



The Association of Dietary Choline and Betaine With the Risk of Type 2 Diabetes: The Atherosclerosis Risk in Communities (ARIC) Study

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OBJECTIVE

To examine the association between dietary intake of choline and betaine and the risk of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Among 13,440 Atherosclerosis Risk in Communities (ARIC) study participants, the prospective longitudinal association between dietary choline and betaine intake and the risk of type 2 diabetes was assessed using interval-censored Cox proportional hazards and logistic regression models adjusted for baseline potential confounding variables.

RESULTS

Among 13,440 participants (55% women, mean age 54 [SD 7.4] years), 1,396 developed incident type 2 diabetes during median follow-up of 9 years from 1987 to 1998. There was no statistically significant association between every 1-SD increase in dietary choline and risk of type 2 diabetes (hazard ratio [HR] 1.01 [95% CI 0.87, 1.16]) nor between dietary betaine intake and the risk of type 2 diabetes (HR 1.01 [0.94, 1.10]). Those in the highest quartile of dietary choline intake did not have a statistically significant higher risk of type 2 diabetes than those in the lowest choline quartile (HR 1.09 [0.84, 1.42]); similarly, dietary betaine intake was not associated with the risk of type 2 diabetes comparing the highest quartile to the lowest (HR 1.06 [0.87, 1.29]). Among women, there was a higher risk of type 2 diabetes, comparing the highest to lowest dietary choline quartile (HR 1.54 [1.06, 2.25]), while in men, the association was null (HR 0.82 [0.57, 1.17]). Nevertheless, there was a nonsignificant interaction between high choline intake and sex on the risk of type 2 diabetes ($P = 0.07$). The results from logistic regression were similar.

CONCLUSIONS

Overall and among male participants, dietary choline or betaine intakes were not associated with the risk of type 2 diabetes. Among female participants, there was a trend for a modestly higher risk of type 2 diabetes among those with the highest as compared with the lowest quartile of dietary choline intake. Our study should inform clinical trials on dietary choline and betaine supplementation in relationship with the risk of type 2 diabetes.

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Type 2 diabetes is a major health problem globally and in the U.S. (1,2). Dietary habit is a leading contributor to morbidity and mortality globally (3) and is a most prominent risk factor for type 2 diabetes in addition to obesity, fat distribution, physical inactivity, age, and family history of diabetes (4,5). There is strong evidence for the association between food groups and the risk of type 2 diabetes, but there remains a huge gap in evidence on the potential association between specific dietary nutrients such as choline and betaine and the risk of type 2 diabetes.

Given the involvement of choline (an essential nutrient) and betaine (main methyl donor) in the one-carbon cycle with possible epigenetic effects, there is a growing interest in the association between dietary choline and betaine intake, both found in a variety of foods, and the risk of diseases in humans. However, there are only a few previous human studies on dietary choline and betaine intake and the risk of type 2 diabetes with inconsistent results. A recent study among men found a lower risk of type 2 diabetes in those with the highest choline intake as compared with those with the lowest choline intake (6). Extant literature points to an association between dietary consumption of phosphatidylcholine (7), as well as plasma, serum, or urine metabolites of choline, and risk of type 2 diabetes (8). Trimethylamine-*N*-oxide (TMAO), a microbiota-dependent metabolite of dietary choline, is found to be directly associated with the risk of type 2 diabetes (9). At the same time, some studies suggest that dietary choline and betaine intakes are associated with better insulin sensitivity in humans (10). The enzymes in the one-carbon cycle that involves choline and betaine are differently expressed in males and females and result in higher choline and betaine metabolites in females than males (11). There are also differences in choline requirements (12).

Given that dietary habits are modifiable, examination of the association of dietary choline intake with the risk of type 2 diabetes may inform dietary modifications, which may help reduce the risk attributable to choline. In this study, we aimed to assess the prospective longitudinal association between dietary choline and betaine intake and the risk of type 2 diabetes among participants of the

Atherosclerosis Risk in Communities (ARIC) cohort.

RESEARCH DESIGN AND METHODS

Study Population

Data for the current study were obtained from the ARIC study cohort (13). The baseline ARIC cohort examination was conducted from 1987 to 1989 and included 15,792 men and women from four U.S. communities including Forsyth County, NC; Jackson, MS; suburbs of Minneapolis, MN; and Washington County, MD. Participants were followed up through annual telephone interviews and review of hospitalizations. Extensive examinations were conducted initially every 3 years, with the latest examination currently underway. Participants were aged 45–64 years at baseline. Participants were excluded from this analysis if they had baseline type 2 diabetes ($n = 2,014$); if they had missing values for dietary choline, dietary betaine, type 2 diabetes, or other relevant covariates ($n = 127$); or if they were of a race other than Black or White ($n = 48$). Participants ($n = 163$) with improbable total calorie intake (≤ 500 kcal/day or $> 5,000$ kcal/day) were also excluded from the analysis (14). All participants ($n = 13,440$) with complete data on exposure variables, covariates, and outcome variables were included in the final analysis. Insulin resistance, an impaired response of peripheral tissues to insulin action, was defined based on the HOMA of insulin resistance, which was calculated using: $(FPI \times FPG)/22.5$, in which FPI is fasting plasma insulin concentration (milliunits per liter) and FPG is fasting plasma glucose (millimoles per liter) (15). An HOMA of insulin resistance cutoff value of ≥ 4.70 (80th percentile) was used to define insulin resistance (16). Impaired glucose tolerance was defined based on fasting blood glucose. Participants with fasting blood glucose of 100–125 mg/dL were classified as meeting the definition of impaired fasting glucose (17). In a sensitivity analysis, we excluded participants with impaired glucose tolerance and insulin resistance conditions with an underlying abnormality of glucose metabolism and considered precursors of diabetes.

Primary Exposures of Interest

Baseline dietary choline and dietary betaine intake were measured using a

66-item version of the Willett semiquantitative food frequency questionnaire (FFQ) (18). The dietary choline and betaine intakes were estimated based on the daily serving sizes of the food items known to have choline and betaine by summing the amount of choline and betaine in each food item per serving size based on the U.S. Department of Agriculture food content database (19,20).

Primary Outcome

Incident type 2 diabetes was the primary outcome of interest in the study and was ascertained at visit 2 (1990–1992), visit 3 (1993–1995), and visit 4 (1996–1998) among those free of type 2 diabetes at baseline. Participants with fasting blood glucose level ≥ 126 mg/dL, taking diabetes medication, or reporting a physician diagnosis of diabetes were considered to have type 2 diabetes, while those with fasting blood glucose level < 126 mg/dL who were not taking diabetes medications were considered free of type 2 diabetes.

Covariates

The covariates in the study collected at study baseline included sociodemographic variables (age, sex, race, and education); BMI; health behaviors (smoking and alcohol use); total cholesterol; and intake of total calories, folate, magnesium, vitamin B6, vitamin B12, methionine, and animal fat.

Statistical Analysis

Baseline participant characteristics across the choline quartiles were compared using ANOVA for normally distributed and Kruskal-Wallis tests for nonnormally distributed continuous variables and χ^2 test for categorical variables. Interval-censored Cox proportional hazards regression models sequentially adjusted for baseline covariates were fit to determine the longitudinal association between dietary choline and betaine intake and the risk of type 2 diabetes considering cubic splines of baseline hazard. Covariates adjusted for in model 1 were age, race, sex, BMI, alcohol intake, and smoking, and the exposure variables (choline and betaine) were mutually adjusted. Model 2 further adjusted for baseline fasting blood glucose and total calorie intake. Model 3 further adjusted for intake of dietary vitamin B6, vitamin B12, folate, methionine, animal fat, and

dietary magnesium. Nonnormally distributed variables were log-transformed. We also conducted logistic regression analysis adjusting for covariates listed from model 1 to model 3. The interactions between the exposure variables and race or sex were tested using the likelihood ratio test. *P* values ≤ 0.05 were considered statistically significant. *P*-trend values were computed using the median of choline and betaine in each quartile as continuous (21,22). A sensitivity analysis excluding participants with insulin resistance at baseline was also conducted. Multicollinearity was examined using variance inflation factor. A variance inflation factor >10 was considered high. Animal protein intake was removed from the models, as it was collinear with methionine intake. All statistical analyses were conducted using R Statistical Software (version 3.5.1) and SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

The mean baseline age of the participants was 54 (SD 7.4) years, and 55% were female. Compared with participants with the lowest quartile of choline intake, those with the highest dietary choline intake had a higher BMI and higher intakes of alcohol, total calories, folate, vitamin B6, vitamin B12, animal fat, animal protein, and dietary magnesium. The proportion of male participants and participants with less than a high school education was greater among those with high as compared with low dietary choline intake (Table 1). We observed that betaine intake was positively correlated with choline intake ($\rho = 0.62$).

Among our 13,440 ARIC participants free of type 2 diabetes at baseline, during a median of 9 years (interquartile range 3 years), 1,396 developed incident type 2 diabetes during the follow-up. In the fully adjusted multivariable model (model 3), we observed no association of dietary choline with the risk of type 2 diabetes (hazard ratio [HR] 1.01 [95% CI 0.87, 1.16] per 1 SD of choline intake). Likewise, betaine intake was not associated with the risk of type 2 diabetes (HR 1.01 [95% CI 0.94, 1.10] per 1 SD of betaine intake) (Table 2). In a sensitivity analysis only among those without insulin resistance at baseline, there was no association between 1-SD increase in dietary choline or betaine intake and the risk of type 2

diabetes (data not shown). The logistic regression analysis result per 1 SD of choline and betaine intake was similar to that of the survival analysis (Supplementary Table 1).

Similarly, comparing those in the highest quartile of dietary choline intake with those in the lowest quartile, there was no significant association between dietary choline intake and the risk type 2 diabetes (HR 1.09 [95% CI 0.84, 1.42]) nor was dietary betaine intake significantly associated with the risk of type 2 diabetes, comparing the highest to the lowest quartile (HR 1.06 [95% CI 0.87, 1.29]) (Table 3). In a sensitivity analysis among study participants without insulin resistance at baseline, effect estimates were similar to those observed in the main analyses (data not shown).

The result from logistic regression was similar to the interval-censored Cox proportional hazards regression analysis for the highest choline quartile compared with the lowest (odds ratio [OR] 1.22 [0.89, 1.66]) and for betaine (OR 0.99 [0.77, 1.26]) (Supplementary Table 2).

In crude models, there were significant effect modifications by sex and race on the association between choline intake and the risk of type 2 diabetes. In fully adjusted models, there was no effect modification by race but there was effect modification by sex. In a sex-stratified fully adjusted analysis, while there was no significant association between 1-SD increase in dietary choline or betaine intake and the risk of type 2 diabetes (Table 2), in analysis comparing the highest quartile to the lowest, dietary choline intake was associated with a higher risk of type 2 diabetes among women (HR 1.54 [1.06, 2.25]; *P*-trend = 0.029) but not among men (HR 0.82 [0.57, 1.17]; *P*-trend = 0.360) (Table 4). The result of the logistic regression was also similar among female participants for the highest choline quartile versus the lowest (OR 1.87 [95% CI 1.17, 3.00]), while among male participants, there was no significant association (OR 0.89 [95% CI 0.58, 1.37]) (Supplementary Table 3). The *P* for interaction between choline and sex was 0.070. In interval-censored Cox proportional regression analysis, there was a direct association between betaine and risk of type 2 diabetes among females (HR 1.31 [0.99, 1.74]; *P*-trend = 0.039) but not among males (HR 0.82 [0.62, 1.08]; *P*-trend = 0.188) (Table 4). In logistic

regression analysis, there was no statistically significant association between dietary betaine intake and the risk of type 2 diabetes in stratified analysis by sex (Supplementary Table 3). The interaction of choline and betaine with race was not statistically significant ($P > 0.05$). In both the overall and sex-stratified multivariate-adjusted models, the association between each nutrient without mutually adjusting for betaine or choline, respectively, and the risk of type 2 diabetes did not materially change. The HR for the highest choline quartile versus the lowest was 1.10 (0.85, 1.42) and for betaine, 1.06 (0.87, 1.29). In sex-stratified analysis, among females, the HR for the highest quartile was 1.57 (1.08, 2.29) for choline and 1.35 (1.02, 1.78) for betaine. Among males, the HR for choline was 0.81 (0.57, 1.16) and for betaine, 0.81 (0.62, 1.07) comparing the highest quartile to the lowest.

CONCLUSIONS

In this large cohort study, we observed no statistically significant association between dietary choline intake and the risk of type 2 diabetes, nor was dietary betaine intake significantly associated with the risk of type 2 diabetes. The associations remained similar among study participants without insulin resistance at baseline. Among female (but not male) participants, there was a trend for a modestly higher risk of type 2 diabetes among those with the highest as compared with the lowest quartile of dietary choline intake.

Choline, an essential nutrient (23,24) present in high quantities in red meat, eggs, beef, low-fat milk, chicken without skin, liver, and mustard (20), is involved in neurotransmission, lipid transport, cell membrane phospholipid synthesis, the one-carbon cycle as a precursor of the methyl donor betaine, and gene methylation (25). Betaine, mainly found in bread, spinach, and cold breakfast cereals (20), is a main methyl donor (one-carbon metabolism) in a variety of body cells (6). The distributions of top 10 food sources of choline and betaine in the ARIC study are listed in Bidulescu et al. (20). Humans can produce choline endogenously in the liver, mostly as phosphatidylcholine, but the amount that the body naturally synthesizes is not sufficient to meet human needs (24).

Table 1—Baseline ARIC cohort participants' characteristics

	Choline quartiles				P value
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
N	3,392	3,392	3,392	3,391	
Choline, mg/day (median [IQR])	175.04 (35.00)	258.71 (20.18)	333.88 (24.37)	487.04 (114.62)	<0.001
Betaine, mg/day (median [IQR])	56.79 (21.62)	74.51 (25.86)	87.65 (27.84)	114.84 (43.06)	<0.001
Age (mean [SD])	53.79 (5.75)	54.07 (5.72)	54.02 (5.74)	54.00 (5.75)	0.175
BMI, kg/m ² (median [IQR])	26.73 (4.94)	27.17 (5.17)	27.44 (5.14)	27.63 (5.11)	<0.001
FBG, mg/dL (median [IQR])	98.15 (9.33)	98.68 (9.14)	98.79 (9.44)	98.97 (9.32)	0.002
Race					0.082
Black	814 (24.0)	782 (23.1)	803 (23.7)	869 (25.6)	
White	2,578 (76.0)	2,610 (76.9)	2,589 (76.3)	2,522 (74.4)	
Sex					<0.001
Female	2,138 (63.0)	1,955 (57.6)	1,836 (54.1)	1,547 (45.6)	
Male	1,254 (37.0)	1,437 (42.4)	1,556 (45.9)	1,844 (54.4)	
Education, n (%)					<0.001
Grade school	305 (9.0)	266 (7.9)	271 (8.0)	299 (8.8)	
Less than high school	471 (13.9)	432 (12.8)	404 (11.9)	495 (14.6)	
High school graduate	1,223 (36.1)	1,107 (32.7)	1,064 (31.4)	1,047 (30.9)	
Vocational school	271 (8.0)	291 (8.6)	311 (9.2)	299 (8.8)	
College	819 (24.2)	921 (27.2)	968 (28.6)	886 (26.2)	
Graduate/professional school	298 (8.8)	370 (10.9)	372 (11.0)	360 (10.6)	
Alcohol, g/week (mean [SD])	39.88 (90.58)	43.30 (93.93)	45.14 (96.68)	50.12 (111.36)	<0.001
Magnesium, mg/day (median [IQR])	176.61 (56.96)	227.98 (64.84)	267.66 (69.79)	343.34 (94.39)	<0.001
Total calorie intake, kcal/day (mean [SD])	1,082.90 (323.77)	1,411.50 (342.88)	1,714.48 (394.38)	2,335.27 (639.38)	<0.001
Cholesterol, mg/dL (median [IQR])	215.78 (42.54)	214.62 (40.99)	213.46 (40.99)	212.69 (40.22)	0.011
Folate intake, μg DFE/day (mean [SD])	157.17 (68.35)	205.20 (77.42)	240.71 (84.35)	306.43 (111.41)	<0.001
Vitamin B6, mg/day (mean [SD])	1.14 (0.41)	1.52 (0.44)	1.82 (0.49)	2.35 (0.67)	<0.001
Vitamin B12, μg/day (mean [SD])	4.70 (2.69)	6.44 (3.16)	8.11 (3.64)	11.10 (4.81)	<0.001
Methionine, g/day (mean [SD])	0.98 (0.26)	1.43 (0.25)	1.81 (0.29)	2.53 (0.62)	<0.001
Animal fat, g/day (mean [SD])	20.52 (8.36)	29.44 (9.60)	38.45 (10.82)	55.82 (18.89)	<0.001
Animal protein, g/day (mean [SD])	29.79 (9.24)	44.58 (9.61)	57.49 (10.97)	81.56 (22.57)	<0.001

DFE, dietary folate equivalent; FBG, fasting blood glucose; IQR, interquartile range.

Therefore, humans must obtain some choline from the diet. The most common sources of choline in foods are the fat-soluble phospholipids phosphatidylcholine and sphingomyelin as well as the water-soluble compounds phosphocholine, glycerophosphocholine, and free choline (20,23–25). When these choline-

containing compounds are ingested, pancreatic and mucosal enzymes liberate free choline (19). Free choline, phosphocholine, and glycerophosphocholine are absorbed in the small intestine, enter the portal circulation, and are stored in the liver, where they are subsequently phosphorylated and distributed throughout

the body (19,24). The remaining fat-soluble phospholipids (phosphatidylcholine and sphingomyelin) are absorbed intact, incorporated into chylomicrons, and secreted into the lymphatic circulation, where they are distributed to tissues and other organs (19,24). Despite the hypothesis that choline might affect cardiovascular outcomes, several large observational studies have found no significant associations between choline intakes and cardiovascular disease risk (20,24,25). Other studies suggest that higher dietary choline might increase cardiovascular disease risk because some choline and other dietary compounds are converted to TMA by intestinal bacteria. TMA is then absorbed and converted by the liver into TMAO, a compound that has been associated with a higher risk of cardiovascular disease (25–27).

Studies investigating the direct association between choline and betaine intake and the risk of type 2 diabetes are

Table 2—HRs and 95% CIs for the association of dietary choline and betaine intake per 1-SD increase as a continuous variable with the risk of type 2 diabetes

	Overall	Female	Male
Choline			
Model 1	1.06 (0.99, 1.12)	1.05 (0.96, 1.15)	1.06 (0.97, 1.15)
Model 2	0.99 (0.91, 1.07)	1.06 (0.97, 1.15)	0.99 (0.91, 1.07)
Model 3	1.01 (0.87, 1.16)	1.16 (0.93, 1.44)	0.93 (0.76, 1.13)
Betaine			
Model 1	0.97 (0.91, 1.04)	1.03 (0.94, 1.14)	0.92 (0.84, 1.00)
Model 2	1.02 (0.95, 1.10)	1.09 (0.98, 1.22)	0.94 (0.85, 1.05)
Model 3	1.01 (0.94, 1.10)	1.05 (0.94, 1.18)	0.98 (0.87, 1.09)

Model 1 mutually adjusted for choline and betaine and further adjusted for age, sex (in overall models), race, log-BMI, education, smoking, and alcohol. Model 2 further adjusted for total calorie intake and fasting blood glucose. Model 3 further adjusted for magnesium, vitamin B6, vitamin B12, methionine, animal fat, animal protein, and folate intake.

Table 3—HRs and 95% CIs for the association of dietary choline and betaine intake with the risk of type 2 diabetes

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Choline quartiles					
Model 1	1.00 (Reference)	1.03 (0.88, 1.20)	1.14 (0.98, 1.33)	1.20 (1.01, 1.43)	0.022
Model 2	1.00 (Reference)	0.98 (0.84, 1.16)	1.02 (0.85, 1.23)	1.09 (0.84, 1.42)	0.685
Model 3	1.00 (Reference)	0.98 (0.84, 1.16)	1.02 (0.85, 1.23)	1.09 (0.84, 1.42)	0.440
Sensitivity analysis					
Model 3	1.00 (Reference)	1.05 (0.83, 1.32)	1.01 (0.77, 1.31)	0.97 (0.67, 1.41)	0.260
Betaine quartiles					
Model 1	1.00 (Reference)	1.03 (0.88, 1.19)	1.09 (0.94, 1.28)	0.99 (0.83, 1.18)	0.923
Model 2	1.00 (Reference)	1.02 (0.87, 1.18)	1.08 (0.93, 1.27)	1.08 (0.89, 1.30)	0.362
Model 3	1.00 (Reference)	1.00 (0.86, 1.17)	1.06 (0.90, 1.25)	1.06 (0.87, 1.29)	0.510
Sensitivity analysis*					
Model 3	1.00 (Reference)	0.92 (0.74, 1.15)	1.11 (0.88, 1.40)	1.15 (0.87, 1.52)	0.176

P value is *P* for trend computed by modeling the quartile median of each nutrient as the predictor variable. Model 1 mutually adjusted for choline and betaine and further adjusted for age, sex, race, log-BMI, education, smoking, and alcohol. Model 2 further adjusted for total calorie intake and baseline fasting blood glucose. Model 3 further adjusted for magnesium, vitamin B6, vitamin B12, methionine, animal fat, and folate intake. *In sensitivity analyses, we limited estimates to participants without insulin resistance at baseline.

scant and contradictory. An animal study showed that choline-deficient mice on a high-fat diet had reduced fasting insulin and improved glucose tolerance, as compared with low-fat diet mice (28). In rodents, low intakes of dietary choline and methionine were related to enhanced insulin sensitivity (29), and intraperitoneal administration of choline increased serum insulin levels, perhaps by augmenting acetylcholine release in the pancreas (30). In other animal models, glucose downregulated methylene tetrahydrofolate reductase, an enzyme in the one-carbon cycle that is involved in the conversion of homocysteine to methionine and upregulated cystathionin

β -synthase, another enzyme in one-carbon metabolism that converts homocysteine to cystathionine. Insulin had the opposite effect (31). This indicates an interaction between glucose metabolism and the one-carbon cycle. High homocysteine level was associated with the risk of type 2 diabetes (32).

In a study that combined results from three large cohorts, those in the highest as compared with the lowest phosphatidylcholine intake had 30% higher risk of type 2 diabetes; however, the authors found that high dietary free choline and betaine were associated with reduced risk of type 2 diabetes (33). In a cross-sectional study, participants with type 2

diabetes had high plasma concentrations of choline and low concentrations of betaine (34). Another study found that dietary choline and betaine intake were inversely correlated with fasting blood glucose and insulin levels (10). In a case-control study, plasma TMAO (a choline metabolite) was associated with 2.6 times higher odds of type 2 diabetes (9). In a metabolomics study conducted among European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam participants, choline-containing metabolites were positively associated with the risk of type 2 diabetes (8). In a Mexican American study sample, betaine and betaine to choline ratio were inversely

Table 4—HRs and 95% CIs for the association of dietary choline and betaine intake with the risk of type 2 diabetes stratified by sex

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Female					
Choline quartiles					
Model 1	1.00 (Reference)	1.09 (0.89, 1.35)	1.22 (0.98, 1.51)	1.28 (0.99, 1.64)	0.044
Model 2	1.00 (Reference)	1.10 (0.89, 1.36)	1.15 (0.92, 1.45)	1.37 (1.03, 1.83)	0.032
Model 3	1.00 (Reference)	1.14 (0.91, 1.43)	1.22 (0.93, 1.59)	1.54 (1.06, 2.25)	0.029
Betaine quartiles					
Model 1	1.00 (Reference)	1.13 (0.91, 1.39)	1.34 (1.08, 1.65)	1.24 (0.96, 1.60)	0.065
Model 2	1.00 (Reference)	1.14 (0.92, 1.41)	1.40 (1.13, 1.74)	1.45 (1.11, 1.90)	0.003
Model 3	1.00 (Reference)	1.11 (0.90, 1.37)	1.33 (1.06, 1.66)	1.31 (0.99, 1.74)	0.039
Male					
Choline quartiles					
Model 1	1.00 (Reference)	0.96 (0.77, 1.20)	1.07 (0.86, 1.34)	1.13 (0.89, 1.44)	0.196
Model 2	1.00 (Reference)	0.85 (0.67, 1.07)	0.88 (0.70, 1.12)	0.80 (0.60, 1.08)	0.219
Model 3	1.00 (Reference)	0.85 (0.67, 1.07)	0.88 (0.68, 1.14)	0.82 (0.57, 1.17)	0.360
Betaine quartiles					
Model 1	1.00 (Reference)	0.92 (0.74, 1.14)	0.86 (0.69, 1.08)	0.77 (0.60, 0.98)	0.032
Model 2	1.00 (Reference)	0.88 (0.71, 1.10)	0.81 (0.65, 1.03)	0.78 (0.60, 1.02)	0.079
Model 3	1.00 (Reference)	0.89 (0.77, 1.02)	0.81 (0.64, 1.03)	0.82 (0.62, 1.08)	0.188

P value is *P* for trend computed by modeling the quartile median of each nutrient as the predictor variable. Model 1 mutually adjusted for choline and betaine and further adjusted for age, race, log-BMI, education, smoking, and alcohol. Model 2 further adjusted for total calorie intake and baseline fasting blood glucose. Model 3 further adjusted for magnesium, vitamin B6, vitamin B12, methionine, animal fat, and folate intake.

associated with BMI and indicators of metabolic stress (35). In a small betaine supplementation study conducted among Japanese men, investigators did not find a statistically significant difference in fasting blood glucose, HbA_{1c} (%), and fasting insulin levels between the supplementation group and placebo group over a 12-week follow-up period (36). In another study, plasma betaine levels were observed to be low among insulin-resistant humans, and betaine supplementation improved glucose homeostasis in animal models (37). In a previous ARIC study by our research group, dietary choline intake but not combination of choline and betaine intake was associated with a nonsignificant higher risk of coronary heart disease (20).

There is an established difference in choline metabolism by sex. Women of childbearing age have higher plasma betaine and choline, and various enzymes in the one-carbon cycle are upregulated or downregulated in women compared with men (38). Specifically, increase in phosphatidylethanolamine *N*-methyltransferase (PEMT) from male value to female value increased both plasma choline and betaine, while increasing betaine-homocysteine methyltransferase from male value to female value decreased betaine from male level (38). The differences are suggested to be the effect of sex hormones. It appears that the effect of choline and betaine persists more in females. PEMT^{+/+} diet-fed mice had 60% increased body mass and displayed insulin resistance, while PEMT^{-/-} mice did not. The PEMT^{-/-} mice had increased energy expenditure and maintained normal insulin sensitivity (39). Whether choline or its metabolites have epigenetic impacts on the risk of type 2 diabetes has not yet been established. Choline is converted to betaine, which donates the methyl group in the one-carbon cycle and thus is involved in DNA methylation. Whether the tendency for the modest association between choline and the risk of type 2 diabetes (as observed in this study among women) can be attributed to methylation of DNA or epigenetic modification is a subject of future investigation.

The strength of this study includes a large sample size, long prospective follow-up, and consistent results from both interval-censored survival analyses and logistic regression analysis. The study

also has some limitations relevant to the interpretation of the results. The dietary intake data were collected using self-report with an FFQ, and measurement errors cannot be ruled out for nutrient intake; however, the FFQs have been validated by dietary records (18), and participants were ranked according to choline and betaine intake reducing the impact of measurement errors. Another limitation is that TMAO levels were not available to adjust for.

Conclusion

In our study, dietary choline or betaine intake were not associated with the increased risk of type 2 diabetes, although differences by sex were present. Our study should inform clinical trials on dietary choline and betaine supplementation in relationship with risk of type 2 diabetes.

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