beneficial effects against cognitive impairment and AD neuropathology probably involve first and foremost the unique ability of caffeine to suppress both enzymes needed for brain A$\beta$ generation, namely $\beta$-secretase and $\gamma$-secretase. Nonetheless, multiple other mechanisms are probably contributing to the therapeutic actions of caffeine in AD animal models, including caffeine's ability to act as: 1) a strong anti-inflammatory agent, 2) an anti-oxidant, 3) a mitochondrial activator, and 4) a stimulator of both neuronal activity and glucose utilization. Although adenosine receptor antagonism should certainly be included in this list, caffeine's actions almost certainly do not involve simply the increase in alertness provided by such receptor blockade. Indeed, if this were a primary mechanism of caffeine's cognitive benefit, normal mice should have benefited cognitively from a lifetime of caffeine administration, but this was not the case [12]. Thus, we propose that caffeine does not act as a general nootropic agent to enhance "normal" cognitive performance. Rather, we believe that *caffeine protects against and minimizes brain cognitive impairment, such as that due to brain A$\beta$ accumulation/aggregation in AD.*

Studies in this paper present evidence that caffeine is providing cognitive benefits to AD transgenic mice *directly*, since a major active metabolite of caffeine (theophylline) was found to be cognitively ineffective. It is important to note, however, that our theophylline administration studies utilized mice of a different transgenic line (A$\beta$PPsw + PS1), bearing a different strain background (C57/BL6), and obtained from a commercial breeder (Jackson Laboratories). By contrast, all of our other behavioral studies with caffeine administration [11–13] involved inbred mice of our own transgenic colonies. We have previously determined that A$\beta$PPsw or A$\beta$PPsw + PS1 mice on a C57/BL6 background (e.g., those from commercial breeders) require considerably longer aging (compared to our inbred mice on a mixed C57/BL6/SW/SJL background) before cognitive impairment becomes apparent. Thus, it is possible that results of this study could have been different had we utilized mice of our own inbred colonies and/or began the study with aged, already-impaired transgenic mice for examining the effects of theophylline administration on cognitive function.

If the inability of long-term theophylline administration to mimic the therapeutic effects of caffeine is independent of the AD mice and treatment paradigm utilized (as we suspect is the case), this would argue for such benefits of caffeine to be independent of adenosine receptors. Supportive of this premise, theophylline is 3 to 5 times more potent than caffeine as an antagonist of both adenosine A1 and A2a receptors. Alternatively, it is possible that brain theophylline levels (which we did not measure in this study) in theophylline-treated Tg mice were insufficient for providing behavioral and neuropathologic benefit. Either scenario would be consistent with a mechanism that does not primarily involve adenosine receptor antagonism.

In our 'acute' studies, AD mice given a single treatment with caffeine exhibited quick reductions in both brain interstitial fluid and plasma A$\beta$ levels (i.e., within 2–3 hours). Since caffeine did not affect the half-life of ISF A$\beta$, caffeine had directly affected brain A$\beta$ production rather than its elimination. Underscoring this premise are our findings that caffeine affects A$\beta$ production through suppression of both $\beta$-secretase (BACE1) and $\gamma$-secretase/PS1 expression [11,12]. Acutely, this caffeine-induced decrease in A$\beta$ production would result in lower soluble A$\beta$ in brain ISF and consequently lower plasma A$\beta$ levels (Fig. 2, Caffeine – Short-Term). This is exactly what we observed in AD mice that were young adults (with no brain A$\beta$ deposition), as well as aged AD mice (bearing robust A$\beta$ deposition). Whether or not this caffeine-induced suppression of A$\beta$ production is direct or involves adenosine receptor blockade/mediation is currently unknown. Evidence against involvement of adenosine receptors is provided by studies showing that acute and chronic caffeine administration to mice given noxious brain conditions (e.g., ischemia, excitotoxin exposure) results in opposite effects (worse or lesser damage) due to antagonism of A1 and A2a receptors, respectively [21]. In sharp contrast, both acute and chronic caffeine administration decrease plasma and brain A$\beta$ levels.

In our long-term "treatment" study in aged/cognitively-impaired AD mice, caffeine reversed their working memory impairment. This 2-month caffeine administration regime not only reduced brain soluble A$\beta$ levels, but also reversed brain A$\beta$ neuropathology. Although the mechanism(s) for caffeine-induced reversal of A$\beta$ deposition requires further investigation, we hypothesize that "chronic" caffeine suppresses A$\beta$ production long-term, resulting in consistently lower brain levels of soluble ISF A$\beta$ (Fig. 2, Caffeine – Long-Term). This

would then induce a flux of insoluble A$\beta$ out of the deposited form and into the soluble form due to dynamic equilibrium. Newly-solubilized A$\beta$ would then be cleared into the plasma via both blood-brain-barrier transport and bulk fluid flow. The dynamic equilibrium between soluble (ISF) and insoluble/deposited A$\beta$ in the brain is underscored by previous work showing that young adult and aged A$\beta$PPsw mice had similar steady-state levels of ISF A$\beta$, but rapid inhibition of A$\beta$ production resulted in a two-fold longer A$\beta$ half-life in the aged, A$\beta$ deposit-bearing mice [22]. We believe this longer A$\beta$ half-life is caused by solubilization of a portion of the deposited A$\beta$ pool due to the dynamic ISF A$\beta$ $\leftrightarrow$ deposited A$\beta$ equilibrium.

The effects of "chronic" caffeine intake on health have been reported in numerous retrospective and prospective studies. A comprehensive review of the literature [23] found that for healthy adults, moderate daily caffeine intake poses no adverse effects on the cardiovascular system, bone status and calcium balance, or incidence of cancer. Prospective studies have not found significant associations between coffee consumption and the risk of coronary heart disease [24, 25]. Contrary to public belief, caffeine intake of 500–600 mg daily does not increase the risk, frequency, or severity of cardiac arrhythmias [26,27]. Indeed, there is a growing list of age-related diseases wherein habitual caffeine intake of 400–600 mg (4–6 cups of coffee) daily reduces the risk. For example, strong data from a number of prospective studies show that both caffeine and coffee reduce the risk of Type 2 Diabetes Mellitus by 35–79% [28]. Both retrospective and prospective studies report a reduced risk of Parkinson's disease with habitual caffeine/coffee intake [29,30]. Risk of liver cirrhosis is significantly reduced by both caffeine and coffee [31]. Thus, far from posing a threat to human health during aging, habitual caffeine/coffee intake provides a number of health benefits.

Finally, it should be underscored that "acute" effects of caffeine or coffee administration on plasma A$\beta$ levels clearly occur in both AD mouse models and humans. This suggests not only that these mouse models are providing valuable insight into development of therapeutics against AD, but also that blood levels of A$\beta$ could be a monitor for anti-A$\beta$ therapeutic effects occurring in the brain, such as with caffeine. An exciting period of clinical research with caffeine and coffee against AD is on the horizon. This research may finally show that a safe and readily available natural agent – caffeine – provides the first effective therapeutic against AD.

In summary, our studies utilized AD transgenic mice to demonstrate that: 1) Caffeine *protects* AD mice against memory loss through mechanisms that directly affect the disease process, 2) Caffeine *reverses* memory loss and AD pathology in "aged" AD mice, and 3) Caffeine and/or caffeinated coffee both affect plasma levels of A$\beta$ in AD mice and humans. Our results warrant the clinical trials in progress with caffeine and caffeinated coffee as safe, inexpensive, and effective therapeutics against AD.

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