Folic Acid and the Prevention of Birth Defects: 30 Years of Opportunity and Controversies

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Abstract

For three decades, the US Public Health Service has recommended that all persons capable of becoming pregnant consume 400 μg/day of folic acid (FA) to prevent neural tube defects (NTDs). The neural tube forms by 28 days after conception. Fortification can be an effective NTD prevention strategy in populations with limited access to folic acid foods and/or supplements. This review describes the status of mandatory FA fortification among countries that fortify ($n = 71$) and the research describing the impact of those programs on NTD rates (up to 78% reduction), blood folate concentrations [red blood cell folate concentrations increased ~1.47-fold (95% CI, 1.27, 1.70) following fortification], and other health outcomes. Across settings, high-quality studies such as those with randomized exposures (e.g., randomized controlled trials, Mendelian randomization studies) are needed to elucidate interactions of FA with vitamin B_{12} as well as expanded biomarker testing.

1. INTRODUCTION

The fall of 2022 marks 30 years since the US Public Health Service [through the efforts of the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the Health Resources and Services Administration, and the National Institutes of

Health] recommended consumption of 400 μg/day of folic acid (FA) for the prevention of neural tube defects (NTDs) (19). The first trial examining FA for the prevention of NTDs took place in 1991 among women with a previous NTD-affected pregnancy ($n = 1,817$) who were randomized to receive a 4,000 μg/day supplement containing only FA; this study showed a 72% reduction (RR, 0.28; 95% CI, 0.12, 0.71) in subsequent pregnancies (116). In 1992, a second trial was conducted among women without a history of an NTD-affected pregnancy ($n = 4,753$) who were randomized to receive a daily multivitamin supplement containing 800 μg/day FA. In this trial, there were six NTD-affected pregnancies, with no cases among women who received the FA-containing supplement ($p = 0.029$) (32). This high-quality evidence (Figure 1) set the stage for the March of Dimes to petition the FDA to mandate FA fortification of products labeled as enriched cereal grain products (authorized in 1996 and fully implemented in 1998) (45).

FA fortification has been heralded as one of the top 10 public health achievements in the USA during the first 10 years of the twenty-first century (20). More than 1,300 NTDs are prevented annually in the USA, and mandatory FA fortification is estimated to provide savings of more than $600 million in direct costs each year (61). Fortification of staple grains is one of the best ways to increase health equity by reaching at-risk populations with an intervention that requires no behavioral change and has nominal costs. While many families and communities have benefited from the success of the program, concerns around safety, interactions with other nutrients, and challenges surrounding fortification implementation both domestically and abroad have arisen and will shape the future of FA fortification.

This review focuses on two areas of exploration: (*a*) monitoring the impact of FA fortification and (*b*) folate interaction with vitamin B$_{12}$ (vB12). First, while FA monitoring and dosages may seem like settled science, the operationalization of the long-standing intake recommendations is remarkably complex, and information on the totality of the homeostatic process between intake and outcomes is still missing some critical steps, including summary estimates of the impact of fortification on biomarkers. Second, the interaction between FA and vB12 on megaloblastic anemia has been a multifaceted story since early clinicians first mistreated it as a fatal blood disorder that had not one but two nutritional etiologies. This review does not cover the safety literature surrounding FA supplementation and other health outcomes (such as cancers), as these conditions are routinely reviewed by multiple expert groups around the globe. To date, these reviews have produced no findings that alter recommendations for FA fortification at the current dosages (44, 50, 51, 67, 145).

2. THE BASICS

2.1. Folate Metabolism

Humans are generally reliant on external sources of one-carbon methyl groups that are necessary for basic cellular processes. Folate is a general term used to describe naturally occurring and synthetic forms of vitamin B9. Folate is essential for cell growth as well as DNA synthesis and methylation, and, if depleted, results in double-stranded breaks in the DNA, triggering apoptosis (30). Folate is particularly important for preventing megaloblastic anemia due to the high folate requirements needed to maintain adequate blood counts. In

addition to the causal link with NTDs, epidemiological studies have shown that low folate status is associated with heart diseases and an increased risk of most cancers (5).

Naturally occurring dietary folate exists in both monoglutamate and polyglutamate forms. To be absorbed through the intestinal mucosa, polyglutamate dietary folate must be converted into 5-methyltetrahydrofolate (5-MTHF), a monoglutamate form of folate that is the primary form taken up by cells in peripheral tissue (5). Once in the cells, 5-MTHF must be polyglutamated to be retained and to function as a one-carbon-cycle coenzyme. To accomplish this task, methionine synthase (MS) converts 5-MTHF to tetrahydrofolate (THF). Synthetic FA is a monoglutamate form of folate that is readily transported across the intestinal epithelium. The enzyme dihydrofolate reductase reduces FA into dihydrofolate and THF (30) (Figure 2).

Once formed from either dietary folate or FA, THF can be converted to 5,10-methylene-THF by serine hydroxymethyltransferase, which is a vitamin B6–dependent reaction. Importantly for the one-carbon pathway, methylenetetrahydrofolate reductase (MTHFR) can catalyze the conversion of 5,10-methylene-THF to 5-MTHF, which serves as a carbon donor in the vB12-dependent conversion of homocysteine to methionine by MS. Methionine is an essential amino acid used for protein synthesis and as a substrate for *S*-adenosylmethionine (SAM), which is required for many methylation reactions for DNA, RNA, lipids, neurotransmitters, and hormones. Additionally, 5,10-methylene-THF is a cofactor for the methylation of deoxyuridylate to thymidylate, which is catalyzed by thymidylate synthase (156). Thymidylate is an essential metabolite necessary for DNA synthesis and repair (53). The folate one-carbon cycle is tightly regulated by several cofactors and metabolites, including SAM, vitamin B2, vitamin B6, and vB12 (53).

2.2.   Folate Vitamers and Neural Tube Defect Prevention

Folate occurs in various forms, known as vitamers. All folate vitamers consist of a common biochemical structure but differ in the number of glutamic acids, oxidation state, and presence of one-carbon groups (e.g., FA, 5-MTHF, THF, 5,10-methylene-THF, dihydrofolate). Although most FA is converted to 5-MTHF during intestinal absorption and first-pass metabolism in the liver, concerns about low levels of circulating unmetabolized FA have led to discussions about the potential advantages of using 5-MTHF instead of FA for food fortification and periconceptional supplementation for reducing NTD risk. This concept is problematic for several reasons. No randomized controlled trials (RCTs), or even less-rigorous studies, have examined any other folate form for the prevention of NTDs. Arguments in favor of 5-MTHF supplements in the form of L-MTHF or (6S)-5-methyltetrahydro-FA claim that 5-MTHF is more bioavailable and more "natural" than FA and not as affected by polymorphisms in the *MTHFR* gene (47, 144). Studies have found that supplementation with L-MTHF or (6S)-5-methyltetrahydro-FA increases red blood cell (RBC) and serum folate concentrations (6, 68, 85). However, RCTs would have to be conducted to determine effectiveness, timing, dosage, stability, and safety in order for 5-MTHF (or a synthetic equivalent) to be recommended. Given its position in the folate metabolism pathway, it is unlikely that 5-MTHF in the presence of lower concentrations of vB12 would be as effective as FA. Studies have found that to reach ideal RBC folate

concentrations, much higher serum/plasma folate concentrations (25.6 versus 34.6 nmol) are necessary in the presence of vB12 deficiency (22). Lastly, despite years of study, the unmetabolized FA that is in circulation has had no confirmed adverse effects, thus it is not necessary to replace a known effective intervention with one that does not have established effectiveness.

FA has a very similar dose–response increase in RBC folate concentrations across *MTHFR* genotypes when supplementing with 400 μg/day (27, 31). A meta-analysis of FA supplementation trials has shown that FA increases RBC folate concentrations by 178% and serum/plasma folate concentrations by 200% at the recommended dosage of 350–500 μg/day (28). Another meta-analysis showed only moderate absolute differences across *MTHFR* genotypes in folate concentrations, with an 18% reduction in CC versus TT (164). This finding suggests that people with *MTHFR* variants increase their folate status adequately to prevent NTDs with the recommended FA intake. Supporting evidence comes from studies in areas with very high rates of *MTHFR C677T* variants, such as northern China (12, 31) and Mexico (100), where FA reduces NTDs even when the variant is the major allele. Although efforts have been made to modify 5-MTHF to increase stability for commercial production (6, 68, 85), few studies have compared FA with these 5-MTHF isomers, and the impact of these modifications on birth defects has not been investigated.

2.3.   Folate in Food and Neural Tube Defect Prevention

The Food and Nutrition Board at the National Academies of Sciences, Engineering, and Medicine (73) has established intake recommendations for nutrients, including folate. To prevent anemia, the current recommended dietary allowance for folate is 400 μg/day of dietary folate equivalents (DFEs) for adults older than 19 years (73). DFEs are units of measurement that account for differences in bioavailability between naturally occurring food folate and synthetic FA (73). One DFE is equal to 0.6 μg of FA from fortified food or a supplement taken with food, or 0.5 μg of FA taken on an empty stomach, highlighting the lower bioavailability of food folate (73). Using DFEs, an individual can consume 400 μg of food folate or 240 μg of synthetic FA to meet the daily requirement of 400 DFEs. While many foods are folate rich, cooking and processing greatly reduce the available folate content in final products (142). To our knowledge, no intervention studies have shown that increasing natural food folate to target a specific intake [e.g., 667 DFEs (400 μg FA equivalent)] reduces NTDs or have elucidated interactions with other vitamins, so it is not possible to recommend an intake of natural food folate that is certain to prevent NTDs.

A meta-analysis of natural folate intake from food suggests a substantial variance in blood folate response as DFE intake increases (99). The modeled dose–response relationship between natural folate and RBC folate concentration showed that an estimated median intake of 350–800 DFEs would be needed to achieve RBC folate concentrations within the optimal range (906–1,500 nmol/L) for NTD reduction (99). Few studies have natural folate intakes in this range (99). Adequate vB12 is needed to effectively use natural folate for DNA replication (Figure 2). The high variance in the meta-analysis of the natural folate intake versus blood folate concentrations might be explained by the variation in vB12 status among

populations. Thus, it is reasonable to assume that adequate vB12 status might be needed for NTD prevention among persons consuming only food folate.

3.   EPIDEMIOLOGICAL RISK FACTORS FOR NEURAL TUBE DEFECTS

The etiologic heterogeneity of NTDs encompasses both modifiable and nonmodifiable risk factors, including maternal conditions, behaviors, genetics, and environmental exposures. Most risk factors for NTDs identified through epidemiological studies are folate sensitive, meaning that the risk for an NTD can be attenuated by FA before and during organogenesis. These include diabetes, obesity, *MTHFR C677T* variant, Hispanic ethnicity, vB12 deficiency, and hyperthermia/fever, as described in detail below.

3.1.   Diabetes

Epidemiological studies have identified pregestational diabetes (types 1 and 2) as an important risk factor for numerous congenital anomalies, including NTDs. Hyperglycemia acts as a teratogen during organogenesis through the induction of oxidative stress, leading to excessive apoptosis in the neural tube and gene expression dysregulation (57). A case–control study found significantly elevated odds of anencephaly, holoprosencephaly, and encephalocele (OR, 3.5–13.1) in offspring of women with pregestational diabetes; the association with spina bifida was not significant (OR, 1.4; 95% CI, 0.8, 2.7) (162). Other studies have found an elevated risk of spina bifida in infants of mothers with pregestational diabetes (1, 115). FA supplementation may weaken the association between diabetes and risk of spina bifida (125).

3.2.   Obesity

Prepregnancy obesity, defined as a body mass index (BMI) of 30 or higher, is associated with an increased risk for NTDs. Obesity affects approximately one-third of women of reproductive age (WRA) in the USA and contributes to the occurrence of NTDs through altered glucose metabolism, inadequate dietary intake, and increased FA requirements (18, 170). A recent meta-analysis of 22 case–control studies observed a maternal BMI dose–response relationship with risk of NTDs, with an odds ratio of 1.04 (95% CI, 1.03, 1.05) for every BMI increase of 1 kg/m^2 in the mother (72). FA supplementation reduces the risk of NTDs among persons with obesity, but the effect might be lower than among those with a normal BMI (170). The population attributable fraction for anencephaly and spina bifida due to obesity was estimated as 2.2% and 9.9%, respectively (1). Elimination of prepregnancy obesity could result in the prevention of 405 spina bifida cases and 95 anencephaly cases per year in the USA (71).

3.3.   *MTHFR C677T* Variant

As shown in Figure 2, *MTHFR C677T* polymorphism reduces the activity of the MTHFR enzyme, resulting in a decrease in plasma/serum and RBC folate concentrations across studies using an appropriate folate assay (microbiologic assay for RBC folate concentrations, where CC>CT>TT) for CC versus TT [plasma/serum, 13%; 95% creditable interval (CrI), 7%, 18%; RBC, 16%; 95% CrI, 12%, 20%] (164). Both maternal and fetal *MTHFR C677T* polymorphisms have been implicated as significant risk factors for NTDs

(164). Depending on the genotype (homozygous versus heterozygous) for *MTHFR C677T*, the odds of an NTD increase by 30% to 80% (OR, 1.3, 1.8) (157, 181). In an analysis of the impact of *MTHFR C677T* polymorphism on RBC folate concentrations, predicted models were elevated for the *MTHFR TT* genotype compared with the *CC* genotype [RR, 1.49; 95% uncertainty interval (UI), 1.33, 1.70] and for the *TT* genotype compared with the *CT* genotype (RR, 1.28; 95% UI, 1.17, 1.39) (27). These data are very similar to those from a meta-analysis of NTD risk from *MTHFR* genotypes, suggesting that the impact of *MTHFR C677T* on NTDs arises mainly from its impact on folate concentrations at lower folate intakes as NTD risk increases dramatically (27).

3.4. Hispanic Ethnicity

In the USA, racial/ethnic disparities in NTDs exist, with a greater burden of NTDs occurring among Hispanic populations (172). The underlying reasons for this disparity might be attributable to less FA supplementation use, reduced consumption of FA-fortified flour, and a greater prevalence of the *MTHFR C677T* polymorphism in Hispanic populations compared with non-Hispanic populations (17, 117, 150, 160). Public health interventions specifically targeted to the Hispanic population, such as fortification of corn masa flour (CMF), may decrease NTDs in this population (161).

3.5. Vitamin B$_{12}$ Deficiency

Maternal vB12 deficiency increases susceptibility to NTDs in offspring (110, 118, 126, 148). vB12 is a key micronutrient that is directly involved as a coenzyme in folate metabolism. Low vB12 status is correlated with lower serum or RBC folate concentrations (22, 118). Additionally, an association between low maternal vB12 levels and NTD risk has been established independently of folate levels (126), but additional studies are needed to elucidate these interactions. FA supplementation prevents NTDs in populations with substantial vB12 deficiency (12, 64, 65).

3.6. Hyperthermia and Fever

A meta-analysis consisting of 15 studies evaluating the association between maternal hyperthermia and the risk of NTDs observed an OR of 1.92 (95% CI, 1.61, 2.29) (111). There is evidence that, in addition to fever, external heat sources that raise core body temperature, including weather-related exposures and hot tubs, can increase the risk of NTDs (4, 38). Similarly, a recent case–control study reported elevated odds of NTDs in infants of mothers who experienced fever in the periconceptional period (OR, 2.4; 95% CI, 1.5, 4.0). This association was modified by consumption of the recommended intake of 400 μg/day of FA. Mothers who consumed 400 μg/day of FA had significantly lower odds of having a child with an NTD (OR, 1.8; 95% CI, 0.8, 4.0) than mothers with FA intake below 400 μg/day (OR, 4.2; 95% CI, 2.2, 8.2) (82).

4. APPROACHES FOR INCREASING FOLATE STATUS

4.1. Introduction

Three approaches for improving folate status among WRA exist: (*a*) eating a diet rich in natural food folate, (*b*) taking a FA supplement, and (*c*) consuming foods fortified with FA.

The first two approaches require more active personal-level action, while the third approach hinges on a population-level intervention.

High consumption of foods rich in natural folate is a beneficial part of a healthy diet, yet the daily personal action needed to obtain sufficient folate levels for NTD prevention requires behavioral change, sustained public education, and accessible and affordable folate-rich food sources (10). Obtaining the equivalent intake of 400 μg/day of FA with natural food folate alone requires consumption of a large quantity of foods not typically consumed. For example, a person would need to consume approximately 12 cups of raw spinach daily to reach the recommended level (119). Therefore, it is extremely difficult to achieve improved folate status at a population level via dietary changes alone (10, 88), and, as previously discussed, achieving improved folate status may require adequate vB12 status.

FA supplements provide excellent bioavailability, but intake of supplements requires a daily behavioral activity during the critical periconceptional period as well as sustained public education. The early closure of the neural tube by 28 days after conception presents a major barrier to the use of FA supplements for NTD prevention. Ideally, intake should occur prior to and during early pregnancy before the closure of the neural tube, which occurs before many women know they are pregnant. Currently, only 23% of WRA and 38% of those planning a pregnancy consume a daily multivitamin in the USA (173). Given that almost half of all pregnancies in the USA are unplanned (48), FA supplementation for NTD prevention requires awareness of FA through sustained public education to reach targeted audiences. However, studies have shown that educational campaigns encouraging women to increase their use of supplements have limited effectiveness at reaching high-risk populations (134). Finally, the cost of purchasing supplements could deter some women from daily intake (92).

Food fortification with FA is a low-cost public health intervention that aims to reach populations at the country or regional level and is often delivered through national programs (33). Food fortification can be either voluntary or mandatory. In the USA, voluntary fortification allows food manufacturers to add FA to foods, as long as they abide by the FDA's food additive regulations (46). Examples of voluntary fortified foods include ready-to-eat breakfast cereals and CMF (42). Alternatively, mandatory fortification requires food manufacturers to add FA to specified foods. The FDA requires FA to be added to products labeled "enriched cereal grain products" as part of these foods' standard of identity (45). This population-level intervention enables WRA to obtain additional FA without requiring active behavior changes or sustained education campaigns. Women consume fortified food regardless of their pregnancy intention (10). As a result, FA food fortification is a cost-effective population-level strategy for improving folate status among WRA to prevent birth defects (62).

4.2. Mandatory Folic Acid Fortification

World Health Organization (WHO) guidelines on food fortification with micronutrients provide a procedure for estimating and selecting feasible FA levels for fortification programs (174). Globally, 71 countries have passed legislation or regulations to mandate fortification of food sources with FA on the basis of information from the Global

Fortification Data Exchange, UN Food and Agriculture Organization, and WHO (see https://fortificationdata.org; see also Supplemental Appendix 1 for specific country data).

4.2.1. Food vehicles.—The primary vehicles for FA fortification are wheat flour, maize (corn) flour, and rice. Wheat flour fortification is used predominantly in countries with mandatory FA fortification. The fortified FA dosage level for wheat flour varies from 100 to 511 μg per 100 g of flour (Supplemental Appendix 1). In the USA, enriched cereal grain products are fortified at 140 μg per 100 g, contributing to a total median daily intake of 220 μg of FA and natural food folate in nonpregnant WRA (29).

Maize flour or cornmeal is the second most common food vehicle (used in 16 countries) for mandatory FA fortification. The fortified FA dosage level in countries with mandatory maize flour fortification varies from 100 to 260 μg per 100 g of flour. Mandatory rice fortification with FA has been used in five countries, with FA dosage levels varying from 100 to 231 μg per 100 g of rice. FA retention in fortified rice ranges between 76% and 96%, depending on the preparation and cooking method, and limited data show that fortified rice may slightly increase serum folate concentrations (175).

In regions where grains are not industrially milled or widely consumed, fortification of alternative nongrain food vehicles with FA might help reduce the prevalence of NTDs. Beverages, condiments, and seasonings have been or are being considered for FA fortification. Costa Rica mandated that all milk products be fortified with 40 μg FA per 250 mL of milk (7) and is the only country to have done so. Seasonings and condiments including salt, sugar, margarine, oil, and fish sauce are being explored for FA fortification. While studies on FA bioavailability in seasonings and condiments are limited (34), fortification of salt with FA might be a promising strategy. Salt is widely consumed and has succeeded as a fortification vehicle through iodization, which led to a significant global reduction in iodine deficiency (103). The efficacy of adding FA to salt has been demonstrated as a double-fortified vehicle with iodine (90, 102); a triple-fortified vehicle with iodine and iron (109); and a quadruple-fortified vehicle with iodine, iron, and vB12 (98). McGee et al. (102) reported stable double-fortified salt with iodine and FA using a single-spray solution that does not require any change in existing salt iodization equipment or procedures. After 12 months, the amount of FA and iodine retained in the salt was >80% and >90%, respectively (102). Additionally, a recent study estimated that 65% of FA-preventable NTD cases could be prevented annually through salt fortification (80).

4.2.2. Fortification of multiple food vehicles.—Safe and effective fortification requires establishing a balance between addressing inadequate nutritional intake and avoiding excessive intake, particularly in populations not at risk for nutritional inadequacies. While fortification of multiple food vehicles with the same micronutrient (e.g., FA) increases the likelihood that the benefits of a fortification program will extend to a broader range of the population, consumption of multiple fortified foods might result in a higher likelihood of nutrient intakes near or above tolerable upper levels. Estimating potential dietary contributions of fortified foods within populations can help determine which food vehicle to fortify and how much of a nutrient should be added to the fortified food.

Implementing fortification of food products not commonly consumed together might extend fortification coverage to diverse populations without overfortifying. For example, the USA allows for FA fortification of both wheat flour (mandatory) and CMF (voluntary). It is unlikely that individuals are "doubling up" on FA intake in a particular meal, given that typical dietary patterns include consumption of either CMF or wheat flour, but not both. Two recent studies (168, 169) that evaluated the usual intake of FA by WRA in the USA using data from the National Health and Nutrition Examination Survey (NHANES) suggest that the current fortification programs have not led to usual intakes above the tolerable upper intake level for FA. The usual intake from mandatory fortification alone for women aged 12–49 is only 115 μg/day [interquartile range (IQR), 79–156]. This moderate intake has very low variance and is ideal for a mandatory fortification program. Among women who consumed FA from all three potential sources of FA (i.e., mandatory enriched cereal grain products, voluntary fortified ready-to-eat cereals, and supplements), the median FA intake was 661 μg/day (IQR, 519–830), which is well within the daily intake recommendations of 400 μg/day by the CDC and 400–800 μg/day by the US Preventive Services Task Force (29). The CMF program was implemented in 2016, and Hispanic women had similar usual intakes pre-CMF (177 μg/day; IQR, 85–299) versus post-CMF (161 μg/day, IQR 71–277) (168), consistent with the general population (169).

5. MONITORING THE IMPACT OF FOLIC ACID FORTIFICATION

5.1. Monitoring the Impact of Folic Acid Fortification Using Biomarkers on Neural Tube Defects

As shown in Figure 1, no RCTs have assessed the impact of FA fortification, because it is not possible to conduct an RCT after the implementation of a fortification program due to universal exposure across the population. The literature is therefore limited to pre- and postfortification implementation studies, which are ecological and subject to the inherent biases of this type of design.

This primary method of monitoring FA fortification's impact on NTD prevalence both pre- and postfortification (Figure 3*a*) (Supplemental Appendix 2) depends on many factors, including baseline NTD rates, folate concentrations, population characteristics, fortification levels, program implementation, and population folate consumption. Since FA fortification was implemented in the USA, analyses of pre- and postfortification data (1995–2011) from 19 population-based birth defect surveillance programs have shown that the prevalence of NTDs decreased by 21% to 35% (172). Several meta-analyses have reported similar reductions in NTD prevalence following mandatory FA fortification in other countries. A meta-analysis of eight population-based, large-scale studies from five countries (Canada, USA, Chile, Argentina, and South Africa) assessed the effect of mandatory FA fortification on the prevalence of NTDs and estimated a 46% risk reduction (RR, 0.54; 95% CI, 0.46, 0.63) (14). Another meta-analysis of eight studies, most of which were conducted in Central or South America, showed a significant postfortification reduction in total NTDs (OR, 0.59; 95% CI, 0.49, 0.73) as well as NTD subtypes, including both anencephaly (OR, 0.49; 95% CI, 0.4, 0.6) and spina bifida (OR, 0.66; 95% CI, 0.53, 0.82) (81). Furthermore, a study of FA fortification status in 123 countries showed that the countries with mandatory FA

fortification had a lower prevalence of spina bifida (3.5 per 10,000 live births) compared with countries without mandatory FA fortification (5.3 per 10,000 live births) (3).

Changes in NTD prevalence from pre- to postfortification show that the percent decrease in NTD rates varies from country to country and that the countries with the highest baseline rates have the largest percent decrease (Figure 3*a*). However, using changes in NTD rates alone as an outcome makes it difficult to attribute the changes to fortification or determine which populations are being missed by a fortification program and is dependent on having an effective surveillance system that assesses birth defects not only among live-born infants but also among pregnancies ending in stillbirths or elective terminations.

In 2015, the WHO established the RBC folate concentration for NTD prevention as a tool to assess the need for or success of a fortification program (25, 163). This five-step process assesses, monitors, and adjusts programs on the basis of RBC folate concentrations among WRA. Several countries and regions, including the USA and Guatemala, have assessed RBC folate concentrations after fortification (29, 138, 163). Data on the USA show that while most WRA have RBC folate concentrations consistent with optimal NTD prevention, more than 22% remain at risk due to folate insufficiency (163). Median FA intake is only 115 μg/day (the predicted NTD prevalence in this group is 8.5 per 10,000 births); with additional sources of FA, it is estimated that up to 700 additional NTDs could be prevented annually in the USA (29). Utilizing the WHO guidelines, the USA also evaluated the addition of a new voluntary FA source (CMF) that was authorized in 2016 to address the higher rate of NTDs among Hispanic populations (168). In Guatemala, researchers demonstrated the success of fortification by showing increased RBC folate concentrations in metropolitan areas, where fortified products were readily available, compared with other regions of the country, where there was poor penetration with fortified products and different food consumption patterns (138). Countries without mandatory FA fortification, such as India, have also used the guidelines to examine recent regional population–based data. Results showed that the prevalence of RBC folate deficiency and insufficiency among WRA was 7.6% and 79.3%, respectively, and the NTD prevalence predicted on the basis of RBC folate concentrations was 20.6 per 10,000 births (95% UI, 16.5, 25.5) (49), suggesting that an FA fortification program would probably be beneficial.

The laboratory gold standard for assessing RBC folate concentrations is a microbiologic assay, as most other assays have established inaccuracies and/or need adjustments (130, 164). Few studies (Figure 3*b*) (Supplemental Appendix 3) have compared pre- and postfortification RBC folate concentrations using microbiological assays. To address this severe limitation, we conducted a systematic search of the literature and modeled the change for studies that used the same assay in both pre- and postfortification periods (for details, see Supplemental Appendix 3); however, the results should be interpreted with caution. Despite the limitations of the available data, changes in RBC folate concentrations are consistent with expected changes observed in supplementation trials. RBC folate concentrations increased approximately 1.47-fold (95% CI, 1.27, 1.70). Likewise, serum folate concentrations, which are not as susceptible to the assay bias, increased approximately 1.89-fold (95% CI, 1.49, 2.45) following FA fortification, also consistent with supplementation data (Figure 3*c*). These findings are reassuring, given their

consistency with the anticipated folate concentration increases of one-and-a-half- to twofold over baseline found in supplementation studies. Pre- and postfortification biomarkers of populations in countries that are implementing new FA fortification programs (e.g., United Kingdom, New Zealand) will be of great interest.

5.2. Impact of Folic Acid Fortification on Other Health Outcomes

While folic acid fortification was implemented to target persons capable of becoming pregnant for the prevention of NTDs, this intervention affects the entire population and multiple health outcomes. Studies have examined the effects of fortification programs on other birth defects, prevention of anemia, cardiovascular disease, and cancer.

5.2.1. Orofacial clefts and congenital heart defects.—In addition to NTDs, there might be an association between FA fortification and reduction of orofacial clefts (OFCs). A meta-analysis of studies comparing the prevalence of OFCs before and after mandatory FA fortification in multiple countries, including Argentina, Brazil, Chile, and the USA, demonstrated that the prevalence of nonsyndromic cleft lip and palate significantly decreased (RR, 0.88; 95% CI, 0.81, 0.96) following fortification, but there were no significant effects of fortification on the prevalence of total OFCs or cleft palate only (105). These results are similar to those from another meta-analysis, which demonstrated a nonstatistically significant decrease in both cleft lip with or without palate (RR, 0.87; 95% CI, 0.76, 1.00) and cleft palate only (RR, 0.90; 95% CI, 0.71, 1.15) (184).

Certain types of congenital heart defects (CHDs) might also be prevented through FA fortification. A Canadian time-trend analysis that compared the prevalence of severe CHDs prefortification (1990–1998) with the prevalence postfortification (1998–2005) demonstrated a decrease from 1.64 cases (95% CI, 1.55, 1.73) per 1,000 births prefortification to 1.47 (95% CI, 1.37, 1.58) per 1,000 live births postfortification, or a 6% decrease per year in the 7 years following the initiation of mandatory FA fortification (74). Another Canadian population-based study of all live births and stillbirths from 1990 to 2011 demonstrated lower rates of conotruncal defects (RR, 0.73; 95% CI, 0.62, 0.85), coarctation of the aorta (RR, 0.77; 95% CI, 0.61, 0.96), ventricular septal defects (RR, 0.85; 95% CI, 0.75, 0.96), and atrial septal defects (RR, 0.82; 95% CI, 0.69, 0.95) following FA fortification (93). Although FA fortification was associated with an overall 11% decrease in the prevalence of CHDs, it was not associated with a significant reduction in severe nonconotruncal heart defects, suggesting a differential effect across subtypes of CHDs (93).

5.2.2. Prevention of anemia.—Folate deficiency megaloblastic anemia is a consequence of inadequate folate intake (73). In the USA, the usual intake of folate from food is only ~170 DFEs, less than half the recommended dietary allowance of 400 DFEs for the prevention of anemia (29, 168). Studies comparing data prefortification (1988–1994) and postfortification (1999–2004) among WRA found a 27.9% reduction in the prevalence of anemia (60), a 50% increase in RBC folate concentrations, and a subsequent decrease in folate deficiency from 38% to 5% (129). The benefits were not limited to WRA. Meta-analyses of four studies and eight studies suggest that FA fortification is associated with significant declines of folate deficiency in broader populations (RR, 0.20; 95% CI, 0.15,

0.25) and with significant increases in plasma folate (standard mean difference, 1.25 nmol/L; 95% CI, 0.5, 1.99), respectively (81). In older adults in the USA, folate deficiency anemia has nearly been eliminated after mandatory FA fortification, consistent with findings in other countries such as Canada and Australia (123).

5.2.3. Cardiovascular disease.—Elevated homocysteine concentrations are consistently associated with cardiovascular disease, and FA fortification has a significant effect on plasma levels (75, 179). Following FA fortification in the USA, there were markedly lower homocysteine levels among middle-aged and older adults enrolled in the Framingham Heart Study, a large epidemiological study of heart disease (75). Data on homocysteine levels from NHANES were used to predict that fortification alone would decrease cardiovascular events by 8% in women and 13% in men (159). A study that evaluated age-adjusted stroke mortality rates in the USA, Canada, England, and Wales from 1990 to 2002 (179) found that the annual decline in stroke mortality was between 0.7% and 1.4% in each of these countries in the prefortification years but following fortification shifted to a 2.7–2.9% annual decline in the USA and a 5.4–5.6% annual decline in Canada (179). In contrast, there was no change in decline of age-adjusted stroke mortality in England and Wales after 1998, suggesting that FA fortification decreases stroke mortality. These types of ecological comparisons need to be interpreted with great caution. However, strengthening the case for this possibility, a large, randomized trial of FA supplementation from China found that intake of FA, in addition to the usual medicine taken for stroke prevention, decreased stroke risk, with greater benefit for individuals with lower initial folate concentrations (89).

5.2.4. Cancer.—Results from studies evaluating the incidence of cancer following FA fortification have been largely reassuring. While an increase in colorectal cancer (CRC) diagnoses in the USA and Canada was observed in 1996 (coinciding with the implementation of voluntary FA fortification), a decline in both CRC diagnoses and mortality has been observed since 1998, after the implementation of mandatory FA fortification (83). Data from the Australian Cancer Database also show a decrease in CRC since 1999, yet this decline became much more pronounced in 2010, when Australia mandated FA fortification (166).

Additionally, two studies used the Pediatric Oncology Group of Ontario's database, which identifies approximately 95% of all pediatric cancers in Ontario, to evaluate the effect of FA fortification on the incidence of childhood cancer (54, 63). FA fortification was associated with a 62% reduction (95% CI, 38–77%) in the incidence of neuroblastoma (i.e., from 1.57 per 10,000 children prefortification to 0.62 cases per 10,000 children postfortification) (54) and a 26% reduction (95% CI, 5–43%) in the incidence of Wilms tumor (i.e., from 1.94 per 100,000 children to 1.43 per 100,000 children) (63). Data from the National Cancer Institute's Surveillance, Epidemiology, and End Results program demonstrated a similar reduction in the incidence of Wilms tumor in the USA (91). These ecological studies should be interpreted with caution.

5.3.   Is There a Need for Continued Supplementation Postfortification?

The current CDC recommendation for 400 μg/day of FA from supplements or fortified foods, or a combination of both, has stood for 30 years (19). Regardless of *MTHFR* genotype, women will reach optimal blood folate concentrations if they consume 400 μg/day for 3–6 months or more; however, this effect is not observed at consumption levels of 100 μg/day (31). Fortification programs add a modest amount of FA to a vehicle (100–260 μg FA per 100 g of product), targeting a usual intake of approximately 200 μg/day. With current US consumption patterns, the median overall intake from all sources (natural food folates, supplements, and mandatory and voluntary fortification) is 237 μg/day (IQR, 146–370); of this amount, 115 μg/day (IQR, 79–156) comes from mandatory fortification alone (29). This intake results in 22.8% of US women having insufficient RBC folate concentrations for optimal NTD prevention (163). Therefore, the US Preventive Services Task Force continues to recommend supplementation of 400–800 μg/day for women planning pregnancy (165).

Higher intakes of FA might be needed in specific situations, such as exposure to agents that require detoxification with one-carbon sources, including heavy metals (e.g., arsenic, lead), or intake of folate agonists. Chronic exposure to inorganic arsenic affects 140 million people worldwide. FA supplementation facilitates arsenic methylation and excretion and is associated with lower levels of serum arsenic (15, 58, 59, 86). A case–control study showed that the protective effect of FA supplementation for NTDs declined as inorganic arsenic concentrations in drinking water increased from 1 μg/L to 25 μg/L (OR from 0.22 to 1.03; 95% CI from 0.13, 0.37 to 0.55, 0.91) (101). Additional research is needed.

5.4.   Cost–Benefit of Folic Acid Fortification

NTDs impose a significant economic burden on health care systems, as lifetime direct and indirect costs are substantial. In addition to costs accrued shortly after birth from hospitalizations and surgeries, persons with NTDs require ongoing care; have high rates of comorbidities; and often need other services, such as special education, developmental services, or mobility-related assistive devices. Caregivers of children with NTDs are affected by reductions in paid work time. Lifetime costs of NTDs vary across countries, likely due to differences in health care prices and availability of treatments and services. In the USA, estimated lifetime direct costs for a person with spina bifida were $791,000 in 2014 (61).

Food fortification is a relatively inexpensive public health intervention to reduce micronutrient deficiencies. The cost of FA fortification is estimated to be $0.20–0.30 per metric ton, or approximately $0.06 per person per year, which equates to $607 million in costs averted each year (61). Chile has reported similar results, with an estimated savings of $11.80 for every $1.00 spent on fortification equating to $89 per disability-adjusted life year averted (95). The Chilean study considered life expenses only up to the age of 22 years, so these cost savings are probably underestimated (95). A study from South Africa reported a cost–benefit ratio of 30 to 1 of FA for the prevention of NTDs (143). More evidence is available from Europe. Using 2009 birth surveillance data from EUROCAT and assuming a 50% reduction in NTDs, researchers estimated that a total of €79.6 million (US$94.2 million) of direct lifetime medical costs could have been prevented annually if Germany had adopted FA fortification policies (122).

6. FOLIC ACID SAFETY AND INTERRELATIONSHIP BETWEEN FOLATE AND VITAMIN B$_{12}$

The effectiveness and safety of FA fortification programs have withstood the test of time. However, theoretical concerns persist, including the effects of interactions between high folate and low vB12 status on anemia (especially among older adults), cognition, and metabolism. There is also heightened interest in the potential effects of antenatal maternal FA exposure on cardiometabolic risk and the development of diabetes in children from populations where vB12 deficiencies are prevalent. It is critical to evaluate all evidence for each of these concerns, keeping in mind the impact of study designs and examining each aspect of folate and vB12 metabolism (intake, absorption, processing, utilization, and excretion) to consider ways to mitigate these concerns.

6.1. Masking of Vitamin B$_{12}$ Deficiency Anemia

Vitamin B$_{12}$ deficiency can cause macrocytic anemias and neurological complications, which, if left untreated, can be irreversible. Neurologic complications can be present with or without a macrocytic anemia. However, early case reports describing the occurrence or progression of neurological complications among vB12-deficient patients treated with FA led to concerns that increased FA exposure through fortification might lead to the masking of vB12 deficiency by correcting macrocytic anemia while allowing neurologic complications to progress. This hypothetical situation is particularly concerning for older adults, who are more likely to have vB12 deficiency (73).

Standard clinical practice includes testing any individual with unexplained neurological complications for vB12 deficiency regardless of anemia status. A Canadian study reported that the prevalence of serum vB12 insufficiency (<150 pmol/L) with high serum folate status (>45 nmol/L) among older Canadian women increased postfortification compared with prefortification (0.09% versus 0.61%) (136). Although this study did not examine folate and vB12 in regard to hematological indicators, results raised concerns about masking of vB12 deficiency by FA. Therefore, some public health and nutrition experts have suggested adding vB12 and FA to supplements and fortified foods, respectively, to reduce this risk.

If increased FA intake truly masks anemia in individuals with vB12 deficiency, then we would expect a drop in vB12 deficiency anemia following FA fortification. Several studies have compared the prevalence of vB12 deficiency without anemia before and after FA fortification (94, 104, 108, 132); only one study from an academic hospital reported an increase in low serum vB12 without macrocytosis among US adults (19 years) after fortification (176). Because macrocytosis is not a specific indicator of vB12 status and could also be a result of folate deficiency, an overall improvement in population folate status may explain the lower prevalence of macrocytosis postfortification. As a whole, the available epidemiological evidence suggests that there is little risk of FA fortification masking vB12 deficiency macrocytosis and adversely affecting the clinical presentation of vB12 deficiency, in particular among older adults. This view agrees with assessments conducted by multiple governmental and independent advisory agencies that support the safety of mandatory fortification with FA (43, 45, 50–52, 67, 145).

As noted in a recent review (9), blood assays for folate and vB12 had not yet been developed in early case reports of vB12-deficient patients being treated with folic acid (37). Routine diagnostic tests used at the time (e.g., hemoglobin, hematocrit, stained blood smears) were not specific for vB12 deficiency and thus would not have been able to distinguish among macrocytic anemias from vB12, folate, or some other nutritional deficiency. Therefore, neurological complications from early reports likely arose from incorrect treatment of vB12 deficiency anemia with FA and should not be used as evidence of FA's toxicity (9, 37). Now that clinical and laboratory tools for diagnosing vB12 deficiency are readily available, incorrect treatment of vB12 deficiency with FA is a medical error.

6.2. Folate and Vitamin B$_{12}$ Interactions

Another concern about the potential health risks associated with increased FA intake is the effect of the interaction between folate and vB12 status on cognition and vB12 metabolism. Three cross-sectional studies (106, 147, 152) reported that abnormalities in total homocysteine and/or methylmalonic acid (MMA, a metabolic indicator of vB12 status) were associated with concurrent higher folate and low vB12 status (defined through different cutoffs and summary measures). Several other studies also assessed the association between high folate and low vB12. For anemia, three cross-sectional studies (two in voluntary fortification settings in the United Kingdom and Ireland and one after mandatory fortification in the USA) also found that higher serum folate concentrations did not affect the association between low vB12 status and anemia or hemoglobin concentrations (24, 66, 107).

6.2.1. Cognition and folate/vitamin B$_{12}$ status.—The US Framingham Heart Study examined older adults' pre- and postfortification serum/plasma folate levels, vB12 status, and cognition. The study found that, among older adults with low levels of plasma vB12 (<258 pmol/L), faster cognitive decline (e.g., annual change in Mini–Mental State Examination scores) was associated with plasma folate in the highest quintile (21.75 nmol/L) in comparison to the lowest quintile (<5 nmol/L) (114). Two cross-sectional analyses of NHANES 1999–2002 (after mandatory fortification) reported an interaction between folate and vB12 status among older adults. In older persons with low vB12 status, poorer cognitive function was associated with higher serum folate concentrations (OR, 2.6; 95% CI, 1.1, 6.1) (112). However, in the same study, higher serum folate protected older adults with normal vB12 status from cognitive impairment (OR, 0.5; 95% CI, 0.2, 0.96). In the second NHANES study of the same population, circulating unmetabolized FA was associated with lower cognitive function among seniors with low vB12 status but not among those with normal vB12 status ($p_{\text{interaction}} = 0.007$) (113).

Other studies could not corroborate these observations of higher folate status affecting the association between low vB12 status and impaired cognition. One cohort study of older adults in the United Kingdom found that while cognitive decline was not associated with either serum folate or vB12, the rate of decline slowed with indicators of active vB12 status (e.g., holotranscobalamin, MMA) (23). This study found no interaction between folate and vB12 status, as higher folate status was not associated with faster cognitive decline among persons with low vB12 status (e.g., low holotranscobalamin concentrations). Four additional

cross-sectional studies found no evidence that higher folate status increased the association between low vB12 status and poor cognitive performance. Under the voluntary fortification setting of the United Kingdom, an analysis of combined data from two cross-sectional studies found that low vB12 status (holotranscobalamin <45 pmol/L) was associated with cognitive impairment among older British adults with normal folate status (OR, 1.45; 95% CI, 1.19, 1.76) but that higher serum folate status (>30 nmol/L) did not increase the association between low vB12 status and cognitive impairment (OR, 1.50; 95% CI, 0.91, 2.46) (24). These associations remained similar even at a higher serum folate cutoff point (>60 nmol/L). The association between low vB12 status and poor cognition (OR, 1.56; 95% CI, 1.30, 1.88) did not worsen in the presence of higher serum folate (OR, 2.46; 95% CI, 0.90, 6.71).

Among a population of older Latinos with low vB12 status in California after mandatory fortification, higher folate concentrations were not associated with poorer cognitive performance (106). Likewise, combined higher plasma folate and low vB12 status were not associated with cognitive impairment among a small group of octogenarian and centenarian African Americans and Caucasians living in the US state of Georgia (66). A more recent analysis using data from NHANES 2011–2014 reported that among older adults (60 years) with low serum vB12 concentrations (258 pmol/L), higher folate status (defined as serum folate 59 nmol/L or RBC folate 1,609 nmol/L) was protective against poor cognition (OR, 0.61; 95% CI, 0.45, 0.83) (139). However, individuals with concurrent higher folate and low vB12 status were not more likely to have poor cognitive performance (OR, 0.89; 95% CI, 0.36, 2.22) (139). In addition, 34% of persons with high folate and low vB12 levels were supplemented with vB12 (139). A moderate association between high MMA/high folate and poor cognition was attenuated after the authors adjusted for kidney function (139). Without increased folic acid intake, poor kidney function has been associated with increased folate concentrations (169). Taken together, these results suggest that both vB12 intake (indicator of possible malabsorption) and kidney function are important confounders of the reported association of high folate and low vB12 (139). Therefore, studies of high folate and low vB12 should be interpreted with caution.

Regardless of the limited evidence, several hypotheses have been suggested for associations between concurrent high folate and low vB12 status and cognition, anemia, and vB12 metabolism. One theory is that excess FA and folate metabolites might directly affect or worsen vB12 deficiency by inducing oxidation of vB12 and reducing the amount of available vB12 for important reactions [i.e., conversion of homocysteine to methionine via MS or the conversion of methylmalonyl–coenzyme A (CoA) to succinyl-CoA in the mitochondria via methylmalonyl-CoA mutase], which might lead to neurologic, hematologic, or abnormal metabolic consequences (147). Whether FA has any oxidative effect on vB12 is unknown.

A second theory is based on the methylfolate trap hypothesis, which centers on vB12 deficiency and the disruption in MS activity (69, 146). Without vB12, MS cannot convert homocysteine to methionine, resulting in a decrease in cellular SAM. This decrease in turn disrupts critical methylation reactions that produce and maintain myelin, the insulating layer surrounding neurons. Disturbances in myelin formation can lead to neurological

complications, including cognitive impairment (171). The disruption in the function of MS would also block the conversion of 5-MTHF to THF, resulting in an accumulation of intracellular 5-MTHF. 5-MTHF can easily move from inside the cell into the blood circulation, so increases in serum folate concentrations would be observed. Thus, elevated serum folate concentrations can be observed among persons with vB12 deficiency (87, 155).

A third hypothesis to explain the outcomes observed among individuals with concurrent high folate and low vB12 status is that this group has an underlying undetected condition that affects the absorption of vB12 (e.g., pernicious anemia, other types of malabsorption) (11). According to this theory, in the absence of underlying disease, supplement users metabolize FA and vB12 normally and, consequently, have higher blood concentrations of both vitamins. However, for individuals with pernicious anemia, the inability to absorb supplemental vB12 combined with the slow progressive nature of the disease eventually depletes the body's vB12 stores. Because these individuals are still able to absorb and metabolize FA normally, an apparent association would be observed between higher blood folate concentrations and clinical and metabolic abnormalities. Consistent with this theory are findings from studies that observed a significantly higher proportion of individuals who took supplements containing vB12 among persons with concurrent higher folate and low vB12 status than among those with concurrent normal folate and low vB12 status. Data from NHANES showed that the majority (>90%) of seniors with serum folate concentrations in the highest quintile were multivitamin supplement users who took not only FA but also enough vB12 (median intake, 16.6 μg) to correct any vB12 insufficiency under normal circumstances (11). Additional analyses from NHANES indicate that adults with higher FA intake and higher serum folate concentrations had higher vB12 intakes and were significantly less likely to have vB12 depletion (180).

Among studies examining the interaction between folate and vB12 status on health outcomes, an important methodological limitation is the inability to establish a causal link between an individual's folate and vB12 status and their risk for anemia, cognitive impairment, and abnormal vB12 metabolism. In our review, most of the evidence comes from cross-sectional studies that are weaker in epidemiologic study design (Figure 1); findings from epidemiologically stronger cohort studies failed to provide consistent results. Cross-sectional studies supporting an association between concurrent high folate/low vB12 status and adverse clinical and metabolic outcomes might be susceptible to reverse causality, making higher folate and low vB12 status the effect rather than the cause of clinical and metabolic abnormalities. This theory is plausible if persons with low vB12 status seeking medical care for their symptoms (e.g., fatigue, cognitive changes, tingling, numbness of extremities) were advised by their providers to take vitamin supplements, thus raising their blood folate concentration and creating a spurious association between higher folate status and anemia, cognitive impairment, and elevated total homocysteine and MMA concentrations. Carefully designed prospective studies, Mendelian randomization, and systematic and ongoing monitoring of populations with greater exposure to FA could enable us to better understand any potential interactions between higher folate status and vB12 deficiency. Furthermore, interpretation of studies examining the effects of fortification may differ from that of studies examining the effects of supplementation, as amounts of FA consumption vary greatly depending on the source (180, 182). Because

the contribution of fortification to higher blood concentrations is minor compared with the contribution of supplements, conclusions drawn from supplementation studies may not apply to fortification.

6.2.2. Antenatal maternal folic acid exposure and risk of diabetes and metabolic disease.—Evidence from epidemiological and animal studies suggests that unbalanced maternal nutrition might lead to changes in the intrauterine environment, thereby affecting the risk of health outcomes in the offspring later in life (78, 121, 124). Studies have suggested that shifts in the availability of FA and vB12 during critical developmental periods in utero can induce long-term modifications in gene expression via epigenetic mechanisms and alter fetal development and metabolic programming (30, 121, 151, 177). A recent topic of interest is the longer-term effect of antenatal FA exposure (i.e., prenatal FA supplementation or higher maternal folate concentration during pregnancy) on diabetes and metabolic disease in children among populations with prevalent vB12 deficiency (13, 16).

6.2.3. Diabetes and metabolic disease.—A population-based cohort study in India reported that mothers with higher maternal RBC folate status during pregnancy (at 28 weeks gestation) were more likely to have children with higher insulin resistance [as measured by homeostasis model assessment of insulin resistance (HOMA-IR)] and greater adiposity, as measured by fat mass and percent body fat at age 6 years (178). Approximately two-thirds of this population had vB12 deficiency. Insulin resistance was highest among children born to mothers with a combination of plasma vB12–deficient status and higher RBC folate concentrations (178). These findings raised concerns that higher folate or FA intake during pregnancy among women who are vB12 deficient might lead to an increased risk of type 2 diabetes in children.

In contrast to these findings, two analyses from a community-based RCT in Nepal reported that antenatal supplementation with FA, either alone or in combination with other micronutrients, did not increase the risk for metabolic disease in children (153, 154). This RCT under the Nepal Nutrition Intervention Project–Sarlahi examined the effects of antenatal micronutrient supplementation on health outcomes in offspring. A total of 4,926 pregnant women were enrolled and randomized into five different supplement groups: vitamin A alone (control); vitamin A and FA; vitamin A, FA, and iron; vitamin A, FA, iron, and zinc; and vitamin A, FA, iron, zinc, and 11 other micronutrients. Apart from those in the control group, the women were given daily supplements containing 400 μg of FA from the time of enrollment in early pregnancy (mean gestation at enrollment, 11 ± 5 weeks) to 3 months postpartum. Approximately 28% of the women had vB12 deficiency (serum vB12 <150 pmol/L) in the first trimester prior to supplementation, and 12% had folate deficiency (serum folate <6.7 nmol/L) (77). Children were followed from birth through age 6–8 years and were assessed for cardiometabolic risk factors, insulin resistance, and peripheral adiposity.

While no differences in insulin, glucose, HOMA-IR, cholesterol, triglycerides, or blood pressure were observed across the supplement groups in comparison to children from mothers in the control group, the risk for metabolic syndrome was significantly lower among children from mothers in the FA-only group (OR, 0.63; 95% CI, 0.41, 0.97; $P = 0.03$) (154).

In comparison to controls, children from mothers in the FA-only group and the FA, iron, and zinc group had a lower risk for microalbuminuria, a well-known marker for kidney and cardiovascular disease. These results suggest that antenatal maternal FA supplementation does not increase the potential for cardiovascular disease and diabetes in children within this population with prevalent vB12 deficiency.

In a substudy of this RCT among women who provided blood samples early (at 10.2 + 4.1 weeks gestation) and late (at 32.6 + 2.9 weeks gestation) in pregnancy, HOMA-IR was 26.7% higher among children whose mothers had vB12 deficiency during early pregnancy compared with children whose mothers did not have vB12 deficiency during early pregnancy ($p = 0.02$) (153). In this analysis, neither early nor late pregnancy maternal folate status was associated with insulin resistance in children, and no interaction was observed between maternal folate and vB12 status relative to childhood insulin resistance. Among children whose mothers had vB12 deficiency early in pregnancy, maternal supplementation with FA alone or in combination with other micronutrients did not change the risk for insulin resistance in children. Furthermore, no interaction was observed between maternal vB12 status and vitamin supplementation for HOMA-IR in children ($p > 0.1$ for all supplementation groups). Taken together, results from these RCT analyses suggest that maternal vB12 deficiency in early pregnancy is an important predictor of childhood insulin resistance and that antenatal supplementation with FA (either alone or in combination with other micronutrients) does not lead to (and may even lower the risk for) diabetes and metabolic disease among their offspring.

The mechanisms by which maternal folate and vB12 status might affect metabolic status and neurodevelopment in children are not well understood. Because both folate and vB12 are important methyl donors in the one-carbon metabolism pathway, they could play a part in epigenetic regulation through DNA and/or histone methylation, controlling when and where genes are expressed and generating significant effects on disease development (30, 55, 56, 76, 121, 149, 151, 177). Further research is required to clarify the role of maternal folate and vB12 nutrition during pregnancy on health outcomes in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUMMARY POINTS

1. Mandatory FA fortification reduces NTD prevalence, increases blood folate concentration less than twofold, and has not resulted in any known increased risk for adverse outcomes at the recommended dosages.

2. To fully establish the effectiveness of FA programs, additional RBC folate concentration population-based studies can be conducted to monitor program reach, as specified in WHO guidelines.

3. Only a few programs have used the WHO tool to identify populations that have not been reached by fortified products in quantities sufficient for NTD prevention.

4. High-quality studies of the interactions of FA and vB12 that carefully measure and control for intake, metabolism, and excretion may help maximize benefit and alleviate concerns about fortification.

Figure 1.

Hierarchy of evidence for folic acid and neural tube defects (NTDs). It is crucial to keep the basics of the hierarchy of evidence in mind when reviewing any new study. Policy changes generally require randomized controlled trials (RCTs) and preferably meta-analyses of multiples, given the lack of causal inference without randomization. The level of evidence is a critical issue in nutrition because both supplementation and nutritional patterns are heavily confounded by innumerable health conditions and demographic characteristics. Folic acid is one of the best-studied molecules in the published literature, with more than 93,000 peer-reviewed publications; however, no fortification studies of NTDs (only initial supplementation trials) have been conducted because of the ethical and logistical issues surrounding withdrawal of an established, successful intervention. Additional trials are generally limited to intermediate biomarkers. In contrast to other nutritional interventions, there are large RCTs that show the prevention of NTDs by periconceptional folic acid supplementation. New studies are weighted not only against these critical metrics but also against the totality of the evidence in the field.
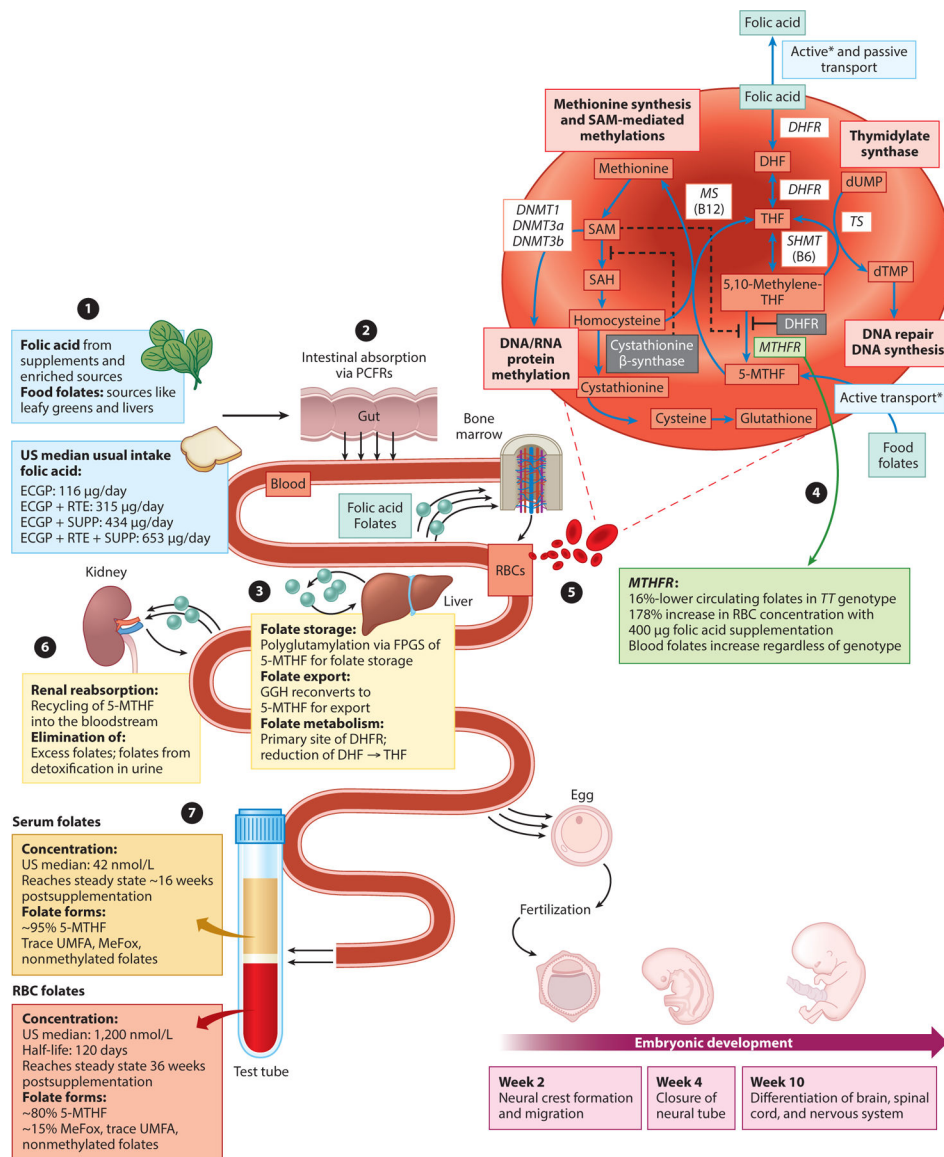
Figure 2.

Folic acid and food folates: metabolic pathway, one-carbon pathway, and biomarker measures. (❶). In addition to naturally occurring food folates from diet (available primarily from leafy greens and liver), it is recommended that all women of reproductive age consume 400 μg/day of folic acid, a synthetic isomer of folate. In the USA, there are three primary sources of folic acid: cereal grain products labeled as enriched (ECGP) containing 140 μg of folic acid per 100 g of flour, ready-to-eat cereals (RTE) containing up to 400 μg of folic acid per serving, and supplements (SUPP) (45). These three sources generate four mutually exclusive folic acid consumption groups with varying median usual intakes of folic acid in adults over 16 years of age: ECGP, ECGP+RTE, ECGP+SUPP, and ECGP+RTE+SUPP (169). (❷) Food folates and folic acid are absorbed primarily from the small intestine via proton-coupled folate receptors (PCFRs), which have a high affinity to folic acid, and reduced folate carriers (RFCs), which have a lower affinity to folic acid (167).

Once absorbed, folates reach systemic circulation and are further processed by peripheral tissue. (❸) Absorbed folates are partially removed by the liver. In the liver, folic acid may undergo biotransformation to 5-methyltetrahydrofolate (5-MTHF) via dihydrofolate reductase (DHFR), where it is highly expressed; however, the final biotransformation to 5-MTHF may be limited by *MTHFR* genotype (❹), leading to ~16%-lower circulating folates in *TT* genotypes (31, 164). 5-MTHF may be partially released into bile, allowing for further reabsorption in the small intestine. Folylpolyglutamate synthetase (FPGS) in the liver enables long-term storage of folates within the liver. γ-Glutamyl hydrolase (GGH) in the liver enables hydrolysis of stored folate polyglutamates back into bioavailable monoglutamate folate forms, which then reenter the systemic circulation via the hepatic vein (183). (❺) Circulating folates are transported from serum into newly created red blood cells (RBCs) in the bone marrow via membrane-associated folate-binding proteins with a half-life of approximately 120 days (5). Serum folates that are not bound to proteins are filtered by the kidney. (❻) Folate receptor α (FRα), which has high affinities to both folic acid and 5-MTHF, is highly expressed along tubule epithelial cells, allowing for efficient reabsorption of folates (79). Excess folates not reabsorbed and reduced folate forms with lower affinities to FRα are then eliminated into the urine by the kidney (79). In pregnant women, FRα is also highly expressed in the placenta, enabling nutritional transfer of folates to the fetus throughout pregnancy (158). (❼) RBC and serum folate measurements reflect the biological processing of dietary food folates and folic acids. The median serum folate concentration following fortification is ~42 nmol/L, and the median RBC folate concentration is ~1,200 nmol/L. Both comprise primarily 5-MTHF (41, 131). Asterisks indicate active transport via membrane-associated folate-binding proteins. Abbreviations: DHF, dihydrofolate; DHFR, dihydrofolate reductase; DNMT, DNA methyltransferase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MS, methionine synthase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase; UMFA, unmetabolized folic acid.
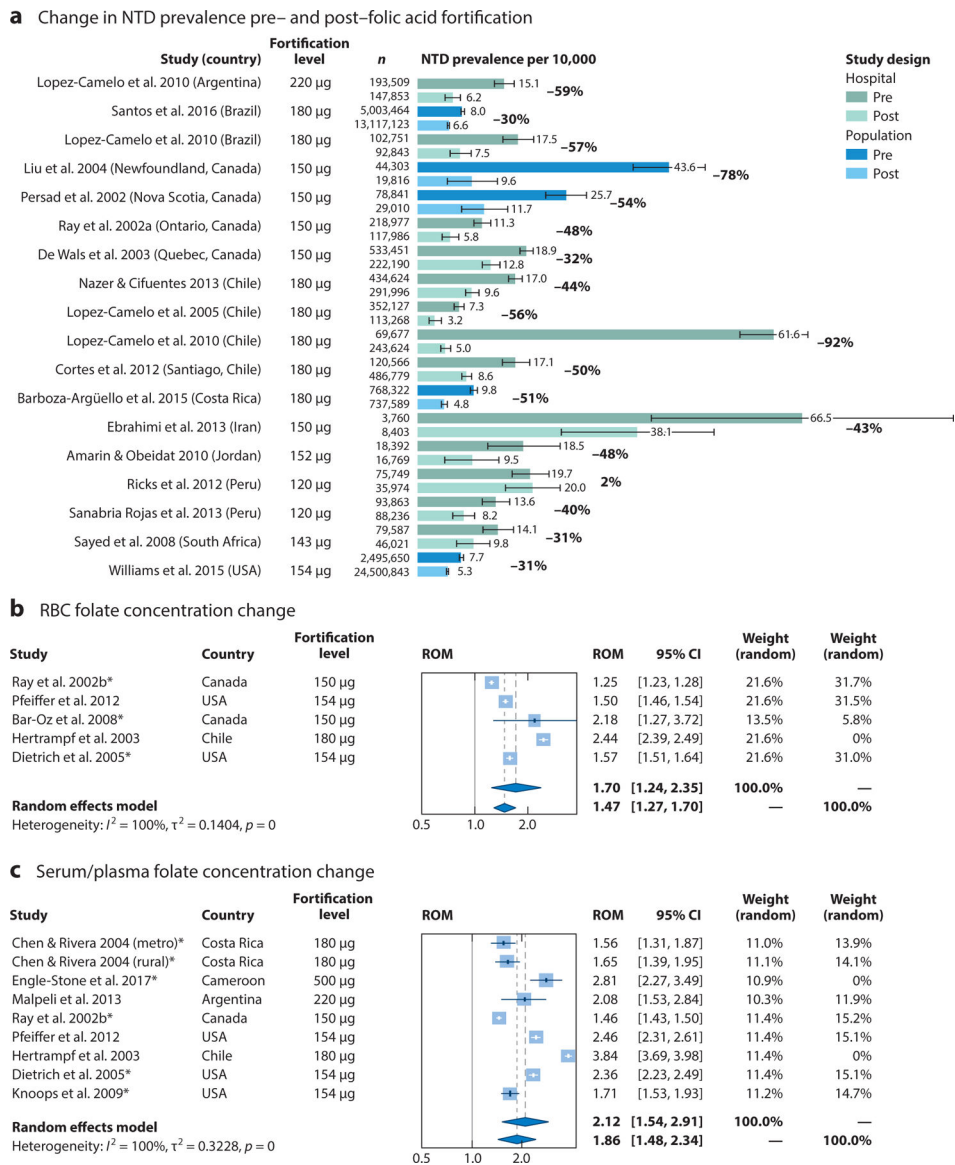
a Change in NTD prevalence pre– and post–folic acid fortification

Study (country)	Fortification level	n	NTD prevalence per 10,000	Change
Lopez-Camelo et al. 2010 (Argentina)	220 µg	193,509 / 147,853	15.1 / 6.2	−59%
Santos et al. 2016 (Brazil)	180 µg	5,003,464 / 13,117,123	8.0 / 6.6	−30%
Lopez-Camelo et al. 2010 (Brazil)	180 µg	102,751 / 92,843	17.5 / 7.5	−57%
Liu et al. 2004 (Newfoundland, Canada)	150 µg	44,303 / 19,816	43.6 / 9.6	−78%
Persad et al. 2002 (Nova Scotia, Canada)	150 µg	78,841 / 29,010	25.7 / 11.7	−54%
Ray et al. 2002a (Ontario, Canada)	150 µg	218,977 / 117,986	11.3 / 5.8	−48%
De Wals et al. 2003 (Quebec, Canada)	150 µg	533,451 / 222,190	18.9 / 12.8	−32%
Nazer & Cifuentes 2013 (Chile)	180 µg	434,624 / 291,996	17.0 / 9.6	−44%
Lopez-Camelo et al. 2005 (Chile)	180 µg	352,127 / 113,268	7.3 / 3.2	−56%
Lopez-Camelo et al. 2010 (Chile)	180 µg	69,677 / 243,624	61.6 / 5.0	−92%
Cortes et al. 2012 (Santiago, Chile)	180 µg	120,566 / 486,779	17.1 / 8.6	−50%
Barboza-Argüello et al. 2015 (Costa Rica)	180 µg	768,322 / 737,589	9.8 / 4.8	−51%
Ebrahimi et al. 2013 (Iran)	150 µg	3,760 / 8,403	66.5 / 38.1	−43%
Amarin & Obeidat 2010 (Jordan)	152 µg	18,392 / 16,769	18.5 / 9.5	−48%
Ricks et al. 2012 (Peru)	120 µg	75,749 / 35,974	19.7 / 20.0	2%
Sanabria Rojas et al. 2013 (Peru)	120 µg	93,863 / 88,236	13.6 / 8.2	−40%
Sayed et al. 2008 (South Africa)	143 µg	79,587 / 46,021	14.1 / 9.8	−31%
Williams et al. 2015 (USA)	154 µg	2,495,650 / 24,500,843	7.7 / 5.3	−31%

Study design: Hospital (Pre, Post); Population (Pre, Post)

b RBC folate concentration change

Study	Country	Fortification level	ROM	ROM	95% CI	Weight (random)	Weight (random)
Ray et al. 2002b*	Canada	150 µg		1.25	[1.23, 1.28]	21.6%	31.7%
Pfeiffer et al. 2012	USA	154 µg		1.50	[1.46, 1.54]	21.6%	31.5%
Bar-Oz et al. 2008*	Canada	150 µg		2.18	[1.27, 3.72]	13.5%	5.8%
Hertrampf et al. 2003	Chile	180 µg		2.44	[2.39, 2.49]	21.6%	0%
Dietrich et al. 2005*	USA	154 µg		1.57	[1.51, 1.64]	21.6%	31.0%
Random effects model				**1.70**	**[1.24, 2.35]**	**100.0%**	**—**
				1.47	[1.27, 1.70]	—	100.0%

Heterogeneity: $I^2 = 100\%$, $\tau^2 = 0.1404$, $p = 0$

c Serum/plasma folate concentration change

Study	Country	Fortification level	ROM	ROM	95% CI	Weight (random)	Weight (random)
Chen & Rivera 2004 (metro)*	Costa Rica	180 µg		1.56	[1.31, 1.87]	11.0%	13.9%
Chen & Rivera 2004 (rural)*	Costa Rica	180 µg		1.65	[1.39, 1.95]	11.1%	14.1%
Engle-Stone et al. 2017*	Cameroon	500 µg		2.81	[2.27, 3.49]	10.9%	0%
Malpeli et al. 2013	Argentina	220 µg		2.08	[1.53, 2.84]	10.3%	11.9%
Ray et al. 2002b*	Canada	150 µg		1.46	[1.43, 1.50]	11.4%	15.2%
Pfeiffer et al. 2012	USA	154 µg		2.46	[2.31, 2.61]	11.4%	15.1%
Hertrampf et al. 2003	Chile	180 µg		3.84	[3.69, 3.98]	11.4%	0%
Dietrich et al. 2005*	USA	154 µg		2.36	[2.23, 2.49]	11.4%	15.1%
Knoops et al. 2009*	USA	154 µg		1.71	[1.53, 1.93]	11.2%	14.7%
Random effects model				**2.12**	**[1.54, 2.91]**	**100.0%**	**—**
				1.86	[1.48, 2.34]	—	100.0%

Heterogeneity: $I^2 = 100\%$, $\tau^2 = 0.3228$, $p = 0$

Figure 3.
Impact of folic acid fortification on neural tube defect (NTD) prevalence, red blood cell (RBC) folate concentrations, and serum/plasma folate concentrations. (*a*) NTD rates per 10,000 live births before and after the implementation of mandatory folic acid fortification from population and hospital surveillance programs. Results for studies that monitored all NTDs in either population or hospital surveillance programs are compared during pre- and postfortification periods. For these studies, the postfortification period lasted at least 2 years following the implementation of mandatory folic acid fortification. The standard error was calculated using a Poisson distribution for rare events. Results for studies comparing pre- and postfortification (*b*) RBC folate concentrations and (*c*) serum folate concentrations are summarized as a ratio of means (ROM) corresponding to the fold change in post-versus prefortification folate concentration levels. Asterisks indicate concentrations measured via radioimmunological assays, as opposed to microbiological assays. Despite the high level of

heterogeneity across studies, the overall effect using a random effects model demonstrated a significant fold change in folate concentration following the introduction of folic acid fortification ($P < 0.001$ in both cases). RBC folate concentrations increased approximately 1.70-fold (95% CI, 1.24, 2.35), and serum folate concentrations increased approximately 2.12-fold (95% CI, 1.54, 2.91). A subanalysis excluding countries that had fortification levels >400 μg per 100 g of product was also conducted; RBC folate concentrations increased approximately 1.47-fold (95% CI, 1.27, 1.70) and serum folate concentrations increased approximately 1.86-fold (95% CI, 1.48, 2.34) following folic acid fortification. For the search strategy and references, see Supplemental Appendix 3.