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Dietary choline and betaine; associations with subclinical markers of cardiovascular disease risk and incidence of CVD, coronary heart disease and stroke: the Jackson Heart Study

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Abstract

Purpose—Several mechanisms have been described through which dietary intake of choline and its derivative betaine may be associated in both directions with subclinical atherosclerosis. We assessed the association of dietary intake of choline and betaine with cardiovascular risk and markers of subclinical cardiovascular disease.

Methods—Data from 3924 Jackson Heart Study (JHS) African-American participants with complete food frequency questionnaire at baseline and follow-up measurements of heart disease measures were used. Multivariable linear regression models were employed to assess associations between choline and betaine intake with carotid intima-media thickness, coronary artery calcium, abdominal aortic calcium and left ventricular mass. Cox proportional hazards regression models were used to estimate associations with time to incident coronary heart disease (CHD), ischemic stroke and cardiovascular disease (CVD).

Results—During an average nine years of follow-up, 124 incident CHD events, 75 incident stroke events and 153 incident CVD events were documented. In women, greater choline intake was associated with lower left ventricular mass ($p = 0.0006$ for trend across choline quartiles) and with abdominal aortic calcium score. Among all JHS participants, there was a statistically significant inverse association between dietary choline intake and incident stroke, $\beta = -0.33$ ($p =$

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0.04). Betaine intake was associated with greater risk of incident CHD when comparing the third quartile of intake with the lowest quartile of intake (HR 1.89, 95 % CI 1.14, 3.15).

Conclusions—Among our African-American participants, higher dietary choline intake was associated with a lower risk of incident ischemic stroke, and thus putative dietary benefits. Higher dietary betaine intake was associated with a nonlinear higher risk of incident CHD.

Keywords

Diet; Choline; Betaine; Subclinical measures of cardiovascular disease; Incident coronary heart disease; The Jackson Heart Study

Introduction

The essential nutrient choline, its metabolite betaine, as well as folate and methionine, are all metabolically interrelated by transmethylation pathways [1, 2]. Low dietary intakes of choline and betaine may alter epigenetic regulation for a series of genes by which the atherogenic process may be accelerated [3, 4]. There is also an important crosstalk between choline/1-carbon metabolism (such as betaine) and the pathways of insulin sensitivity, fat deposition and energy metabolism through epigenetic modifications. When choline stores are inadequate, there is a diminished capacity to methylate homocysteine to methionine and plasma homocysteine increases [5]. Elevated homocysteine has been associated with greater risk of several chronic diseases and conditions including cardiovascular disease [6], cancer [7], cognitive decline [8] and bone fracture [9].

Several mechanisms have been described through which dietary intake of choline and its derivative betaine may be associated with subclinical atherosclerosis. Acute choline deficiency in rodent models causes lipid accumulation in liver, heart and arterial tissues [10]. Human studies have indicated that plasma levels [11, 12] or diets at both the lower end (≤150 mg/day) or higher end (>500 mg/day) of normal intake for choline may have adverse health consequences [13]. Moreover, decreased choline results in increased metabolic rate and increased insulin sensitivity, while increased betaine also results in increased metabolic rate and increased insulin sensitivity. The differentiation between the effects of the two related metabolites, choline and betaine could lie in mechanisms that choline can participate in but betaine cannot [14].

High intakes of choline and betaine were inversely associated with inflammation in one observational study [15]. Data from the atherosclerosis risk in communities study (ARIC) showed a nonsignificant association of higher choline and betaine intakes with higher CVD risk [16]. The same association was also not statistically significant after adjustment for CVD risk markers [13]. Therefore, in the current study, we aimed to assess the association of choline and betaine with incident CHD and stroke among the African-American participants enrolled in the Jackson Heart Study (JHS), a group with high prevalence of CVD. We also aimed to assess the association between choline and betaine and subclinical markers of CV disease such as carotid intima-media thickness (cIMT), coronary artery calcium (CAC) score, abdominal aortic calcium (AAC) score and left ventricular mass (LVM).

Subjects and methods

We used data from baseline and follow-up examinations of the JHS. The JHS is a single-site, prospective cohort study of risk factors and causes of heart disease in African-American adults [17, 18]. A sample of 5301 adults, aged 21–94 years, residing in a three-county area surrounding the city of Jackson, MS were recruited, interviewed and examined by certified technicians according to standardized protocols during the baseline examination (Exam 1) in 2000–2004. The clinical visits included collection of data on socio-demographics, anthropometry, medical history, cardiovascular and behavioral risk factors, and blood and urine for biological risk factors. The present study included 3924 JHS participants (65 % women; mean age, 48 ± 11 years) with available dietary data on choline and betaine, and available data on subclinical markers of cardiovascular disease. Among the JHS participants, 1377 did not have available dietary data from which to estimate the quantities of choline and betaine. Dietary assessment (JHS Exam 1) was accomplished with a validated region-specific food frequency questionnaire (FFQ). The FFQ, adapted from a longer questionnaire developed for use in the Mississippi Delta region of the USA, included 158 food items aggregated into 31 predefined food groups based on their nutrient profiles [19]. This FFQ has been validated previously and thus has relatively low misclassification bias/measurement error.

For this study, we created a choline and betaine dietary intake dataset by linking foods on the FFQ with published values for these nutrients. In general, food items in the JHS FFQ corresponded well with food items in a published food list [20], and in the current USDA choline/betaine food content listing [21]. If more than one food from the published data was a close match for the FFQ food line item, their values for choline and for betaine were averaged. If the FFQ item was composed of more than one basic food from the USDA database, a recipe for the item was added, including item weights previously specified based on frequency of use in the Southern US Delta region. Therefore, weighted averages for the values of choline and betaine were applied, based on the proportion of food contribution to the total weight of the recipe. For each of the JHS FFQ items, the choline and betaine content per composite 100 g/food line item was added to the database. The average daily intake for each study participant was obtained as the nutrient content for each FFQ food line item times its frequency and portion size, summed over all FFQ items. The nutrient content of each food item was calculated as the product of the food micronutrient content (expressed in mg per 100 grams of food) and the food quantity, expressed in grams, in each FFQ food item. The quantities of food, expressed in grams, in each of the JHS FFQ items were estimated using the Nutrition Data System for Research (NDS-R), developed by the University of Minnesota [22], as detailed in our previous publications [16, 23].

To determine the occurrence of CVD events, all JHS participants were followed from the first examination until December 31, 2011, through periodic examinations at the JHS and a review of hospital and physician office visit records. The CVD events included ischemic stroke, angina, myocardial infarction (MI), intermittent claudication, congestive heart failure (CHF), stroke death and other CVD death. Angina was defined by the presence of chest pain or discomfort. MI was defined by a combination of the presence of cardiac pain, a change in enzymes and electrocardiographic findings [24]. Ischemic stroke was defined based on

ICD-9 code 435 and ICD-10 code G45 [24]. Hospitalized MI was defined using ICD-9 codes 402, 410–414, 427, 428 and 518.4 [24]. The outcome for our study was the first incidence of any CVD event, CHD event and stroke, respectively. Proportional food contributions to choline and betaine intake were assessed for the total group by dividing the nutrient intake from each food group by the total intake of that nutrient and multiplying by 100. These were then ranked using the RANK procedure in SAS.

Written consent was obtained from each participant before the collection of data. The institutional review boards of the participating JHS institutions, including the University of Mississippi Medical Center, Jackson State University and Tougaloo College, approved the study protocol.

Measurements

Our main variables were measured during JHS Exam 1, from 2000 to 2004 [17, 18, 25]. They included age, sex, body mass index (BMI), waist circumference, smoking, alcohol intake, physical activity, blood pressure, hypertension medication, plasma glucose and lipids, diabetes (type 2 only) status, education and estimated glomerular filtration rate (eGFR). eGFR was calculated using the modification of diet in renal disease (MDRD) study equation [$GFR = 186.3 \times (PCr)^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$], where PCr is serum creatinine [26]. The following variables were also measured at Exam 1. They included dietary intakes of total energy, choline, methionine, betaine, folate, vitamins B6 and B12, as well as plasma homocysteine concentration. LVM by echocardiography [27] was also measured during Exam 1. Subclinical cardiovascular disease variables at Exam 2 (2004–2007) included carotid intima-media thickness (cIMT) by ultrasound, coronary artery calcium (CAC) score and abdominal aortic artery calcium (AAC) score (Agatston score) by non-contrast CT-scan [28]. The total calcium scores for coronary artery and abdominal aortic artery were calculated based on the number, areas and peak Hounsfield computed tomographic numbers of the calcified lesions detected using computed tomography [29]. Considering that beer and wine are main sources of betaine, and that alcohol intake is associated with cardiovascular disease in a nonlinear (U-shaped) manner [30], dietary alcohol intake was used as a categorical variable constructed as follows. We assigned the value ‘0’ for non-drinkers, the value ‘1’ for moderate drinkers (up to 1 glass/day for women and up to 2 glasses/day for men; calculated from grams of intake per day, assuming 14 grams per glass) and the value ‘2’ for heavy drinkers (above the cut point of the value ‘1’) [31]. Incident events (CHD and stroke) were centrally adjudicated using JHS surveillance data until December 31, 2011, with an average 9 years of follow-up [24].

Statistical analysis

For the statistical analyses, CAC and AAC score were logarithmically transformed after adding 1 to avoid null values. LVM was expressed as an index by its ratio to height, raised to a power of 2.7, as is often done to adjust for differences in height [32, 33]. We compared proportions of categorical variables such as smoking, type 2 diabetes, hypertension, alcohol and ACE inhibitor use using Chi-square tests. We compared continuous variables using *t* test.

Multivariable linear regression models were used to assess associations between choline and betaine with cIMT, CAC and AAC scores, and with LVM per one standard deviation increase. In model 1, we adjusted for age, BMI, smoking and total energy intake; in model 2, we adjusted for the variables in model 1 plus systolic BP, hypertension medication, fasting plasma glucose, HDL-cholesterol, triglycerides, eGFR, and in model 3, for the variables in model 2 plus alcohol intake (as a categorical variable), plasma homocysteine, and energy-adjusted dietary intakes of folate, methionine, vitamin B6 and B12. Cox proportional hazards regression models with a backward selection procedure run in a stepwise fashion (from the minimal to a full adjustment) were used to estimate associations between choline and betaine intake with time to incident CHD and stroke events, after testing for the assumption of proportionality of the hazard ratios. Adjustment for total energy intake was done with the residual method before categorizing these nutrient intakes into quartiles. The most parsimonious models (indicated by the backward selection procedure; with a p value of 0.10 as the cut point) were used for the analyses, with final adjustment for age, sex, smoking status, systolic blood pressure, antihypertensive medication, fasting plasma glucose, total to HDL-cholesterol concentration, homocysteine, methionine and total energy intake. Sensitivity analyses using the dietary folate equivalent were also performed. Statistical analyses were conducted using SAS software [34]. All p values were two-tailed, and alpha value of 0.05 was used for interpretation of results.

Results

Among the 3924 participants in this study, the mean (standard deviation) intakes of choline were 278 (±126) and 357 (±147) mg/day among women and men, respectively, and the average intakes of betaine were 115 (±74) and 139 (±83) mg/day among women and men, respectively (Table 1). The majority of covariates differed statistically by sex (Table 1). Among women, greater choline intake was associated with lower LVM index ($p = 0.0001$ for trend across the choline quartiles). There was also an inverse association between choline and AAC score among women ($\beta = -0.35$, $p = 0.004$) that remained statistically significant when adjusting for other dietary variables ($\beta = -0.31$, $p = 0.049$) (Table 2). There were no statistically significant associations between choline, betaine, or both together, and cIMT or CAC score (log transformed) (Table 2).

During an average nine years of follow-up, 124 incident CHD events (75 among women; 49 among men), 75 incident ischemic stroke events (50 among women; 25 among men) and 153 incident CVD events (103 among women; 50 among men) were documented. Among all JHS participants, there was a statistically significant inverse association between dietary choline intake and incident stroke, $\beta = -0.33$ ($p = 0.04$) (Table 3). While this inverse linear association with incident stroke was overall statistically significant in the continuous model (Table 3, when categorizing the choline distribution in quartiles, there was a nonsignificant association among those participants with the highest intake of choline. Specifically, choline intake was inversely associated with incident stroke when comparing the second and third quartiles of intake with the lowest quartile of intake (HR 0.51, 95 % CI 0.26, 0.99, and HR 0.42, 95 % CI 0.20, 0.90, respectively; Table 3), but not when the fourth quartile was used. Betaine intake was associated with increased risk of incident CHD when comparing the third quartile of intake with the lowest quartile of intake (HR 1.89, 95 % CI 1.14, 3.15; Table 3),

but not when the second and the fourth quartile were used; thus, a curvilinear relationship was present. Among women, betaine intake was associated with increased risk of both incident CHD and incident CVD events, when comparing the third quartile with the first quartile (HR = 3.16, 95 % CI 1.65, 6.05, and HR = 1.80, 95 % CI 1.05, 3.12, respectively), but not when comparing the fourth quartile with the first quartile (Table 4). Thus, similar curvilinear relationships were present. Similar associations were found in sensitivity analyses using folate dietary equivalent estimated variable. Specifically, the association between dietary choline and incident CHD remained nonsignificant for all quartiles compared to the lowest quartile. Comparing the highest quartile versus the lowest quartile, HR = 0.83 (0.32, 2.15). The results for betaine were also similar, with the third quartile statistically significant when compared with the first quartile, HR = 2.19 (1.29–3.73).

The principal foods that contributed to choline intake were non-fried eggs (12.4 %), fried fish (5.5 %), corn bread or muffins (2.9 %), fried beef (2.8 %) and whole milk (2 %) (Table 5). The principal foods that contributed to betaine intake were whole wheat bread (16.2 %), other high-fiber cereals (12.7 %), white bread (9.1 %), cracked wheat bread (7.2 %), beer (4 %), pasta (4 %), mixed dishes with beef (4 %), fried fish (4 %), sweet potato (3.6 %) and macaroni and cheese (3.4 %) (Table 5).

Discussion

Among African-American participants in the JHS, higher dietary choline intake was associated with lower LVM and AAC score in women, and with a lower risk of incident ischemic stroke among all participants. Higher dietary betaine intake was associated with a curvilinear higher risk of incident CHD among all participants, and with a curvilinear higher risk of incident CHD and incident CVD among women. No associations with CAC score or with carotid intima-media thickness were detected.

Choline, an essential nutrient for humans [35], is associated with several compounds that are methyl donors. Supplementation in the dietary intake range of betaine, a methyl-donor continuously produced from choline [36], leads to lowering of plasma homocysteine, a putative CHD risk factor [6, 37]. Homocysteine, which has a cytotoxic effect on vascular endothelium [38], is a sulfur amino acid whose metabolism stands at the intersection of two pathways [39]. One catalyzes the synthesis of the amino acid cysteine and the other is a remethylation reaction to form methionine, a process that requires folate and vitamin B_{12}. In an alternative reaction, betaine, the oxidative by-product of choline, serves as a donor of methyl groups to homocysteine to form methionine [40]. Thus, the two metabolic pathways provide alternate mechanisms for removal of homocysteine. The increase in plasma homocysteine after a methionine load [5] and consequent vascular cytotoxicity, or the aberrant methylation produced by a low plasma choline and plasma betaine with possible increased atherogenesis [3, 4], provides the putative mechanisms that could explain an increase in CHD or stroke risk when not enough choline is available in the circulation. Until recently, it was not possible to estimate dietary choline intake in humans and there are still no nationally representative estimates of this intake from food, because the choline content of foods had not been included in major nutrient databases until lately [20]. There is a recommended adequate intake for choline (550 mg/day in adult men and 425 mg/day in

adult women), but in several human cohorts, choline intake has been estimated to vary as much as threefold [23, 41]. Thus, our investigation conducted among African-Americans adds information for this ethnicity with a high prevalence of cardiovascular disease and obesity. As mentioned above, the mean intakes of choline were 278 (±126) and 357 (±147) mg/day among women and men, respectively, in this cohort, which is well below the recommended adequate intake, and the average intakes of betaine were 115 (±74) and 139 (±83) mg/day among women and men.

Choline and betaine are at the crossroad of several important metabolic pathways, as they are involved in the formation of specific phosphatidylcholine species such as the endogenous peroxisome proliferator-activated receptor α ligand (PPAR-α). PPAR-α is involved in fatty acid oxidation, gluconeogenesis, lipid transport and ketogenesis. Sterol regulatory element-binding protein 1 (SREBP-1) regulates genes of fatty acid, phospholipid, and triacylglycerol synthesis and also induces multiple genes to synthesize S-adenosyl methionine (SAM). [14] In the liver, SREBP-1 inhibits insulin receptor substrate-2 (IRS-2) expression and inhibits insulin signaling. Betaine increases SAM, down regulates SREBP-1 activity, increased IRS-2 expression and insulin sensitivity. [42, 43] Down regulation of SREBP-1 has been shown to reverse insulin resistance in animal studies [44]. It remains to be clarified, in experimental studies, if a compensatory increase in betaine concentration is the explanation for the association among JHS women participants between betaine dietary intake and higher incident CVD events. As major sources of betaine include whole grains, it is also possible that women at greater CVD risk had recently adopted these foods, based on medical advice, which would bias the result due to reverse causation. The inverse association that we found between choline and AAC score among women deserves further investigations.

As presented, there is substantial evidence suggesting that there is important crosstalk between choline/1-carbon metabolism and the pathways of insulin sensitivity, fat deposition and energy metabolism. Yet, the extant literature on dietary choline is small [13, 15, 16, 45–49] with the current study being the first to explore choline as well as betaine in relationship with cardiovascular outcomes among African-Americans. Our previous investigation in the Atherosclerosis Risk in Communities (ARIC) Study showed no association between choline or betaine intake and incident coronary heart disease or stroke while controlling for the other dietary covariates [16]. Regular dietary intakes of folate, betaine and choline were also not associated with CVD risk in 16,165 female breast cancer screening participants in the PROSPECT-EPIC cohort (one of the two Dutch contributions to the European Prospective Investigation into Cancer and Nutrition, EPIC). Although neither folate, betaine or choline intakes were associated with CVD, high folate and choline intakes were significantly associated with lower homocysteine [13]. A cross-sectional survey (the ATTICA Study) with 1514 men (18–87 years) and 1528 women (18–89 years) with no history of cardiovascular disease, found that participants who consumed >310 mg/day of choline had 22 % lower C-reactive protein (CRP), 26 % lower interleukin-6 (IL-6), and 6 % lower tumor necrosis factor-alpha (TNF-α) relative to those with choline intake <250 mg/day (all p values statistically significant). Similarly, participants who consumed >360 mg/day of betaine had, on average, 10 % lower homocysteine, 19 % lower CRP, and 12 % lower TNF-α than those who consumed <260 mg/day (all statistically significant p values) [15]. Thus, previous studies have shown improvement in plasma inflammatory markers and

homocysteine with higher intakes of dietary choline and betaine, but they have not shown relationships with incident CVD. Therefore, our investigation showing divergent risks of CHD and stroke might be explained by the different cardiometabolic risk profile of our African-American sample, still unaccounted for in the multivariate analyses, or by specific genetic factors pertaining to this ethnicity.

The interest in choline and betaine was augmented recently when it was shown that dietary supplementation of mice with choline, choline derivatives such as trimethylamine *N*-oxide (TMAO), or betaine promoted upregulation of multiple macrophage scavenger receptors linked to atherosclerosis, and that supplementation with choline or TMAO promoted atherosclerosis [50]. Therefore, choline and betaine stores may be linked to both an increased and a decreased atherosclerotic process, and thus explain why the few studies conducted so far have been equivocal. Our study indicating protective associations for choline and direct negative associations for betaine on CVD risk adds to the studies aiming to clarify the mechanisms. We do not have the plasma values for the specific choline derivatives such as phosphatidylcholine, and thus cannot comment on those correlations that are supposed to be higher than those with dietary choline/betaine concentrations. We, nevertheless, estimated the correlation coefficients for plasma homocysteine with dietary choline and dietary betaine; they were both relatively small when partially adjusted for age, sex, and intakes of folate and vitamin B12 ($p = 0.12$ and $p = 0.48$, for choline and betaine, respectively).

There are several limitations of the present study. Our participants are exclusively African-Americans, and our study sample is localized to one geographical area and one ethnic group, so generalizability is limited. Dietary intake was available only at baseline (JHS Exam 1), and diets may have changed over time, although (as in the majority of middle-aged individuals) there is a tendency for relative consistency. We were not able to separate the dietary beer intake (one of the main contributors to choline intake) from the total alcohol intake, and thus, we were not able to specifically adjust for this dietary variable.

There are a series of strength of our study. Our analyses are based on an extended follow-up of the largest African-American cohort of US adults, with the added strengths of validated CHD outcomes and a standardized collection of covariate information. These are elements that support the internal validity of the findings. The FFQ used in our study has also been validated in previous studies [19].

Significance

The observation of lower risk of incident stroke with higher choline intake, coupled with a higher risk of incident CHD with higher betaine requires further investigation. It appears that exposure to these nutrients operates in complex ways, and in balance with other nutrients. The implication of our findings is that those at risk of stroke might benefit from higher dietary intake of choline, but our findings should be confirmed in other studies for conclusive dietary recommendations. Such recommendations would then have the potential to prevent several disease endpoints such as various types of cancer and cardiovascular disease. Therefore, additional mechanistic studies as well as observational studies may be needed to better understand their effects on CVD risk.

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Table 1

Descriptive characteristics (mean (SD) and percentage) of study participants, by sex (n = 3924)

Variable	Women (n = 2613)	Men (n = 1311)	p value
Age	55.1 (12.4)[†]	54.1 (12.4)	0.02
Choline intake, mg/day	278 (126)	357 (147)	<0.0001
Betaine intake, mg/day	115 (74)	139 (83)	<0.0001
Homocysteine concentration, μmol/L	8.9 (5.2)	10 (3.3)	<0.0001
cIMT, mm	0.7 (0.17)	0.76 (0.19)	<0.0001
CAC score	99 (295)	169 (415)	<0.0001
AAC score	754 (1403)	746 (1503)	0.9
LVM, grams	137 (35)	166 (43)	<0.0001
LVM index ($LVM^{2.7}$)	23.9 (7.1)	22.9 (7.2)	0.002
Folate intake, μg/day	300 (128)	325 (129)	<0.0001
Vitamin B6 intake, mg/day	3.8 (13.3)	3.4 (11.1)	0.36
Vitamin B12 intake, μg/day	31.9 (106)	23.2 (84)	0.009
Methionine intake, g/day	1.5 (0.7)	1.8 (0.8)	<0.0001
BMI (kg/m^2)	32.7 (7.4)	29.9 (6.2)	<0.0001
Triglycerides (mg/dl)	101 (67)	115 (79)	<0.0001
HDL-cholesterol (mg/dl)	55 (14)	46 (12)	<0.0001
LDL-cholesterol (mg/dl)	127 (36)	131 (37)	0.003
Total cholesterol (mg/dl)	202 (39)	199 (40)	0.02
Fasting glucose (mg/dl)	99.1 (31.9)	99.2 (29.1)	0.97
Total physical activity score	8.3 (2.5)	8.7 (2.6)	<0.0001
Smoking	8.7 %	14.2 %	<0.0001
Hypertension status	62.9 %	58.0 %	0.003
Systolic blood pressure (mmHg)	126 (18)	128 (17)	0.03
Diastolic blood pressure (mmHg)	78 (10)	82 (10)	<0.0001
Alcohol intake, g/day	1.22 (4.72)	6.44 (14.34)	<0.0001
Alcohol drinking past year	38.7 %	58.5 %	<0.0001
Alcohol drinking status			
Non-drinkers	27 (1.0 %)	5 (0.4 %)	
Moderate drinkers	2533 (96.9 %)	1212 (92.5 %)	
Heavy drinkers	53 (2.0 %)	94 (7.2 %)	<0.0001
Type 2 diabetes	18.0 %	15.2 %	0.03
Obesity BMI (kg/m^2) ≥30	59.6 %	41.0 %	<0.0001
Total energy intake, kJ/day	7450 (2791)	8721 (2829)	<0.0001
eGFR (ml/min/1.73 m^2)	85.3 (18.1)	88.4 (16.4)	<0.0001
ACE inhibitor medication	12.8 %	14.2 %	0.28

cIMT carotid intima-media thickness, *CAC* coronary artery calcium score, *AAC* abdominal aortic calcium score and *LVM* left ventricular mass, *eGFR* estimated glomerular filtration rate, *SD* standard deviation

[†]Values are means and standard deviations

Table 2

Associations of dietary choline and betaine with AAC, LVMI, cIMT and CAC by sex

	Choline[a]		Betaine[a]		Choline + Betaine[a]	
	Women	**Men**	**Women**	**Men**	**Women**	**Male**
Abdominal aortic calcium (AAC) score						
Model 1	−0.295 (0.013)	0.113 (0.401)	−0.063 (0.395)	−0.053 (0.543)	−0.252 (0.022)	0.03 (0.819)
Model 2	−0.358 (0.004)	0.03 (0.837)	−0.006 (0.94)	−0.128 (0.21)	−0.242 (0.037)	−0.087 (0.55)
Model 3	−0.274 (0.085)	−0.084 (0.651)	0.05 (0.549)	−0.085 (0.441)	−0.1 (0.474)	−0.155 (0.388)
Left ventricular mass index (LVMI)						
Model 1	−0.416 (0.122)	0.221 (0.495)	−0.186 (0.315)	0.123 (0.601)	−0.439 (0.088)	0.277 (0.402)
Model 2	−0.416 (0.141)	0.386 (0.259)	−0.033 (0.863)	0.214 (0.411)	−0.312 (0.245)	0.488 (0.171)
Model 3	0.082 (0.826)	0.925 (0.035)	−0.042 (0.831)	0.342 (0.217)	−0.001 (0.998)	1.081 (0.015)
Carotid intima-media thickness (cIMT)						
Model 1	0.001 (0.81)	−0.0003 (0.969)	−0.004 (0.276)	0.002 (0.704)	−0.002 (0.613)	0.001 (0.838)
Model 2	0.002 (0.771)	−0.005 (0.487)	−0.004 (0.334)	−0.004 (0.477)	−0.003 (0.588)	−0.007 (0.232)
Model 3	−0.009 (0.216)	−0.006 (0.482)	−0.002 (0.603)	−0.003 (0.623)	−0.008 (0.226)	−0.008 (0.386)
Coronary artery calcium (CAC) score						
Model 1	0.027 (0.782)	0.163 (0.208)	−0.078 (0.21)	−0.03 (0.723)	−0.091 (0.325)	0.088 (0.485)
Model 2	−0.107 (0.307)	0.042 (0.76)	−0.024 (0.726)	−0.109 (0.273)	−0.092 (0.348)	−0.06 (0.67)
Model 3	−0.163 (0.224)	−0.138 (0.443)	−0.02 (0.774)	−0.08 (0.645)−	0.12 (0.311)	−0.187 (0.278)

Model 1: age, BMI, smoking and total caloric intake

Model 2: model 1 variables plus systolic BP, antihypertensive medication, fasting plasma glucose, HDL-cholesterol, triglycerides and blood creatinine level

Model 3: model 2 variables plus alcohol (categorical), plasma homocysteine, dietary intake of folate, methionine and vitamin B6 and B12

Values presented are linear regression coefficients with *p* values in parentheses

AAC and CAC scores were log-transformed

No effect measure modification detected for homocysteine, folate and methionine

[a]Choline, betaine and choline + betaine values are per one standard deviation

Table 3

Associations between dietary intakes of choline and betaine (by quartiles and continuous) with incident CHD, incident stroke and incident CVD events

	Proportion at risk	Choline	Proportion at risk	Betaine
Incident CHD events				
Quartile 1	39/822	1.00	32/829	1.00
Quartile 2	30/860	0.88 (0.53, 1.48) *	23/852	0.92 (0.52, 1.62)
Quartile 3	34/843	0.96 (0.53, 1.81)	41/836	1.89 (1.14, 3.15)
Quartile 4	21/840	0.66 (0.27, 1.60)	28/848	1.20 (0.68, 2.11)
Continuous variable (beta coefficient and *p* value)	124/3489	−0.08 ($p = 0.55$)	130/3489	0.12 ($p = 0.16$)
Incident stroke events				
Quartile 1	28/813	1.00	25/816	1.00
Quartile 2	15/831	0.51 (0.26, 0.99)	16/822	0.71 (0.37, 1.35)
Quartile 3	14/823	0.42 (0.20, 0.90)	18/817	0.76 (0.40, 1.46)
Quartile 4	18/805	0.41 (0.16, 1.09)	16/817	0.56 (0.28, 1.14)
Continuous variable (beta coefficient and *p* value)	75/3347	−0.33 ($p = 0.04$)	75/3347	−0.17 ($p = 0.14$)
Incident CVD events				
Quartile 1	48/781	1.00	39/788	1.00
Quartile 2	35/812	0.73 (0.46,1.16)	29/807	0.85 (0.53, 1.39)
Quartile 3	40/790	0.85 (0.51, 1.43)	45/784	1.35 (0.86, 2.12)
Quartile 4	30/787	0.58 (0.28,1.20)	40/791	1.07 (0.66, 1.73)
Continuous variable (beta coefficient and *p* value)	153/3323	0.13 ($p = 0.25$)	153/3323	0.06 ($p = 0.42$)

Models were adjusted for age, sex, smoking status, systolic blood pressure, antihypertensive medication, fasting plasma glucose, total to HDL-cholesterol concentration, methionine and total energy intake

*Values are hazard ratios and associated 95 % confidence intervals

†The ranges of intake (mg/day) of choline and betaine quartiles (from quartile 1 to 4, respectively) are (51.34, 199.182), (199.18, 279.22), (279.34, 384.72), (384.84, 1076.02) for choline and (9.89, 71.21), (71.21, 103.52), (103.56, 153.09), (153.18, 803.3) for betaine

Table 4

Associations by sex between dietary intakes of choline and betaine (by quartiles and continuous) with incident CHD, incident stroke and incident CVD events

	Choline		Betaine	
	Women	**Men**	**Women**	**Men**
Incident CHD events				
Quartile 1	1.00	1.00	1.00	1.00
Quartile 2	0.83 (0.43, 1.59) *	1.04 (0.41, 2.67)	1.39 (0.68, 2.82)	0.40 (0.15,1.10)
Quartile 3	1.46 (0.67, 3.20)	0.56 (0.20, 1.62)	3.16 (1.65,6.05)	0.71 (0.30, 1.66)
Quartile 4	0.30 (0.06, 1.39)	0.90 (0.26, 3.04)	1.53 (0.69,3.38)	0.66 (0.28, 1.52)
Continuous variable (beta coefficient and *p* value)	−0.03, $p = 0.88$	−0.11, $p = 0.58$	0.23, $p = 0.0362$	−0.08, $p = 0.57$
Incident stroke events				
Quartile 1	1.00	1.00	1.00	1.00
Quartile 2	0.67 (0.31, 1.43)	0.28 (0.07, 1.17)	0.98 (0.47, 2.03)	0.23 (0.05, 1.13)
Quartile 3	0.59 (0.23, 1.51)	0.22 (0.06, 0.89)	0.91 (0.41, 1.99)	0.54 (0.17, 1.74)
Quartile 4	0.58 (0.16, 2.09)	0.25 (0.05, 1.18)	0.63 (0.26, 1.51)	0.38 (0.11, 1.34)
Continuous variable (beta coefficient and *p* value)	−0.21, $p = 0.30$	−0.48, $p = 0.0832$	−0.13, $p = 0.32$	−0.23, $p = 0.29$
Incident CVD events				
Quartile 1	1.00	1.00	1.00	1.00
Quartile 2	0.71 (0.41, 1.23)	0.77 (0.31, 1.92)	1.12 (0.63, 1.98)	0.43 (0.16, 1.15)
Quartile 3	1.03 (0.55, 1.93)	0.61 (0.23, 1.60)	1.80 (1.05, 3.12)	0.79 (0.35, 1.79)
Quartile 4	0.43 (0.16, 1.17)	0.76 (0.25, 2.30)	1.17 (0.63, 2.19)	0.76 (0.34, 1.70)
Continuous variable (beta coefficient and *p* value)	−0.13, $p = 0.37$	−0.10, $p = 0.58$	0.10, $p = 0.27$	−0.023, $p = 0.87$

Models were adjusted for age, smoking status, systolic blood pressure, antihypertensive medication, fasting plasma glucose, total to HDL-cholesterol concentration, methionine and total energy intake

*Values presented are hazard ratios (95 % confidence interval)

†The ranges of intake (mg/day) of choline and betaine quartiles (from quartile 1 to 4, respectively) are (51.34, 199.182), (199.18, 279.22), (279.34, 384.72), (384.84, 1076.02) for choline and (9.89, 71.21), (71.21, 103.52), (103.56, 153.09), (153.18, 803.3) for betaine

Table 5

Principal foods that contributed to the total dietary intake of choline and betaine

Choline	Percentage contribution	Mean (SE) mg/day	Betaine	Percentage contribution	Mean (SE) mg/day
Eggs, not fried	12.4	53.9 (0.9)	Whole wheat bread	16.2	22.4 (0.4)
Fried fish	5.5	22.0 (0.4)	High-fiber cereal	12.7	34.7 (1.4)
Fried eggs	3.3	14.5 (0.2)	White bread	9.1	12.6 (0.2)
Corn bread, muffins	2.9	11.7 (0.2)	Cracked wheat bread	7.8	10.8 (0.2)
Fried beef	2.8	11.5 (0.3)	Beer	4.0	13.7 (1.0)
Whole milk	2.0	9.13 (0.3)	Pasta, mixed dishes with beef	4.0	5.6 (0.1)
Beer	1.7	17.0 (1.3)	Fried fish	4.0	5.6 (0.1)
2 % Low-fat Milk	1.7	8.1 (0.3)	Sweet potato	3.6	5.0 (0.1)
Fried liver	1.6	8.2 (0.3)	Macaroni and cheese	3.4	4.8 (0.1)
Chicken/turkey, light meat, broiled, without skin	1.6	9.4 (0.2)	Corn bread, muffins	2.6	3.6 (0.1)
Regular ice cream	1.6	6.8 (0.2)	Hamburgers, fast food	1.7	2.4 (0.1)
Orange juice	1.5	6.4 (0.1)	Plain pizza	1.6	3.8 (0.1)
Black-eyed cowpeas	1.5	5.8 (0.1)	Ramon noodles	1.3	2.0 (0.1)
Regular processed meat	1.5	6.0 (0.1)	Fried beef	1.3	1.8 (0.1)
Fish, not fried	1.5	5.8 (0.1)	Biscuits	1.1	1.6 (0.0)
Chicken/Turkey, light meat, broiled, with skin	1.3	7.7 (0.2)	Fast food fish	1.0	1.4 (0.0)

Values presented are mean and standard error