Nutrient Requirements

Ethnicity and Race Influence the Folate Status Response to Controlled Folate Intakes in Young Women1,2

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ABSTRACT  Population-based studies report differences in folate status indicators among Mexican American (MA), African American (AA) and Caucasian (CA) women. It is unclear, however, whether these differences are due to variations in dietary folate intake. The present study was designed to investigate the influence of ethnicity/race on folate status parameters in MA, AA, and CA women (18–45 y; n = 14 in each group) under conditions of strictly controlled folate intake. In addition, the adequacy of the 1998 folate U.S. recommended dietary allowance (RDA), 400 μg/d as dietary folate equivalents (DFE), for non-Caucasian women was assessed. Subjects (n = 42) with the methylenetetrahydrofolate reductase 677 CC genotype consumed a low-folate diet (135 μg DFE/d) for 7 wk followed by repletion with 400 (7 MA, 7 AA, 7 CA) or 800 μg DFE/d (7 MA, 7 AA, 7 CA) for 7 wk. AA women had lower (P ≤ 0.05) blood folate concentrations and excreted less (P ≤ 0.05) urinary folate throughout folate depletion and repletion with 400 and/or 800 μg DFE/d compared with MA and/or CA women. MA women had lower (P ≤ 0.05) plasma total homocysteine (tHcy) throughout folate depletion and during repletion with 400 μg DFE/d relative to the other ethnic/racial groups. Repletion with the 1998 folate U.S. RDA led to normal blood folate and plasma tHcy for all 3 ethnic/racial groups. Collectively, these data demonstrate that ethnicity/race is an important determinant of folate status under conditions of strictly controlled dietary folate intake and support the adequacy of the 1998 folate U.S. RDA for the 3 largest ethnic/racial groups in the United States.  J. Nutr. 134: 1786–1792, 2004.

KEY WORDS:  ● ethnicity ● race ● homocysteine ● folate ● humans ● RDA

Folate, a water-soluble B-vitamin, functions to accept and donate 1-carbon units (1). In this capacity, folate is essential for the de novo synthesis of certain amino acids (i.e., methionine) and nucleic acids. Folate also serves as the primary de novo source of methyl groups transferred by S-adenosylmethionine to a wide variety of essential biological substances including phospholipids, proteins, DNA, and neurotransmitters (1). Methylene tetrahydrofolate reductase (MTHFR)4 is a key regulatory enzyme in folate-dependent 1-carbon metabolism (2). By catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, MTHFR directs 1-carbon units toward methylene genesis at the expense of nucleotide synthesis.

Suboptimal folate status, which contributes to hyperhomocysteinemia (3), altered DNA methylation (4–6), and imbalanced DNA synthesis and repair (7), is linked to several adverse health outcomes including certain cancers (8–10), vascular disease (11–14), and cognitive impairments (15). Inadequate or marginal folate status is also associated with numerous unfavorable birth outcomes including neural tube and other congenital defects (16,17), Down Syndrome (18,19), very low birth weights, and premature births (20). Ethnic/racial disparities in health and birth outcomes were described between Caucasian, African American, and Hispanic, the major ethnic/racial groups residing in the United States. African American women experience a higher incidence and/or mortality rate from colorectal, breast, and cervical cancers (21–23) and have a greater risk of dying from cardiovascular disease (24,25). For birth outcomes, African American women experience more low-birth-weight and preterm births (26), whereas Hispanic women have the highest incidence of neural tube defects (27).

One of the numerous factors that may contribute to the health disparities among the major ethnic/racial groups is folate status. In the United States, Caucasian women have higher serum folate and RBC folate concentrations than Mexican and African American women (28–30). Paradoxically, however, Mexican American women and adolescent girls have the lowest homocysteine concentrations (31,32). These differences in folate status cannot readily be explained by differ-

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4 Abbreviations used: DFE, dietary folate equivalents; DRI, dietary reference intakes; MTHFR, methylenetetrahydrofolate reductase; RDA, recommended dietary allowance; tHcy, total homocysteine.
ences in dietary folate intake (29,31–33) and thus are suggestive of possible differences in folate requirements among the 3 major ethnic/racial groups. The U.S. folate recommended dietary allowance (RDA), 400 μg/d as dietary folate equivalents (DFE; 34), was based primarily on 2 controlled folate feeding studies (35,36) whose subjects were mostly Caucasian women (n = 27, 21 Caucasian, 4 African American, 1 Hispanic and 1 Polynesian women). Thus, it is uncertain whether the 1998 U.S. folate RDA is sufficient for non-Caucasian women.

The purpose of this study was to investigate the influence of ethnicity/race on folate status indicators in Mexican American, African American, and Caucasian women consuming controlled intakes of folate. Further, the adequacy of the 1998 U.S. folate RDA, 400 μg DFE/d, for sustaining sufficient folate nutriture in women differing in ethnicity/race was assessed.

SUBJECTS AND METHODS

Subjects. Women of self-reported Mexican American (n = 14), African American (n = 14), and Caucasian (n = 14) descent, defined as having 2 parents possessing the same ethnicity/race, were selected for participation in this study and were recruited among staff and students at Cal Poly Pomona University and the surrounding community from January 2000 through December 2002. At the initial screening, potential subjects completed a health history questionnaire and gave a fasting blood sample for determination of MTHFR C677T genotype. For those possessing the MTHFR 677 CC genotype, another fasting blood sample was obtained for clinical chemistry evaluation. In addition to the MTHFR 677 CC genotype, inclusion criterion was nonlactating, and a normal blood chemistry profile. The screening evaluation. In addition to the MTHFR 677 CC genotype, inclusion criteria were nonsmoker, nonanemic, not using supplements, no chronic drug or alcohol use, no antifolate medication use, no history of chronic disease, nonpregnant and not planning a pregnancy, nonlactating, and a normal blood chemistry profile. The screening and experimental procedures were reviewed and approved by the Institutional Review Board at Cal Poly Pomona University, and informed consent was obtained from each participant.

Experimental design. This was a 14-wk controlled folate feeding study consisting of a 7-wk folate depletion phase (135 μg DFE/d derived from naturally occurring food folate) followed by a 7-wk repletion phase with 400 or 800 μg DFE/d. During repletion, subjects (n = 42) were randomly assigned to receive 400 (n = 21) or 800 (n = 21) μg DFE/d derived from the low-folate diet plus 156 or 391 μg/d synthetic folic acid, respectively. Subjects consumed their breakfast and dinner daily in the Human Nutrition's metabolic kitchen at Cal Poly Pomona University. Lunch and snacks along with 14 meals (breakfast and/or dinner) were allowed to be consumed off-site. Weight was monitored weekly and any deviation ±5% from baseline was addressed by modifying energy intake with folate-free items such as sodas, gelatin, whipped topping, and margarine. The principal investigator and/or trained graduate student had daily contact with the subjects to help ensure compliance.

Diet and supplements. The 5-d low-folate rotation menu utilized in the present study was described previously (37) and provided 135 ± 9 μg/d (mean ± SEM) folate, as determined by trienzyme methodology on 3 separate occasions. Subjects were given supplements to provide the 1998 dietary reference intake (DRI) for choline and the B vitamins (except folate) and the 1989 RDA for all of the other essential nutrients not met by the diet. The diet and supplements provided ~90–120% of the 1989 RDA or 1998 DRI for all essential vitamins (except folate) and minerals. Commercially available folic acid (Sigma Chemical) was used to prepare the folic acid supplement (37) and was consumed during the morning and evening meals throughout repletion, under the supervision of the investigators. The subjects and investigators administering the folic acid supplements had no knowledge of the amount of folic acid in the supplements.

Sample collection and processing. Baseline and weekly venous blood samples were collected from fasting subjects (10 h) into serum separator gel and clot-activator tubes (SST, Vacutainer; Becton Dickinson) and EDTA tubes (Vacutainer). Serum, whole blood, plasma, and peripheral leukocytes for serum folate, RBC folate, plasma total homocysteine (tHcy), and MTHFR C677T genotype determination, respectively, were processed and stored as previously described (37). Baseline and weekly 24-h urine collections were also obtained, processed, and stored as detailed by Guinote et al. (37).

Analytical methods

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, mixed in a blender with 150 mL of cold 0.1 mol potassium phosphate buffer/L (pH 6.3) containing 57 mmol ascorbic acid/L, dispersed into 50-mL conical tubes, and stored at ~20°C. Duplicates of the blender-mixed samples were thawed, homogenized, and subjected to trienzyme treatment (38) and double extraction (39). Total folate content was analyzed microbiologically (40).

MTHFR C677T genotype. DNA for genotyping was extracted from leukocytes using a commercially available kit (DNAeasy Tissue Kit; Qiagen) and determination of the MTHFR genotype was via PCR and HinfI restriction enzyme digestion as described by Frost et al. (41) with minor modifications (42).

Plasma tHcy. An HPLC-method with electrochemical detection as described by the manufacturer (ESA) was used to measure plasma tHcy concentrations in duplicate at baseline, after folate depletion (wk 7) and after folate repletion with 400 or 800 μg DFE/d (wk 14). The intra- and interassay CV for the internal control were 3 and 10%, respectively.

Blood and urinary folate. Microrotator plate adaptation with Lactobacillus casei as described by Tamura (40) was used to measure serum, erythrocyte, and urinary folate concentrations. The intra- and interassay CV for the internal control were 10 and 12% respectively.

Statistical analysis. Baseline (wk 0) differences in serum folate, RBC folate, and urinary folate (all log-transformed) as well as BMI (untransformed) were analyzed by 1-way ANOVA. If a significant ethnicity/racial effect was detected by ANOVA, Tukey's Honestly Significant Difference (HSD) test was used for mean separation. For tHcy, normality could not be achieved by data transformation, and the Kruskal-Wallis test was employed.

Differences in serum folate, RBC folate, and urinary folate mean concentrations throughout folate depletion were analyzed by two-factor ANOVA with Tukey's HSD test for multiple comparisons. The factors were 1) week with 8 levels (wk 0 through wk 7) and 2) ethnic/racial group with 3 levels (Mexican American, African American, and Caucasian). In this analysis, the mean value of the response variable for each ethnic group at each week was used rather than individual subject values. Using the mean emphasizes differences among the ethnic groups, a principal goal of this study, rather than the individual differences within the ethnic groups. For the repletion phase (wk 8–14), a 2-factor ANOVA was used similarly to that used for depletion; however, separate analyses for each folate response variable were performed for the 400 and 800 μg DFE/d groups.

Differences in plasma tHcy among the 3 ethnic groups after folate depletion (wk 7) were analyzed by 1-factor ANOVA. Similarly, differences among ethnic groups after folate repletion (wk 14) were tested by 1-factor ANOVA, but separate analyses were made for the 400 and 800 μg DFE/d groups.

All data summarization and analyses were generated using SAS/STAT software, version 8.2 of the SAS System for Windows (SAS Institute). Significance was set at P ≤ 0.05. Data are presented as means (untransformed) ± SEM in the text, table, and figures.

RESULTS

Subject characteristics and baseline measurements. The final study group included 42 women with the following ethnicity/race: 14 Mexican American, 14 African American, and 14 Caucasian. The mean age of the women was 24.6 ± 19–44 y, and mean BMI (kg/m²) was 25.5 ± 19.2–32.1. No differences (P > 0.05) were detected in age or body weight between the women from each ethnic/racial group at baseline or upon completion of the study. Body weights were maintained within 5% of baseline in all but 13 subjects (3.
Mexican American, 4 African American, and 6 Caucasian) who lost ~9% (range = 6.1–13.3%) of baseline weight. A total of 17 women (5 Mexican American, 6 African American, and 6 Caucasian) reported using oral contraceptives during the study period. At baseline, no differences (P > 0.05) existed in serum folate or urinary folate between ethnicities/races (Table 1). However, baseline plasma tHcy concentrations between ethnicities/races tended to be different (P = 0.052) and baseline RBC folate concentrations were lower (P = 0.018) in the African American women than in the Caucasian women (Table 1).

**Serum folate.** Folate depletion decreased (P ≤ 0.05) serum folate concentration 57%, from 31.9 ± 2.1 to 13.6 ± 1.1 nmol/L. Throughout depletion, African American women had lower (P ≤ 0.05) serum folate concentrations compared with Mexican American and Caucasian women (Fig. 1A). Folate repletion with 400 µg DFE/d increased serum folate concentration 19%, although this was not significant (P = 0.07), from 14.3 ± 1.7 to 17.0 ± 0.6 nmol/L. Although differences (P ≤ 0.05) were detected among all ethnic groups throughout repletion with 400 µg DFE/d, African Americans had the lowest (P ≤ 0.05) serum folate concentrations (Fig. 1B). Folate repletion with 800 µg DFE/d increased (P ≤ 0.05) serum folate concentration 221%, from 13.1 ± 0.9 to 42.1 ± 3.2 nmol/L. Throughout repletion with 800 µg DFE/d, African American women had lower (P ≤ 0.05) serum folate concentrations than Mexican American and Caucasian women (Fig. 1C).

**RBC folate.** Folate depletion decreased (P ≤ 0.05) RBC folate concentration 26%, from 1517 ± 103 to 1117 ± 66 nmol/L. Throughout depletion, Mexican American and African American women had lower (P ≤ 0.05) RBC folate concentrations than Caucasian women (Fig. 2A). Folate repletion with 400 µg DFE/d did not change (P = 0.12) RBC folate concentration, 1101 ± 32 (wk 8) vs. 1151 ± 17 (wk 14) nmol/L. Throughout repletion with 400 µg DFE/d, no differences (P > 0.05) among the groups were detected (Fig. 2B). Folate repletion with 800 µg DFE/d increased (P ≤ 0.05) RBC folate concentration 15%, from 1134 ± 103 to 1298 ± 96 nmol/L. Differences (P ≤ 0.05) among all groups were detected during repletion with 800 µg DFE/d with the African American women having the lowest (P ≤ 0.05) RBC folate concentrations (Fig. 2C).

**Urinary folate.** Folate depletion decreased (P ≤ 0.05) urinary folate excretion 73%, from 71 ± 8 to 19 ± 3 nmol/d. During depletion, the African American women excreted less (P ≤ 0.05) urinary folate than the Caucasian women. The response of the Mexican American women was intermediate (Fig. 3A). Folate repletion with 400 µg DFE/d resulted in similar (P = 0.07) urinary folate excretion, 19 ± 1 (wk 8) vs. 17 ± 2 (wk 14) nmol/d. Throughout repletion with 400 µg DFE/d, African American women excreted less (P ≤ 0.05) urinary folate than Caucasian women. The urinary folate excretion of the Mexican American women was intermediate (Fig. 3B). Folate repletion with 800 µg DFE/d increased (P ≤ 0.05) urinary folate excretion 332%, from 19 ± 5 to 82 ± 10 nmol/d. Throughout repletion with 800 µg DFE/d, no differences (P > 0.05) in urinary folate excretion were detected among the groups (Fig. 3C).

**Homocysteine.** Folate depletion increased (P ≤ 0.05) plasma tHcy concentration 28%, from 6.3 ± 0.4 to 8.1 ± 0.6 µmol/L. After depletion, plasma tHcy concentrations were higher (P ≤ 0.05) in African American and Caucasian women than in Mexican American women (Fig. 4A). Folate repletion with 400 µg DFE/d did not change (P = 0.2) plasma tHcy concentrations, 8.0 ± 0.7 (wk 7) vs. 7.7 ± 0.7 (wk 14) µmol/L. After repletion with 400 µg DFE/d, African American and Caucasian women had higher (P ≤ 0.05) plasma tHcy concentrations relative to Mexican American women (Fig. 4B). Folate repletion with 800 µg DFE/d decreased (P ≤ 0.05) plasma tHcy concentration 17%, from 8.2 ± 0.5 to 6.8 ± 0.2 µmol/L. After repletion with 800 µg DFE/d, plasma tHcy concentrations did not differ (P > 0.05) between the ethnic groups (Fig. 4C).

**DISCUSSION**

Large, population-based studies report differences in folate status indicators among Mexican American, Caucasian and African American women (28–31). It is unclear, however, whether these differences are due to variations in dietary folate intake (29,31). Further, it is not known whether the 1998 folate U.S. RDA, 400 µg DFE/d, is adequate for non-Caucasian women because this recommendation was based on studies whose subjects were primarily Caucasian (34–36). The present study was designed to assess the influence of ethnicity/race on indicators of folate status under conditions of controlled folate intake and to evaluate the adequacy of the 1998 folate U.S. RDA in non-Caucasian women. Because the MTHFR 677C→T variant modulates blood folate and homocysteine concentrations (37,43), only women with the MTHFR 677 CC genotype were included.

The results of the present study demonstrate that ethnicity, itself, is an important determinant of folate status. African American women had lower (P ≤ 0.05) serum folate concentrations than Mexican American and Caucasian women throughout folate depletion and repletion with 400 or 800 µg DFE/d. African American women also had lower (P ≤ 0.05) RBC folate concentrations throughout folate depletion and repletion with 800 µg DFE/d compared with Caucasian women. Further, African American women excreted less (P ≤ 0.05) urinary folate through folate depletion and repletion.

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<th>TABLE 1</th>
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| **Baseline concentrations of serum folate, RBC folate, urinary folate, and plasma tHcy in women differing in ethnicity/race**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ethnicity/Race</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mexican American</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>34.9 ± 4</td>
</tr>
<tr>
<td>RBC folate, nmol/L</td>
<td>1450 ± 77&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary folate, nmol/d</td>
<td>56 ± 14</td>
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<tr>
<td>tHcy, µmol/L</td>
<td>5.7 ± 0.3</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SEM, n = 14/group.
<sup>2</sup> Means in a row with superscripts without a common letter differ, P ≤ 0.05.
though our data clearly demonstrate that ethnicity influences folate status, it is important to recognize the presence of variation within ethnic groups and that some African American women had numerically higher folate status than some Caucasian women.

Fewer differences in blood folate concentrations and no differences in urinary folate excretion were detected between Mexican American and Caucasian women. Mexican Ameri-
can women had higher (P ≤ 0.05) serum folate concentrations throughout repletion with 400 μg DFE/d, whereas Caucasian women had higher (P ≤ 0.05) RBC folate concentrations throughout folate depletion and repletion with 800 μg DFE/d.

These data are not entirely consistent with findings from large, population-based studies that report lower serum folate and RBC folate concentrations in Mexican American compared with Caucasian women (28–30). In the present study, women with the MTHFR 677 C→T polymorphism, which is more prevalent in Mexican Americans (42), were excluded, and this may explain the lack of differences or even higher serum folate concentrations in Mexican American relative to Caucasian women. Another possibility is that subjects who participated in this study were not truly representative of the general population because of the small sample size.

The results of the present study also demonstrate that ethnicity/race is an important determinant of plasma tHcy concentrations. Plasma tHcy concentration was lowest (P ≤ 0.05) in Mexican American women and did not differ (P ≤ 0.05) from each other in this study. These findings support the importance of considering ethnicity/race in the evaluation of plasma tHcy concentrations and the potential clinical implications of these differences.
reaches a plateau with folic acid doses of 317 nmol/L, levels deemed to reflect folate deficiency (34). Thus, data from the present study, together with the observation that consumption of 400 \( \mu \)g DFE/d led to normal folate nutriture in women possessing the MTHFR 677 TT genotype (37,43), support the sufficiency of the 1998 folate U.S. RDA for women differing in ethnicity as well as the MTHFR 677C→T polymorphism.

To summarize, ethnicity/race is an important determinant of folate and homocysteine status when dietary folate intake as well as other micronutrients are strictly controlled. The ethnicity-folate connection may provide important insights into certain health disparities that exist among ethnic/racial groups. Additional work, however, is warranted to ascertain whether these differences are biological and, if so, identify the nature of these biological factors. Notably, the 1998 U.S. folate RDA was adequate in maintaining normal folate nutriture in all study participants regardless of ethnicity/race.

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LITERATURE CITED


