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Methylenetetrahydrofolate reductase 677C→T polymorphism affects DNA methylation in response to controlled folate intake in young women

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Abstract

DNA methylation is critical for normal genomic structure and function and is dependent on adequate folate status. A polymorphism (677C→T) in a key folate enzyme, methylenetetrahydrofolate reductase (MTHFR), may impair DNA methylation when folate intake is inadequate and may increase the risk of reproductive abnormalities. The present study was designed to evaluate the effect of the MTHFR 677C→T polymorphism on changes in global DNA methylation in young women consuming a low folate diet followed by repletion with the current Recommended Dietary Allowance (RDA). Women (age 20–30 years) with the TT (variant; $n = 19$) or CC ($n = 22$) genotype for the MTHFR 677C→T polymorphism participated in a folate depletion-repletion study (7 weeks, 115 μg DFE/day; 7 weeks, 400 μg DFE/day). DNA methylation was measured at baseline, week 7, and week 14 using a [^{3}H]methyl acceptance assay and a novel liquid chromatography tandem mass spectrometry assay of the DNA bases methylcytosine and cytosine. [^{3}H]Methyl group acceptance tended to increase ($P = 0.08$) during depletion in all subjects, indicative of a decrease in global DNA methylation. During repletion, the raw change and the percent change in the methylcytosine/total cytosine ratio increased ($P = 0.03$ and $P = 0.04$, respectively) only in the subjects with the TT genotype. Moderate folate depletion in young women may cause a decrease in overall DNA methylation. The response to folate repletion suggests that following folate depletion women with the MTHFR 677 TT genotype have a greater increase in DNA methylation with folate repletion than women with the CC genotype.

Keywords: Folic acid; MTHFR; Genotype; Young women; Feeding study; DNA methylation

1. Introduction

Folate functions in the formation of S-adenosylmethionine (SAM), the methylating agent for DNA methylation, a key epigenetic modification critical for genome stability [1], gene expression [2], and thus normal development [3,4]. To ensure maintenance of normal DNA methylation, it is important to understand the interrelationship among folate status, genetic factors that may impair the synthesis of folate coenzymes, and the methyl group donor SAM. SAM synthesis is dependent in part on the availability of 5-methyltetrahydrofolate (5-methylTHF), which may be limited because of dietary folate restriction or reduced activity of methylenetetrahydrofolate reductase (MTHFR), the enzyme that converts 5,10-methyleneTHF to 5-methylTHF. MTHFR activity is affected negatively by a common genetic variant in the gene that codes for MTHFR. A C→T base transition at base pair 677 causes an alanine to valine substitution [5], which impairs stability as a result of less avid binding of FAD to the variant form of the MTHFR enzyme under conditions of low folate concentration [6]. The MTHFR 677C→T polymorphism affects a large percentage of the population with an estimated frequency of ~12% for the homozygous (TT) genotype with considerable variation among different ethnic groups [7,8]. The potential for the MTHFR 677C→T transition to reduce global DNA methylation when coupled with poor folate status has been evaluated in observational, population-based

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studies [9,10]. There are, however, no previous reports of the effect of the MTHFR 677C→T polymorphism on global DNA methylation in response to controlled dietary folate intake. Because the intake of other nutrients involved in one carbon metabolism may affect DNA methylation, it is important to evaluate the effect of dietary folate intake alone in individuals with the TT or CC MTHFR 677C→T genotype under controlled dietary conditions. The responsiveness of DNA methylation to changes in folate status under controlled metabolic conditions has not been previously reported for women of reproductive age.

Inadequate folate status has been linked to abnormal fetal growth and development [11] as well as pregnancy complications [12]. In addition, it is well established that periconceptional folic acid supplementation significantly reduces the risk for neural tube defects [13–15]. Although the mechanism behind the relationship between folate status and pregnancy outcome is unknown, one possibility could be the role of folate in SAM synthesis. A decrease in folate status may affect SAM availability, resulting in decreased DNA methylation [16]. Reduced global DNA methylation has been associated with impaired fetal development and viability [3,4], emphasizing the crucial need to maintain normal DNA methylation in women of reproductive age.

The potential for impaired folate status coupled with the MTHFR 677 TT genotype to negatively influence DNA methylation [9,10] provided the incentive for this study. We investigated the differences in response to folate depletion and repletion with the current RDA in leukocyte DNA methylation between women of childbearing age (20–30 years) with the TT or CC MTHFR 677C→T genotype. This is the first study in young women in which changes in DNA methylation were evaluated in response to controlled folate intake.

2. Methods and materials

2.1. Subjects

Young (age 20–30 years), healthy, nonpregnant women were recruited and screened for this study. Only women with the TT or CC MTHFR 677 genotype were eligible. Exclusion criteria were chronic use of alcohol or tobacco products; use of medications including oral contraceptives; recent use of vitamin–mineral supplements; history of chronic disease or major surgery; body weight >120% of ideal; and abnormal blood chemistry profile. Serum and red blood cell folate, plasma vitamin B_{12}, pyridoxal phosphate, and homocysteine concentrations were normal at baseline for all subjects (i.e., ≥7 nmol/L, ≥317 nmol/L, ≥125 pmol/L, ≥20 nmol/L, and ≤14 μmol/L, respectively). The ethnicity of the subjects was reported to be 93% non-Hispanic white, and 7% non-Hispanic African American. A total of 41 women (19 TT, 22 CC) completed the 7-week depletion phase, and 20 women (10 TT, 10 CC) completed the entire 14-week depletion–repletion protocol as previously described [17]. The University of Florida Institutional Review Board approved the study, and each subject provided written informed consent.

2.2. Experimental design and diet

The 98-day experimental protocol was divided into two consecutive 7-week periods during which time the subjects consumed a depletion diet followed by a repletion diet. The low-folate diet consumed by the subjects provided 115 ± 20 μg dietary folate equivalents (DFE)/day during the first 7 weeks of the study. During repletion, the subjects consumed the depletion diet plus folic acid, which provided 400 μg DFE/day (115 + 285 μg DFE [168 μg folic acid × 1.7 = 285 μg DFE]) [18]. The diet consisted of conventional foods, and all food items containing cereal-grain products were prepared onsite using unenriched flour purchased from Kansas State University (Manhattan, KS). Details of preparation of the low-folate diet have been described previously [17]. The Minnesota Nutrient Data System (version 4.03; Nutrition Coordinating Center at the University of Minnesota; Minneapolis, MN) was used to estimate the nutrient content of the diet with the exception of folate, which was analyzed directly using a microbiological procedure [19,20] following a trienzyme extraction [21]. The diet provided an average energy intake of 9.87 MJ (2358 kilocalories/day; 63% carbohydrate, 11% protein, and 26% fat). A vitamin–mineral supplement was custom-formulated (Westlab Pharmacy; Gainesville, FL) to provide the RDA for all nutrients with the exception of folate and choline. The dietary choline content (285 mg/day), which was determined by direct analysis [22], provided 67% of the Adequate Intake [23]. A separate calcium supplement (Citracal, Mission Pharmacal; San Antonio, TX) (200 mg of calcium as calcium citrate) also was consumed daily to provide the RDA. The body weights of the subjects were maintained within 5% of baseline by modifying the intake of foods with relatively little or no nutrient value aside from calories (i.e., margarine, candy, Jello® gelatin dessert, and sweetened beverages).

2.3. Specimen collection and processing

Leukocytes for DNA extraction were collected at baseline, week 7, and week 14 by using whole blood collected in tubes with EDTA (Vacutainer, Becton Dickinson, Rutherford, NJ). Iced blood was centrifuged at 2000 × *g* for 30 minutes. After removal of plasma, 500 μL of leukocytes were removed and stored at −30°C until analysis. A commercially available kit (Aquapure, Bio-Rad Laboratories, Hercules, CA) was used to extract genomic DNA from the leukocyte layer.

2.4. Determination of MTHFR 677C→T genotype

Polymerase chain reaction followed by restriction enzyme analysis with *Hin*f1 was used to determine the presence of the MTHFR 677C→T allele [5].

2.5. DNA methylation analyses

Two methods were used to determine DNA methylation, [^{3}H]methyl group incorporation and liquid chromatography tandem mass spectrometry, as the [^{3}H]methyl acceptance assay is considered to be semiquantitative [10]. The first method was a methyl acceptance assay based on the ability of DNA to accept methyl groups when extracts are incubated with [^{3}H-methyl] SAM in the presence of *Sss*I bacterial methylase. The method used was a modification of the original method of Balaghi and Wagner [24] as previously described by our research group [25]. Although this method usually has high variability, intra- and inter-assay coefficients of variation (CV) for this study were 10% and 7%, respectively.

The second approach to estimate global DNA methylation involved the measurement of deoxymethylcytidine (mCyt) and deoxycytidine (Cyt) in enzymatically hydrolyzed DNA samples by liquid chromatography tandem mass spectrometry [26]. Before analysis, DNA was hydrolyzed enzymatically using a modified method of Crain [27]. To hydrolyze the DNA, 1 μg of denatured DNA for each subject at weeks 0, 7, and 14 was mixed with 5 μL of 0.1 mol/L ammonium acetate (pH 5.3) and incubated with 6 U (3 μL) P_1 nuclease (Sigma Chemical Co., St. Louis, MO) at 50°C to nick various phosphate bonds of the DNA. Quantities of 6 μL of 1 mol/L ammonium bicarbonate (pH 7.75) were incubated with 3.25 mU (2.5 μL) phosphodiesterase (Sigma Chemical Co.) at 37°C for 2 hours to complete the digestion of the phosphate backbone. Finally, samples were incubated with 0.5 U (2.5 μL) alkaline phosphatase (Sigma Chemical Co.) at 37°C for 1 hour to cleave the sugar-phosphate bonds leaving only the nucleosides. Samples were then chromatographed on a 5-μ Discovery C18 column (100 × 4.6 mm; Supelco; Bellefonte, PA) eluted with a 50-mmol/L ammonium formate (solvent A): methanol (solvent B) gradient. The analysis was started using an eluent of 95% A and 5% B for 4 minutes, followed by a gradient from 5% B to 65% B over 4 minutes at a flow rate of 0.6 mL/min, then maintained at 65% solvent B for 3 minutes, reversed to the original composition (95% solvent A, 5% solvent B) over 1 minute, and re-equilibrated at that composition for 2 minutes. mCyt and Cyt had retention times of 6.99 and 3.96 minutes, respectively. Mass spectrometry was performed in the selective reaction monitoring (SRM) mode using a Finnigan TSQ 7000 (Thermo Finnigan, San Jose, CA) mass spectrometer. SRM monitoring of the ion transitions from m/z 241.6 (protonated molecular ion) to 125.8 and 227.6 to 111.8 was performed. To quantify the mCyt and Cyt in the samples, an external standard consisting of 6% mCyt was prepared by adding 23.5 μL deoxymethylcytidine (Sigma Chemical Co.) at a concentration of 10 ng/mL and 15 μL deoxycytidine (Acros Organics, Fisher Scientific, Pittsburg, PA) at a concentration of 100 ng/mL to 961.5 μL sterile filtered water. Sample mCyt and Cyt concentrations were quantified by comparing their peak areas to standard curves calculated from the peak areas of deoxycytidine (18.75–150 ng/mL; $r = 0.99$) and deoxymethylcytidine (0.29–2.35 ng/mL; $r = 0.99$). Ratios were calculated by dividing the mCyt concentration by the total Cyt (tCyt) concentration (mCyt + Cyt) and expressed as the mCyt/tCyt ratio. The intra-assay CV for this method was 4%.

2.6. Statistical methods

One-way analysis of variance (ANOVA) was used to test for differences in all indices including serum and red blood cell folate, plasma homocysteine, and DNA methylation ([^{3}H]methyl group acceptance and mCyt/tCyt ratio) at week 0. To account for subject variability on entry into the study, analysis of covariance (ANCOVA) was used to evaluate genotype group differences for all indices (i.e., serum and red blood cell folate and plasma homocysteine concentrations and DNA methylation) at weeks 7 and 14 with adjustment for baseline or week 7 values, respectively. Least-square means were used to describe the magnitude of the differences between each group and were evaluated at the average covariate value (week 0 for weeks 7 and 14 analyses, week 7 for week 14 analysis). As a secondary analysis, ANOVA was performed on the raw and percent change values from week 0 to week 7, week 7 to week 14, and week 0 to week 14 for DNA methylation indices. To calculate overall mean raw or percent change over the depletion (week 0 to week 7) and repletion (week 7 to week 14) periods, the raw or percent change score for each subject was calculated and the mean of these values was determined.

Pearson correlations were used to evaluate the strength of the relationships between the dependent variables at each point in time (weeks 0, 7, and 14). A sign test for proportion of trends analysis [28] was used to compare the expected and observed combination of trends for DNA methylation indicators and plasma homocysteine, serum folate, and red blood cell folate concentrations over the depletion and repletion periods. To evaluate the strength of the relationship between each status indicator over the depletion and repletion phases, regression and Pearson correlation techniques were used. Specifically, linear regression was used to determine the slope parameters for each variable for each subject. Correlations of combinations of these coefficients were then determined to assess the magnitude of the relationship.

To compare the combined trends of methylation and plasma homocysteine, and serum and red blood cell folate, the expected and observed proportions of trends from week

Table 1
DNA [^{3}H]methyl group acceptance at baseline, postdepletion, and postrepletion for all subjects and by MTHFR 677C→T genotype

	Genotype		
	CC	TT	Overall
Baseline	470 ± 80 (n = 22)	500 ± 70 (n = 19)	490 ± 70 (n = 41)
Postdepletion	490 ± 80 (n = 22)	520 ± 70 (n = 19)	500 ± 80 (n = 41)
Postrepletion	550 ± 150 (n = 10)	470 ± 70 (n = 10)	510 ± 120 (n = 20)

Values are mean ± SD, [^{3}H]methyl group acceptance (dpm × 10^2/0.5 μg DNA).
MTHFR = methylenetetrahydrofolate reductase.

0 to week 7 and from week 7 to week 14 were evaluated. Specifically, regression was used to determine the slope for each subject's depletion (week 0 to week 7) and repletion (week 7 to week 14) phase for methylation, plasma homocysteine, and serum and red blood cell folate. The signs of the slopes (positive or negative) were then tallied for each trial phase and this observed proportion was tested against the proportion that would be expected by chance alone (0.25) using a sign test.

Statistical methods using ANOVA models with main factors for genotype and serum folate status (median status and depletion status separately) as well as the interaction term were used to evaluate potential differences in [^{3}H]methyl group acceptance, mCyt/tCyt ratio, and plasma homocysteine concentration at each time point (week 0, week 7, and week 14). In addition, one-way ANOVA was used as a secondary analysis to evaluate potential differences in [^{3}H]methyl group acceptance, mCyt/tCyt ratio, and plasma homocysteine concentration by serum folate status (median status and depletion status separately) within each genotype group (TT and CC separately) at each point in time (weeks 0, 7, and 14). For these analyses, the α value was adjusted as α/n, where n = the number of comparisons. The adjusted α was therefore 0.05/12 = 0.004. For all other comparisons, the α level was set a priori to 0.05. All statistics were computed using SAS version 8.00 (SAS Institute, Cary, NC).

3. Results

3.1. Serum and red blood cell folate and plasma homocysteine concentrations

The results for serum and red blood cell folate and plasma homocysteine concentrations at baseline, postdepletion, and postrepletion by genotype and overall have been previously described [17].

3.2. DNA methylation

No differences were detected between the means ± SD for [^{3}H]methyl group acceptance (Table 1) at weeks 0, 7, or 14 between genotype groups ($P > 0.05$). The overall mean percent change in [^{3}H]methyl group acceptance tended (P = 0.08) to increase from baseline to postdepletion (5% ± 19%), suggesting a trend for DNA methylation to decrease in all subjects during depletion (weeks 0–7) (Fig. 1). In addition, although both genotype groups had nonsignificant percent increases in [^{3}H]methyl group acceptance during depletion (Fig. 1), only subjects with the TT genotype had a decrease in [^{3}H]methyl group acceptance during repletion (Fig. 1). This is in contrast to subjects with the CC genotype who had an increase during repletion. Over the depletion and repletion phases of the study, an inverse relationship between serum folate or red blood cell folate concentrations and [^{3}H]methyl group acceptance was expected (i.e., it was expected that serum folate concentrations would decrease and [^{3}H]methyl group acceptance would increase with folate depletion). Conversely, a direct relationship between plasma homocysteine concentration and [^{3}H]methyl group acceptance was expected in response to folate depletion and repletion. Based on the sign test for trend analysis, an inverse relationship between serum folate concentration and [^{3}H]methyl group acceptance was observed in 68% and 55% of the TT (P = 0.0004) and CC (P = 0.006) genotype groups, respectively, over the depletion phase. An inverse relationship also was observed in 68% of the TT and 50% of the CC genotype groups (P = 0.0005 and P = 0.02, respectively) between red blood cell folate concentration and

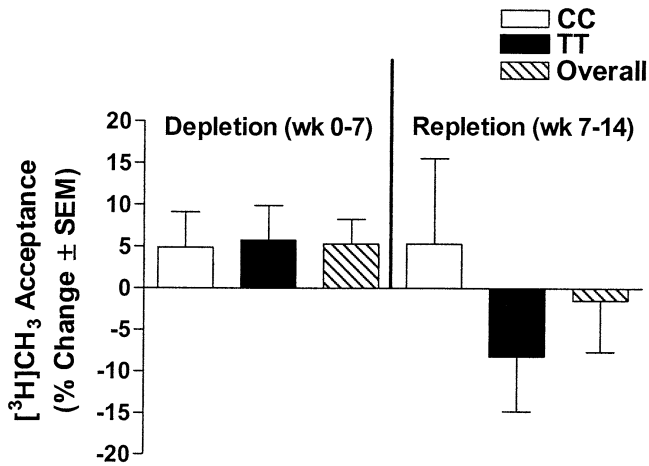

Fig. 1. Percent (%) change in [^{3}H]methyl group acceptance for all subjects and by methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype during depletion and repletion.

Table 2
Percentage (%) of methylated cytosine at baseline, postdepletion, and postrepletion for all subjects and by MTHFR 677C→T genotype as measured by liquid chromatography tandem mass spectrometry

	Genotype		
	CC	TT	Overall
Baseline	4.7 ± 0.5 (n = 22)	4.8 ± 1.0 (n = 19)	4.7 ± 0.8 (n = 41)
Post-depletion	4.6 ± 0.7 (n = 22)	4.6 ± 0.7 (n = 19)	4.6 ± 0.7 (n = 41)
Post-repletion	4.6 ± 0.4 (n = 10)	4.5 ± 0.5 (n = 10)	4.6 ± 0.5 (n = 20)

Values are mean ± SD, % of methylated cytosine.
MTHFR = methylenetetrahydrofolate reductase.

[^{3}H]methyl group acceptance during depletion. A direct relationship was observed between plasma homocysteine concentration and [^{3}H]methyl group acceptance in 68% and 55% of the TT (P = 0.0005) and CC (P = 0.006) genotype groups, respectively.

In response to folate repletion, the inverse relationship between serum folate and [^{3}H]methyl group acceptance was observed in 60% of both the subjects with the TT and CC genotypes (P = 0.03), whereas there was a trend (P = 0.08) for a direct relationship between plasma homocysteine and [^{3}H]methyl group acceptance in 50% of both genotype groups (sign test for trend). Based on the Pearson correlation, plasma homocysteine concentration tended (P = 0.05) to be correlated positively (r = 0.44) with [^{3}H]methyl group acceptance in subjects with the CC genotype only during the repletion phase. In addition, a significant inverse relationship was found between red blood cell folate concentrations and [^{3}H]methyl group acceptance for all subjects (r = −0.37; P = 0.02) postdepletion based on Pearson correlation analysis.

A significant difference in the percentage of methylated cytosine (± SD) was not detected ($P > 0.05$) at weeks 0, 7, or 14 among genotype groups (Table 2). At baseline, a significant (P = 0.03) interaction between mCyt/tCyt ratio and folate status was detected in all subjects. Specifically, in subjects with a serum folate concentration below the median (40 nmol/L) the mCyt/tCyt ratio tended to be lower (P = 0.01) than in subjects with serum folate concentrations above the median based on an adjusted α of 0.004.

In response to depletion, there were direct relationships ($P < 0.05$) between the mCyt/tCyt ratio and both serum and red blood cell folate concentrations in the CC genotype group only (50% and 45%, respectively, sign test for trend). Plasma homocysteine concentration was inversely (P = 0.02) related to the mCyt/tCyt ratio in 50% of the CC genotype group only.

The positive mean percent change in the mCyt/tCyt ratio in response to folate repletion (weeks 7–14) was significant (P = 0.04) for subjects with the TT genotype with no change ($P > 0.05$) observed for subjects with the CC genotype (Fig. 2). The positive raw change in the mCyt/tCyt ratio also was significant (P = 0.03) during repletion for subjects with the TT but not the CC genotype.

4. Discussion

The primary objective of this metabolic study was to evaluate the influence of the MTHFR 677C→T variant on DNA methylation response to a low-folate diet followed by repletion with the current folate RDA for nonpregnant women using a controlled feeding protocol. Previous studies have been primarily observational, population-based studies in which the combined effect of the MTHFR polymorphism and folate status was evaluated. The women in the current study had baseline folate values well above what is considered to be deficient (<13.6 nmol/L) [29]. In addition, none of the women were severely deficient (<7 nmol/L) [29] postdepletion, which is in contrast to our previous study of elderly women following the same protocol [30]. Repletion with the current RDA for folate did not restore blood folate values back to baseline in the young women. These results indicate that these women may have been consuming large quantities of folic acid in fortified foods since the United States Food and Drug Administration mandated folic acid fortification in 1998 [31]. Perhaps longer depletion and repletion phases may have resulted in more significant changes in folate status. The negative in-

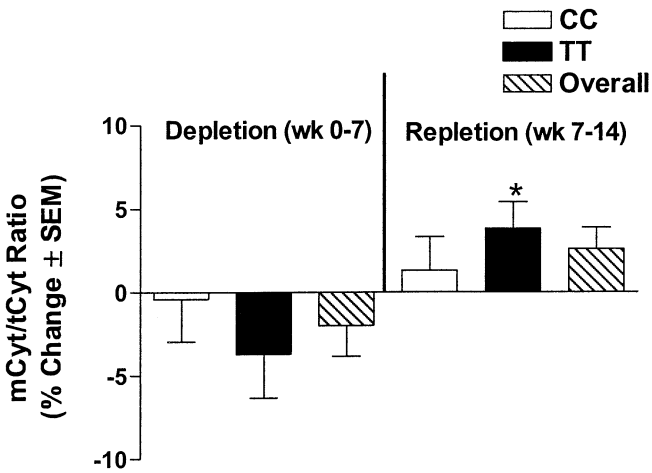

Fig. 2. Percent (%) change in mCyt/tCyt ratio for all subjects and by methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype during depletion and repletion, as measured by liquid chromatography–tandem mass spectrometry. *Significant percent change during repletion for subjects with the TT genotype ($P < 0.05$).

fluence of the MTHFR 677C→T variant when coupled with poor folate status on genomic DNA methylation has been reported in U.S. [9] and European [10] population-based studies. In contrast to these observational studies, metabolic protocols such as that used in the present study provide the opportunity to control dietary intake of folate and other nutrients that may significantly influence plasma homocysteine concentration and DNA methylation in individuals with the MTHFR polymorphism.

DNA methylation has been used successfully as a biomarker of response to controlled folate intake in two previously published studies [25,32]. Jacob et al. [32] found a significant decrease in global DNA methylation in postmenopausal women after consumption of a low-folate diet for 91 days. In a previous study with elderly women by our research group, global DNA methylation significantly decreased in response to a low-folate diet for 7 weeks [25]. In contrast, a significant decrease in DNA methylation was not detected after a 7-week folate depletion in the young women in the present study. This difference between our previous folate-controlled feeding study in which the same low folate diet was consumed may be explained by the fact that 21% of the elderly women in the previous study had severe deficiencies (<7 nmol/L) by week 7 [30] compared to none of the young women in the present study. The prestudy folate status for both groups were comparable (serum folate 46 vs 47 nmol/L), suggesting that factors other than prior folate intake were involved in the observed age-specific differences in DNA methylation response. An important difference between the women in the present study (age 20–30 years) and earlier studies in elderly women (>60 years) [25] is the age difference. An effect of age on folate status has been demonstrated in an animal model [33]. Specific age-dependent effects on DNA methylation have been reported as previously reviewed, and include endogenous (i.e., altered expression of DNA methyltransferases) and exogenous (i.e. medications and diet) factors [35]. Additional studies are needed to further characterize the potential age-related effects on DNA methylation.

The main objective of the present study was to investigate genotypic differences in DNA methylation between subjects with the TT and CC MTHFR 677C→T genotypes. Although 59% of subjects with the TT genotype had low folate status (<13.6 nmol/L) at week 7 compared to only 15% of subjects with the CC genotype [17], a significant difference in labeled methyl group acceptance between genotypes was not detected at any time point in the present study. This is in contrast to the findings of Stern et al. [9], who reported significantly higher labeled methyl group incorporation in individuals with the TT compared to the CC genotype. The inclusion by Stern et al. [9] of older subjects (age range 25–75 years) may have contributed to the significant difference between genotypes, although this conclusion cannot be made definitively because methylation status was not evaluated by age. The study by Stern et al. [9] may have been conducted before folic acid fortification, since the baseline serum folate concentration of subjects in the present study was more than 2-fold higher than that of the subjects in the Stern et al. [9] study for both the TT and CC genotypes (42 vs 21 nmol/L and 52 vs 24 nmol/L, respectively).

The trend for an increase in DNA [^{3}H]methyl group acceptance, indicative of diminished DNA methylation, was consistent with our previously reported significant decreases in this population of young women in serum and red blood cell folate concentrations and increase in plasma homocysteine concentration during depletion [17]. Also in the young women, a significant inverse correlation was detected between labeled methyl group acceptance in subjects with the TT genotype and red blood cell folate concentrations postdepletion, indicating an effect of folate status on DNA methylation in subjects with this genotype.

In an observational study of an Italian population, a liquid chromatography–mass spectrometry method was used to compare the DNA methylcytosine content in subjects with the TT and CC genotypes for the MTHFR 677C→T polymorphism [10]. Significantly less DNA methylation in subjects with the TT genotype compared to those with the CC genotype was reported in conjunction with significantly lower serum folate concentration [10]. In contrast, a significant difference in DNA methylation between genotypes was not detected in the present study of young women in the U.S. using a liquid chromatography–tandem mass spectrometry method. These conflicting findings may be a result of consumption of folic acid fortified foods by our population compared to the Italian population, where lower folate intakes were chronically consumed. Differences in folate status due to consumption of fortified foods in the U.S. compared to European countries are illustrated by the fact that the serum folate concentrations in the Italian population were much lower than those in the present study (~11.6 vs 47.2 nmol/L). The significant increase in DNA methylation in subjects in this study with the TT genotype during repletion is consistent with the results by Friso et al. [10], who found significantly more DNA methylation in subjects with the TT genotype with plasma folate values in the highest tertile compared to the lowest tertile. This indicates that as folate status is improved, DNA methylation is enhanced. In the present study, a change in DNA methylation during repletion was not detected in subjects with the CC genotype, a finding that is consistent with data by Friso et al. [10], who did not find a significant difference in DNA methylation among tertiles of plasma folate in subjects with the CC genotype. These data suggest that individuals with the TT genotype may have a greater increase in DNA methylation with folate repletion following a low folate intake than individuals with the CC genotype.

In summary, the results of the present study indicate that DNA methylation in young women may be affected by inadequate folate intake. In addition, women with the homozygous genotype (TT) for the MTHFR 677C→T polymorphism may benefit more from folate supplementation

(i.e., improved methylation status) than women with the CC genotype. Future research is needed to delineate the effect of chronic consumption of low-folate diets on DNA methylation in individuals with the MTHFR 677C→T polymorphism. In addition, more research is warranted to investigate the potential influence of aging on DNA methylation.

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