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Choline status is not a reliable indicator of moderate changes in dietary choline consumption in premenopausal women

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Abstract

For the prevention of liver dysfunction in women, a choline adequate intake of 425 mg/day was established. To date, the relationship between dietary choline intake and plasma concentrations of choline moieties remains relatively unexplored. As an extension of our previous work, this 14-week controlled feeding study investigated the relationship between moderate changes in dietary choline intake and blood indicators of status. The influences of folate intake and the methylenetetrahydrofolate reductase (MTHFR) C677T genotype were also considered. Healthy premenopausal women (n=45, 18–46 years) with the MTHFR 677CC (n=28) or TT (n=17) genotype consumed a folate-restricted diet for 2 weeks followed by randomization to one of four dietary treatments (n=6–9/group) differing in total choline (344–486 mg/day), betaine (122–349 mg/day) and/or folate (400–800 μg dietary folate equivalents/day) content for 12 weeks. Responses to treatment were assessed as changes in the plasma levels of choline moieties (i.e., betaine, choline, phosphatidylcholine and sphingomyelin) and/or leukocyte global DNA methylation between pretreatment (Week 2) and posttreatment (Week 14) values. No significant changes were detected in the measured variables in response to dietary increases in choline (i.e., 41% increase) or betaine (i.e., 286% increase) intake. However, the MTHFR C677T genotype, alone or together with a diet, influenced betaine (P=.03) and phosphatidylcholine (P=.03). These data suggest that choline status is not a reliable indicator of moderate changes in dietary choline intake possibly due to the engagement of compensatory mechanisms. In addition, the MTHFR C677T genotype appears to influence the direction and use of choline moieties in this group of women.

Keywords: Choline; Phosphatidylcholine; MTHFR; Betaine; Women; Folate

1. Introduction

While the nutritional importance of choline was described over 70 years ago [1], the dietary essentiality of this nutrient was only recently recognized with an establishment of an adequate intake (AI) level of 425 and 550 mg of choline/day for women and men, respectively [2]. Phosphatidylcholine is the primary form of choline in food and in the body. In mammalian cells, phosphatidylcholine may be synthesized from choline via the cytidine diphosphate (CDP) choline pathway in which cytidine triphosphate (CTP): phosphocholine cytidylyltransferase (CT) serves as the rate-limiting and regulating enzyme (reviewed in Ref. [3]). In addition, the liver and a few other tissues make phosphatidylcholine via the methylation of phosphatidylethanolamine, a reaction catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) and involving the sequential transfer of three methyl groups from *S*-adenosylmethionine [4].

Phosphatidylcholine is the primary phospholipid of all classes of lipoproteins in mammals [5]. The active synthesis of phosphatidylcholine appears to be required for the secretion of very low-density lipoprotein and high-density lipoprotein [6,7], although gender-specific differences have been described [7–11]. In addition to its important role as a phospholipid precursor, choline can be acetylated to the

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Table 1
Study design

Description	Dietary treatment group			
	I	II	III	IV
MTHFR C677T genotype	7 CC	6 CC	7 CC, 8 TT	8 CC, 9 TT
Dietary choline (mg/day)[a]	169	169	237	311
Supplemental choline (mg/day)[b]	175	175	175	175
Total choline (mg/day)	344	344	412	486
Betaine (mg/day)	122	122	267	349
Folate intake (μg DFE/day)	400	800	400	800

[a] Includes dietary phosphatidylcholine, glycerophosphocholine, choline and lysophosphatidylcholine.
[b] Choline (350 mg/day) (from choline bitartrate; TwinLab, Twin Laboratories) was consumed every other day, yielding an average supplemental choline intake of 175 mg/day.

neurotransmitter acetylcholine or oxidized to the methyl donor betaine [12].

Folate and choline are interrelated as either metabolite may serve as a methyl donor for the conversion of homocysteine to methionine. In a depletion–repletion study, folate restriction was associated with significant declines in plasma concentrations of phosphatidylcholine, whereas folate treatment with 800 μg/day as dietary folate equivalents (DFE) was linked to significant increases [10]. Although the roles of folate in disease and developmental conditions have been extensively investigated (reviewed in Ref. [13]), fewer studies have assessed the role of choline in these disease processes. Nonetheless, choline and/or betaine insufficiency is associated with the accumulation of lipid in liver and liver dysfunction [8], neural tube defect risk [14], hyperhomocysteinemia [15], DNA damage [16], muscle dysfunction [17] and altered DNA methylation patterns [18,19].

Given the establishment of a choline AI and a growing body of literature suggesting that suboptimal choline intake/status may increase the risk of certain diseases, it is essential to delineate the relationship between the dietary intake of choline moieties and blood status indicators. Thus, as an extension of our previous work [20,21], this 14-week controlled feeding study conducted in healthy premenopausal women sought to investigate the relationship between moderate changes in dietary choline/betaine intake and blood status indicators. In doing so, we considered the influences of folate intake and methylenetetrahydrofolate reductase (MTHFR) C677T genotype as these factors may influence choline status [10].

2. Methods

2.1. Subjects

Healthy premenopausal female subjects aged 18 to 46 years, preselected for the MTHFR 677CC or TT genotype, were recruited between January 2002 and April 2003 from the staff and student population at Cal Poly Pomona, Pomona, CA, as well as the surrounding community as previously detailed [20,21]. The study was approved by the Cal Poly Pomona's institutional review board for human subjects, and informed consent was obtained from each participant.

2.2. Study design

This was a 14-week controlled feeding study. During the first 2 weeks of the study, participants consumed a folate-restricted diet that, together with supplements, provided 133 μg/day of DFE, 344 mg/day of choline (169 and 175 mg/day from the diet and supplement, respectively), 122 mg/day of betaine and all other nutrients in recommended amounts. For the remaining 12 weeks of the study (i.e., Week 3 through 14), subjects with the MTHFR 677CC genotype were randomized to one of four treatment groups: Group I and II consumed 344 mg of total choline, 122 mg of betaine and 400 or 800 μg of DFE/day respectively; Group III consumed 412 mg of total choline, 267 mg of betaine and 400 μg of DFE/day; and Group IV consumed 486 mg of total choline, 349 mg of betaine and 800 μg of DFE/day (Table 1). In addition, subjects with the MTHFR 677TT genotype were randomized to groups III or IV (Table 1). For every treatment group, supplemental choline provided 350 mg every other day or an average of 175 mg of choline/day. The 400 and 800 μg of DFE/day were derived from varying amounts of supplemental folic acid (prepared in-house) and/or naturally occurring food folate as previously described [20,21].

2.3. Diets and supplements

The 5-day diet described in detail previously [20,21] provided 169±11 mg/day of total choline, 122±9 mg/day of betaine and 133±8 μg/day of naturally occurring food folate

Table 2
Choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, lysophosphatidylcholine sphingomyelin, betaine and folate content of the menus[a,b]

Metabolite	Menus		
	A	B	C
Choline (mg/day)	25.6±1.5	50.9±9.1	105.0±11.7
Glycerophosphocholine (mg/day)	18.2±1.7	29.5±5.1	42.0±5.5
Phosphocholine (mg/day)	5.6±0.9	7.6±1.7	7.2±0.5
PtdCho (mg/day)	63±7	79±6	99±5
LysoPtdCho (mg/day)	47±5	61±13	51±7
Sphingomyelin (mg/day)	8.2±1.2	8.5±1.7	7.3±1.2
Total choline (mg/day)	169±11	237±22	311±18
Betaine (mg/day)	122±9	267±42	349±57
Folate (μg DFE/day)	133±8	426±44	835±33

PtdCho, phosphatidylcholine; LysoPtdCho, lysophosphatidylcholine.
[a] Values are means±S.E.M., n=5 days.
[b] Menu A was consumed by all subjects during the first 2-week baseline phase of the study. During the 12-week treatment phase, Menu A was consumed by Groups I and II, Menu B by Group III and Menu C by Group IV.

(Menu A); 237±22 mg/day of total choline, 267±42 mg/day of betaine and 426±44 μg/day of folate (Menu B); or 311±18 mg/day of total choline, 349±57 mg/day of betaine and 835±33 μg/day of folate (Menu C) (Table 2). Menu A was consumed by all subjects during the first 2-week baseline phase of the study. During the 12-week treatment phase, Menu A was consumed by Groups I and II, Menu B by Group III and Menu C by Group IV. The diets provided ~2086 to 2300 kcal/day with 57% to 66% from carbohydrate, 13.2% to 13.4% from protein and 21% to 30% from fat (ESHA Food Processor Nutrient Data Base, version 7.81; ESHA Research, Salem, OR). All foodstuffs were weighed to the nearest gram. Throughout the 14-week study, subjects consumed morning and evening meals in the metabolic kitchen under the supervision of the investigators 7 days per week. Lunches and snacks were provided as take-aways, and subjects were instructed to consume all food items received.

Based on the nutrient content of the baseline diet as estimated using ESHA Food Processor Nutrient Data Base (version 7.81; ESHA Research), all subjects were given supplements to meet the dietary reference intakes (recommended dietary allowance [RDA] or AI [2,22,23]) or 1989 RDA [24] for essential nutrients. The supplements were consumed throughout the 14-week study and included a multimineral (LifeTime; Nutritional Specialties, Anaheim, CA) given everyday, a multivitamin without folic acid (Trader Darwin's Stress Vitamin; Trader Joe's, South Pasadena, CA) cut into thirds and given every 4 days, vitamin K (KAL; Nutraceutical, Park City, IT) given every other day, choline from choline bitartrate (TwinLab, Twin Laboratories, Ronkonkoma, NY) given every other day and iron (TwinLab, Twin Laboratories) given as needed (based on weekly hematocrit measures). Beginning Week 3 of the study, folic acid supplements prepared in-house [20] were also administered to study participants consuming the low-folate diet (i.e., 133 μg of DFE/d) in order to achieve total folate intakes of 400 or 800 μg DFE/day. All supplements were consumed at the morning meals under the supervision of the investigators.

2.4. Sample collection and processing

Baseline and weekly fasting (10 h) venous blood samples were collected in serum separator gel and clot-activator tubes (SST, Vacutainer; Becton Dickinson, Rutherford, NJ) and EDTA tubes (Vacutainer), and processed as previously described [25]. The EDTA whole blood was also used for mononuclear cell isolation and subsequent DNA methylation analysis.

3. Analytical methods

Folate content of the diet. The folate content of the diet was determined before starting the study and twice during the study. Each meal, including beverage, was prepared as for the subject's consumption, blended with 150 ml of cold 0.1 mol potassium phosphate buffer/L (pH 6.3) containing 57 mmol ascorbic acid/L, dispensed into 50 ml of conical tubes and stored at −20° C. Duplicates of the blended samples were thawed, homogenized and subjected to trienzyme treatment [26] and double extraction [27]. Total food folate was then measured microbiologically [28].

Serum folate. Microtiter plate adaptation with *Lactobacillus casei* as described by Tamura [28] was used to measure serum folate in triplicate for Weeks 0, 2 and 14. The intra- and interassay coefficient of variation (CV) for the positive control (i.e., pooled serum) was <12%.

Plasma total homocysteine. High-performance liquid chromatography with fluorometric detection [29,30] was used to measure plasma total homocysteine (tHcy) concentrations in duplicate for Weeks 0, 2 and 14. The intra- and interassay CV for the positive control was <9%.

MTHFR C677T genotype. DNA for genotyping was extracted from leukocytes using a commercially available kit (DNeasy Tissue Kit; Qiagen, Valencia, CA), and determination of the MTHFR genotype was via polymerase chain reaction and *Hin*f1 restriction enzyme digestion as described by Frosst et al. [31].

Dietary and plasma choline measurements. Plasma and food betaine, choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, sphingomyelin and food lysophosphatidylcholine were determined using the method developed by Koc et al [32], with modifications based on our instrumentation [10]. Because of the large sample number, the samples were run in batches. Each batch contained all four dietary treatments, both MTHFR 677C→T genotypes, Weeks 2 and 14 for each subject, and a positive control (i.e., pooled human plasma). Baseline measurements were performed separately and included both MTHFR 677C→T genotypes. All samples were run in duplicate. The intra- and inter-CVs for each analyte measured ranged from 5% to 13% based on the pooled plasma control.

3.1. Global DNA methylation

The cytosine extension assay [33] with minor modifications [34] was used to assess global DNA methylation. DNA was extracted from mononuclear cells, and genomic DNA (0.75 μg) was digested with an excess of methylation-sensitive *Hpa*II restriction endonuclease (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. A second DNA aliquot (0.75 μg) was incubated without restriction enzyme and served as a background control. The [^{3}H]deoxyCTP incorporation into DNA was expressed as mean disintegrations per minute (dpm) per 0.75 μg of DNA. In addition to running the undigested control, we included an aliquot of lambda DNA as a positive control suitable for high levels of [^{3}H]deoxyCTP incorporation, an aliquot of lambda DNA that had been methylated completely in vitro by the action of M●SssI as a negative control suitable for low levels of [^{3}H]deoxyCTP incorporation and an aliquot of human pooled DNA as an intermediate control. Because

of the large sample number, the samples were run in batches. All batches contained the controls described above and were blinded to the investigator performing biochemical tests. For each subject within a batch, Week 0, 2 and 14 were analyzed simultaneously. All samples were run in duplicate. The interassay CVs were 7%, 15% and 9% based on the negative control, pooled plasma and the positive control, respectively.

3.2. Statistical analysis

Each dependent variable of interest (i.e., phosphatidylcholine) was tested for normality with the Shapiro–Wilks test (SAS PROC UNIVARIATE). Variables that were not normally distributed were transformed to normal by log transform, Box–Cox method (SAS PROC TRANSREG) or an exponent suggested by the Box-Cox procedure. All transformations were successful with the exception of choline during the treatment period (i.e., Week 2–14). Transformed values were used in all analysis of variance (ANOVA) procedures.

To test for baseline differences (Week 0) in the dependent variables among the various treatment groups, we performed a two-factor ANOVA (treatment group and MTHFR C677T genotype). Post hoc mean separations were accomplished using Tukey's HSD procedure. The effect of folate restriction on the measured analytes during the initial 2 weeks of the experimental period was assessed using a paired *t* test. Comparisons among the treatment groups consuming various levels of choline, betaine and folate were made by two-factor ANOVA (dietary treatment and MTHFR C677T genotype) using the Week 2 to 14 difference as the response. Because the study design was incomplete (i.e., Groups I and II did not include the MTHFR 677TT genotype) (Table 1), Type IV sums of squares were used for evaluating the factors and their interactions. All data summarization and analyses were performed by use of SAS/STAT software, version 9.1.3 of the SAS System for Windows. Differences were considered to be significant at $P<.05$. Data are presented as mean±S.E.M.

4. Results

4.1. Subject characteristics and baseline measures

The final study group was comprised of 45 women with a mean age of 24.2 years (range, 18–46 years) and a mean body mass index of 24 kg/m^2 (range, 19.2–36.6 kg/m^2). No differences ($P>.05$) were detected between the dietary treatment groups or between MTHFR C677T genotypes in age or body weight at the beginning or end of the study. A total of 9 women reported using oral contraceptives during the study period (one CC Group I, one CC Group II, one CC Group III, three CC and three TT Group IV). The dietary treatment groups were similar in regard to ethnicity/race with one to three Mexican Americans, one to two African Americans, zero to two Caucasians and one to three Asians in each group among the CC genotypes and four to seven Mexican Americans, zero African Americans, one Caucasian, one to two Asians and one Arabian in each group among the TT genotypes. Table 3 shows the baseline concentrations of the measured analytes as well as the serum folate and plasma tHcy, which were previously reported [20,21]. At baseline (Week 0), no differences ($P>.05$) in the measured analytes were detected among the dietary treatment groups (i.e., choline moieties and DNA methylation), although women with the MTHFR 677TT genotype had lower serum folate ($P=.04$) than women with the CC genotype.

4.2. Influence of folate restriction (Week 0 vs. 2) on the measured metabolites

Folate restriction during the first 2 weeks of the study was associated with decreased serum folate (28–21 nmol/L, $P<.001$), decreased plasma phosphatidylcholine (1644 to 1548 μmol/L, $P<.001$), decreased plasma sphingomyelin (495 to 447 μmol/L, $P=.0035$) and increased plasma tHcy (5.8 to 6.3 μmol/L, $P<.001$). Based on the transformed data, a statistically significant decline was also observed in plasma betaine ($P<.001$); however, numerically, there appeared to be

Table 3
Baseline (Week 0) measures of plasma concentrations (μmol/L) of betaine, choline, phosphatidylcholine, sphingomyelin and tHcy, as well as serum folate (nmol/L) and leukocyte global DNA methylation (DNA-CH_3; [^{3}H]-dCTP incorporation, dpm/0.75 μg DNA) among women with the MTHFR 677CC ($n=28$) or TT ($n=17$) genotype for each treatment group ($n=6–9$) [a]

Group	Betaine	Choline	PtdCho	Sphingomyelin	tHcy	Serum folate	DNA-CH_3 [b]
CC, I	35.7±3.5	7.4±1.1	1575±85	550±60	5.1±0.3	34.4±5.7	12 617±1132
CC, II	43.6±10.0	9.2±0.7	1575±139	506±38	5.4±0.2	31.5±4.3	13 092±1822
CC, III	36.9±6.3	8.1±1.1	1638±86	453±35	5.2±0.2	35.3±4.3	12 746±836
CC, IV	31.3±3.2	8.5±0.9	1614±175	417±31	6.0±0.4	22.9±2.6	13 766±1200
CC, ALL	36.4±2.9	8.3±0.5	1601±62	480±23	5.4±0.2	30.6±2.3	13 163±639
TT, III	40.3±2.2	7.7±0.8	1609±91	516±55	5.9±0.6	24.7±2.7	14 119±956
TT, IV	29.3±4.2	7.6±0.6	1821±88	510±22	6.7±0.4	20.2±2.7	11 909±574
TT, ALL	34.5±2.8	7.6±0.5	1721±67	513±27	6.4±0.4	22.4±2.0 [c]	12 829±592

For all other variables, no differences ($P>.05$) were detected between MTHFR C677T genotypes and/or dietary treatment groups. PtdCho, phosphatidylcholine.

[a] Values are means±S.E.M.

[b] For DNA methylation, only a subsample of the study participants was included in the analysis: $n=4–7$ for Groups I through IV; $n=20$ with the MTHFR 677CC genotype and 12 with the TT genotype.

[c] Women with the MTHFR 677TT had lower ($P=.04$) serum folate than women with the 677 CC genotype (ANOVA, Tukey's test).

Table 4
Plasma concentrations (μmol/L) and the percentage change (Week 14 vs. 2) of the measured variables among women with the MTHFR 677 CC (n=28) or TT (n=17) genotype for each treatment group (n=6–9/group) at the beginning (Week 2) and end (Week 14) of the treatment phase[a,b,c]

Group	Betaine			Choline			Phosphatidylcholine			Sphingomyelin		
	Week 2	Week 14	%Chg[d]	Week 2	Week 14	% Chg	Week 2	Week 14	%Chg[d]	Week 2	Week 14	%Chg
CC, I	39±7	40±5	2.6±13	6.0±0.8	6.4±0.6	6.7±11	1568±99	1609 ±146	2.6±9.8	519±38	487±48	−6.2±9.7
CC, II	47±9	45±6	−4.3±12	7.0±1.2	5.6±1.3	−20±20	1462±116	1464 ±95	0.1±6.5	395±20	465±33	17.7±8.3
CC, III	34±5	33±4	−2.9±13	7.7±1.5	8.3±1.4	7.8±19	1514±19	1479±40	−2.3±2.7	409±26	401±31	−2.0±7.5
CC, IV	32±4	30±5	−6.3±14	6.9±0.5	7.1±0.9	2.9±12	1502±113	1564 ±118	4.1±7.9	421±36	429±47	1.9±11
CC, ALL	37±3	36±3	−2.7±6.9	6.9±0.5	6.9±0.5	0.0±7.7	1512± 45	1530±52	1.2±3.4	435±18	442±20	1.6±4.7
TT, III	36±3	44±3	22±8	6.7±1.1	7.3±0.9	9.0±14	1452± 48	1601±89	10.3±6.1	480±34	476±36	−0.8±7.5
TT, IV	29±5	33±4	14±15	7.6±1.0	7.7±0.8	1.3±11	1736±178	1597±81	−8.0±4.6	466±28	443±32	−4.9±6.8
TT, ALL	33±3	39±3	18±9	7.2±0.7	7.5±0.6	4.2±8.6	1602±101	1599±58	−0.2±3.6	473±21	458±23	−3.2±5.0

%Chg, percentage change.
[a] Values are means±S.E.M. or %Chg±S.E.M.
[b] Total choline (mg/day), betaine (mg/day) and folate (μg/day) for Groups I, II, III and IV were 344/122/400, 344/122/800, 412/267/400, and 486/349/800 respectively.
[c] Neither choline or betaine intake influenced (P>.05) the response of the measured variables.
[d] Women with the MTHFR 677TT genotype responded differently to the dietary regimen compared to women with the CC genotype for plasma phosphatidylcholine (genotype×diet, P=.03) and betaine (time×genotype, P=.03).

little effect (36 to 35 μmol/L). A tendency toward a decline in plasma choline was also observed (8.0 to 7.0 μmol/L, P=.1). In contrast, folate restriction did not influence leukocyte global DNA methylation (13 038 to 13 011 dpm/0.75 μg DNA, P=.90). The response of the measured variables to folate restriction did not differ (P>.05) between the MTHFR 677CC and TT genotypes.

4.3. Influence of the dietary treatment (Week 2 vs. 14) on the measured metabolites

Table 4 displays the plasma concentrations of the measured choline moieties at Weeks 2 and 14 and shows the percentage change of each analyte (i.e., Week 14 vs. 2). For betaine, the response of the dietary groups to treatment was similar (P=.87). However, the response of betaine between Weeks 2 and 14 differed between women with the MTHFR CC or TT genotypes regardless of dietary treatment (time × genotype, P=.03). In this regard, a relative increase (+18%) in betaine was observed among women with the TT genotype, whereas betaine remained relatively unchanged (i.e., −2.3% change) in women with the CC genotype. For choline, the response of the dietary groups and MTHFR C677T genotypes to treatment was similar (P>.86), and no interactions (P=.98) between the diet and genotype were observed. For phosphatidylcholine, the overall change between Weeks 2 and 14 was similar among the four dietary groups (P=.51), and no main effect of the MTHFR C677T genotype was detected (P=.64). However, the response of women with the MTHFR 677TT genotype to the dietary treatment differed from the response of the CC genotype (diet×MTHFR, P=.03). For women with the MTHFR 677TT genotype, a relative increase in phosphatidylcholine (i.e., 10.3%) was observed in the group consuming less choline, betaine and folate (Group III), and a relative decrease (i.e., −8.0%) was observed in the group consuming more choline, betaine and folate (Group IV). For women with the CC genotype, there was a general lack of response to the dietary treatment (i.e., changes ranging from −2.3% to 4.1%). For sphingomyelin, the response of the dietary groups to treatment between Weeks 2 and 14 differed (P=.04). Specifically, the response of Group I (i.e., 17.7% change) differed from that of Group II (−6.2% change). However, these two groups differed only in their consumption of folate, not in their consumption of choline moieties. For the subgroup in which DNA methylation was assessed, no differences in global leukocyte DNA methylation were detected between dietary treatments (P=.23) or between the MTHFR 677CC or TT genotypes (P=.75), nor did these two factors interact to affect leukocyte global DNA methylation (P=.68, Table 5).

Table 5
Global leukocyte DNA methylation, expressed as [^{3}H]-dCTP incorporation (dpm/0.75 μg DNA), and the percentage change (Week 14 vs. 2) among women with the MTHFR 677 CC (n=20) or TT (n=12) genotype for each treatment group (n=4–7/group) at the beginning (Week 2) and end (Week 14) of the treatment phase[a,b,c]

Group	DNA methylation		
	Week 2	Week 14	%Chg
CC, I	12 591±1597	12 632±795	0.3±8.2
CC, II	13 426±1463	12 227±689	−8.9±5.5
CC, III	12 472±1345	12 599±1288	1.0±13
CC, IV	13 428±1039	14 072±1098	4.8±8.5
CC, ALL	13 069±616	13 028±512	−0.3±4.0
TT, III	13 996±1149	14 185±512	1.4±4.3
TT, IV	12 145±765	12 324±462	1.5±4.0
TT, ALL	12 916±680	13 099±429	1.4±3.4

[a] Values are means±S.E.M. or %Chg±S.E.M.
[b] Total choline (mg/day), betaine (mg/day) and folate (μg/day) for Groups I, II, III and IV were 344/122/400, 344/122/800, 412/267/400, and 486/349/800 respectively.
[c] Neither choline or betaine intake influenced (P>.05) DNA methylation response.

The influence of the dietary treatment on serum folate and plasma tHcy has been described in detail previously [20,21]. Briefly, no differences ($P>.05$) among the dietary treatment groups were detected for plasma tHcy. However, a main effect of the dietary treatment was detected for serum folate, which was higher ($P<.05$) in the 800-μg DFE/day groups (II and/or IV) relative to the 400-μg DFE/day groups (I and/or III). Furthermore, a main effect of the MTHFR C677T genotype was detected for serum folate and plasma tHcy, with the TT genotype having lower folate and higher homocysteine concentrations relative to the CC genotype. The response of the genotypes to the diets was similar for both variables (diet×genotype, $P>.05$).

5. Discussion

For most nutrients, a positive relationship exists between dietary intakes and plasma concentrations [2]. As such, plasma concentrations are generally accepted as useful indicators of status especially under conditions of controlled intake. Although declines in plasma choline and/or phosphatidylcholine in response to severe choline restriction have been reported [8], the response of plasma-free choline or its derivatives (i.e., phosphatidylcholine) to moderate changes in dietary intake is unknown.

As an extension of our previous work [20,21], this controlled feeding study sought to examine the relationship between moderate changes in dietary choline/betaine intake and blood status parameters. The influences of folate intake and the MTHFR C677T genotype on this relationship were also considered. Compliance to the study protocol was previously demonstrated [20,21] by the response of serum folate, a specific and sensitive marker of recent folate intake. In this regard, serum folate declined ($P<.001$) in response to folate restriction (Week 0–2) and was higher ($P<.05$) among women consuming 800 vs. 400 μg of DFE/day at the end of the treatment phase (Week 14).

The study findings suggest that there is no association between moderate changes in the dietary intake of choline moieties and indicators of blood choline status. Specifically, a 41% increase in total choline intake (i.e., 346 vs. 486 mg/day) and a 286% increase in betaine intake (i.e., 122 vs. 349 mg/day) did not influence plasma concentrations of the measured choline moieties (Table 4). In addition, a doubling of folate intake (400 vs. 800 μg of DFE/day) did not influence plasma levels of choline moieties, nor did choline or betaine intake appear to influence plasma levels of each other. Interestingly, however, the short period of folate restriction (i.e., 2 weeks) was associated with significant declines in plasma phosphatidylcholine and sphingomyelin, suggesting that suboptimal folate intake can perturb choline homeostasis.

Choline differs from most other vitamins in that it can be synthesized de novo. Since folate is a primary source of methyl groups, folate insufficiency is likely diminishing the availability of methyl groups used by PEMT for phosphatidylcholine synthesis. The apparent lack of an effect of choline intake on choline status under conditions of folate adequacy is consistent with data obtained from studies in rodents showing a reciprocal relationship between choline intake and PEMT activity [35,36]. Specifically, PEMT expression and activity increased under conditions of suboptimal choline intake, thereby allowing for greater choline synthesis [35,36]. Additional compensatory mechanisms aimed at maintaining choline nutriture include changes in catabolism and excretion [37]. Nonetheless, to more fully assess the relationship between choline consumption patterns and plasma concentrations in healthy populations, we needed more studies using a wider range of dietary choline intakes as well as studies in men and postmenopausal women, groups that may respond differently [38].

The lack of relationship between betaine intake and plasma levels is consistent with the prior work. Lever et al. [39–41] reported that human circulating betaine is homeostatically controlled within a distinct range (20–60 μmol/L) for each individual and is not normally affected by dietary betaine intake; plasma betaine is affected when betaine is severely restricted [38] and/or supplemented in amounts that exceed dietary intakes [42].

Data from the present study also suggest that the MTHFR C677T genotype influences the response of plasma betaine and phosphatidylcholine to the dietary regimen. The relative increase in plasma betaine among women with the MTHFR 677TT (+18%) vs. the CC (−6%) genotype was unexpected, but it may represent differences in choline oxidation rates between the MTHFR C677T genotypes (i.e., increased rates of choline oxidation in the TT vs. the CC genotype). The relative increase (i.e., 10.3%) and decrease (i.e., −8.0%) in plasma phosphatidylcholine for the groups consuming less (Group III) and more (Group IV) choline, betaine and folate, respectively, among women with the MTHFR 677TT genotype was also unexpected. It is possible that the activities of CT and/or PEMT were enhanced in the group consuming less choline, as these adaptations occur in hepatocytes obtained from choline-deficient mice and lead to increased phosphatidylcholine synthesis [36]. In women with the MTHFR 677CC genotype, phosphatidylcholine remained relatively unchanged throughout the treatment period. Taken together, these data suggest that the MTHFR C677T genotype alone and/or together with dietary methyl group intake (i.e., folate, choline and/or betaine) may influence the metabolic flux of choline moieties and is consistent with other study findings [10,43].

In our previous work [20], we utilized the U.S. Department of Agriculture (USDA) choline database [44] to estimate the choline and betaine content of the diets used in this study. For total choline, the estimated and measured amounts were relatively close (i.e., 188±21 vs. 169±11 mg/day, respectively, for Menu A and 359±25 vs. 311±18 mg/day, respectively, for Menu C). These results support earlier

work demonstrating that the laboratory analyses of choline in whole-diet aliquots match the estimated amounts calculated from the individual foods contained in the USDA database [45]. For betaine, the estimated and measured amounts were comparable in terms of the relative amounts of betaine in Menu A vs. Menu C. Specifically, the betaine content of Menu C was ~3 to 4.5 times higher than that of Menu A. However, the estimated and measured absolute amounts were markedly different (220±17 vs. 122±8.8 mg/day, respectively, for Menu A and 1020±187 vs. 349±57 mg/day, respectively, for Menu C). Since betaine was measured in whole-diet aliquots that had been stored at −20°C for approximately 3 years in the present study, we measured the betaine content of freshly prepared whole-diet aliquots consisting of the same food items as our frozen samples. Our results showed that the betaine content of freshly prepared whole-diet aliquots was similar to those of the stored samples, suggesting that storage time/conditions had minimal effect on betaine content. The possibility that measurements of betaine from individual foods yield different results than measurement from whole-diet aliquots is currently under investigation in our laboratory.

DNA methylation is of interest to scientists largely because of its association with transcriptional silencing of genes, genomic stability and/or cancer risk (reviewed in Ref. [46]). Early studies performed in postmenopausal women reported significant declines in global leukocyte DNA methylation in response to folate restriction [47,48] and increases in response to folate treatment [47]. However, subsequent studies reported no influence of folate restriction and/or treatment on global leukocyte DNA methylation in premenopausal women [49–51], although interactions with the MTHFR C677T genotype were observed [49,50]. The findings of the present study confirm the lack of an effect of folate intake on global leukocyte DNA methylation in well-nourished premenopausal women and extend these observations to choline/betaine.

In conclusion, data from the present study suggest that blood status indicators of choline may be poor predictors of dietary intake possibly due to the engagement of compensatory mechanisms. If future work confirms the lack of relationship between moderate changes in dietary intake and blood levels, careful interpretation of data generated by studies assessing the relationship between blood levels of choline/betaine and disease risk is needed. In this regard, a lack of difference in plasma levels of choline moieties between an experimental and control group should not necessarily be construed as evidence of a condition that is unrelated to choline. In such cases, the dietary intake of choline moieties may be more informative of the relationship between choline and disease risk than blood levels. While severe choline deficiency may be assessed through indicators of liver and muscle dysfunction [8,17] and/or DNA damage [16], a sensitive and specific biomarker of choline intake for populations that are consuming normal but varied diets is sorely needed.

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