Folic Acid Fortification Increases Red Blood Cell Folate Concentrations in the Framingham Study¹,²

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ABSTRACT In 1996 the Food and Drug Administration (FDA) issued a regulation to take effect in January 1998 that all enriched cereal grain products include 140 µg of folic acid/100 g. The present cross-sectional study was undertaken to assess the effect of this fortification on RBC folate concentrations in the Framingham Offspring Cohort. Among those who did not take B-vitamin supplements, we compared RBC folate in 561 individuals who were examined before implementation of the FDA mandatory folic acid fortification (not exposed) vs. 354 individuals who were examined after implementation of fortification (exposed). We calculated the prevalence of deficient (<160 µg/L, 362.6 nmol/L) and acceptable (>200 µg/L, 453.2 nmol/L) RBC folate concentrations in both groups. Those exposed to folic acid fortification had a mean RBC folate of 450.0 µg/L (1019.7 nmol/L), a value 38% higher than the mean RBC folate of 325.3 µg/L (737.1 nmol/L) in those who were not exposed to fortification (P < 0.001). The prevalence of individuals with deficient RBC folate was 4.9% in the group not exposed to fortification compared with 1.9% in the group exposed to fortification (P < 0.02), and the prevalence of individuals with acceptable RBC folate was 87.0% in the group not exposed to fortification compared with 96.1% in the group exposed to fortification (P < 0.001). Similar results were seen in individuals who used supplements containing B-vitamins. The results of this study showed that in this cohort, the introduction of folic acid fortification significantly improved folate nutritional status measured as RBC folate. J. Nutr. 131: 3277–3280, 2001.

KEY WORDS: • folate • folic acid • fortification • erythrocyte folate • nutritional status • humans

In September 1992 the Centers for Disease Control and Prevention (CDC)⁴ issued a recommendation that all women of childbearing age consume 400 µg of folic acid per day to decrease the risk of having a neural tube defect (NTD)-affected pregnancy (1,2). The proportion of women in the United States achieving this recommendation before conceiving was very small and a considerable proportion of all pregnancies were not planned (3,4). Consequently, the Food and Drug Administration (FDA) issued a regulation in March 1996 to take effect by January 1998 that all enriched cereal grain products (flour, rice, breads, rolls and buns, pasta, corn grits, corn meal, farina, macaroni and noodle products) be enriched with folic acid in addition to the iron, thiamin, riboflavin and niacin already added to these products. The amount of folic acid added to each product was to range from 95 to 309 µg folic acid/100 g of product. This range of fortification was selected on the basis of an overall fortification level of 140 µg folic acid/100 g of the cereal grain product (5). Using dietary modeling, the projected average daily increase of folic acid intake in the general population was estimated to be 100 µg (6), or one quarter of the recommended amount.

Using plasma samples from the Framingham Offspring Cohort Study, our research group recently showed that the implementation of folic acid fortification resulted in the doubling of plasma folate concentrations and an ~50% reduction in the prevalence of hyperhomocysteinemia among individuals who did not use B-vitamin supplements (7). The present study was undertaken to assess the effect of folic acid fortification on RBC folate concentrations in the same cohort. RBC folate is believed to be a good measure of long-term tissue folate status (8); it is the measure of folate status used by many investigators, particularly in research that addresses the protective effect of folic acid on NTD.

SUBJECTS AND METHODS

Study population. The Framingham Heart Study (FHS) is an epidemiologic study of heart disease established in Framingham, MA, between 1948 and 1950. This cohort originally included 5209 individuals and among them, 1644 husband and wife pairs. Their offspring and their spouses were recruited and invited to participate in the Framingham Offspring Study. Also, offspring who had only one parent participating in the FHS were invited to participate with their spouses in the Framingham Offspring Study if that parent had either abnormal lipoprotein patterns or coronary heart disease at the 1970

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biennial examination of the FHS. The first examination of the offspring cohort was in 1971, and they have typically been examined every 3–4 y. The ethnicity of the participants is almost exclusively non-Hispanic Caucasian (9).

Data from the 6th examination of the Framingham Offspring Cohort were used for this cross-sectional study. This examination started in January 1995, finished in August 1998, and 3532 individuals were examined. We focused our analyses on data from 872 individuals whose examination date preceded the implementation of folic acid fortification and from 626 individuals whose examination date was at least 2 mo after the implementation of this fortification. Details of the selection of both groups are explained elsewhere (7). Briefly, the folic acid fortification regulation gave two years notice to food manufacturers to allow time to change the labels and product formulations. In New England, there were very few products forti

ded by July 1997 (7). Thus, participants of the Framingham Offspring Study whose 6th examination occurred between January 1995 and September 1996 were considered as the group that was examined before implementation of folic acid fortification (or group not exposed to fortification). Those who attended the 6th examination between September 1997 and August 1998 were considered as the group that was examined after implementation of folic acid fortification (or group exposed to fortification). Individuals whose examination was in between those dates (October 1996–August 1997) were not considered in the analysis. This study was approved by the Human Investigations Review Committee at New England Medical Center and by the Institutional Review Board for Human Research at Boston University Medical Center.

Biochemical measurements. Individuals who participated in the 6th examination of the Framingham Offspring Cohort had a fasting blood sample taken in a supine position from an antecubital vein after a 12-h fast. Plasma was separated by centrifugation (800 g for 10 min) and plasma and red cell aliquots were frozen at −80° C until analyzed. Frozen RBC samples were extracted by suspension in a buffer containing 12.5 g/L sodium ascorbate and heating for 15 min in a boiling water bath. After cooling on ice, samples were centrifuged and the supernatant fraction was used to measure RBC folate content by the microbial assay (Lactobacillus casei) with conjugase treatment (10,11). RBC folate was measured as ng of folate/g hemoglobin (Hb). Values were converted to μg folate/L packed cells using the mean cell Hb concentration as described by O’Broin et al. (12).

Statistics. We performed separate analyses according to the use of B-vitamin supplements. RBC folate data were positively skewed; therefore these values were log-transformed before analysis. To determine the effect of folic acid fortification on RBC folate, we compared RBC folate geometric means in individuals exposed and not exposed to folic acid fortification using SAS PROC GLM program (13) with adjustment for age and number of cigarettes smoked per day. We also adjusted for folate intake calculated using the food composition database not modified to reflect folic acid fortification of enriched cereal grain products. In this way, we adjusted RBC folate values for dietary patterns related to folate intake (to ensure that any differences seen in RBC folate were due to fortification and not to differences in dietary patterns of folate or folic acid–containing foods). We also calculated the prevalence of deficient and acceptable RBC folate values in the group exposed and in the group not exposed to folic acid fortification. Deficient RBC folate values were defined as values <160 μg/L packed cells (<362.6 nmol/L) and acceptable RBC folate values as values ≥200 μg/L packed cells (>453.2 nmol/L) (14).

RESULTS

Results and characteristics of the population studied are summarized in Table 1. In this cohort, we measured RBC folate in a group of 872 individuals not exposed and in another group of 626 individuals exposed to folic acid fortification. We categorized them according to their B-vitamin supplement use. The mean age in each group was between 58 and 60 y old and the proportion of women within nonusers and users of B-vitamin supplements was similar between those exposed and not exposed to fortification, although women were more likely to use B-vitamin supplements. Red blood cell folate geometric means are reported; means and prevalences were adjusted by

TABLE 1

<table>
<thead>
<tr>
<th>B-vitamin supplement use</th>
<th>Nonexposed</th>
<th>Exposed</th>
<th>Nonexposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>561</td>
<td>354</td>
<td>311</td>
<td>272</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>58.0 (32–80)</td>
<td>60.1 (33–85)</td>
<td>58.3 (29–78)</td>
<td>59.6 (33–85)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Female</td>
<td>47.2</td>
<td>48.0</td>
<td>56.6</td>
<td>59.6</td>
</tr>
<tr>
<td>RBC folate,1,2,3 μg/L</td>
<td>325.3 (314.1–336.9)</td>
<td>450.0* (430.6–470.3)</td>
<td>548.1 (523.2–574.2)</td>
<td>679.5* (646.5–714.2)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RBC folate&lt;160 μg/L</td>
<td>4.9</td>
<td>1.9**</td>
<td>1.6</td>
<td>0**</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(3.4–6.4)</td>
<td>(0–3.8)</td>
<td>(0.6–2.6)</td>
<td>(0–1.1)</td>
</tr>
<tr>
<td>% RBC folate≥200 μg/L</td>
<td>87.0</td>
<td>96.1*</td>
<td>97.2</td>
<td>99.2</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(84.6–89.3)</td>
<td>(93.1–99.1)</td>
<td>(95.7–98.7)</td>
<td>(97.6–100)</td>
</tr>
</tbody>
</table>

1 RBC folate is log transformed, geometric means reported.
2 Means and prevalences were adjusted for age, number of cigarettes smoked per day and folate-related dietary patterns as explained in Subjects and Methods.
3 To convert values from μg/L to nmol/L, multiply by 2.666.
4 CI, confidence interval.

* P < 0.001 for the comparison with group not exposed to folic acid fortification; ** P < 0.05 for the comparison with group not exposed to folic acid fortification.
age, number of cigarettes smoked per day and folate-related dietary patterns. Sex and body mass index were not significant predictors of RBC folate and were not included in the final model.

Among individuals who did not use B-vitamin supplements, RBC folate concentrations were 38% higher in the group that was exposed to folic acid fortification ($P < 0.001$). The prevalence of individuals with deficient RBC folate concentrations ($<160 \mu g/L$, 362.6 nmol/L) was 4.9% in the group that was not exposed to folic acid fortification and 1.9% in the group that was exposed to fortification ($P < 0.02$). The prevalence of individuals with acceptable RBC folate concentrations ($>200 \mu g/L$, 453.2 nmol/L) was 87.0% in the group that was not exposed to fortification and 96.1% in the group that was exposed to fortification ($P < 0.001$).

In individuals who used B-vitamin supplements, RBC folate concentrations were 24% higher in the group that was exposed to folic acid fortification ($P < 0.001$). The prevalence of individuals with deficient RBC folate concentrations was 1.6% in the group not exposed to fortification and 0% in the group exposed to fortification ($P < 0.05$). The prevalence of individuals with acceptable RBC folate concentrations was 97.2% in the group not exposed to fortification and 99.2% in the group exposed to fortification ($P = 0.08$).

**DISCUSSION**

In the Framingham Offspring Cohort, the introduction of folic acid fortification significantly improved folate nutritional status measured as RBC folate. We demonstrated previously in this cohort that folic acid fortification resulted in an increase in plasma folate concentrations from 4.6 to 10.0 $\mu g/L$ (10.4–22.7 nmol/L) among individuals not taking B-vitamins supplements and from 11.7 to 18.9 $\mu g/L$ (26.5–42.8 nmol/L) among supplement users, and a decrease in plasma total homocysteine concentrations from 10.1 to 9.4 $\mu mol/L$ among those not taking B-vitamin supplements (7). The present results confirm our earlier work using RBC folate, which is considered to be a good measure of tissue folate status and was chosen as the main indicator of folate nutritional status and folate sufficiency to establish the new Dietary Reference Intakes (DRI) (15,16).

We defined folate deficiency as RBC folate concentrations $<160 \mu g/L$ (362.6 nmol/L) and folate adequacy as RBC folate concentrations $>200 \mu g/L$ (453.2 nmol/L) (14). However, there is no accepted standard for folate deficiency or inadequacy based on RBC folate concentrations. The new DRI suggests a cut-off point of 140 $\mu g/L$ (317.2 nmol/L) of RBC folate to assess folate deficiency and adequacy (16). Even by our more conservative definitions, folate deficiency was uncommon in this cohort among those who did not take B-vitamin supplements (1.9%) and among those who took B-vitamins supplements (0%) after implementation of folic acid fortification. The prevalence of individuals with acceptable RBC folate status after implementation of folic acid fortification was very high, 96.1% in those who did not take B-vitamin supplements and 99.2% in B-vitamin supplements users.

It is possible that the observed differences in RBC folate status stemmed from variations in folate intake that were unrelated to fortification. However, we adjusted RBC values for consumption of folate and folic acid-rich foods in the groups exposed and not exposed to fortification, and therefore we are confident that the changes seen in RBC folate status can be ascribed to folic acid fortification.

Because this study sample included only seven women $\leq 45$ y old who were exposed to fortification and who did not take B-vitamin supplements, we could not assess the effect of folic acid fortification in women of childbearing age for which this fortification was targeted. This analysis was recently carried out by the CDC using data from the third National Health and Nutrition Examination Survey (NHANES III; 1988–1994) and NHANES 1999 (17). Among women of childbearing age (15–44 y old) who did not use supplements, mean plasma folate was 4.7 $\mu g/L$ (10.7 nmol/L) in women not exposed to fortification vs. 12.6 $\mu g/L$ (28.6 nmol/L) in women exposed to fortification. Among supplement users, mean plasma folate was 8.4 $\mu g/L$ (19.0 nmol/L) in women not exposed to fortification vs. 20.0 $\mu g/L$ (45.3 nmol/L) in women exposed to fortification. Mean RBC folate from all women was 181 $\mu g/L$ (410.1 nmol/L) compared with 315 $\mu g/L$ (713.8 nmol/L) in women not exposed and exposed to fortification, respectively (17). However, the benefits of higher folate intake may extend beyond protection against NTD. Mild folate deficiency has been associated with increased concentrations of plasma homocysteine, which is an independent risk factor for vascular disease (18,19). It has also been associated with increased risk of certain cancers (20) and increased risk of cognitive impairment in the elderly (21).

Results of this study and others (7,17) suggest that American adults have reached unprecedented levels of folic acid intake and that folate deficiency has essentially been eliminated in the general U.S. population. Nevertheless, some suggest that higher fortification levels are required to optimally prevent NTD (22–25). Before any changes in the levels of fortification are made, it is essential to evaluate the effect of the current levels of fortification on NTD rates (26). The difference in mean RBC folate levels between nonsupplement users not exposed to fortification and those exposed (325 vs. 450 $\mu g/L$) was comparable to the change in RBC folate observed by Daly et al. (27) in women who received 200 $\mu g$ of folic acid/d for 6 mo (from 311 to 475 $\mu g/L$). In the latter study, a reduction in NTD risk of 41% was estimated among those women (27). A recently published study reported a 19% decrease in NTD birth prevalence in the United States after implementation of folic acid fortification (28). However, another aspect that should be considered in evaluating the effect of folic acid fortification is the effect (positive and negative) of the current levels of fortification on the health of the population that were not targeted for this fortification, mainly children and the elderly (26). The current levels of fortification may expose a large proportion of American children to daily amounts of folic acid that surpass their Tolerable Upper Intake Levels for folic acid (29). Similarly, a large proportion of older Americans may be at risk because high levels of folic acid may mask the anemia associated with vitamin B-12 deficiency which, if left untreated, can cause irreversible neurologic damage. It has also been proposed that folic acid may induce or increase the rate and severity of neuropsychiatric disorders caused by B-12 deficiency (30,31). Changes in the current fortification levels should await the necessary data to assess and balance the potential benefits and risks of increased folic acid intakes.

**LITERATURE CITED**


