Associations of circulating choline and its related metabolites with cardiometabolic biomarkers: an international pooled analysis

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ABSTRACT

Background: Choline is an essential nutrient; however, the associations of choline and its related metabolites with cardiometabolic risk remain unclear.

Objective: We examined the associations of circulating choline, betaine, carnitine, and dimethylglycine (DMG) with cardiometabolic biomarkers and their potential dietary and nondietary determinants.

Methods: The cross-sectional analyses included 32,853 participants from 17 studies, who were free of cancer, cardiovascular diseases, chronic kidney diseases, and inflammatory bowel disease. In each study, metabolites and biomarkers were log-transformed and standardized by means and SDs, and linear regression coefficients (β) and 95% CIs were estimated with adjustments for potential confounders. Study-specific results were combined by random-effects meta-analyses. A false discovery rate <0.05 was considered significant.

Results: We observed moderate positive associations of circulating choline, carnitine, and DMG with creatinine [β (95% CI): 0.136 (0.084, 0.188), 0.106 (0.045, 0.168), and 0.128 (0.087, 0.169), respectively, for each SD increase in biomarkers on the log scale], carnitine with triglycerides (β = 0.076; 95% CI: 0.042, 0.109), homocysteine (β = 0.064; 95% CI: 0.033, 0.095), and LDL cholesterol (β = 0.055; 95% CI: 0.013, 0.096), DMG with homocysteine (β = 0.068; 95% CI: 0.023, 0.114), insulin (β = 0.068; 95% CI: 0.043, 0.093), and IL-6 (β = 0.060; 95% CI: 0.027, 0.094), but moderate inverse associations of betaine with triglycerides (β = −0.146; 95% CI: −0.188, −0.104), insulin (β = −0.106; 95% CI: −0.130, −0.082), homocysteine (β = −0.097; 95% CI: −0.149, −0.045), and total cholesterol (β = −0.074; 95% CI: −0.102, −0.047). In the whole pooled population, no dietary factor was associated with circulating choline; red meat intake was associated with circulating carnitine [β = 0.092 (0.042, 0.142) for a 1 serving/d increase], whereas plant protein was associated with circulating betaine [β = 0.249 (0.110, 0.388) for a 5% energy increase]. Demographics, lifestyle, and metabolic disease history showed differential associations with these metabolites.

Conclusions: Circulating choline, carnitine, and DMG were associated with unfavorable cardiometabolic risk profiles, whereas circulating betaine was associated with a favorable cardiometabolic risk profile. Future prospective studies are needed to examine the associations of these metabolites with incident cardiovascular events. *Am J Clin Nutr* 2021;114:893–906.

Keywords: choline, betaine, carnitine, dimethylglycine, cardiometabolic disease, biomarkers

Introduction

Choline and its related metabolites—betaine, carnitine, and dimethylglycine (DMG)—are major nutrients in human health and disease (1–5). As an essential nutrient, choline can be

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obtained from dietary sources (e.g., eggs, red meat, and soy foods) or synthesized de novo through methylation of phosphatidylethanolamine to phosphatidylcholine (1–3). While betaine, carnitine, and DMG can be obtained from dietary sources (e.g., spinach and whole-wheat products for betaine, red meat for carnitine, and beans and cereal grains for DMG) (4, 5), they are also products of choline metabolism. In addition to dietary intakes and the choline metabolism pathway, concentrations of choline and betaine in the human body have been reported to be affected by age, sex, menopausal status, metabolic health status such as glucose and cholesterol metabolism, and kidney excretion (3, 6, 7). In addition, choline, betaine, and carnitine are major precursors of trimethylamine-*N*-oxide (TMAO), a gut microbiota–derived metabolite that has been linked to cardiovascular disease (CVD) events and mortality (8).

Despite their potential roles in cardiometabolic health and disease, population studies have reported inconsistent findings regarding choline, betaine, carnitine, or DMG with cardiometabolic risk factors. Some cross-sectional studies showed higher plasma choline and carnitine and lower betaine concentrations were associated with unfavorable cardiometabolic risk profile (e.g., higher systolic blood pressure, insulin resistance, and BMI, and lower HDL cholesterol) (9–11). However, a review of 6 prospective studies found that elevated circulating concentrations of choline, carnitine, and betaine were all associated with increased risks of adverse CVD events (12). In addition, a review on dietary choline and betaine observed no clear evidence of positive associations with incident CVD but noted that high choline intake may be linked to increased CVD mortality (13). More recently, our prospective analyses among >200,000 Black and White Americans and Chinese adults showed that, while dietary sources of choline varied across ethnic groups,

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Supplemental Tables 1–18 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AIRWAVE, Airwave Health Monitoring Study; CRP, C-reactive protein; COMETS, Consortium of Metabolomics Studies; CVD, cardiovascular disease; DBP, diastolic blood pressure; DMG, dimethylglycine; FHS, Framingham Heart Study; HbA1c, glycated hemoglobin; MESA, Multi-Ethnic Study of Atherosclerosis; NAFLD, nonalcoholic fatty liver disease; NHS, Nurses' Health Study; oz, ounce(s); SBP, systolic blood pressure; SCCS, Southern Community Cohort Study; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; TMAO, trimethylamine-*N*-oxide; TMCS, Tsuruoka Metabolomics Cohort Study; WHR, waist-to-hip ratio.

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high choline intake was consistently associated with elevated cardiometabolic mortality (14).

Given the previous inconsistent findings and potential differences in dietary intakes (14–16) and cardiometabolic conditions across populations (17–19), large-scale analyses among ethnically and geographically diverse populations may provide more robust evidence regarding the associations of choline and related metabolites with cardiometabolic disease risk. Since circulating choline, betaine, carnitine, and DMG can be more objectively quantified than their dietary intakes and reflect integrated levels of dietary intake, endogenous biosynthesis, metabolism, and excretion, the analyses of their circulating concentrations with cardiometabolic biomarkers may more accurately capture their cardiometabolic effects. Furthermore, prior studies have shown that dietary choline and betaine correlated weakly with their plasma concentrations (20, 21), suggesting that circulating choline and related metabolites may be substantially affected by nondietary factors. To address the knowledge gap, we conducted a pooling project to examine the associations of cardiometabolic biomarkers with circulating choline, betaine, carnitine, and DMG using data from 17 studies in the United States, Europe, and Asia. Second, we evaluated potential dietary and nondietary factors that may influence the circulating concentrations of these metabolites.

Methods

Study design and population

Our study comprised cross-sectional analyses of data collected for the TMAO Pooling Project, which is primarily based on the Consortium of Metabolomics Studies (COMETS) (22). We also contacted studies that were not members of the COMETS but had data on blood TMAO and related metabolites (choline, betaine, carnitine, or DMG). In total, we pooled data from 17 studies, including 10 in the United States [the Coronary Artery Risk Development in Young Adults Study (CARDIA) (23); Framingham Heart Study (FHS) (24); Health Professionals Follow-Up Study (HPFS) (25); Insulin Resistance Atherosclerosis Family Study (IRASFS) (26); Multi-Ethnic Study of Atherosclerosis (MESA) (27); Nurses' Health Study (NHS) (28); NHS II (28); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) (29); Southern Community Cohort Study (SCCS) (30); and Women's Health Initiative (WHI) (31)]; 3 in Europe [the Airwave Health Monitoring Study (AIRWAVE) (32), Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) (33), and UK Adult Twin Registry (TwinsUK) (34)]; and 4 in Asia [the Guangzhou Nutrition and Health Study (GNHS) (35), Shanghai Men's Health Study (SMHS) (36), Shanghai Women's Health Study (SWHS) (37), and Tsuruoka Metabolomics Cohort Study (TMCS) (38)]. The pooling project was approved by the COMETS Steering Committee, the study committees of participating studies, and the Institutional Review Board of Vanderbilt University Medical Center. Since the work on the primary outcome, circulating TMAO, was reported in a separate paper (39), our current analyses were focused on circulating choline and its related metabolites—that is, betaine, carnitine, and DMG.

For the current analyses, we only included adult participants (>18 y) with valid data on plasma or serum choline, betaine, carnitine, or DMG. To reduce the effects of severe underlying diseases and their treatments on the concentrations of these metabolites, we excluded participants who had a history of cancer (except for nonmelanoma skin cancer), CVD (coronary artery disease, stroke, and heart failure), chronic kidney disease (physician diagnosis, or estimated glomerular filtration rate <45 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2} in case of no physician diagnosis), or inflammatory bowel disease. A total of 32,853 adults were included in the final analyses (**Supplemental Figure 1**).

Metabolites and cardiometabolic biomarkers

Circulating concentrations of choline, betaine, carnitine, and DMG were measured in each study using targeted or untargeted assays. Metabolomics platforms and analytical techniques in 17 individual studies were detailed in the profile paper for the COMETS (22) and briefly presented in **Supplemental Table 1**. Targeted assays were performed by the Bevital A/S (Bergen, Norway) (40), Broad Institute (Boston, MA, USA) (41), Keio University (Tsuruoka, Japan) (42), University of North Carolina Nutrition Obesity Research Center (Kannapolis, NC, USA) (43), and Sun Yat-sen University (Guangzhou, China) (35), whereas untargeted assays were performed by the Imperial College London's National Phenome Centre (London, UK) (44), and Metabolon, Inc. (Morrisville, NC, USA) (45). The majority of studies utilized GC-MS and/or LC-MS from the Broad Institute or Metabolon, Inc. The measurements of choline-related metabolites agreed well between the Broad Institute and Metabolon, Inc., platforms (Spearman correlation coefficients were 0.91, 0.75, 0.62, and 0.90 for choline, betaine, carnitine, and DMG) (22). To account for interstudy differences and improve normality, all metabolites were natural log-transformed and standardized using study-specific means and SDs.

Cardiometabolic biomarkers were measured, including glucose (milligrams/deciliter), insulin (microunits/milliliter), glycated hemoglobin (HbA1c; % of total hemoglobin), systolic blood pressure (SBP; millimeters of mercury), diastolic blood pressure (DBP; millimeters of mercury), total cholesterol (milligrams/deciliter), HDL cholesterol (milligrams/deciliter), LDL cholesterol (milligrams/deciliter), triglycerides (milligrams/deciliter), C-reactive protein (CRP; milligrams/liter), IL-6 (picograms/milliliter), creatinine (milligrams/deciliter), and homocysteine (micromoles/liter). Prior to statistical analyses, they were harmonized to the same units and natural log-transformed and standardized by the mean and SD in each study (SBP and DBP were not log-transformed due to their normal distributions).

Collection of survey data

Data on demographics, diet and lifestyle, anthropometrics, and medical history were collected at the time of blood sample collection (i.e., mostly at enrollment) in individual studies. Variables harmonized across studies include age at blood collection, sex, self-identified ethnicity, BMI, waist-to-hip ratio (WHR), smoking status, alcohol consumption, physical activity, menopausal status and hormone therapy (women only), history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease (NAFLD), as well as dietary information.

Disease history was self-reported based on doctor diagnosis or medication use. Dietary intakes of major foods and nutrients per day were estimated in 15 studies using validated food-frequency questionnaires and country- or region-specific food-composition tables (data were not available from the AIRWAVE and TMCS) and were standardized to intakes per 2000 kcal/d (46). Food-portion sizes for transformations were defined as follows: red meat, processed meat, poultry, total fish, and shellfish [1 serving = 4 ounces (oz)/113.4 g]; eggs (50 g); dairy foods [milk/cottage cheese (8 fluid oz/240 g), firm cheese (50 g), and ice cream (100 g)]; soy products [soy milk (8 fluid oz/240 g) and tofu/soybeans/soy meats (4 oz/113.4 g)]; legumes (50 g in dry weight); nuts (30 g in dry weight); vegetables (80 g); fruits (80 g); and whole grains (50 g in dry weight). In the statistical analyses, food intakes were modeled as each 1-serving/day increase, macronutrients were modeled as each 5%-energy/day intake from them, and dietary fiber was modeled as each 5-gram/day increase.

Statistical analysis

A standard analytic protocol was followed to analyze data from all participating studies. Linear regressions were used to estimate β-coefficients and 95% CIs for the associations of cardiometabolic biomarkers with circulating metabolites. Since metabolites and biomarker concentrations were all log-transformed and standardized by their means and SDs in each study, β-coefficients indicate changes in SD units on the log scale. Covariates were determined *a priori* and adjusted in 3 models. In model 1, we adjusted for age (years), sex (men and women), ethnicity (White, Black, Hispanic, Asian, and others), and fasting time (<6 and ≥6 h). In model 2, we additionally adjusted for education (<high school, high school graduation, post–high school training or some college, and ≥college graduation), obesity [BMI (kg/m^2) <18.5, 18.5–24.9, 25.0–29.9, and ≥30.0], central obesity (normal, moderate, high, and very high defined per WHO criteria; WHR <0.90, 0.90–0.94, 0.95–0.99, and ≥1.00 for non-Asian men; <0.85, 0.85–0.89, 0.90–0.94, ≥0.95 for Asian men; and <0.75, 0.75–0.79, 0.80–0.84, and ≥0.85 for all women), tobacco smoking status (never, former, and current), alcohol drinking (none, >0 to ≤1, >1 to ≤2, and >2 drinks/d; 1 drink = 14 g ethanol), total physical activity (study- and sex-specific tertiles of total physical activity), use of multivitamins (yes or no), menopausal status and hormone therapy (yes or no, for women only), and intakes of red meat, eggs, and fish (study- and sex-specific quintiles). In model 3, we further adjusted for metabolic disease status, including diabetes, hypertension, dyslipidemia, and NAFLD (yes or no). Missing values of covariates were coded as an unknown category in the analyses (proportions of missing are provided in **Supplemental Table 2**). We conducted 3 sensitivity analyses by *1*) additionally adjusting for creatinine to minimize the effects of renal function (FHS, MESA, SCCS, SMHS, SWHS, and TMCS only), *2*) excluding recent antibiotics users to control for the effects of microbiota metabolism of nutrients (FHS, MESA, SCCS, SMHS, SWHS, and TMCS only), and *3*) additionally adjusting for TMAO to control for its cardiometabolic effects (FHS, SMHS, SWHS, and TMCS only). We also evaluated whether the associations of choline and related metabolites with cardiometabolic biomarkers were modified by TMAO concentrations (FHS, SMHS, SWHS, and TMCS only). We further conducted stratified analyses by age, sex, ethnicity, region of cohorts, fasting time, obesity, central obesity, metabolic disease status (diabetes, hypertension, dyslipidemia, and NAFLD), and intakes of red meat, fish, fiber, and vegetables/fruits.

Using the same linear regression models, we also examined associations of circulating choline and related metabolites with demographics (age, sex, and ethnicity), lifestyle factors (smoking, alcohol drinking, and physical activity), metabolic conditions (obesity, central obesity, diabetes, hypertension, dyslipidemia, and NAFLD), and dietary intakes. In diet-related analyses, we excluded participants with implausible energy intakes (beyond ±5 SDs of the study- and sex-specific mean) and additionally adjusted for total energy intake in all 3 models.

Study-specific estimates were combined using random-effects meta-analyses (47), and heterogeneity across studies was assessed based on I^2. Subgroup heterogeneity was assessed using meta-regression. P values were corrected for multiple comparisons using the Benjamini-Hochberg procedure (48), and false discovery rate–adjusted P values (Q values) were estimated, with threshold set at 0.05. Study-specific analyses were conducted by using SAS version 9.4 (SAS Institute, Inc.), and meta-analyses were performed by using Stata 16.0 (StataCorp).

Results

Basic characteristics of study participants

Of the 32,853 participants from 17 studies (Supplemental Table 1), ages ranged from 19 to 84 y, 20,030 (61.0%) were women, 16,393 (49.9%) were White, 13,293 (40.5%) were Asian, 1779 (5.4%) were Black, and 1252 (3.8%) were Hispanic. Details of participants from each individual study are provided in Supplemental Table 2.

Associations of circulating choline and related metabolites with cardiometabolic biomarkers

Circulating choline showed positive associations with HbA1c, blood pressure, total cholesterol, triglycerides, CRP, and creatinine (**Figure 1** and **Supplemental Table 3**). After adjustments for sociodemographic, lifestyle, and dietary factors and metabolic conditions, the βs (95% CIs) corresponding to a 1-SD increase in biomarker on the log scale, in descending order of magnitude, were 0.136 (95% CI: 0.084, 0.188) for creatinine, 0.050 (0.027, 0.073) for triglycerides, 0.041 (0.017, 0.066) for HbA1c, 0.040 (0.014, 0.067) for total cholesterol, 0.036 (0.020, 0.053) for CRP, 0.032 (0.018, 0.046) for SBP, and 0.026 (0.006, 0.046) for DBP (all $Q \leq 0.020$; **Figure 2**A).

In contrast, circulating betaine showed inverse associations with glucose, insulin, blood pressure, total cholesterol, triglycerides, and homocysteine (Figure 1 and Supplemental Table 3). The βs (95% CIs) corresponding to a 1-SD increase in biomarker on the log scale, in descending order of magnitude, were −0.146 (−0.188, −0.104) for triglycerides, −0.106 (−0.130, −0.082) for insulin, −0.097 (−0.149, −0.045) for homocysteine, −0.074 (−0.102, −0.047) for total cholesterol, −0.039 (−0.069, −0.009) for DBP, −0.037 (−0.061, −0.013) for SBP, and −0.032 (−0.056, −0.008) for glucose (all $Q \leq 0.019$; Figure 2B).

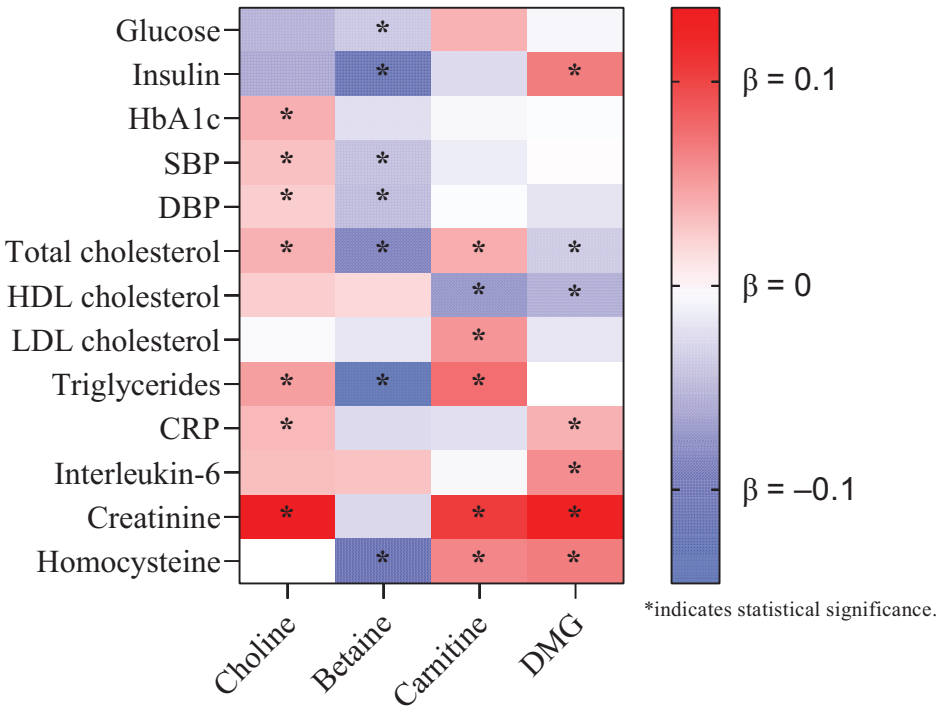


FIGURE 1 Heat map for the associations of choline and related metabolites with cardiometabolic biomarkers. Linear regression coefficients (β) were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. βs indicate the increase or decrease in SD units of choline, betaine, carnitine, and DMG on the log scale. Statistical significance is indicated by false discovery rate–adjusted *P* values (Q values) <0.05. CRP, C-reactive protein; DBP, diastolic blood pressure; DMG, dimethylglycine; HbA1c, glycated hemoglobin; SBP, systolic blood pressure.

Similar to choline, circulating carnitine was positively associated with creatinine (β = 0.106; 95% CI: 0.045, 0.168), triglycerides (0.076; 0.042, 0.109), homocysteine (0.064; 0.033, 0.095), LDL cholesterol (0.055; 0.013, 0.096), and total cholesterol (0.042; 0.007; 0.077), but negatively associated with HDL cholesterol (−0.061; −0.086, −0.037; Figure 1, Figure 2C, and Supplemental Table 3). Circulating DMG was positively related to creatinine (0.128; 0.087, 0.169), homocysteine (0.068; 0.023, 0.114), insulin (0.068; 0.043, 0.093), interleukin-6 (0.060; 0.027, 0.094), and CRP (0.040; 0.019, 0.060), but negatively related to HDL cholesterol (−0.046; −0.069, −0.023), and total cholesterol (−0.029; −0.047, −0.010; Figure 1, Figure 2D, and Supplemental Table 3).

Sensitivity analyses with further adjustment for creatinine, exclusion of recent antibiotic users, or additional adjustment for TMAO did not show appreciable changes in the results from the main analyses (**Supplemental Tables 4** and **5**). In addition, the associations between these metabolites and cardiometabolic biomarkers remained robust in a series of analyses stratified by demographics, CVD risk factors, dietary factors, and TMAO concentrations, and no significant interactions were found (**Supplemental Tables 6–9**).

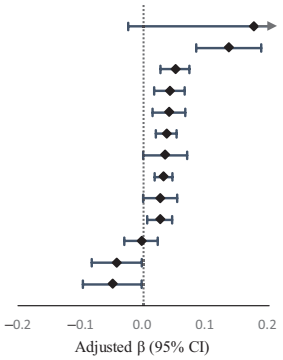
Associations of circulating choline and related metabolites with dietary intakes and nondietary characteristics

In our pooled analyses, circulating choline was not significantly associated with any dietary factors (**Figure 3** and **Supplemental Table 10**). In a subset of data from 3 studies (n = 2809), we also did not find a significant correlation between total dietary choline and circulating choline (partial correlation coefficient = 0.024, Q = 0.207; **Supplemental Table 11**). However, among US studies, circulating choline was positively associated with red meat intake (β = 0.066 per 1-serving/d increase), whereas among Asian studies, choline concentration was positively associated with egg intake and inversely with intakes of legumes and fruits (β = 0.215, −0.083, and −0.043, respectively, per 1-serving/d increase; data not shown in tables/figures). Among nondietary factors, circulating choline was positively associated with older age (β = 0.014 per 1-year increase), high physical activity (β = 0.065 vs. low physical activity), obesity (β = 0.108 vs. normal weight), high WHR (β = 0.067 vs. normal WHR), and hypertension (β = 0.068 vs. normal blood pressure), and was lower among Black than White participants in the US studies (β = −0.244) (Figure 3 and **Supplemental Table 12**).

Circulating betaine was positively associated with intakes of plant protein (β = 0.249 per 5% energy/d increase), fiber (β = 0.050 per 5 g/d increase), whole grains (β = 0.040 per 1 serving/d increase), and carbohydrate (β = 0.026 per 5% energy/d increase) but inversely with intakes of saturated fat (β = −0.108 per 5% energy/d increase), monounsaturated fat (β = −0.056), and animal fat (β = −0.056; **Figure 4** and **Supplemental Table 13**). Circulating betaine was positively associated with high physical activity (β = 0.044) and negatively associated with overweight and obesity (β = −0.097 and −0.160), dyslipidemia

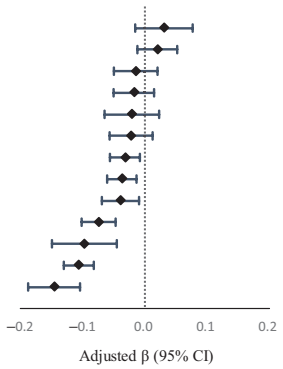
A. Choline

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Homocysteine, 1 SD increase	4,720	0.176 (–0.024, 0.376)	0.092	97.7%
Creatinine, 1 SD increase	19,749	0.136 (0.084, 0.188)	<0.001	87.4%
Triglycerides, 1 SD increase	18,842	0.050 (0.027, 0.073)	<0.001	24.1%
HbA1c, 1 SD increase	14,580	0.041 (0.017, 0.066)	0.003	17.8%
Total cholesterol, 1 SD increase	24,235	0.040 (0.014, 0.067)	0.004	62.4%
CRP, 1 SD increase	15,340	0.036 (0.020, 0.053)	<0.001	0.0%
Interleukin-6, 1 SD increase	4,850	0.034 (–0.001, 0.069)	0.066	16.4%
SBP, 1 SD increase	21,922	0.032 (0.018, 0.046)	<0.001	0.0%
HDL cholesterol, 1 SD increase	22,404	0.026 (–0.001, 0.054)	0.066	55.0%
DBP, 1 SD increase	21,922	0.026 (0.006, 0.046)	0.020	32.0%
LDL cholesterol, 1 SD increase	17,327	–0.004 (–0.031, 0.023)	0.766	46.3%
Glucose, 1 SD increase	21,245	–0.043 (–0.083, –0.003)	0.053	80.7%
Insulin, 1 SD increase	8,001	–0.050 (–0.097, –0.003)	0.053	66.7%



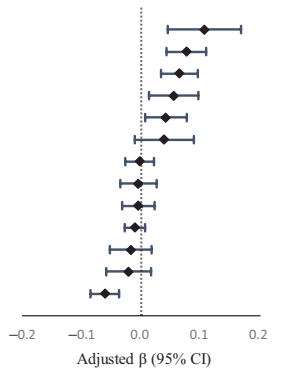
B. Betaine

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Interleukin-6, 1 SD increase	4,852	0.031 (–0.016, 0.077)	0.289	48.0%
HDL cholesterol, 1 SD increase	22,407	0.020 (–0.012, 0.052)	0.289	68.2%
LDL cholesterol, 1 SD increase	17,330	–0.015 (–0.050, 0.020)	0.409	70.3%
HbA1c, 1 SD increase	14,584	–0.018 (–0.050, 0.015)	0.336	47.5%
CRP, 1 SD increase	15,343	–0.021 (–0.065, 0.023)	0.377	79.9%
Creatinine, 1 SD increase	19,753	–0.022 (–0.057, 0.012)	0.289	73.1%
Glucose, 1 SD increase	21,248	–0.032 (–0.056, -0.008)	0.019	46.4%
SBP, 1 SD increase	21,925	–0.037 (–0.061, –0.013)	0.005	47.3%
DBP, 1 SD increase	21,925	–0.039 (–0.069, –0.009)	0.019	68.6%
Total cholesterol, 1 SD increase	24,238	–0.074 (–0.102, –0.047)	<0.001	67.2%
Homocysteine, 1 SD increase	4,724	–0.097 (–0.149, –0.045)	<0.001	69.5%
Insulin, 1 SD increase	8,004	–0.106 (–0.130, –0.082)	<0.001	0.0%
Triglycerides, 1 SD increase	18,845	–0.146 (–0.188, –0.104)	<0.001	75.8%



C. Carnitine

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Creatinine, 1 SD increase	19,665	0.106 (0.045, 0.168)	0.003	91.3%
Triglycerides, 1 SD increase	16,084	0.076 (0.042, 0.109)	<0.001	47.1%
Homocysteine, 1 SD increase	4,620	0.064 (0.033, 0.095)	<0.001	0.0%
LDL cholesterol, 1 SD increase	15,917	0.055 (0.013, 0.096)	0.026	72.0%
Total cholesterol, 1 SD increase	23,786	0.042 (0.007, 0.077)	0.043	78.3%
Glucose, 1 SD increase	18,101	0.039 (–0.011, 0.089)	0.234	83.2%
DBP, 1 SD increase	19,411	–0.003 (–0.027, 0.021)	0.822	42.3%
Interleukin-6, 1 SD increase	7,228	–0.005 (–0.035, 0.026)	0.822	19.7%
HbA1c, 1 SD increase	15,885	–0.005 (–0.032, 0.022)	0.822	26.0%
SBP, 1 SD increase	19,411	–0.011 (–0.028, 0.006)	0.358	7.8%
CRP, 1 SD increase	17,049	–0.018 (–0.053, 0.017)	0.417	70.0%
Insulin, 1 SD increase	7,488	–0.021 (–0.059, 0.016)	0.389	46.9%
HDL cholesterol, 1 SD increase	21,317	–0.061 (–0.086, –0.037)	<0.001	38.4%



D. DMG

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Creatinine, 1 SD increase	20,581	0.128 (0.087, 0.169)	<0.001	79.4%
Homocysteine, 1 SD increase	5,307	0.068 (0.023, 0.114)	0.006	50.9%
Insulin, 1 SD increase	7,487	0.068 (0.043, 0.093)	<0.001	3.6%
Interleukin-6, 1 SD increase	7,227	0.060 (0.027, 0.094)	<0.001	31.6%
CRP, 1 SD increase	17,513	0.040 (0.019, 0.060)	<0.001	23.7%
SBP, 1 SD increase	19,410	0.002 (–0.022, 0.025)	0.920	38.9%
Triglycerides, 1 SD increase	16,560	0.001 (–0.016, 0.017)	0.920	0.0%
HbA1c, 1 SD increase	15,885	–0.003 (–0.033, 0.027)	0.920	36.8%
Glucose, 1 SD increase	18,732	–0.006 (–0.041, 0.029)	0.920	68.9%
LDL cholesterol, 1 SD increase	16,393	–0.015 (–0.044, 0.015)	0.488	48.6%
DBP, 1 SD increase	19,410	–0.016 (–0.043, 0.012)	0.423	56.4%
Total cholesterol, 1 SD increase	24,262	–0.029 (–0.047, –0.010)	0.004	29.5%
HDL cholesterol, 1 SD increase	21,793	–0.046 (–0.069, –0.023)	<0.001	37.2%

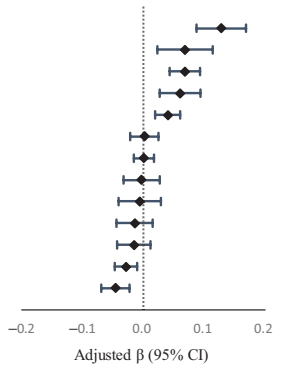
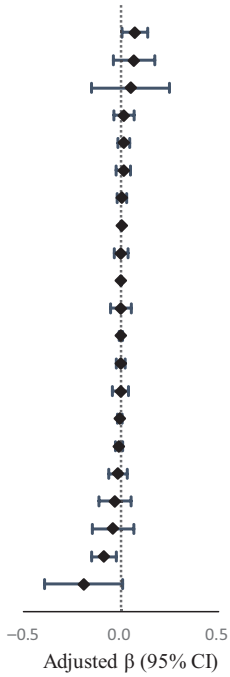


FIGURE 2 Circulating choline (A), betaine (B), carnitine (C), and DMG (D) in relation to cardiometabolic biomarkers. Regression coefficients (β) and 95% CIs were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; and history of diabetes, hypertension, dyslipidemia, and nonalcoholic fatty liver disease. βs indicate the increase or decrease in SD units of choline, betaine, carnitine, and DMG on the log scale. *Q* values represent corrected *P* values for each group of analyses by controlling the false discovery rate. CRP, C-reactive protein; DBP, diastolic blood pressure; DMG, dimethylglycine; HbA1c, glycated hemoglobin; SBP, systolic blood pressure.

A. Dietary factors (per day)

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Eggs, per 1 serving	14,674	0.071 (0.005, 0.137)	0.184	62.8%
Processed meat, per 1 serving	13,829	0.067 (–0.039, 0.173)	0.760	50.8%
Shellfish, per 1 serving	13,829	0.048 (–0.151, 0.248)	0.889	0.0%
Monounsaturated fat, per 5% energy	15,530	0.015 (–0.037, 0.067)	0.848	50.2%
Red meat, per 1 serving	14,674	0.014 (–0.016, 0.044)	0.831	48.1%
Saturated fat, per 5% energy	15,530	0.012 (–0.025, 0.048)	0.848	13.2%
Whole grains, per 1 seving	11,708	0.004 (–0.020, 0.029)	0.942	49.0%
Wheat, per 1 serving	13,829	0.001 (0, 0.003)	0.184	0.0%
Animal protein, per 5% energy	11,971	0.001 (–0.035, 0.036)	0.987	0.0%
Dairy foods, per 1 serving	13,829	0 (0, 0)	0.063	0.0%
Total fish, per 1 serving	14,674	0 (–0.054, 0.053)	0.987	61.7%
Vegetables, per 1 serving	13,829	–0.001 (–0.009, 0.008)	0.987	23.8%
Carbohydrate, per 5% energy	15,530	–0.002 (–0.024, 0.021)	0.987	72.4%
Legume, per 1 serving	13,509	–0.003 (–0.044, 0.038)	0.987	29.5%
Fruits, per 1 serving	13,829	–0.006 (–0.018, 0.006)	0.803	45.4%
Fiber, per 5 grams	15,530	–0.010 (–0.029, 0.008)	0.803	17.6%
Poultry, per 1 seving	13,829	–0.016 (–0.063, 0.032)	0.848	36.3%
Plant protein, per 5% energy	11,971	–0.031 (–0.113, 0.052)	0.848	0.0%
Polyunsaturated fat, per 5% energy	15,530	–0.041 (–0.147, 0.066)	0.848	72.1%
Nuts, per 1 serving	11,708	–0.087 (–0.151, –0.024)	0.074	0.0%
Soy products, per 1 serving	13,829	–0.192 (–0.392, 0.008)	0.252	56.8%



B. Non-dietary factors

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Obese vs. normal weight	3,231/13,827	0.108 (0.030, 0.186)	0.038	73.2%
Very high WHR vs. normal WHR	3,616/3,665	0.084 (0.014, 0.154)	0.068	39.9%
Hypertension vs. none	7,041/19,673	0.068 (0.024, 0.112)	0.017	67.4%
High WHR vs. normal WHR	3,470/3,665	0.067 (0.017, 0.117)	0.038	20.8%
High physical activity vs. low	8,855/8,463	0.065 (0.017, 0.113)	0.038	69.9%
Underweight vs. normal weight	2,057/13,827	0.057 (–0.033, 0.148)	0.446	59.2%
Overweight vs. normal weight	7,573/13,827	0.050 (–0.012, 0.112)	0.288	81.9%
Moderate WHR vs. normal WHR	3,556/3,665	0.046 (–0.010, 0.102)	0.288	37.2%
Current smokers vs. never smokers	5,849/15,459	0.036 (–0.012, 0.083)	0.321	42.6%
>2 drinks/day vs. none	3,332/11,661	0.035 (–0.075, 0.146)	0.738	87.8%
Diabetes vs. none	1,936/24,780	0.032 (–0.030, 0.093)	0.594	52.1%
Moderate physical activity vs. low	8,420/8,463	0.022 (–0.026, 0.071)	0.620	68.2%
Age, years	26,740	0.014 (0.010, 0.019)	<0.001	77.6%
Hispanic vs. White	1,253/10,391	0.014 (–0.524, 0.552)	0.988	91.5%
>1 to ⩽ 2 drinks/day vs. none	2,539/11,661	0.013 (–0.070, 0.097)	0.865	74.4%
Dyslipidemia vs. none	5,412/17,588	0.012 (–0.016, 0.041)	0.620	22.4%
Menopause vs. pre-menopause	5,480/4,171	0.009 (–0.048, 0.065)	0.865	19.4%
Hormone therapy vs. none	1,725/11,645	0.003 (–0.003, 0.009)	0.620	0.0%
NAFLD vs. none	1,114/12,119	0.002 (–0.076, 0.079)	0.988	0.0%
Former smokers vs. never smokers	5,348/15,459	0 (–0.042, 0.043)	0.988	41.6%
>0 to ⩽ 1 drinks/day vs. none	3,332/11,661	–0.008 (–0.045, 0.029)	0.821	31.9%
Asian vs. White	13,283/10,391	–0.055 (–0.287, 0.178)	0.821	0.0%
Others vs. White	64/10,391	–0.103 (–0.389, 0.183)	0.706	0.0%
Black vs. White	1,749/10,391	–0.244 (–0.389, –0.100)	0.013	50.6%
Women vs. men	16,372/10,368	–0.268 (–0.540, 0.004)	0.166	98.4%

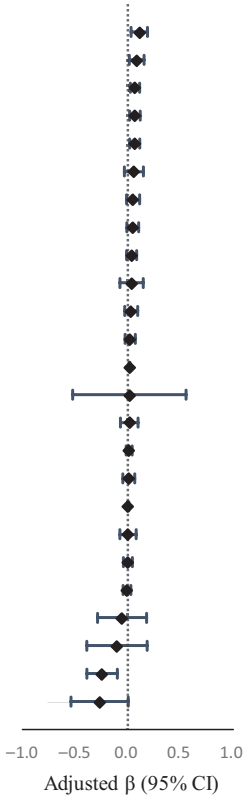
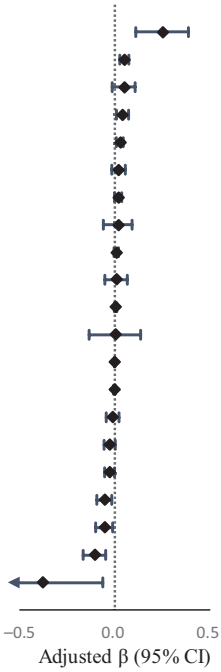


FIGURE 3 Circulating choline in relation to dietary (A) and nondietary (B) factors. Regression coefficients (β) and 95% CIs were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; history of diabetes, hypertension, dyslipidemia, and NAFLD; and total energy. Dietary covariates were mutually adjusted for other foods and included in the model as the cohort- and sex-specific quintiles. Total energy intake was additionally adjusted for in diet-related analyses. βs indicate the increase or decrease in SD units of choline on the log scale. *Q* values represent corrected *P* values for each group of analyses by controlling the false discovery rate. NAFLD, nonalcoholic fatty liver disease; WHR, waist-to-height ratio.

A. Dietary factors (per day)

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Plant protein, per 5% energy	11,975	0.249 (0.110, 0.388)	<0.001	65.7%
Fiber, per 5 grams	15,533	0.050 (0.026, 0.074)	<0.001	54.1%
Eggs, per 1 serving	14,677	0.046 (–0.014, 0.107)	0.233	59.6%
Whole grains, per 1 seving	11,711	0.040 (0.008, 0.072)	0.042	74.7%
Carbohydrate, per 5% energy	15,533	0.026 (0.008, 0.044)	0.021	61.3%
Legume, per 1 serving	13,512	0.019 (–0.016, 0.055)	0.352	23.2%
Fruits, per 1 serving	13,832	0.017 (–0.002, 0.036)	0.143	82.4%
Polyunsaturated fat, per 5% energy	15,533	0.015 (–0.061, 0.091)	0.769	49.9%
Wheat, per 1 serving	13,832	0.007 (–0.004, 0.019)	0.317	25.9%
Nuts, per 1 serving	11,711	0.006 (–0.053, 0.066)	0.873	0.0%
Vegetables, per 1 serving	13,832	0.004 (–0.003, 0.010)	0.344	11.8%
Soy products, per 1 serving	13,832	0 (–0.136, 0.136)	0.999	31.2%
Processed meat, per 1 serving	13,832	–0.001 (–0.002, 0)	0.317	0.0%
Poultry, per 1 seving	13,832	–0.003 (–0.007, 0.002)	0.352	0.0%
Total fish, per 1 serving	14,677	–0.012 (–0.045, 0.022)	0.571	29.5%
Red meat, per 1 serving	14,677	–0.027 (–0.057, 0.002)	0.143	50.0%
Dairy foods, per 1 serving	13,832	–0.028 (–0.054, –0.002)	0.086	71.1%
Animal protein, per 5% energy	11,975	–0.056 (–0.095, –0.017)	0.021	20.4%
Monounsaturated fat, per 5% energy	15,533	–0.056 (–0.101, –0.012)	0.042	39.8%
Saturated fat, per 5% energy	15,533	–0.108 (–0.167, –0.048)	<0.001	67.2%
Shellfish, per 1 serving	13,832	–0.380 (–0.697, –0.063)	0.050	46.2%



B. Non-dietary factors

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Black vs. White	1,779/16,393	0.248 (0.157, 0.339)	<0.001	0.0%
Asian vs. White	13,293/16,393	0.233 (0.020, 0.447)	0.080	0.0%
Others vs. White	136/16,393	0.118 (–0.158, 0.393)	0.496	0.0%
Underweight vs. normal weight	4,177/16,526	0.072 (–0.004, 0.149)	0.121	47.3%
Current smokers vs. never smokers	6,431/18,672	0.049 (–0.002, 0.100)	0.121	51.0%
High physical activity vs. low	10,586/10,815	0.044 (0.024, 0.065)	<0.001	3.4%
Menopause vs. pre-menopause	7,602/5,325	0.039 (–0.089, 0.168)	0.624	83.1%
Hispanic vs. White	1,252/16,393	0.033 (–0.167, 0.233)	0.775	48.2%
Moderate physical activity vs. low	10,450/10,815	0.024 (–0.004, 0.053)	0.163	22.2%
Age, years	32,853	0.006 (0.002, 0.010)	0.022	80.4%
Former smokers vs. never smokers	7,606/18,672	–0.005 (–0.029, 0.020)	0.752	6.1%
>2 drinks/day vs. none	4,309/13,459	–0.007 (–0.094, 0.081)	0.883	78.2%
>0 to ⩽ 1 drinks/day vs. none	9,694/13,459	–0.014 (–0.043, 0.016)	0.496	15.6%
>1 to ⩽ 2 drinks/day vs. none	3,742/13,459	–0.022 (–0.075, 0.031)	0.496	39.5%
Moderate WHR vs. normal WHR	4,124/4,475	–0.026 (–0.085, 0.033)	0.496	46.6%
Hypertension vs. none	8,438/24,389	–0.038 (–0.071, –0.005)	0.061	41.7%
High WHR vs. normal WHR	3,782/4,475	–0.056 (–0.135, 0.023)	0.243	65.9%
Very high WHR vs. normal WHR	3,867/4,475	–0.065 (–0.153, 0.024)	0.241	65.9%
Dyslipidemia vs. none	7,193/21,920	–0.068 (–0.118, –0.019)	0.022	71.2%
NAFLD vs. none	1,114/12,121	–0.073 (–0.151, 0.005)	0.121	0.0%
Overweight vs. normal weight	8,499/16,526	–0.097 (–0.147, –0.048)	<0.001	71.1%
Diabetes vs. none	2,054/30,775	–0.110 (–0.218, –0.003)	0.102	88.4%
Obese vs. normal weight	3,599/16,526	–0.160 (–0.254, –0.066)	0.004	84.1%
Women vs. men	20,032/12,821	–0.564 (–0.849, –0.279)	<0.001	98.6%
Hormone therapy vs. none	2,629/14,309	–0.684 (–1.049, –0.318)	<0.001	98.8%

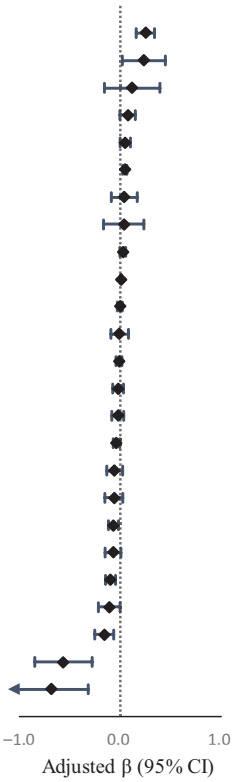


FIGURE 4 Circulating betaine in relation to dietary (A) and nondietary (B) factors. Regression coefficients (β) and 95% CIs were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; history of diabetes, hypertension, dyslipidemia, and NAFLD; and total energy. Dietary covariates were mutually adjusted for other foods and included in the model as the cohort- and sex-specific quintiles. Total energy intake was additionally adjusted for in diet-related analyses. βs indicate the increase or decrease in SD units of betaine on the log scale. *Q* values represent corrected *P* values for each group of analyses by controlling the false discovery rate. NAFLD, nonalcoholic fatty liver disease; WHR, waist-to-height ratio.

($\beta = -0.068$), and hormone therapy ($\beta = -0.684$) (Figure 4 and **Supplemental Table 14**). Circulating betaine was lower in women than in men ($\beta = -0.564$) and higher in Black than in White participants in the US studies ($\beta = 0.248$).

Circulating carnitine was positively associated with intake of red meat ($\beta = 0.092$ per 1 serving/d increase) and inversely associated with plant protein ($\beta = -0.132$ per 5% energy/d increase; **Figure 5** and **Supplemental Table 15**). Circulating carnitine was positively associated with current and former smoking ($\beta = 0.160$ and 0.082), alcohol drinking ($\beta = 0.053$ and 0.184 for >0 to ≤1 and >2 drinks/d), overweight and obesity ($\beta = 0.072$ and 0.155), very high WHR ($\beta = 0.101$), hypertension ($\beta = 0.065$), and menopause ($\beta = 0.161$) but negatively associated with diabetes ($\beta = -0.150$) and hormone therapy ($\beta = -0.235$; Figure 5 and **Supplemental Table 16**). Circulating carnitine was lower in women than in men ($\beta = -0.359$) and higher in Asian than in White participants in the US studies ($\beta = 0.476$).

Circulating DMG was positively associated with plant protein ($\beta = 0.201$ per 5% energy/d increase), age ($\beta = 0.011$ per 1-y increase), current smoking ($\beta = 0.147$), obesity ($\beta = 0.070$), and hypertension ($\beta = 0.098$), but negatively associated with alcohol drinking ($\beta = -0.059$, -0.099, and -0.165 for >0 to ≤1, >1 to ≤2, and >2 drinks/d), diabetes ($\beta = -0.077$), and hormone therapy ($\beta = -0.300$; **Figure 6** and **Supplemental Tables 17** and **18**). Circulating DMG was lower in women than in men ($\beta = -0.372$) and higher in Asian than in White participants in the US studies ($\beta = 0.388$).

Discussion

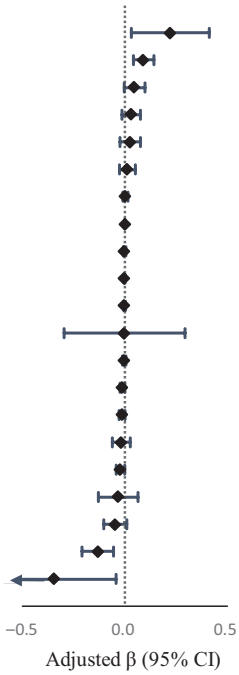
In this large international pooling project, circulating choline, carnitine, and DMG were associated with an unfavorable cardiometabolic risk profile, while betaine showed a beneficial cardiometabolic risk profile. Circulating choline was positively associated with creatinine, total cholesterol, triglycerides, HbA1c, blood pressure, and CRP. Similarly, circulating carnitine was positively associated with creatinine, an unfavorable blood lipid profile (low HDL and high total cholesterol, LDL cholesterol, and triglycerides), and homocysteine. DMG was positively associated with creatinine, insulin, CRP, IL-6, and homocysteine, but was inversely associated with total and HDL cholesterol. In contrast, circulating betaine was associated with markers of favorable glycemic control (lower blood glucose and insulin) and lower concentrations of total cholesterol and triglycerides and homocysteine and lower blood pressure.

Our findings were generally consistent with previous reports regarding associations of choline and betaine with cardiometabolic risk factors. Three population-based studies have reported contrasting associations for choline and betaine among >7000 Norwegian adults and nearly 2000 American adults in the Multiethnic Cohort Adiposity Phenotype Study and the Nutrition, Aging, and Memory in Elders cohort (9–11). Across studies, poor lipid profiles were related to higher circulating choline and lower betaine, and higher choline and lower betaine were also suggestively associated with higher blood pressure, poorer glucose control, and higher BMI. Consistent with findings from observational studies (9, 11), phosphatidylcholine supplementation (providing 2.6 g/d of choline) for 2 wk increased serum triglycerides in an analysis of 4 clinical trials (49). However, the same analysis also showed that betaine supplementation of 6 g/d for 6 wk increased total cholesterol, LDL cholesterol, and triglycerides (49), and another meta-analysis of 6 clinical trials among 233 participants showed that betaine supplementation of >4 g/d for 6–24 wk moderately increased total cholesterol (mean difference of 0.34 mmol/L compared with placebo) (50). Thus, while the triglyceride-increasing effect of choline supplementation has been consistent in observational and intervention studies, the adverse effects of betaine supplementation on blood lipids remain contradictory (51). On the other hand, our finding of a negative association of circulating betaine with homocysteine concentrations was supported by evidence from observational and intervention studies (9, 52). A meta-analysis of 5 clinical trials among 206 healthy adults showed a reduction of 1.23 μmol/L in plasma homocysteine for >4 g/d betaine supplementation for 6–24 wk compared with placebo (52). In another trial among 35 participants with mildly elevated homocysteine, daily supplementation of 6 g betaine for 6 wk decreased plasma homocysteine concentrations by 11% (53). Consistently, a trial among 76 participants with high homocysteine concentrations showed that 1.5, 3, and 6 g/d for 6 wk could reduce fasting plasma homocysteine concentration by 12%, 15%, and 20%, respectively (54), suggesting that a moderate dose of betaine may render substantial homocysteine-lowering effects. Of note, usual dietary intakes of choline are ~400–600 mg/d and of betaine are ~200–400 mg/d (55), much lower than the doses used in most clinical trials. In addition, previous observational studies and our current data (Supplemental Table 11) indicated weak correlations between dietary intake and circulating concentrations of choline and betaine (20, 21), which may partially explain conflicting results for dietary choline intake versus circulating choline concentrations in association with cardiometabolic biomarkers (10, 55–57). Thus, caution should be taken when comparing our findings with those from clinical trials or observational studies that evaluated self-reported dietary intakes of choline and betaine. Since prior clinical trials mostly had high doses of supplementation, small sample sizes, and short follow-ups, and observational studies were mostly cross-sectional and could not determine causality, future prospective studies and large trials on sustaining low-dose supplementation are still needed to confirm the associations of choline and betaine with cardiometabolic factors.

Although our pooling analyses showed unfavorable cardiometabolic risk profiles for higher carnitine and DMG, very few studies examined similar topics. In the Multiethnic Cohort Adiposity Phenotype Study, plasma carnitine showed positive associations with higher insulin resistance, poorer blood lipids (higher triglycerides and lower HDL), and higher CRP (11), which was consistent with our findings. While DMG was rarely studied for its relation with cardiometabolic risk factors, it was associated with higher incidence of acute myocardial infarction in patients with stable angina pectoris (58) and total or CVD mortality in patients with coronary artery disease (59). Of note, DMG can be produced from betaine during the remethylation of homocysteine to methionine, which is catalyzed by betaine-homocysteine methyltransferase (5), and the betaine-homocysteine methyltransferase can enhance the hepatic production of VLDL and apolipoprotein-B (60), which are related to atherosclerosis and CVD (61, 62). The collective evidence so far suggests that DMG might be involved in CVD development. Inconsistently, circulating

A. Dietary factors (per day)

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Processed meat, per 1 serving	16,117	0.222 (0.032, 0.412)	0.131	75.4%
Red meat, per 1 serving	16,962	0.092 (0.042, 0.142)	<0.001	84.4%
Animal protein, per 5% energy	14,886	0.048 (–0.003, 0.098)	0.173	57.1%
Saturated fat, per 5% energy	16,117	0.031 (–0.014, 0.076)	0.378	39.4%
Poultry, per 1 seving	16,117	0.026 (–0.024, 0.076)	0.574	28.2%
Monounsaturated fat, per 5% energy	16,117	0.012 (–0.026, 0.051)	0.686	11.8%
Legume, per 1 serving	16,117	0.003 (–0.010, 0.015)	0.772	0.0%
Dairy foods, per 1 serving	16,117	0 (0, 0)	0.772	0.0%
Wheat, per 1 serving	16,117	–0.001 (–0.007, 0.005)	0.782	4.1%
Eggs, per 1 serving	16,962	–0.001 (–0.003, 0.001)	0.574	0.0%
Whole grains, per 1 seving	13,996	–0.001 (–0.006, 0.003)	0.686	70.3%
Shellfish, per 1 serving	16,117	–0.002 (–0.297, 0.294)	0.992	0.0%
Fruits, per 1 serving	16,117	–0.003 (–0.012, 0.005)	0.668	17.7%
Vegetables, per 1 serving	16,117	–0.012 (–0.024, 0.000)	0.173	58.4%
Carbohydrate, per 5% energy	16,117	–0.014 (–0.028, 0.001)	0.173	27.2%
Total fish, per 1 serving	16,962	–0.017 (–0.061, 0.026)	0.668	41.9%
Fiber, per 5 grams	16,117	–0.022 (–0.042, –0.001	0.173	23.4%
Nuts, per 1 serving	13,996	–0.033 (–0.129, 0.064)	0.686	9.9%
Polyunsaturated fat, per 5% energy	16,117	–0.047 (–0.103, 0.009)	0.238	0.0%
Plant protein, per 5% energy	14,886	–0.132 (–0.209, –0.055)	0.011	0.0%
Soy products, per 1 serving	16,117	–0.345 (–0.647, –0.043)	0.131	35.2%



B. Non-dietary factors

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Asian vs. White	11,561/13,611	0.476 (0.249, 0.704)	<0.001	0.0%
Others vs. White	121/13,611	0.426 (0.086, 0.765)	0.023	100.0%
>2 drinks/day vs. none	3,802/11,127	0.184 (0.067, 0.301)	0.007	90.5%
Menopause vs. pre-menopause	6,117/4,252	0.161 (0.053, 0.269)	0.008	74.3%
Current smokers vs. never smokers	5,668/15,104	0.160 (0.066, 0.254)	0.004	86.8%
Obese vs. normal weight	2,597/14,214	0.155 (0.060, 0.250)	0.004	81.5%
NAFLD vs. none	331/11,507	0.102 (–0.008, 0.212)	0.103	0.0%
Very high WHR vs. normal WHR	2,639/3,273	0.101 (0.024, 0.179)	0.018	48.1%
Former smokers vs. never smokers	6,544/15,104	0.082 (0.028, 0.136)	0.008	69.2%
Overweight vs. normal weight	6,510/14,214	0.072 (0.019, 0.125)	0.017	71.1%
>1 to ⩽ 2 drinks/day vs. none	3,194/11,127	0.069 (–0.028, 0.165)	0.225	85.7%
Hypertension vs. none	7,172/20,219	0.065 (0.025, 0.104)	0.004	59.9%
High WHR vs. normal WHR	2,672/3,273	0.055 (–0.003, 0.113)	0.097	30.7%
>0 to ⩽ 1 drinks/day vs. none	8,307/11,127	0.053 (0.014, 0.092)	0.017	40.7%
Dyslipidemia vs. none	6,016/17,984	0.050 (–0.029, 0.130)	0.286	90.3%
Black vs. White	852/13,611	0.047 (–0.131, 0.225)	0.723	16.5%
Underweight vs. normal weight	4,066/14,214	0.022 (–0.079, 0.124)	0.727	82.1%
High physical activity vs. low	8,835/9,177	0.009 (–0.032, 0.049)	0.727	55.2%
Age, years	27,393	0.007 (0.002, 0.013)	0.018	87.5%
Moderate physical activity vs. low	8,736/9,177	0.007 (–0.027, 0.040)	0.727	35.8%
Moderate WHR vs. normal WHR	3,023/3,273	0.004 (–0.026, 0.033)	0.805	4.0%
Hispanic vs. White	1,248/13,611	–0.057 (–0.160, 0.046)	0.344	0.0%
Diabetes vs. none	1,726/25,667	–0.150 (–0.240, -0.059)	0.004	77.0%
Hormone therapy vs. none	2,411/13,340	–0.235 (–0.410, -0.060)	0.017	95.4%
Women vs. men	17,088/10,305	–0.359 (–0.575, -0.143)	0.004	96.8%

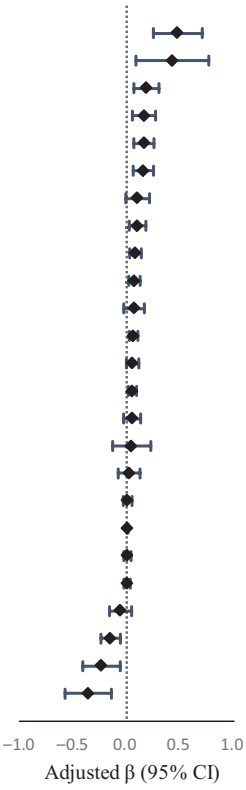
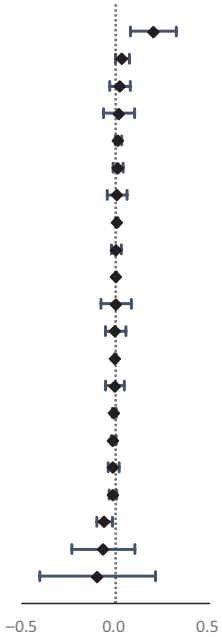


FIGURE 5 Circulating carnitine in relation to dietary (A) and nondietary (B) factors. Regression coefficients (β) and 95% CIs were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; history of diabetes, hypertension, dyslipidemia, and NAFLD; and total energy. Dietary covariates were mutually adjusted for other foods and included in the model as the cohort- and sex-specific quintiles. Total energy intake was additionally adjusted for in diet-related analyses. βs indicate the increase or decrease in SD units of carnitine on the log scale. *Q* values represent corrected *P* values for each group of analyses by controlling the false discovery rate. NAFLD, nonalcoholic fatty liver disease; WHR, waist-to-height ratio.

A. Dietary factors (per day)

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Plant protein, per 5% energy	15,206	0.201 (0.079, 0.324)	0.021	58.8%
Eggs, per 1 serving	17,908	0.036 (–0.001, 0.074)	0.284	17.0%
Processed meat, per 1 serving	17,063	0.023 (–0.032, 0.078)	0.716	5.8%
Nuts, per 1 serving	14,942	0.018 (–0.065, 0.101)	0.878	0.0%
Carbohydrate, per 5% energy	17,063	0.015 (-0.002, 0.032)	0.315	52.4%
Fiber, per 5 grams	17,063	0.013 (–0.014, 0.040)	0.649	58.3%
Monounsaturated fat, per 5% energy	17,063	0.008 (–0.045, 0.061)	0.900	55.7%
Wheat, per 1 serving	17,063	0.006 (–0.001, 0.012)	0.315	5.0%
Red meat, per 1 serving	17,908	0.004 (–0.022, 0.030)	0.900	38.9%
Whole grains, per 1 seving	14,942	0.003 (0, 0.006)	0.284	29.7%
Polyunsaturated fat, per 5% energy	17,063	0.003 (–0.078, 0.084)	0.995	53.6%
Legume, per 1 serving	16,743	0 (–0.054, 0.055)	0.997	50.4%
Vegetables, per 1 serving	17,063	–0.002 (–0.006, 0.002)	0.605	4.1%
Saturated fat, per 5% energy	17,063	–0.004 (–0.054, 0.046)	0.970	54.0%
Poultry, per 1 seving	17,063	–0.007 (–0.018, 0.004)	0.434	0.6%
Fruits, per 1 serving	17,063	–0.010 (–0.023, 0.002)	0.333	54.4%
Total fish, per 1 serving	17,908	–0.011 (–0.040, 0.019)	0.719	14.6%
Dairy foods, per 1 serving	17,063	–0.015 (–0.035, 0.005)	0.394	56.6%
Animal protein, per 5% energy	15,206	–0.059 (–0.100, –0.017	0.063	39.7%
Soy products, per 1 serving	17,063	–0.066 (–0.234, 0.102)	0.717	5.5%
Shellfish, per 1 serving	17,063	–0.096 (–0.405, 0.212)	0.757	17.1%



B. Non-dietary factors

Characteristics	No. of participants	β (95% CI)	*Q*-values	I^2
Asian vs. White	11,561/14,055	0.388 (0.162, 0.613)	0.004	0.0%
Current smokers vs. never smokers	5,952/15,488	0.147 (0.065, 0.230)	<0.001	82.5%
Hypertension vs. none	7,669/20,704	0.098 (0.047, 0.149)	<0.001	78.4%
Black vs. White	1,395/14,055	0.078 (–0.057, 0.213)	0.375	13.4%
Hispanic vs. White	1,252/14,055	0.071 (–0.065, 0.208)	0.425	0.0%
Obese vs. normal weight	2,943/14,509	0.070 (0.025, 0.116)	0.007	26.1%
Others vs. White	136/14,055	0.060 (–0.559, 0.679)	0.884	74.9%
Very high WHR vs. normal WHR	2,760/3,350	0.060 (0.003, 0.117)	0.081	22.0%
Menopause vs. pre-menopause	6,420/4,348	0.048 (–0.021, 0.117)	0.283	39.1%
Underweight vs. normal weight	4,079/14,509	0.033 (–0.024, 0.090)	0.375	41.7%
NAFLD vs. none	331/11,507	0.029 (–0.081, 0.139)	0.690	0.0%
Former smokers vs. never smokers	6,852/15,488	0.021 (–0.002, 0.045)	0.152	6.4%
High WHR vs. normal WHR	2,768/3,350	0.014 (–0.037, 0.064)	0.690	19.8%
Overweight vs. normal weight	6,833/14,509	0.013 (–0.031, 0.057)	0.690	56.8%
High physical activity vs. low	9,072/9,377	0.013 (–0.005, 0.031)	0.270	0.0%
Age, years	28,399	0.011 (0.007, 0.014)	<0.001	71.9%
Moderate physical activity vs. low	8,948/9,377	0.005 (–0.013, 0.023)	0.690	0.0%
Dyslipidemia vs. none	6,228/18,431	0.001 (–0.027, 0.029)	0.940	22.1%
Moderate WHR vs. normal WHR	3,116/3,350	–0.003 (–0.023, 0.017)	0.829	0.0%
>0 to ⩽ 1 drinks/day vs. none	8,514/11,449	–0.059 (-0.099, –0.019	0.013	45.5%
Diabetes vs. none	1,906/26,469	–0.077 (–0.136, –0.017)	0.030	44.9%
>1 to ⩽ 2 drinks/day vs. none	3,227/11,449	–0.099 (–0.177, –0.021)	0.030	77.1%
>2 drinks/day vs. none	3,894/11,449	–0.165 (–0.256, –0.075)	<0.001	83.5%
Hormone therapy vs. none	2,591/13,688	–0.300 (–0.467, –0.133)	<0.001	95.1%
Women vs. men	17,807/10,592	–0.372 (–0.661, –0.082)	0.030	98.3%

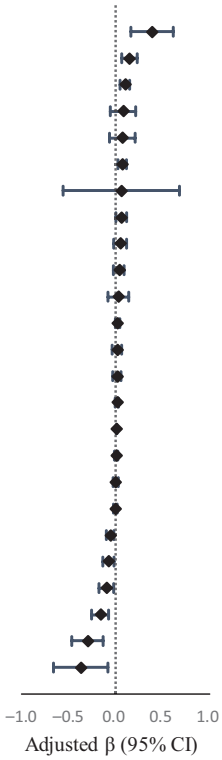


FIGURE 6 Circulating DMG in relation to dietary (A) and nondietary (B) factors. Regression coefficients (β) and 95% CIs were adjusted for age; sex; ethnicity; fasting time; education; obesity; central obesity; smoking status; alcohol drinking; physical activity level; use of multivitamins; menopausal status and hormone therapy in women; intakes of red meat, eggs, and fish; history of diabetes, hypertension, dyslipidemia, and NAFLD; and total energy. Dietary covariates were mutually adjusted for other foods and included in the model as the cohort- and sex-specific quintiles. Total energy intake was additionally adjusted for in diet-related analyses. βs indicate the increase or decrease in SD units of DMG on the log scale. *Q* values represent corrected *P* values for each groups of analyses by controlling the false discovery rate. DMG, dimethylglycine; NAFLD, nonalcoholic fatty liver disease; WHR, waist-to-height ratio.

carnitine or DMG concentrations were not associated with most of the glucose control biomarkers or blood pressure in our current study, so the unfavorable cardiometabolic risk profiles associated with circulating carnitine and DMG warrant further examination.

Since choline, betaine, and carnitine are precursors of TMAO (63, 64), whether their cardiometabolic associations were independent of TMAO is worth exploring. In a 3-y follow-up study among 3903 patients undergoing coronary angiography, increased plasma choline and betaine at baseline were associated with incident major adverse cardiac events only when TMAO was also elevated (65), which suggests that the CVD effects of choline and betaine may be modified by TMAO. However, after we further adjusted for TMAO concentrations or antibiotic use (as a proxy of the status of gut microbiota that impacts TMAO), the associations of circulating choline, betaine, and carnitine with cardiometabolic biomarkers were not attenuated substantially. In addition, we did not find statistical evidence of significant effect modifications in analyses stratified by TMAO concentrations. Thus, the observed associations of choline and related metabolites with cardiometabolic biomarkers are, at least in part, independent of TMAO.

Our findings with regard to dietary factors were generally consistent with previous reports. Despite no overall associations in the pooled analyses, we observed significant positive associations of circulating choline with red meat intake among US studies and with egg intake among Asian studies. In our earlier study, while some dietary sources of choline (i.e., red meat and eggs) were common in Chinese, Black Americans, and White Americans, other major dietary sources varied across ethnic groups: poultry and processed meat in Black Americans, dairy and poultry in White Americans, and soy foods and fish in Chinese (14). Similar variations in sources of dietary choline were previously reported in a US multiethnic cohort (15), and variations in dietary choline levels were also noted across countries (16). Circulating betaine was associated with intake of whole grains, carbohydrates, plant protein, and fiber, whereas carnitine was associated with higher red meat intake and lower plant protein intake, which was consistent with the current understanding of their dietary sources (4, 5, 66). Since circulating DMG is a product from betaine (5), its current association with higher plant protein intake might reflect both its plant food source and connection with betaine. As choline and related metabolites can also be endogenously synthesized, the dietary correlations should not be interpreted as causality. Meanwhile, these metabolites were correlated with various nondietary factors in our study, including age; sex; ethnicity; lifestyle factors such as physical activity, smoking, and alcohol drinking; and metabolic conditions such as diabetes, dyslipidemia, and obesity. This suggests that the prior identified associations of these metabolites with cardiometabolic biomarkers could be due to the impact of cardiometabolic conditions on homeostasis of choline and related metabolites, and that the directionality of the associations needs to be validated in prospective studies.

To our knowledge, this is the largest international study on the associations of circulating choline, betaine, carnitine, and DMG with cardiometabolic biomarkers. However, several limitations need to be acknowledged. First, this is a cross-sectional study and could not imply causality nor the directionality of the associations. Although we minimized the reverse causality by excluding prevalent cancer, CVD, chronic kidney disease, and inflammatory bowel disease from our analyses, our findings should still be evaluated in future prospective studies. Second, since our study was a pooling project using data from multiple studies, there existed measurement heterogeneity, errors, and misclassifications associated with different methods for data collection and platforms for biomarker/metabolite measurements. In particular, relative concentrations of choline and related metabolites were measured, so that we could not directly compare their concentrations across studies. We used standardized data harmonization and analytical protocols to minimize potential errors. Third, there could be residual confounding. Given that carnitine was mostly from foods of animal sources while dietary betaine was mostly from plant sources, their concentrations could reflect different lifestyles, whose effects on cardiometabolic risk factors could not be fully adjusted for in statistical models. Finally, our study did not aim to elucidate the underlying mechanisms, but our findings provided evidence that choline and related metabolites are linked to certain cardiometabolic risk factors that could potentially contribute to cardiometabolic disease.

In conclusion, we documented differential associations of circulating choline, betaine, carnitine, and DMG with cardiometabolic risk factors. Circulating choline, carnitine, and DMG were linked to unfavorable cardiometabolic risk profiles, while circulating betaine was related to a favorable cardiometabolic risk profile. Our findings should be validated in future large prospective studies, ideally with clinical cardiometabolic events as the research outcomes.

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The authors' responsibilities were as follows—X-OS and DY: designed the study; X-FP and JJY: analyzed the data; X-FP: drafted the manuscript; JJY, X-OS, SCM, NDP, MG-F, DMH, SH, HE, TJW, REG, DA, IT, IK, PE, HZ, LEW, WZ, HC, QC, CEM, CM, KAM, LPL, JO, MF, CMU, and DY: provided critical revisions of the manuscript for important intellectual contents; DY: is the guarantor of the manuscript; and all authors: contributed to the interpretation of the data and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

The data described in the manuscript, code book, and analytic codes will be made available upon reasonable request and approval of the corresponding author of the paper and collaborating investigators of each included cohort.

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