

Are dietary choline and betaine intakes determinants of total homocysteine concentration?^{1–4}

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ABSTRACT

Background: Elevated homocysteine concentrations are associated with an increased risk of cardiovascular disease and a decline in cognitive function. Intakes of choline and betaine, as methyl donors, may affect homocysteine concentrations.

Objective: The objective was to examine whether choline and betaine intakes, assessed from food-frequency questionnaires, are associated with total plasma homocysteine concentrations under both fasting and post-methionine-load conditions in both pre- and post-folic acid fortification periods in the United States.

Design: We assessed the association between choline and betaine intakes and fasting and post-methionine-load homocysteine concentrations using the US Department of Agriculture revised food-composition tables and evaluated whether the associations varied by folic acid fortification periods in 1325 male and 1407 female participants in the sixth examination (1995–1998) of the Framingham Offspring Study.

Results: A higher choline-plus-betaine intake was associated with lower concentrations of post-methionine-load homocysteine; the multivariate geometric means were 24.1 $\mu\text{mol/L}$ (95% CI: 23.4, 24.9 $\mu\text{mol/L}$) in the top quintile of intake and 25.0 $\mu\text{mol/L}$ (95% CI: 24.2, 25.7 $\mu\text{mol/L}$) in the bottom quintile (P for trend = 0.01). We found an inverse association between choline-plus-betaine intake and fasting homocysteine concentrations; the multivariate geometric mean fasting homocysteine concentrations were 9.6 $\mu\text{mol/L}$ (95% CI: 9.3, 9.9 $\mu\text{mol/L}$) in the top quintile and 10.1 $\mu\text{mol/L}$ (95% CI: 9.8, 10.4 $\mu\text{mol/L}$) in the bottom quintile (P for trend < 0.001). When we stratified by plasma folate and vitamin B-12 concentrations, the inverse association was limited to participants with low plasma folate or vitamin B-12 concentrations. In the postfortification period, the inverse association between choline-plus-betaine intake and either fasting or post-methionine-load homocysteine was no longer present.

Conclusions: Choline and betaine intakes were associated with both fasting and post-methionine-load total homocysteine concentrations, especially in participants with low folate and vitamin B-12 status. The inverse association between choline and betaine intakes and homocysteine concentrations was no longer present in the post-fortification period. *Am J Clin Nutr* 2010;91:1303–10.

INTRODUCTION

Choline is important for the structural integrity of cell membranes, methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid and cholesterol transport and metabolism, because it is a precursor for acetylcholine, phospholipids, and the methyl donor betaine (1). Like 5-methylte-

trahydrofolate, once choline is oxidized to betaine, it can provide the necessary one-carbon unit in the conversion of homocysteine to methionine, thus generating *S*-adenosylmethionine (SAM)—the universal methyl donor (1). These folate and choline metabolic pathways are closely interrelated (2, 3). It has been estimated that $\approx 60\%$ of methyl groups are derived from choline, 20% from methionine, and 10–20% from folate (3), which supports the central role of choline as a methyl donor.

Elevated homocysteine concentrations have been associated with low concentrations of B vitamins involved in one-carbon metabolism, including folate, vitamin B-12, and vitamin B-6 (3–6). Elevated homocysteine concentrations in individuals with B vitamin deficiency may indicate a disturbance in one-carbon metabolism (3, 7, 8). In addition to fasting homocysteine concentrations, the effect of an oral methionine load on homocysteine concentrations can be used to test the capacity of the homocysteine removal pathways; an abnormal rise in homocysteine concentrations after a methionine load indicates inadequate capacity for methylation or transsulfuration of the excess homocysteine generated from methionine (7, 9). Post-methionine-load homocysteine concentrations may be more sensitive than fasting concentrations as a marker of hyperhomocysteinemia (10). High concentrations of homocysteine are

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associated with an elevated risk of stroke (11, 12), coronary heart disease (13), and cognitive decline (14–16).

Because of the role of choline and betaine as methyl donors in the one-carbon metabolism, epidemiologic studies evaluating dietary choline and betaine intakes in relation to risk of several chronic diseases (17–23) found an elevated risk of colorectal adenoma (17) and prostate cancer (23) and lower concentrations of inflammation markers (22) with higher choline intakes, an inverse association between choline intake and breast cancer (20), and no association between choline or betaine intake and premenopausal breast cancer (19) and cardiovascular disease (18, 21).

We previously found that choline and betaine intakes predicted fasting homocysteine concentrations, which supports the biological role of choline and betaine in one-carbon metabolism. However, neither our previous study (24) nor those by other researchers (18, 25–29) evaluated the association between choline and betaine and homocysteine concentrations in populations receiving folic acid fortification, which is more relevant to current situations in the United States and some other countries. Therefore, in our current study, using blood samples from the sixth examination of the Framingham Offspring Study cohort, we examined the association between choline and betaine intakes and fasting and post-methionine-load homocysteine concentrations and sought to determine whether the associations were affected by folic acid fortification.

SUBJECTS AND METHODS

Study populations

The Framingham Offspring Study consisted of offspring and their spouses of the participants of the Framingham Heart Study, which was established in Framingham, MA, between 1948 and 1950 with a cohort of 5209 men and women aged 30–59 y. By 1971, the original cohort included 1644 husband-wife pairs and 1921 individuals without a spouse in the cohort. The offspring of the original cohort and the offsprings' spouses were invited to participate in the Framingham Offspring Study, and 5315 of the 6838 eligible individuals participated in the first examination in 1971–1974 (30). The offspring cohort has subsequently undergone follow-up examinations approximately every 3–4 y. The sixth offspring cohort examination began in January 1995 and was completed in August 1998. In March of 1996, the US Food and Drug Administration mandated that all enriched flour and uncooked cereal grains be fortified with 140 μg folic acid/100 g flour per grain by January 1998. In New England, most of the targeted products were fortified with folic acid by July 1997 (31). Therefore, the sixth examination covered both the pre- and postfortification periods of folic acid. We excluded participants who did not have valid food-frequency questionnaires (FFQs), did not have data on either fasting homocysteine or post-methionine-load homocysteine, had a diagnosis of cardiovascular disease, or were taking medications that might alter homocysteine concentrations (32). A total of 1325 men and 1407 women aged 29–86 y were included in the current analyses. The procedures and protocols of the study were approved by the Institutional Review Board for Human Research at Boston Medical Center.

Measurements

Dietary information was collected from the participants by using validated FFQs that included ≈ 130 food items (33). Before the sixth examination, the FFQs were mailed to the participants, who were asked to complete the form and bring it to their appointment. The participants were asked how frequently, on average, during the past year they consumed one standard serving of a specific food item in 9 categories (<1/mo, 1–3/mo, 1/wk, 2–4/wk, 5–6/wk, 1/d, 2–3/d, 4–5/d, or ≥ 6 /d). Responses on frequencies of a specified serving size for each food item were converted to average daily intake. An individual's intakes of choline, betaine, and B vitamins from foods were calculated by multiplying the reported intake frequency of each food by the nutrient content of one serving of that food (34–36). The choline and betaine composition of individual foods was added to the FFQ's nutrient database (Harvard University Food Composition Database) with the use of values published by Zeisel et al (34) and from the recent US Department of Agriculture's choline database with updated betaine values (35). We used the regression-residual method to adjust nutrient intakes for a total energy intake of 1777.5 kcal/d (median energy intake in this population) (37). We took into account the participants' current use of B vitamin supplements and the type of breakfast cereal they consumed to calculate vitamin B intake. For participants who provided FFQs after folic acid fortification was fully implemented (September 1997 to August 1998) (31), folic acid from fortified foods was included in their total folate intake.

Blood samples were obtained during the sixth examinations from participants who had fasted for >10 h to determine the concentrations of homocysteine, folate, vitamin B-12, and pyridoxal 5'-phosphate (PLP)—an active form of vitamin B-6. After the fasting phlebotomy, methionine (100 mg/kg) was immediately administered in 200 mL fruit juice. Two hours later, repeat plasma samples were obtained for homocysteine measurement. Both homocysteine concentrations were measured by HPLC with fluorescence detection (38). Plasma folate was measured with a microbial (*Lactobacillus casei*) assay in a 96-well plate (39). Plasma PLP was measured with an enzymatic procedure using radioactive tyrosine and the apo-enzyme tyrosine decarboxylase (40). Plasma vitamin B-12 was measured with a commercially available radioimmunoassay kit (Quanta-phase II; Bio-Rad, Hercules, CA). All assays were conducted at the laboratory of Jacob Selhub at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. The mean intraassay CVs were 8% for homocysteine, 13% for plasma folate, 16% for PLP, and 7% for plasma vitamin B-12. Laboratory personnel were blinded to the quality-control status and the participants' choline and betaine intakes.

Statistical analyses

We examined the correlations between intakes of betaine, total choline, individual choline compounds, folate, and vitamins B-6 and B-12 and homocysteine concentrations using Spearman correlations. Geometric mean and 95% CIs of plasma homocysteine concentrations were calculated based on exponentiation of log-transformed values according to quintiles of choline and betaine intakes. To test for trend across quintiles, participants



were assigned the median value of their quintile level. This variable was entered as a continuous term, the coefficient for which was evaluated by the Wald test. In the multivariate analyses, we adjusted for age, sex, smoking (none or currently 1–15, 16–25, or ≥ 26 cigarettes/d), total energy intake (continuous), alcohol intake (continuous), serum creatinine concentrations (continuous), and intakes of folate (continuous), vitamin B-6 (continuous), and vitamin B-12 (continuous), which may influence homocysteine concentrations. We examined whether the associations between choline-plus-betaine intake and homocysteine concentrations differed by periods based on folic acid fortification (prefortification period: January 1995 to September 1996; transition period: October 1996 to August 1997; and postfortification period: September 1997 to August 1998) (31), folate intake, alcohol intake, age, sex, and concentrations of plasma folate, PLP, and vitamin B-12 by introducing a cross-product term (choline-plus-betaine \times the factor of interest) in the multivariate model. $P < 0.05$ (2 sided) was considered significant. All statistical analyses were performed by using SAS 9.1 (SAS Institute, Cary, NC) (41).

RESULTS

The energy-adjusted means (\pm SDs) were 437 ± 78 mg/d for choline-plus-betaine, 308 ± 56 mg/d for choline intake, and 129 ± 49 mg/d for betaine intake (**Table 1**). Post-methionine-load homocysteine concentrations were 2.5-fold higher than fasting concentrations; the means (\pm SDs) were 25 ± 8 and 10 ± 4 $\mu\text{mol/L}$, respectively ($P < 0.001$). Choline and betaine intakes were weakly correlated ($r = 0.15$, $P < 0.001$). For choline from individual sources, choline from phosphatidylcholine and choline from sphingomyelin were highly correlated. The correlation coefficients between total choline or betaine intake and dietary one-carbon nutrients ranged from 0.09 to 0.28 ($P < 0.001$).

Higher choline-plus-betaine intake was associated with lower fasting homocysteine concentrations, as in our previous study (24) (**Table 2**); the geometric means of fasting homocysteine concentrations were 10.1 (95% CI: 9.8, 10.4) $\mu\text{mol/L}$ in the bottom quintile of choline-plus-betaine intake and 9.6 (95% CI: 9.3, 9.9) $\mu\text{mol/L}$ in the top quintile (P for trend < 0.001). Choline and betaine intakes were each inversely associated with fasting homocysteine concentrations. Of choline from different sources, choline from glycerophosphocholine, choline from phosphocholine, and choline from sphingomyelin were each inversely associated with fasting homocysteine concentrations (data not shown).

Like with fasting homocysteine, we also observed a statistically significant inverse association between choline-plus-betaine intake and post-methionine-load homocysteine concentrations (**Table 2**). The results with adjustment for intake of folate, vitamin B-6, and vitamin B-12 (**Table 2**) were similar to those without adjustment for intakes of these other one-carbon nutrients (data not shown). The geometric means of post-methionine-load homocysteine concentrations were 25.0 (95% CI: 24.2, 25.7) $\mu\text{mol/L}$ in the bottom quintile and 24.1 (95% CI: 23.4, 24.9) $\mu\text{mol/L}$ in the top quintile (P for trend = 0.01). Higher betaine intake was also significantly associated with lower post-methionine-load homocysteine concentrations. However, the association between choline intake and post-methionine-load homocysteine concentrations was not statistically significant

(P for trend = 0.11). When we examined choline from different sources, only choline intake from glycerophosphocholine (major food sources: milk, fish, beer, coffee, and yogurt) was significantly inversely associated with post-methionine-load homocysteine concentrations (data not shown).

We examined whether the associations between choline-plus-betaine intake and fasting and post-methionine-load homocysteine concentrations differed according to folic acid fortification (prefortification period, transition period, and postfortification period), folate intake (< 250 , 250 – < 400 , 400 – < 600 , ≥ 600 mcg/d), alcohol intake (none, 0.1 to < 15 , 15 to < 30 , and ≥ 30 g/d), age (≤ 50 , 51–60, 61–65, and > 65 y), sex (men, women), plasma folate (tertiles), plasma PLP (tertiles), and plasma vitamin B-12 (tertiles) (**Table 3**). We found a significant inverse association between choline-plus-betaine intake and fasting or post-methionine-load homocysteine concentrations in the prefortification period (P for trend < 0.05). The association between choline-plus-betaine intake and fasting homocysteine concentrations was more pronounced in participants whose plasma concentrations of folate or vitamin B-12 were low (P for interaction = 0.05 for plasma folate and 0.02 for plasma vitamin B-12). When we limited participants to those with low plasma folate (bottom tertile), low plasma vitamin B-12 (bottom tertile), and alcohol intake > 15 g/d ($n = 121$), the geometric means (95% CI) of fasting homocysteine concentrations were 14.4 (95% CI: 12.1, 17.3) $\mu\text{mol/L}$ in the bottom quintile and 12.8 (95% CI: 10.5, 15.6) $\mu\text{mol/L}$ in the top quintile (P for trend = 0.12). Likewise, the inverse association between choline-plus-betaine intake and post-methionine-load homocysteine concentrations was limited to the prefortification period (P for interaction = 0.06) or those with low plasma vitamin B-12 concentrations (P for interaction = 0.06). The association between choline-plus-betaine intake and fasting or post-methionine-load homocysteine concentrations was more pronounced in men than in women (P for interaction = 0.05 for fasting and 0.09 for post-methionine-load homocysteine).

We further examined whether the associations between choline-plus-betaine intake and fasting and post-methionine-load homocysteine concentrations varied by factors related to one-carbon pathways in the post-folic acid fortification period. We found that the associations did not vary by plasma concentrations of PLP or vitamin B-12 (P for interaction ≥ 0.15).

DISCUSSION

We found an inverse association between choline and betaine intakes and either fasting or post-methionine-load homocysteine concentrations in the pre-folic acid fortification period, but not after fortification. We also observed that inverse associations between choline-plus-betaine intake and fasting or post-methionine-load homocysteine concentrations were limited to participants whose folate and vitamin B-12 status was low. Our findings suggest that high folate levels attenuated the association between choline-plus-betaine and homocysteine concentrations.

In our previous study (24), choline and betaine intakes showed an inverse relation to fasting total plasma homocysteine concentrations, particularly in participants with low folate intakes or who consumed alcohol, using blood samples from the fifth examination (January 1991 to December 1994) of the study cohort. Inverse associations in that study (24), which was conducted



TABLE 1
Mean (\pm SD) intakes and Spearman correlations for energy-adjusted intakes of choline compounds, betaine, and vitamin B and concentrations of homocysteine in 1325 male and 1407 female participants in the Framingham Offspring Study¹

Variable (mean \pm SD)	Choline + betaine	Choline	Betaine	Free choline	Choline from glycerophosphocholine	Choline from phosphocholine	Choline from phosphatidylcholine	Choline from sphingomyelin	Folate	Vitamin B-6	Vitamin B-12	Fasting homocysteine	Post-methionine-load homocysteine
Choline + betaine intake (437 \pm 78 mg/d)	1	0.80 ²	0.66 ²	0.56 ²	0.45 ²	0.49 ²	0.50 ²	0.41 ²	0.23 ²	0.24 ²	0.28 ²	-0.10 ²	-0.07 ²
Choline intake (308 \pm 56 mg/d)		1	0.15 ²	0.52 ²	0.48 ²	0.57 ²	0.72 ²	0.65 ²	0.09 ²	0.19 ²	0.28 ²	-0.07 ²	-0.03 ³
Betaine intake (129 \pm 49 mg/d)			1	0.33 ²	0.18 ²	0.11 ²	-0.02 ³	-0.09 ²	0.27 ²	0.17 ²	0.11 ²	-0.10 ²	-0.08 ²
Individual choline intake				1	0.49 ²	0.37 ²	0.02 ³	-0.01 ³	0.19 ²	0.22 ²	0.09 ²	-0.008 ³	0.01 ³
Free choline (76 \pm 18 mg/d)					1	0.56 ²	-0.11 ²	0.07 ²	0.15 ²	0.17 ²	0.21 ²	-0.11 ²	-0.08 ²
Choline from glycerophosphocholine (53 \pm 21 mg/d)						1	0.22 ²	0.40 ²	0.28 ²	0.31 ²	0.25 ²	-0.20 ²	-0.08 ²
Choline from phosphocholine (14 \pm 5 mg/d)							1	0.72 ²	-0.04 ⁴	0.05 ⁴	0.20 ²	-0.004 ³	0.006 ³
Choline from phosphatidylcholine (147 \pm 40 mg/d)								1	-0.06 ⁴	0.08 ²	0.18 ²	-0.08 ²	-0.01 ³
Choline from sphingomyelin (18 \pm 6 mg/d)									1	0.74 ²	0.60 ²	-0.31 ²	-0.20 ²
Folate intake (550 \pm 296 μ g/d)										1	0.69 ²	-0.33 ²	-0.21 ²
Vitamin B-6 intake (7 \pm 23 mg/d)											1	-0.25 ²	-0.15 ²
Vitamin B-12 intake (10 \pm 15 μ g/d)												1	0.54 ²
Fasting homocysteine (10 \pm 4 μ mol/L)													1
Post-methionine-load homocysteine (25 \pm 8 μ mol/L)													

¹ Major food sources of choline: free choline (coffee, potato, beer, milk, and chicken), choline from glycerophosphocholine (milk, fish, beer, coffee, and yogurt), choline from phosphocholine (milk, chicken, broccoli, potato, and tomato), choline from phosphatidylcholine (red meat, chicken, eggs, fish, and shellfish), choline from sphingomyelin (chicken, red meat, milk, eggs, and fish), and betaine (spinach, pasta, white bread, and cold breakfast cereal).

² $P < 0.001$.

³ $P < 0.05$.

⁴ $0.001 < P < 0.05$.

TABLE 2

Multivariate-adjusted geometric mean (95% CI) fasting and post-methionine-load homocysteine concentrations ($\mu\text{mol/L}$) by quintile of energy-adjusted choline and betaine intakes in 1325 male and 1407 female participants in the Framingham Offspring Study: 1995–1998¹

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> for trend ²
Fasting homocysteine						
Choline + betaine (mg/d)						
Median \pm SD	344 \pm 34	395 \pm 12	433 \pm 11	473 \pm 13	550 \pm 52	
Geometric mean (95% CI)	10.1 (9.8, 10.4)	10.0 (9.7, 10.3)	9.7 (9.5, 10.0)	9.7 (9.4, 10.0)	9.6 (9.3, 9.9)	<0.001
Choline (mg/d)						
Median \pm SD	234 \pm 25	278 \pm 9	305 \pm 7	334 \pm 10	379 \pm 36	
Geometric mean (95% CI)	10.1 (9.8, 10.4)	10.1 (9.8, 10.4)	9.7 (9.5, 10.0)	9.7 (9.4, 9.9)	9.7 (9.4, 9.9)	0.001
Betaine (mg/d)						
Median \pm SD	77 \pm 12	102 \pm 5	120 \pm 5	143 \pm 8	203 \pm 50	
Geometric mean (95% CI)	10.1 (9.8, 10.4)	9.9 (9.6, 10.2)	9.7 (9.4, 10.0)	9.8 (9.5, 10.1)	9.6 (9.3, 9.9)	<0.001
Post-methionine-load homocysteine						
Choline + betaine						
Geometric mean (95% CI)	25.0 (24.2, 25.7)	25.0 (24.2, 25.8)	24.3 (23.6, 25.1)	24.2 (23.5, 25.0)	24.1 (23.4, 24.9)	0.01
Choline						
Geometric mean (95% CI)	24.5 (23.8, 25.3)	25.6 (24.8, 26.4)	24.0 (23.3, 24.8)	24.4 (23.7, 25.2)	24.3 (23.6, 25.0)	0.11
Betaine						
Geometric mean (95% CI)	24.9 (24.1, 25.7)	24.8 (24.1, 25.6)	24.3 (23.6, 25.1)	24.6 (23.9, 25.4)	24.0 (23.2, 24.8)	0.04

¹ Values were adjusted for age, sex, smoking, alcohol intake, total energy intake, serum creatinine concentration, and intakes of folate, vitamin B-6, and vitamin B-12.

² *P* for trend (2-sided) was calculated by using the Wald test statistic.

before the implementation of mandatory folic acid fortification of grain products, were generally stronger than those in the present study, which covers both pre- and postfortification periods. Another study of 1477 women in the Nurses' Health Study found an inverse association between choline-plus-betaine intake and fasting homocysteine concentrations (29), and a Dutch study including 903 women found no association for betaine intake, but a weak inverse association between choline intake and homocysteine concentrations in blood samples collected after a fast of ≥ 2 -h (18). None of these studies evaluated choline intake in relation to post-methionine-load homocysteine concentrations or during the post-folic acid fortification period.

Our data support the contention that individuals with low folate and vitamin B-12 status may benefit from intake of choline and betaine. This concurs with animal studies indicating that folate and choline metabolism intersect at the remethylation of homocysteine to form methionine in one-carbon pathways (2). Studies report a reduction in hepatic choline content in rats fed a folate-deficient diet compared with controls (42). Similarly, total hepatic folate content (43, 44) or hepatic SAM content (45) decreased in rats fed a choline-deficient diet compared with controls. Methylene-tetrahydrofolate reductase or cystathionine- β -synthase knockout mice, which accumulate homocysteine, have depleted choline and betaine pools in the liver (46, 47). Methionine adenosyltransferase knockout mice, which have impaired formation of SAM and activated gene expression of betaine-homocysteine methyltransferase, also have increased dietary choline requirements (48).

Elevated concentrations of homocysteine may be a marker of diminished capacity of the remethylation or transsulfuration pathways (7). Although some randomized controlled trials reported no effect of B vitamin supplementation associated with reduction in homocysteine concentrations on myocardial infarction (12, 49), or deaths from cardiovascular disease (12) in patients with existing cardiovascular diseases, a meta-analysis of prospective studies that included only participants who did not

have preexisting cardiovascular diseases showed an 18% increased risk of coronary events associated with a 5- $\mu\text{mol/L}$ increment of homocysteine concentrations (13). Another meta-analysis (11) found an 11% lower risk of ischemic heart disease and a 19% lower risk of stroke associated with 25% lower homocysteine concentrations.

Several crossover studies or randomized trials have shown that a diet deficient in choline increased fasting homocysteine concentrations (50) and that supplementation with choline or betaine either decreased fasting homocysteine concentrations (26, 27) or attenuated the rise in post-methionine-load homocysteine concentrations (25–27). Our study of >2700 free-living healthy participants provides further evidence that the range of choline and betaine intakes from foods still predicts both fasting and post-methionine-load homocysteine concentrations, especially given low folate and vitamin B-12 status.

Of the individual sources of choline, the esters glycerophosphocholine, phosphocholine, and sphingomyelin were associated with fasting homocysteine concentrations, but only glycerophosphocholine was statistically significantly associated with post-methionine-load homocysteine concentrations. Our previous study using the 5th examination of the Framingham Offspring Study found significant inverse associations for free choline, glycerophosphocholine, phosphocholine, and sphingomyelin (24). The study in the Nurses' Health Study also found a significant inverse association for glycerophosphocholine and phosphocholine in relation to fasting homocysteine concentrations (29). The consistent inverse association for glycerophosphocholine found in these 3 studies merits further examination, given that the differences in the metabolism and specific roles of individual choline compounds remain unknown.

We found a stronger association in men than in women, consistent with our previous study (24). It is possible that women are less reliant on dietary sources of choline because of an enhanced capacity for endogenous synthesis of choline due to estrogen-induction of the gene for this pathway (51, 52).

TABLE 3Multivariate-adjusted geometric mean (95% CI) fasting and post-methionine-load homocysteine concentrations ($\mu\text{mol/L}$) by quintile of energy-adjusted choline-plus-betaine intake and categories of other factors in 1325 male and 1407 female participants in the Framingham Offspring Study, 1995–1998¹

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> for interaction ²
Fasting homocysteine						
Folic acid fortification status ³						
Prefortification (<i>n</i> = 1252)	10.5 (10.0, 11.0)	10.0 (9.5, 10.4)	10.0 (9.6, 10.5)	10.2 (9.7, 10.6)	9.8 (9.4, 10.3)	0.95
Transition period (<i>n</i> = 785)	10.1 (9.6, 10.6)	10.2 (9.7, 10.8)	9.7 (9.2, 10.2)	9.2 (8.8, 9.7)	9.5 (9.0, 10.0)	
Postfortification (<i>n</i> = 695)	9.6 (9.1, 10.2)	9.9 (9.4, 10.4)	9.6 (9.1, 10.1)	9.6 (9.1, 10.1)	9.3 (8.8, 9.8)	
Folate intake						
<250 $\mu\text{g/d}$ (<i>n</i> = 284)	11.8 (11.0, 12.7)	11.1 (10.2, 12.0)	10.7 (9.8, 11.7)	10.8 (9.6, 12.3)	11.7 (10.3, 13.3)	0.44
≥ 250 to <400 $\mu\text{g/d}$ (<i>n</i> = 770)	10.8 (10.2, 11.4)	10.7 (10.2, 11.3)	11.0 (10.4, 11.6)	11.0 (10.4, 11.6)	10.3 (9.7, 11.0)	
≥ 400 to <600 $\mu\text{g/d}$ (<i>n</i> = 680)	9.5 (8.9, 10.1)	9.4 (8.9, 10.0)	9.4 (8.9, 9.9)	9.1 (8.6, 9.7)	9.3 (8.8, 9.9)	
≥ 600 $\mu\text{g/d}$ (<i>n</i> = 998)	8.7 (8.3, 9.2)	9.1 (8.6, 9.5)	8.6 (8.1, 9.0)	8.5 (8.1, 9.0)	8.5 (8.1, 8.9)	
Alcohol intake						
0 g/d (<i>n</i> = 718)	9.7 (9.2, 10.2)	9.4 (8.9, 9.9)	9.7 (9.1, 10.3)	9.6 (9.0, 10.2)	9.5 (8.9, 10.0)	0.21
>0 to <15 g/d (<i>n</i> = 1401)	9.9 (9.5, 10.3)	10.0 (9.6, 10.5)	9.6 (9.2, 10.0)	9.4 (9.0, 9.8)	9.4 (9.0, 9.8)	
15 to <30 g/d (<i>n</i> = 247)	11.2 (9.9, 12.7)	10.9 (9.7, 12.3)	9.8 (8.8, 11.0)	10.2 (9.1, 11.5)	9.8 (8.7, 11.0)	
≥ 30 g/d (<i>n</i> = 366)	11.5 (10.6, 12.5)	10.7 (9.9, 11.5)	10.3 (9.4, 11.2)	10.9 (10.1, 11.8)	10.4 (9.7, 11.1)	
Age						
≤ 50 y (<i>n</i> = 602)	9.9 (9.3, 10.4)	9.1 (8.6, 9.7)	9.2 (8.7, 9.8)	8.9 (8.4, 9.5)	9.2 (8.7, 9.8)	0.79
51 to ≤ 60 y (<i>n</i> = 978)	9.5 (9.1, 10.0)	9.8 (9.4, 10.3)	9.2 (8.8, 9.6)	9.5 (9.0, 9.9)	9.1 (8.7, 9.5)	
61 to ≤ 65 y (<i>n</i> = 448)	10.3 (9.6, 11.2)	10.0 (9.3, 10.8)	9.8 (9.0, 10.6)	9.7 (9.0, 10.5)	9.4 (8.7, 10.2)	
> 65 y (<i>n</i> = 704)	10.8 (10.1, 11.5)	10.9 (10.2, 11.6)	10.9 (10.1, 11.7)	10.5 (9.8, 11.3)	10.5 (9.8, 11.2)	
Sex						
Male (<i>n</i> = 1325)	11.0 (10.5, 11.4)	10.8 (10.4, 11.3)	10.5 (10.1, 10.9)	10.4 (10.0, 10.8)	10.1 (9.7, 10.5) ^d	0.05
Female (<i>n</i> = 1407)	9.3 (8.9, 9.7)	9.2 (8.8, 9.6)	9.0 (8.6, 9.4)	9.0 (8.6, 9.4)	9.0 (8.6, 9.4)	
Plasma folate						
<6 ng/mL (<i>n</i> = 908)	12.0 (11.4, 12.6)	11.5 (11.0, 12.1)	11.8 (11.2, 12.4)	11.3 (10.8, 11.9)	11.1 (10.5, 11.7) ^d	0.05
6 to <12 ng/mL (<i>n</i> = 916)	9.6 (9.2, 10.0)	9.7 (9.3, 10.1)	9.3 (8.9, 9.6)	9.2 (8.8, 9.6)	9.3 (9.0, 9.8)	
≥ 12 ng/mL (<i>n</i> = 908)	8.6 (8.1, 9.1)	8.5 (8.0, 9.0)	8.3 (7.8, 8.8)	8.4 (7.9, 8.9)	8.2 (7.8, 8.7)	
Plasma PLP						
<48 nmol/L (<i>n</i> = 911)	10.9 (10.4, 11.5)	10.8 (10.3, 11.3)	10.9 (10.4, 11.5)	10.3 (9.8, 10.9)	10.7 (10.1, 11.3)	0.32
48 to <80 nmol/L (<i>n</i> = 910)	10.0 (9.5, 10.5)	9.9 (9.4, 10.4)	9.6 (9.2, 10.1)	9.7 (9.3, 10.3)	9.6 (9.1, 10.1)	
≥ 80 nmol/L (<i>n</i> = 911)	8.9 (8.4, 9.4)	8.7 (8.3, 9.2)	8.3 (7.8, 8.8)	8.6 (8.1, 9.0)	8.2 (7.8, 8.6)	
Plasma vitamin B-12						
<334 pg/mL (<i>n</i> = 910)	11.5 (10.9, 12.1)	11.4 (10.8, 12.0)	10.9 (10.3, 11.5)	10.7 (10.2, 11.3)	10.4 (9.8, 11.0) ^d	0.02
334 to <459 pg/mL (<i>n</i> = 911)	9.9 (9.4, 10.3)	9.8 (9.3, 10.2)	10.0 (9.5, 10.4)	9.7 (9.2, 10.1)	9.9 (9.5, 10.4)	
≥ 459 pg/mL (<i>n</i> = 911)	9.0 (8.5, 9.5)	9.0 (8.5, 9.4)	8.5 (8.1, 9.0)	8.8 (8.3, 9.2)	8.6 (8.2, 9.0)	
Post-methionine-load homocysteine						
Folic acid fortification status ³						
Prefortification (<i>n</i> = 1252)	25.7 (24.6, 26.9)	24.8 (23.6, 25.9)	24.9 (23.7, 26.1)	24.6 (23.5, 25.7)	24.1 (23.1, 25.3)	0.06
Transition period (<i>n</i> = 785)	25.1 (23.8, 26.6)	26.8 (25.3, 28.4)	25.0 (23.6, 26.5)	23.7 (22.3, 25.1)	24.7 (23.3, 26.2)	
Postfortification (<i>n</i> = 695)	23.5 (22.1, 25.0)	23.7 (22.4, 25.1)	23.0 (21.7, 24.5)	24.2 (22.8, 25.6)	23.2 (21.8, 24.7)	
Folate intake						
<250 $\mu\text{g/d}$ (<i>n</i> = 284)	28.4 (26.6, 30.3)	26.1 (24.2, 28.2)	26.5 (24.3, 28.8)	25.3 (22.5, 28.4)	27.7 (24.6, 31.2)	0.26
≥ 250 to <400 $\mu\text{g/d}$ (<i>n</i> = 770)	26.1 (24.7, 27.7)	27.1 (25.6, 28.6)	27.2 (25.7, 28.8)	26.8 (25.3, 28.3)	25.5 (23.9, 27.2)	
≥ 400 to <600 $\mu\text{g/d}$ (<i>n</i> = 680)	23.5 (22.0, 25.2)	23.4 (21.9, 25.0)	23.3 (21.9, 24.8)	23.0 (21.6, 24.6)	23.4 (21.9, 25.0)	
≥ 600 $\mu\text{g/d}$ (<i>n</i> = 998)	22.6 (21.3, 23.9)	23.6 (22.3, 24.9)	22.0 (20.8, 23.4)	22.2 (21.0, 23.4)	22.4 (21.3, 23.6)	
Alcohol intake						
0 g/d (<i>n</i> = 718)	23.3 (22.0, 24.5)	22.8 (21.6, 24.2)	22.7 (21.3, 24.2)	22.6 (21.3, 24.1)	23.0 (21.6, 24.4)	0.82
>0 to <15 g/d (<i>n</i> = 1401)	24.6 (23.5, 25.7)	25.2 (24.1, 26.4)	24.4 (23.3, 25.5)	23.8 (22.8, 24.8)	23.7 (22.6, 24.8)	
15 to <30 g/d (<i>n</i> = 247)	29.3 (26.0, 32.9)	27.7 (24.8, 31.0)	26.5 (23.8, 29.4)	28.3 (25.3, 31.7)	27.4 (24.4, 30.7)	
≥ 30 g/d (<i>n</i> = 366)	29.4 (26.9, 32.0)	28.0 (25.8, 30.3)	26.7 (24.4, 29.2)	27.6 (25.5, 30.0)	27.5 (25.6, 29.5)	
Age						
≤ 50 y (<i>n</i> = 602)	24.8 (23.4, 26.4)	23.5 (22.1, 25.0)	24.4 (23.0, 25.9)	23.5 (22.0, 25.0)	24.3 (22.8, 25.9)	0.38
51 to ≤ 60 y (<i>n</i> = 978)	24.0 (22.8, 25.2)	25.0 (23.7, 26.2)	24.1 (22.9, 25.3)	24.2 (23.0, 25.4)	24.0 (22.8, 25.3)	
61 to ≤ 65 y (<i>n</i> = 448)	26.0 (23.9, 28.3)	26.1 (24.0, 28.3)	24.2 (22.2, 26.5)	24.2 (22.3, 26.4)	23.1 (21.2, 25.1)	
>65 y (<i>n</i> = 704)	25.5 (23.9, 27.2)	25.7 (24.1, 27.5)	24.6 (22.9, 26.3)	24.8 (23.2, 26.6)	24.7 (23.1, 26.4)	
Sex						
Male (<i>n</i> = 1325)	24.3 (23.3, 25.3)	24.2 (23.2, 25.2)	23.4 (22.4, 24.4)	23.5 (22.6, 24.5)	23.2 (22.3, 24.1)	0.09
Female (<i>n</i> = 1407)	25.2 (24.0, 26.4)	25.6 (24.4, 26.8)	25.0 (23.9, 26.2)	24.7 (23.5, 25.9)	24.9 (23.8, 26.2)	

(Continued)

TABLE 3 (Continued)

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	<i>P</i> for interaction ²
Plasma folate						0.13
<6 ng/mL (<i>n</i> = 908)	27.8 (26.6, 29.2)	27.7 (26.4, 29.1)	28.8 (27.4, 30.4)	26.7 (25.4, 28.2)	26.6 (25.2, 28.2)	
6 to <12 ng/mL (<i>n</i> = 916)	24.2 (23.0, 25.4)	25.1 (23.8, 26.4)	23.2 (22.1, 24.4)	23.7 (22.6, 24.9)	23.9 (22.8, 25.2)	
≥12 ng/mL (<i>n</i> = 908)	22.4 (21.0, 23.8)	21.7 (20.4, 23.0)	21.2 (19.9, 22.6)	21.6 (20.3, 23.0)	21.4 (20.2, 22.7)	
Plasma PLP						0.63
<48 nmol/L (<i>n</i> = 911)	26.5 (25.3, 27.8)	27.3 (25.9, 28.6)	27.1 (25.7, 28.5)	26.2 (24.9, 27.6)	26.2 (24.8, 27.6)	
48 to <80 nmol/L (<i>n</i> = 910)	24.5 (23.2, 25.9)	24.4 (23.1, 25.7)	24.0 (22.7, 25.4)	23.8 (22.5, 25.1)	23.8 (22.4, 25.2)	
≥80 nmol/L (<i>n</i> = 911)	22.2 (21.0, 23.6)	21.7 (20.4, 23.0)	20.5 (19.4, 21.8)	21.3 (20.1, 22.5)	21.0 (19.8, 22.1)	
Plasma vitamin B-12						0.06
<334 pg/mL (<i>n</i> = 910)	27.7 (26.3, 29.2)	27.7 (26.2, 29.2)	27.2 (25.7, 28.8)	26.8 (25.3, 28.3)	26.2 (24.7, 27.8)	
334 to <459 pg/mL (<i>n</i> = 911)	24.4 (23.3, 25.6)	25.0 (23.7, 26.3)	24.0 (22.9, 25.2)	24.0 (22.9, 25.3)	24.3 (23.1, 25.5)	
≥459 pg/mL (<i>n</i> = 911)	22.6 (21.4, 24.0)	22.6 (21.5, 23.8)	22.1 (20.9, 23.4)	22.1 (21.0, 23.3)	22.3 (21.2, 23.5)	

¹ Values were adjusted for age, sex, smoking, alcohol intake, total energy intake, serum creatinine concentration, and intakes of folate, vitamin B-6, and vitamin B-12. PLP, pyridoxal 5'-phosphate.

² *P* for interaction (2-sided) was calculated by using the Wald test statistic.

³ Prefortification period: January 1995 to September 1996; transition period: October 1996 to August 1997; postfortification period: September 1997 to August 1998.

⁴ *P* for trend <0.05.

Our study had several limitations. Because the assessment of choline and betaine intakes through FFQs and blood collection were made within a short period of time, our study may not fully reflect the temporal relation between choline and betaine intakes and homocysteine concentrations. However, because the FFQs we used were designed to assess long-term dietary intake (53), choline and betaine intakes in our study might reflect choline and betaine intakes before homocysteine measurement.

The strengths of our study include a large sample size and measurements of both fasting and post-methionine-load homocysteine concentrations as well as circulating concentrations of folate, vitamin B-6, and vitamin B-12. Inclusion of >2700 participants gave us considerable statistical power to evaluate whether the associations varied according to several factors related to the one-carbon pathway, including vitamin B status and alcohol intake. Plasma concentrations of folate and vitamins B-6 and B-12 may reflect the bioavailability of these B vitamins, including malabsorption and intakes from fortified foods, unfortified foods, and supplements. Because some participants' blood samples were collected after folic acid fortification began (*n* = 695), we were able to examine the association between choline and betaine intakes and homocysteine concentrations during the folic acid fortification era, which could be applied to the current US general population.

In conclusion, choline and betaine intakes were associated with both fasting and post-methionine-load total homocysteine concentrations, especially in participants with low folate and vitamin B-12 status. Choline and betaine intakes were associated with both fasting and post-methionine-load homocysteine concentrations in the pre-folic acid fortification period, but the association was no longer present in the postfortification period. Intake of choline and betaine may still be important in populations who do not receive folic acid fortification and for individuals with low folate or vitamin B-12 status.

The authors' responsibilities were as follows—PFJ, JS, SHZ, and EC: study design; PFJ, LD, JS, and EC: data collection; JEL: data analysis;

and JEL, PFJ, LD, JS, EG, SHZ, and EC: data interpretation and manuscript preparation. No personal or financial conflicts of interest were declared.

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