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REVIEW ARTICLE

Will Increasing Folic Acid in Fortified Grain Products Further Reduce Neural Tube Defects without Causing Harm?: Consideration of the Evidence

RICHARD B. JOHNSTON, JR.

Department of Pediatrics, University of Colorado School of Medicine and National Jewish Medical and Research Center, Denver, CO 80262

ABSTRACT: To reduce neural tube defects (NTDs), the U.S. Food and Drug Administration (FDA) mandated that by January 1998 all enriched grain products should contain 140 μg of folic acid (FA)/100 g of flour. Groups concerned with optimal prevention of NTDs had argued that the level should be 350 μg/100 g. However, when it appeared that the debate might delay implementation of any fortification, these groups petitioned the FDA to implement fortification at the originally proposed level of 140 μg/100 g, anticipating that the FDA might consider increasing the level at a later time. Mandated FA fortification (FAF) has now been in place in the United States for 9 y. The impact of this important public health intervention on NTD rates, the possible benefit to other disease conditions, and potential harms have been evaluated. As background for a possible request that the FDA consider increasing FAF, evidence bearing on the question of whether an increase can further reduce NTD births without causing harm is reviewed here. The published data indicate that it is appropriate that the FDA conduct or commission a balanced analysis of the evidence by scientists who will act on that evidence to decide this important question. **(*Pediatr Res* 63: 2–8, 2008)**

Neural tube defects (NTDs) result from failure of normal neural tube closure by approximately 28 d after conception, before most women know they are pregnant. In 1991, the British Medical Research Council (MRC) Vitamin Study, a randomized controlled trial (RCT), showed that administration of folic acid (FA), 4000 μg/d before pregnancy, could reduce by 72% the recurrence of NTDs in women with a prior NTD pregnancy (1). The MRC has identified this report as one of its most influential (2). A second RCT showed that a multivitamin with FA could prevent the first occurrence of NTDs (3).

On the basis of the available evidence, the U.S. Public Health Service recommended in 1992 that all women of childbearing age consume at least 400 μg of synthetic FA/d (4). In spite of continuing efforts by the Centers for Disease Control and Prevention (CDC), the March of Dimes, and others to encourage adherence to this recommendation, regular Gallup surveys have indicated that the fraction of nonpregnant women 18–45 y old who regularly take a FA supplement has remained at approximately 30% over the last decade (5). Moreover, in annual examinations of women of reproductive age in the United States, the physician discusses the importance of FA intake only about half the time, and about half of all pregnancies are unplanned (6).

To increase FA intake among women of childbearing potential, the U.S. Food and Drug Administration (FDA) mandated that by January 1998, FA should be added to all "enriched" cereal-grain products at a level of 140 μg/100 g of flour (7). The FDA's compulsory fortification with FA has been praised as possibly ″the most important science-driven intervention in nutrition and public health in decades" (8).

During the period of scientific review that preceded the FDA's decision, the CDC, American Medical Association (AMA), American Academy of Pediatrics (AAP), American College of Obstetricians and Gynecologists (ACOG), American College of Medical Genetics (ACMG), March of Dimes, and Spina Bifida Association had argued for a higher level of fortification (350 μg/100 g) on the basis of models that predicted that the mandated dose would reduce the rate of NTDs by only 20% (7,9,10). However, when it appeared that the fortification initiative was stalled over this debate, the AAP, ACOG, ACMG, and March of Dimes petitioned the FDA to ratify the proposed level of 140 μg/100 g, with the anticipation that the FDA would review the possibility of increasing fortification in the future.

This article reviews current evidence related to the potential benefits and harms of increasing FA fortification (FAF) as a background for considering whether it is appropriate now to petition the FDA to address scientifically the question of the optimal safe level of FAF. The information contained here might also inform deliberations in other countries considering the implementation of FAF.

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Correspondence: Richard B. Johnston, Jr., M.D., Office of the Dean, C290, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, CO 80262.

Abbreviations: CVD, cardiovascular disease; **FA,** folic acid; **FAF,** folic acid fortification; **MTX,** methotrexate; **NHANES,** National Health and Nutrition Examination Survey; **NTD,** neural tube defect; **RCT,** randomized controlled trial

EFFECT OF FAF ON RATES OF NTDS

A CDC analysis of births from 23 states and Puerto Rico compared NTD rates before and after FAF (11). The prevalence of NTDs was reduced by 26% in programs without prenatal diagnosis and by 27% in programs that included data from all NTD-affected pregnancies (live births, still births, fetal deaths, and elective terminations) (11). The reduction in NTD rates in Canada, which instituted FAF at 150 μg/100 g in 1998, have varied by province from 32% to 78%, and the higher the prefortification NTD level, the greater the reduction (12). In pooled data from seven Canadian provinces, FAF has resulted in reduction in NTD-affected pregnancies by 46% (from 1.58/1000 births to 0.86/1000 births) (12).

FAF in the United States has been estimated to avert $145 million/y in costs for the care of children born with spina bifida and to produce a net economic benefit of $422 million/y (2002 dollars) (13). The prevalence of NTDs in European countries that have depended on education to promote the use of FA supplements rather than FAF has not clearly declined (14,15).

The somewhat greater than predicted reduction in NTD prevalence in the Untied States because of fortification is reflected in the early reports of plasma and blood cell folate levels (16). These levels were estimated to reflect an increase in the average daily FA intake of approximately 200 μg, twice the intake originally projected (7,9,10,17).

The reason for these higher than expected folate concentrations and rates of NTD prevention is not clear, but a 1999 survey of fortified products identified higher than mandated levels in several products (18). In addition, FA has been voluntarily added to a variety of other foods. On the other hand, two recent analyses of National Health and Nutrition Examination Survey (NHANES) data (19,20), one of which used a correction for the usual measurement error inherent in dietary intake surveys (19), indicate that folate intake has increased by 100–130 μg/d after fortification, as predicted. In addition, a recent comparison of NHANES data on women of childbearing age found that serum and erythrocyte folate levels fell 16% and 8%, respectively, from 1999 to 2000 to 2003 to 2004 (21). Perhaps related is the report that the folate content of several breads was reduced during 2000–2003 (22).

In 2000, Chile required fortification of the wheat flour used for bread with FA at 220 μg/100 g (23). Chilean women eat about twice as much bread as do American women, and this level was chosen to increase FA consumption by an average of 400 μg/d. Rates of NTD live and still births after fortification decreased by 43%, and the overall stillbirth rate decreased by 20% (24,25). Unregistered induced abortions are uncommon in this population (26). FAF of wheat flour in 1997 and corn flour in 1999 reduced NTD births by 35% in Costa Rica (27).

OTHER POSSIBLE BENEFITS OF FAF

Evidence that FA can prevent NTDs is considered convincing. The near elimination since mandated fortification of the low blood folate levels associated with anemia (16) makes folate deficiency anemia unlikely in the United States, an important health benefit for individuals across the general population. However, evidence that FA has other health effects has not received general acceptance.

Vascular disease. Attention has focused particularly on the relationship between FA intake, homocysteine, and vascular disease, stimulated by the report of McCully (28) in 1969 that individuals with the metabolic disorder homocystinuria die early in life from severe atherosclerosis. Meta-analyses of association studies have concluded that elevated homocysteine is an independent risk factor for cardiovascular disease (CVD) and stroke (29–32).

Attempts to understand the underlying mechanism indicate that homocysteine has a direct effect on endothelial structure and function (33,34). Raising blood folate lowers blood homocysteine and reduces markers of oxidant stress and inflammation (34). FA can also directly correct endothelial dysfunction in type-1 or type-2 diabetes, coronary artery disease, and other disorders exclusive of its homocysteine-lowering effect (35–37).

Population homocysteine levels (16,38) and deaths caused by stroke in the United States and Canada (39) have declined significantly since the advent of FAF. There are abundant data that associate lower population homocysteine levels and greater FA intake with protection against CVD and stroke (29–32,40). Three large RCTs have examined the effect of lowering homocysteine with large doses of FA in combination with B6 or B12, or both, on further vascular events in individuals with a history of acute myocardial infarction, stroke, vascular disease, or diabetes (41–43). One of these trials reported marginally significant protection against stroke (43), but none of the three showed protection against other CVDs, and one trial showed a trend (not significant) toward more myocardial infarctions (42). Median follow-up periods were 24, 40, and 60 mo. A meta-analysis of RCTs that studied the effect of FA supplementation on prevention of CVDs in persons with preexisting cardiovascular or renal disease also did not show a preventive effect (44).

Possible explanations for the discordant results between the observational studies and trials include the greater risk of confounding factors in the former; the possibility that the RCTs lack the statistical power to detect the expected 10–20% reduction in cardiovascular endpoints because of the wide availability of fortified foods; or the difference between observing the effects of primary prevention in the early stages of the disease process and attempts to intervene after demonstrated vascular damage (33,38,43–46).

Other birth defects. The balance of available data, including a recent meta-analysis, strongly supports the conclusion that periconceptional use of multivitamins containing FA can reduce the risk of certain birth defects other than NTDs, especially congenital cardiac anomalies, orofacial clefts, and combined birth defects exclusive of NTDs (3,47–52). Orofacial clefts have not declined since the introduction of fortification in Chile (26), but mandated fortification in the United States has been associated with modest yet statistically significant decreases in several birth defects (52). Higher levels of FA than those achieved by fortification or the inclusion of other vitamins may be needed to prevent other birth defects optimally (47).

Cognitive decline. Elevated plasma homocysteine, low blood folate, or low folate intake has been associated in several large studies, including a recent RCT (53), with more rapid cognitive decline and increased rates of dementia and Alzheimer disease in the elderly (53–59). This relationship has not been consistent, however (60–62).

Summary. RCTs, as designed and conducted to date, have not shown clear and consistent proof that increasing FA consumption can prevent more than NTDs. However, there are strong observational and some RCT data that support additional health benefits from FA, and the question of other salutary effects of FA is far from settled (46).

IS THERE EVIDENCE THAT FA CAUSES ADVERSE EFFECTS?

At the time FAF was mandated, no additional funding was made available for the systematic study of possible adverse effects (8). Nevertheless, data on safety have emerged.

In establishing new recommendations for dietary folate intake in 1998, the Institute of Medicine (IOM) Food and Nutrition Board set its tolerable upper level of synthetic FA at 1000 μg/d on the basis of the risk that high FA intake could correct the anemia of pernicious anemia and thus "mask" the diagnosis yet permit or promote neurologic damage (63). In the absence of controlled, dose-response data, the lowest-observed adverse effect level (LOAEL) was fixed at 5000 μg/d based on case reports from 1947 to 1960 in which neurologic manifestations progressed on high-dose folate therapy. The relatively high uncertainty factor of 5 was then applied to the LOAEL to derive the figure of 1000 μg (63). The issue of high-dose folate therapy in patients with pernicious anemia is reviewed in 64.

In 1573 adults with abnormally low B12 levels, the proportion of subjects with B12 deficiency but no anemia before FAF (39.2%) did not differ from that after fortification (37.6%) (65). Other evidence also favors the conclusion that FA in doses currently consumed does not cure the anemia of B12 deficiency (reviewed in 62). NHANES data indicate that the percentage of U.S. adults over 64 y who consume more than 1000 μg folate/d has increased since fortification but has remained below 5% in all groups (19).

No adverse effects were demonstrated in the many studies that evaluated the periconceptional use of FA in doses of up to 5000 μg/d (1) or in the recently completed homocysteine-lowering RCTs in which daily folic FA doses were 800–2500 μg (34,41–43). The periconceptional studies were generally not designed to assess adverse effects, but such effects were specifically addressed in the trials.

CONCERNS ABOUT POSSIBLE ADVERSE EFFECTS OF FAF

Masking B12 deficiency. In setting the tolerable upper level for synthetic FA intake at 1000 μg/d for adults, the IOM Food and Nutrition Board evaluated all potential hazards associated with high folate intake and found reason for concern only on the issue of masking B12 deficiency and consequent neurologic damage (63). This concern continues to be the major reason offered for delaying fortification, although no evidence has emerged to demonstrate that the fear is justified.

Intake in children. Concern has been expressed that children are likely exceeding the IOM's age-related tolerable upper level for FA since the advent of fortification. The IOM found no data to suggest that other life-stage groups are more susceptible to the adverse effects of high folate intake than adults, and lacking utilization and metabolic turnover data, upper levels for children were adjusted down on the basis of relative body weight (63). The 1000 μg adult limit, established because of the concern for exacerbating neuropathy, was used as the starting point, although this risk in children is negligible. In consideration of the rapid growth that characterizes childhood, it is possible that relatively higher doses of FA would be beneficial.

Unmetabolized FA. Related to this concern are reports that unmetabolized FA, not the natural form of folate, appeared in the circulation after ingestion of 800 μg FA/d given as fortified breakfast cereal and bread or as 300–400 μg given in a single dose (66), or in cord blood from 11 infants born to Irish mothers (67). The 11 mothers had not consumed FA supplements, but some foods are fortified with FA in Ireland. The authors assumed that the circulating unmetabolized FA represents excess that could harm the baby.

FA is converted to its physiologic form in tissues, and excess folates are excreted (68). It is not known what harm, if any, circulating unmetabolized FA might cause. Surveys since 1980 indicate that approximately one quarter of adults in the United States regularly consume a multivitamin containing FA (69,70). Moreover, almost all pregnant women in the United States consume prenatal vitamins that contain 600–800 μg of FA, and some consume additional FA supplements of several milligrams (71). Thus, it is likely that most American infants born within recent decades have had unmetabolized FA circulating at birth and that many Americans have had unmetabolized FA circulating for years. No untoward effects of this condition have been suggested.

Decreased natural killer cell activity. In a group of 109 overweight or obese women, those with higher levels of circulating unmetabolized FA had 23% lower natural killer (NK) cell activity than did women with lower levels (72). It is not clear that a drop in NK cell activity of this magnitude has biologic meaning. The authors raise the possibility that such a decline could compromise host defense, but NK cell activity is particularly sensitive to up-regulation by inflammatory cytokines (73), and lower activity could reflect less inflammation.

Cancer promotion. Folate is required for one-carbon transfer (methylation) reactions, including those involved in the biosynthesis of DNA and RNA. Folate deficiency in humans leads to DNA strand breaks, known to predispose to cancer, and these can be corrected by increasing FA intake (74). The available epidemiologic data collectively support the concept that higher folate status offers relative protection against several cancers, including colorectal, cervical, and breast, and leukemia (75). Preconception and prenatal vitamin use has been reported to reduce the risk in offspring of brain tumors, neuroblastoma, and leukemia by 30–60% (76–79); infant

neuroblastoma in Ontario has decreased by 60% since the introduction of FAF (80).

On the other hand, folate antagonists such as methotrexate (MTX) inhibit the rapidly dividing malignant cells of established tumors, which has led to the concern that dose and timing of folate intervention might determine whether folate prevents or promotes cancer (reviewed in 81–83). In two mouse models of spontaneously developing intestinal adenomas, folate supplementation at doses four to 10 times above basal requirements suppressed or enhanced the development of adenomas depending on whether folate was given before or after adenoma foci were established (84,85). However, the results differed with the two mouse strains, the mice were genetically programmed to develop adenomas, and the FA doses were comparably higher than those to which North Americans would be regularly exposed even if taking a FA supplement. In an RCT of individuals with a recent history of colorectal adenomas, FA, 1000 μg/d, was associated with a higher risk of having three or more adenomas at the 6–8-y follow-up (75), in agreement with the concerns raised in adenoma-prone mice.

Epigenetic hypermethylation. Folate's role in methylation reactions has also raised concern that increasing blood folate could increase the methylation of DNA cytosine residues that control promoter activity. This "epigenetic" hypermethylation could silence promoter activity and thereby inactivate tumor-suppressor genes or genes that play a role in cell-cycle control, repair of DNA damage, or other functions that constrain carcinogenesis (82). Humans given FA, 5000–10,000 μg/d for 3–12 mo, had increased methylation of genomic DNA (genes not identified) in resected adenomas (reviewed in 82), and adults given 400 μg of FA/d for 10 wk had increased methylation in leukocytes of 31% (86). On the other hand, FA, 2000 μg/d for 6 mo did not modulate genomic DNA methylation in lymphocytes from healthy adults (82).

In a mouse strain carrying the abnormal agouti gene for coat color, dams fed a diet containing four added methyl donors and cofactors, one of which was FA, delivered offspring with hypermethylation of the agouti gene and a change in coat color (87). This experiment demonstrates the biologic principle that diet during pregnancy can modify the phenotype of the offspring, at least under certain circumstances; but its relevance to humans and human disease is uncertain. In summary, there is no conclusive evidence at this time that FA intake at levels expected with fortification and supplements can cause meaningful epigenetic changes in humans.

Interference with antifolate treatment. Concern has been raised that FAF could interfere with the therapeutic effect of antifolates such as MTX on malignant and inflammatory diseases. However, MTX is used over wide dose ranges, and dosage is carefully tailored to each individual patient because of its potential for serious toxicity (88). In the event of such, some form of folate is given parenterally in doses much higher than those available through fortification and supplements (88). FA supplements (commonly 1000 μg/d) are routinely given along with MTX to prevent or treat toxicity (89), to reduce toxicity-related discontinuation of MTX in rheumatoid arthritis (90), and to allow effective use of MTX to involute ectopic fetuses (91). It appears unlikely that fortification at present or higher levels can adversely affect the application or efficacy of antifolate therapy.

The antiseizure medication phenytoin and folates interact bidirectionally, and either can reduce the circulating level of the other, raising concern that fortification could lead to poorer seizure control. Again, however, dosages are individualized across a range to obtain optimal seizure management. Moreover, blood phenytoin levels in a large Canadian sample were not changed by FAF (92).

Multiple births and miscarriages. Early studies suggested that periconceptional multivitamin use was associated with a higher prevalence of multiple births and miscarriages, but confounding by older maternal age, ovarian stimulation, assisted reproduction, or previous miscarriage was not eliminated (reviewed in 51). Consumption of 4000 μg/d FA with or without other vitamins in the MRC RCT (1), or as FA alone, 400 μg/d, in the 250,000-subject China-US Collaborative Project (93–95) was not associated with an increased risk of multiple births or miscarriages. In one study, low blood folates were associated with increased risk of miscarriage, but normal levels were not (96). Twinning rates have not been affected by mandated fortification in the United States (97–101) or Chile (25). Higher blood folates were associated with better embryo survival in multiple embryo transfer but not with rates of natural twinning (102).

Summary. It is vitally important to consider possible risks of FAF at any level, and this should be an ongoing process (17). However, the evidence available to date does not support the conclusion that foods fortified with FA or FA supplements cause harm.

COULD HIGHER LEVELS OF FAF PREVENT MORE NTDS?

Inherent in the question of the optimal level of FAF is the question of the percentage of NTDs that can be prevented with periconceptional FA at any dose. It has been assumed that the majority of NTDs are caused by dietary folate deficiency, but other causes of NTDs are recognized. It is possible that some of these can be prevented by FA at doses currently recommended or higher (103).

The MRC Vitamin Study had sufficient cases to estimate the magnitude of the preventive effect of a specific dose of FA. In the main analysis based on all women randomized, including 7% of the total who had stopped taking their pills before they became pregnant, the rate of prevention was 72% (1). That figure has commonly been taken as a lower boundary to the preventable fraction in a typical Western population. If the analysis was restricted to women who took their pills and were not already pregnant at the time of randomization (80% of the total sample), the rate of prevention was 83% (1). In the large China-US study, consumption of 400 μg/d FA reduced NTD births by 79% in a northern region where NTD rates were high and by 41% in a southern region where the rates were lower (93).

A two-stage model based on published evidence has been developed to analyze the relationship between prevention of

NTDs and FA intake in doses relevant to fortification (10). Data were used from 13 publications that reported the effect on serum folate of consumption of FA doses of up to 1000 μg/d. A given rise in FA intake was associated with a constant rise in serum folate from any starting concentration. In the second step, data from a large cohort study (104) were plotted to compare serum folate levels and risk of NTDs. The plotted association indicated a constant proportional relationship between serum folate and risk. According to the model, prevention of NTDs increases in a dose-responsive fashion as intake of FA increases.

Mandated fortification has been associated with a reduction in the NTD rate of 27% in U.S. programs that surveyed all NTD-affected pregnancies (live births, still births, fetal deaths, and elective terminations) and with a reduction of 26% in programs without prenatal ascertainment (11). Reductions have varied from 32 to 54% in various Canadian provinces, except in Newfoundland, where prefortification NTD rates were exceptionally high and prevention was 78% (12). Most Canadian provinces surveyed all NTD pregnancies as well as NTD births. The decrease in Chile was 43% (23,24). Whether the study analyzed the preventive effect of supplements or fortification, the higher the baseline NTD rate, the higher the percentage of cases prevented.

The reduction in NTD rates attained by fortification to date, 27% in the United States (11), is well below the evidence-based projected achievable level (105), and the available data support the conclusion that increasing FAF would further reduce NTDs. More effort will be needed to achieve the 2010 national health objective of reducing NTDs by 50% (105). In agreement with this concept, the AMA recommended in 2006 that the FDA be urged to increase FAF to 350 μg/100 g of grain (106).

CONCLUSIONS

The possibility that higher FA intake may pose a risk to the general population must be taken seriously. The risks that have been postulated carry varying degrees of biologic plausibility, and these should be explored; but, at present, these risks are theoretical. It will not be possible to prove the negative in this case that increasing FAF will never cause harm. However, there is no known evidence that consumption of FA through dietary supplements and fortification as available in the United States has harmed individuals in any age group. The question of whether to add more FA to fortified grains is too important to public health to remain unexamined. It is imperative that the FDA convene or commission the convening of a review body that will evaluate the evidence and use that evidence to decide on this important question.

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