Original Article

Folic acid fortified milk increases blood folate to concentrations associated with a very low risk of neural tube defects in Singaporean women of childbearing age

Magdalin Cheong MSc[1], Huang Ying Xiao MSc[1], Vicky Tay PhD[2], Crystal D Karakochuk MSc[3], Yazheng Amy Liu MSc[3], Sarah Harvey MSc[3], Yvonne Lamers PhD[3], Lisa A Houghton PhD[4], David D Kitts PhD[3], Tim J Green PhD[3]

[1]*Dietetic & Food Services Department, Changi General Hospital, Singapore*
[2]*Sprim Advanced Life Sciences Ltd, Singapore*
[3]*Food, Nutrition, and Health, the University of British Columbia, Vancouver, Canada*
[4]*Department of Human Nutrition, University of Otago, Dunedin, New Zealand*

Background and Objectives: Folic acid (400 μg/d) taken during the periconceptional period reduces neural tube defect (NTD) risk by >75%. Achieving red cell folate (RCF) or plasma folate (PF) >905 nmol/L and >35 nmol/L, respectively, has been associated with a low risk of NTDs. We determined whether daily consumption of folic acid fortified milk increases blood folate concentrations to levels associated with a low risk of NTDs in Singaporean women of childbearing age. **Methods and Study Design:** In this double-blind placebo-controlled trial, 70 non-pregnant women (21-35 y) were randomly assigned to receive fortified milk (FM) powder providing 400 μg folic acid per day or unfortified placebo milk (PM) powder for 12 weeks. Blood samples were collected at baseline and at 6 and 12 weeks. **Results:** At 12 weeks, mean (95% CI) RCF and PF concentrations were 376 (240, 512) and 39 (26, 51) nmol/L higher in the FM group compared with the PM group (p<0.001). Of the women receiving FM, 71% (n=25) and 86% (n=30) achieved a RCF and PF associated with a very low risk of NTDs, respectively. **Conclusion:** Folic acid fortified milk increased blood folate concentrations in women of childbearing age to levels associated with a reduced risk of an NTD-affected pregnancy.

Key Words: folate, folic acid, fortification, milk, neural tube defect

INTRODUCTION

Folic acid (~400 μg/day) taken around the time of conception significantly reduces the risk of a neural tube defect (NTD)-affected pregnancy.[1] Strategies to reduce NTDs with folic acid include supplement use and food fortification.[2] One strategy for NTD prevention is the use of fortified foods targeted for use by women planning a pregnancy. Milk powders fortified with folic acid and other micronutrients designed for use prior to and during pregnancy are available.[3] Whether folic acid fortified milk (FM) will lower NTD risk is not known and it would be unethical to conduct studies in women who could become pregnant. However, in a case-control study in Ireland, the risk of NTD was inversely associated with maternal red cell folate (RCF) and plasma folate (PF) concentrations in early pregnancy, with the lowest NTD risk associated with a RCF >905 nmol/L or a PF >35 nmol/L.[4] Accordingly, if FM increases blood folate concentrations, it could be expected to decrease the risk of NTDs.

In a study in New Zealand, we randomized non-pregnant women to milk powder providing 400 μg/day folic acid (fortified milk) or an unfortified powder.[3] After 12 weeks, RCF and PF concentration were over 500 nmol/L and 35 nmol/L higher in the women receiving the fortified milk group compared with the placebo milk. Further, over 90% of women had achieved a RCF >905 nmol/L in the fortified milk group compared with only 18% in the placebo milk group. However, participants in this study were primarily of European ethnicity; whereas, milk powders are mainly available in Asia where milk consumption, while increasing, is less common. Further, there is a common perception of milk intolerance among Asian populations. Previous studies have documented prevalence of lactose malabsorption and intolerance among Asian adults,[5-8] which may present in individuals with symptoms that cause the avoidance of milk-based products. These factors may be affecting the acceptance and consumption of these fortified milks in this population.[9] As such, it is important to determine the efficacy and acceptability of fortified milk at increasing blood folate concentrations in this population. Our main object-

Corresponding Author: Dr Tim J Green, Food, Nutrition, and Health, The University of British Columbia, 2205 East Mall, Vancouver, BC, V6T 1Z4, Canada.
Tel: 604-822-0421; Fax: 604-822-5143
Email: tim.green@ubc.ca; tigreen@mail.ubc.ca
Manuscript received 18 November 2014. Initial review completed 22 December 2014. Revision accepted 10 January 2015.
doi: 10.6133/apjcn.2016.25.1.08

ive was to determine whether consumption of fortified milk providing 400 µg/d folic acid increases blood folate to concentrations associated with a low NTD risk in Singaporean women of childbearing age.

These milks are typically fortified with other vitamins including B-12, D and B-6, which are also important during pregnancy. A lack of vitamin B-12 has been associated with an increased risk of NTDs.[10,11] Poor vitamin D status during pregnancy may affect calcium homeostasis and skeletal mineralization of the unborn child and has been associated with an increased risk of pre-eclampsia.[12,13] In addition, vitamin B-6 supplementation may help alleviate symptoms of morning sickness such as nausea and vomiting which can occur in early pregnancy.[14] Therefore our secondary objective was to determine the effect of fortified milk compared to placebo milk on serum B-12, plasma 25 hydroxyvitamin D (25OHD), and plasma pyridoxal 5'-phosphate (PLP), which are indicators of vitamin B-12, D, and B-6 status, respectively.

MATERIALS AND METHODS

Participants

Women were recruited from Changi General Hospital, Singapore through advertisement and word-of-mouth. To be eligible to participate women had to be: between 21-35 y; able to understand potential risks and side effects; willing to consent to study participation and to comply with study requirements; and non-pregnant as confirmed by a pregnancy test at baseline. Women were excluded if they had: taken supplements known to contain folic acid in the previous 6 months; a chronic disease (e.g., diabetes mellitus, cardiovascular disease); used medications known to interfere with folate metabolism (e.g., methotrexate, sulfasalazine, or anti-convulsive medications); milk and/or lactose-intolerance; been pregnant in the last 12 months, planning a pregnancy, or had a prior history of a NTD-affected pregnancy. The Singhealth Centralised Institutional Review Board approved the study and all participants gave informed written consent. This trial was registered at www.clinicaltrials.gov (NCT 01712165).

Intervention

This was a 12 week double-blind randomized placebo-controlled study. At baseline, participants attended a morning clinic following an overnight fast where blood was collected by venipuncture. A questionnaire was used to obtain demographic information. Weight and height were measured using standardized methods. A statistician not involved in the study prepared the randomization scheme. Subjects were randomized in equal proportions (1:1) using a blocked randomization list to one of two treatment groups: Fortified Milk, or Placebo (standard [unfortified] Milk). The Sponsor labeled all study products with a product code before the study commenced. Both products were identified using a blinded product code and the product identity of the product code was not known to the investigators. Study personnel received the randomization list of subject code numbers in ascending order with allocation to a product number and assigned subject numbers sequentially to the subjects as they enrolled.

Women were provided with verbal and written instructions on how to prepare the milk powder. Women were telephoned weekly for the first two weeks and then fortnightly thereafter. Women were asked to return to the clinic after 6 weeks and 12 weeks where blood was collected, weight measured, and compliance assessed. Compliance was assessed as the self-reported number of servings of study product consumed during the study divided by the number of possible servings.

Milk powder

The products to be used in this study were Anmum™ Materna fortified milk (referred to as "FM") and unfortified placebo milk ("PM"). The FM and PM powder were identical except that the FM contained added micronutrients. Milk powders were identical in color, taste, and smell, and were manufactured by Fonterra Brands (Singapore) PTE LTD. Women were asked to consume 75 g milk powder daily as 37.5 g powder in 200 mL water twice daily (morning and evening) during the 12 week supplementation period. Both milks contained (naturally) per 75 g: 38 µg folate; 1.2 µg B-12; 0.4 µg vitamin D; and 0.4 mg vitamin B-6. The FM contained an additional 400 µg folic acid, 4.6 µg vitamin D_3, 1.4 µg vitamin B-12 (cyanocobalamin), 1.2 mg vitamin B-6 (pyridoxine), and 1.05 x 10^7 cfu probiotic DR10™ (*Bifidobacterium Lactis HN019*).

Laboratory assessment

Blood was collected into two vacutainer tubes, one that contained EDTA and one that contained no anticoagulant. A complete blood count was performed using an automated hematology analyzer (Sysmex XT-169 1800i, Sysmex Corp). For the whole blood folate analyses, an aliquot of EDTA whole blood was diluted 10-fold in 1% ascorbic acid to lyse the cells and protect folate from oxidation. The evacuated tubes were centrifuged, the plasma and serum removed, aliquoted, and stored at -80°C until analysis.

PF and whole blood folate concentrations were determined using the microtiter technique described by O'Broin and Kelleher with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism.[15] RCF was calculated from whole blood folate by subtracting PF and correcting for hematocrit. The inter-assay CV for the folate assay was 8.7% on the basis of repeated measurements of a pooled control. Serum vitamin B-12 concentrations were assayed using the Elecsys® 2010 (Roche Diagnostics, Switzerland) automated electrochemiluminescence immunoassay. The control samples provided by the manufacturer were within the recommended range and the inter-assay CV based on a pooled serum was 8.3% (n=13).

Plasma total 25OHD serum aliquots were batch analyzed for $25OHD_2$ and $25OHD_3$ by isotope-dilution liquid chromatography tandem mass spectrometry method using an API 3200 instrument (Applied Biosystems) connected to a Dionex Ultimate 3000 HPLC system.[16] To assess accuracy and inter-assay variability, external quality control serum material (UTAK Laboratories) containing low and medium $25(OH)D_3$ and $25(OH)D_2$ were analyzed with every run. The $25OHD_3$ low control, verified value 29.9 nmol/L, mean was 28.3 nmol/L (SD 0.7); CV 2.6%,

and the medium control, verified value 79.9 nmol/L, mean was 78.8 nmol/L (SD 3.6); CV4.5%. For $25OHD_2$ the low control, verified value 26.6 nmol/L, mean was 25.2 nmol/L (SD 0.9); CV 3.5% and the medium control, verified value 77.5 nmol/L, mean was 74.8 nmol/L (SD 2.7); CV 3.7%. Internal quality control pooled serum samples were also analyzed, the inter-assay CV for $25OHD_3$ was 3.7% at 46.7 nmol/L (n=8). The level of $25OHD_2$ in the internal controls was below the limit of quantification. Plasma PLP (as a marker of vitamin B-6 status) was measured using HPLC according to the method by Ubbink et al[17] with modifications.

The low, medium, and high external controls for plasma PLP (IRIS Technologies International GmbH) showed an inter-assay variability (CV%) of 7.8%, 5.0%, and 5.8%, respectively. Mean (±SD) plasma PLP was 47.1 (±3.7) nmol/L for the low control, verified value 45.7 nmol/L; 84.4 (±4.2) nmol/L for the medium control, verified value of 85.4 nmol/L; and 118.5 (±5.8) nmol/L for the high control, verified value of 125 nmol/L.

Statistical analysis

The sample size was calculated based on an expected mean difference in RCF concentration of at least 140 nmol/L between the two groups. A sample size of 30 per group was estimated to be sufficient to detect this difference with 80% power assuming a standard deviation of 80 nmol/L and a two-sided alpha of 0.05. We assumed 15% attrition over the period of study, thus 70 women were recruited in total.

Data were analyzed as intent-to-treat (ITT) where, in the case of missing values, the last available value for that participant was carried forward. As treated (AT) analysis was also carried out where dropouts were excluded from the analysis. Continuous variables were checked for normality. Plasma PLP was not normally distributed and was therefore transformed using the natural log (ln). The differences in blood vitamin concentration between treatment groups at 12 weeks determined using ANOVA and least significant differences test after adjustment for baseline vitamin concentration. Fisher's Exact Test was used to compare the percentage of women achieving a RCF >905 nmol/L and PF >35 nmol/L at 6 and 12 weeks. Results were considered significant at $p<0.05$. All analyses were performed using SPSS Statistics 18.0 for Macintosh (Armonk, USA). Values in the text are means±SDs or n (%) unless otherwise indicated.

RESULTS

Seventy-six women were screened for eligibility and 6 did not meet the inclusion and exclusion criteria. Seventy women were randomized to treatment with 35 each to the FM or PM groups. By 6 weeks, 6 women had withdrawn from the study: 4 in the FM group and 2 in the PM group. By 12 weeks, 3 additional women had withdrawn: 2 in the FM group and 1 in the PM group. Of the 9 withdrawals, 6 complained of gastrointestinal complications (usually diarrhea and/or bloating), 1 complained of lethargy, 1 a sore throat, and 1 woman was lost to follow-up. The overall drop out rate was 12%. The participant flow and follow-up is shown in Figure 1. Of the 61 women who completed the study, compliance was high with these

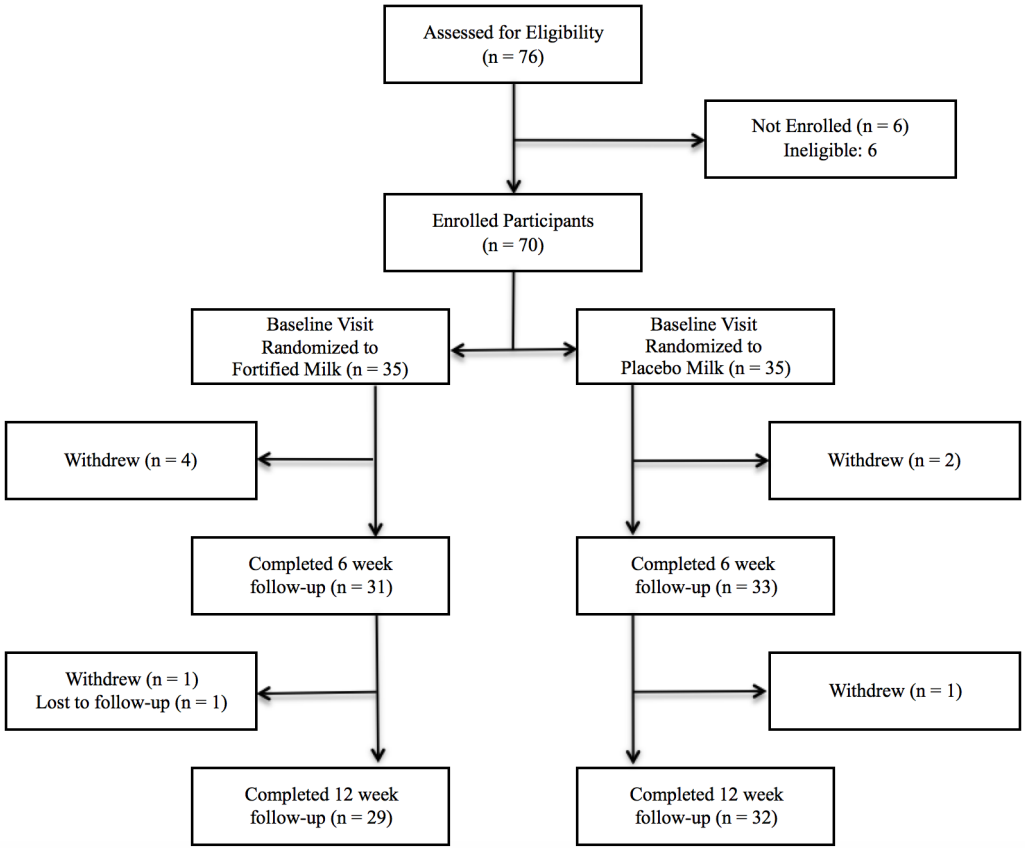

Figure 1. Participant flow and follow-up.

women consuming >90% of the milk on average. Only 5 women reported consuming <80% of the milk.

Participant characteristics by treatment group are shown in Table 1. The overall mean age of the participants was 27 y with a range of 21-35 y. Most of the women were Chinese (67%, n=47, followed by South Asian (11%, n=8), Malay (10%, n=7) and Other (11% overall; Filipino: n=6, Indonesian: n=1, and Burmese: n=1). There were very few smokers, most women had a normal BMI, and just over 30% had a prior pregnancy. At baseline there was no biochemical evidence of B-12 (<150 pmol/L) or B-6 (PLP <20 nmol/L) deficiency.[18] There was no biochemical evidence of folate deficiency (PF <6.8 nmol/L; RCF <227 nmol/L),[19] however, only 15% and 28% of the women had a RCF and PF consistent with a very low risk of NTDs (>905 nmol/L and >35 nmol/L, respectively). Based on a 25OHD concentration <30 nmol/L, only 7% of women had biochemical evidence of vitamin D deficiency. Almost 65% of women had a 25OHD consistent with vitamin D insufficiency (<50 nmol/L).[20] Mean (95% CI) bodyweight was 55 g (-600, 700 g) higher at 12 weeks in the PM group than the FM group after adjustment for baseline bodyweight (p=0.671). There was no significant differences in weight gain between groups over the 12 weeks.

The main outcomes of the intervention using ITT anal-

Table 1. Participant characteristics[†]

Characteristic	Fortified milk (n=35)	Placebo milk (n=35)
Age, y	27±3	28±4
Ethnicity		
Chinese	25 (72)	22 (63)
South Asian	2 (6)	6 (17)
Malay	4 (11)	3 (9)
Other	4 (11)	4 (11)
Married	6 (17)	15 (43)
Smoker	2 (6)	2 (6)
BMI, kg/m^2	22±5	23±5
Underweight (<18.5)	4 (11)	8 (22)
Normal weight (18.5-25)	28 (80)	16 (45)
Overweight (25-30)	1 (3)	6 (17)
Obese (≥30)	2 (6)	5 (14)
Prior pregnancy	10 (29)	12 (34)
Red cell folate, (nmol/L)		
<300	0	0
<905	31 (89)	28 (80)
Serum 25 hydroxyvitamin D (nmol/L)		
<30	2 (6)	3 (9)
<50	23 (66)	22 (63)
Plasma vitamin B-12 (pmol/L)		
<150	0	0
Plasma pyridoxal 5'phosphate (nmol/L)		
<20	0	0

[†]Total n=70. Values are mean±SD or n (%).

Table 2. Vitamin concentrations in women of childbearing age consuming fortified or placebo milk for 12 weeks using intent to treat analysis[†]

Outcome	n	Baseline	6 weeks	12 weeks	Difference (95% CI)	p
Red cell folate (nmol/L)						
Fortified milk	35	672±316	890±245	1115±347	376 (240, 512)[‡]	<0.001
Placebo milk	35	709±316	700±245	744±205		
Plasma folate (nmol/L)						
Fortified milk	35	27±13	65±25	72±35	39 (26, 51)[‡]	<0.001
Placebo milk	35	30±17	30±15	34±14		
Plasma vitamin B-12 (pmol/L)						
Fortified milk	35	393±146	-	475±167	50 (11, 89)[‡]	<0.001
Placebo milk	35	337±90	-	370±111		
Serum 25-hydroxyvitamin D (nmol/L)						
Fortified milk	35	46±14	-	52±13	5 (1, 8)[‡]	0.01
Placebo milk	35	47±15	-	48±14		
Plasma pyridoxal 5'-phosphate (nmol/L)						
Fortified milk	35	71±39[§]	-	82±38	34 (16, 52)[¶]	<0.001
Placebo milk	35	58±25	-	52±22		

[†]Values are mean±SD unless otherwise stated.
[‡]Difference at 12 weeks relative to placebo milk, adjusted for baseline (ANOVA and least significant differences test).
[§]Geometric mean.
[¶]Percentage difference at 12 weeks relative to placebo milk, adjusted for baseline.

ysis are shown in Table 2. At 12 weeks, mean (95% CI) RCF and PF concentrations were 376 nmol/L higher (240, 512 nmol/L) and 39 nmol/L higher (26, 51 nmol/L) in the FM group compared with the PM group, respectively (p<0.001). Likewise, plasma vitamin B-12, serum 25OHD and plasma PLP were significantly higher in the FM group compared with the PM group.

The outcomes using AT analysis, where women whom withdrew from the study were excluded (n=6), are shown in Table 3. At 12 weeks, mean (95% CI) RCF and PF concentrations were 475 nmol/L higher (347, 600 nmol/L) and 47 nmol/L higher (35, 58 nmol/L) in the FM group compared with the PM group, respectively (p<0.001). There was a higher mean difference in RCF and PF concentrations among the FM and PM groups using AT analysis, as compared to ITT analysis. Plasma vitamin B-12, serum 25OHD and plasma PLP were also significantly higher in the FM group compared with the PM group.

Figure 2a presents the percentage of women by treatment group achieving a RCF >905 nmol/L using ITT analysis. At 6 weeks, 57% (n=20) of women in the FM group had a RCF >905 nmol/L compared with only 17% (n=6) in the PM group. By 12 weeks, the corresponding percentages were 71% (n=25) and 26% (n=9). In those who completed the study (AT analysis), 84% (n=25) of women in the FM group had a RCF >905 nmol/L compared with only 27% (n=9) in the PM group. Figure 2b presents the percentage of women by treatment group achieving a PF >35 nmol/L using ITT analysis. At 6 weeks, 80% (n=28) of women in the FM group had a PF >35 nmol/L compared with only 29% (n=10) in the PM group. By 12 weeks, the corresponding percentages were 86% (n=30) and 26% (n=9). In those who completed the study (AT analysis), 93% (n=27) of women in the FM group had a PF >35 nmol/L compared with 50% (n=16) in the PM group.

DISCUSSION

Severe folate deficiency has been previously shown to be rare in Southeast Asia. However, many women do not have blood folate concentrations associated with a low risk of an NTD-pregnancy.[21] Blood folate concentration during early pregnancy has been inversely related to NTD-risk. We have previously shown that fortified milk powder increased RCF in non-pregnant women to levels associated with a very low risk of NTDs in New Zealand women of childbearing age.[3] Fortified milk powders are popular in Asia. However, we lack data on the efficacy of these milks in Asian women where acceptability and tolerance, and therefore acceptance, may be lower. Here we have shown that milk fortified with 400 µg/d folic acid as well as other micronutrients is well tolerated, did not increase body weight, and is effective at increasing RCF concentration in Singaporean women to optimal levels by 12 weeks. RCF concentrations were 376 nmol/L higher using ITT analysis in the FM group than the PM group, an amount that would be expected to decrease NTD risk, if these women became pregnant. These findings are somewhat lower than those we reported for New Zealand women where after 12 weeks, RCF was 540 nmol/L higher in the FM group than the PM group.[3] The reason for this is not clear. One potential explanation is that RCF was ~150 nmol/L lower at baseline in our study as compared to the New Zealand study. However, we have shown that folic acid, either as a supplement or in fortified milk, increases RCF concentration irrespective of baseline folate status.[3, 20]

One of our concerns was that Asian women would dropout or not comply in a study that involved 12 weeks of milk consumption. The dropout rate was only 5% higher (15% vs 10%) in this study compared with our previous study of New Zealand women, which is minor. However, when we included only women who did not complete the trial (AT analysis), the difference between the FM and PM was 475 nmol/L, which is more similar to our New Zealand findings.

The increase in RCF in the current study is similar to that achieved with supplements and other fortified foods.

Table 3. Vitamin concentrations in women of childbearing age consuming fortified or placebo milk for 12 weeks using as treated analysis[†]

Outcome	n	Baseline	6 weeks	12 weeks	Difference (95% CI)	p
Red cell folate (nmol/L)						
Fortified milk	29	614±186	927±242	1200±311	475 (347, 600)[‡]	<0.001
Placebo milk	32	717±329	716±249	744±205		
Plasma folate (nmol/L)						
Fortified milk	29	28±14	71±16	82±32	47 (35, 58)[‡]	<0.001
Placebo milk	32	30±18	31±20	34±14		
Plasma vitamin B-12 (pmol/L)						
Fortified milk	28	376±115	-	475±153	62 (20, 103)[‡]	<0.001
Placebo milk	32	338±86	-	373±140		
Serum 25-hydroxyvitamin D (nmol/L)						
Fortified milk	29	43±10	-	50±9	4 (1, 8)[‡]	0.02
Placebo milk	32	48±15	-	50±13		
Plasma pyridoxal 5'-phosphate (nmol/L)						
Fortified milk	29	69±35[§]	-	79±35	35 (14, 56)[¶]	0.001
Placebo milk	32	58±26	-	51±23		

[†]Values are mean±SD unless otherwise stated. As treated analysis where dropouts were excluded.
[‡]Difference at 12 weeks relative to placebo milk, adjusted for baseline (ANOVA and least significant differences test).
[§]Geometric mean.
[¶]Percentage difference at 12 weeks relative to placebo milk, adjusted for baseline.

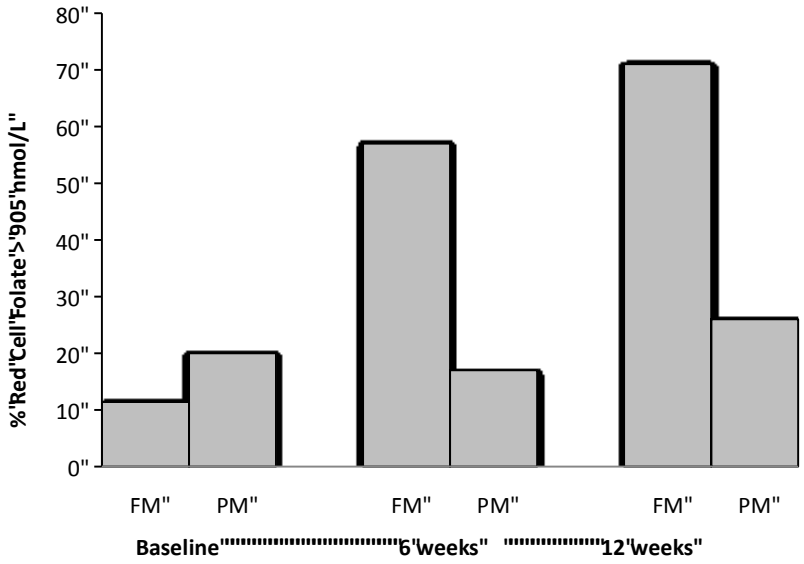

Figure 2a. Percentage of women (n=70) by treatment group achieving a red cell folate (RCF) >905 nmol/L using intent to treat analysis. FM: fortified milk; PM: placebo milk.

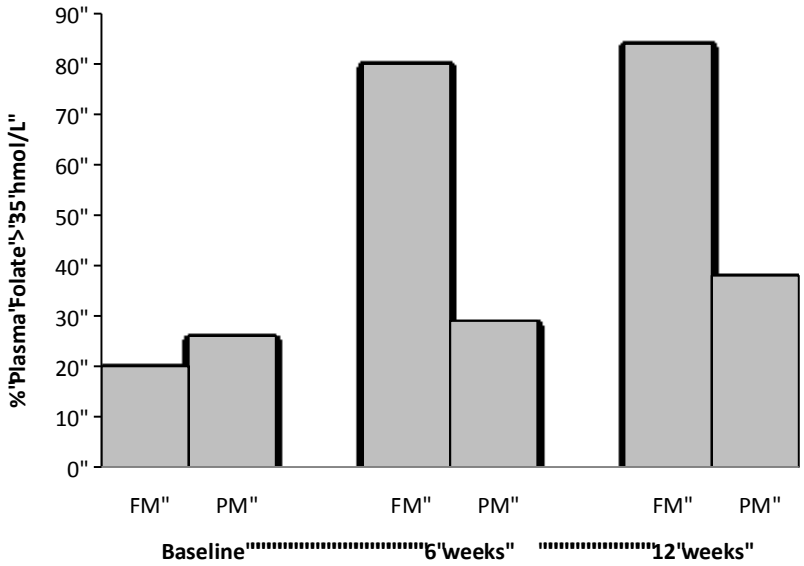

Figure 2b. Percentage of women (n=70) by treatment group achieving a plasma folate (PF) >35 nmol/L using intent to treat analysis. FM: fortified milk; PM: placebo milk

We previously reported RCF concentrations were 411 nmol/L higher in women taking 400 µg/d folic acid supplement compared to placebo after 12 weeks.[22] Similarly, Cuskelly et al[23] reported that a daily 400 µg/d folic acid, or consumption of fortified foods containing ~400 µg/d folic acid over 12 weeks increased RCF by 320 nmol/L and 392 nmol/L, respectively.

Based on a case-control study in Ireland, it has been suggested that achieving a RCF >905 nmol/L was associated with a very low risk of NTD.[4] In the current study, 71% (ITT analysis) and 84% (AT analysis) of women had achieved a RCF >905 nmol/L by 12 weeks, whereas, all but 1 woman failed to achieve this in the New Zealand study. The reason for this is likely that the Singaporean women had a lower baseline RCF. It is important to note that achieving a RCF of 905 nmol/L is not a threshold for NTD prevention. Rather, it was merely the lower bound of the highest quintile reported in a single case-control study in Ireland; the relationship between RCF and NTD risk is continuous. Thus, any increase in RCF would be predicted to further decrease NTD risk.

In the current study, it appears that RCF did not reach a threshold after 12 weeks. This is not surprising, as others have shown that it takes more than 12 weeks to reach a steady-state RCF on 400 µg/d folic acid.[24] In contrast, PF appears to have reached a steady concentration by 12 weeks in the current study. While RCF is often used as an indicator of NTD risk in epidemiological studies because it is more stable and a better indicator of long-term folate status, it is plasma that supplies the fetus with folate.[25] PF responds more quickly to supplementation and therefore may be a better indicator of a woman's NTD-risk in cases where a woman is taking additional folic acid prior to

becoming pregnant. A PF >35 nmol/L was associated with a very low risk in the case-control study in Ireland.[4] In our study, all but 14% (n=5) of women achieved a PF >35 nmol/L in the FM group after 12 weeks (using IT analysis).

Despite a lack of B-12 deficiency (<150 pmol/L) at baseline there was a 50 pmol/L (ITT analysis) higher plasma B-12 at 12 weeks in the FM group compared with the PM group. Plasma B-12 and other indicators of B-12 status have been shown to be inversely associated with the risk of NTDs. Molloy et al[11] suggest that women achieve a plasma B-12 of 221 pmol/L before becoming pregnant for NTD prevention. FM could help women achieve this. The FM provided in our trial provided 2.6 μg/d, which is based on the US recommended dietary allowance (RDA) for pregnant women. There are no studies of vitamin B-12 fortified milks, but our findings are similar to a study in older people where 2.5 μg/day vitamin B-12, as a supplement, increased serum B-12 by 64 pmol/L over 16 weeks.[26]

Serum 25OHD was significantly higher in FM milk group at 12 weeks but only by 5 nmol/L, which may not be clinically meaningful. The FM provided 5 μg/day vitamin D, which is 100% of the RDA for many Asian countries. Our results are consistent with dose response studies that indicate that for each 1 μg/d of vitamin D3, 25OHD concentrations increase by 0.7 nmol/L.[27] The Institute of Medicine (IOM) has stated that a serum 25OHD of 50 nmol/L is associated with the US RDA (15 μg/day), which meets or exceeds the needs of most people in Canada and the US, including pregnant women.[20] Other groups have called for higher levels of 25OHD during pregnancy, such as 75 nmol/L.[28] After 12 weeks on FM, almost 63% (n=22) of women had not achieved a serum 25OHD >50 nmol/L and only 1 woman had a serum 25OHD >75 nmol/L. Consideration should be given to raising the amount of vitamin D in fortified milk to 15 μg/day, as per the current US IOM recommendations. This is especially important in Asia, where other dietary sources of vitamin D are scarce and several studies have shown high levels of vitamin D deficiency among women of childbearing age, even in countries with high sun exposure located near the equator.[29-31]

There was no evidence of vitamin B-6 deficiency at baseline, but plasma PLP was significantly higher in the FM group. Results about the clinical significance of vitamin B-6 deficiency in pregnancy are inconclusive in a non-deficient population. Marginal vitamin B-6 deficiency (defined as having a plasma PLP concentration of between 20-30 nmol/L) was associated with a higher risk of preterm birth in Chinese women.[32] Vitamin B-6 adequacy in early pregnancy and prior to conception seems crucial, since low vitamin B-6 status was associated with low conception rate and a higher risk of early pregnancy loss.[33] Vitamin B-6 supplementation during pregnancy has been used to prevent vomiting and nausea during pregnancy but usually at much higher (10-25 mg every 8 hours) amounts than found in FM.[34]

We acknowledge two limitations of our study. First, women in our study were non-pregnant. However, it would be unethical to recruit and randomize pregnant women to a placebo that didn't contain folic acid. Further, the neural tube closes early in pregnancy at around four weeks, so it is the period prior to pregnancy and very early pregnancy that is most relevant. Second, we are relying on a study conducted in an Irish population to justify the association between blood folate and NTD risk and this may not apply to other groups. However, mandatory flour fortification in several countries has increased RCF concentrations and reduced the incidence of NTDs in the population, providing additional evidence for the association between RCF and NTD risk. Further, in Northern China NTD rates were high and RCF concentrations were low, whereas in Southern China a low rate of NTDs were accompanied by higher RCF concentrations, which suggests this relationship applies in the Asian context.[1,35]

In conclusion, we have shown that Singaporean women tolerated and complied with fortified milk in the ration of 75 g milk powder daily as 37.5 g powder in 200 ml water twice daily. This was effective at increasing their RCF to concentrations associated with a very low risk of an NTD-affected pregnancy. Fortified milk also improved vitamin D, B-12, and B-6 status in women, however consideration should be given to further increasing the amount of vitamin D in fortified milk.

ACKNOWLEDGMENTS

We gratefully acknowledge the women in Singapore who participated in our study.

AUTHOR DISCLOSURES

Disclaimers: Tim Green was a paid consultant for Fonterra Brands New Zealand Ltd. Sources of support: Fonterra Brands (Singapore) PTE LTD. All other authors declare no conflicts of interest.

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Original Article

Folic acid fortified milk increases blood folate to concentrations associated with a very low risk of neural tube defects in Singaporean women of childbearing age

Magdalin Cheong MSc[1], Huang Ying Xiao MSc[1], Vicky Tay PhD[2], Crystal D Karakochuk MSc[3], Yazheng Amy Liu MSc[3], Sarah Harvey MSc[3], Yvonne Lamers PhD[3], Lisa A Houghton PhD[4], David D Kitts PhD[3], Tim J Green PhD[3]

[1]*Dietetic & Food Services Department, Changi General Hospital, Singapore*
[2]*Sprim Advanced Life Sciences, Singapore*
[3]*Food, Nutrition, and Health, the University of British Columbia, Vancouver, Canada*
[4]*Department of Human Nutrition, University of Otago, Dunedin, New Zealand*

新加坡育龄妇女通过服用叶酸强化牛奶提高血液叶酸浓度，与低神经管缺陷风险有关

背景与研究目的：围孕期服用叶酸（400 微克/天）可以降低患有神经管缺陷的几率 75%以上。红细胞叶酸（RCF）含量大于 905 nmol/L 或血浆叶酸（PF）大于 35 nmol/L 时与神经管缺陷的低风险有关。我们确定育龄新加坡妇女每天摄入叶酸强化牛奶是否会增加血液中叶酸浓度，与神经管畸形的低风险水平有关。**方法与研究设计**：在为期 12 周的双盲安慰剂对照试验中，70 名非孕期妇女（21 至 35 岁），分别随机给予添加了含量为每天 400 微克叶酸的奶粉（FM）和未添加叶酸的安慰剂奶粉（PM）。分别在实验初始、6 周以及 12 周对研究对象进行血液样本采集。**结果**：在 12 周时，相比于 PM 组，FM 组研究对象的红细胞叶酸与血浆叶酸浓度的平均值（95% CI）分别高出 376（240，512）和 39（26，51）nmol/L （p<0.001）。在 FM 组中，分别有 71%（n=25）以及 80%（n=30）的研究对象实现了 RCF 和 PF 与极低神经管缺陷风险有关。**结论**：育龄妇女服用添加了叶酸的牛奶可以提高血液叶酸含量并与降低神经管缺陷影响的怀孕有关。

关键词：叶酸盐、叶酸、强化、奶、神经管缺陷