



Folic acid and human reproduction—ten important issues for clinicians

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Abstract

This article presents data on the current best evidence-based clinical practices and controversies surrounding folic acid supplementation/fortification for the prevention of neural tube defects (NTDs) during early pregnancy. Formatted as a series of ten clinical questions, answers and extensive discussion are provided for each point. We assess the history and evidence behind supplementation and fortification, racial/ethnic disparities in NTDs on a global scale, and present information on risk factors for NTDs other than dietary folic acid deficiency. Also discussed are public health challenges, including disparities in NTD rates, population-wide monitoring of NTDs, and tracking safety data in the post-fortification era. Emerging data are also reviewed regarding the role folic acid may play in malignant processes, cardiovascular disease, male fertility, and other medical conditions.

Introduction

Neural tube defects (NTDs) are common and devastating congenital malformations of the central nervous system. The two most common, anencephaly (a total or partial absence of the brain tissue, skull, and overlying skin) and spina bifida (herniation of spinal cord, meninges, or both through a defect in the spine), comprise >90% of cases. Both arise from incomplete closure of the neural tube early in gestation, often before a woman is even aware that she might be pregnant [1]. NTDs are a worldwide problem, with approximately 300,000 affected newborns every year and 3,000 cases per year in the United States alone [2]. A 2005 report from the Centers for Disease Control (CDC) estimated the United States rates for spina bifida and anencephaly at 17.96 and 11.11 per 100,000 live births, respectively [3].

NTDs cause substantial morbidity and mortality for newborns and lead to staggering financial and emotional costs. Prenatal diagnosis is now widely available in the United States, with second trimester maternal alpha-fetoprotein analysis and fetal ultrasound identifying >80% of cases. Anencephaly results in *in utero* death, or death within a few days of birth. In contrast, children afflicted with spina bifida suffer from physical disabilities including paralysis, bowel and bladder incontinence, learning disabilities, and excess mortality well into childhood and adult years,

despite advances in medical and surgical care [4]. The financial cost of NTDs, principally the medical and surgical care of spina bifida, is high, with estimates approximating \$250,000 lifetime cost per case in the United States [2].

Although the exact etiology of these defects is not yet fully understood, it is likely multifactorial involving a complex interplay of genetic and environmental components. Support for the genetic component comes from the well-documented higher recurrence rate among families with a previously affected pregnancy, as well as among twins [5]. Specific polymorphisms have been identified that may increase risk [6], but the higher risk observed in certain ethnicities, such as the Irish [7] and Mexican Americans [8] provides further evidence suggesting a genetic component. In addition, siblings of affected individuals have a 10-fold increase in having a NTD affected pregnancy themselves. Finally, couples with a prior affected pregnancy have a risk of NTD in a subsequent pregnancy that is 3- to 5-fold higher than couples with no prior history [9]. Evidence for environmental factors comes from the variation in risk based on socioeconomic status and neighborhood conditions [10,11], seasonal variation [12], and geography [7,13–15].

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The most well studied of the numerous potential environmental factors contributing to NTDs is the protective effect of folic acid delivered during the periconceptual period. Folate is a water soluble B vitamin that acts as a cofactor in one-carbon transfer reactions and plays a central role in nucleic acid biosynthesis [16]. Exactly how folic acid works to prevent NTDs is currently unclear; many questions remain about the processes underlying normal and abnormal neural development [17]. What is well established, however, is that maternal folic acid intake during the periconceptual period is effective in reducing both recurrence and first occurrence of NTDs [18–20].

Few opportunities are present in the public health arena where a primary prevention such as folic acid supplementation can effectively target the catastrophic health outcome of NTD. Unfortunately, putting prevention into practice has been much more difficult than initially anticipated, and numerous controversies exist about some of the most important folic acid related issues facing women and public health authorities worldwide. Some of these contentious issues include the optimal dose of supplemental folic acid, the safety of folic acid, the optimal level of fortification (and if this is even effective), and the root causes of NTDs in different populations. This report addresses these issues as well as highlights areas of emerging research.

1. What are the DRIs (Dietary Reference Intakes) for folic acid?

The recommended daily allowance of folic acid for NTD prevention depends on obstetrical history. For women with a prior history of NTD affected pregnancy, a 4.0 mg daily dose starting at least one month prior to conception and continuing throughout the first trimester is the current United States recommendation [21]. This dose was initially recommended in 1991 after the results of the MRC trial were published, which showed that a 4.0 mg daily dose was effective in preventing NTD in women with a prior affected pregnancy [20]. For women with no prior affected pregnancy, the CDC broadened its guidelines in 1992 to include a recommended 0.4 mg daily dose for all women of childbearing age for primary prevention of NTD [22]. This 0.4 mg dose was based on several case control and cohort studies (cited as above) as well as a 1992 Hungarian RCT that used 0.8 mg daily for primary prevention. In 1999, Berry *et al* provided additional evidence for 0.4 mg supplementation when a study carried out in China showed significant primary prevention of NTDs with that dose [14,15]. Although it is possible that a lower dose could provide similar levels of protection as

0.4 mg, a RCT assessing different doses will likely never be done due to ethical constraints.

Despite many countries throughout the world having similar recommendations, no international consensus has been developed and some countries still have no formal recommendations. For example, one study of European folic acid policies performed in 2005 found that although 13 countries had a policy in place, some of these recommendations had only been disseminated ten years after the initial CDC recommendation. Two countries, Malta and Finland, recommended dietary sources of folate only (non-synthetic, dietary form), and five others had no official recommendation [23]. Interestingly, Canada's Motherisk program based in the Hospital for Sick Children, Ontario, recently changed its folic acid guidelines to 5mg/d for all women. This was after a recent study determined that 40% of Ontario women were not achieving optimally protective levels, despite fortification and supplementation [24]. Additionally, the 2007 guidelines from the Motherisk program and the Society of Obstetricians and Gynecologists of Canada call for 5mg/d for women with a variety of medical and social indications, including minority status, epilepsy, obesity, substance abuse, poor medication compliance, and lack of birth control [25]. These recommendations are not reflective of those that are generally available in other jurisdictions, however.

This 5mg/d recommendation is notable because it is so far above most other recommendations and because it well exceeds the generally accepted tolerable upper limit (TUL) of folic acid of 1.0 mg daily. This limit is based on concerns of possible masking of B₁₂ deficiency anemia (pernicious anemia) through the use of high dose folic acid, leading to the progression of irreversible neurologic defects resulting from B₁₂ deficiency. Studies performed in the 1950s showed the level of folic acid needed to correct the B₁₂ deficiency-related anemia was about 5.0 g per day [26]. The Institute of Medicine then established the TUL at a somewhat arbitrary level 5 times lower than this, at 1 mg per day. Of note, vitamin B₁₂ levels are now routinely and easily measured in patients with unexplained neurological symptoms [27]. Additionally, studies performed post-fortification have shown no significant change in B₁₂ levels after fortification [28], nor has fortification increased the percentage of people with B₁₂ deficiency presenting in the absence of anemia [29].

These observations are important for two reasons. The first is that a "safety concern" is frequently mentioned throughout the literature as an argument against widespread folic acid use [4]. Indeed, potential masking of B₁₂ deficiency was a key reason United Kingdom health regulators decided against fortification there in 2002; this

has likewise been a contentious issue in Australia, Switzerland, and other countries that continue to debate fortification [30]. Secondly, the TUL was a main factor behind the fortification policy implemented in the United States. The fortification level was chosen to ensure that while the majority of people would get an extra 0.1 g/d, almost no one would cross the 1.0 mg/d threshold [30].

2. What is the minimum effective dose for preventing NTD?

This important question is difficult to answer and has been the subject of much debate [31,32]. A RCT focusing on a range of doses of folic acid with incidence of NTDs as the primary endpoint would be unethical, yet the clinical dose-response effect is a critical issue when implementing fortification policies and supplementation recommendations. This question is also challenging given the multifactorial etiology of NTDs. The minimum effective dose may depend on several population-based factors, such as baseline folate status or the particular folate pathway polymorphisms found in a certain racial/ethnic group, for example. Thus, the evolving understanding of the complexity of NTDs makes a universal answer to this question elusive.

Nevertheless, some researchers have attempted to address this question within the limits placed by ethics and the difficulties of studying such a complex process. In one study, Daly *et al* (1997) looked at the amount of daily folic acid supplementation needed to deliver protective levels of red blood cell (RBC) folate [31]. These were previously determined and showed an inverse relationship between RBC folate levels and NTD risk [33]. Women with RBC folate levels <150mcg/L were at high risk for a NTD pregnancy, whereas women with levels >400 mcg/L had a 60% reduction in risk. The study focused on women of childbearing years, but excluded women who were either planning or at risk of pregnancy. Administration of 100, 200, and 400 mcg/d were associated with a 22%, 41%, and 47% reduction in NTD risk, respectively. The authors concluded that further fortification to increase levels to 400 mcg/d or higher would offer little further benefit [31].

These findings are in contrast to a 2001 investigation that found a continuous inverse dose response relationship and concluded that all women should take a 5mg supplement daily. Wald *et al* reviewed 13 studies that assessed the dose of folic acid and resulting serum concentrations, and then used data from a previous study that correlated serum levels with NTD risk [34]. Significantly, however, the 13 studies only examined doses of up to 1mg/d. The resulting model found that risk decreases proportionally to dose, with diminishing returns at increasingly higher levels. The

authors cite risk reductions of 18%, 35%, and 53% at 100, 200, and 400 mcg/d folic acid levels, respectively. This model also found that the effect depended on the baseline level of serum folic acid. For example, 0.4 mg daily could result in a protective effect between 23–54%, with a greater effect seen with a lower baseline. The model also extrapolated high doses of folic acid, and found an 85% protective effect with a 5 mg/d dose of folic acid. The authors advocated granting immediate access to 5mg tablets for all women, recommending this higher dose to all women of childbearing potential, and increasing fortification levels to 0.6–0.8 mg per day [32,34]. Although these recommendations have not been widely implemented, the results have been used to advocate increased fortification along with 5mg/d supplements [35] and to increase Canadian recommendations to 5mg/d through the Motherisk program [24]. However, caution is warranted in interpreting these data because the model cannot incorporate the heterogeneous nature of NTDs, nor can it assess the clinical response to folic acid that likely varies between populations. In addition, the CDC estimates that only 50% of NTDs are folic acid sensitive [22], a factor that is not addressed by the model. Additional dose-response clinical data would be helpful, and it is unfortunate that other case control studies performed since then [36] were limited by low sample size. If the 5 mg/d recommendations are successfully followed in Canada, surveillance data from that population may eventually shed some light on the issue.

3. Why and when did the United States government mandate fortification with folic acid?

By the time the FDA issued its recommendation (1992) that every woman capable of pregnancy should consume 0.4mg/d folic acid for NTD prevention, evidence supporting this intervention had been accumulating for years—data mostly from observational studies and nonrandomized trials. However, it was not until the publication of two landmark randomized controlled trials that the evidence was strong enough for the United States Preventive Health Service (USPHS) to issue its initial recommendation [22]. The Vitamins Research Council study [20] demonstrated recurrence protection with 4mg/d, and a Hungarian RCT showed occurrence protection with 0.8 mg/d [18]. The evidence was subsequently strengthened by a large study in China, demonstrating a protective effect for first-occurrence NTD with 0.4mg/d of folic acid [14]. The magnitude of the effect varied, but the figure often cited on the basis of these trials is that 50–70% of NTDs can be prevented with folic acid supplementation.

Unfortunately, while supplementation in theory represents a straightforward method of primary prevention, the

practice of actually translating these recommendations into an effective public health outreach has not been easy. A major public health campaign was undertaken soon after the 1992 CDC recommendation, including educational and media efforts to raise awareness of folic acid and increase supplement use [37]. Despite this, a Gallup poll undertaken in 1997 showed that while 66% of women had “heard of” folic acid, just 10% knew that it could prevent birth defects, only 32% of women reported taking it daily, and a dismal 6% knew that it needed to be taken before pregnancy [38]. These low rates of knowledge and supplement use, combined with the reality that approximately 50% of all pregnancies in the United States are unplanned [39], led to a general understanding that an emphasis on supplementation as the sole means of increasing folic acid consumption was likely not going to work.

Thus, in March 1996, the FDA advised folic acid fortification of enriched grains, such as flour, bread, farina, cornmeal, rice, and pastas, with the requirement that all be enriched by January 1998. Whole grain products were not considered for fortification because they contain natural folate. Although many organizations, including the CDC, American Academy of Pediatrics, and March of Dimes, lobbied for fortification of 350mg folic acid per 100gm flour, the FDA required a much lower concentration of 140mg per 100g flour, with the understanding that this would lead to a modest 0.1 mg increase in folic acid levels in the average American [27].

After the United States became the first country to mandate fortification with folic acid, similar programs were adopted in other countries. Canada adopted a nearly identical program to that of the United States, fortifying with 150mg per 100g flour less than a year later. As of 2008, wheat flour in 67 countries has been fortified with folic acid, representing about 30% of the world’s wheat flour, reaching about 27% of the world’s population [40]. Six countries fortify both wheat and maize flour, reflecting national dietary preferences. The levels of fortification vary widely throughout the world. In contrast to the United States and Canada, Chile mandated fortification with 2.2g per 100 g flour with the expectation that this would lead to an additional intake of 0.4mg/d [41]. After a lengthy debate in Australia, the country started fortifying all bread flour products in September 2009 at a level of 120 mcg per 100g flour [42]. Despite these advances in fortification, approximately 150 countries, including those of the European Union, have no requirement for fortification, mostly because of safety concerns [40].

4. How many American women of childbearing age do not get sufficient folic acid?

Despite a dramatic increase in average serum folate concentrations across women of all races/ethnicities since

fortification began, most nonpregnant women in the United States still fall short of the 0.4mg/d recommendation [43,44]. Additionally, disparities in folate levels seen during the pre-fortification era among women of different racial/ethnic groups continue post-fortification [45]. The 2003–2004 National Health and Nutrition Examination Survey (NHANES) data showed a doubling in serum folate concentration pre- vs. post-fortification for the population as a whole [46]. Using data from the 2001–2002 NHANES, Yang *et al* found that approximately two-thirds of women of childbearing age were not getting the RDA for folic acid, a number well below the 80% goal set in the *Healthy People 2010* objectives (Objectives 16–15 and 16–16) [47]. Among women who did consume 0.4mg/d, most (76%) achieved this with use of a daily supplement. When data were analyzed by racial/ethnic groups, significant disparities existed. Whereas 40.5% of non-Hispanic white women consumed >0.4 mg/d, this was reduced to 21.0% among Hispanic women and 19.1% among non-Hispanic black women. Another study comparing pre-fortification (1991–1994) and post-fortification (1999–2002) NHANES data identified a similar trend [45]. Despite all racial/ethnic groups benefiting from fortification, low RBC folate status is more concentrated among poor and non-Hispanic black populations [45].

The reasons behind the persistent racial and ethnic variance may be complicated. First, evidence shows that minority women are less likely to take daily supplements than are non-Hispanic whites. For example, NHANES data documented rates of supplement use of 43.9%, 20.8%, and 19.3%, respectively, among non-Hispanic white, Hispanic, and non-Hispanic black women [44]. Another community-based study found that Spanish-speaking pregnant women were much less likely to have used a multivitamin periconceptually (3.8%) than were their English-speaking counterparts (22.4%) [48]. Chacko *et al* (2003) studied young minority women treated in reproductive health clinics in Texas, and found that only 9% of women were taking a daily multivitamin [49]. This is significant because only 8% of women analyzed through NHANES reached 0.4mg/d through fortified foods alone, making supplement use a crucial determinate of adequate folic acid intake. Other research looking at diet and nutrition in minority populations also found lower intakes of fruits and vegetables in these groups, and a growing body of literature addresses the role of neighborhood resources in contributing to ongoing disparities [50,51].

Second, data from NHANES 2003–2004 revealed a statistically significant drop in folate levels in 2003–2004, compared to 1999–2000. The reasons for this drop are

unknown. It has been suggested that the rise in popularity of low-carbohydrate diets during the last decade is partly responsible [46]. Another possibility is that serum folate levels immediately post-fortification increased almost twice as much as originally anticipated because of fortification. Authorities initially predicted an increase of 80–130mcg/d of folic acid from fortification, although NHANES data shows larger increases of 219mcg/d and 190mcg/d in supplement users and nonusers, respectively. Because manufacturers are allowed to add “reasonable overages” of folic acid to foods to ensure that the minimum required levels are present throughout the shelf life of a product [52], foods often contained an estimated 160–175% of the mandated amount shortly after folate fortification was initiated, although levels are now decreasing [53]. As of the present writing, the United States does not maintain a monitoring system for the amount of folic acid added to foods. Some studies show manufacturer’s food labels to be unreliable [54,52] thus making monitoring trends in NTD rates in response to fortification difficult. Ultimately, however, the drop in folate levels is small compared to the magnitude of the initial rise after fortification was initiated and affected mostly people at the higher end of serum folate levels [46].

5. How well has fortification worked to reduce NTDs?

It is difficult to assess the efficacy of fortification in preventing NTDs for several reasons. Problems with surveillance systems, scientific uncertainty, and confounding background trends in NTD rates unrelated to fortification all combine to produce conflicting results. The resulting confusion makes it difficult for countries attempting to assess current fortification levels and for others initiating their own programs.

One fact making it difficult to measure effectiveness of folic acid fortification is that the proportion of NTDs that are actually folate sensitive is unknown. Decades of research have failed to show exactly how folic acid prevents NTDs. Early investigations using case control studies found a protective effect of 50–60% with supplementation, but these studies used different methods of case reporting and differing amounts of folate [55–57]. Additionally, this effect varied depending on the population studied, with one study of a low prevalence population showing no effect [58]. More rigorous evidence from the MRC RCT showed a protective effect of 72%. Although this trial is referenced most often for the widely quoted figure of 70% of NTDs preventable with folic acid [20], there are several potential problems with this figure. One is that the MRC study’s confidence interval was wide (29–88%). Another is that the study assessed recurrence risk

in women who already had a previous NTD pregnancy. But because most NTDs (90%) occur in women with no history of NTD, the level of effect shown by this study may not necessarily be generalizable to the background population. Other investigators have estimated the percentage of folate sensitive NTDs at 75% globally [40] or 50% in the United States [22]. Because the actual number of folate sensitive defects is unknown, it is difficult to assess if current fortification levels are adequate to give the maximum protective benefit.

It must also be acknowledged that NTD rates both in the United States and elsewhere began falling before the initiation of folic acid fortification [59,60]. The reasons for this decline are unclear. In Ireland, a country with historically high rates of NTDs, prevalence has been falling for decades and cannot be accounted for by prenatal diagnosis and pregnancy termination because abortion is not legal in that country [61]. England and Wales have seen NTD rates decline by 96% since the early 1970s, with 40% attributable to prenatal diagnosis and termination and 56% attributable to a decline in incidence [62]. A study performed in Atlanta (USA) using active case ascertainment methods for NTD detection found that rates of NTDs declined sharply between 1968 and 2003; it was impossible to assess the impact of fortification in the setting of such falling rates [59].

An additional problem stems from the inherent difficulties in using United States surveillance data to track prevalence changes of NTDs [63]. If data used to track NTDs are obtained only from vital records and hospital discharges, then NTDs that are diagnosed prenatally (and terminated or spontaneously aborted before 20 weeks) would be missed. Recently, Stoll *et al* reported the prenatal diagnosis rate for anencephaly and spinal bifida was 96.4% and 68.6%, respectively, along with respective termination rates of 73.2% and 40.7% that varied among regional and ethnic groups [64]. Vital records can have sensitivities of 86% and 40% for anencephaly and spina bifida, respectively [65], so accurate surveillance for NTDs is particularly challenging. Ideally, multisource methods of case ascertainment capable of tabulating prenatally diagnosed, terminated cases, cases of fetal death, and live birth should be utilized to allow for meaningful comparisons, but these methods do not exist everywhere in the United States.

These surveillance issues are important because the picture of the impact of fortification on NTDs changes quite dramatically based on the completeness of case ascertainment [66]. Studies that relied mostly on vital statistics reporting found rate reductions in the range of 19–26% in the United States, with the greater benefit seen in studies

with more complete surveillance data [67,68]. In announcing the decision to fortify, the CDC had projected a 50% reduction in incidence, so these rates were met with some disappointment and a call for higher levels of fortification. By way of comparison, Canada with a fortification program nearly identical to that of United States has a more complete method of case ascertainment. After the initiation of fortification, rates there dropped much more substantially, with reductions of 32% in Quebec [69], and 47–48% in Ontario. Again, studies with the most complete case ascertainment also showed the greatest benefit [70, 71]. Nova Scotia, for example, which had one of the highest reported NTD prevalence rates in the world, had a greater magnitude of reduction of 54%. Fortification in Canada has essentially erased geographic differences in NTD rates across that country [69]. This phenomenon, in which areas with the greatest NTD incidence also show the greatest benefit from folic acid, also has been documented elsewhere [14]. Areas with highest NTD incidence may also have the greatest number of folate sensitive NTDs.

It is interesting that a recent study using data from the National Birth Defects Prevention Study showed no association between folic acid (from supplements) or natural folate (from dietary sources) and NTD risk. Because this study looked at pregnancies in the post-fortification era, the authors hypothesized that most folate-sensitive defects have been prevented through fortification [72]. This issue will likely remain unresolved until there is a better understanding of the etiology of NTDs, the role of folic acid in the development of NTDs, and further improvements in birth defect surveillance systems.

6. What are the racial/ethnic differences in NTD rates in this country?

Both before and after fortification, Hispanics had the highest rates of NTD affected pregnancies, non-Hispanic blacks and Asians the lowest, and non-Hispanic whites rates that were intermediate between the two. A 2005 study by Williams *et al* showed that after fortification there was a statistically significant decline in NTDs among non-Hispanic whites and Hispanics but not among non-Hispanic blacks. For spina bifida, rates among Hispanics (PR: 0.64; 95% CI 0.56–0.74) declined 36% pre- to post-fortification and 34% among non-Hispanic white births (PR: 0.66 95% CI 0.60–0.72), but showed only borderline significance among non-Hispanic blacks (PR: 0.81; 95% CI 0.67–1.00) [73]. For anencephaly, the picture was similar, with both Hispanics and non-Hispanic whites showing a similar decrease but with no significant decline in non-Hispanic blacks [73].

The reasons for such disparities are unclear, and the involved factors are likely go far beyond folic acid status in different racial/ethnic groups. Indeed, one only has to compare rates of NTD with the NHANES data on folate status to appreciate the degree of complexity of this issue. NTD rates by race/ethnicity are ordered as follows: Hispanics > non-Hispanic whites > non-Hispanic blacks/Asians, but the serum and RBC folate levels as reported by NHANES data are different: non-Hispanic whites > Hispanics > non-Hispanic blacks [46].

The Hispanic population long has been suspected of carrying a large burden of the NTD cases in the United States. Investigators have shown risk increases of 50% to 200% in Hispanics compared to non-Hispanic whites and blacks [6,74]. In particular, women living on the Texas-Mexico border are at very high risk, and NTDs are endemic to this region. In 1991, Cameron County along the border in Texas experienced an outbreak of cases of anencephaly. The cause of this cluster remains unknown, although environmental exposure to a mycotoxin, fumonisin, which infected corn flour, is suspected [75]. In an attempt to decipher genetic vs. environmental factors influencing risk, research has focused on differences between Mexico-born women and women of Mexican descent born in the United States. Some studies show that NTD rates are higher in women born in Mexico than women of Mexican descent born in the United States [6,74]. Others show rates of U.S.-born Mexican women to be intermediate between Mexico-born Mexican women and U.S.-born white women, supporting environmental rather than genetic causes [76]. Still other studies have found no association, confounding the ability to draw definitive conclusions at this time [77].

Research into possible genetic causes underlying the increased risk in Hispanics is ongoing. One of the best studied genetic polymorphisms in the folate pathway involves 5, 10-methylenetetrahydrofolate reductase (MTHFR) that encodes the enzyme responsible for production of the major circulating form of folate. One particular polymorphism, the C677T allele, results in reduced enzymatic activity and has been shown to increase the rates of NTDs [78]. This allele has the highest frequency in Hispanics, followed by non-Hispanic whites, and finally by non-Hispanic blacks who have the lowest frequency. These data correlate well with the racial/ethnic trends in NTD rates. Yet, analysis of the frequency of this allele and NTD rates worldwide presents a more confusing picture. Although the C677T allele is common in Mexico (and northern China) where rates are high, it also is common in southern Italy where NTD rates are low [79,80]. According to Botto, one possible explanation for this is

that the ultimate fetal phenotype depends on the maternal nutritional status and/or polymorphisms in other genes in the folic acid and related metabolic pathways [78].

Other genes besides MTHFR have been associated with high NTD rates in different populations, and interest in studying an array of polymorphisms of genes in the folate pathway and other related metabolic pathways is growing. This is challenging research for several reasons. Gene-gene [81] and gene-environment/nutrient [82] interactions are likely both important determinants of risk, and genes involved in NTDs may work at the level of the maternal and fetal genotypic levels as well as interact together and with environmental factors [83,84]. Much of the work to date is characterized by small, underpowered studies that are difficult to replicate. NTDs are complex, non-Mendelian traits that arise sporadically and often have only one or two affected members even in large families. No single gene has yet emerged as the most important genetic risk factor. Increased efforts at gathering larger cohorts, use of animal models, or meta-analysis may help address some of these issues [9].

No matter what the relative contributions of genetic and environmental factors, cultural/dietary practices among Hispanics are also likely important. Given the heavy consumption of legumes throughout Mexico and Central and South America, the Hispanic diet is quite rich in natural food folate [76]. However, supplement use (described above) is low. Moreover, Hispanic women also are less likely to benefit from folic acid added to prepared foods and less likely than non-Hispanic white women to eat breakfast cereals, an important source of folic acid with some providing 0.4mg in one serving [85]. Of even greater importance is that the Hispanic diet is based primarily on corn flour rather than the wheat flour of the Western diet.

This is relevant because corn flour was omitted from the mandatory fortification policy in the United States. Hamner *et al* developed a model to assess the projected effect of fortification of corn masa flour, a main ingredient in many commonly consumed food items in Hispanic cuisine, such as tamales and tortillas. This model predicted a 19.9–33.1% increase in folic acid levels among Hispanic women of childbearing years with the addition folic acid to corn masa. The model also predicted that although fortification of corn masa flour would effectively target Hispanics more than other ethnic groups, predicted serum folic acid increases among non-Hispanic white and non-Hispanic blacks would only be 4.0% and 3.6%, respectively. Accordingly, even with corn masa flour fortification, daily intake for most Hispanics would remain well below the 0.4mg recommendation; it is uncertain if this intervention would translate into improved reproductive

outcomes [86]. However, it does show how important it is to acknowledge dietary needs of different racial/ethnic groups when countries develop their fortification policies.

It has been proposed that Hispanics may not be as sensitive to folic acid as are other ethnic groups, or may need higher amounts for protection [8]. However, these studies are few in number and the rates of supplement use so low that it is impossible to draw any meaningful conclusions. Mandatory fortification programs and studies providing supplements also tell a different story. Indeed, NTD rates dropped by 40% after fortification was mandated in Chile [28]. Spina bifida rates at birth in the National Hospital dropped 74% after mandatory fortification in Costa Rica [87], and an intervention study providing a free 5mg/week supplement to women in a resource poor area of Mexico was associated with a decline in NTD-affected pregnancies of 43% [88]. Although most of the evidence showing efficacy of folic acid is derived from study populations of largely non-Hispanic whites, no conclusive evidence suggests that folic acid is not equally effective in Hispanics.

It is presently unclear, however, how much of the reported racial/ethnic differences may be attributable to differences in prenatal diagnosis and termination rates and the type of surveillance system used. Termination and prenatal diagnosis rates vary by race/ethnicity [89]. If certain racial/ethnic groups have lower rates of prenatal diagnosis or if certain groups are less likely to terminate affected pregnancies (because of cultural or religious reasons), then these practices pose a serious confounding effect for surveillance data. As one example, Williams *et al* [73] used 21 birth defects surveillance systems data to analyze NTD rates by race ethnicity. Only nine of these systems included prenatal case ascertainment. When these nine were analyzed separately, the prevalence of NTDs among Hispanics was 10% higher compared to non-Hispanic whites, but this observation was of borderline statistical significance (PR 1.10 95% CI 1.00–1.21). This difference rose to 42% when using data from surveillance systems lacking prenatal case ascertainment (PR 1.42 95% CI 1.33–1.52).

7. Does the modern diet provide sufficient folate?

A fundamental issue in answering this question is the difference between natural food folates and synthetic folic acid. Natural food folates are ~50% less bioavailable compared to synthetic folic acid used in supplements and fortification. This diminished bioavailability results from several factors: 1) the nature of food matrix, with some folates remaining bound and hence unavailable within the plant material; 2) factors affecting deconjugation of the

polyglutamate form of folates into the monoglutamyl form (the latter form is required for absorption in the small intestine); and 3) losses incurred during harvesting, processing, and preparation of foods [90]. Synthetic folic acid is highly stable, but leafy green vegetables and legumes undergo a loss of 50–89% of folate after cooking [91]. To adjust for these differences, Dietary Folate Equivalents (DFE)—defined as natural food folate + 1.7 times the dietary folic acid—were introduced in the United States as a tool for measuring overall folate status from both natural and synthetic sources.

In theory, today's American diet could provide a sufficient intake of folate, yet the foods needed to obtain this level would likely represent a significant departure from normal dietary habits of most women and may be impractical for daily consumption. The usual Western diet contains about 200 mcg natural folate. The 1992 CDC guideline recommending 0.4mg/d of folic acid means that a woman would have to consume 1000 mcg/day of natural food folates to obtain that level, taking into account the bioavailability difference [35]. Because of this, the 1998 Food and Nutrition Board of the Institute of Medicine restated the findings to recommend 400mcg of synthetic folic acid in addition to the natural folates consumed in a normal, varied diet [92]. It also is important to note that most of the evidence showing a protective effect looked at synthetic folic acid, not at the natural food folates. How effectively natural food folates might work to reduce NTD risk is unknown [26].

Of course, the importance of overall nutritional status during pregnancy cannot be overstated, and uncertainty about folate levels in foods should not deter the clinician from a discussion about healthy diet before and during pregnancy. A balanced, high nutrient diet contributes greatly to fetal growth and development, promotes positive maternal health outcomes, including healthy levels of pregnancy weight gain, and may help prevent adult onset diseases later in the infant's life [93]. Although there is a surfeit of literature for women to read about diet and lifestyle during pregnancy, many studies show that only a very small percentage of readers actually follow this advice, making nutrition an ongoing area of concern [94, 95]. Patient educational literature about folic acid has increased dramatically since the 1992 CDC recommendation, and high-risk women such as Hispanics are increasingly being targeted by culture-specific literature [96]. Good sources of dietary folate that are often cited include legumes, orange juice, leafy green vegetables, broccoli, and whole grains [93]. Unfortunately patient education materials often fail to distinguish between the wide variations in folate content in common foods. For

example, broccoli has widely different folate values depending on whether it is cooked, raw, or frozen.

8. Besides maternal folic acid status, what other known risk factors exist for NTD?

Whereas folic acid status is the most widely studied determinant of NTD risk, other factors contribute to increased risk. Understanding these factors is important for two reasons. The first is that most (90%) of NTDs occur in women with no personal history of an NTD-affected pregnancy, making it impossible to use medical history as a means of targeting all high-risk women for intervention. The second reason lies in the ability or inability of the clinician and the patient to modify some of these risk factors.

Obesity and diabetes have emerged as significant risk factors for NTDs. The link between diabetes and congenital anomalies has been observed for decades, with about 10% of babies born to diabetic mothers displaying birth defects. These defects are wide ranging, but NTDs are among the most common, with a 2-fold increase in risk for spina bifida and a 3-fold increase risk for anencephaly in infants of diabetic mothers [97]. The degree of maternal hyperglycemia during the first trimester is among the most important determinants of risk, with tight glucose control during that time leading to a reduced incidence of anomalies [97]. Even in women without diabetes or obesity, abnormal glycemic control is associated with increased risk [98]. Obesity is also a likely risk factor, with the risk increasing in a dose dependant fashion. Rates of prepregnancy obesity have skyrocketed in the last two decades, with a 70% increase from 1992–2004. Among women ages 20–39, 30% are obese. A recent meta-analysis found that compared to women of normal weight, the OR for NTD was 1.22 (95% CI .99–1.49), 1.70 (95% CI 1.34–2.15), and 3.11 (95% CI 1.75–5.46) among overweight, obese, and severely obese women, respectively [99]. Why obesity leads to increases in NTD risk is unknown, but may be related to altered glucose metabolism [98] or consumption of a diet high in calories but low in micronutrients [100].

Deficient or inadequate levels of vitamin B₁₂ have been implicated in NTD risk. Metabolically related to folic acid, vitamin B₁₂ is a significant element in the NTD equation, with B₁₂ deficiency increasing risk of NTDs independent of folic acid status [101–103]. In one study that stratified risk according to B₁₂ levels, most of the risk was found in women with levels below 250mcg/L, and women with levels <200mcg/L were 3 times more likely to have a NTD-affected pregnancy than were those who had levels >400 mcg/L. It is unknown how many cases of NTD might be prevented by increasing B₁₂ levels in a

population exposed to folate fortification, although one study conducted in post-fortification Canada estimated that 34% of remaining NTDs could be attributable to low B₁₂ levels [103]. Debate is ongoing about adding B₁₂ to food through fortification, similar to folic acid, and/or adding it to supplements [104]. Many questions about B₁₂, including an unexplored safety profile, the dose needed for protection, clinical outcomes data from RCTs, and technical issues related to fortification remain unanswered [102,105].

A wide variety of maternal exposures during pregnancy also is associated with NTD risk. For example, maternal hyperthermia in early pregnancy (OR 1.92, 95% CI 1.61–2.29) increases risk, reflecting the possible teratogenic nature of heat during embryogenesis [106]. Maternal diarrheal illness around the periconceptual period is an independent risk factor as well, likely though decreased absorption of micronutrients during the illness (OR 3.7, 95% CI 1.8–7.6) [107]. Attempting to explain the large geographic variations in NTD risk, researchers have studied other exposures such as secondhand smoke, pesticides, proximity to hazardous waste sites, and urban vs. rural residence with conflicting results [108–110]. The host of potential risk factors cited by the literature underlines the uncertain, complex etiology of NTDs.

9. What are the rates for supplement use among pregnant women periconceptually and among women who could become pregnant, and what are the barriers to supplement use and strategies to increase use?

Many academic medical agencies endorse supplementation with folic acid at 0.4mg/d for all women ages 18–44 who are capable of becoming pregnant. Supplementation is a United States Preventive Service Task Force (USPSTF) Grade A recommendation (2009) and is endorsed by the American College of Obstetricians and Gynecologists (ACOG), the American Academy of Pediatrics (AAP), the American Academy of Family Practitioners (AFP), and the American Academy of Neurology (AAN) [111–112].

Despite the strong USPHF recommendation in 1992 and public awareness campaigns carried out by the March of Dimes, the CDC, and the Spina Bifida Association, rates of supplement use by women remain persistently low. A 2006 report by the March of Dimes, for example, tracked awareness and use of folic acid supplements from 1995–2005 and found that, by 2005, 80% of women had heard of folic acid but only 19% of women had specific knowledge that folic acid prevents birth defects and even less (7%) knew that it should optimally be taken before con-

ceiving. The percentage of women taking a daily supplement increased modestly during this same decade, with 28% in 1995 and 33% in 2005 reporting use, with the latter number representing a disheartening decrease from the 40% high in 2004 [38]. Globally, rates of folic acid use also are low. A systematic review in 2004 reported rates throughout the world of 0.9–50%. Predictors of low use included young age, single status, history of unplanned pregnancy, immigrant status, non-White race, low educational attainment, and lower income level [113–115]. Although mass media campaigns improved rates, usage never rose above 50% [113]. These findings demonstrate a need for further education and addressing the barriers that exist in translating this knowledge into practice.

Several barriers to regular supplement use exist. Many women believe there is no need to take a supplement if they are not planning pregnancy or that folate present in diet alone is sufficient [38]. Additionally, although 86% of women surveyed in 2005 said that they would be willing to take a supplement if advised by their doctor, only 26% of women said that they had heard about folic acid from a health-care provider [38]. A study of supplement knowledge among physician and nonphysician women's health providers found a high level of knowledge about folic acid in general, but only 58% of respondents knew the correct daily recommended dose, less than a third knew the correct dose for a woman with a history of NTD pregnancy, and only 45% of family medicine/general internal medicine physicians recommended daily supplement use to their patients [116].

Education that specifically targets women of lower educational attainment and lower SES must be made a priority, and culturally specific literature, such as that developed for Mexican American women, must be made widely available [96]. Because more than 50% of pregnancies are unplanned, providers must make a concerted effort to educate women during well-women visits and other pre-conception contact with the health-care system [114,116]. Further research is needed on the best means to educate women and promote behavioral changes. Such efforts likely will involve a combination of interventions, support from health-care providers, media attention, and creative strategies. One approach recommends adding folic acid to oral contraceptive pills (OCPs), in an effort to target some of the unplanned pregnancies. This idea is being explored by Johnson & Johnson, maker of several types of OCPs [35].

Changing women's behavior is a challenge in almost every setting, but it is particularly difficult in resource-poor areas. One potentially promising solution is the use of once-a-week high-dose folic acid supplement. A recent

study showed that, although a once-a-week 2800mcg supplement was somewhat less effective at increasing RBC folate concentrations than was daily use, it nevertheless increased levels to concentrations that are associated with a reduced risk of NTDs [117]. When once-a-week 5mg supplements were used in Nuevo Leon, Mexico, a significant reduction in NTD cases was observed over the course of two years [88]. Further research investigating the degree of expected benefit and the increase in compliance over daily supplement use, if any, is needed.

10. What questions remain unanswered?

Many questions remain surrounding folic acid, fortification policy, supplementation, and likely or projected impact on NTDs, public health, and a host of other diseases. Some of the most pressing issues involve recent questions about the role of folic acid in cancer promotion and prevention. Recent data from the United States, Canada, and Chile [118–121] describe a significant increase in colon cancer cases observed shortly after the initiation of fortification in each country. This change cannot be accounted for by increased screening. Although these data show only a correlation in time, the trends are disturbing and may fit with the emerging biological role of folic acid in cancer promotion and prevention. Emerging research shows that folic acid may play dual and opposite roles in relation to cancer promotion [120–122]. Because folate deficiency long has been suspected to promote cancer through genomic destabilization perhaps via increased homocysteine levels, impaired methylation, uracil misincorporation, or double strand DNA breaks leading to chromosomal damage [123,124], adequate levels of folate could be protective against carcinogenesis by dampening these effects. However, given the critical role of folate in nucleic acid biosynthesis, it also may have a role in cancer promotion in susceptible individuals. In patients harboring preneoplastic and neoplastic lesions, exposure to extra folate (through supplementation or fortification) could promote cellular proliferation and cancer growth [121, 125]. Additionally, studies have shown conflicting or equivocal results for the role of folic acid in other cancers, including breast [126,127], prostate [128], and neuroblastoma [129].

The role of folic acid in cardiovascular diseases also is an area of active research. Folic acid long has been assumed to promote cardiovascular health. Observational epidemiologic studies show an inverse relationship between folate intake and CVD [130]. Several RCTs have shown that increases in folic acid led to dramatic reduction in plasma homocysteine levels, which long have been linked to CVD [131]. Recent studies failed to show evidence of a protective effect, and one recent meta-analysis showed

no reduced risk of cardiovascular or all-cause mortality in patients with preexisting CV disease [132]. A 2006 RCT looking at the benefits of folic acid supplementation in women at high risk of CV disease showed no reduction in CV events, despite evidence of reduced plasma homocysteine in the supplementation group [133]. More studies, especially those that focus on the role of folic acid in primary prevention of CV disease and studies that follow cohorts over long time periods are needed to address this issue.

Other ongoing areas of uncertainty relate to a variety of mostly pediatric diseases and other congenital malformations distinct from NTD. A recent meta-analysis included studies that looked at the effect of prenatal multivitamins containing folic acid on rates of birth defects and found decreased risk for congenital heart defects, cleft palate, oral cleft, urinary tract anomalies, and congenital hydrocephalus in women who took prenatal supplements [134]. Additionally, other data suggest that, along with a decrease in rates of congenital anomalies, the severity of the remaining defects has decreased [135]. A reported rise in autism which coincided with the beginning of fortification in the United States has been hypothesized to be a result of increased *in utero* exposure to folic acid [136, 137]. Additionally, a link between the rise in asthma and allergic diseases of childhood and the increase in prenatal folic acid use [138] has been suggested by studies in both humans [139] and mice [140]. Although intriguing, these issues will require further research before any definitive statements can be made.

Additional issues

Fortification—Not all foods that are currently fortified would facilitate increasing folic acid intakes among all women of reproductive age to a specific level, because numerous women of specific ethnic heritages do not eat fortified foods on a regular basis, if ever. Additionally, government-mandated fortification of food products with folic acid to reduce the incidence of birth defects, although well-established in cereal and bakery products, appears to be conspicuously ignored by the dairy, beverage, and prepared food industries. One reason for this may be because of the decline in taste and flavor caused by folic acid at fortification levels in such foods.

Cereals and cereal-based foods meet the necessary criteria of being technically amenable to folic acid fortification under existing fortification legislation. Yogurt and other dairy products, however, deteriorate in taste and flavor with the addition of folic acid at fortification levels. The food industry continues to be challenged in making

healthy products taste good while concurrently addressing a new market need – new avenues for folic acid fortification. In addition, the increasing levels of wheat and gluten intolerance and individual preferences are creating a need for new ingredient technologies to facilitate including folic acid in new food groups.

The provision of foods fortified with folic acid does not come without significant costs for the food industry. Generally speaking, cereal, juice, and dairy markets are extremely competitive and leave little financial incentive for developing marketing programs to educate target audiences. Additionally, not all cereal foods are fortified (e.g., corn tortillas). There also is the issue of allergy to synthetic folic acid, the ingredient of choice in most fortified foods. Although rare, allergies to synthetic folic acid are likely to become more common, given the rate of fortification with this vitamin. Physicians should consider folic acid allergies in the differential diagnosis of idiopathic anaphylaxis in patients with suspected grain allergies [141].

Because folate supplementation is particularly important at least three months before and after conception, the U.S. Food and Drug Administration (FDA) recently approved a new oral contraceptive, Beyaz™ (drospirenone/ethinyl estradiol/levomefolate calcium tablets and levomefolate calcium tablets) to increase folate levels in women who choose an oral contraceptive as their preferred method of birth control. Putting folate in birth control pills addresses the public health need for increased folate levels in young women and reduces the risk of a neural tube defect in a pregnancy conceived while taking it or shortly after discontinuing it. The popularity of Beyaz among young women for its effect on acne and Premenstrual Dysphoric Disorder also may help with regular folate intake for this population.

Cancer—Some evidence indicates that folic acid may facilitate the preliminary stages of specific malignant processes. Hospitalization rates for colon cancer among men and women age 45 and older in Chile more than doubled after folic acid fortification was introduced in the country in 2000 [142,143]. Additionally, two Norwegian studies, the Norwegian Vitamin Trial and the Western Norway B Vitamin Intervention Trial, found that supplementation with 800mcg/d of folic acid, B₁₂, and B₆ for more than three years increased the risk of lung cancer by 21%. An analysis of these latter two studies, designed to study the effects of higher dose folic acid and vitamin B₁₂ on reducing cardiovascular deaths by lowering plasma homocysteine levels, showed that high dose (synthetic) folic acid supplementation unexpectedly may increase cancer and

all-cause mortality [144]. It is important to note, however, that the dose used in these two trials is twice that recommended on an international basis for pregnancy-related intakes (see above). Systematic studies of the safety of high doses of folic acid are lacking, and it is axiomatic that absence of data does not imply assurance of safety. No single agency is tasked with the responsibility of monitoring the long-term or overall safety of the fortification program. The lack of systematic safety studies means uncertainty about which outcomes are the most sensitive predictors of risk. The issue is proving to be of great urgency to researchers, governments, and industry, given the level of mandatory folic acid fortification in the United States and other countries and the many years of education on the health benefits associated with folic acid that has been directed at consumers.

Conclusion

Because of ongoing and substantial efforts and collaboration of scientists, public-health authorities, nonprofit groups, and governmental agencies, women in many areas of the world now benefit from significant improvements in their folic acid status through the use of folic acid supplementation and fortification, alone or together. There efforts should continue, with a particular focus on innovative public-health strategies that target population specific barriers to supplement use. Additionally, the relationship folic acid to the health of people of all ages and with many different health conditions, especially cancer, is among the field's most pressing questions.

Despite all efforts, many women may remain at risk of having a folic acid-preventable NTD-affected pregnancy. Additional investigation into gene-gene and gene-environment risk factors is an exciting avenue of research that has only just begun. Eventually this research could lead to tailoring of prevention measures to specific risk groups. Work on potentially modifiable risk factors, including vitamin B₁₂ status, is a very promising avenue of investigation as research expands beyond folic acid to explore other determinants of these complex anomalies. Surveillance systems must be in place to detect as many cases as possible, particularly those that are prenatally diagnosed. Women must have access to second trimester screening and advanced diagnosis, at no cost to the patient. This is becoming even more important given the emerging options for affected babies, including promising fetal surgery for spina bifida-affected neonates. Research on NTDs in different racial/ethnic groups, especially in the Hispanic population, also must continue. Minority groups in the United States too often are disproportionately burdened by poor health outcomes. Public health authorities

must ensure that minority women are able to reap the maximum benefit from NTD prevention, detection, and treatment efforts. This multifaceted approach may lead to a time when no woman will have to suffer through a NTD-affected pregnancy.

Finally, although this review has focused on the effect of folic acid intake and supplementation in women, recent evidence suggests that it may be of great importance in men, not only in terms of potential cancer and/or other associated risks, but in terms of improving sperm quality in men who are partners to women who are considered subfertile. In particular, men who take supplements containing folic acid have improved sperm counts, motility, and decreased numbers of abnormal forms [145].

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