The relation of dietary choline to cognitive performance and white-matter hyperintensity in the Framingham Offspring Cohort[1–4]

Coreyann Poly, Joseph M Massaro, Sudha Seshadri, Philip A Wolf, Eunyoung Cho, Elizabeth Krall, Paul F Jacques, and Rhoda Au0

ABSTRACT

Background: Choline is the precursor to the neurotransmitter acetylcholine. Loss of cholinergic neurons is associated with impaired cognitive function, particularly memory loss and Alzheimer disease (AD). Brain atrophy and white-matter hyperintensity (WMH) are also associated with impaired cognitive function and AD.

Objective: The objective was to determine whether a relation exists between dietary choline intake, cognitive function, and brain morphology in a large, nondemented community-based cohort.

Design: A dementia-free cohort of 1391 subjects (744 women, 647 men; age range: 36–83 y; mean ± SD age: 60.9 ± 9.29 y) from the Framingham Offspring population completed a food-frequency questionnaire administered from 1991 to 1995 (exam 5; remote intake) and from 1998 to 2001 (exam 7; concurrent intake). Participants underwent neuropsychological evaluation and brain MRI at exam 7. Four neuropsychological factors were constructed: verbal memory (VM), visual memory (VsM), verbal learning, and executive function. MRI measures included WMH volume (WMHV).

Results: Performance on the VM and VsM factors was better with higher concurrent choline intake in multivariable-adjusted models for VM (average change in neuropsychological factor per 1-unit change in choline = 0.60; 95% CI: 0.29, 0.91; $P < 0.01$) and VsM (0.66; 95% CI: 0.19, 1.13; $P < 0.01$). Remote choline intake was inversely related to log-transformed WMHV (average change in log WMHV per 1-unit change in choline = −0.05; 95% CI: −0.10, −0.01; $P = 0.02$). Furthermore, an inverse association was observed between remote higher choline intake and presence of large WMVH (OR: 0.56; 95% CI: 0.34, 0.92; $P = 0.01$).

Conclusion: In this community-based population of nondemented individuals, higher concurrent choline intake was related to better cognitive performance, whereas higher remote choline intake was associated with little to no WMHV. *Am J Clin Nutr* 2011;94:1584–91.

INTRODUCTION

The global prevalence of AD[5] is predicted to quadruple by 2050 to >100 million, at which time 1 in 85 persons worldwide will be living with the disease (1, 2). More than 40% of those cases will be in late-stage AD, which causes a significant burden to caregivers because activities of daily living decline in this demented population (2). Previous epidemiologic studies have shown strong associations between generalized brain atrophy and increased WMH to cognitive impairment and AD (3–8). Cognitive impairments that precede the onset of AD have been related to alterations in brain neurotransmission systems, mainly cholinergic deficits (9, 10). The cause of these morphologic changes, which lead to cognitive deterioration and are associated with this disease, remains unknown.

Choline is an essential nutrient necessary for several biological functions in the human body. In addition to being the precursor to acetylcholine, choline also serves as the precursor to sphingomyelin and phosphatidylcholine—structural components of cell membranes. When oxidized, choline forms the methyl donor betaine for the conversion of homocysteine to methionine (11–13). High homocysteine concentrations have been shown to be related to both cognitive impairments in nondemented samples and an increased risk of AD (14, 15). The US Institute of Medicine has estimated an AI of choline of 550 mg/d for men and 425 mg/d for women (11).

The neurotransmitter acetylcholine is intricately connected with the cholinergic neural networks associated with memory

[1] From the Department of Neurology, Boston University School of Medicine, Boston, MA (CP, SS, and RA); the Department of Biostatistics, Boston University School of Public Health, Boston, MA (JMM); the Department of Mathematics & Statistics, Boston University, Boston, MA (JMM); the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (PAW and EC); the Department of Health Policy & Health Services Research, Boston University Henry M Goldman School of Dental Medicine, Boston, MA (EK); and the USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA (PFJ).

[2] PFJ and RA are senior authors.

[3] Supported by the National Heart, Lung, and Blood Institute's Framingham Heart Study, the NIH (NIH/NHLBI contract N01-HC-25195), the National Institute on Aging (NIA 5R01-AG 16495 and NIA 5R01-AG08122), National Institute of Neurological Disorders and Stroke (NINDS 5R01-NS17950), and the USDA, Agricultural Research Service (agreement no. 58-1950-7-707).

[4] Address reprint requests and correspondence to R Au, Department of Neurology (Neurological Epidemiology and Genetics Division), Boston University School of Medicine, 715 Albany Street, B-608, Boston, MA 02118-2526. E-mail: rhodaau@bu.edu.

[5] Abbreviations used: AD, Alzheimer disease; AI, adequate intake; apo E, apolipoprotein E; EF, executive function; FFQ, food-frequency questionnaire; FSRP, Framingham Stroke Risk Profile; TBV, total brain volume; TCBV, total cranial brain volume; TCV, total cranial volume; TE, echo time; VL, verbal learning; VM, verbal memory; VsM, visual memory; WMH, white-matter hyperintensity; WMHV, white-matter hyperintensity volume.

Received November 26, 2010. Accepted for publication September 2, 2011.
First published online November 9, 2011; doi: 10.3945/ajcn.110.008938.

(16) and is synthesized from choline and acetyl-CoA through the action of choline acetyltransferase. Neurons obtain choline from 2 sources: uptake from serum choline mainly derived from dietary intake and de novo synthesis (17). The loss of cholinergic neurons and choline acetyltransferase activity is consistent with abnormalities in AD and is thought to contribute to the learning and memory deficits associated with AD (16–18). Adequate concentrations of choline in the brain are believed to protect against age-related cognitive decline and certain types of dementia, including AD (19, 20) because adequate concentrations potentially preserve neurons, brain volume, and neuronal transmissions.

In animal models, prenatal choline supplementation has led to an improvement in memory function in rats. The behavioral effects of prenatal choline-supplemented rats were long-lasting and persisted beyond the age of 2 y—an age at which a rat is developmentally old. Thus, prenatal supplementation with choline seems protective against normally observed memory decline due to old age (21, 22). Other studies examined the effects of choline supplementation on the cognitive impairment of aged rats and also found that supplementation attenuates age-related cognitive deficits (23, 24).

Most human evidence comes from pharmacologic research that has shown cognitive improvement in mild-to-moderate AD after choline treatment. The loss of cholinergic function in the hippocampus and neocortex is evident in AD. It was therefore hypothesized that choline precursor loading may offer therapeutic benefit to those who suffer with this progressive neurologic degenerative disorder. The pharmacologic therapies tested include intervention with acetylcholine precursors, stimulation of acetylcholine release, and use of muscarinic or nicotinic receptor agonists and acetylcholinesterase. Treatment goals included improvement in cognitive function, control of behavioral disturbances, and slowing down the progression of the disease.

However, only temporary improvement has been found with these treatments (20, 25, 26). The relation between dietary choline intake and cognition in a relatively young, well-nourished population, where potential cases of dementia are still in the preclinical stage, are unknown. Human studies are needed to determine the effects of dietary choline on cognition and brain morphology.

Given the role choline plays in brain function and the protective effects of choline against age-related cognitive deficits shown in animal models, we postulate that lower dietary choline intake may be related to deficit performance on neuropsychological tests and to brain structures independent of other dietary factors. The relation between dietary choline intake, cognition, and brain morphology in a nondemented population has not been examined to our knowledge.

SUBJECTS AND METHODS

Subjects

The Framingham Heart Study Offspring Study cohort, recruited in 1971, has undergone periodic examinations for >30 y to identify risk factors for cardiovascular disease and stroke (27, 28). From 1999 to 2001, surviving members of this cohort were invited to participate in a call-back study on cognition and brain imaging. Of the 2187 participants who agreed to enroll in the study, 1889 were administered a neuropsychological test battery and had a brain MRI scan. In addition, for inclusion in the analysis, participants were required to have completed the Harvard FFQ administered from 1991 to 1995 (exam 5) and again from 1998 to 2001 (exam 7). Exclusion criteria included prevalent dementia, clinical stroke, multiple sclerosis, or certain other neurologic conditions (n = 69), those who did not have complete and reliable FFQs at exam 7 (n = 429), and those who did not have both a neuropsychological test battery and brain MRI scan (n = 298). After all exclusions, our sample of participants with completed the FFQ, the neuropsychological test battery, and the brain MRI totaled 1391 (744 women, 647 men; age range: 36–83 y; mean ± SD age: 60.9 ± 9.29 y.)

Measurement of dietary choline

The Harvard FFQ is a well-validated instrument (29, 30). FFQs provide a relatively simple, cost-effective method for assessing habitual dietary intake over a specified period of time. In the current study, participants were asked to report how often they had eaten each particular food from a standard list of foods for the prior 12-mo period.

Studies have shown that choline intake was associated with plasma choline concentrations (31–33). Cho et al (34) showed that choline intake from the 131 food item Harvard FFQ was predictive of fasting homocysteine concentrations under conditions of low folate intake, providing validation of dietary choline intake derived from this FFQ.

A nutrient database was derived from the responses on the FFQs and included the composition of choline-contributing compounds (free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin). Total choline intake was calculated by summing these compounds.

Participants were excluded from further analysis if reported energy intakes were <2.51 MJ/d (600 kcal/d) or >16.74 MJ/d (4000 kcal/d) for women and >17.57 MJ/d (4200 kcal/d) for men or if ≥12 food items were left blank on the questionnaire. Participants who met the energy intake criteria and had <12 blank items were included in analyses and were considered to be nonconsumers of the blank items. Line items that contributed largely to total choline intake included eggs, meat, bread, and dairy products. We used dietary choline intake at exam 7 to examine the association between concurrent choline intake, cognitive function, and brain morphology and choline intake at exam 5 to examine the association of remote choline intake on cognitive function and brain morphology.

Neuropsychological tests

Neuropsychological tests were administered to participants according to standard protocols. Because the protocol consists of numerous tests, a factor analysis was conducted by using criteria such as eigenvalues to identify domain-specific factors. Within a sex, each of the neuropsychological measures (log transformed as necessary) was regressed onto age and education (group), and the residuals were standardized to have a mean of 0 and an SD of 1. Similar results were obtained if the factor analysis was repeated restricting to those participants aged ≥65 y. Within each factor, loadings on each of the variables were similar, so the variables were summed to create the factor composite scores. As

an example, factor 1 is a linear combination of logical memory-immediate and logical memory-delayed, with nearly identical coefficients; therefore, we defined factor 1 as the sum of the 2 coefficients. Finally, we applied z score transformations to the factors.

After the factor analysis, 4 factors were identified for which, within each factor, the variable loadings were similar to each other or near 0 and were reflective of the cognitive domain. Thus, it was decided to sum the variables with the non-zero loadings to create the factors. So, for example, factor 1 is the sum of logical memory-immediate and logical memory-delayed standardized scores. The 4 domain-specific factors and the individual test measures that compose each (35–40) are listed in **Table 1**.

Brain MRI measures

The MRI measures used were previously described (34). In summary, subjects received brain imaging from a Magnetom 1-T field strength machine (Siemens). T2-weighted sequences were performed with double spin-echo coronal imaging, 4-mm contiguous slices from nasion to occiput with a repetition time of 2420 ms, a TE of TE1 20/TE2 90 ms, an echo train length of 8, a field of view of 22 cm, and an acquisition matrix of 192_256 interpolated to 256_256 with excitation. Images were analyzed and interpreted blindly to subject data and in random order by using a custom-designed image analysis package.

TCBV

DeCarli et al (42) described the quantification of TCBV. Briefly, brain volume was determined by manually outlining the intracranial vault to determine TCV. Once nonbrain elements from the image were removed, mathematical modeling was performed to determine TBV. The ratio of TBV to TCV (TCBV) was used for this analysis.

WMHV

WMH measures for the Framingham Heart Study were previously published (3, 5, 43). WMHV was expressed as a proportion of TCV to correct for head size (WMHV = WMH/TCV), and this value was designated as WMHV. The distribution of WMHV was markedly skewed; hence, natural log transformation was applied for all regression analyses. We used log-transformed WMHV as a continuous variable in our multilinear regression models assessing the relation of WMHV to choline intake. We further dichotomized WMHV into nonlarge and large WMHV (3, 43). Participants were categorized by age group and determined as having large WMHV when the residual from a regression of natural log-transformed WMHV compared with age was >1 SD of the mean residual for the participant's age group. In our multivariate logistic regression models relating WMHV to choline intake, we used large WMH (yes or no) as a dichotomous outcome.

Statistical analyses

The analyses focused on assessing the relation between neuropsychological factors, WMHV, and TCBV measured at exam 7 compared with choline intake measured at each of exams 5 and 7. Variables are presented as means $\pm$ SDs (continuous) or number and percentage (categorical). In multivariate linear regression models, we used the natural log-transformed values for choline intake, which provided the best-fitting model for the analyses in which they were treated as continuous variables.

Age and sex-adjusted linear regression models were first used to test the relation between each neuropsychological factor (primary dependent variable) and log-transformed dietary choline intake as measured by the FFQ (primary independent variable), followed by multivariable-adjusted linear regression. Candidates for entry as covariates into the multivariable models were determined based on current literature for those variables that affect cognitive function, brain morphology, and/or the metabolic functions of choline in the body. Variables that were candidates for entry into the model were age, sex (age and sex were forced into the model), education, BMI, homocysteine concentration, apo E, the FSRP (a composite score of cardiovascular disease risk factors that predict the 10-y probability of a stroke), and total energy, saturated fat, vitamin B-12, and vitamin B-6 intakes (15, 44–54). A level of entry and stay of 0.05 was used to develop the stepwise model.

A similar regression approach was used to test the multivariable-adjusted linear trend of each of TCBV and log-transformed WMHV

TABLE 1
Neuropsychological test battery

Cognitive factors	Cognitive domains assessed	Performance measures	Unstandardized range
Factor 1: verbal memory			
WMS[1] logical memory	Verbal memory	Immediate recall	0–24
		Delayed recall	0–24
Factor 2: visual memory			
WMS visual reproductions	Visual memory	Immediate recall	0–14
		Delayed recall	0–14
Factor 3: verbal learning			
WMS paired associates	Verbal learning	Total score at immediate recall	0–21
		Total score at delayed recall	0–21
Factor 4: executive function			
Trail Making Test A[2]	Attention	Time to completion in seconds	0–300
Trail Making Test B[2]	Executive function	Time to completion in seconds	0–300

[1] WMS, Wechsler Memory Scale.
[2] Halstead Reitan Trail Making Test.

(dependent variables) across quartiles of log-transformed dietary choline intake. As secondary analyses, we used similar multivariate-adjusted linear regressions to analyze a linear trend in individual neuropsychological factors across quartiles of choline intake. In separate multivariate logistic regression models, we assessed the trend in WMHV as a dichotomous variable (presence of large WMHV) across quartiles of choline intake.

All statistical analyses were performed by using Statistical Analyses System software 9.1 (SAS Institute). Two-sided P values ≤ 0.05 were considered statistically significant. Given the exploratory nature of the study, no multiple comparison adjustment was made.

RESULTS

Demographics, average choline intake, and other covariate used in the analyses are summarized in **Table 2**. In age- and sex-adjusted models, dietary choline intake at exam 7 (concurrent intake) was positively associated with the cognitive factors VM (adjusted average change in neuropsychological factor per 1-unit change in choline = 0.55; 95% CI: 0.25, 0.85; $P < 0.01$), VsM (0.35; 95% CI: 0.05, 0.64; $P = 0.02$), and VL (0.51; 95% CI: 0.21, 0.80; $P < 0.01$), but not with EF ($P = 0.23$). In multivariate-adjusted models, choline intake at exam 7 remained significantly related to VM (0.60; 95% CI: 0.29, 0.91; $P < 0.01$) and VsM (0.66; 95% CI: 0.19, 1.13; $P < 0.01$), but not to VL ($P = 0.48$) or EF ($P = 0.27$; **Table 3**). No significant association was observed between remote choline intake (exam 5) and neuropsychological factors (data not shown).

To further investigate the association between exam 7 neuropsychological factors and exam 7 choline intake, multivariate-adjusted linear regressions were performed to assess the linear trend in individual neuropsychological items across choline quartiles. These models showed that a higher choline intake was significantly and positively related to VM in both the immediate (adjusted average change across choline quartiles = 0.28, 95% CI: 0.05, 0.51; $P = 0.02$) and delayed (0.30; 95% CI: 0.13, 0.46; $P < 0.01$) recall. Higher choline intake was also significantly positively related to VsM in both the immediate (0.25; 95% CI: 0.05, 0.45; $P = 0.01$) and delayed (0.26; 95% CI: 0.05, 0.47; $P = 0.01$) recall. We also noted an inverse association between the EF Trail Making Test A and higher choline intake (−0.01; 95% CI: −0.03, 0.00; $P = 0.05$). No significant relation was found between choline and the EF Trail Making Test B ($P = 0.32$; **Table 4**).

No significant association between TCBV and choline intake was found at exam 5 ($P = 0.82$) or exam 7 ($P = 0.32$). Log-transformed WMHV was significantly and inversely related to choline intake at exam 5 in multivariate-adjusted models (adjusted average change in log-transformed WMHV across choline quartiles = −0.05; 95% CI: −0.10, −0.01; $P = 0.02$. However, no such significant relation between WMHV and choline was seen for concurrent exam 7 choline intake ($P = 0.29$; **Table 5**).

A significant inverse relation was observed between large WMHV and higher choline intake at exam 5. For example, the multivariable adjusted OR for large WMHV for choline quartile 4 compared with quartile1 is 0.56 (95% CI: 0.34, 0.92; $P = 0.01$ for linear trend across all 4 quartiles; **Table 6**). No other significant relations were observed for brain measures and choline intake at exam 5 or exam 7. The results were unaffected by age or sex (interaction age-by-choline intake and sex-by-choline intake P values ≥ 0.2).

TABLE 2
Characteristics and risk factors of the study sample ($n = 1391$) and average choline intake

Characteristic	Value
Female (%)	53.4
Age at neuropsychological exam (y)	60.8 ± 9.3[1]
Education (%)	
At most some high school	0.2
High school graduate	2.5
Some college or vocational	31.6
College graduate/postgraduate	65.6
BMI (kg/m^2)	27.8 ± 5.1
Total energy (kcal/d)	1857.9 ± 595.9
Saturated fat (g/d)	22.4 ± 10.1
Folate intake (μg/d)	605.3 ± 300.6
Vitamin B-6 intake (mg/d)	8.4 ± 24.0
Vitamin B-12 intake (μg/d)	13.1 ± 24.8
Average choline intake, exam 5 (mg/d)	322.7 ± 106.2
Average choline intake, exam 5 (log)	5.7 ± 0.3
Average choline intake, exam 7 (mg/d)	321.1 ± 105.2
Average choline intake, exam 7 (log)	5.7 ± 0.3
Homocysteine (μmol/L)	8.3 ± 3.1
Framingham Stroke Risk Profile[2]	0.1 ± 0.1
APOE gene	0.2 ± 0.4

[1] Mean ± SD (all such values).
[2] Provides the estimated stroke risk over the subsequent 10-y period.

DISCUSSION

In this study, the principal findings show that better memory performance is related to a higher concurrent choline intake (exam 7), whereas remote choline intake (exam 5) is associated with a significant inverse relation to larger WMH in a large, nondemented, community-based population.

VM and VsM were found to be strongly associated with choline intake in both age- and sex-adjusted models as well as final models. Dietary choline intake was significantly associated with verbal learning in an age- and sex-adjusted model, but lost its significance when saturated fat was added into the model. EF was not related to choline intake in covariate-adjusted models. Further investigation of the individual cognitive tests for each factor confirmed a significant positive association between choline intake and VM and VsM. Memory impairment is a hallmark sign of AD (2, 4, 8, 13, 18, 55). Preservation of the neurologic pathways associated with memory may be key in preventing adverse morphologic changes in the brain that lead to AD.

WMHs are patchy areas with increased signal on T2-weighted and fluid-attenuated inversion recovery MRI sequences of the brain and seen in up to 90% of persons with vascular dementia and AD. Researchers have found that subjects with large WMHV had significantly poorer cognitive function and brain atrophy (7, 43, 54). Large amounts of WMH are, therefore, pathologic in nature and prevention is important. Whereas WMH is thought to be a measure of subclinical vascular disease and thus a potential biomarker of vascular dementia (55), researchers have also shown that changes in white matter are also present in up to 70% of persons with AD (3, 47, 56).

Our findings show that early higher choline intake is significantly related to smaller WMHV, which suggests that choline

TABLE 3
Results of 4 domain-specific cognitive factors in stepwise linear regression of neuropsychological factor compared with continuous log-transformed choline adjusted for age and sex and significant covariates at exam 7[1]

Domain-specific factors	Age- and sex-adjusted: regression coefficient (95% CI)[2]	*P* value[3]	Final models: regression coefficient (95% CI)[2]	*P* value[3]
Verbal memory	0.55 (0.25, 0.85)	<0.01	0.60 (0.29, 0.91)	<0.01
Visual memory	0.35 (0.05, 0.64)	0.02	0.66 (0.19, 1.13)	<0.01
Verbal learning	0.51 (0.21, 0.80)	<0.01	0.14 (−0.23, 0.51)	0.48
Executive function	0.16 (−0.09, 0.42)	0.23	0.14 (−0.11, 0.39)	0.27

[1] Candidates for entry included age, sex, education, BMI, homocysteine concentration, apolipoprotein E, Wide Range Achievement Test, the Framingham Stroke Risk Profile, and total energy, saturated fat, vitamin B-12, and vitamin B-6 intakes.
[2] Regression coefficient represents the change in the factor (created from summing individual standardized test items) score per 1-unit change in log choline.
[3] Significance indicated at $P \leq 0.05$.

intake at midlife may be neuroprotective. The mechanism by which choline affects WMH is unknown. Yoshita et al (6) report that an increase in WMH is positively associated with cognitive impairment and AD. In another prospective cohort study, WMHV was determined to be a risk factor for the incidence of mild cognitive impairment (5). Possible biological mechanisms underlying our findings include choline's role as the precursor to the neurotransmitter acetylcholine, which is essential to normal cognition and brain function (17). Some evidence indicates that acetylcholine depletion occurs in AD and contributes to the cognitive decline observed in AD (9, 13, 16, 57).

Animal studies have shown that choline supplementation is neuroprotective. Researchers have shown that prenatal supplementation of choline improved memory function in rats well into adulthood (21, 22, 58). Teather and Wurtman (23) examined the effects of dietary supplementation of cytidine (5′)-diphosphocholine, a source of choline, on memory impairment in aged rats and found supplementation to be protective against age-related memory deficits.

Choline's metabolites are important for the structural integrity of cell membranes and for cholinergic transmission and signaling during the development of neuron cells (57). Several studies suggest that pharmacologic doses of choline and choline derivatives may have clinical efficacy in elderly patients with cognitive deficits, inefficient memory, and early-stage AD (59–61). Magil et al (31) showed that dietary intake of choline in the form of lecithin elevated blood choline, brain choline, and brain acetylcholine concentrations significantly (31).

Neuropathologic studies of brains showed reductions in cortical cholinergic markers correlated significantly with AD (9, 13, 62). The postmortem samples of people who have died of AD show an accelerated rate in turnover of membrane

TABLE 4
Relation between dietary choline intake and individual adjusted mean (2-sided 95% CI) neuropsychological measures of significant neuropsychological measure factors at exam 7[1]

	Choline intake quartile					
Domain-specific factors	1	2	3	4	Average change (95% CI) across quartiles	*P* value[2]
Verbal memory						
Immediate recall[3]	11.0 (10.6, 11.4)	11.5 (11.2, 11.8)	11.7 (11.4, 12.0)	11.9 (11.4, 12.2)	0.28 (0.05, 0.51)	0.02
Delayed recall[4]	10.1 (9.7, 10.4)	10.6 (10.3, 11.0)	10.8 (10.5, 11.2)	11.0 (10.6, 11.4)	0.30 (0.13, 0.46)	<0.01
Visual memory						
Immediate recall[5]	8.8 (8.4, 9.1)	8.9 (8.6, 9.2)	9.3 (9.0, 9.6)	9.4 (9.1, 9.8)	0.25 (0.05, 0.45)	0.01
Delayed recall[5]	7.8 (7.4, 8.2)	8.1 (7.8, 8.4)	8.5 (8.2, 8.8)	8.5 (8.1, 8.9)	0.26 (0.05, 0.47)	0.01
Verbal learning						
Immediate recall[6]	13.8 (13.5, 14.2)	13.9 (13.6, 14.2)	14.1 (13.8, 14.4)	13.9 (13.6, 14.3)	0.05 (−0.12, 0.23)	0.55
Delayed recall[6]	8.3 (8.2, 8.5)	8.2 (8.1, 8.4)	8.3 (8.2, 8.5)	8.4 (8.2, 8.6)	0.04 (−0.04, 0.11)	0.38
Executive function						
Trail Making Test A[7]	0.57 (0.54, 0.59)	0.54 (0.52, 0.56)	0.53 (0.51, 0.55)	0.52 (0.50, 0.55)	−0.01 (−0.03, 0.00)	0.05
Trail making Test B[8]	1.37 (1.31, 1.43)	1.34 (1.28, 1.40)	1.31 (1.25, 1.37)	1.33 (1.27, 1.39)	−0.01 (−0.04, 0.01)	0.32

[1] Data derived from the final multivariate linear regression model. Candidates for entry included age, sex, education, BMI, apolipoprotein E, Wide Range Achievement Test, the Framingham Stroke Risk Profile, and intakes of total energy, saturated fat, vitamin B-12, vitamin B-6, and homocysteine.
[2] *P* value is based on a linear regression of continuous item score compared with continuous natural log-transformed choline. Significance indicated at $P \leq 0.05$.
[3] Adjusted for age, sex, education, BMI, energy and saturated fat intakes, and Wide Range Achievement Test.
[4] Adjusted for age, sex, education, BMI, folate intake, apolipoprotein E, and Wide Range Achievement Test.
[5] Adjusted for age, sex, education, energy intake, Framingham Stroke Risk Profile, and Wide Range Achievement Test.
[6] Adjusted for age, sex, saturated fat intake, and Wide Range Achievement Test.
[7] Adjusted for age, sex, education, energy intake, homocysteine concentration, and Framingham Stroke Risk Profile.
[8] Adjusted for age, sex, education, homocysteine concentration, apolipoprotein E, Framingham Stroke Risk Profile, and Wide Range Achievement Test.

TABLE 5
Relation between dietary choline intake and covariate-adjusted mean (2-sided 95% CI) TCBV and WMHV at exams 5 and 7 (n = 1414)[1]

	Choline quartile					
Final models exam	1	2	3	4	Average change across quartiles	P value
Exam 5						
Natural log(WMHV)[2]	−7.52 (−7.62, −7.42)	−7.65 (−7.75, −7.55)	−7.60 (−7.69, −7.50)	−7.71 (−7.81, −7.61)	−0.05 (−0.10,-0.01)	0.02
TCBV[3]	79.5 (79.2, 79.8)	79.5 (79.2, 79.8)	79.4 (79.1, 79.7)	79.6 (79.3, 79.9)	0.02 (−0.12, 0.15)	0.82
Exam 7						
Natural log(WMHV)[2]	−7.62 (−7.72, −7.52)	−7.54 (−7.64, −7.44)	−7.65 (−7.75, −7.55)	−7.67 (−7.77, −7.57)	−0.02 (−0.07, 0.02)	0.29
TCBV[4]	79.6 (79.3, 79.9)	79.5 (79.2, 79.8)	79.5 (79.2, 79.8)	79.4 (79.2, 79.7)	−0.07 (−0.21, 0.07)	0.32

[1] P values were based on a linear regression of continuous volume compared with continuous natural log-transformed choline. Significance indicated at $P \leq 0.05$. TCBV, total cranial brain volume; WMHV, white-matter hyperintensity volume.
[2] Adjusted for age and sex.
[3] Adjusted for age, sex, BMI, vitamin B-12, and the Framingham Stroke Risk Profile.
[4] Adjusted for age, sex, BMI, folate, and Framingham Stroke Risk Profile.

phosphatidylcholine and decreased choline availability throughout the brain. It has been suggested that cholinergic neurons autocannibalize the choline-containing membrane, thus resulting in their demise (16, 63). Conversely, adequate concentrations of acetylcholine in the brain are believed to be protective against certain types of dementia, including AD.

It is important to note that the reported average dietary choline intake in this study is significantly lower than the Food and Nutrition Board's recommended adequate intake. Therefore, these results may not fully reflect choline's potential role regarding brain preservation and cognitive function. Consequently, to better understand the relation between choline intake and the development of age-related diseases and cognitive impairment, it is important to either have multiple measures of choline intake over the appropriate exposure period and/or show that choline intake is relatively consistent within individuals over time. We therefore assessed the stability of choline intake in this cohort using the same choline-validated instrument. Mean choline intake between exams was virtually identical. Although a small proportion of individuals had relatively large changes over time, the average difference was essentially zero. We also showed that individuals who consumed the highest or lowest intakes at exam 5 also tended to consume these same amounts at exam 7. The correlations of choline compounds and kilocalories that we observed in this study between exams 5 and 7 were comparable with those reported previously in reproducibility studies for other nutrients, in which the questionnaires were administered 1 y apart (29, 30).

Although choline is synthesized in small amounts in the body, studies indicate that additional choline from the diet is needed for normal health (12). These results support the hypothesis that dietary choline intake is neuroprotective over time and promotes improved cognitive function. We posit that an increase in dietary choline intake ensures adequate acetylcholine concentrations for cholinergic neurotransmission and prevents cell breakdown by preserving phosphatidylcholine within the cell membrane as a result of more choline available to cross the blood-brain barrier. This is confirmed by our findings that past choline intake was significantly associated with changes in WMHV in the brain, whereas cognitive function was only affected by concurrent choline intake.

A key strength of this study was the application to a large relatively young and cognitively healthy community-based cohort. However, this study had several limitations that must be considered. Our study focused on TCBV, global brain volume, and did not include examination of medial temporal regions (64, 65) that have been linked to early stages of AD and mild cognitive impairment—a prodromal phase of AD (64, 66). This analysis was based on cross-sectional data and would require further studies to confirm these findings. Cognitive performance data were only available at exam 7 for this offspring cohort; therefore, choline data could not be compared with cognitive measures at

TABLE 6
Adjusted ORs (compared with quartile 1, reference quartile) of large WMHV (dichotomous variable) and choline intake as a continuous variable (n = 1414)[1]

	Choline quartile				
Exam	1	2	3	4	P value
Adjusted for age and sex					
Exam 5	1	0.64 (0.40, 1.04)	0.79 (0.50, 1.25)	0.57 (0.34, 0.93)	0.01
Exam 7	1	1.01 (0.63, 1.63)	0.70 (0.42, 1.17)	1.08 (0.68, 1.74)	0.98
Adjusted for age, sex, and multivariates[2]					
Exam 5	1	0.64 (0.40, 1.04)	0.79 (0.50, 1.25)	0.56 (0.34, 0.92)	0.01
Exam 7	1	1.04 (0.65, 1.66)	0.72 (0.43, 1.20)	1.08 (0.67, 1.74)	0.98

[1] P values were based on a logistic regression of dichotomous volume compared with continuous natural log-transformed choline. Significance indicated at $P \leq 0.05$. WMHV, white-matter hyperintensity volume.
[2] Only the stroke risk profile was significantly related to the outcome of large WMHV and hence is the only covariate that was used in final model to assess the relation of WMHV with choline at exam 5 and exam 7.

exam 5. Also limited by having brain volume measures from only one period of time, we were unable to look at choline's effect on brain volume over a period of time. Another limitation was the lack of a biomarker for choline, such as serum choline concentrations, that might have eliminated the limitation of potential bias related to self-reporting on the FFQs. Finally, the Framingham Offspring cohort consisted primarily of whites, which did not permit generalization of these results to other populations.

Preserving cognitive function and TBV are related to a decreased risk of AD. The goal of this research was to potentially identify a dietary, and therefore modifiable, risk factor for preventing decline in cognitive function and identifying a potential mechanism for decreasing the risk of dementia. Further study is necessary to determine whether an adequate dietary intake of choline is related to improved cognitive function throughout the life span and to determine the role it plays regarding the preservation of brain health.

We thank the study participants for their endless dedication, participation, and commitment to the Framingham Heart Study.

The authors' responsibilities were as follows—PAW and RA: involved in the collection of the outcome measures; PFJ: created the choline measures based on existing dietary data; CP, RA, and PFJ: responsible for the study design; and CP, RA, PFJ, and JMM: responsible for the analysis and interpretation of the data. All authors were responsible for writing and critically revising the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Ferri CP, Prince M, Brayne C, Brodaty H, Fratigioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;366:2112–7.
2. Alzheimer's Association's International Conference on Prevention of Dementia. Newest estimate of worldwide prevalence of Alzheimer's disease = 26.6 million. Washington, DC, June 10, 2007. Available from: http://www.alz.org/preventionconference/pc2007/releases/61007_12am_worldprev.asp (cited January 2005).
3. Au R, Massaro JM, Wolf PA, Young M, Beiser A, Seshadri S, D'Agostino RB, DeCarli C. Association of white matter hyperintensity volume with decreased cognitive functioning. Arch Neurol 2006;63:246–50.
4. Burton EJ, Kenny RA, O'Brien J, Stephens S, Bradbury M, Rowan E, Kalaria R, Firbank M, Wesnes K, Ballard C. White matter hyperintensities are associated with impairment of memory, attention, and global performance in older stroke patients. Stroke 2004;35:1270–5.
5. Smith EE, Egorova S, Blackner D, Killiany RJ, Muzikansky A, Dickerson BC, Tanzi RE, Albert MS, Greenberg SM, Gutmann C. Magnetic imaging white matter hyperintensities and brain volume in the prediction of mild cognitive impairment and dementia. Arch Neurol 2008;65:94–100.
6. Yoshita M, Fletcher E, Harvey D, Ortega M, Martinez O, Mungas DM, Reed BR, DeCarli CS. Extent and distribution of white matter hyperintensities in normal aging, MCI and AD. Neurology 2006;67:2192–8.
7. DeCarli C, Murphy DG, Tranh M, Grady CL, Haxbym JV, Gillette JA, Salerno JA, Gonzales-Aviles A, Horwitz B, Rapoport SI. The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. Neurology 1995;45:2077–84.
8. Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST, Ganguli M. Patterns of cognitive decline in presymptomatic Alzheimer disease: a prospective community study. Arch Gen Psychiatry 2001;58:853–8.
9. Davis KL, Mohs RC, Marin D, Purohit DP, Perl DP, Lantz M, Austin G, Haroutunian V. Cholinergic markers in elderly patients with early signs of Alzheimer disease. JAMA 1999;281:1401–6.
10. Alvarez XA, Laredo M, Corzo D, Fernandez-Novoa L, Mouzo R, Perea JE, Daniele D, Cacbelos R. Citicoline improves memory performance in elderly subjects. Methods Find Exp Clin Pharmacol 1997;19:201–10.
11. Howe JC, Williams J, Holden JM, Zeisel SH, Mar M. USDA database for the choline content of common foods. Home page. 2004. Available from: http://www.nal.usda.gov/fnic/foodcomp (cited March 2006).
12. Zeisel SH, Da Costa KA, Franklin PD, Alexander EA, Lamont JT, Sheard NF, Beiser A. Choline, an essential nutrient for humans. FASEB J 1991;5:2093–8.
13. Hunt S, Groff JL. In: Gomez J, ed. Advanced nutrition and human metabolism. St. Paul, MN: West Publishing Company 1990;221-222:437–8.
14. Elias MF, Sullivan LM, D'Agostino RB, Elias PK, Jacques PF, Selhub J, Seshadri S, Au R, Beiser A, Wolf PA. Homocysteine and cognitive performance in the Framingham Offspring Study; age is important. Am J Epidemiol 2005;162:644–53.
15. Seshadri S, Beiser A, Selhub J, Jacques P, Rosenberg IH, D'Agostino RB, Wilson P, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
16. Davies P. Challenging the cholinergic hypothesis in Alzheimer's disease. JAMA 1999;281:1433–4.
17. Zeisel SH, Blusztajn JK. Choline and human nutrition. Annu Rev Nutr 1994;14:269–96.
18. Muir JL. Acetylcholine, aging, and Alzheimer's disease. Pharmacol Biochem Behav 1997;56:687–96.
19. Tomimoto H, Ohtani R, Shibata M, Nakamura N, Ihara M. Loss of cholinergic pathways in Vascular Dementia of the Binswager Type. Dement Geriatr Cogn Disord 2005;19:282–8.
20. Amenta F, Parnetti L, Gallai V, Wallin A. Treatment of cognitive dysfunction associated with Alzheimer's Disease with cholinergic precursors. Ineffective treatments or inappropriate approaches? Mech Ageing Dev 2001;122:2025–40.
21. Meck WH, Williams CL. Choline supplementation during prenatal development reduces proactive interference in spatial memory. Brain Res Dev Brain Res 1999;118:51–9.
22. Meck WH, Williams CL. Metabolic imprinting of choline by its availability during gestation: implication for memory and attentional processing across the lifespan. Neurosci Biobehav Rev 2003;27:385–99.
23. Teather LA, Wurtman RJ. Dietary (5′)-diphosphocholine supplementation protects against development of memory deficits in aging rats. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:711–7.
24. Dickinson-Anson H, Winkler J, Fisher LJ, Song HJ, Poo M, Gage FH. Acetylcholine-secreting cells improved age-induced memory deficits. Mol Ther 2003;8:51–61.
25. Tayebati SK, Di Tullio MA, Amenta F. Effect of treatment with the cholinesterase inhibitor rivastigine on vesicular acetylcholine transporter and choline acetyltransferase in rat brain. Clin Exp Hypertens 2004;26:363–73.
26. Parnetti L, Amenta F, Gallai V. Choline alphoscerate in cognitive decline and in acute cerebrovascular disease: an analysis of published clinical data. Mech Ageing Dev 2001;122:2041–55.
27. Feinleib M, Kannel WB, Garrison RJ, et al. The Framingham Offspring Study. Design and preliminary data. Prev Med 1975;4:518–25.
28. Garrison RJ, Kannel WB, Stokes J III, Castelli WP. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev Med 1987;16:235–51.
29. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
30. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
31. Magil SG, Zeisel SH, Wurtman RJ. Effects of ingesting soy or egg lecithins on serum choline, brain choline and brain acetylcholine. J Nutr 1981;111:166–70.
32. Christie JE, Blackburn IM, Glen AM, Zeisel S, Shering A, Yates CM. Effects of choline and lecithin on CSF choline levels and on cognitive function in patients with presenile dementia of the Alzheimer type. In: Barbeau A, Growdon J, Wurtman R, eds. Nutrition and the brain. Vol 5. New York, NY: Raven Press, 1979;377–87.
33. Wurtman RJ, Regan M, Ulus I, Yu L. Effect of oral CDP-choline on plasma choline and uridine levels in humans. Biochem Pharmacol 2000;60:989–92.
34. Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA, Willet WC. Dietary choline and betaine assessed by food frequency

questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. Am J Clin Nutr 2006;83:905–11.
35. Wechsler D. Weschsler Adult Intelligence Scale-III. San Antonio, TX: The Psychological Corporation, 1997.
36. Wechsler D. A standardized memory scale for clinical use. J Psychol 1945;19:87–95.
37. Wechsler D. Manual for the Wechsler Adult Intelligence Scale. New York, NY: Psychological Corp, 1955.
38. Caffarra P, Scaglioni A, Malvezzi L, Girotti F, Soliveri P, Musicco M, et al. Fuld's formula and WAIS subtests in differential diagnosis of dementia. Funct Neurol 1991;6:23–7.
39. Gfeller JD, Meldrum DL, Jacobi KA. (1995) The impact of constructional impairment on the WMS-R visual reproduction subtests. J Clin Psychol 199;51(1):58–63.
40. Reitan RM. Validity of the Trail Making test as an indicator of organic brain damage. Percept Mot Skills 1958;8:271–6.
41. Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, Herman G, Atlas S, Grossman R, Berrett D, et al. Gender differences in age effect on brain atrophy measured by magnetic resonance imaging. Proc Natl Acad Sci USA 1991;88:2845–9.
42. DeCarli C, Massaro J, Harvey D, Hald J, Tullberg M, Au R, Beiser A, D'Agostino R, Wolf P. Measures of brain morphology and infarction in the Framingham Heart Study: establishing what is normal. Neurobiol Aging 2005;26:491–510.
43. DeCarli C, Fletcher E, Ramey V, Harvey D, Jagust WJ. Anatomical mapping of white matter hyperintensities. Stroke 2005;36:50–5.
44. Annerbo S, Wahlund L, Lokk J. The significance of thyroid-stimulating hormone and homocysteine in the development of Alzheimer's disease in mild cognitive impairment: a 6-year follow-up study. Am J Alzheimers Dis Other Demen 2006;21:182–8.
45. Elias MF, Sullivan LM, D'Agostino RB, Elias PK, Beiser A, Au R, Seshadri S, DeCarli C, Wolf PA. Framingham stroke risk profile and lowered cognitive performance. Stroke 2004;35:404–9.
46. Filley CM, Cullum CM. Education and cognitive function in Alzheimer's disease. Neuropsychiatry Neuropsychol Behav Neurol 1997; 10:48–51.
47. Seshadri S, Wolf PA, Beiser A, Elias M, Au R, Kase CS, D'Agostino RB, DeCarli C. Stroke risk profile, brain volume, and cognitive function: the Framingham Offspring Study. Neurology 2004;63:1591–9.
48. Yehuda S, Rabinovitz S, Mostofsky DI. Mediation of high fat diet following stress and inflammation. Nutr Neurosci 2005;8:309–15.
49. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Schneider J, Wilson RS. Dietary fats and the risk of incident Alzheimer disease. Arch Neurol 2003;60:194–200.
50. Quadri P, Fragiacomo C, Pezzati R, Zanda E, Forloni G, Tettamanti M, Lucca U. Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. Am J Clin Nutr 2004;80:114–22.
51. Morris MC, Evans DA, Bienias JL, Tangney CC, Wilson RS. Dietary fat intake and 6-year cognitive change in an older biracial community population. Neurology 2004;62:1573–9.
52. Greenwood CE, Winocur G. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty-acid intake. Behav Neurosci 1996;110:451–9.
53. Tucker KL, Qiao N, Scott T, Rosenberg I, Spiro A. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affair Normative Aging Study. Am J Clin Nutr 2005;82: 627–35.
54. Jeerakathil T, Wolf PA, Beiser A, Massaro J, Seshadri S, D'Agostino RB, DeCarli C. Stroke risk profile predicts white matter hyperintensity volume: The Framingham Heart Study. Stroke 2004;35:1857–61.
55. Murray AD, Staff RT, Shenkin SD, Deary IJ, Starr JM, Whalley LJ. Brain white matter hyperintensities: relative importance of vascular risk factors in nondemented elderly people. Radiology 2005;237:251–7.
56. Burke JR, Morgenlander JC. Update on Alzheimer's disease: promising advances in detection and treatment. Postgrad Med 1999;106: 85–86.
57. Wurtman RJ. Choline metabolism as a basis for the selective vulnerability of cholinergic neurons. Trends Neurosci 1992;15:117–22.
58. Li Q, Guo-Ross S, Lewis DV, Turner D, White AM, Wilson WA, Swartzwelder HS. Dietary prenatal choline supplementation alters postnatal hippocampal structure and function. J Neurophysiol 2004;91: 1545–55.
59. Alvarez XA, Mouzo R, Pichel V, Perez P, Laredo M, Fernandez-Novoa L, Corzo L, Zas R, Alcarez M, Secades J, et al. Double-blind placebo-controlled study with citicoline in APOE genotyped Alzheimer's disease patients. effects on cognitive performance, brain bioelectrical activity and cerebral perfusion. Methods Find Exp Clin Pharmacol 1999;21:633–44.
60. Conant R, Schauss AG. Therapeutic applications of citicoline for stroke and cognitive dysfunction in the elderly: a review of the literature. Altern Med Rev 2004;9:17–31.
61. De Jesus Moreno Moreno M. Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. Clin Ther 2003;25:178–93.
62. Geula C, Mesulam M. Systematic regional variations in the loss of cortical cholinergic fibers in Alzheimer's disease. Cereb Cortex 1996; 6:l65–77.
63. Wurtman RJ, Zeisel SH. Brain choline: its sources and effects on the synthesis and release of acetylcholine. In: Corkin S, Growdon JH, Wurtman RJ, eds. Alzheimer's disease: a report of progress. New York, NY: Raven Press, 1982:303–13.
64. Petersen RC, Smith GE, Waring SC, Ivnik RI, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcomes. Arch Neurol 1999;56:303–8.
65. Jack CR Jr, Petersen RC, Xu YC, O'Brien PC, Smith GE, Ivnik RJ, Boeve BF, Waring SC, Tangalos EG, Kokmen E. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. Neurology 1999;52:1397–403.
66. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004;256:183–94.