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RESEARCH ARTICLES

Additional food folate derived exclusively from natural sources improves folate status in young women with the MTHFR 677 CC or TT genotype☆

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Abstract

The effectiveness of additional food folate in improving folate status in humans is uncertain particularly in people with the common genetic variant (677 C→T) in the methylenetetrahydrofolate reductase (MTHFR) gene. To examine the effect of a doubling of food folate consumption on folate status response variables, women ($n=32$; 18–46 years) with the MTHFR 677 CC or TT genotype consumed either 400 ($n=15$; 7 CC and 8 TT) or 800 ($n=17$; 8 CC and 9 TT) μg/day of dietary folate equivalents (DFE) derived exclusively from naturally occurring food folate for 12 weeks. A repeated measures two-factor ANOVA was used to examine the effect of the dietary treatment, the MTHFR C677T genotype and their interactions on serum folate, RBC folate and plasma total homocysteine (tHcy) during the last 3 weeks of the study. Consumption of 800 μg DFE/day resulted in serum folate concentrations that were 67% ($P=.005$) higher than consumption of 400 μg DFE/day (18.6±2.9 vs. 31.0±2.7 nmol/L, respectively) and RBC folate concentrations that were 33% ($P=.001$) higher (1172±75 vs. 1559±70 nmol/L, respectively). Serum folate ($P=.065$) and RBC folate ($P=.022$) concentrations were lower and plasma tHcy was higher ($P=.039$) in women with the MTHFR 677 TT genotype relative to the CC genotype. However, no genotype by dietary treatment interaction was detected. These data suggest that a doubling of food folate intake will lead to marked improvements in folate status in women with the MTHFR 677 CC or TT genotype.

Keywords: Folate; Food folate; Dietary folate; Methylenetetrahydrofolate reductase (MTHFR); Homocysteine; Folate status

1. Introduction

Suboptimal folate status is associated with vascular disease, certain cancers, impaired cognition, pregnancy complications and fetal malformations including neural tube defects or NTDs [1,2]. In an effort to increase folate consumption among women of childbearing age and reduce the incidence of NTD-affected pregnancies, the United States and a limited number of other countries (i.e., Canada and Chile) mandated folic acid fortification of staple food items [3]. Folic acid is the oxidized monoglutamate form of folate and is about twice as bioavailable as naturally occurring food folates [4,5], most of which exist in the reduced form as a mixture of mono- and polyglutamates [6]. In the United States, folic acid fortification has resulted in substantial improvements in the folate status of women of childbearing age [7,8] and has virtually eliminated folate deficiency in American adults [9]. However, most of the world's population is not exposed to widespread folic acid fortification and, therefore, must rely on supplemental folic acid or increased consumption of foods with a high content of natural folate to optimize their folate intake/status.

A valid concern of relying exclusively on increased consumption of folate-rich foods to optimize folate status is the efficacy of naturally occurring food folate. Studies providing about onefold additional food folate report percent changes ranging from +15% to 85% for serum folate, nonsignificant to +18% for RBC folate and nonsignificant to −16% for plasma total homocysteine (tHcy) [10–16]. The

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short-term nature (i.e., ≤5 weeks) of these studies and/or the lack of controlled folate intake may have contributed to the poor response and/or to the large amount of variability observed overall.

The question as to whether additional food folate is effective in improving folate status may be especially critical in people with genetic variations that alter folate requirements. A common polymorphism in methylenetetrahydrofolate reductase (MTHFR), a key regulatory gene in folate metabolism, occurs at nucleotide 677 and involves a cytosine-to-thymine transition [17]. People with the MTHFR 677 TT genotype have lower blood folate and higher plasma tHcy concentrations than those with the CC genotype [18], particularly under conditions of folate insufficiency, and are at greater risk for NTD-affected pregnancies and other pathologies [19,20]. While folic acid administration is efficacious in persons with the MTHFR 677 TT genotype [21,22], only limited data are available on the usefulness of additional food folate in improving their folate nutriture.

The objective of this study was to examine the effect of a doubling of food folate consumption on serum folate, RBC folate and plasma tHcy in women with the MTHFR 677 CC ($n=15$) or TT ($n=17$) genotype under conditions of controlled folate intakes.

2. Methods

2.1. Subjects

Healthy female subjects aged 18–46 years were recruited between January 2002 and April 2003 from the staff and student population at Cal Poly Pomona University as well as the surrounding community. The subjects were eligible for inclusion if the following criteria were fulfilled: no history of vascular, gastrointestinal, renal or hepatic disease; normal blood glucose and lipid concentrations; normal blood chemistry; MTHFR 677 CC or TT genotype; nonsmoker; not taking drugs known to interfere with folate metabolism; not a current user (within the past 3 months) of supplemental vitamins/and or minerals; not pregnant or planning a pregnancy; and not lactating. The study was approved by the Institutional Review Board of California State Polytechnic University, Pomona, and informed consent was obtained from each participant.

2.2. Experimental design

This was a 14-week controlled folate feeding study. Subjects ($n=32$) with the MTHFR 677 CC or TT genotype consumed a low folate diet (135 μg/day food folate) for 2 weeks followed by randomization to 400 ($n=15$; 7 CC and

Table 1
Five-day cycle menus consumed by women assigned to 400 μg DFE/day[a–e]

Day 1	Day 2	Day 3	Day 4	Day 5
Breakfast				
Whole wheat waffle	Shredded wheat cereal	Whole wheat waffle	Granola cereal	2 muffins
Strawberries, frozen (35 g)	Raisins	Boysenberries, frozen (35 g)	Raisins	Strawberries, frozen (30 g)
Syrup	Hazelnuts (15 g)	Syrup	Almonds (10 g)	Boysenberry yogurt (227 g)
Spiced applesauce	Strawberry yogurt (227 g)	Cranapple juice	Peach yogurt (227 g)	Crangrape juice
Cranapple juice	Crangrape juice		Crangrape juice	
Lunch				
Turkey	Ham	Tuna	Turkey	Chicken salad
Swiss cheese	Cheddar cheese	Whole wheat pita	Swiss cheese	American cheese
Whole wheat pita	Whole wheat bread	Fruit cocktail, canned	Whole wheat bread	Whole wheat pita
Fruit cocktail, canned	Fruit cocktail, canned	Soda	Fruit cocktail, canned	Peaches, canned
Broccoli, frozen (60 g)	Soda		Soda	Soda
Kidney beans, canned (30 g)				
Soda				
Dinner				
Tuna casserole	Chicken enchilada with spinach, frozen (30 g)	Pizza with ham and spinach, frozen (60 g)	Spaghetti with spinach, frozen (100 g)	Chicken breast
Asparagus, canned (180 g)	Green beans, frozen (120 g)	Broccoli, frozen (120 g)	Asparagus, canned (60 g)	Barbeque sauce
Lemon juice	Refried beans, canned (120 g)	Cauliflower, frozen (60 g)	Lemon juice	Brown rice
Cranapple juice	Strawberry (55 g) crisp	Cranapple juice	Whole wheat biscuit	Spinach, frozen (100 g)
	Cranapple juice		Cranapple juice	Strawberry (55 g) crisp
				Cranapple juice
Snacks				
Rice cake	Popcorn	S'mores granola bar	No snack	Oat/honey granola bar

[a] Menus analyzed by trienzyme folate extraction method [25,26] provided a mean of 426 μg DFE/day.
[b] Menu items prepared with unfortified flour were strawberry muffins and pizza dough. Unfortified flour was also used in sauces for tuna casserole and chicken enchiladas and for the strawberry crisp topping.
[c] Menu items prepared with whole-wheat flour and unfortified flour (1:1) were waffles and biscuits.
[d] For folate-rich menu items, gram amounts are given in parentheses.
[e] Foods allowed in unlimited amounts included soda, jello and whipped topping; also two cups of coffee or tea were allowed per day.

8 TT) or 800 ($n=17$; 8 CC and 9 TT) μg DFE/day derived exclusively from food folate for 12 weeks. No folic acid-fortified foods were utilized at any time throughout the study duration. The purpose of folate restriction was to acclimatize the subjects to the study protocol and aid in assessing the specificity and sensitivity of the measured variables to folate intake. It also served to decrease the variability observed at baseline within and between treatment groups. Treatment with 400 and 800 μg DFE/day allowed for investigation of a doubling of folate intake on folate status parameters.

2.3. Diet and supplements

The folate-restricted diet utilized during the first 2 weeks was a slightly modified version of the menus previously described [18] and provided 133±18 μg/day (mean±S.D.) food folate. For the 133 μg/day treatment diet, certain foods were thrice boiled in order to reduce folate content, and unfortified flour (Kansas State University, Manhattan, KS) was used to make flour-containing food items [18]. The folate content of the 400 and 800 μg DFE/day treatment diets (Tables 1 and 2) was 426±44 and 835±73 μg food folate and provided ~2086–2300 kcal/day with 57–66% from carbohydrate, 13.2–13.4% from protein and 21–30% from fat (ESHA Food Processor Nutrient Data Base, version 7.81; ESHA Research, Salem, OR). The folate-rich foods selected for this study included whole wheat flour, strawberries, boysenberries, orange juice, broccoli, asparagus, cauliflower, spinach, kidney beans, refried beans, sunflower seeds, almonds, hazelnuts and wheat nuts. Foods utilized to increase folate consumption from 400 to 800 μg DFE/day included orange juice, sunflower seeds, almonds, wheat nuts and increased amounts of strawberries, boysenberries, hazelnuts, kidney beans, broccoli and asparagus. To offset the additional calories, low-folate food items were omitted including cookies, soda, cranapple juice, crangrape juice, popcorn, canned fruit, spiced applesauce, rice cakes and/or granola bars. All foodstuffs were weighed to the nearest gram. Subjects consumed morning and evening meals in the metabolic kitchen under the supervision of the investigators. Lunch, snacks and an additional 14 meals of the subjects choosing were provided by the investigators as "take away" items and consumed off-site.

The nutrient content of each diet was estimated using ESHA Food Processor Nutrient Data Base (version 7.81; ESHA Research) or the USDA choline data base [23,24]. Based on the nutrient content of the 133 μg DFE/day diet, supplements were given in order to provide ≥85% of the recommended intakes for the nutrients analyzed as detailed

Table 2
Five-day cycle menus consumed by women assigned to 800 μg DFE/day[a–e]

Day 1	Day 2	Day 3	Day 4	Day 5
Breakfast				
Whole wheat waffle	Shredded wheat cereal	Whole wheat waffle	Granola cereal	2 muffins
Strawberries, frozen (70 g)	Raisins	Boysenberries, frozen (70 g)	Raisins	Strawberries, frozen (30 g)
Syrup	Hazelnuts (15 g)	Syrup	Almonds (10 g)	Boysenberry yogurt (227 g)
Orange juice (233 g)	Strawberry yogurt (227 g)	Orange juice (233 g)	Peach yogurt (227 g)	Orange juice (233 g)
	Orange juice (233 g)		Orange juice (233 g)	
Lunch				
Turkey	Ham	Tuna	Turkey	Chicken salad
Swiss cheese	Cheddar cheese	Whole wheat pita	Swiss cheese	American cheese
Whole wheat pita	Whole wheat bread	Asparagus, canned (60 g)	Whole wheat bread	Whole wheat pita
Broccoli, frozen (60 g)	Asparagus, canned (60 g)	Lemon juice	Broccoli, frozen (60 g)	Broccoli, frozen (60 g)
Kidney beans, canned (30 g)	Lemon juice	Orange juice (384 g)	Kidney beans, canned (30 g)	Kidney beans, canned (30 g)
Orange juice (384 g)	Orange juice (384 g)		Orange juice (384 g)	Orange juice (384 g)
Dinner				
Tuna casserole	Chicken enchilada with spinach (30 g)	Pizza with ham and spinach, frozen (60 g)	Spaghetti with spinach, frozen (100 g)	Chicken breast
Asparagus, canned (180 g)	Green beans, frozen (120 g)	Broccoli, frozen (120 g)	Asparagus, canned (120 g)	Barbeque sauce
Lemon juice	Canned refried beans (120 g)	Cauliflower, frozen (60 g)	Lemon juice	Brown rice
Orange juice (384 g)	Strawberry (90 g) crisp	Orange juice (384 g)	Whole wheat biscuit	Spinach, frozen (100 g)
	Orange juice (384 g)		Orange juice (384 g)	Strawberry (90 g) crisp
				Orange juice (384 g)
Snacks				
Sunflower seeds (60 g)	Wheat nuts (30 g)	Wheat nuts (30 g)	No snack	Wheat nuts (30 g)
Hazelnuts (30 g)	Almonds (15 g)	Almonds (15 g)		Almonds (15 g)

[a] Menus analyzed by trienzyme folate extraction method [25,26] provided a mean of 835 μg DFE/day.
[b] Menu items prepared with unfortified flour were strawberry muffins and pizza dough. Unfortified flour was also used in sauces for tuna casserole and chicken enchiladas and for the strawberry crisp topping.
[c] Menu items prepared with whole-wheat flour and unfortified flour (1:1) were waffles and biscuits.
[d] For folate-rich menu items, gram amounts are given in parentheses.
[e] Foods allowed in unlimited amounts included soda, jello and whipped topping; also two cups of coffee or tea were allowed per day.

previously [5]. The supplements were administered at the morning meals throughout folate restriction and treatment under the supervision of the investigators. The supplements included a multimineral (LifeTime, Nutritional Specialities, 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 fourth day; vitamin K (KAL, Nutraceutical, Park City, UT) given every other day; choline (TwinLab, Twin Laboratories, Ronkonkoma, NY), given every other day; and iron (TwinLab, Twin Laboratories) given as needed (based on weekly hematocrit measures).

2.4. Sample collection and processing

Baseline and weekly fasting (9 h) venous blood samples were collected in serum separator gel and clot-activator tubes (SST, Vacutainer; Becton Dickinson, Rutherford, NJ) and EDTA tubes (Vacutainer). Serum, whole blood, plasma and peripheral leukocytes for serum folate, whole blood folate, plasma tHcy and MTHFR C677T genotype determinations, respectively, were processed and stored as previously described [18]. The EDTA whole blood was also used for hematocrit determination.

2.5. Analytical methods

2.5.1. Folate content of 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 conical tubes and stored at −20°C. Duplicates of the blended samples were thawed, homogenized and subjected to trienzyme treatment [25] and double extraction [26]. Total food folate was measured microbiologically [27].

2.5.2. 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 in duplicate via polymerase chain reaction and *Hin*f1 restriction enzyme digestion as described by Frosst et al. [17].

2.5.3. Plasma tHcy

An HPLC method with fluorometric detection [28,29] was used to measure plasma tHcy concentrations in duplicate for Weeks 0, 2, 12–14. The intra- and interassay CV for the pooled plasma (6.3 μmol/L) performed in triplicate over 8 days were 7% and 9%, respectively.

2.5.4. Serum and RBC folate

Microtiter plate adaptation with *Lactobacillus casei* as described by Tamura [27] was used to measure serum and erythrocyte folate in triplicate for Weeks 0, 2, 12–14. The intra- and interassay CV for the pooled serum (26 nmol/L) and whole blood (29 nmol/L) performed in quadruplet over 20 days were 10% and 12%, respectively.

2.6. Statistical analysis

All data summarization and analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL). Data are presented as means±S.E.M. in the text, tables and figures. To test for baseline (Week 0) and post-folate restriction (Week 2) differences in BMI, age, serum folate, RBC folate and/or plasma tHcy among the four groups, two-way ANOVA was performed using dietary folate and MTHFR C677T genotype as factors. Mean separations were accomplished using Tukey's HSD procedure. Comparisons between the dietary treatment groups during Weeks 12, 13 and 14 were made using the repeated-measures ANOVA facility of the GLM procedure with one within factor (Weeks 12, 13 and 14) and two between factors (dietary treatment and MTHFR C677T genotype).

3. Results

3.1. Subject characteristics

The final study group was composed of 32 women with a median age of 22 years (range: 19–46 years) and a median

Table 3
Serum folate (SF), RBC folate (RBCF) and plasma tHcy concentrations in women with the MTHFR 677 CC or TT genotype assigned to the 400 or 800 μg DFE/day groups at baseline (Week 0) and after folate restriction (Week 2) with 135 μg DFE/day*,**

Variable	Folate level	Baseline (Week 0)			After folate restriction (Week 2)		
		CC	TT	CC+TT	CC	TT	CC+TT
SF, nmol/L	400	36.5±3.9 (7)	24.7±2.9 (8)	30.1±2.7 (15)[a]	22.2±3.4 (7)	17.0±3.2 (8)	19.5±2.3 (15)
	800	22.9±2.7 (8)	20.4±2.7 (9)	21.5±1.8 (17)[b]	21.3±2.7 (8)	17.2±1.6 (9)	19.0±1.6 (17)
	Total	29.2±2.7 (15)[+]	22.4±2.0 (17)[++]	25.4±1.8 (32)	21.8±2.0 (15)	17.0±1.6 (17)	19.3±1.4 (32)
RBCF, nmol/L	400	1704±163 (7)	1421±120 (8)	1552±102 (15)	1584±143 (7)	1260±88 (8)	1409±88 (15)
	800	1561±159 (8)	1412±125 (9)	1482±97 (17)	1554±145 (8)	1355±118 (9)	1448±93 (17)
	Total	1627±111 (15)	1416±84 (17)	1516±70 (32)	1568±97 (15)[+]	1310±75 (17)[++]	1430±63 (32)
Plasma tHcy, μmol/L	400	5.2±0.2 (7)	5.9±0.6 (8)	5.6±0.3 (15)	5.9±0.2 (7)	6.9±0.6 (8)	6.4±0.3 (15)
	800	6.0±0.4 (8)	6.8±0.4 (9)	6.4±0.3 (17)	6.5±0.5 (8)	7.0±0.4 (9)	6.8±0.3 (17)
	Total	5.6±0.3 (15)	6.4±0.4 (17)	6.0±0.2 (32)	6.2±0.3 (15)	7.0±0.3 (17)	6.6±0.2 (32)

* Values are means±S.E.M. (*n*).

** Values with different superscript letters denote differences ($P = .05$) in the measured response variable between dietary treatment groups. Values with different superscript symbols ([+], [++]) denote differences ($P \leq .05$) in the measured variable between the MTHFR 677 CC and TT genotypes.

body mass index (BMI) (kg/m^2) of 23.0 (range: 19.1–36.0). The 400 μg DFE/day group consisted of 15 subjects: 7 with the MTHFR 677 CC genotype (1 African American, 3 Asians, 1 Caucasian and 2 Mexican Americans) and 8 with the MTHFR 677 TT genotype (1 Arabian, 2 Asians, 1 Caucasians and 4 Mexican Americans). The 800 μg DFE/day group consisted of 17 subjects: 8 with the MTHFR 677 CC genotype (2 African Americans, 2 Asians, 2 Caucasians and 2 Mexican Americans) and 9 with the MTHFR 677 TT genotype (1 Asian, 1 Caucasian and 7 Mexican Americans). No differences ($P>.05$) were detected in age among the four groups. No differences ($P>.05$) were detected in body mass index between dietary treatment groups or MTHFR C677T genotypes. However, subjects with the MTHFR TT genotype assigned to 800 μg DFE/day had a higher mean BMI (28.4) than the mean BMI of the 400 μg DFE/day group (22.5). Body weights were maintained within 5% of baseline in all but 3 subjects (1 CC 400, 1 TT 400 and 1 TT 800) who lost ~6.9% (range: 6.0–7.6%) of baseline weight. A total of 9 women reported using oral contraceptives during the study period (2 CC 400, 3 CC 800, 4 TT 800).

3.2. Serum folate

At baseline, women with the MTHFR 677 TT genotype had lower ($P=.024$) serum folate concentrations than women with the MTHFR 677 CC genotype (Table 3). In addition, women assigned to 800 μg DFE/day had lower ($P=.006$) serum folate than women assigned to the 400 μg DFE/day treatment (Table 3). At Week 2, serum folate did not differ between the dietary treatment groups but tended ($P=.09$) to remain lower in women with the MTHFR 677 TT genotype (Table 3). Throughout the last 3 weeks of the intervention (Weeks 12–14), serum folate was higher ($P=.005$) in women consuming 800 vs. 400 μg DFE/day (Fig. 1, Panel A). In addition, serum folate tended ($P=.065$) to be lower in women with the MTHFR 677 TT genotype relative to the 677 CC genotype (Fig. 1, Panel A). An interaction between the MTHFR C677T genotype and folate treatment across the last 3 weeks of intervention was not detected, and no significant ($P>.05$) effects of week or its interaction with diet or MTHFR C677T genotype were detected.

3.3. RBC folate

At baseline, RBC folate did not differ ($P>.05$) between dietary groups or between MTHFR C677T genotypes (Table 3). At Week 2, RBC folate did not differ ($P>.05$) between dietary groups but was lower ($P=.045$) in women with the MTHFR 677 TT relative to the CC genotype. Throughout the last 3 weeks of the intervention (Weeks 12–14), RBC folate was higher ($P=.001$) in women consuming 800 vs. 400 μg DFE/day (Fig. 1, Panel B). In addition, RBC folate was lower ($P=.022$) in women with the MTHFR 677 TT genotype relative to the 677 CC genotype (Fig. 1, Panel B). An interaction between the MTHFR C677T genotype and folate treatment across the last 3 weeks of intervention was not detected, and no significant ($P>.05$) effects of week or its interaction with diet or MTHFR C677T genotype were detected.

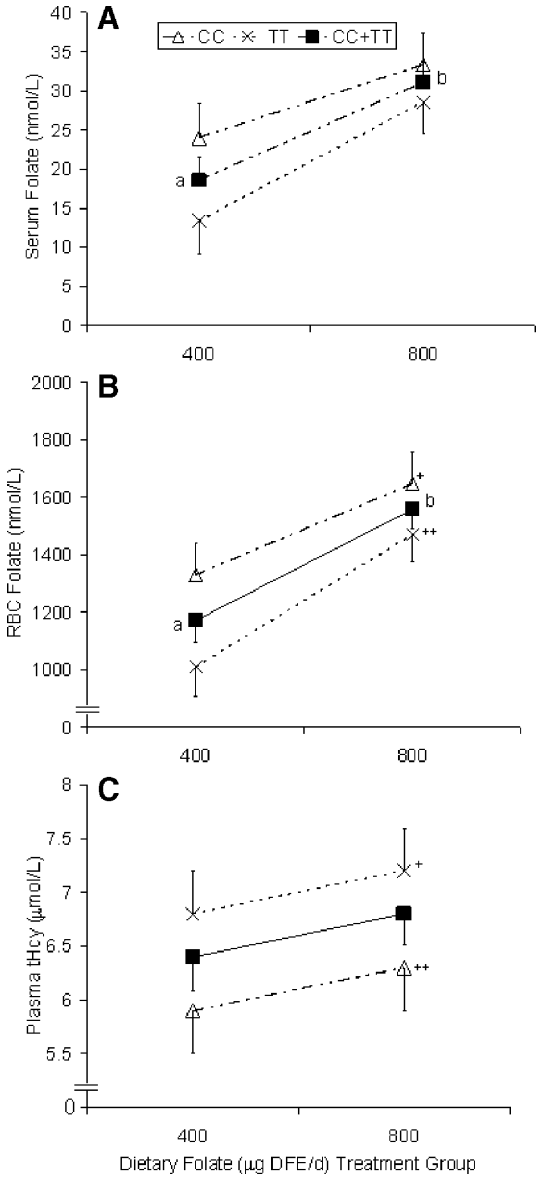

Fig. 1. The effect of a doubling of food folate intake (400 vs 800 μg/day as dietary folate equivalents) derived exclusively from natural sources during the last 3 weeks of the study (Weeks 12–14) on serum folate (A), RBC folate (B) and plasma tHcy (C) in women with the MTHFR 677 CC ($n=7$–8 per dietary treatment group) or TT ($n=8$–9 per dietary treatment group) genotype. Values are means of Week 12–14±S.E.M. Lines with different beginning and ending superscript letters denote differences ($P\leq.05$) in the measured variable between women consuming 400 ($n=15$) and 800 ($n=17$) μg DFE/day. Lines with different symbols (+, ++) denote differences ($P\leq.05$) in the measured variable between MTHFR 677 CC and TT genotypes. A diet by genotype interaction was not detected. The data were analyzed by the use of a repeated measures two-way ANOVA.

3.4. Plasma tHcy

At baseline and Week 2, plasma tHcy concentrations did not differ ($P>.05$) between the dietary groups or MTHFR C677T genotypes (Table 3). Throughout the last 3 weeks of intervention (Weeks 12–14), plasma tHcy did not differ

($P>.05$) between the dietary treatment groups (Fig. 1, Panel C). However, plasma tHcy was higher ($P=.039$) in women with the MTHFR 677 TT genotype relative to the 677 CC genotype. An interaction between the MTHFR C677T genotype and folate treatment across the last 3 weeks of intervention was not detected, and no significant ($P>.05$) effects of week or its interaction with diet or MTHFR C677T genotype were detected.

4. Discussion

This is the first long-term controlled folate feeding study to examine the effect of a doubling of food folate consumption on folate status in women with the MTHFR 677 CC or TT genotype. Because this study was conducted after folic acid fortification of staple food items, comparisons were made between the 400 and 800 μg DFE/day diet using data obtained during the final 3 weeks of folate treatment (Weeks 12–14) for serum folate, RBC folate and plasma tHcy. Changes from baseline (Week 0) or even after folate restriction (Week 2) in response to 400 or 800 μg DFE/day were not assessed because of the high blood folate concentrations and low plasma tHcy concentrations at either time point.

Our data demonstrate that a doubling of food folate derived exclusively from natural sources is effective in improving folate nutriture in young women. Consumption of 800 μg DFE/day resulted in serum folate and RBC folate concentrations that were 67% ($P=.005$) and 33% ($P=.001$) higher, respectively, than consumption of 400 μg DFE/day. Brouwer et al. [11] observed a 48% increase ($P\leq.05$) in serum folate and an 18% increase ($P\leq.05$) in RBC folate after ~2.5 times basal intake for 4 weeks. Riddell et al. [13] reported serum folate concentrations that were 52% higher ($P\leq.05$) than the control group after providing 2.0–2.3 times basal intake achieved via dietary counseling for 12 weeks. The relatively greater blood folate response to additional food folate observed in the present study (2.0 times increased) may reflect the long treatment period (12 weeks) combined with strictly controlled folate intakes.

In addition to dietary folate, the MTHFR 677 C→T polymorphism influenced folate status. Specifically, RBC folate concentrations were lower ($P=.022$) and plasma tHcy concentrations higher ($P=.039$) in women with the MTHFR 677 TT genotype than in women with the MTHFR 677 CC genotype. However, an interaction between dietary folate and MTHFR C677T genotype was not observed. This suggests that the response of women with the MTHFR 677 TT genotype to increased consumption of naturally occurring food folate is similar to the response of women with the MTHFR 677 CC genotype.

Only one other study has investigated folate status response to additional food folate in women with the MTHFR 677 TT genotype. Silaste et al. [14] observed a 50% increase ($P<.05$) in serum folate and an 18% decrease ($P<.05$) in plasma tHcy after 5 weeks of treatment with 600 vs. 220 μg/day food folate, which is consistent with the present study. In addition, two other studies have shown that individuals with the MTHFR 677 TT genotype respond well to supplementation with synthetic folic acid [21,22]. Taken together, these data suggest that women with the MTHFR TT genotype respond well to additional folate regardless of the source (i.e., naturally occurring folate or synthetic folic acid).

In the present study, plasma tHcy concentrations were low at baseline (i.e., <8 μmol/L) and remained low throughout the study regardless of folate intake. While it is recognized that plasma tHcy plateaus with folic acid intakes approximating 400–500 μg/day [30], the amount of food folate required to reach this plateau is unknown. The results of the present study suggest that consumption of 400 μg DFE/day derived exclusively from food folate is enough to achieve, or at least maintain, this plateau and that no further reductions are likely with higher intakes.

This study used folate intake levels, 400 and 800 μg DFE/day, that may result in greater urinary folate excretion and possible underestimation of what the response would be to a doubling of folate intake with lower quantities of food folate (i.e., 200 vs. 400 μg DFE/day). However, urinary folate excretion ranged from 0.9% to 2.9% of the total dose (data not shown), indicating that urinary folate excretion was small and would have minimal effect on the measured responses. Given the linear relationship between folate intake and blood folate concentrations [31,32], similar percent changes in blood folate concentrations are likely following a doubling of folate intake with lower levels of folate.

Data from the present study support and extend upon our previous work [18], suggesting that the 1998 folate RDA, 400 μg DFE/day, is sufficient in achieving/maintaining normal folate nutriture in women differing in MTHFR C677T genotype. In a previous study [18], 400 μg DFE/day derived primarily from folic acid was administered to women with the CC ($n=7$), CT ($n=6$) or TT ($n=9$) genotype following a 7-week period of folate restriction with 135 μg DFE/day. At Week 14, mean serum folate, RBC folate and plasma tHcy concentrations were 14.3±2.0 nmol/L, 773±47 nmol/L and 6.0±0.4 μmol/L for the TT genotype and 19.3±1.4 nmol/L, 952±39 nmol/L and 5.2±0.08 μmol/L for the CC genotype, respectively. These values are above the levels deemed to reflect folate insufficiency [4] and are consistent with data from the present study in which naturally occurring food folate provided the entire 400 μg/day. However, in both studies, 400 μg DFE/day was not enough to restore folate status to baseline values nor was it enough to overcome the influence of the MTHFR 677 C→T polymorphism.

This study also demonstrates that folate consumption levels of 400 and 800 μg DFE/day can be achieved by consuming folate-rich foods such as orange juice, strawberries, asparagus, broccoli, spinach, whole wheat flour, nuts and legumes (Tables 1 and 2). These data may be particularly important for countries where folic acid fortification and

supplement use are limited, and women of reproductive age, and others, must rely on increased consumption of food folate as the means by which to optimize their status. In addition, increased consumption of natural sources of food folate will not mask a vitamin B_{12} deficiency, a concern with high intakes of synthetic folic acid [4].

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