Choline and its metabolites are differently associated with cardiometabolic risk factors, history of cardiovascular disease, and MRI-documented cerebrovascular disease in older adults[1,2]

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ABSTRACT

Background: There is a potential role of choline in cardiovascular and cerebrovascular disease through its involvement in lipid and one-carbon metabolism.

Objective: We evaluated the associations of plasma choline and choline-related compounds with cardiometabolic risk factors, history of cardiovascular disease, and cerebrovascular pathology.

Design: A cross-sectional subset of the Nutrition, Aging, and Memory in Elders cohort who had undergone MRI of the brain (n = 296; mean ± SD age: 73 ± 8.1 y) was assessed. Plasma concentrations of free choline, betaine, and phosphatidylcholine were measured with the use of liquid-chromatography–stable-isotope dilution–multiple-reaction monitoring–mass spectrometry. A volumetric analysis of MRI was used to determine the cerebrovascular pathology (white-matter hyperintensities and small- and large-vessel infarcts). Multiple linear and logistic regression models were used to examine relations of plasma measures with cardiometabolic risk factors, history of cardiovascular disease, and radiologic evidence of cerebrovascular pathology.

Results: Higher concentrations of plasma choline were associated with an unfavorable cardiometabolic risk-factor profile [lower high-density lipoprotein (HDL) cholesterol, higher total homocysteine, and higher body mass index (BMI)] and greater odds of large-vessel cerebral vascular disease or history of cardiovascular disease but lower odds of small-vessel cerebral vascular disease. Conversely, higher concentrations of plasma betaine were associated with a favorable cardiometabolic risk-factor profile [lower low-density lipoprotein (LDL) cholesterol and triglycerides] and lower odds of diabetes. Higher concentrations of plasma phosphatidylcholine were associated with characteristics of both a favorable cardiometabolic risk-factor profile (higher HDL cholesterol, lower BMI, lower C-reactive protein, lower waist circumference, and lower odds of hypertension and diabetes) and an unfavorable profile (higher LDL cholesterol and triglycerides).

Conclusion: Choline and its metabolites have differential associations with cardiometabolic risk factors and subtypes of vascular disease, thereby suggesting differing roles in the pathogenesis of cardiovascular and cerebral large-vessel disease compared with that of small-vessel disease. *Am J Clin Nutr* 2017;105:1283–90.

Keywords: cardiometabolic risk, cerebrovascular disease, choline, older adults, magnetic resonance imaging, nutrition

INTRODUCTION

Choline is an essential nutrient necessary for the structural integrity and signaling of cells and is a precursor to the neurotransmitter acetylcholine (1). Much attention has been given to the potential role of choline in cardiovascular disease. Although the main focus has been on the microbial metabolism of phosphatidylcholine and the production of the proatherosclerotic metabolite trimethylamine *N*-oxide (2), there have also been links between choline and more-traditional cardiometabolic risk factors. The biosynthesis of phosphatidylcholine in the liver is critical for the synthesis and secretion of VLDLs (3, 4) and is an essential component of lipoproteins. In addition, supplementation with the choline metabolite betaine has been reported to increase plasma LDL-cholesterol concentrations relative to the effect of a placebo and to increase the total-cholesterol:HDL-cholesterol ratio (5).

Choline may be oxidized to form the methyl donor betaine for the conversion of homocysteine to methionine. Hyperhomocysteinemia has been associated with increased risk of cardiovascular disease (6). As part of the one-carbon metabolism pathway, choline, folate, and vitamins B-12 and B-6 intersect at the point of homocysteine methylation. Both betaine and 5-methyltetrahydrofolate can be used to remethylate homocysteine, whereas vitamin B-6 is involved in the transmethylation of homocysteine (7). Thus, a fluctuation in the concentration of any of these nutrients has the potential to alter the utilization and concentrations of the other nutrients, and all nutrients should be taken into account when assessing the relations between choline and cardiovascular or cerebrovascular health.

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[2] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Both choline and betaine can be obtained from food or synthesized de novo. Konstantinova et al. (8) showed differing associations between plasma choline and betaine and components of metabolic syndrome with an unfavorable cardiovascular disease risk profile being associated with high-choline and low-betaine plasma concentrations. To our knowledge, whether this same relation holds true for cerebrovascular disease has not previously been evaluated. In this study, we evaluated the associations of plasma choline and choline-related compounds with cardiometabolic risk factors, history of cardiovascular disease, and cerebrovascular pathology.

METHODS

Subjects and study design

The study involved a secondary analysis of a subset of the NAME (Nutrition, Aging, and Memory in Elders)[7] cohort who had undergone MRI and cognitive testing and had archived plasma (n = 296) (**Supplemental Figure 1**). The NAME study's design, subject recruitment, and data-collection methodology have been previously described in detail (9). Briefly, the NAME study was a cross-sectional study that was designed to identify relations between micronutrient status and cognitive capacity and cerebrovascular pathology in older adults. The study cohort consisted of community-based elders who were living in the greater Boston, Massachusetts, area, and were aged ≥60 y. Subjects were visited in their homes by trained research staff who administered a neuropsychological testing battery. Fasting blood samples (overnight fast; 0000−0800) and a range of health and behavior questionnaires and anthropometric and blood pressure measures were obtained in the participants' homes by trained research staff. MRI was done at Tufts Medical Center, Boston, Massachusetts. The study was approved by the Tufts University Health Sciences Campus Institutional Review Board.

Plasma choline and related compounds

Fasting plasma samples that were collected between 2003 and 2007 were stored in a freezer at −80°F. Plasma aliquots were shipped on dry ice to the University of North Carolina Chapel Hill Nutrition Research Institute for the assay of free choline, betaine, and phosphatidylcholine. The lipid fraction was separated with the use of standardized methods (10). Aqueous and organic compounds were analyzed and quantified with the use of liquid-chromatography–stable-isotope dilution–multiple-reaction monitoring–mass spectrometry according to previously published methods (11).

Cardiometabolic risk factors and cardiovascular disease

Plasma HDL-cholesterol, LDL-cholesterol, triglyceride, and glucose concentrations were assessed with the use of Beckman Coulter AU400 standard operating procedures (Beckman Coulter American Inc.). Plasma total homocysteine concentrations were determined with the use of HPLC with fluorescence detection (12). Folate and vitamin B-12 concentrations were measured with the use of the Quantaphase II radioassay kit (Bio-Rad Laboratories). Pyridoxal-*P* (PLP; vitamin B-6) concentrations were determined with the use of the tyrosine decarboxylase apoenzyme method (13). High-sensitivity C-reactive protein was measured with the use of a solid-phase, 2-site, chemiluminescent, immunometric assay on the IMMULITE 2000 immunoassay analyzer (Siemens Healthcare Diagnostics) with intra-assay and interassay CVs of 3.5% and 5%, respectively. Diabetes status was defined with the use of guidelines from the American Diabetes Association (14). Hypertension was assessed according to the guidelines of the Joint National Committee on Prevention (15). BMI (in kg/m^2) was calculated as weight divided by height squared. Cardiovascular disease was self-reported and included histories of congestive heart failure, coronary heart disease, and myocardial infarction.

Cerebrovascular disease and MRI

Cranial MRI was performed with a 1.5-T Symphony superconducting magnet (Siemens). The MRI protocol included *1*) T1-weighted axial images, *2*) intermediate and T2-weighted conventional spin-echo axial images, and *3*) fluid-attenuation inversion recovery (FLAIR) turbo spin-echo axial images. Analyze image-analysis software (1986–2004; Biomedical Imaging Resource) was used to quantify brain segmentation and a region-of-interest analysis. Analysts worked with de-identified data.

White-matter hyperintensities (WMHIs), which are indicators of vascular pathology, were defined as hyperintense changes on intermediate-intensity/FLAIR and T2-weighted images with no corresponding T1 abnormality. Quantitative measurements involved histogram-analysis procedures as previously described (16). High-resolution images were reviewed with the use of primary and secondary trained analysts under the supervision of a neuroradiologist. The interrater reliability was r = 0.92 ($P < 0.001$).

Brain infarcts were identified and measured with the use of a neuroradiologist to quantify subclinical and clinical cardiovascular disease. Large-vessel infarcts were defined as cortical infarcts of any size and subcortical infarcts >1.5 cm in maximum dimension, whereas small-vessel infarcts were defined as subcortical infarcts from 3 mm to 1.5 cm. To qualify for an infarct, the lesion had an increased signal on T2 and FLAIR images. For subcortical infarcts in white matter, additional low-signal on T1-weighted images was required to be considered as an infarct (17).

Statistical methods

Data are presented as means ± SDs or frequencies (percentages). Multiple linear regression models and logistic regression models were used to examine relations between plasma concentrations of choline and choline-related metabolites and cardiometabolic risk factors, B vitamins, and odds of cardiometabolic-related diseases and cerebrovascular pathology. Independent variables were examined as continuous variables and as categorical data (tertiles) when the outcome variable was dichotomous. When continuous data were not normally distributed, values were log transformed before the analysis. We

[7] Abbreviations used: FLAIR, fluid-attenuation inversion recovery; NAME, Nutrition Aging, and Memory in Elders; PLP, pyridoxal-*P*; WMHI, white-matter hyperintensity.

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TABLE 1
Population characteristics

Variable	*n*	Value
Age, y	296	73.6 ± 8.1 (59.0–103.0)[1]
BMI, kg/m^2	280	30.7 ± 7.7 (15.4–75.0)
Plasma		
HDL cholesterol, mg/dL	295	50.6 ± 15.0 (21.0–122.0)
LDL cholesterol, mg/dL	291	108.3 ± 35.3 (13.6–271.4)
Triglycerides, mg/dL	295	144.6 ± 97.8 (41.0–905.0)
Glucose, mg/dL	295	111.4 ± 36.8 (51.0–440.0)
Folate, ng/mL	293	15.3 ± 10.6 (2.4–109.6)
PLP,[2] nm/L	295	83.0 ± 81.0 (8.3–492.6)
Vitamin B-12, pg/mL	294	575.9 ± 330.4 (166.0–3444.0)
Total homocysteine, μmol/L	295	11.8 ± 5.5 (4.5–44.3)
Sex, F, %	296	72.6
Hypertension, %	291	84.2
Diabetes, %	295	30.5
Self-reported cardiovascular disease, %	287	35.2
Smoking status (self-reported cigarette use), %	294	
Never		30.6
Past		45.2
Current		24.2
Drinking status (self-reported alcoholic beverage intake), %	294	
Never		10.5
Past		47.0
Current		42.5
Education, %	296	
None to fourth grade		1.4
Fifth to eighth grades		7.5
9th to 11th grades		14.3
12th grade or high school graduate		32.6
Some college or bachelor's degree		36.7
Graduate school		7.5

[1] Mean ± SD; range in parentheses (all such values).
[2] PLP, pyridoxal-*P*.

assessed linear trends across categories of plasma concentrations of choline and choline-related metabolites by assigning each participant the median value for the category and modeling this value as a continuous variable.

Three regression models were used to assess relations while controlling for *1*) basic covariates [age, sex, smoking, and drinking status (self-reported alcohol and cigarette use defined as never, current, or past)]; *2*) one-carbon metabolism covariates (age, sex, smoking and drinking status, plasma total homocysteine, PLP, and folate), and *3*) lipid metabolism covariates (age, sex, smoking and drinking status, LDL cholesterol, HDL cholesterol, triglycerides, and BMI). Subjects with missing covariate data or outliers for plasma choline, betaine, or phosphatidylcholine concentrations were excluded from the analyses (betaine: $n = 2$; phosphatidylcholine: $n = 3$) (see Supplemental Figure 1 for details). Results were considered significant at $P \leq 0.05$.

RESULTS

Population characteristics

The mean ± SD age was 73 ± 8.1 y, and participants were predominantly women (73%) (**Table 1**). The mean BMI was in the obese range (30.7 ± 7.7), and there was a high prevalence of hypertension (84%). The attained level of education was moderate with 33% of subjects being high-school graduates, and 44% of subjects having attended at least some college. The mean betaine concentration was greater in men than in women, whereas the mean phosphatidylcholine concentration was greater in women than in men ($P \leq 0.05$) (**Table 2**).

Plasma choline and cardiometabolic risk factors

LDL cholesterol and triglyceride were inversely associated with plasma betaine and positively associated with plasma phosphatidylcholine concentrations (**Table 3**). HDL cholesterol was inversely associated with plasma choline and positively associated with phosphatidylcholine concentrations. There were no significant relations between blood glucose and plasma choline and choline-related metabolite concentrations.

TABLE 2
Plasma concentration of choline and choline-related compounds[1]

	Men	Women	All
Choline, μM	10.3 ± 3.8	9.6 ± 3.2	9.7 ± 3.4
Betaine, μM	46.1 ± 25.2	38.8 ± 15.3	40.8 ± 18.9[2]
Phosphatidylcholine, μM	2030 ± 418	2315 ± 417	2237 ± 435[2]

[1] All values are means ± SDs. $n = 296$ (81 men and 215 women).
[2] Significant difference between men and women, $P \leq 0.05$ (independent *t* test).

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TABLE 3
Multiple linear regression coefficients for cardiometabolic risk factors in relation to plasma concentrations of choline, betaine, and phosphatidylcholine[1]

	Choline			Betaine			Phosphatidylcholine		
	n	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*
Plasma									
LDL cholesterol	287	−0.05 ± 0.06	0.40	285	−0.13 ± 0.06	0.04	284	0.0002 ± 0.00002	<0.0001
HDL cholesterol	291	−0.11 ± 0.05	0.03	289	−0.06 ± 0.05	0.28	288	0.0001 ± 0.00002	<0.0001
Triglycerides	291	0.07 ± 0.09	0.46	289	−0.31 ± 0.09	0.001	288	0.0002 ± 0.00003	<0.0001
Glucose	291	−0.05 ± 0.05	0.31	289	−0.07 ± 0.05	0.14	288	−0.00001 ± 0.00002	0.40
tHcy	291	0.64 ± 0.16	<0.0001	289	−0.20 ± 0.16	0.23	288	−0.00009 ± 0.00006	0.12
BMI	277	0.13 ± 0.04	0.002	275	0.01 ± 0.04	0.85	275	−0.00006 ± 0.00001	<0.0001
CRP	291	0.43 ± 0.23	0.06	289	−0.03 ± 0.23	0.90	288	−0.0002 ± 0.00008	0.01
Waist circumference	252	0.04 ± 0.30	0.20	250	−0.02 ± 0.03	0.56	250	−0.17 ± 0.05	0.001

[1] Multiple linear regression analysis was adjusted for age, sex, smoking status, and drinking status. All variables except for phosphatidylcholine were log transformed for the statistical analysis. CRP, C-reactive protein; tHcy, total homocysteine.

Plasma total homocysteine was positively associated with the plasma choline concentration. BMI was positively associated with plasma choline and inversely associated with the plasma phosphatidylcholine concentration. Waist circumference and the concentration of C-reactive protein were both inversely associated with the plasma phosphatidylcholine concentration.

Plasma choline and B vitamins

A multiple linear regression was used to assess potential relations between plasma B-vitamin and choline and choline-related metabolite concentrations while controlling for age and sex (**Table 4**). Plasma PLP was positively associated with the phosphatidylcholine concentration. Plasma folate had a weak positive relation with choline and betaine concentrations. There were no significant relations between plasma vitamin B-12 and plasma choline, betaine, or phosphatidylcholine concentrations.

Plasma choline and associations with cardiometabolic-related disease

Odds of self-reported cardiovascular disease were greater in subjects with plasma choline concentrations in the second or third tertile than in the first tertile after controlling for age, sex, and smoking and alcohol use (**Table 5**, model 1). These relations remained significant when controlling for plasma total homocysteine, PLP, and folate concentrations (model 2) as well as for BMI and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations (model 3). The linear trend across tertiles was also significant in all models, which indicated a positive association.

Odds of hypertension were lower in subjects with plasma phosphatidylcholine concentrations in the third tertile than in the first tertile when controlled for age, sex, smoking, and alcohol use. These relations did not remain significant when plasma total homocysteine, PLP, and folate concentrations were included (model 2) or when BMI and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations were added to the model (model 3). However, the linear trend across tertiles was significant in all models, which indicated an inverse association.

Odds of diabetes were lower in subjects with betaine concentrations in the third tertile than in the first tertile after controlling for age, sex, smoking and alcohol use, and plasma total homocysteine, PLP, and folate concentrations (model 2). The linear trend across tertiles was also significant in this model and after controlling for BMI and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations (model 3), which indicated an inverse relation between plasma betaine and the presence of type 2 diabetes. Odds of diabetes were also lower in subjects with plasma phosphatidylcholine concentrations in the third tertile than in the first tertile when controlling for age, sex, and smoking and alcohol use (model 1). These relations remained significant when controlling for plasma total homocysteine, PLP,

TABLE 4
Multiple linear regression coefficients for plasma concentrations of B vitamins in relation to plasma concentrations of choline, betaine, and phosphatidylcholine[1]

	Choline			Betaine			Phosphatidylcholine		
	n	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*
PLP[2]	295	0.06 ± 0.16	0.70	293	0.16 ± 0.16	0.31	292	0.0001 ± 0.00005	0.05
Vitamin B-12	294	−0.08 ± 0.09	0.33	292	−0.02 ± 0.09	0.80	291	−0.00005 ± 0.00003	0.12
Folate	293	0.31 ± 0.11	0.004	291	0.32 ± 0.11	0.003	290	0.000004 ± 0.00004	0.90

[1] Multiple linear regression analysis was adjusted for age and sex. All variables except for phosphatidylcholine were log transformed for the statistical analysis.
[2] PLP, pyridoxal-*P*.

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TABLE 5
Multivariate-adjusted ORs for cardiometabolic-related disease risk factors across tertile categories of plasma choline-related compounds[1]

	Choline					Betaine					Phosphatidylcholine				
	Tertile (μM range)					Tertile (μM range)					Tertile (μM range)				
	n	1 (3.6–8.1)	2 (8.12–10.4)	3 (10.42–26.9)	*P*-trend	*n*	1 (13.6–33.3)	2 (33.32–43.66)	3 (43.662–239.4)	*P*-trend	*n*	1 (1093.2–2044.7)	2 (2044.8–2383.0)	3 (2383.1–3856.5)	*P*-trend
CVD															
Model 1	283	1.00 (—)[2]	2.34 (1.20, 4.58)	3.26 (1.67, 6.36)	0.001	281	1.00 (—)	1.08 (0.58, 2.04)	1.24 (0.65, 2.37)	0.51	280	1.00 (—)	0.83 (0.45, 1.54)	0.66 (0.34, 1.27)	0.21
Model 2	280	1.00 (—)	2.28 (1.16, 4.48)	3.08 (1.55, 6.13)	0.002	278	1.00 (—)	1.12 (0.59, 2.15)	1.24 (0.62, 2.34)	0.59	277	1.00 (—)	0.89 (0.48, 1.67)	0.75 (0.38, 1.48)	0.41
Model 3	263	1.00 (—)	2.35 (1.15, 4.78)	3.06 (1.50, 6.22)	0.002	261	1.00 (—)	1.21 (0.62, 2.39)	1.23 (0.61, 2.48)	0.57	261	1.00 (—)	0.74 (0.34, 1.59)	0.44 (0.15, 1.26)	0.12
Hypertension															
Model 1	287	1.00 (—)	0.59 (0.28, 1.25)	2.25 (0.85, 5.93)	0.15	285	1.00 (—)	1.10 (0.50, 2.42)	1.25 (0.54, 2.89)	0.60	284	1.00 (—)	0.89 (0.35, 2.22)	0.42 (0.18, 0.98)	0.03
Model 2	284	1.00 (—)	0.54 (0.25, 1.17)	1.59 (0.58, 4.33)	0.56	282	1.00 (—)	1.15 (0.51, 2.61)	1.11 (0.45, 2.70)	0.81	281	1.00 (—)	0.98 (0.38, 2.56)	0.43 (0.18, 1.06)	0.04
Model 3	267	1.00 (—)	0.59 (0.27, 1.31)	1.93 (0.71, 5.26)	0.30	265	1.00 (—)	1.08 (0.47, 2.48)	1.25 (0.50, 3.11)	0.63	265	1.00 (—)	0.68 (0.22, 2.08)	0.27 (0.06, 1.15)	0.05
DM															
Model 1	291	1.00 (—)	0.75 (0.39, 1.43)	0.91 (0.48, 1.72)	0.75	289	1.00 (—)	1.26 (0.67, 2.35)	0.51 (0.25, 1.03)	0.06	288	1.00 (—)	0.86 (0.46, 1.59)	0.39 (0.19, 0.79)	0.01
Model 2	289	1.00 (—)	0.72 (0.37, 1.38)	0.82 (0.42, 1.60)	0.55	287	1.00 (—)	1.22 (0.64, 2.30)	0.43 (0.21, 0.89)	0.02	286	1.00 (—)	0.88 (0.47, 1.65)	0.41 (0.20, 0.83)	0.01
Model 3	272	1.00 (—)	0.61 (0.31, 1.24)	0.73 (0.36, 1.46)	0.36	270	1.00 (—)	1.13 (0.57, 2.23)	0.47 (0.22, 1.00)	0.05	270	1.00 (—)	0.96 (0.44, 2.12)	0.39 (0.12, 1.22)	0.09

[1] Model 1 was a logistic regression that was adjusted for age, sex, smoking status, and drinking status. Model 2 was a logistic regression that was adjusted as for model 1 and for plasma (log) total homocysteine, (log) pyridoxal-*P*, and (log) folate. Model 3 was a logistic regression that was adjusted as for model 1 and for plasma (log) LDL cholesterol, (log) HDL cholesterol, and (log) triglycerides and (log) BMI. CVD, cardiovascular disease; DM, diabetes mellitus.
[2] OR; 95% CI in parentheses (all such values).

and folate concentrations (model 2) but not after controlling for BMI and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations (model 3). The linear trend across tertiles was also significant in models 1 and 2, which indicated an inverse relation.

Plasma choline and cerebrovascular pathology

Relations between plasma choline and choline-related compounds and MRI volumetric measurements are presented in **Tables 6** and **7**. There were no significant associations between concentrations of plasma choline-related compounds and the volume of WMHIs. The odds of a small-vessel infarct were lower in individuals with plasma choline concentrations in the second or third tertiles than in the first tertile after controlling for age, sex, smoking and alcohol use, and plasma total homocysteine, PLP, and folate concentrations (model 2). These relations were NS after controlling for age, sex, smoking and alcohol use, BMI, and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations (model 3). The linear trend across tertiles was also significant in models 1 and 2 ($P \leq 0.05$), which indicated an inverse relation between plasma choline concentrations and the presence of cerebral small-vessel infarcts.

Conversely, odds of a large-vessel infarct were greater in individuals with plasma choline concentrations in the second or third tertile than in the first tertile after controlling for age, sex, smoking and alcohol use, and plasma total homocysteine, PLP, and folate concentrations (model 2) as well as when controlling for age, sex, smoking and alcohol use, BMI, and plasma LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations (model 3). The linear trend across tertiles was also significant in all models ($P \leq 0.05$), which indicated a positive association between plasma choline concentrations and the presence of large-vessel infarcts.

DISCUSSION

In a population of community-dwelling elders, choline and its metabolites had differential associations with cardiometabolic risk factors and subtypes of vascular disease. We showed that higher plasma choline concentrations were associated with an unfavorable cardiometabolic risk-factor profile (lower HDL cholesterol, higher total homocysteine, and higher BMI) and greater odds of large-vessel cerebral vascular disease or a history of cardiovascular disease but lower odds of small-vessel cerebral vascular disease. Conversely, higher plasma betaine concentrations were associated with a favorable cardiometabolic risk-factor profile (lower LDL cholesterol and triglycerides) and lower odds of diabetes. Higher plasma phosphatidylcholine concentrations were associated with characteristics of both a favorable cardiometabolic risk-factor profile (higher HDL cholesterol, lower BMI, lower C-reactive protein, lower waist circumference, and lower odds of hypertension and diabetes) and an unfavorable profile (higher LDL cholesterol and triglycerides). Our findings that plasma phosphatidylcholine concentrations were associated with both favorable and unfavorable cardiometabolic risk factors (higher HDL cholesterol, LDL cholesterol, and triglycerides) were not unexpected because phosphatidylcholine biosynthesis in the liver is

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involved in the assembly and secretion of lipoprotein particles. Any disruption would result in the impairment of hepatic VLDL- and HDL-cholesterol secretions and an increase in hepatic steatosis (18).

In general, our findings were consistent with previous reports of the relation between choline and its metabolites and cardiovascular disease and metabolic disorders. Higher betaine concentrations were associated with a favorable risk-factor profile, and higher choline concentrations were associated with an unfavorable risk-factor profile (8). Plasma choline and betaine concentrations were comparable with those previously reported (8, 19–22). Compared with the overall US population aged ≥65 y, the NAME population has a higher percentage of women, more college graduates, higher prevalences of diabetes and hypertension, and higher reported current alcohol consumption and cigarette use (23). In addition, the NAME population consisted of community-dwelling elders in contrast with patient populations who have been studied by other authors. For example, a study of a cohort with acute coronary syndrome concluded that high and low plasma betaine concentrations were associated with increased risks of secondary heart failure and acute myocardial infarction, respectively (24). A second study in the same cohort concluded that lower plasma betaine concentrations were associated with higher concentrations of triglycerides and non-HDL cholesterol (25). In contrast, in the NAME population, although there was an inverse relation between plasma betaine and both LDL-cholesterol and triglyceride concentrations, there was no significant relation between plasma betaine concentrations and odds of cardiovascular or cerebrovascular disease. However, our findings were similar to those of a large-cohort study of patients with stable angina pectoris that observed no association between plasma betaine and acute myocardial infarction or all-cause mortality (26).

Minor discrepancies between our results and findings from some other studies may have been partially due to whether the analyses were based on plasma concentrations or dietary intakes of choline and its metabolites. Dietary choline does not account fully for both the endogenous and exogenous pathways; however, circulating choline and metabolites may not be sensitive markers of choline status (27) because disturbances in circulating choline metabolites arise early in disease development (28), and lipid overload (indicated by elevated BMI) may perturb choline metabolism, thereby resulting in reduced plasma betaine concentrations (20). For example, we showed that subjects with highest plasma phosphatidylcholine concentrations had lower odds of type 2 diabetes, which somewhat countered the findings of a brief report on dietary intake data from 3 ongoing cohorts in the United States that showed an increase in the risk of type 2 diabetes that was associated with increased dietary choline from phosphatidylcholine (29). However, our data were consistent with recent prospective studies from both a Norwegian sample of patients with suspected stable angina pectoris (30) and an American general-population sample (31) that showed an inverse relation between plasma betaine and the presence of type 2 diabetes. In addition, analyses of 4 other cohorts showed no relation between dietary intake of betaine or choline and the incidence of peripheral artery disease (32), incidence of coronary heart disease (33), or cardiovascular disease risk (34). However, an analysis of a large prospective cohort with stable angina pectoris showed increased risk of acute myocardial infarction in individuals with plasma choline concentrations in the highest quartile than in the lowest quartile (26). Although this relation was only shown in smokers, in the NAME population, subjects with choline concentrations in the upper 2 tertiles had significantly greater odds of self-reported cardiovascular disease than did subjects with plasma choline concentrations in the lower tertile in all models (which included smoking status as a covariate).

The possibility cannot be ruled out that the relation between choline and vascular disease is different when considering the location of blood vessels, cerebrovascular or cardiovascular, as well as the size of the blood vessels. This possibility may have been the case in the NAME population in whom we showed that the odds of a small-vessel infarct were inversely related with the plasma choline concentration, whereas the odds of a large-vessel infarct were positively related with the plasma choline concentration. Similarly, a longitudinal study showed a significant inverse relation between dietary choline intake and the volume of cerebral WMHIs, which is a measure of myelin integrity that has been associated with small-vessel disease (35). Furthermore, results from a prospective cohort study of African Americans recently reported that higher dietary choline intake was associated with lower risk of incident ischemic stroke (36). These results support the concept that the relation between choline and its related compounds and vascular disease is dependent on the vessel location and size. Coronary artery disease is a large-vessel disease, whereas the volume of WMHIs is

TABLE 6
Multiple linear regression coefficients for the volume of white-matter hyperintensities in relation to plasma concentrations of choline, betaine, and phosphatidylcholine[1]

	Choline			Betaine			Phosphatidylcholine		
	n	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*	*n*	$\beta \pm$ SE	*P*
Model 1	292	0.08 ± 0.21	0.70	290	−0.04 ± 0.21	0.86	289	0.00005 ± 0.00008	0.48
Model 2	289	0.10 ± 0.23	0.68	287	−0.03 ± 0.22	0.88	286	0.00006 ± 0.00008	0.45
Model 3	272	0.24 ± 0.29	0.73	270	−0.03 ± 0.23	0.89	270	0.00009 ± 0.0002	0.58

[1] Model 1 was a logistic regression that was adjusted for age, sex, smoking status, and drinking status. All variables except for phosphatidylcholine were log transformed for the statistical analysis. Model 2 was a logistic regression that was adjusted as for model 1 and for plasma (log) total homocysteine, (log) pyridoxal-*P*, and (log) folate. Model 3 was a logistic regression that was adjusted as for model 1 and for plasma (log) LDL cholesterol, (log) HDL cholesterol, and (log) triglycerides and (log) BMI.

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TABLE 7
Multivariate-adjusted ORs for the presence of cerebrovascular infarct across tertile categories of plasma choline related compounds[1]

	Choline					Betaine					Phosphatidylcholine				
	Tertile (μM range)					Tertile (μM range)					Tertile (μM range)				
	n	1 (3.6–8.1)	2 (8.12–10.4)	3 (10.42–26.9)	*P*-trend	*n*	1 (13.6–33.3)	2 (33.32–43.66)	3 (43.662–239.4)	*P*-trend	*n*	1 (1093.2–2044.7)	2 (2044.75–2383.0)	3 (2383.1–3856.5)	*P*-trend
SVINF															
Model 1	287	1.00 (—)[2]	0.44 (0.21, 0.90)	0.51 (0.25, 1.02)	0.05	285	1.00 (—)	0.80 (0.40, 1.60)	0.57 (0.27, 1.18)	0.13	284	1.00 (—)	0.55 (0.26, 1.15)	0.89 (0.44, 1.82)	0.81
Model 2	284	1.00 (—)	0.41 (0.20, 0.87)	0.44 (0.21, 0.93)	0.03	282	1.00 (—)	0.83 (0.40, 1.70)	0.63 (0.29, 1.37)	0.25	281	1.00 (—)	0.57 (0.27, 1.21)	0.90 (0.43, 1.89)	0.83
Model 3	267	1.00 (—)	0.48 (0.23, 1.02)	0.52 (0.25, 1.11)	0.08	265	1.00 (—)	0.77 (0.37, 1.59)	0.53 (0.24, 1.15)	0.10	265	1.00 (—)	0.43 (0.18, 1.05)	0.55 (0.18, 1.70)	0.42
LVINF															
Model 1	287	1.00 (—)	3.16 (1.07, 9.34)	3.29 (1.12, 9.66)	0.04	285	1.00 (—)	1.29 (0.52, 3.18)	0.76 (0.28, 2.02)	0.54	284	1.00 (—)	0.60 (0.24, 1.49)	0.87 (0.35, 2.14)	0.78
Model 2	284	1.00 (—)	3.30 (1.11, 9.84)	3.73 (1.24, 11.16)	0.02	282	1.00 (—)	1.48 (0.59, 3.76)	0.90 (0.33, 2.49)	0.79	281	1.00 (—)	0.61 (0.24, 1.54)	0.89 (0.35, 2.25)	0.83
Model 3	267	1.00 (—)	4.15 (1.22, 14.05)	5.74 (1.69, 19.50)	0.01	265	1.00 (—)	1.06 (0.39, 2.87)	0.72 (0.25, 2.04)	0.50	265	1.00 (—)	0.77 (0.25, 2.41)	0.94 (0.22, 4.02)	1.00

[1] Model 1 was a logistic regression that was adjusted for age, sex, smoking status, and drinking status. Model 2 was a logistic regression that was adjusted as for model 1 and for plasma (log) total homocysteine, (log) pyridoxal-*P*, and (log) folate. Model 3 was a logistic regression that was adjusted as for model 1 and for plasma (log) LDL cholesterol, (log) HDL cholesterol, and (log) triglycerides and (log) BMI. LVINF, large-vessel infarct; SVINF, small-vessel infarct.

[2] OR; 95% CI in parentheses (all such values).

associated with small-vessel disease. It is also possible that this discrepancy may have arisen because of different kinds of bias that were introduced in an elderly population with high rates of diabetes and hypertension (which would have put them at increased stroke risk), because patients who were dying from a large-vessel infarct may have been underrepresented in the cross-sectional analysis, or because cerebral infarctions that are related to large-vessel disease were related to embolic rather than thrombotic events.

The major limitations of this study were its cross-sectional nature and specific subject population, which prevented causal conclusions or the generalization of results and may have caused the study to be more prone to bias than a prospective study would have been. We were also limited by the plasma metabolites that were measured, including measurements of plasma dimethylglycine [which is an indicator of the methylation capacity from choline (37)] and trimethylamine *N*-oxide [which is produced when bacteria in the large intestines digest dietary phosphatidylcholine and has been associated with cardiovascular disease (2)], that would have provided a more complete picture of choline metabolism. Single nucleotide polymorphisms in genes that are involved in choline metabolism may modify these relations and have been shown to be associated with odds of cerebrovascular disease (38) or cardiovascular disease (39) as well to alter the susceptibility to choline deficiency (40, 41).

In conclusion, our findings that choline and its metabolites have differential associations with cardiometabolic risk factors and subtypes of vascular disease suggest differing roles in the pathogenesis of small-and large-vessel cardiovascular and cerebrovascular disease. These results help to provide metabolic insight into the processes that contribute to or protect from these disease outcomes and suggest a need to further explore these associations to better understand the underlying factors.

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