

Effect of cow milk on food folate bioavailability in young women¹⁻³

Mary Frances Picciano, Sheila G West, Amanda L Ruch, Penny M Kris-Etherton, Guixiang Zhao, Kelley E Johnston, Deborah H Maddox, Valerie K Fishell, Douglas B Dirienzo, and Tsunenobu Tamura

ABSTRACT

Background: The findings of animal and human studies suggest that a certain component in milk enhances food folate bioavailability.

Objective: The objective was to evaluate whether cow milk enhances the bioavailability of food folate in humans.

Design: Thirty-one young women were fed low-folate diets on a 4-d rotation with ($n = 17$) or without ($n = 14$) cow milk for 8 wk. Plasma and erythrocyte folate and plasma total homocysteine (tHcy) concentrations were monitored.

Results: Plasma folate concentrations steadily declined in both the milk and the nonmilk groups; however, the declines were not significantly different between groups ($P = 0.09$). In contrast, erythrocyte folate concentrations declined only in the nonmilk group and remained unchanged in the milk group; the difference between the 2 groups was significant ($P = 0.01$). Plasma tHcy concentrations significantly increased in the nonmilk group and remained unchanged in the milk group; the difference between the 2 groups was significant ($P = 0.02$).

Conclusion: The inclusion of cow milk in the diet enhanced the bioavailability of food folate as assessed by the changes in erythrocyte folate and plasma tHcy concentrations but not in plasma folate concentrations. The mechanisms of action by the components of cow milk remain to be investigated. *Am J Clin Nutr* 2004;80:1565-9.

KEY WORDS Folate, milk, bioavailability, food, humans

INTRODUCTION

The importance of adequate folate nutrition for the maintenance of health and the prevention of various diseases, such as neural tube defects and occlusive vascular disease, has been well recognized in recent years (1, 2). However, it is difficult to accurately assess the dietary folate intake of the general population because of the inadequate availability of accurate food folate values (3). Furthermore, information on food folate bioavailability and the factors affecting it in our diets is extremely scarce (4).

Several groups of researchers using various animal models have evaluated the influence of the components of milk on the bioavailability of folate (5-10). Colman et al (6) reported that the uptake of folic acid (pteroylglutamic acid) bound to goat milk folate-binding protein (FBP) was twice that of unbound folic acid in the isolated rat intestinal mucosal cells. Mason and Selhub (7) reported a similar observation using an in vivo intestinal loop technique. Iwai et al's group (8, 9) subsequently suggested that bovine milk FBP plays an important role in folate bioavailability in rats. Swiatlo et al (10) later showed that the addition of human or cow milk to diets increased folate bioavailability in rats, although what constituent in milk has such an effect is unknown.

Recently, an Australian group showed that FBP stabilizes tetrahydrofolates in vitro and increases dietary folate bioavailability in vivo in rats when it is given with other whey proteins or soluble casein (11, 12). Furthermore, Said et al (13) and Verwei et al (14), using the everted-rat-intestinal sac and an artificial gastrointestinal model, respectively, reported findings suggesting the role of milk FBP in regulating intestinal folate absorption and bioavailability. In addition to the evaluation of folate absorption at the site of the intestinal mucosa, Ford (15) reported the possible importance of milk FBP in the economy of the vitamin in the intestine through its interaction with gut flora.

To our knowledge, however, only one human study has been performed to evaluate the effect of milk on folate bioavailability. Smith et al (16) showed in infants that folate bioavailability in human or cow milk is higher than that in foods without milk products. The data of that study together with the findings of the animal studies reviewed above suggest that a certain component in milk enhances folate absorption and bioavailability, that milk folate may be more bioavailable than is folate in nonmilk-containing food, or both.

In the current study, we hypothesized that the bioavailability of food folate is enhanced by the presence of cow milk. To test this hypothesis, we conducted a study to evaluate the effect of cow milk on folate bioavailability in young women who were fed low-folate diets with or without cow milk for 8 wk. This was estimated by monitoring plasma and erythrocyte folate concentrations, the most commonly used means to assess folate nutritional adequacy and plasma total homocysteine (tHcy) concentrations—a folate functional test.

SUBJECTS AND METHODS

Subjects and blood samples

The study was approved by the Pennsylvania State University, Office for Regulatory Compliance, and signed informed consent

¹ From the Departments of Nutrition (MFP, ALR, PMK-E, GZ, DHM, and VKF) and Biobehavioral Health (SGW), Pennsylvania State University, University Park; the National Dairy Council, Rosemont, IL (DBD); and the Department of Nutrition Sciences, University of Alabama at Birmingham (KEJ and TT).

² Supported by a grant from the National Dairy Council.

³ Address reprint requests to MF Picciano, Office of Dietary Supplements, 6100 Executive Boulevard, Bethesda, MD, 20892-7517. E-mail: piccianm@od.nih.gov.

Received August 13, 2003.

Accepted for publication July 16, 2004.

TABLE 1

Characteristics of subjects in the milk and the nonmilk groups at enrollment¹

	Milk group (<i>n</i> = 17)	Nonmilk group (<i>n</i> = 14)
Age (y)	23.1 ± 3.7	21.4 ± 1.2
Height (cm)	166 ± 7	162 ± 7
Weight (kg)	61.3 ± 7.0	57.8 ± 9.6
BMI (kg/m ²)	22.3 ± 2.1	21.8 ± 2.9
Hematocrit (%)	40.1 ± 2.4	39.1 ± 1.3
Hemoglobin (g/L)	136 ± 8	134 ± 3
Mean corpuscular volume (fL)	92.0 ± 3.7	90.4 ± 4.5

¹ All values are $\bar{x} \pm SE$. There were no significant differences between the groups (Student's *t* test).

was obtained from each subject. The study was performed between February and November 1999. Two African American and 29 white women (mean age: 22.3 y; range: 19–33 y) participated in the study. Women were excluded if they were pregnant, were lactating, were a smoker, were users of oral contraceptives or other medications that could possibly interfere with folate metabolism, were unwilling to discontinue taking nutritional supplements, had abnormal results from clinical tests (including plasma folate and vitamin B-12 concentrations <11 nmol/L and 150 pmol/L, respectively), and had a medical history indicating serious disease. The subjects were asked to refrain from the consumption of alcoholic drinks during the study, because alcohol intake is known to perturb folate metabolism (17). Pregnancy tests were done at the beginning of the study and at 4 wk of the study.

The subjects were randomly assigned to 1 of 2 dietary groups. One group received diets with skim milk (0% fat; milk group, *n* = 17) and the other received diets containing no milk, which was replaced with apple juice and egg substitutes (nonmilk group, *n* = 14) for 8 wk. The characteristics of the subjects were similar in both groups (**Table 1**).

Fasting blood samples were initially collected at the beginning of the first week (baseline) and then at the end of each week with the use of evacuated tubes containing EDTA (BD Vacutainer; Preanalytical Solutions, Franklin Lake, NJ). Blood samples were kept on ice, and portions of samples were separated for hematocrit and erythrocyte folate analyses before plasma separation. Samples were kept at -70 °C until assayed.

Meals and supplements

At the Metabolic Diet Study Center at Pennsylvania State University, 4-d rotation meals (3 meals and 1 snack daily) were prepared by using the NUTRITIONIST V database (First Data-Bank, San Bruno, CA). The ingredients low in folate were selected to keep the total daily folate intake <0.45 μmol (200 μg), and any product fortified with folic acid was avoided. Meals prepared for the milk group contained three 245-mL servings of skim milk daily, which was replaced with three 245-mL servings of apple juice and egg substitutes (20 g egg whites and 78 g Egg Beaters; ConAgra Foods, Omaha) in the nonmilk group. Examples of the 2 menus are shown in **Table 2**. These meals contained daily energy supplies ranging from 8640 to 11 500 kJ (15% of energy from protein, 55% from carbohydrates, and 30% from fat), and the energy content of the meals given depended on the body weight of the subjects. Unit foods (muffins, 420 kJ each)

TABLE 2

Menus (8640 kJ) for the milk and the nonmilk groups

Food items	Milk group	Nonmilk group
	<i>g</i>	
Breakfast		
Skim milk	245	0
Apple juice	0	120
Butter	15	15
Muffin	100	100
Jam	28	14
Fat- and cholesterol-free egg product ¹	0	78
Egg whites	0	20
Lunch		
Skim milk	245	0
Apple juice	0	120
Pasta	112	112
Sliced tomato	50	50
Sweet peppers	50	50
Onions, red	20	20
Black olives	28	28
Ham, cubed	28	28
Salad dressing	25	25
Peaches, diced	113	125
Wheat-free crackers	33	23
Dinner		
Skim milk	245	0
Apple juice	0	120
Beef	61	110
Gravy	60	60
Potato, canned	150	125
Olive oil	7.2	7.8
Egg yolk, boiled	2.5	0
Tomato, sliced	50	50
Cucumber, peeled and sliced	50	50
Salad dressing	20	20
Apple sauce	150	155
Snack		
Potato chips	18	18

¹ Egg Beaters; ConAgra Foods, Omaha.

were used to further adjust energy intake to maintain the initial weight. The subjects consumed breakfast and dinner at the Metabolic Diet Study Center during weekdays, whereas lunches and weekend meals were provided to be eaten away from the center. The subjects received a custom-formulated dietary supplement (Tishcon, Salisbury, MD) that contained all essential micronutrients with the exception of folic acid. The supplement provided 6.0 μg cyanocobalamin/d and 2.0 mg vitamin B-6 hydrochloride/d. In addition, the subjects in the nonmilk group received calcium supplements (250 mg as calcium carbonate 3 times daily) to substitute for the amount of calcium in each glass of milk.

Food folate analysis

Four whole-day meals were set aside for folate analyses at 6 time points during the study. Homogenates of a total of 24 meals were prepared by using a Waring blender with 2 volumes (≈ 2000 mL) of potassium phosphate buffer containing ascorbic acid (1.0 g/100 mL) with a final pH of 7.0 and were shipped in aliquots on dry ice to the University of Alabama at Birmingham. Folate contents were measured by microbiological assay with the use of

Lactobacillus casei after trienzyme extraction (α -amylase, protease, and folate conjugase treatments) or folate conjugase treatment alone (3, 18, 19). The within-day CV of the food folate assay after trienzyme extraction was $\approx 14.2\%$ in slices of 63 brands of sandwich bread (KE Johnston and T Tamura, unpublished observations, 2004).

Laboratory analyses

Plasma and erythrocyte folate concentrations were measured by *L. casei* microbiological assay as previously described (19, 20). Plasma vitamin B-12 concentrations were analyzed by using a MAGIC Vitamin B-12 [^{57}Co] Radioassay kit (Ciba-Corning, Medfield, MA), and plasma pyridoxal-5'-phosphate (PLP) concentrations were measured by using [^3H]-tyrosine (Moravsek, Brea, CA) as substrate based on the method described by Camp et al (21) with slight modifications (22). Plasma tHcy was measured by an HPLC-fluorescence method with homocysteine thiolactone as a standard (23). Folate analyses were performed weekly, whereas the assays for vitamin B-12, PLP, and tHcy were done with the use of samples obtained at baseline and at the end of the fourth and eighth weeks. The interassay CVs measured with the use of pooled plasma or control samples provided by the manufacturer were $\approx 10\%$, 8% , 11% , and 8% for folate, vitamin B-12, PLP, and tHcy, respectively (23).

Statistical analyses

Student *t* tests were used to compare the 2 groups on baseline concentrations of each dependent variable measured before the subjects were randomly assigned to a group. Because direct folate analyses of the 2 diets indicated that the milk group received a lower daily dietary folate intake than did the nonmilk group (mean: 0.30 compared with $0.41 \mu\text{mol}$, respectively; $P < 0.001$, see Results), dietary folate intake was used as a covariate in all analyses of treatment-related changes. We conducted a series of mixed-models analyses on change scores (posttreatment value minus pretreatment value) for each variable, with time (week) and group (milk and nonmilk) as fixed effects with the use of SAS PROC MIXED (version 8; SAS Institute Inc, Cary, NC). When significant main effects and interactions were found, Tukey-Kramer adjusted *P* values were used to examine the source of these effects. To test whether the groups showed similar rates of change over time, the analyses of change scores were repeated, and the group factor was removed from the model. Significant interactions of group and time from this reduced model indicate that the rate of change is not identical in the 2 groups. Interrelations between the variables were estimated by using Pearson's correlation coefficients. Tables and figures depict least-squares means \pm SEs, and *P* values ≤ 0.05 were considered statistically significant.

RESULTS

Mean (\pm SE) folate contents of the 24 whole-day meals were $0.30 \pm 0.01 \mu\text{mol}$ ($134 \pm 3 \mu\text{g}$) and $0.41 \pm 0.01 \mu\text{mol}$ ($182 \pm 3 \mu\text{g}$) for the milk group and the nonmilk group, respectively. The difference between the groups was significant ($P < 0.001$), although we intended to make folate contents in the diets similar for both groups by using ingredients with the same lot numbers to formulate the diets. Thus, the nonmilk group consumed 36% more folate than did the milk group. These values were obtained by microbiological assay after trienzyme extraction, and they

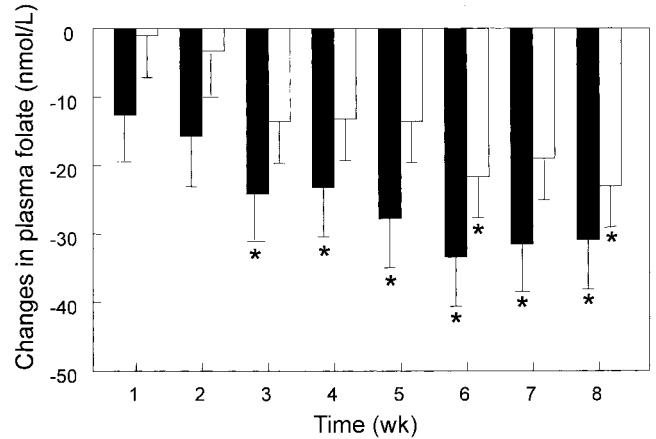


FIGURE 1. Adjusted mean (\pm SE) changes in plasma folate concentrations in the milk (\square ; $n = 17$) and the nonmilk (\blacksquare ; $n = 14$) groups. *Significantly different from baseline, $P < 0.003$ (Bonferroni-adjusted post hoc test). Baseline plasma folate concentrations were 49 ± 4 and $58 \pm 5 \text{ nmol/L}$ in the milk and the nonmilk groups, respectively. When change scores were analyzed, there was a significant effect of time ($P = 0.0001$). Neither the group main effect nor the time \times group interaction was significant. In the group as a whole, plasma folate concentrations were significantly different from baseline during weeks 2–8 ($P \leq 0.004$).

were 48% and 56% higher than those measured after the traditional treatment with folate conjugase alone in the meals for the milk and the nonmilk groups, respectively.

There were no significant group differences in plasma (Figure 1) and erythrocyte (Figure 2) folate, plasma vitamin B-12, PLP, and tHcy concentrations before randomization (Table 3). Plasma folate significantly decreased during the 8-wk period in both groups, and this decline became larger as the study progressed. The decline in mean plasma folate in the nonmilk group was 45% larger than in the milk group; however, this group difference was not statistically significant. The mean declines in plasma folate concentrations were 2.9 and 3.9 nmol/L per week in the milk and

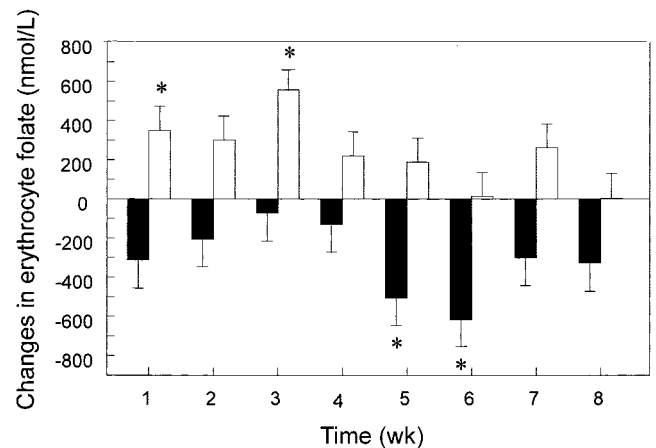


FIGURE 2. Adjusted mean (\pm SE) changes in erythrocyte folate concentrations in the milk (\square ; $n = 17$) and the nonmilk (\blacksquare ; $n = 14$) groups. Baseline erythrocyte folate concentrations were 1372 ± 96 and $1478 \pm 106 \text{ nmol/L}$ in the milk and the nonmilk groups, respectively. When change scores were analyzed, there were significant effects of group ($P = 0.01$) and time ($P = 0.0001$). When group was removed from the model, there was a significant group \times time interaction ($P = 0.02$), which indicated that change in the groups was not parallel over time. *Significantly different from baseline, $P < 0.003$ (Bonferroni-adjusted post hoc test).

TABLE 3

Vitamin and total homocysteine concentrations at baseline and change scores at 4 and 8 wk of dietary intervention in the milk and the nonmilk groups¹

	Milk group (n = 17)			Nonmilk group (n = 14)		
	Baseline	4 wk	8 wk	Baseline	4 wk	8 wk
Vitamin B-12 (pmol/L) ²	300 ± 27	41 ± 27	76 ± 27 ³	246 ± 31	35 ± 33	24 ± 33
Pyroxidal-5'-phosphate (nmol/L) ⁴	76 ± 10	16 ± 17	14 ± 17	63 ± 11	44 ± 20	28 ± 20
Total homocysteine (μmol/L) ^{5,6}	6.1 ± 0.3	-0.0 ± 0.5	-0.1 ± 0.5	5.4 ± 0.3	2.5 ± 0.6 ³	2.0 ± 0.6 ³

¹ All values are least-squares $\bar{x} \pm \text{SE}$.² Significant group \times time interaction, $P = 0.03$ (mixed-models analysis of change scores).³ Significant change from baseline, $P \leq 0.05$ (Bonferroni adjustment).⁴ Significant main effect of time, $P = 0.05$ (mixed-models analysis of change scores).⁵ Significant main effect of group, $P = 0.02$ (mixed-models analysis of change scores).⁶ Significant group \times time interactions in a model in which group was removed, which indicated that the change in the 2 groups was not parallel over time, $P = 0.04$.

the nonmilk groups, respectively, with a mean decline of 3.4 nmol/L per week as a whole. As shown in Figure 2, erythrocyte folate concentrations declined substantially in the nonmilk group only over the 8-wk period, whereas the milk group showed increases in erythrocyte folate concentrations. This difference between the 2 groups was indicated by a significant effect of the diets on erythrocyte folate concentrations ($P = 0.01$).

There was a significant group \times time interaction for change in plasma vitamin B-12 ($P = 0.03$). Only the milk group showed significant increases in plasma vitamin B-12 concentrations ($P = 0.008$), and this effect was only significant at the end of the 8-wk period. In the sample as a whole, plasma PLP concentrations increased over the 8-wk period. There was no significant group difference in plasma PLP changes.

As shown in Table 3, changes in plasma tHcy concentrations showed a different pattern, which increased significantly in the nonmilk group (by $2.3 \pm 0.6 \mu\text{mol/L}$; $P = 0.0004$) but did not change significantly in the milk group ($-0.07 \pm 0.05 \mu\text{mol/L}$; $P = 0.89$). Furthermore, the group difference in change in tHcy concentrations was statistically significant ($P = 0.02$).

Regardless of the type of diets consumed during the 8-wk period, subjects with the largest reductions in plasma folate concentrations at the end of 8 wk had the largest increases in tHcy concentrations ($r = -0.51$, $P = 0.004$) and the largest decreases in erythrocyte folate concentrations ($r = 0.40$, $P = 0.05$). In addition, changes in plasma vitamin B-12 were inversely correlated with changes in plasma tHcy ($r = -0.42$, $P = 0.02$) and changes in erythrocyte folate concentrations ($r = -0.48$, $P = 0.02$).

DISCUSSION

We found that the inclusion of skim milk to the diet enhanced the bioavailability of food folate as assessed by the changes in erythrocyte folate and plasma tHcy concentrations in a total of 31 young women who were fed diets with or without milk for 8 wk. As for the two 4-d rotation diets, the subjects received mean daily folate intakes of 134 and 182 μg in the milk and the nonmilk groups, respectively. The main difference between the 2 groups was the substitution of skim milk with apple juice and egg substitutes. The reason for the difference in folate content in the 2 diets is unknown because the folate content of each food was not independently assayed. We found that folate values in the meals were on average $\approx 50\%$ higher after trienzyme treatment than

after folate conjugase treatment alone. The data suggest that for the folate assay in the mixed diet, the trienzyme-extraction method should be used to obtain the highest values detectable as suggested previously (3, 18).


Because we provided low-folate diets, plasma folate concentrations steadily declined in both the milk and the nonmilk groups during the 8-wk study period (Figure 1). Because the dietary folate intakes were different between the groups, we analyzed the decline in plasma folate with folate intake as a covariate; we found that the declines were similar in the milk and the nonmilk groups ($P = 0.09$). In contrast, the decline in erythrocyte folate was found only in the nonmilk group (Figure 2), and erythrocyte folate concentrations remained unchanged over the 8-wk period in the milk group. This difference between the 2 groups indicated a significant effect of the diets on erythrocyte folate concentrations ($P = 0.01$). Considering that the life span of erythrocytes is 120 d, it was unexpected that such a large difference in erythrocyte folate, but not in plasma folate, was observed between the 2 groups after only an 8-wk period (56 d) because only $<50\%$ of erythrocytes turned over during the study. However, the wide variation in both plasma and erythrocyte folate concentrations is the likely cause of such an inconsistency. The difference in plasma folate response to the 2 diets was large; however, variability was also high. As a result, our study may not have been adequately powered to test the effects of the diets on plasma folate concentrations with 14–17 subjects per group. Retrospective analyses showed that statistical power was 0.21 for testing group differences in plasma folate in the current study. In contrast, variability was substantially lower and power was much higher (0.90) for testing group differences in erythrocyte folate concentrations. Future studies are needed to examine the sources of individual variation in plasma folate concentrations, and subsequent studies may need to include larger samples to compensate for variability in plasma folate.

Furthermore, it remains to be determined whether continuing treatment for a longer period of time would have produced significant changes in plasma folate. The interpretation of data might have been easier if the folate intakes from the 2 diets had been similar. Would it have been possible to observe the difference in plasma folate concentrations between the groups if we had used 2 diets containing an equal amount of folate or diets with slightly higher amounts of folate than used in the current study? In well-controlled folate-depletion experiments, such as reviewed below, investigators have used only one folate intake (24–26).

In addition, plasma tHcy concentrations, a functional test for folate nutriture, showed a significant increase in the nonmilk group but no change in the milk group. Of the 3 indexes of folate nutriture measured in this study, 2 indexes (erythrocyte folate and plasma tHcy concentrations) indicated that the subjects in the milk group had better folate nutriture at the end the 8-wk period, despite the lower folate intake than in those in the nonmilk group. These findings agree with those of several studies, which showed that a component of milk enhances folate absorption and bioavailability in both animals (6–12, 14) and humans (16).

Our observation of a decline in plasma folate (3.5 nmol/L per week) is similar to the results found by others, who used the trienzyme extraction method to obtain dietary folate values. In 8 postmenopausal women, Jacob et al (24) reported that the consumption of a low-folate diet containing 152 μg folate for 21 d resulted in a significant reduction in plasma folate concentrations at a rate of ≈ 3.7 nmol/L per week. The folate content in the diets used by Jacob et al (24) was similar to the content in the diet used in the current study. Also, Kauwell et al (25) conducted a study in 17 elderly women who were fed a diet containing 118 μg folate/d for 7 wk; they found that plasma folate decreased at a weekly rate of 3.8 nmol/L. In a 7-wk study in 41 young females, Shelnutt et al (26) found that the mean plasma folate concentration declined at a rate of ≈ 4.2 nmol/L per week when the subjects were fed diets providing 115 μg folate/d. These data together indicate that plasma folate concentrations decline at the rate of 3.5–4.2 nmol/L when subjects are fed a low-folate diet providing 115–160 μg folate/d.

We observed increases in both plasma vitamin B-12 and PLP concentrations. This may indicate that the daily doses of vitamins B-12 and B-6 (6.0 μg as cyanocobalamin and 2.0 mg as B-6 hydrochloride) in addition to the diets were sufficient to improve their baseline nutriture of the vitamins among our subjects.

In summary, the inclusion of cow milk to the diet enhances the bioavailability of food folate as assessed by the changes in erythrocyte folate and plasma tHcy concentrations, but not plasma folate concentrations, in a total of 31 young women who were fed diets with or without milk for 8 wk. The mechanisms of such action by the components of cow milk remain to be explored. Furthermore, future studies should address how much milk is needed to affect dietary folate bioavailability. 

MFP served as Principal Investigator, designed the study, and oversaw its implementation including manuscript preparation. SGW supervised the statistical analyses, data review, and presentation in tables and figures and wrote sections of the manuscript. ALR assisted with the implementation of the study as her undergraduate honor thesis project. PMK-E provided study oversight, helped plan the test diets, and assisted with the data presentation and interpretation. GZ conducted the statistical analyses. KEJ performed all of the laboratory analyses. DHM supervised subject recruitment and adherence to the study design as the Clinical Coordinator. VKF planned the preparation of the experimental diets. DBD helped to establish the conceptual aspect of the study. TT supervised the laboratory analyses and wrote the manuscript. DBD is an employee of the National Dairy Council. None of the other authors had a financial or personal conflict of interest related to the funding of or the outcome of this research.

REFERENCES

1. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* 1991; 338:131–7.
2. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999;354:407–13.
3. Tamura T. Determination of food folate. *J Nutr Biochem* 1998;9:285–93.
4. Gregory JF III. Bioavailability of folate. *Eur J Clin Nutr* 1997;51:S54–9.
5. Izak G, Galewski K, Rachmilewitz M, Grossowicz N. The absorption of milk-bound pteroylglutamic acid from small intestine segments. *Proc Soc Exp Biol Med* 1972;140:248–50.
6. Colman N, Hittiarachy N, Herbert V. Detection of a milk factor that facilitates folate uptake by intestinal calls. *Science* 1981;211:1427–9.
7. Mason JB, Selhub J. Folate-binding protein and the absorption of folic acid in the small intestine of the suckling rats. *Am J Clin Nutr* 1988;48:620–5.
8. Tani M, Fushiki T, Iwai K. Influence of folate-binding protein from bovine milk on the absorption of folate in gastrointestinal tract of rat. *Biochim Biophys Acta* 1983;757:274–81.
9. Tani M, Iwai K. Some nutritional effects of folate-binding protein in bovine milk on the bioavailability of folate to rats. *J Nutr* 1984;114:778–85.
10. Swiatlo N, O'Connor DL, Andrews J, Picciano MF. Relative folate bioavailability from diets containing human, bovine and goat milk. *J Nutr* 1990;120:172–7.
11. Jones ML, Nixon PF. Tetrahydrofolates are greatly stabilized by binding to bovine milk folate-binding protein. *J Nutr* 2002;132:2690–4.
12. Jones ML, Treloar T, Nixon PF. Dietary interactions influence the effects of bovine folate-binding protein on the bioavailability of tetrahydrofolates in rats. *J Nutr* 2003;133:489–95.
13. Said HM, Horne DW, Wagner C. Effect of human milk folate binding protein on folate intestinal transport. *Arch Biochem Biophys* 1986;251:114–20.
14. Verwei M, Arkbåge K, Havenaar R, van der Berg H, Witthöft C, Schaafsma G. Folic acid and 5-methyltetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic in vitro gastrointestinal model. *J Nutr* 2003;133:2377–83.
15. Ford JE. Some observations on the possible nutritional significance of vitamin B₁₂- and folate-binding proteins in milk. *Br J Nutr* 1974;31:243–57.
16. Smith AM, Picciano MF, Deering RH. Folate intake and blood concentrations of term infants. *Am J Clin Nutr* 1985;41:590–8.
17. Tamura T, Halsted CH. Folate turnover in chronically alcoholic monkeys. *J Lab Clin Med* 1983;101:623–8.
18. Tamura T, Mizuno Y, Johnston KE, Jacob RA. Food folate assay with protease, α -amylase, and folate conjugase treatments. *J Agri Food Chem* 1997;45:135–9.
19. Tamura T. Microbiological assay of folates. In: Picciano MF, Stokstad ELR, Gregory JF III, eds. Folic acid metabolism in health and disease. New York: Wiley-Liss, 1990:121–37.
20. Tamura T, Freeberg LE, Cornwell PE. Inhibition by EDTA of growth of *Lactobacillus casei* in the folate microbiological assay and its reversal by added manganese or iron. *Clin Chem* 1990;36:1993.
21. Camp VM, Chipponi J, Faraj BA. Radioenzymatic assay for direct measurement of pyridoxal-5-phosphate. *Clin Chem* 1983;29:642–4.
22. Tamura T, Bergman SM, Morgan SL. Homocysteine, B vitamins, and vascular-access thrombosis in patients treated with hemodialysis. *Am J Kidney Dis* 1998;32:475–81.
23. Tamura T, Johnston KE, Bergman SM. Homocysteine and folate concentrations in blood from patients treated with hemodialysis. *J Am Soc Nephrol* 1996;7:2414–8.
24. Jacob RA, Gretz DM, Taylor PC, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr* 1998;128:1204–12.
25. Kauwell GPA, Lippert BL, Wilsky CE, et al. Folate status of elderly women following moderate folate depletion responds only to a higher folate intake. *J Nutr* 2000;130:1584–90.
26. Shelnutt KP, Kauwell GPA, Chapman CM, et al. Folate status response to controlled folate intake is affected by the methylenetetrahydrofolate reductase 677C \rightarrow T polymorphism in young women. *J Nutr* 2003;133:4107–11.