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Consumption of Folic Acid–Fortified Bread Improves Folate Status in Women of Reproductive Age in Chile[1]

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ABSTRACT Since January 2000 the Chilean Ministry of Health has required the fortification of wheat flour with folic acid (FA) at a concentration of 2.2 mg FA/kg in order to reduce the risk of neural tube defects (NTD) in newborns. This policy was expected to result in a mean additional intake of ~ 400 μg FA/d. We assessed the effectiveness of the FA flour fortification program on bread folate content and on blood folate concentration in women of childbearing age in Santiago, Chile. The prefortification folate status of 751 healthy women of reproductive age was assessed. The folate content of 100 bread samples bought at retail bakeries was measured, average wheat flour consumption was estimated and postfortification FA dietary intake was calculated. The effect of flour fortification on blood folate concentration in this group of women (n = 605) was evaluated in a follow-up study. Blood folate concentrations of the 605 women in the follow-up group increased (P < 0.0001) following fortification. Before fortification the mean serum and red blood cell folate concentrations were 9.7 ± 4.3 and 290 ± 102 nmol/L, respectively, compared with 37.2 ± 9.5 and 707 ± 179 nmol/L postfortification, respectively. The mean FA content of bread was 2020 ± 940 μg/kg. The median FA intake of the group evaluated postfortification was 427 μg/d (95% CI 409–445) based on an estimated intake of 219 g/d (95% CI 201–229) of wheat flour, mainly as bread. Fortification of wheat flour substantially improved folate status in a population of women of reproductive age in Chile. The effect of the FA fortification program on the occurrence of NTD is currently being assessed. **J. Nutr. 133: 3166–3169, 2003.**

KEY WORDS: • folic acid • fortification • folate status • neural tube defects

Periconceptional consumption of folic acid (FA)[3] by women significantly reduces the risk of neural tube defects (NTD) in their infants 1,2). The addition of 400 μg of FA to the daily diet in the form of supplements or fortified foods is recommended to reduce the risk of NTD (3). In the United States and Canada, mandated FA fortification has been associated with significant increases in blood folate concentrations (4–6) and reported reductions in the incidence of NTD (7–10).

In Chile, wheat flour has been fortified with iron and B vitamins by legislation since the 1950s, and in January 2000 the addition of 2.2 mg/kg of FA to wheat flour was mandated. Based on estimates of wheat flour consumption, this intervention was expected to result in an additional daily intake of ~400 μg FA by women of reproductive age. The effect of consumption of FA-fortified wheat flour on blood folate concentrations of young women in Santiago, Chile was assessed and is the focus of this report.

METHODS

Assay for total folate in bread. The Chilean wheat flour fortification program monitors concentrations of iron and B vitamins but not FA. To determine the FA content of the type of bread typically consumed in Chile (770 g wheat flour/kg bread), 1 kg of FA-fortified bread was purchased from each of 50 randomly selected retail bakeries at 3 and 6 mo after initiation of the FA fortification program (i.e., 100 samples, 50 bakeries sampled twice). The samples, consisting of ~11.5 loaves of bread per sample (~87 g/loaf), were transported to the laboratory for processing. The loaves of bread comprising each sample were diced, and about 20 g from each loaf was put in a preweighed paper bag to yield a representative composite. The 100 sample composites were then weighed, dried in an oven at 105°C for 24 h, cooled in a desiccator, reweighed, ground and transferred to coded plastic bags in 30-g portions. The sample composites were frozen and shipped to the Food and Science and Human Nutrition Department at the University of Florida, where folate was extracted from the samples in duplicate using a modification of the tri-enzyme extraction method (11). Folate content was measured using the microplate adaptation of the microbiological assay (12).

Subjects. The ethics committee of the Institute of Nutrition and Food Technology approved the study protocol, and written informed consent was obtained from each of the participants. Serum and RBC folate concentrations in 751 women attending the Maternal and Infant Health Program of the National Health Service at three outpatient clinics in Santiago, Chile were measured before flour fortification began (October to December 1999). The women were of

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[3] Abbreviations used: FA, folic acid; NHANES, National Health and Nutrition Examination Survey; NTD, neural tube defects.

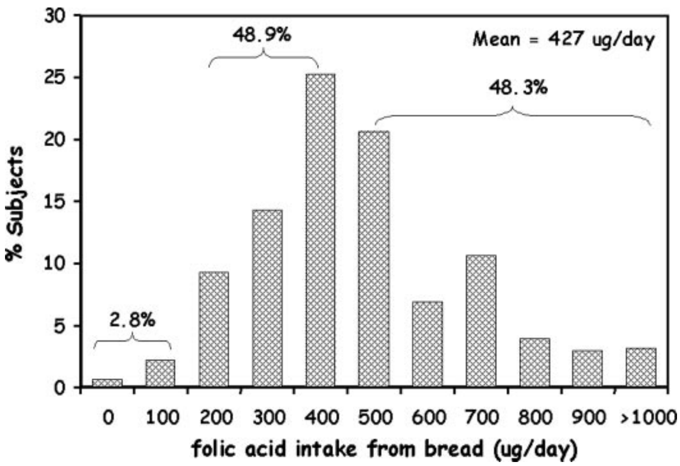

FIGURE 1 Estimates of daily folic acid intake from bread by 605 women in Chile after fortification of wheat flour, calculated from bread folate content and daily bread consumption.

reproductive age (29.6 ± 7 y) with no history of an NTD-affected pregnancy, and all were from low socioeconomic households. Other characteristics of the group included the following: *1*) mean body mass index of 26.4 ± 5.1, *2*) multiparous reproductive history (2.2 ± 1.2 children), *3*) 12.6% were anemic (Hb < 120 g/L), *4*) 75% breastfed their last child > 6 mo, *5*) 23% used an oral contraceptive agent and *6*) ~60% did not smoke or consume alcohol. During the postfortification follow-up period (October to December 2000), folate status was reassessed in 605 women (81% of the initial group).

Procedures. In each outpatient clinic, potential participants were asked to volunteer for the study by research staff. Dietary intake data were obtained in the clinic using a combination of a 24-h recall and a food frequency questionnaire specifically designed to assess the intake of bread and other wheat flour–based foods, FA-fortified foods and vitamin supplements. Estimated FA intake was calculated from the mean bread folate content and bread consumption estimates derived from the mean value of data obtained from both the 24-h recalls and the food frequency questionnaires. Venous blood samples were drawn, immediately placed on ice, transported to the laboratory, processed and stored frozen. Contact with participants was maintained during the following month with three home visits. Follow-up involved collection of the same food consumption data and blood samples for status assessment after 1 y. Frozen blood samples were shipped on dry ice to the Centers for Disease Control and Prevention in Atlanta, GA, for analysis. Serum and RBC folate and serum vitamin B-12 concentrations were analyzed using the Bio-Rad Laboratories QuantaPhase II Folate Assay kit (Hercules, CA).

Statistical analysis. Statistica for Windows version 4.5 (StatSoft, Tulsa, OK) was used, and results were expressed as mean ± SD and median (95% CI). Changes in blood folate concentration following FA fortification were evaluated with Wilcoxon tests for continuous variables. Proportions and correlations were compared using chi-square and Pearson's correlation. All tests for significance were two tailed and performed at $\alpha = 0.05$.

RESULTS

The mean folate concentration of the 100 bread samples was 2020 ± 940 μg/kg (range 220 to 4160 μg/kg). Of the fortified bread samples, 18 contained less FA than the lower limit set by law (1540 μg/kg), with 9 of them (2 from May and 7 from August) containing <370 μg/kg, suggesting they were made from unfortified flour; 30 samples contained more FA than the upper limit established by law (2610 μg/kg). Comparable estimates of bread consumption were obtained from the 24-h recalls and the food frequency questionnaires. The group consumed a median of 219.5 g of wheat flour (24-h recall), 78% as bread, 14% as pasta, and the remaining 8% as other domestic preparations. The estimated median bread intake was the same before and after fortification (245 vs. 239 g/d). These values correlate positively ($r = 0.58$, $P < 0.001$). On a daily basis, 98% of the women consumed bread, and 89% consumed >180 g/d. Of the bread consumed, 97% was industrially processed, corresponding to the type of bread typically consumed in Chile and analyzed for this study. None of the subjects consumed other FA-fortified foods, and none took FA supplements. Mean FA intake was 427 ± 18 μg/d (95% CI 409–445), calculated from estimates of the daily intake of FA from fortified bread based on reported consumption by the women studied (**Fig. 1**). Almost half of the subjects (48%) consumed >400 μg FA/d. Only 3% consumed <100 μg/d. The intake of FA from bread for the rest of the group (49%) ranged from 100 to 400 μg/d.

Both serum and RBC folate concentrations increased significantly ($P < 0.0001$) between the pre- and postfortification periods for the 605 women (2.8- and 1.4-fold, for serum and RBC folate, respectively) (**Table 1**). As expected, vitamin B-12 concentrations did not change during this time. Postfortification, none of the women were classified as folate-deficient compared to some evidence of low blood folate concentrations prefortification.

The distribution curves for serum and RBC folate concentrations before and after fortification showed a striking shift to the right (**Fig. 2***A* and *B*), in contrast with the vitamin B-12 distribution curves, which showed little change (Fig. 2*C*).

The RBC folate concentration was significantly higher in women consuming >400 μg FA/d than in women consuming 100 to 400 μg FA/d (689 ± 170 vs. 732 ± 159 nmol/L, $P = 0.006$).

DISCUSSION

To our knowledge, this is the first study specifically designed to measure the effect of an FA fortification program in a population group. Furthermore, all available data are from industrialized countries. Our findings demonstrate that regular

TABLE 1

Serum and RBC folate concentrations and serum vitamin B-12 concentration in women of reproductive age in Chile pre- and postfortification of wheat flour with folic acid[1]

Parameter	Prefortification	Postfortification
Status indicators[2]		
Serum folate, *nmol/L*	9.7 ± 4.32	37.2 ± 9.5[a]
RBC folate, *nmol/L*	290 ± 102	707 ± 179[a]
Blood vitamin B-12, *pmol/L*	266 ± 105	268 ± 165
Below reference range, %		
Serum folate,[3] < 3.2 nmol/L	1.3	0
RBC folate,[3] < 181 nmol/L	10.6	0
Low blood vitamin B-12,[4] < 148 pmol/L	9.0	10.4
Borderline blood vitamin B-12,[4] 149–185 pmol/L	12.8	13.0

[1] The group was examined before exposure to flour fortified with folic acid and 10 months after fortification was legally mandated, *n* = 605.

[2] Values are means ± SD. Means with a superscript differ significantly from prefortification values ($P < 0.0001$).

[3] Reference ranges from the National Health and Examination Survey (6).

[4] Reference ranges from IOM report.

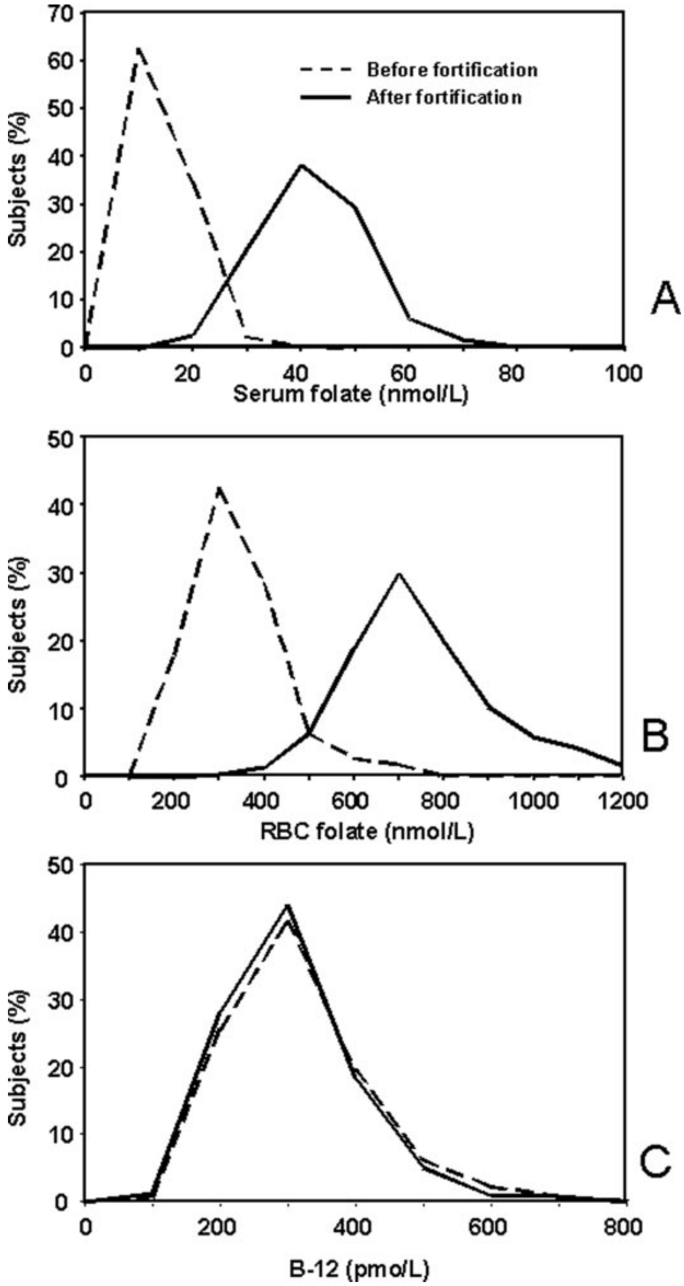

FIGURE 2 Serum folate, red cell folate, and serum vitamin B-12 distribution curves for women of reproductive age in Chile, before and after folic acid fortification of wheat flour.

consumption of an FA-fortified staple food is highly effective in improving folate status in women of childbearing age. Serum and RBC folate concentrations significantly increased after consuming FA-fortified wheat flour for 10 mo.

Estimated daily FA consumption in Chile after mandatory wheat flour fortification (2.2 mg FA/kg) was 427 μg. The changes in blood folate concentrations measured in our study group before and after fortification were similar to the changes observed in all U.S. women of reproductive age monitored by the third National Health and Nutrition Examination Survey (NHANES 1994–1998) compared with NHANES 1999, in which the mean serum folate concentration increased from 14.3 to 36.7 nmol/L, and the mean RBC folate concentration increased from 410 to 714 nmol/L (12). The corresponding blood folate concentration increases in this study were 9.7 to 37.2 nmol/L and 290 to 707 nmol/L for serum and RBC folate, respectively. The blood folate analyses for both this study and NHANES were done in the same laboratory (CDC, Atlanta, GA) using the same analytical technique. This is an important consideration when comparing folate data from different studies due to the recognized variability of analytical methodology (13).

In the United States, mandatory FA enrichment of all cereal grain products was expected to result in an average increased intake of ~100 μg FA/d (14). However, recent estimates indicate that the actual increase due to fortification was approximately twice that figure (~200 μg/d) (15). This apparently relates to the fact that a considerable number of enriched products contain significantly higher concentrations of FA than required by law (16). Other factors that may contribute to the increase in postfortification blood folate concentrations in the United States are the increased availability and consumption of FA-fortified ready-to-eat breakfast cereals (17–18) and the small increase in the number of women using vitamin supplements containing folic acid during those years (12). In contrast, in Chile, the increase in blood folate concentration was mostly attributable to the increase in consumption of FA fortified wheat flour. The study group in Chile did not consume other FA-fortified foods, such as breakfast cereals, because they are not culturally accepted, are scarce and are economically out of reach. In addition, none of the study subjects took FA supplements because they have not been mandated or made available to this low-income population group by the Chilean public health service. Therefore, the FA-fortified wheat bread was the main source of folate in the population studied.

Significant increases in serum folate levels after 6 mo of fortification were also reported in a group of Chilean elderly who were the subjects of a follow-up to another study at the same time that the FA fortification program was begun (19). Elderly populations are considered to be at a higher risk of presenting with masked vitamin B-12 deficiency—deficiency in vitamin B-12 without anemia because of the masking effect of FA—when they increase their consumption of FA, because of their higher overall rate of low blood vitamin B-12 concentration. Interestingly, in a recent study, a group of 1573 U.S. elderly showed no evidence of an increase in low blood vitamin B-12 concentration without anemia after fortification of cereals with folic acid (20). In Chile, based on data suggesting that the elderly population (19) and women of fertile age (this study) are at risk of vitamin B-12 deficiency, fortification of wheat flour with this vitamin has been proposed.

The results of this evaluation of the impact of FA fortification on folate intake and blood folate concentrations lead us to expect that this intervention will significantly reduce the risk of NTD-affected pregnancies from January 2001 onward (21,22). To measure the effect of FA fortification of wheat flour in the prevention of NTD, we are comparing the frequencies of NTD in all births in the nine public hospitals in Santiago, 2 y before and 2 y after fortification (60,000 births per year).

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