Original Article

Correlation between dietary theobromine intake and low cognitive performance in older adults in the United States: A cross-sectional study based on the National Health and Nutrition Examination Survey

Longzhu Zhao MD[1], Runqing Zhan PhD[2], Xueting Wang BD[1], Ruihan Song MD[1], Miaomiao Han MD[1], Xiaoli Shen PhD[1]

[1]Department of Epidemiology and Health Statistics, School of Public Health, Qingdao University, Qingdao, Shandong Province, China
[2]Qingdao Haici Hospital, Qingdao, Shandong Province, China

Background and Objectives: Few studies have investigated the effects of dietary theobromine intake on the cognitive performance of older adults. Therefore, we investigated these effects in older adults in the United States. **Methods and Study Design:** In this cross-sectional study, we used data (2011–2014) from the National Health and Nutrition Examination Survey. Intake of theobromine intake was obtained through two 24-h dietary recall interviews and was adjusted by energy. Cognitive performance was assessed using the animal fluency test, Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD), and Digit Symbol Substitution Test (DSST). Logistic regression and restricted cubic spline models were constructed to evaluate the correlation between the dietary intake of theobromine from different sources and the likelihood of low cognitive performance. **Results:** The fully adjusted model revealed that compared with the lowest quintile, the odds ratios (with 95% confidence intervals) of cognitive performance in the CERAD test were 0.42 (0.28–0.64), 0.34 (0.14–0.83), 0.25 (0.07–0.87), and 0.35 (0.13–0.95) for the highest quintile of total theobromine intake and that from chocolate, coffee, and cream, respectively. Dose–response relationship analysis indicated nonlinear correlations between the likelihood of low cognitive performance and dietary theobromine (total intake and that from chocolate, coffee, and cream). An L-shaped relationship was observed between total theobromine intake and cognitive performance in the CERAD test. **Conclusions:** The dietary intakes of theobromine (total and that from chocolate, coffee, and cream) may protect older adults, particularly men, against low cognitive performance.

Key Words: cognitive performance, theobromine, dietary intake, dose-response relationship, phytochemicals

INTRODUCTION

With the increase in the average lifespan of individuals, age-related cognitive decline has emerged as a major public health concern.[1] Without preventative or intervention measures, cognitive decline may gradually lead to mild cognitive impairment (MCI) and dementia.[2] Alzheimer's disease (AD) has been identified as the leading cause of dementia.[3] In 2060, approximately 13.85 million adults aged ≥65 years will have clinical AD in the United States, and the number of individuals with MCI will approximately be 21.55 million.[4] The development of AD from cognitive decline is irreversible. To our knowledge, no effective treatment is available to slow or stop the development of AD.[5-7] Thus, new pharmacological approaches are urgently required to prevent MCI and AD. Studies have shown that certain phytochemicals, such as lutein, flavonoids, lignans, and polyphenols, exert positive effects on cognitive performance.[8-10] However, few studies have investigated the correlation between theobromine intake and cognitive performance.

Theobromine, a primary methylxanthine found in cocoa products, is widely used in beverages and confections.[11,12] The primary sources of theobromine are chocolate,[13] cocoa products, tea,[14] and cola nut.[15] Cacao beans contain approximately 1% theobromine.[14] The primary mechanisms underlying the effects of theobromine include the blockade of adenosine receptors, the inhibition of phosphodiesterases,[11] the upregulation of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) pathway, an increase in intracellular cyclic adenosine monophosphate (cAMP) expression level, the induction of cerebral brain-derived neurotrophic factor (BDNF) expression, and the restoration of A1 purinergic receptor expression.[16-18] Theobromine serves as a cardiac, respire-

Corresponding Author: Dr Xiaoli Shen, Department of Epidemiology and Health Statistics, Medical College of Qingdao University, No. 308 Ningxia Rd, Qingdao 266071, China.
Tel: 0532-8299171
Email: shenxiaoli@qdu.edu.cn
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tory, and brain stimulant; a diuretic and spasmolytic drug; a vasodilator; a hepatoprotective agent; and an anticancer agent.[11,19-23] This compound can traverse the blood–brain barrier,[17] thus affecting the central nervous system.

Animal studies have revealed the positive effects of theobromine on cognitive function.[17,24] Fernández-Fernández et al fed mice a theobromine-rich natural diet for 20 days, and this diet prevented aging-related cognitive decline and AD by regulating the expression of acetylcholine-associated enzymes, dopamine, and norepinephrine.[24] Yoneda et al demonstrated that the oral administration of 0.05% theobromine in mice for 30 days augmented the cAMP/cAMP-response element–binding protein (CREB)/BDNF pathway in their brain, which is essential for learning and memory.[17] Epidemiological studies on the correlation of theobromine intake with cognitive performance have reported inconsistent findings.[25,26] Travassos et al indicated that in the cerebrospinal fluid (CSF) of patients with AD, theobromine levels were inversely correlated with those of amyloid-β (Aβ).[25] By contrast, a double-blind placebo-controlled trial conducted by Mitchell et al. revealed no improvement in the Digit Symbol Substitution Test (DSST) scores of 24 healthy women after theobromine intake.[26]

Few studies have investigated the correlation between dietary theobromine intake and low cognitive performance in a large sample comprising older adults or the correlation between the dietary intake of theobromine from different sources (chocolate, coffee, and cream) and the likelihood of low cognitive performance. In the present study, through the use of a large data set from the National Health and Nutrition Examination Survey (NHANES), we investigated the correlation between the dietary intake of theobromine (total and that derived from chocolate, coffee, and cream) and the likelihood of low cognitive performance in older adults in the United States. In addition, we explored the relevant dose–response relationships.

METHODS

Data collection and study population

The NHANES is a 2-year-cycle cross-sectional survey conducted by the Centers for Disease Control and Prevention to assess the health and nutritional status of the noninstitutionalized civilian population of the United States. In the present study, the NHANES participants were selected through a complex, layered, and multistage probabilistic sampling approach to represent the general population of the United States. The participants were first interviewed at their homes; subsequently, they underwent health examinations at a mobile examination center (MEC). This study was approved by the Institutional Review Board of the National Center for Health Organization. All participants provided informed consent before the interview and health examination.

We combined data from two consecutive cycles of the NHANES (2011 and 2012 as well as 2013 and 2014). In total, 19,931 participants were identified. Individuals aged ≥60 years who participated in the MEC cognitive function survey (n=3,632) were excluded from this analysis. Individuals with incomplete or unreliable cognitive performance data (n=698) and 24-h recall data (n=1,267) were also excluded. Furthermore, individuals with missing covariant data were excluded (n=145). Finally, a total of 1,522 individuals aged ≥60 years (men and women) were included in our analysis. Figure 1 depicts participant

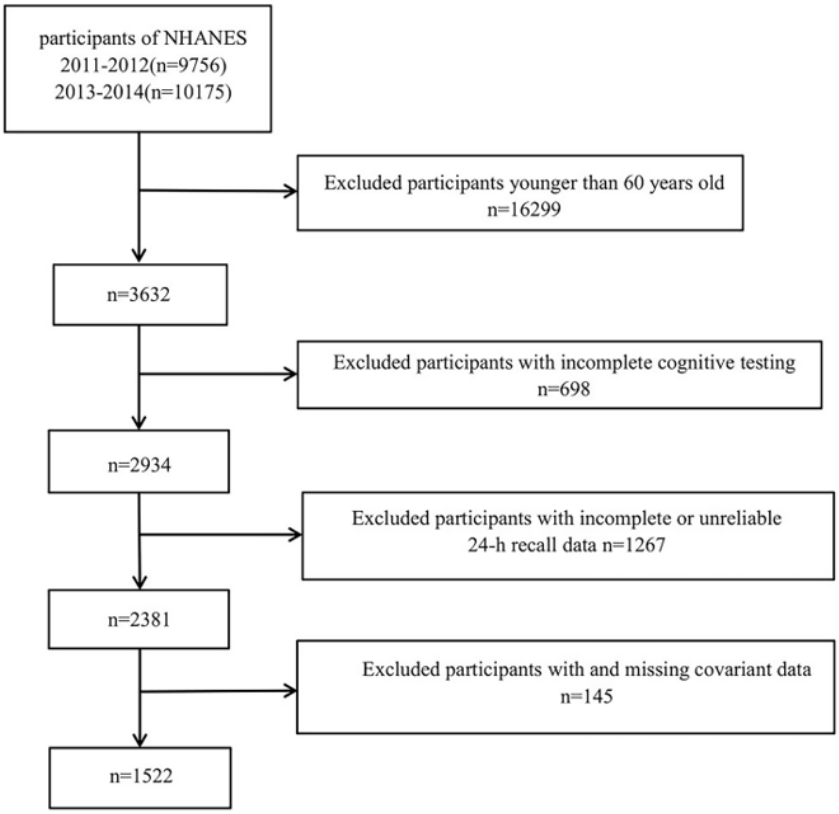


Figure 1. Flowchart for participant inclusion.

inclusion.

Cognitive performance assessment

Several cognitive performance tests were used in the 2011–2012 and 2013–2014 cycles of the NHANES to assess the performance of the participants aged ≥60 years. Cognitive performance was assessed by trained interviewers at the end of face-to-face private interviews at the MEC. The following tests were used for evaluation: the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) Word Learning subtest, the animal fluency test, and the DSST test. These tests, which have been widely used in large-scale screenings as well as epidemiological and clinical studies, helped evaluate the participants' working memory, language, processing speed, and executive functioning. Consent for recording (audio) the test sessions was obtained from the participants for quality control purposes. Two interviewers transcribed the participants' responses from the audio recordings of interviews conducted in English and Spanish and scored them in the CERAD test, the animal fluency test, and the DSST test. Transcription and scoring were generally performed on the same day. If necessary, inconsistent scores were adjudicated by a third party. In addition, the audio recordings were reviewed for consistency in terms of interviewer instructions and to determine the accuracy of test scores. Approximately 10% of the recorded interviews were independently reviewed during data collection.[27]

The CERAD test helped assess the participants' immediate and delayed abilities to learn new verbal information (memory subdomain). This test comprises three consecutive learning trials and a delayed recall. For the learning trials, participants were instructed to read aloud 10 unrelated words, one at a time, as they were presented. Immediately following the word presentation, the participants recalled as many words as possible. The order of the 10 words was altered in each of the three learning trials. Participants who could not read because of literacy or visual impairment were requested to repeat each word after it was read by the interviewer. Delayed word recall was conducted after the other two cognitive exercises (animal fluency test and DSST) were completed (approximately 8–10 min from the initiation of the word learning trials). The score on each trial ranged from 0 to 10, and the total score in the CERAD test was calculated by summing the scores on the three learning trials and the delayed recall trial.

The animal fluency test, which helps assess a vital component of executive function, is used in large-scale screenings and epidemiological studies to assess categorical verbal fluency.[5-7] The participants were required to name as many animals as possible in 1 min. One point is given for each animal name. The total score was calculated in terms of the number of correct answers. In the NHANES, the participants were first requested to name three items of clothing (representing another verbal fluency category) as a practice test. The participants failing to name three articles of clothing were not subjected to the animal fluency test.

The DSST test, a performance module of the Wechsler Adult Intelligence Scale, is frequently used to assess participants' processing speed, sustained attention, and working memory. The test was conducted using a piece of paper showing a key (at the top) containing nine numbers paired with symbols. Participants had 2 min to match the right symbols in the 133 boxes with the numbers. The total score was calculated in terms of the number of the correct matches and ranged from 0 to 133.

To our knowledge, there is no gold standard on the cutoff point for the animal fluency test, CERAD test, and DSST test to indicate low cognitive performance or cognitive decline. Therefore, we selected the 25th percentile of the score for each test, which is the lowest quartile, as the cutoff point, which is consistent with the methods used in a relevant study.[28] Age markedly affects cognitive performance, and therefore, we divided the participants into three age groups: group 1, 60 to <70 years; group 2, 70 to <80 years; and group 3, ≥80 years. The lowest quartile for each group was calculated and used (stratified by age groups) as the cutoff score to define the cognitive performance status of the participants.[6] For the animal fluency test, the cutoff scores indicating low cognitive performance were 11, 10, and 9 for groups 1, 2, and 3, respectively; by contrast, the cutoff scores were 18, 17, and 13, respectively, for the CERAD test and 28, 24, and 23, respectively, for the DSST. For each cognitive test, the participants were divided into the following two groups: individuals with low cognitive performance (those who scored below the lowest quartile in each age group) and those with normal cognitive performance (the others).

Theobromine intake assessment

Data regarding the dietary intakes of theobromine were obtained through two 24-h dietary recall interviews. The first dietary recall was collected by a face-to-face interview at the MEC, and the second one was completed by a telephonic interview conducted by trained interviewers after 3 to 10 days. Interviewers in the office at the study center conducted the telephonic follow-up interviews. The total dietary intake of theobromine was calculated according to the US Department of Agriculture Food and Nutrient Database for Dietary Studies (2011–2012 and 2013–2014). The dietary interview and data processing were conducted following the relevant information available on the NHANES website.[29] For a participant who completed both 24-h recall interviews, the average dietary theobromine intakes were included in the analysis; otherwise, a single dietary recall value was used. The dietary intakes of theobromine (mg/day) were divided into quintiles. Notably, theobromine intake was calculated on the basis of dietary intake alone; the use of relevant supplements was not considered in the NHANES 2011–2014 data. We determined the participants' theobromine intake from different dietary sources according to the relevant food codes.

Study covariates

Some potential confounders associated with cognitive performance were investigated in the present study. The participants' demographics included age (60–70, 70–80 years, and ≥80 years), sex (male and female), race (Mexican American, other Hispanic, non-Hispanic White, non-

Hispanic Black, and other race), marital status (married, living with a partner, widowed, divorced, separated, and never married), and education level (below high school, high school, and above high school). Other covariates included smoking status (smoking at least 100 cigarettes in life or not), alcohol consumption (consuming at least 12 alcoholic beverages per year or not), hypertension (yes or no), diabetes (yes or no), and heart attack (yes or no). Patients were determined to have hypertension based on mean systolic blood pressure of >130 mmHg or a mean diastolic blood pressure of >80 mmHg, the recent administration of blood pressure drugs, or a self-reported hypertension diagnosis. Patients were determined to have diabetes based on a fasting plasma glucose level of ≥7.0 mmol L−1, a 2-h plasma glucose level of ≥11.1 mmol L−1, a glycosylated hemoglobin level of ≥6.5%, the receipt of diabetes medication (pills or insulin), or a self-reported diagnosis of diabetes. A heart attack was confirmed through a self-reported history of a physician-diagnosed heart attack. Data regarding the total energy intake were obtained through 24-h dietary recall interviews.

Statistical analysis

All statistical analyses were performed using Stata (version 15.0; Stata Corporation, USA) to account for the complex sampling design. Appropriate sampling weights, primary sampling unit, and strata information were considered in this analysis to ensure the national representation. Because the 2-year cycles of the NHANES continuous data were combined, 4-year weights for NHANES 2011–2012 and 2013–2014 data were calculated by dividing the 2-year weights by 2 in accordance with the analytical guidelines.

The Kolmogorov–Smirnov normality test was performed to test the normal distribution of the continuous variables. Normally distributed continuous variables are expressed in terms of mean and standard deviation values, whereas non-normally distributed variables are expressed in terms of median and interquartile range values. Conversely, categorical variables are presented in terms of number and frequency values. To compare low and normal cognitive performance levels in terms of continuous variables, an independent samples t test was performed for normally distributed data and a Mann–Whitney U test was used for non-normally distributed data. For categorical variables, between-group differences were evaluated using chi-squared tests. The dietary intake of theobromine was stratified on the basis of quintiles (quintile 1, <20th percentile; quintile 2, ≥20th to 40th percentile; quintile 3, ≥40th to 60th percentile; quintile 4, ≥60th to 80th percentile; and quintile 5, ≥80th percentile).

Logistic regression models were constructed to investigate the correlations between the dietary intakes of theobromine (total intake and that from chocolate, coffee, and cream) and the likelihood of low cognitive performance. The lowest quintile was used as the reference. Cognitive performance was analyzed as a binary variable. The crude model was not adjusted for any confounders. By contrast, Model 1 was adjusted for age and sex, whereas Model 2 was adjusted for age, sex, race, marital status, education, total energy, smoking status, alcohol consumption, diabetes, hypertension, and heart attack. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from logistic regression analyses. Multicollinearity of the independent variables was analyzed using variance inflation factors. Trend tests for dietary and total nutrient intakes; were also carried out in this study, in which each measure of low cognitive performance were used the continuous measures value of these variables by assigning the middle value to each quartile. The *p* value for nonlinearity was calculated by testing the theobromine intake value of the second spline at 0.

In the present study, a restricted cubic spline with three knots was used to investigate the dose–response relationships between the dietary intakes of theobromine (total intake and that from chocolate, coffee, and cream) and the various measures of low cognitive performance. The restricted cubic spline model was adjusted for the aforementioned confounders to determine the accuracy of the observed correlations. All reported probabilities (p value) were two-sided, and a *p* value of <0.05 indicated statistical significance.

RESULTS

Participants characteristics

Table 1 summarizes the characteristics of all participants included in the present study. Participants with low cognitive performance and those with normal cognitive performance in the CERAD test, animal fluency test, and DSST differed significantly in terms race, education level, and diabetes status ($p<0.01$). Individuals with low cognitive performance in all three tests were more likely to be men, non-Hispanic Black individuals, and unmarried (or not living with a partner); to have a low education level; and to exhibit a high prevalence of diabetes, hypertension, and heart attack. By contrast, individuals with low cognitive performance in all three tests were likely to have a relatively low total theobromine intake and that from chocolate, coffee, and cream. In the animal fluency test and DSST, the total energy intake was significantly lower in participants with low cognitive performance than in those with normal cognitive performance ($p<0.01$). In the CERAD test, the rates of smoking and alcohol consumption were significantly lower in individuals with low cognitive performance than in those with normal cognitive performance ($p<0.01$).

Negative correlation between the dietary intakes of theobromine and the likelihood of low cognitive performance

Table 2 presents the correlations between the dietary intakes of theobromine (total intake and that from chocolate, coffee, and cream) and the likelihood of low cognitive performance. As indicated previously, the lowest quintile served as the reference. In the CERAD test, the crude ORs with 95% CIs were 0.45 (0.30–0.69), 0.23 (0.10–0.53), 0.29 (0.09–0.96), and 0.27 (0.11–0.70) in the highest versus lowest quintile of total dietary intakes of theobromine and theobromine intake from chocolate, coffee, and cream, respectively. For Model 1, the results were similar to those of the crude model; however, for Model 2, the corresponding values were 0.42 (0.28–0.64), 0.34 (0.14–0.83), 0.25 (0.07–0.87), and 0.35 (0.13–0.95)

Table 1. Characteristics of the population by cognitive performance status, NHANES 2011–2014 (n=1522)

	Animal fluency test			CERAD test			DSST test		
	Low cognitive performance	Normal cognitive performance	*p* value	Low cognitive performance	Normal cognitive performance	*p* value	Low cognitive performance	Normal cognitive performance	*p* value
Number of subjects (%)	428 (28.1)	1094 (71.9)		427 (28.1)	1095 (71.9)		390 (25.6)	1132 (74.4)	
Age group (%)†			0.507			0.549			0.851
60-70 years	167 (50.9)	649 (54.4)		187 (55.7)	629 (53)		198 (54)	618 (53.5)	
70-80 years	100 (30.5)	347 (29.1)		98 (29.2)	349 (29.4)		110 (30)	337 (29.2)	
≥80 years	61 (18.6)	198 (16.6)		51 (15.2)	208 (17.5)		59 (16.1)	200 (17.3)	
Sex, n (%)†			0.981			<0.001			<0.001
Male	156 (47.6)	567 (47.5)		210 (62.5)	513 (43.3)		214 (58.3)	509 (44.1)	
Female	172 (52.4)	627 (52.5)		126 (37.5)	673 (56.7)		153 (41.7)	646 (55.9)	
Race, n (%)†			<0.001			<0.001			<0.001
Mexican American	26 (7.9)	84 (7.0)		34 (10.1)	76 (6.4)		49 (13.4)	61 (5.3)	
Other Hispanic	31 (9.5)	77 (6.4)		37 (11.0)	71 (6.0)		50 (13.6)	58 (5.0)	
Non-Hispanic White	120 (36.6)	740 (62.0)		153 (45.5)	707 (59.6)		116 (31.6)	744 (64.4)	
Non-Hispanic Black	111 (33.8)	216 (18.1)		91 (27.1)	236 (19.9)		142 (38.7)	185 (16.0)	
Other race	40 (12.2)	77 (6.4)		21 (6.3)	96 (8.1)		10 (2.7)	107 (9.3)	
Marital status, n (%)†			0.231			0.432			<0.001
Married/living with partner	188 (57.3)	728 (61.0)		196 (58.3)	720 (60.7)		182 (49.6)	734 (63.5)	
Widowed/divorced/separated /never married	140 (42.7)	466 (39.0)		140 (41.7)	466 (39.3)		185 (50.4)	421 (36.5)	
Education, n (%)†			<0.001			<0.001			<0.001
< High school	112 (34.1)	198 (16.6)		124 (36.9)	186 (15.7)		182 (49.6)	128 (11.1)	
High school	95 (29.0)	268 (22.4)		90 (26.8)	273 (23.0)		94 (25.6)	269 (23.3)	
>High school	121 (36.9)	728 (61.0)		122 (36.3)	727 (61.3)		91 (24.8)	758 (65.6)	
Smoking, n (%)†	165 (50.3)	617 (51.7)	0.660	190 (56.5)	592 (49.9)	0.032	207 (56.4)	575 (49.8)	0.027
Alcohol consumption status, n (%)†	212 (64.6)	863 (72.3)	0.007	239 (71.1)	836 (70.5)	0.820	242 (65.9)	833 (72.1)	0.024
Diabetes, n (%)†	102 (31.1)	258 (21.6)	<0.001	101 (30.1)	259 (21.8)	0.002	122 (33.2)	238 (20.6)	<0.001
Hypertension, n (%)†	218 (66.5)	714 (59.8)	0.028	207 (61.6)	725 (61.1)	0.874	248 (67.6)	684 (59.2)	0.004
Heart attack, n (%)†	41 (12.5)	99 (8.3)	0.019	38 (11.3)	102 (8.6)	0.129	48 (13.1)	92 (8.0)	0.003
Total energy intake (kcal/day)‡	1691 (724)	1880 (765)	<0.001	1843 (789)	1829 (819)	0.536	1648 (724)	1875 (765)	<0.001
Total theobromine intake (mg/day)‡	21 (28.5)	32.5 (51)	0.001	25.5 (30)	30 (51)	0.085	25.5 (28.5)	30.25 (51)	0.040
Chocolate theobromine intake (mg/day)‡	20.8 (39)	36.5 (57.5)	0.001	25.5 (39.5)	34.5 (60.5)	0.004	25.5 (46)	34.25 (54.5)	0.044
Coffee theobromine intake (mg/day)‡	19 (38)	33 (50.3)	0.007	24 (38)	30.5 (51)	0.003	25.5 (45)	28.8 (48.5)	0.246
Cream theobromine intake (mg/day)‡	17.5 (38)	31.5 (48)	0.001	21.5 (37.3)	30.5 (50)	0.006	25.5 (39)	37.5 (51)	0.380

CERAD: Consortium to Establish a Registry for Alzheimer's disease Word Learning sub-test; DSST: Digit Symbol Substitution Test.
Data is number of subjects (percentage) or medians (interquartile ranges).
†Chi-square test was used to compare the percentage between participants with and without low cognitive performance.
‡Mann-Whitney U test was used to compare the mean values between participants with and without low cognitive performance.

Table 2. Weighted ORs and 95% CIs of low cognitive performance according to the quintiles of dietary theobromine intake, NHANES 2011-2014

	Number of subjects (%)	Animal fluency test			CERAD test		
		Crude[†] OR (95% CI)	Model 1[‡] OR (95% CI)	Model 2[§] OR (95% CI)	Crude[†] OR (95% CI)	Model 1[‡] OR (95% CI)	Model 2[§] OR (95% CI)
Total theobromine, mg/d							
Q1: <7.5	301/1522	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 7.5 to <20.0	306/1522	0.86 (0.53-1.40)	0.84 (0.53-1.34)	0.90 (0.53-1.53)	0.86 (0.55-1.34)	0.84 (0.52-1.37)	0.90 (0.53-1.52)
Q3: 20.0 to <39.0	301/1522	0.59 (0.38-0.92)*	0.52 (0.33-0.81)**	0.55 (0.34-0.91)*	0.79 (0.49-1.27)	0.72 (0.43-1.21)	0.79 (0.43-1.43)
Q4: 39.0 to <75.5	309/1522	0.53 (0.30-0.92)*	0.47 (0.27-0.81)**	0.52 (0.30-0.91)*	0.72 (0.45-1.17)	0.67 (0.40-1.12)	0.74 (0.42-1.29)
Q5: ≥75.5	305/1522	0.59 (0.37-0.95)*	0.55 (0.34-0.88)*	0.65 (0.40-1.06)	0.45 (0.30-0.69)**	0.40 (0.25-0.63)**	0.42 (0.28-0.64)**
p-trend		0.048	0.031	0.158	< 0.001	< 0.001	< 0.001
Theobromine in chocolate, mg/d							
Q1: <9.5	112/589	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 9.5 to <23.5	123/589	1.11 (047-2.62)	1.09 (0.47-2.54)	1.18 (0.48-2.93)	0.69 (0.32-1.46)	0.70 (0.38-1.29)	0.65 (0.29-1.48)
Q3: 23.5 to <42.5	118/589	0.48 (0.25-0.96)*	0.40 (0.21-0.74)**	0.45 (0.24-0.86)*	0.89 (0.42-1.89)	1.04 (0.48-2.29)	0.99 (0.38-2.57)
Q4: 42.5 to <78.0	117/589	0.59 (0.17-2.02)	0.53 (0.18-1.60)	0.71 (0.24-2.12)	0.55 (0.25-1.19)	0.63 (0.28-1.41)	0.64 (0.27-1.53)
Q5: ≥78.0	119/589	0.53 (0.23-1.23)	0.50 (0.23-1.10)	0.65 (0.28-1.48)	0.23 (0.10-0.53)**	0.26 (0.11-0.61)**	0.34 (0.14-0.83)**
p-trend		0.062	0.043	0.222	< 0.001	< 0.001	< 0.001
Theobromine in coffee, mg/d							
Q1: <8.0	103/537	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 8.0 to <20.5	110/537	2.09 (0.89-4.90)	1.99 (0.85-4.70)	2.65 (0.97-7.26)	0.96 (0.43-2.13)	0.98 (0.43-2.22)	1.00 (0.44-2.33)
Q3: 20.5 to <39.0	108/537	0.71 (0.29-1.73)	0.61 (0.24-1.56)	0.67 (0.25-1.83)	1.28 (0.52-3.17)	1.18 (0.49-2.83)	1.14 (0.39-3.33)
Q4: 39.0 to <69.5	108/537	0.89 (0.36-2.24)	0.82 (0.33-2.00)	1.00 (0.35-2.90)	0.70 (0.30-1.62)	0.66 (0.28-1.55)	0.70 (0.27-1.77)
Q5: ≥69.5	108/537	1.03 (0.39-2.68)	0.95 (0.35-2.57)	1.24 (0.43-3.61)	0.29 (0.09-0.96)*	0.25 (0.07-0.90)*	0.25 (0.07-0.87)*
p-trend		0.194	0.162	0.297	0.005	0.004	0.003
Theobromine in cream, mg/d							
Q1: <7.5	89/493	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 7.5 to <21.0	106/493	1.12 (0.52-2.39)	1.13 (0.54-2.35)	1.33 (0.56-3.17)	0.94 (0.49-1.81)	0.98 (0.51-1.89)	1.16 (0.54-2.47)
Q3: 21.0 to <37.5	98/493	0.43 (0.19-0.98)*	0.39 (0.18-0.86)*	0.44 (0.15-1.25)	0.96 (0.38-2.44)	0.90 (0.36-2.25)	0.97 (0.35-2.64)
Q4: 37.5 to <68.5	101/493	0.44 (0.16-1.22)	0.40 (0.14-1.13)	0.46 (0.15-1.39)	0.83 (0.31-2.28)	0.75 (0.27-2.13)	0.98 (0.34-2.86)
Q5: ≥68.5	99/493	0.60 (0.26-1.39)	0.57 (0.23-1.37)	0.68 (0.21-2.19)	0.27 (0.11-0.70)**	0.25 (0.10-0.63)**	0.35 (0.13-0.95)*
p-trend		0.095	0.076	0.180	0.007	0.004	0.029

Q1: quintile 1; Q2: quintile 2; Q3: quintile 3; Q4: quintile 4; Q5: quintile 5.
OR: odds ratios, CI: confidence interval; CERAD: Consortium to Establish a Registry for Alzheimer's disease Word Learning sub-test; DSST: Digit Symbol Substitution Test.
[†]Crude model did not adjust any confounders.
[‡]Model 1 adjusted for age, sex.
[§]Model 2 adjusted for age, sex, race, marital status, educational level, alcohol drinking, smoking status, hypertension, diabetes, heart disease, and total daily energy intake.
*p<0.05; **p<0.01.

Table 2. Weighted ORs and 95% CIs of low cognitive performance according to the quintiles of dietary theobromine intake, NHANES 2011-2014 (cont.)

	DSST test		
	Crude[†] OR (95% CI)	Model 1[‡] OR (95% CI)	Model 2[§] OR (95% CI)
Total theobromine, mg/d			
Q1: <7.5	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 7.5 to <20.0	0.69 (0.46-1.05)	0.68 (0.45-1.02)	0.79 (0.50-1.27)
Q3: 20.0 to <39.0	0.86 (0.50-1.49)	0.80 (0.47-1.35)	1.07 (0.54-2.14)
Q4: 39.0 to <75.5	0.84 (0.42-1.66)	0.78 (0.39-1.56)	1.16 (0.54-2.50)
Q5: ≥75.5	0.55 (0.32-0.95)*	0.51 (0.29-0.90)*	0.80 (0.42-1.52)
p-trend	0.061	0.043	0.524
Theobromine in chocolate, mg/d			
Q1: <9.5	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 9.5 to <23.5	0.72 (0.32-1.64)	0.70 (0.31-1.57)	0.63 (0.21-1.90)
Q3: 23.5 to <42.5	0.99 (0.43-2.29)	0.89 (0.39-2.04)	1.26 (0.43-3.75)
Q4: 42.5 to <78.0	0.45 (0.17-1.22)	0.42 (0.15-1.18)	0.71 (0.24-2.14)
Q5: ≥78.0	0.52 (0.18-1.47)	0.50 (0.17-1.42)	1.20 (0.31-4.62)
p-trend	0.144	0.132	0.741
Theobromine in coffee, mg/d			
Q1: <8.0	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 8.0 to <20.5	1.44 (0.67-3.09)	1.34 (0.66-2.71)	2.42 (1.12-5.23)
Q3: 20.5 to <39.0	1.58 (0.66-3.79)	1.34 (0.56-3.19)	2.33 (0.72-7.59)
Q4: 39.0 to <69.5	1.02 (0.51-2.06)	0.92 (0.44-1.95)	2.17 (0.81-5.80)
Q5: ≥69.5	0.79 (0.36-1.75)	0.71 (0.33-1.52)	2.42 (1.01-5.78)
p-trend	0.154	0.099	0.216
Theobromine in cream, mg/d			
Q1: <7.5	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 7.5 to <21.0	1.11 (0.39-3.14)	1.14 (0.41-3.15)	1.83 (0.82-4.09)
Q3: 21.0 to <37.5	1.49 (0.48-4.64)	1.38 (0.45-4.23)	2.44 (0.86-6.91)
Q4: 37.5 to <68.5	0.84 (0.35-2.03)	0.78 (0.32-1.91)	1.64 (0.74-3.62)
Q5: ≥68.5	0.80 (0.29-2.15)	0.74 (0.27-2.00)	2.41 (0.94-6.21)
p-trend	0.033	0.014	0.265

Q1: quintile 1; Q2: quintile 2; Q3: quintile 3; Q4: quintile 4; Q5: quintile 5.
OR: odds ratios, CI: confidence interval; CERAD: Consortium to Establish a Registry for Alzheimer's disease Word Learning sub-test; DSST: Digit Symbol Substitution Test.
[†]Crude model did not adjust any confounders.
[‡]Model 1 adjusted for age, sex.
[§]Model 2 adjusted for age, sex, race, marital status, educational level, alcohol drinking, smoking status, hypertension, diabetes, heart disease, and total daily energy intake.
*$p<0.05$; **$p<0.01$.

for the highest versus lowest quintile of the total dietary intakes of theobromine and that from chocolate, coffee, and cream, respectively. No multiplicity was noted after the multicollinearity test.

In the animal fluency test, the crude OR with 95% CI value of low cognitive performance was 0.59 (0.37–0.95) for the highest versus lowest quintile of total theobromine intake. For Model 1, the results were similar to those of the crude model. In the DSST, the crude OR and 95% CI value of low cognitive performance were 0.55 and 0.32–0.95, respectively, for the highest versus lowest quintile of total theobromine intake. For Model 1, the results were similar to those of the crude model. No significant correlations were identified between the dietary intakes of theobromine from chocolate, coffee, and cream and the scores in the animal fluency test or DSST.

Results of stratified analysis

Stratified analyses (by sex) were performed to investigate for the correlations between total theobromine intake and low cognitive performance in the three cognitive tests (Table 3). In the CERAD test, the OR and 95% CI value of low cognitive performance were 0.25 and 0.12–0.51, respectively, for the highest versus lowest quintile of total theobromine intake in the multivariate analysis (Model 2) for men. In the DSST, the corresponding value was 0.39 (0.17–0.93) for the highest versus lowest quintile of total theobromine intake in men. For Model 1, the results were similar to those of the crude model. No significant correlation was identified between total theobromine intake and low cognitive performance in the DSST for men. In the animal fluency test, compared with quintile 1, the OR and 95% CI of low cognitive performance for quintile 2 were 0.43 and 0.19–0.99, respectively, in Model 1 for men.

For women, after adjustment for multiple covariates, no significant correlation was identified between total theobromine intake and low cognitive performance in the CERAD test or DSST. In the animal fluency test, compared with quintile 1, the ORs of low cognitive performance for quintile 4 were 0.50 (95% CI, 0.28–0.91) and 0.45 (95% CI, 0.25–0.82) in the crude model and Model 1, respectively, for women.

Dose–response relationship between the dietary intakes of theobromine and the likelihood of low cognitive performance

In the restricted cubic spline model, an L-shaped relationship was observed between the dietary intakes of total theobromine and the likelihood of low cognitive performance in the CERAD test (Figure 2). With an increase in the dietary intakes of total theobromine beyond 120 mg/kg, no reduction was observed in the likelihood of low cognitive performance in the CERAD test; furthermore, no prominent correlation was observed between a total theobromine intake of <5.0 (OR: 0.96; 95% CI: 0.93–0.99) or >268 (OR: 0.47; 95% CI: 0.24–0.94) mg/day and the likelihood of low cognitive performance.

Figure 3 illustrates the dose–response relationships between the dietary intakes of theobromine from chocolate, coffee, and cream and the likelihood of low cognitive performance in the CERAD test. The dietary intakes of theobromine from the three aforementioned sources exhibited nonlinear inverse correlations with the likelihood of low cognitive performance in the CERAD test ($p_{\text{chocolate theobromine for nonlinearity}} = 0.001$; $p_{\text{coffee theobromine for nonlinearity}} = 0.0001$; and $p_{\text{cream theobromine for nonlinearity}} = 0.0347$). The likelihood of low cognitive performance decreased with an increase in the dietary intakes of theobromine from chocolate, coffee, and cream. Markedly negative correlations were noted between the likelihood of low cognitive performance in the CERAD test and the dietary intakes of theobromine from chocolate, coffee, and cream when the intake levels were >86.0 (OR: 0.40; 95% CI: 0.16–0.98), >114 (OR: 0.35; 95% CI: 0.12–0.97), and >112 (OR: 0.38; 95% CI: 0.14–0.99) mg/day, respectively. Because no strong correlations were observed between the dietary intakes of theobromine from chocolate, coffee, or cream and the likelihood of low cognitive performance in the animal fluency test or DSST in Model 2, these correla-

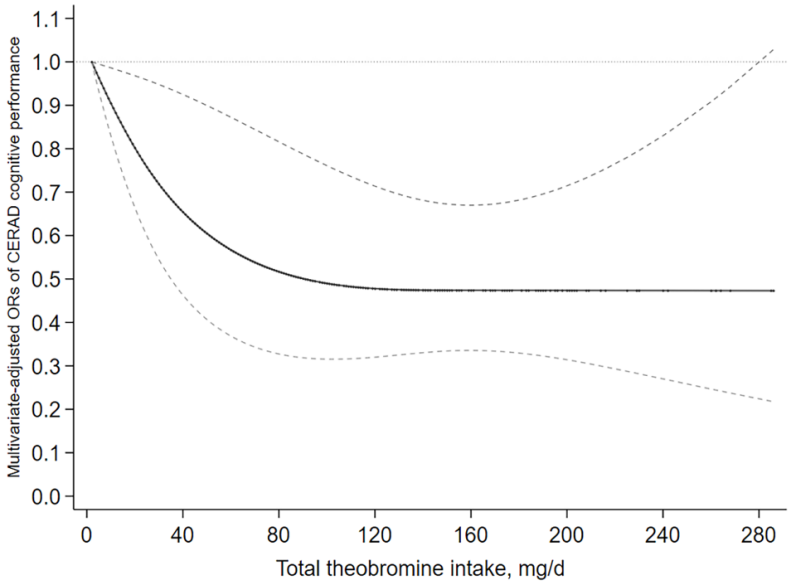


Figure 2. Dose–response relationship between the total dietary intake of theobromine and the likelihood of low cognitive performance in the Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest.

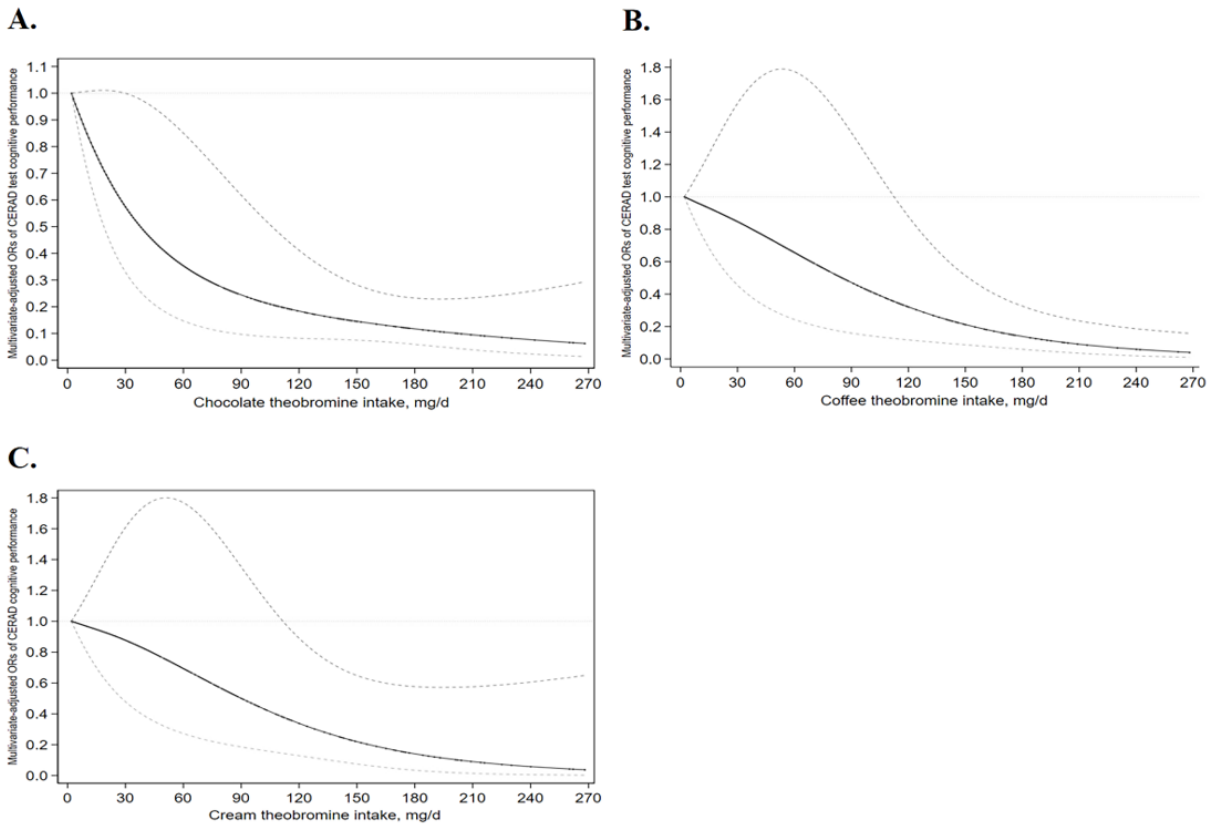


Figure 3. Dose–response relationship between the dietary intake of theobromine from chocolate, coffee, and cream and the likelihood of low cognitive performance in the CERAD test. (A) Correlation between the dietary intake of theobromine from chocolate and the likelihood of low cognitive performance in the CERAD test. (B) Correlation between the dietary intake of theobromine from coffee and the likelihood of low cognitive performance in the CERAD test. (C) Correlation between the dietary intake of theobromine from cream and the likelihood of low cognitive performance in the CERAD test. CERAD, Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest.

tions were not presented in the current study.

Figure 4 presents the results of the restricted cubic spline analyses performed after sex-based stratification. As shown in Figure 4, a nonlinear negative correlation was identified between the likelihood of low cognitive performance in the CERAD test and the dietary intake of total theobromine in men ($p_{\text{total theobromine for nonlinearity}}$=0.0025). Theobromine exerted a considerable protective effect on cognitive performance when the total dietary intake was >75 mg/day (OR: 0.52; 95% CI: 0.27–0.99) in men. By contrast, in women, no prominent correlation was identified between the total dietary intake of theobromine and the likelihood of low cognitive performance in the CERAD test.

DISCUSSION

The present study, by using two cycle data from NHANES 2011–2014, firstly showed an inverse correlation between dietary intake of theobromine and low cognitive performance in older adults. In the CERAD test, the dietary intakes of theobromine (total, chocolate, coffee, and cream) were inversely correlated with low cognitive performance. However, no prominent correlations were noted in the DSST or animal fluency test. The dose–response relationship analysis revealed an L-shaped relationship between the total dietary intake of theobromine and the likelihood of low cognitive performance in the CERAD test. With an increase in the total dietary intake of theobromine beyond 120 mg/kg, no reduction was noted in the likelihood of low cognitive performance in the CERAD test. In the stratified analysis (by sex), a higher level of total theobromine intake was correlated with a lower likelihood of low cognitive performance in the CERAD test in men but not in women.

Several studies have reported similar findings. For example, Yoneda et al revealed that orally administered theobromine upregulated the cAMP/CREB/BDNF signaling pathway and augmented motor learning in mice by functioning as a phosphodiesterase inhibitor in the brain.[17] Mendiola-Precoma et al demonstrated that at dose of 30 mg per liter of drinking water, theobromine restored A1 receptor expression, decreased Aβ expression level, and improved cognitive functions in a rat model of AD.[18] The present results are partially consistent with those of relevant epidemiological studies. Travassos et al studied patients with MCI and those with AD and indicated that theobromine activity in the CSF and plasma was correlated with a relatively low expression level of Aβ42 in the CSF, indicating that theobromine might have a protective role in AD.[25] In their parallel-group randomized trial, Sumiyoshi et al reported that dark chocolate—the main source of theobromine in the human diet—improves human cognitive performance.[30] However, in their study including a total of 24 healthy volunteers, Mitchell et al. demonstrated that cognitive function remained unaffected after theobromine intake at various nutritionally relevant doses.[26]

Table 3. Weighted ORs and 95% CIs of low cognitive performance according to the quintiles of dietary total theobromine intake, stratified by sex, NHANES 2011–2014

	Number of subjects	Animal fluency test			CERAD test		
		Crude[†] OR (95%CI)	Model 1[‡] OR (95%CI)	Model 2[§] OR (95%CI)	Crude[†] OR (95%CI)	Model 1[‡] OR (95%CI)	Model 2[§] OR (95%CI)
Male, mg/d							
Q1: <8.0	139/723	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 8.0 to <21.5	149/723	1.01 (0.39-2.57)	1.02 (0.39-2.63)	1.23 (0.50-3.02)	0.86 (0.43-1.72)	0.86 (0.43-1.72)	0.98 (0.44-2.17)
Q3: 21.5 to <42.5	145/723	0.48 (0.22-1.08)	0.43 (0.19-0.99)*	0.49 (0.17-1.35)	0.82 (0.41-1.65)	0.81 (0.41-1.62)	0.90 (0.41-1.97)
Q4: 42.5 to <85.5	145/723	0.55 (0.21-1.45)	0.49 (0.20-1.19)	0.58 (0.24-1.38)	0.74 (0.36-1.52)	0.73 (0.35-1.51)	0.78 (0.35-1.74)
Q5: ≥85.5	145/723	0.50 (0.21-1.19)	0.46 (0.19-1.11)	0.63 (0.23-1.69)	0.25 (0.14-0.47)**	0.25 (0.14-0.47)**	0.25 (0.12-0.51)**
p-trend		0.057	0.042	0.223	< 0.001	< 0.001	< 0.001
Female, mg/d							
Q1: <6.5	149/799	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 6.5 to <18.5	162/799	0.76 (0.50-1.16)	0.74 (0.48-1.15)	0.71 (0.40-1.25)	0.83 (0.46-1.48)	0.81 (0.45-1.46)	0.81 (0.39-1.66)
Q3: 18.5 to <37.0	162/799	0.69 (0.38-1.24)	0.61 (0.33-1.14)	0.65 (0.34-1.24)	0.63 (0.33-1.22)	0.60 (0.31-1.16)	0.68 (0.32-1.45)
Q4: 37.0 to <71.5	166/799	0.50 (0.28-0.91)*	0.45 (0.25-0.82)*	0.54 (0.28-1.02)	0.61 (0.30-1.22)	0.58 (0.29-1.18)	0.74 (0.33-1.68)
Q5: ≥71.5	160/799	0.70 (0.39-1.25)	0.65 (0.36-1.16)	0.71 (0.36-1.41)	0.74 (0.32-1.73)	0.72 (0.31-1.68)	0.88 (0.42-1.87)
p-trend		0.240	0.158	0.423	0.531	0.500	0.944

	DSST test		
	Crude[†] OR (95%CI)	Model 1[‡] OR (95%CI)	Model 2[§] OR (95%CI)
Male, mg/d			
Q1: <8.0	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 8.0 to <21.5	0.72 (0.36-1.44)	0.72 (0.36-1.45)	0.96 (0.47-1.94)
Q3: 21.5 to <42.5	0.79 (0.39-1.62)	0.77 (0.39-1.53)	1.02 (0.42-2.47)
Q4: 42.5 to <85.5	0.82 (0.35-1.90)	0.80 (0.35-1.85)	1.16 (0.44-3.08)
Q5: ≥85.5	0.39 (0.17-0.93)*	0.39 (0.17-0.89)*	0.61 (0.24-1.55)
p-trend	0.029	0.023	0.206
Female, mg/d			
Q1: <6.5	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2: 6.5 to <18.5	0.65 (0.38-1.12)	0.63 (0.37-1.07)	0.61 (0.30-1.26)
Q3: 18.5 to <37.0	0.90 (0.48-1.67)	0.82 (0.45-1.51)	1.22 (0.54-2.77)
Q4: 37.0 to <71.5	0.81 (0.34-1.92)	0.75 (0.31-1.84)	1.34 (0.49-3.70)
Q5: ≥71.5	0.73 (0.31-1.71)	0.69 (0.29-1.64)	1.21 (0.39-3.73)
p-trend	0.693	0.627	0.395

CERAD: Consortium to Establish a Registry for Alzheimer's disease Word Learning sub-test; DSST: Digit Symbol Substitution Test.
[†]Crude model did not adjust any confounders
[‡]Model 1 adjusted for age, sex, race, marital status, and educational level
[§]Model 2 adjusted for age, sex, race, marital status, educational level, alcohol drinking, smoking status, hypertension, diabetes, heart disease, and total daily energy intake
*p<0.05; **p<0.01

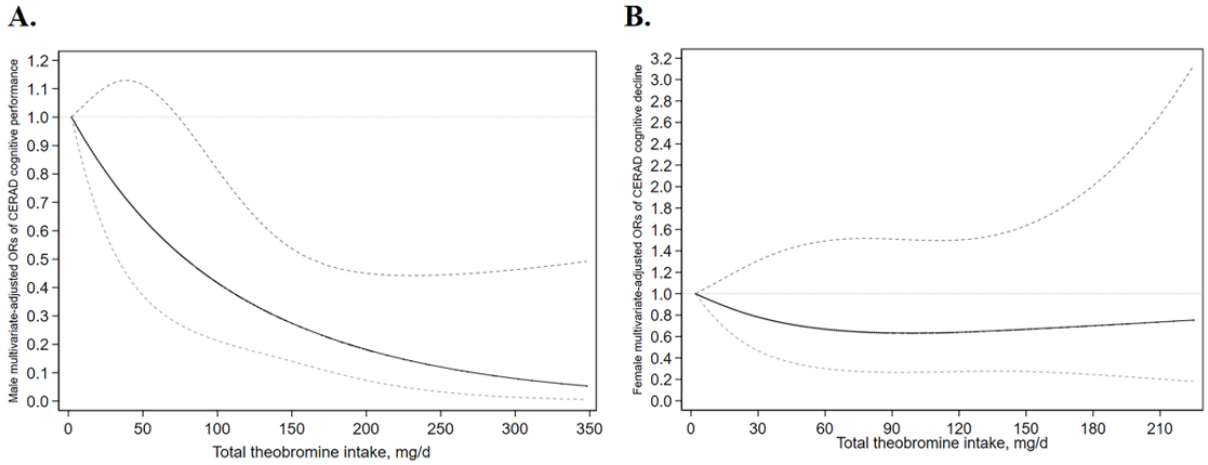


Figure 4. Dose–response relationship between the total dietary intake of theobromine and the likelihood of low cognitive performance in CERAD test after sex-based stratification. (A) Correlation between the total dietary intake of theobromine and the likelihood of low cognitive performance in the CERAD test for men. (B) Correlation between the total dietary intake of theobromine and the likelihood of low cognitive performance in the CERAD test for women.

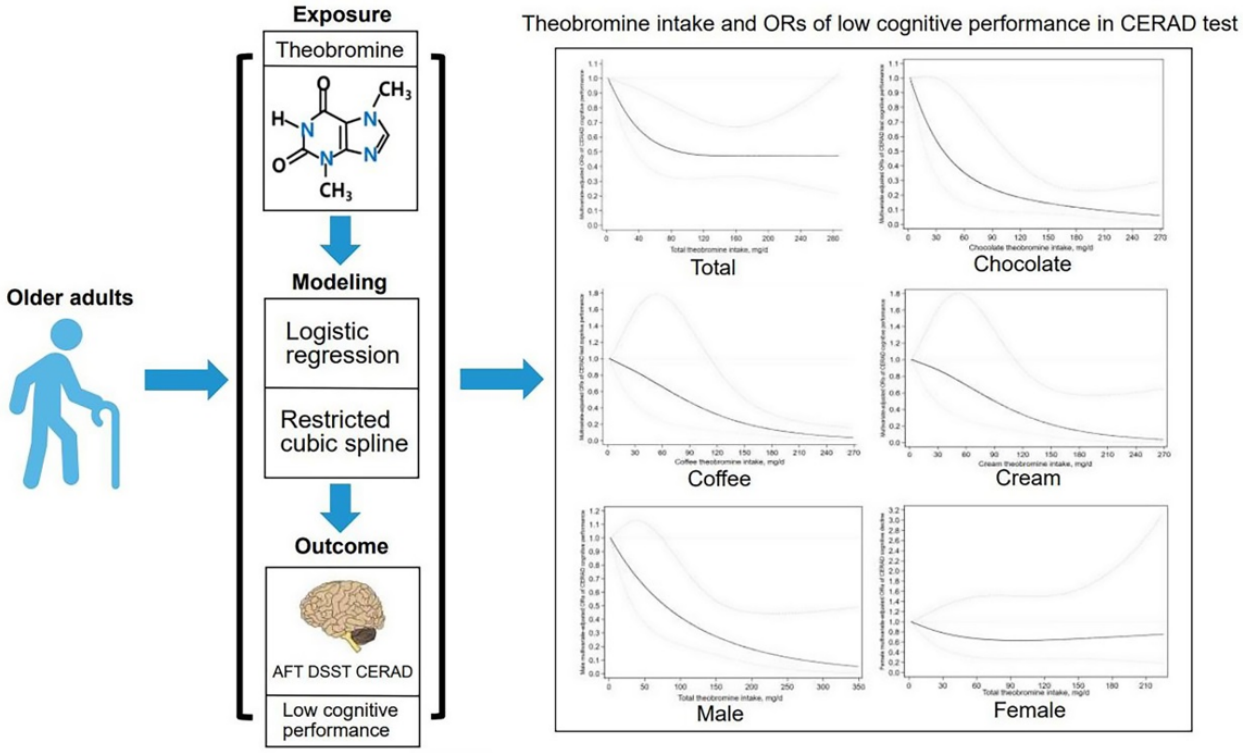
Although the mechanisms underlying the correlation between dietary theobromine intake and low cognitive performance remain unclear, several hypotheses have been proposed. First, owing to its antioxidant property, theobromine may help restore the expression of A1 purinergic receptor in the hippocampus.[18] If the expression level of A1 purinergic receptors are reduced in the hippocampus, their function in inhibiting A2A excitotoxicity are reduced, which leads to the increased formation of Aβ.[31,32] Second, theobromine can traverse the blood–brain barrier and enter the plasma and cerebral.[17] In the brain, theobromine functions as a phosphodiesterase inhibitor and increases the concentrations of intracellular cAMP.[17] Increased expression level of cAMP induce CREB through protein kinase A, and in turn, BDNF is releasesed.[33-35] The cAMP/CREB/BDNF pathway mediates synaptic plasticity, such as long-term potentiation, which facilitates learning and memory.[17,34] Third, theobromine upregulates the CaMKII pathway in the medial prefrontal cortex. CaMKII phosphorylates the transcription factor CREB and transforms it into its active form—phosphorylated CREB, which initiates the transcription and translation of proteins/receptors essential for neuronal plasticity. Theobromine-induced improvements in learning ability and memory are positively correlated with the expression levels of memory-related substrates p-CaMKII and p-CREB.[36-38]

The present study has several advantages. First, to our knowledge, this study is the first to explore the correlation between dietary theobromine intake and low cognitive performance in older adults in the United States. Second, this study showed the dose–response relationships between the dietary intake of theobromine (total and that from chocolate, coffee, and cream) and the likelihood of low cognitive performance. Third, a large nationally representative sample of older adults in the United States was used in this study, and the NHANES results were of high quality owing to the survey method and quality control. Finally, a multidimensional assessment approach (including the CERAD test, animal fluency test, and DSST) was adopted in this study to evaluate cognitive performance.

However, the current study also has several limitations. First, because of the cross-sectional design of this study, it was difficult to make causal inferences between dietary theobromine intake and low cognitive performance, which necessitates prospective longitudinal studies in the future. Second, the intake of theobromine from cocoa and tea was not included in the analysis, because such data were available for very few participants. Third, the dietary intake of theobromine (total and that from chocolate, coffee, and cream) might not have completely reflected the participants' long-term intake at the individual level because the relevant data were collected through only two interviews on 24-h dietary recall, which represented acute intake.[39] Fourth, although the current study controlled for a quantity of potential confounders, it could not fully exclude the residual confusion caused by unmeasured confounders cannot be ignored. Finally, through the use of NHANES data, cognitive performance was evaluated at a single time point and in selected domains; therefore, the results neither substituted a diagnosis based on clinical examinations nor covered all domains of cognitive performance.

Conclusions

The current cross-sectional study suggests that the dietary intakes of theobromine (total and that from chocolate, coffee, and cream) and the likelihood of low cognitive performance in the CERAD test in older adults, particularly men, in the United States. Furthermore, an L-shaped dose–response relationship was noted between the total dietary intake of theobromine and the likelihood of low cognitive performance in the CERAD test. This may have important implications for protecting the elderly against low cognitive performance, especially in immediate and delayed abilities to learn new information, in older adults. Future prospective cohort studies are warranted to discover the actual effects of theobromine on the cognitive performance of older adults and elucidate the underlying mechanisms.



Graphical abstract

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AUTHOR DISCLOSURES

No potential conflict of interest was reported by the authors.

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