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Caffeine, Cognitive Functioning, and White Matter Lesions in the Elderly: Establishing Causality from Epidemiological Evidence

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Abstract. The present study examines the epidemiological evidence for a causal relationship between caffeine consumption and cognitive deterioration in the elderly. Using a population of 641 elderly persons, we examined cognitive functioning, caffeine consumption, magnetic resonance imaging volumetrics, and other factors known to affect cognitive performance. Our findings demonstrate the association between caffeine consumption and lower cognitive change over time to be statistically significant for women only, taking into account multiple confounders, to be dose-dependent and temporarily related (caffeine consumption precedes cognitive change). Mean log transformed white matter lesion/cranial volume ratios were found to be significantly lower in women consuming more than 3 units of caffeine per day after adjustment for age (-1.23 SD $= 0.06$) than in women consuming 2–3 units (-1.04 SD $= 0.04$) or one unit or less (-1.04 SD $= 0.07$, -35% in cm^3 compared to low drinkers). This observation is coherent with biological assumptions that caffeine through adenosine is linked to amyloid accumulation and subsequently white matter lesion formation. The significant relationship observed between caffeine intake in women and lower cognitive decline is highly likely to be a true causal relationship and not a spurious association.

Keywords: Caffeine, cognitive decline, cohort study, white matter lesions

INTRODUCTION

Caffeine consumption has long been associated with enhancement of mood and cognitive functions, notably learning, memory, and speed of information processing [1]. While having multiple biological effects, including increased cortical activity, the non-selective antagonism of adenosine receptors, particularly A_1 and A_{2A} receptors, is the only known central pharmacological effect that occurs in the dose-range of voluntary caffeine intake [2,3]. There is also growing evidence that caffeine may not only provide cognitive enhancement in normal elderly but also symptomatic benefit for persons with Alzheimer's disease (AD). Blockade of adenosine A_{2A} receptors appears to attenuate damage caused by amyloid-β (Aβ), the toxic peptide that accumulates in the brain of patients with Alzheimer's disease (AD) [4,5]. Acute or long-term caffeine administration protects AD transgenic mice against cognitive impairment while limiting brain Aβ levels and increasing brain adenosine levels [6]. Memory restoration and reversal of AD pathology was also observed in mice with preexisting Aβ burden [7].

Cognitive loss and neurodegenerative disorders in the elderly constitute a major public health problem, and the possibility that a readily available, cheap

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and relatively side-effect free therapy such as caffeine may provide symptomatic and perhaps even disease-modifying effects is very seductive. However, while the role of caffeine has been clearly documented in both animal and human studies, it has been more difficult to generalize from these findings to demonstrate cognitive benefits in the general population. There is firstly the question of whether it is caffeine or one of its metabolites which enhances cognition, but more importantly there is the problem that caffeine use is a complex behavior which is linked to numerous behavioral and health variables which may produce erroneous correlations.

Epidemiological research has two important functions in this context: firstly, by taking large population samples it is able to represent a full range of cognitive impairments due to multiple causes, and is not restricted to small homogenous groups used for clinical studies, and secondly, by obtaining extensive biological and clinical data on a large number of subjects, can take into account confounding, i.e., that a false association may be concluded due to the presence of a third and truly causal variable. Caffeine consumption is significantly associated with a wide range of variables also associated with cognitive decline (age, education, gender, depressive symptoms, hypertension, cardiovascular disease, anti-cholinergic medication, hormone replacement therapy, smoking, alcohol use, social involvement) [8], and these variables, rather than caffeine consumption itself, could explain the association with cognitive performance. In order for epidemiological findings to be credible, however, they must not only eliminate confounding variables, but also establish causality and not simply a statistical association.

Epidemiological research on caffeine has to date been fairly limited, being principally cross-sectional and therefore unable to indicate causality. A small case-control study found lower caffeine intake during the preceding 20 years in AD patients compared to controls [9], and a prospective study found regular consumption of coffee but not tea to be associated with a reduced risk of AD at 5 year follow-up [10]. The long-term effect of caffeine consumption at midlife on AD in late life was recently confirmed [11]. Most of these studies were not able, however, to take into account the many other potential confounding variables likely to mediate cognitive decline. Two large cross-sectional population studies found a significant positive association between regular coffee intake and cognitive performance in older subjects (55+) [12], and in women [13]. An early prospective population study [14] reported a cross-sectional association between caffeine and verbal memory and improvement over time in psychomotor speed only [15]. However, this study did not stratify by gender or adequately adjust for other potential causes of cognitive change. In a previous report [8] we used data from a longitudinal population study to demonstrate an association between caffeine consumption in the elderly and decreased decline in cognitive functioning over time in women only, taking into account all known potential confounding variables. Having examined the question of confounding, the other central issue is whether our data confirms the likelihood of a causal relationship rather than a random association. Taking the relevant criteria for causality in epidemiological studies established by Hill [16], namely strength (effect size), temporality (the cause preceding the effect), biological gradient (dose-response relationship), and plausibility (coherence with a biological hypothesis), we were able to conclude (i) that there was significant evidence for causality in relation to strong effect size (although for women only) (OR=0.67, CI=0.53–0.85) for over 300 mg per day, (ii) that the cause preceded the effect based on the data obtained from follow-up examinations, and (iii) that there is a 'dose-response' relationship, with women consuming over three units per day having greater protection than those consuming 2 to 3 units, and those consuming under two daily units having no significant protective effect at all. The present study examines the final criterion for causality, that of biological coherence.

In addition to the data used in the previous report we have also examined the results from neuroimaging in relation to the last criterion. On the basis of previous animal and human studies cited above, indicating that blockade of adenosine A_{2A} receptors limits brain Aβ levels [4,5], we may assume high caffeine consumers to have lower levels of plasma Aβ. While our study did not include direct measures of either plasma or brain amyloid levels, volumetric measures of microvascular brain injury (WML) were obtained by structural magnetic resonance imaging (MRI), which have previously been shown to be related to plasma Aβ [17] in both mild cognitive disorder and dementia [18]. Our hypothesis is that higher rates of caffeine consumption will be associated with lower total WML volume.

MATERIALS AND METHODS

Study population

Subjects were recruited as part of a multi-site cohort study of community-dwelling persons aged 65 years

and over from the electoral rolls of three French cities (Bordeaux, Dijon and Montpellier) between 1999 and 2001 (the Three City Study). The study design has been described elsewhere [19]. The study protocol was approved by the Ethical Committee of the University-Hospital of Bicêtre (France) and written informed consent was obtained from each participant. The mean age (SD) of the whole sample was 74.2 (5.6) years for men and 74.4 (5.6) years for women. The present study concerns only the 641 persons (324 women and 317 men) from the Montpellier site, for whom MRI imaging was available with estimations of WML volume and caffeine consumption.

Caffeine consumption

Questions relating to caffeine consumption were part of a standardized interview administered by either psychologists or research nurses at baseline and several days before the MRI examination. Caffeine consumption referred to habitual current intake (over the past few months independently of periods of illness). Number of cups normally consumed per day of tea and coffee were noted. Other forms of caffeine (e.g., colas, cocoa) were consumed too rarely by this elderly cohort to warrant inclusion, as already reported [14]. Calculations were made on the assumption of one cup of coffee containing 100 mg of caffeine and one cup of tea 50 mg [20], the total average consumption per day being calculated per subject in caffeine units (one unit=100 mg).

Estimation of white matter lesion volume

MRI structural imaging was carried out using fast multislice double echo T2-weighted 2D axial acquisition, 4 mm thick slices, with 0.4 mm between slice spacing covering the whole brain (30 slices, the upper slice passing through the brain vertex). Fast SPGR 3D T1-weighted axial acquisition, with 2 excitations; 124 slices (1 mm thick) was used. WML volume was estimated using a semi-automatic method [17, 21]. Areas of supratentorial white matter hyperintensity (WMH) were segmented on T2 sequences using MRIcro software [22]. A first layer of region of interest (ROIs) corresponding to WMH was created by a semi-automated technique based on intensity thresholding. A second layer of ROIs was then manually outlined on each slide by gross contouring of all WMH. The intersection of the first and second layer was then manually inspected and automatic total volume of WMH obtained, irrespective of underlying cause. Persons with extensive damage due to stroke were excluded. An experienced reader examined all scans. Another experienced neurologist examined 80 randomly chosen scans to assess inter-rater reliability. Inter-reader and intrareader-intraclass correlations coefficients showed good to excellent agreement (0.79 and 0.95 respectively). T1-weighted anatomical images were segmented with SPM 5 (Wellcome Department of Cognitive Neurology) to derive volumes for grey matter, white matter, and cerebrospinal fluid.

Socio-demographic and clinical adjustment variables

A standardized interview included questions on demographic characteristics, education level (classified in four groups corresponding to 5, 9, 12, and 12+ years of education), mobility, and confinement to home and neighborhood, height, and weight. Information was obtained on type and quantity of alcohol consumption (number of units of alcohol per day; 0, 1–12, 13–36, $>$ 36 g/day) and tobacco use (classified as past, present or never users). History of respiratory disorders, cancer, hypertension, hypercholesterolemia, diabetes, stroke, angina pectoris, myocardial infarction, cardiac and vascular surgery, was established according to standardized questions with additional information where necessary from general practitioners. For persons who reported the occurrence of vascular events during follow-up, further medical data were obtained from general practitioners, specialists, and hospital records. The interview also included an inventory of all drugs used during the preceding month, noting those with potential anticholinergic effects [23]. Medical prescriptions and, where feasible, the medications themselves were seen by the interviewer. Depressive symptomatology was assessed by the Center for Epidemiological Studies-Depression scale (CES-D) [24] with a 16 cut-off point.

Statistical analyses

In order to adjust for brain size, total WML volume was expressed as a ratio of total cranial volume (CV: calculated as the sum of total white matter + total grey matter + cerebrospinal fluid). The ratio and the WML values were transformed by a $\log_{10}$ (x+0.01) function given their highly asymmetric distribution and possible null values. Student t test was used to compared log transformed WML and WML to CV ratio according to gender. Mean WML to CV volume ratios ($\log_{10}$) were compared in relation to caffeine use ($\leqslant$ 1, 2–3, and

Table 1
WM and WML volumes, ratio of WML to cranial volume and their log10 transformations

	N	Volumes	Median	25th Pctl	75th Pctl	Minimum	Maximum	Mean	SD	Gender-difference p-value
Men	317	WML (cm^3)	0.900	0.300	3.300	0	126.600			
		WM (cm^3)	454.144	420.994	496.014	308.038	681.090			
		WML/CV (%)	0.058	0.021	0.212	0	7.954			
		WML (log10)	−0.041	−0.509	0.520	−2.000	2.102	0.004	0.752	
		WML/CV(log10)	−1.169	−1.502	−0.653	−2.000	0.901	−1.041	0.594	
Women	324	WML (cm^3)	0.800	0.300	2.900	0	78.200			
		WM (cm^3)	418.270	386.021	452.862	298.412	566.042			
		WML/CV (%)	0.055	0.020	0.207	0	5.748			
		WML (log10)	−0.091	−0.509	0.464	−2.000	1.893	−0.101	0.786	0.08
		WML/CV(log10)	−1.190	−1.521	−0.663	−2.000	0.760	−1.092	0.586	0.28

WML: white matter lesions; WM: white matter; CV: cranial volume.

Table 2
Mean WML/CV volumes (log10) as a function of daily caffeine use

	1 cup or less		2–3 cups		More than 3 cups		p-value
Men $n = 317$	Mean	SD	Mean	SD	Mean	SD	
Univariate	−1.062	0.563	−1.016	0.583	−1.077	0.659	0.72
Adjusted by age	−1.068	0.064	−1.028	0.046	−1.042	0.071	0.88
Women $n = 324$							
Univariate	−1.031	0.516	−1.046	0.622	−1.241	0.547	0.027
Adjusted for age	−1.040	0.067	−1.047	0.044	−1.232	0.064	0.040
Multi-adjusted*	−0.897	0.216	−0.862	0.210	−1.050	0.220	0.046

*Means adjusted for age, alcohol, and disability (covariates selected with p-value < 0.10 in univariate models).

more than 3 units per day) by cross-sectional analysis of variance or covariance adjusted by age and stratified by gender, as gender differences were observed in our previous analyses in the association between caffeine consumption and cognition.

RESULTS

Within this elderly community-dwelling sub-sample 23.6% consumed over 3 units per day, 51.8% 2 to 3 units and 24.6% one unit or less. Mean age (SD) was 70.8 (3.9) years in women and 71.7 (4.1) years in men. High consumers of caffeine were 25.3% of women and 21.8% of men, but the difference was not statistically significant ($p = 0.29$). Table 1 shows total white matter (WM) and WML volume and log transformed values by gender. Mean log transformed WML in men tended to be significantly higher than in women ($p = 0.08$), but this difference disappeared when the ratio WML/CV($\log_{10}$) is considered ($p = 0.28$).

WML/CV ratios are significantly associated with age-adjusted caffeine consumption in women only ($p = 0.04$), with high rates of consumption (over 300 mg per day) being associated with less white matter lesion volume (-35% in cm^3 compared to low drinkers) (Table 2). In women the associations between the following potential confounding factors and the mean WML/CV ratio were tested: depression ($p = 0.89$), cardiovascular diseases ($p = 0.32$), alcohol ($p = 0.07$), disability ($p = 0.0007$), hormone replacement therapy ($p = 0.12$), anticholinergic drugs ($p = 0.47$), body mass index ($p = 0.23$), diabetes ($p = 0.79$), and education ($p = 0.58$). When caffeine consumption was adjusted for age, alcohol and disability the association was found to persist ($p = 0.046$).

DISCUSSION

Our previous research conducted on this database has demonstrated that caffeine consumption of over 300 mg per day reduces the rate of decline over time on a verbal fluency task, which is sensitive to dysfunction in most cortical areas [8]. This protective effect was found to be true for women only. The advantage of large epidemiological data is that it may take into account multiple biological, environmental, and clinical confounding factors which may have obscured the true cause of this association. We reported that these results persisted even when all known potential confounding factors (age, education, gender, depressive symptoms,

hypertension, cardiovascular disease, anti-cholinergic medication, smoking, and alcohol use) were taken into account. In order for this observation to be clinically valid, there should also be evidence of causality. Taking Hill's criteria [16] as a theoretical construct for the demonstration of causality within the context of population research, we have already shown that three of these conditions have been met, namely strength (effect size), temporality (the cause preceding the effect), and biological gradient (a dose-response relationship). In this study we have explored the remaining criterion of plausibility, coherence with a biological hypothesis (in this instance an association of caffeine use with amyloid reduction as indicated by WML volume).

Results from a sub-population of our original cohort for whom MRI was available allowed us to confirm our initial hypothesis that higher caffeine consumption would be associated with lower WML volume. Furthermore, this observation is dose-dependent, providing further evidence of coherence. Education, depression, cardiovascular disease, body mass index, diabetes, cholesterol levels, anticholinergic medication, disability, and alcohol use were all considered to be potential confounders. In women only alcohol use and disability were significantly associated with WML, and in a final model integrating these variables, caffeine consumption was still found to be significantly inversely associated with WML volume. Our results suggest the importance for future studies of adjusting WML volume by total cranial volume to eliminate structural gender differences due to brain size. Our working assumption has been that caffeine has led to a decrease in amyloid accumulation and hence reduced the risk for WML. There are, however, numerous biological hypotheses linking caffeine intake and WML. For example, adenosine-associated decreases in blood brain barrier permeability may reduce the amount of amyloid passing into the brain, WMLs being linked to both clinical and silent stroke, changes in extracellular adenosine during ischemia may provide a neuroprotective response. Our finding that protective effects are observed in women only is consistent with our previous results [8] and another epidemiological study [13], but the reasons of gender differences remain unclear. They could involve differences in caffeine metabolism or in sensitivity to the pharmacologic effect, as well as hormonal factors which could not be evaluated in the present study, but warrant further examination.

It is, however, not the purpose of this preliminary epidemiological study to demonstrate the biological pathways which underlie this observation, this very complex relationship having been reviewed elsewhere [25], but rather to test the coherence of epidemiological findings with current biological research as a means of establishing the likelihood of true causality. Overall our findings suggest that the observed significant relationship between high caffeine intake in women and lower cognitive decline over time is independent of potential confounding factors, is dose-dependent, temporarily related, and coherent with biological observations. On this basis we assume that this relationship is highly likely to be a true causal relationship and not a spurious association.

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