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Circulating folic acid in plasma: relation to folic acid fortification

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Abstract

Background—The implementation of folic acid fortification in the United States has resulted in unprecedented amounts of this synthetic form of folate in the American diet. Folic acid in circulation may be a useful measure of physiologic exposure to synthetic folic acid, and there is a potential for elevated concentrations after fortification and the possibility of adverse effects.

Objective—We assessed the effect of folic acid fortification on circulating concentrations of folic acid and 5-methyltetrahydrofolate in the Framingham Offspring Cohort.

Design—This is a cross-sectional study that used plasma samples from fasting subjects before and after fortification. Samples were measured for folate distribution with the use of an affinity-HPLC method with electrochemical detection.

Results—Among nonsupplement users, the median concentration of folic acid in plasma increased from 0.25 to 0.50 nmol/L ($P < 0.001$) after fortification, and among supplement users the median increased from 0.54 to 0.68 nmol/L ($P = 0.001$). Among nonsupplement users, the prevalence of high circulating folic acid (≥85th percentile) increased from 9.4% to 19.1% ($P = 0.002$) after fortification. Among supplement users, the prevalence of high circulating folic acid increased from 15.9% to 24.3% ($P = 0.02$). Folic acid intake and total plasma folate were positively and significantly related to high circulating folic acid after adjustment for potential confounding factors (P for trend < 0.001).

Conclusions—Folic acid fortification has resulted in increased exposure to circulating folic acid. The biochemical and physiologic consequences of this are unknown, but these findings highlight the need to understand the effects of chronic exposure to circulating folic acid.

INTRODUCTION

Since January 1998, folic acid fortification of flour and cereal grain has been mandatory in the United States and has also been implemented in Canada and other countries (1). The amount of fortification in the United States was set at 140 μg/100 g of cereal grain product, which was expected to increase the average folic acid intake attributable to fortification by

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≈100 μg/d, although studies have indicated the increase to be closer to 200 μg/d (2, 3). Since the start of folic acid fortification, there has been a virtual elimination of folate deficiency and ≈50% decrease in the prevalence of hyperhomocysteinemia (4). These biochemical effects of fortification are temporally associated with a decrease in the incidence of neural tube defects (5, 6) and stroke associated mortality (7) but also with an increased incidence of colorectal cancer (8) and possible harm to large segments of the population with low vitamin B-12 status (9, 10). Considering that all Americans are now exposed to fortification and the uncertainty about its risks and benefits beyond those relating to the prevention of neural tube defects, it is imperative to clarify the long-term effects of fortification on unintended health outcomes (11).

Folic acid, the synthetic form of the vitamin folate, is not found in nature. Its conversion to the active form requires reduction to tetrahydrofolate, a reaction that is catalyzed by dihydrofolate reductase, which is expressed in the intestine and other peripheral tissues (12). Dihydrofolate reductase activity in the intestine converts absorbed folic acid to dihydrofolate, which is both reduced and methylated to 5-methyltetrahydrofolate (5MeTHF), the form which is predominantly found in blood. However, because the capacity of this conversion is limited, excessive intake of folic acid saturates this capacity and results in its appearance unaltered in circulation (13, 14). Thus, the presence of residual circulating folic acid under fasting conditions is indicative of saturating folic acid intake.

In the present study we used a highly sensitive HPLC method to analyze plasma samples from the sixth examination of the Framingham Offspring Cohort. Because these samples were taken before and after fortification was implemented, we could determine the prevalence of circulating folic acid associated with folic acid fortification.

SUBJECTS AND METHODS

Study population

The Framingham Heart Study is an epidemiologic study of heart disease established in Framingham, MA, in 1948. The original Framingham cohort included 1644 husband-wife pairs and 378 persons whose parents had developed cardiovascular disease (15). The offspring of those subjects and the spouse of the offspring were invited to participate in the Framingham Offspring Study in 1971, and 5135 of the 6838 eligible persons participated in the first examination (16). The Offspring cohort undergoes repeat examination approximately every 3–4 y.

Between January 1995 and August 1998, members of the Offspring cohort (n = 3532) participated in the sixth study examination. Fortification of enriched cereal grain products was phased in over a 2-y period during this examination cycle (March 1996–January 1998). On the basis of discussions with food distributors in southern New England, we determined that there were few products fortified before September 1996 but that most products were fortified by July 1997. Exposure to fortification was determined on the basis of the examination date with the information noted above from the local food distributor. Participants whose sixth examination occurred before September 1996 are considered to be unexposed to fortification (before fortification). Participants who attended the examinations between September 1997 and August 1998 are considered as exposed to fortification (after fortification). Individuals who were examined between October 1996 and August 1997 (around fortification) were excluded from these analyses because of uncertainty of their folic acid intake. Consequently, the present cross-sectional analysis included participants whose examination date met our definitions of before fortification (n = 1103) or after fortification (n = 600). The Institutional Review Boards for Human Research at Boston University and Tufts-New England Medical Center approved the protocol.

Dietary data

Dietary intake was assessed by a semiquantitative food-frequency questionnaire (FFQ) (17). Subjects were asked to report on frequency of food consumption in the past year, vitamin and mineral supplements, and type of breakfast cereal most commonly consumed. To estimate intake of specific nutrients, the frequency of consumption was multiplied by the nutrient content of the specified portions. If ≥12 food items were left blank or if reported energy intakes were <600 kcal or >4000 kcal, the information was excluded from analysis.

To determine folic acid intake associated with fortification, folate intake from the FFQ was modified to reflect fortification as previously described (2). Briefly, foods enriched with folic acid after mandatory fortification was implemented were recalculated with the use of data from a study that measured the folate content of foods after fortification (18). The foods that were recalculated included bread, corn grits, rice, pasta, corn meal, muffins, pancakes, crackers, pizza, cookies, brownies, doughnuts, cakes, sweet rolls, and pie. Ready-to-eat cereals that were fortified before mandatory fortification were also modified if the measured folic acid content exceeded published database values. For all these enriched cereal grain products, the original total folate values used in the FFQ database were replaced by the more recently measured total folate values, and a new variable representing measured folic acid intake was created. Folic acid intake from supplements was assessed with the use of data from the FFQ that asks about supplement brand, type, and frequency of consumption. Analyses were separate for nonsupplement and supplement users.

After applying these modifications, total folate intake as micrograms of dietary folate equivalents (DFEs) was also calculated. This is done by adding the amount of folate consumed as natural folate (in μg) to the amount of folate consumed as folic acid (in μg) multiplied by 1.7 to account for the increased bioavailability of folic acid.

Biochemical methods

As part of the sixth examination, blood samples were taken from subjects who had fasted for ≥10 h. Folic acid and 5MeTHF concentrations were determined with the use of the affinity-HPLC method with electrochemical (coulometric) detection method (19) with the following modifications to increase throughput. Plasma samples (0.2 mL) were diluted with 1.2 mL of 50 mmol/L potassium tetraborate in 1% sodium ascorbate and vortexed. Twenty picomoles of ethyltetrahydrofolate was added as an internal standard. The mixture was boiled for 30 min and then cooled on ice and kept covered overnight at 4 °C. The mixture was centrifuged for 20 min at 4 °C at 36 000 × g and filtered. One milliliter of the supernatant fluid fraction was injected onto the affinity-HPLC system.

The revised affinity-HPLC system consisted of 2 affinity columns that were connected by a 10-port, 2-position switching valve to an analytic column (Betasil Phenyl 250 × 4.6 mm; Keystone Scientific Inc, Bellefonte, PA). This allows the first affinity column to be loaded with the sample and subsequently washed with a pH 7.0 phosphate buffer, while the second affinity column is eluted into the analytic column with an acetonitrile gradient at acid pH and into the electrochemical detector. At the end of the run, a signal is sent to switch the direction of the mobile phases to allow the elution of the loaded column for folate analysis and simultaneous loading of the second column with the new sample.

Folate activity was determined with the use of an ESA Four-Channel CoulArray Detector (Bioscience Inc, Chelmsford, MA). Quantification and identification of individual folates was done by comparison to external and internal folate standards of known concentration. The CV for folate detection by the described method ranged between 5.2% and 8.6% (intraassay) and 3.2% and 7.3% (interassay). The limit of quantification for folic acid was

0.19 nmol/L, and the limit of detection was 0.18 nmol/L. For these analyses, the limit of detectable folic acid (0.18 nmol/L) was used to describe detectable folic acid.

Statistical analysis

The statistical analyses were performed with SAS (version 9.1; SAS Institute, Cary, NC). Analyses assessing the effect of fortification on circulating folic acid, 5MeTHF, and total folate were calculated separately for users of B vitamin supplements and for users of non-B vitamin supplements. Data for total folate and 5MeTHF concentrations were positively skewed; therefore, these values were log transformed before analysis and reported as geometric means with 95% CIs. Folic acid intake values were positively skewed, and a square root transformation was used to normalize values. Circulating folic acid concentrations were skewed toward low values, and not all samples had detectable concentrations; thus, median concentrations and prevalence of detectable and high concentrations were reported. Detectable was defined as >0.18 nmol/L, the limit of detection for the method, and high was defined as ≥1.35 nmol/L, the 85th percentile for the cohort at the sixth examination cycle.

To determine the effect of folic acid fortification on circulating folic acid we compared median values in subjects both exposed and unexposed to fortification with the use of the Kruskal-Wallis test. SAS PROC GLM was used to assess the differences in prevalence of detectable and high circulating folic acid with adjustment for age and sex with the use of the Tukey's adjustment for multiple comparisons. We also used SAS PROC GLM to compare 5MeTHF and total plasma folate geometric means in subjects exposed and unexposed to folic acid fortification. To ensure values were related to fortification and not to differences in dietary patterns of folate- or folic acid-containing foods, we also adjusted for folate intake with the use of the food composition database not modified to reflect folic acid fortification.

To identify determinants of circulating folic acid ≥ 85% for this cohort, age- and sex-adjusted logistic regression analysis was used to calculate odds ratios for high circulating folic acid. We created categories for the continuous variables of age, body mass index (in kg/m^2), number of cigarettes smoked per day, and alcohol intake and for the quartile categories of intakes of caffeine, natural folate, synthetic folic acid, vitamin B-6, and vitamin B-12 and the plasma concentrations of folate, vitamin B-6, and vitamin B-12. Relations between dietary vitamin intakes were also adjusted for total energy intake, and supplement users were excluded from these analyses. *P* values reported are for the comparison between the reference value and each of the categories. A test for a trend was performed by entering independent variables as continuous variables in the logistic regression model. The *P* for trend was the resulting *P* values for the associated logistic regression coefficient.

To describe graphically the relation between circulating folic acid and folate intake, we plotted predicted circulating folic acid concentrations and 95% CIs by folic acid intake with the use of SAS PROC LOESS with a smoothing value of 0.3. A test for a trend was performed by entering folic acid intake as a continuous variable in the model with the use of PROC GLM and adjusting for age, sex, and total energy.

We also plotted the prevalence and 95% CIs of high circulating folic acid by categories of DFE intake with the use of SAS PROC GLM. Within each DFE intake category, the average percentage of total DFE intake attributed to natural folate and synthetic folic acid was also calculated. Analyses were adjusted for age, sex, and total energy. *P* for trend was calculated by entering DFE intake as a continuous variable in the regression model relating high circulating folic acid to folic acid intake as a percentage of total folate intake.

RESULTS

Characteristics of the population

Circulating concentrations of folic acid, total plasma folate, and 5MeTHF were measured in 705 nonsupplement users and 398 supplement users not exposed to fortification and in 355 nonsupplement users and 245 supplement users exposed to fortification (**Table 1**). At the time of the sixth examination the range of ages was 29–86 y and the average in each group was between 57 and 60 y. The proportion of men within users of non-B vitamin supplements and of B vitamin supplements was similar for before and after fortification, but men were less likely to use B vitamin supplements than were women. The proportion of subjects who used supplements increased after fortification. Increased prevalence of supplement use during this time period was reported in other cohorts, particularly in older white populations (20), which describes a large proportion of our cohort. We are not able to assess why more people used supplements; it may be due to a perceived health benefit.

Folic acid fortification: relation to plasma folate

Folate in fasting plasma is mostly made up of 5MeTHF. Residual folic acid is a minor constituent of plasma folate that is not always detectable (Table 1). Concentrations are skewed toward lower values. Exposure to fortification significantly increased circulating concentrations of folic acid, total plasma folate, and 5MeTHF (Table 1). Among subjects who did not use B vitamin supplements, median circulating concentrations of folic acid increased after fortification (0.25 before compared with 0.50 after fortification, $P < 0.001$). The prevalence of detectable circulating folic acid was 55.0% before fortification and 74.7% after fortification ($P < 0.001$). The prevalence of high circulating folic acid was 9.4% before fortification and 19.1% after fortification ($P = 0.002$). Compared with concentrations before fortification, total plasma folate concentrations were 91.0% higher after fortification ($P < 0.001$), and 5MeTHF concentrations were 92.0% higher after fortification ($P < 0.001$).

Among subjects who did use B vitamin supplements, the median circulating concentrations of folic acid increased after fortification compared with before fortification (0.54 before compared with 0.68 after fortification, $P = 0.001$) (Table 1). The prevalence of detectable circulating folic acid was 72.5% before fortification and 80.7% after fortification ($P = 0.13$). The prevalence of high circulating folic acid was 15.9% before fortification and 24.3% after fortification ($P = 0.02$). Compared with before fortification, total plasma folate concentrations were 29.9% higher after fortification ($P < 0.001$), and 5MeTHF concentrations were 28.7% higher ($P < 0.001$) after fortification.

Relation between circulating folic acid and folate intakes

Predicted circulating concentrations of folic acid by folic acid intake for the entire population is shown in **Figure 1**. A positive linear association was observed between folic acid intake and circulating folic acid concentrations (P for trend < 0.001).

The proportion of subjects with high circulating folic acid status (≥85 percentile for the entire cohort) according to folate intake expressed as DFEs is shown in **Figure 2**. A trend was observed for an increased prevalence of high circulating folic acid as the total folate intake increased (P for trend < 0.001). Subjects consuming >1000 μg of folate as DFEs had a 77.3% higher prevalence of circulating folic acid ≥85% than did subjects consuming between 400 and 1000 μg of folate. The increase appears to be attributable to folic acid intake and not to natural folate intake.

Determinants of high circulating folic acid

The statistically significant determinants for the prevalence of high circulating folic acid are shown in **Table 2**. Analyses were adjusted for age and sex, and analyses for dietary vitamin intakes excluded supplement users and were also adjusted for total energy intake. After adjustment, neither age, sex, body mass index, smoking, alcohol intake, caffeine intake, natural folate intake, nor plasma concentrations and intake of vitamins B-6 and B-12 were related to the prevalence of high circulating folic acid (data not shown). Folic acid intake, total folate intake, use of B vitamin supplements, and total plasma folate were the only significant determinants identified in the present study. Among nonsupplement users, the prevalence of high circulating folic acid was 2.16 times greater in the highest quartile category of folic acid intake than in the lowest quartile category after multivariate adjustment (95% CI: 1.22, 3.84). The odds ratio of high circulating folic acid was 2.84 for participants in the highest quartile category compared with the lowest quartile category for total folate intake as DFEs (95% CI: 1.51, 5.35). The odds ratios for high circulating folic acid were 2.52 (95% CI: 1.58, 4.03) in the third quartile category of total plasma folate and 4.94 (95% CI: 3.16, 7.72) in the fourth quartile category. Supplement users were 2.28 (95% CI: 1.7, 3.01) times more likely to have high circulating folic acid than were nonsupplement users.

DISCUSSION

The present study used a modified affinity-HPLC with electrochemical detection method to determine the effect of folic acid fortification on the folic acid and folate distributions in plasma from a population-based sample of Americans. In agreement with previous data (4), fortification resulted in the doubling of plasma (total) folate. As expected from fasting plasma samples, 5MeTHF was the predominate form in circulation. However, circulating folic acid was detectable in 67% of the samples tested. The data showed that fortification was associated with a significant increase in the percentage of samples with detectable folic acid and a doubling of those with high circulating folic acid in nonsupplement users. Exposure to fortification also increased the prevalence of high circulating folic acid in participants who used B vitamin supplements, and this group had the highest median concentrations of folic acid. Our analyses were adjusted for intakes of folate and folic acid without accounting for the added folic acid from fortification to ensure that changes in folate status observed were attributable to fortification and not a consequence of differences in folate-related dietary patterns.

Folic acid in circulation is almost certainly derived from synthetic folic acid from fortified foods and supplements. Given the positive relation between folic acid intake and circulating folic acid, it is not surprising that participants taking supplements and exposed to fortification had the highest concentrations. Although even in this group, concentrations tended to be low. This is probably because plasma samples were taken from fasted participants, which will not represent total exposure to synthetic folic acid. Folic acid is eventually converted to 5MeTHF in the peripheral tissues, so the amount measured in fasted plasma represents the residual unmetabolized folic acid. With the use of nonfasted samples, Pfieffer et al (21) has reported that those with high total serum folate (>50 nmol/L) had a higher percentage of folic acid as total folate than did samples in which total serum folate was <50 nmol/L. Taken together, these results imply that higher folic acid intake will increase exposure to circulating folic acid.

Because circulating folic acid was never quantified in a population sample, the effect of fortification on folic acid concentrations was previously unknown. An earlier study by Sweeney et al (22) predicted the current fortification program in the United States, which was estimated to supply an average of ≈100 μg of folic acid, would be less likely to have

folic acid in circulation than would a program that supplies 400 μg/d. However, our findings show that approximately three-quarters of persons using supplements, which typically provide 400 μg of folic acid, in the time before fortification and persons not using supplements in the time after fortification, have detectable circulating folic acid. The median circulating folic acid concentration and the prevalence of high folic acid concentrations was also comparable in these 2 groups.

One possible explanation for the higher than expected prevalence of detectable folic acid as a result of fortification is the way "detectable" folic acid is defined. Our assay for folic acid can detect folic acid at concentrations of ≥0.18 nmol/L, whereas the assay used in the Sweeney study can only detect concentrations ≥0.71 nmol/L (22). A second possible explanation for the difference in expected prevalence of detectable folic acid concentrations is that the estimated consumption of folic acid from fortification is higher than the 100 μg/d assumed by Sweeney et al (22) in their study. Finally, another important factor to consider is how long the population has been exposed to fortification. The exposure time in the present study was 1–2 y, compared with 14 wk in the study of Sweeney et al (22). Some evidence shows that repeated consumption of folic acid leads to accumulation in circulation (22, 23), which could mean even small intakes over a long period of time will result in more circulating folic acid. In the present study, 55% of persons before fortification and not taking supplements still had detectable circulating folic acid, despite a low mean intake of folic acid in these subjects. There is no evidence that folic acid in circulation could occur naturally, so detectable concentrations before fortification is probably from consumption of fortified breakfast cereals. Approximately 64% of nonsupplement users unexposed to fortification reported consuming fortified breakfast cereals in the FFQ.

It seems as if most of the population is now exposed to circulating folic acid, and evidence suggests that higher intakes will further increase concentrations. There has been much speculation about whether this poses a health risk (24). Because of a lack of information on what concentration is normal or may pose risk, we arbitrarily defined high as >85th percentile. In studies that have found an adverse effect of folate, it is in persons with the highest total folate status (9, 10) or the highest folic acid intakes (25–27). In the one study that did measure circulating folic acid, there was a trend toward lower natural killer cell cytotoxicity with increased concentrations in plasma in women >60 y (28). This association was independent of 5MeTHF and total folate concentrations.

In conclusion, this study indicated both supplement use and folic acid fortification are associated with increased exposure to circulating folic acid. It also raises the possibility that prolonged exposure and high intakes may further increase concentrations. Given the high prevalence of supplement use in the United States and increased folic acid intake attributable to fortification, ongoing monitoring of folic acid status is needed. To evaluate all the potential benefits and risks and optimal intakes of folic acid for all segments of the population, the physiologic and safety ramifications of lifetime exposure to circulating folic acid need to be elucidated.

REFERENCES

1. Food and Drug Administrations. Food standards: amendment of standards of identity for enriched cereal grain products to require addition of folic acid. Federal Registrar. 1996; 61:8781–97.
2. Choumenkovitch SF, Selhub J, Wilson PW, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. J Nutr. 2002; 132:2792–8. [PubMed: 12221247]
3. Quinlivan EP, Gregory JF III. Effect of food fortification on folic acid intake in the United States. Am J Clin Nutr. 2003; 77:221–5. [PubMed: 12499345]

4. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med. 1999; 340:1449–54. [PubMed: 10320382]
5. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA. 2001; 285:2981–6. [PubMed: 11410096]
6. Mills JL, Signore C. Neural tube defect rates before and after food fortification with folic acid. Birth Defects Res A Clin Mol Teratol. 2004; 70:844–5. [PubMed: 15468072]
7. Yang Q, Botto LD, Erickson JD, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation. 2006; 113:1335–43. [PubMed: 16534029]
8. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev. 2007; 16:1325–9. [PubMed: 17626997]
9. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. Am J Clin Nutr. 2007; 85:193–200. [PubMed: 17209196]
10. Selhub JMM, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci U S A. 2007; 104:19995–20000. [PubMed: 18056804]
11. Rosenberg IH. Science-based micronutrient fortification: which nutrients, how much, and how to know? Am J Clin Nutr. 2005; 82:279–80. [PubMed: 16087969]
12. Shane, B. Folate chemistry and metabolism.. In: Bailey, LB., editor. Folate in health and disease. Marcel Dekker; New York, NY: 1995. p. 1-22.
13. Lucock M, Wild J, Smithells R, Hartley R. Biotransformation of pteroylmonoglutamic acid during absorption: implications of Michaelis-Menten kinetics. Eur J Clin Nutr. 1989; 43:631–5. [PubMed: 2606094]
14. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J Clin Nutr. 1997; 65:1790–5. [PubMed: 9174474]
15. Dawber TR, Moore MF, Mann GV. Coronary heart disease in the Framingham Study. Am J Public Health. 1957; 47:4–24.
16. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study: design and preliminary data. Prev Med. 1975; 4:518–25. [PubMed: 1208363]
17. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol. 1992; 135:1114–26. discussion 1127–36. [PubMed: 1632423]
18. Rader JI, Weaver CM, Angyal G. Total folate in enriched cereal grain products in the United States following fortification. Food Chemistry. 2000; 70:275–289.
19. Bagley PJ, Selhub J. Analysis of folate from distribution by affinity followed by reversed-phase chromatography with electrical detection. Clin Chem. 2000; 46:404–11. [PubMed: 10702529]
20. Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999–2000. Am J Epidemiol. 2004; 160:339–49. [PubMed: 15286019]
21. Pfeiffer CM, Fazili Z, McCoy L, Zhang M, Gunter EW. Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. Clin Chem. 2004; 50:423–32. [PubMed: 14670827]
22. Sweeney MR, McPartlin J, Scott J. Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. BMC Public Health. 2007; 7:41. [PubMed: 17378936]
23. Sweeney MR, McPartlin J, Weir DG, Daly L, Scott JM. Postprandial serum folic acid response to multiple doses of folic acid in fortified bread. Br J Nutr. 2006; 95:145–51. [PubMed: 16441927]

24. Wright AJ, Dainty JR, Finglas PM. Folic acid metabolism in human subjects revisited: potential implications for proposed mandatory folic acid fortification in the UK. Br J Nutr. 2007; 98:667–75. [PubMed: 17617936]

25. Morris MC, Evans DA, Bienias JL, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. Arch Neurol. 2005; 62:641–5. [PubMed: 15824266]

26. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003; 348:891–9. [PubMed: 12621133]

27. Stolzenberg-Solomon RZ, Chang SC, Leitzmann MF, et al. Folate in-take, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Am J Clin Nutr. 2006; 83:895–904. [PubMed: 16600944]

28. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr. 2006; 136:189–94. [PubMed: 16365081]

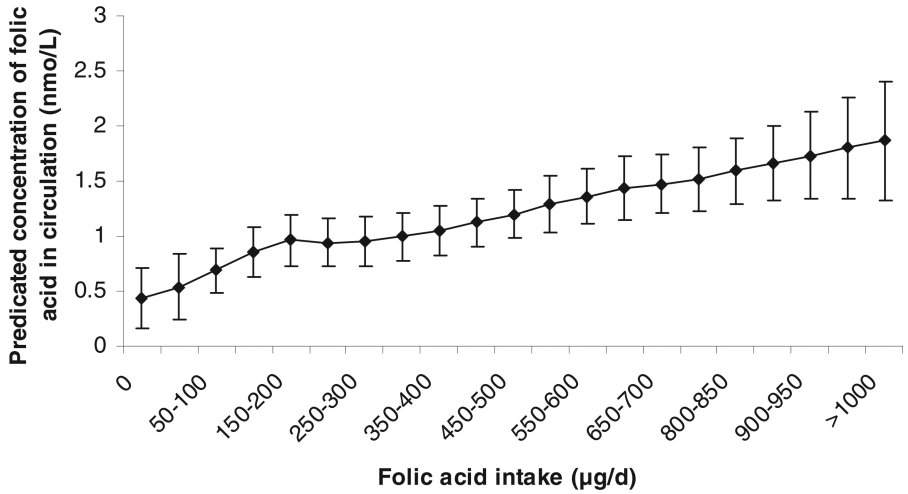

FIGURE 1.

Predicted concentrations of circulating folic acid (error bars are 95% CIs) by folic acid intake. The number of subjects in each group starting at 0 μg was 251, 193, 177, 100, 123, 119, 92, 47, 92, 110, 110, 49, 36, 28, 18, 15, 25, 18, 16, 10, and 29. A nonparametric loess smoother was fitted to the data, and 95% CIs were calculated with the use of PROC LOESS with a smooth value of 0.3. A test for a trend was performed by entering folic acid intake as a continuous variable in the model with the use of PROC GLM and adjustment for age, sex, and total energy. *P* for trend was < 0.001 for the relation between folic acid intake and circulating folic acid concentration.

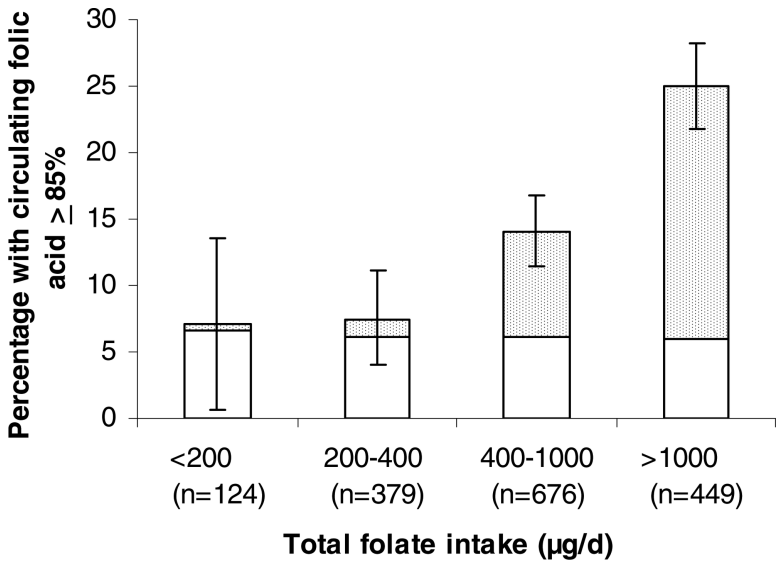

FIGURE 2.

The proportion of subjects (error bars are 95% CIs) with high circulating folic acid status (≥85%) according to folate intake. Folate intake is expressed as the μg/d of dietary folate equivalents (DFEs). Total DFE intake = (1.7 × μg of folic acid intake/d) + μg/d of natural folate intake. Within each DFE category, the average percentage of total DFE intake attributed to natural folate (white bars) and synthetic folic acid (black dots) was also calculated. The results were calculated with the use of PROC GLM adjusted for age, sex, and total energy. *P* for trend was < 0.001 for the relation between the percentage of folic acid intake as total folate intake in DFEs with prevalence of high circulating folic acid.

TABLE 1

5-Methyltetrahydrofolate (5MeTHF) and circulating folic acid (FA) status in subjects from the Framingham Offspring Study before and after fortification, by B vitamin supplement use[1]

	No B vitamin supplements		B vitamin supplements	
	Before	**After**	**Before**	**After**
Subjects (*n*)	705	355	398	245
Male (%)	53.3	51.5	40.6	43.4
Age (y)[2]	57.4 (32–80)	59.9 (33–86)	58.0 (29–78)	59.6 (33–85)
FA intake (μg/d)[3,4]	32.4 (27.9, 37.0)[a,5]	241.4 (224.1, 259.2)[b]	399.4 (378.2, 421.2)[c]	601.4 (568.6, 635.1)[d]
Total plasma folate (nmol/L)[4,6,7]	19.5 (18.8, 20.4)[a]	37.2 (35.3, 39.4)[b]	30.8 (29.2, 32.9)[c]	40.6 (37.8, 43.5)[b]
Total 5MeTHF (nmol/L)[4,6,7]	19.0 (18.3, 19.9)[a]	36.3 (34.2, 38.3)[b]	30.1 (28.5, 31.9)[c]	39.2 (36.5, 41.9)[b]
Circulating FA (nmol/L)[8]	0.25 (0–15.18)[a]	0.50 (0–24.11)[b]	0.54 (0–19.78)[b]	0.68 (0–33.94)[c]
Subjects with detectable FA (%)[4,6,7,9]	55.0 (51.1, 58.9)[a]	74.7 (69.5, 79.9)[b]	72.5 (66.9, 78.1)[b]	80.7 (74.2, 87.2)[b]
Subjects with high FA (%)[4,7,10]	9.4 (6.4, 12.4)[a]	19.1 (15.1, 23.1)[b,c]	15.9 (11.6, 20.2)[a,b]	24.3 (19.2, 29.3)[c]

[1] To convert values for folate to nanograms per milliliter, divide by 2.266.

[2] $\bar{x}$; range in parentheses.

[3] Folic acid intake square root transformed. Values were also adjusted for total energy.

[4] Values in a row without common superscript letters are significantly different, $P < 0.05$. Differences were adjusted for age and sex with the use ANCOVA with Tukey's post hoc tests.

[5] $\bar{x}$; 95% CIs in parentheses (all such values).

[6] Values were log transformed; geometric $\bar{x}$ reported.

[7] Adjusted for age, sex, and total folate intake (total folate does not include FA added from fortification of grain products, other than ready-to-eat breakfast cereals).

[8] Median; range in parentheses. Values in a row without common superscript letters are significantly different as determined with the Kruskal-Wallis test.

[9] Detectable FA was defined as ≥0.18 nmol/L.

[10] High FA was defined as ≥1.35 nmol/L.

TABLE 2

Determinants of circulating folic acid (FA) concentrations ≥ 85%[1]

Categories of exposure	Subjects	OR (95%CI)	*P*
	n		
Dietary FA intake (μg/d)[2]			
≤6.86	265	1.0 (ref)	
6.86–70.18	263	0.79 (0.41, 1.54)	0.49
70.18–203.65	267	1.32 (0.74, 2.38)	0.35
>203.65	265	2.16 (1.22, 3.84)	0.007
P for trend[3]			<0.001
DFE intake (μg/d)[2]			
≤276.94	265	1.0 (ref)	
276.94–417.49	265	1.25 (0.65, 2.40)	0.50
417.49–630.23	265	1.67 (0.89, 3.16)	0.11
>630.23	265	2.84 (1.51, 5.35)	0.001
P for trend[3]			<0.001
B vitamin supplement use[4]			
No	1060	1.0 (ref)	
Yes	643	2.28 (1.73, 3.01)	<0.001
Plasma folate (nmol/L)[4]			
≤10.74	427	1.0 (ref)	
10.74–20.17	424	1.56 (0.94, 2.57)	0.08
20.17–33.72	425	2.52 (1.58, 4.03)	<0.001
>33.72	425	4.94 (3.16, 7.72)	<0.001
P for trend[3]			<0.001

[1] Ref, referent; DFE, dietary folate equivalent; calculated as {natural folate intake (in μg) + [folic acid intake (in μg) × 1.7]}.

[2] Multivariate adjusted for age, sex, and total energy with logistic regression analysis. Does not include data from subjects who consume B vitamin supplements.

[3] Calculated by modeling exposures as continuous variables.

[4] To convert values for folate to nanograms per milliliter, divide by 2.266.