FOLIC ACID AND VITAMIN B-12 BEFORE 1997

Before FA fortification, consensus was limited about what concentrations of FA would be optimally protective. Historically, FA supplementation recommendations for women with a previous NTD pregnancy were not based on dose-response data. Before FA fortification, therefore, FA supplementation was set at an approximate maximal dosage of 5 mg/d for at-risk mothers. The case for minimizing NTDs through voluntary supplementation, however, is limited by a ceiling compliance rate of about 50%, leaving many women and their embryos “unprotected” (4).

Until 1998, serum folate concentrations sampled from an elderly Canadian female population were in the order of 15 nmol/L. Data from this population was considered an accurate reflection of general trends in serum folate concentrations of most adult Canadians. Soon after the 1998 FA fortification initiative, serum folate concentrations rose in the same population and reached a plateau of ~24 nmol/L, a relative increase of nearly 60% (5).

FA may act through reduction of the intermediary metabolite, homocysteine [measured as total serum homocysteine (tHcy); Figure 1], but this is by inference only, and hard evidence for the “homocysteine hypothesis” has been limited. In this pathway, folate is first converted into 5,10-methylenetetrahydrofolate by 5,10-methylenetetrahydrofolate reductase before it acts as a co-factor in the synthesis of methionine from tHcy by methionine synthase, thereby accelerating remethylation and limiting tHcy concentrations (1–4).

FOLIC ACID AND VITAMIN B-12 BEFORE 1997

Before FA fortification, consensus was limited about what concentrations of FA would be optimally protective. Historically, FA supplementation recommendations for women with a previous NTD pregnancy were not based on dose-response data. Before FA fortification, therefore, FA supplementation was set at an approximate maximal dosage of 5 mg/d for at-risk mothers. The case for minimizing NTDs through voluntary supplementation, however, is limited by a ceiling compliance rate of about 50%, leaving many women and their embryos “unprotected” (4).

Until 1998, serum folate concentrations sampled from an elderly Canadian female population were in the order of 15 nmol/L. Data from this population was considered an accurate reflection of general trends in serum folate concentrations of most adult Canadians. Soon after the 1998 FA fortification initiative, serum folate concentrations rose in the same population and reached a plateau of ~24 nmol/L, a relative increase of nearly 60% (5).

FA may act through reduction of the intermediary metabolite, homocysteine [measured as total serum homocysteine (tHcy); Figure 1], but this is by inference only, and hard evidence for the “homocysteine hypothesis” has been limited. In this pathway, folate is first converted into 5,10-methylenetetrahydrofolate by 5,10-methylenetetrahydrofolate reductase before it acts as a co-factor in the synthesis of methionine from tHcy by methionine synthase, thereby accelerating remethylation and limiting tHcy concentrations (1–4).

INTRODUCTION

Open neural tube defects (NTDs) are due to failure of closure of the neural tube after the third or fourth week of gestation. NTDs include spina bifida, anencephaly, and encephalocele. Cause and prevention of NTDs have both been subjects of intense research (1). Epidemiologic data about the beneficial effects of folic acid (FA) supplementation in reducing the risk of NTDs were greatly enhanced by a prospective study performed by Rogozinski et al (2). That study showed an 86% reduction of recurrence in NTDs among previously affected mothers who had subsequently taken FA supplements. Later, studies, such as those of Cziezel et al (3), showed that NTDs could be prevented by the use of periconceptional FA supplements.

On the basis of high-quality data for the efficacy of FA supplements, FA fortification of cereal grains was mandated in the United States and Canada by mid-1997 and early 1998. The estimated increase in daily dietary intake of FA was approximately 0.1–0.2 mg, with the primary intention of lowering the frequency of NTD pregnancies (1).

FOLIC ACID AND VITAMIN B-12 BEFORE 1997

Before FA fortification, consensus was limited about what concentrations of FA would be optimally protective. Historically,
with FA occurred in the following standardized way. Synthetic FA was added at dose of 1.5 mg/kg (1.5 ppm) to white flour and some cornmeal products. There was a high degree of compliance by the Canadian National Millers Association (4).

As in the general US population, Canada’s national FA fortification program has enhanced folate status in women of reproductive age. In our own study, a comparison of data before and after fortification from a large Canadian cohort showed that the geometric mean of the red blood cell (RBC) folate concentrations rose from 527 to 741 nmol/L in women of reproductive age. These data indicated that FA fortification on a population-wide basis significantly increased RBC folate concentrations. These protective effects may not be universal, however, because withdrawal of dietary FA for as little as 12 wk can significantly reduce RBC folate concentrations in women (4).

Subsequently, we sought to determine the effect of supplementation on prevalence of open NTDs in the province of Ontario with the use of the maternal serum screening (MSS) database. Among the 336,963 women who underwent the MSS over 77 mo, the prevalence of open NTDs declined from 1.13/1000 pregnancies before fortification to 0.58/1000 pregnancies thereafter (prevalence ratio: 0.52; 95% CI: 0.40, 0.67). At a population level, therefore, FA food fortification was associated with a pronounced reduction in open NTDs (6).

FOLATE AND OTHER NTD RISK FACTORS

Although a large proportion of NTDs may be preventable through FA tablet supplementation or flour fortification, risk factors for NTDs that are independent of folate metabolism may also include the elements that make up the metabolic syndrome, characterized by the presence of abdominal obesity, dyslipidemia, diabetes mellitus, and nonwhite ethnicity (7). Because 40% of Western teenagers exhibit a sedentary lifestyle (8) and 16% are obese (9, 10), metabolic syndrome is now found in 15% of women who are in their reproductive years (9).

Maternal obesity has been reported to be a significant risk factor for NTDs (11, 12). In our study, although 10% of maternal cases exhibited 2 features of the metabolic syndrome, maternal obesity alone nearly doubled the risk of NTDs (11, 12). Among those described as maternally obese in conjunction with one other risk factor for the metabolic syndrome (13), we found a 6-fold higher risk of NTDs. Interestingly, we observed that women of First Nations ancestry had about a 5 times higher risk of NTD than did “white” women, which was not so among women of other “nonwhite” ethnic origins (14).

OTHER EFFECTS OF FOLATE FORTIFICATION IN PREGNANCY

Polymorphisms in maternal genes responsible for normal folate metabolism have been associated with an increased risk of fetal...
trisomy 21 (15). Indeed, well-defined polymorphisms of folate-dependent enzymes that remethylate tHcy to methionine have been associated with trisomy 21 in some (16) but not all (17) studies. Moreover, offspring affected by trisomy 21 are themselves reported to have impaired folate-dependent remethylation of methionine (17). Thus, it was of interest to know whether FA fortification was associated with a decrease in the birth prevalence rate of trisomy 21. In particular, we investigated whether the frequency of antenatally and postnatally detected trisomy 21 changed before and after FA food fortification. A total of 218,977 women underwent second trimester MSS for trisomy 21 in the 48 mo before fortification, whereas in the 29 mo after fortification 117,986 women were screened. The rate of trisomy 21 before (1.71/1000) and after (1.70/1000) fortification was not different [prevalence ratio (PR): 0.99; 95% CI: 0.84, 1.18], even after adjusting for maternal age (PR: 0.99; 95% CI: 0.82, 1.19) (15).

Similarly, a potential decline in the risk of cleft lip and cleft palate [orofacial clefts (OFCs)] has also been reported in experimental animals (18) and human case-control studies (19) as a result of periconceptional FA and multivitamin exposure. We examined the risk of OFCs among 336,963 women who underwent antenatal MSS. Their prevalence before (1.15/1000) and after (1.21/1000) fortification did not change (PR: 1.06; 95% CI: 0.86, 1.30). However, the latter study did not assess other potential risk factors for OFCs, including preconceptional FA tablet use, exposure to common teratogenic substances, syndromic cases, or other structural defects not detected during the first few days of extrauterine life (20).

**VITAMIN B-12 AND NTDs**

If the mechanism of action of FA in preventing NTDs includes in some way the metabolism of homocysteine, it is evident that other dietary intakes may also be significant. In particular, it can be seen that vitamin B-12 is a key cofactor in the methionine reductase complex that catalyzes homocysteine remethylation from the methyltetrahydrofolate donor (Figure 1). Moreover, vitamin B-12 deficiency causes the same sort of increase in circulating tHcy as does FA deficiency (21). Thus, low vitamin B-12 was postulated to be another potential risk factor for NTDs.

Both FA and vitamin B-12 are nutrients with significant roles in the pathobiology of growth and development. A deficiency of either vitamin may lead to impaired DNA methylation. In the presence of poor oral intake or gastrointestinal malabsorption of vitamin B-12, however, excess FA consumption may hypothetically mask the hematologic (eg, megaloblastic) and biochemical (eg, hyperhomocysteinemic) manifestations of the maternal vitamin B-12 insufficiency that may place the fetus at risk of NTDs. Thus, it has been argued for some time now that the addition of vitamin B-12 to FA supplementation or fortification might further lower the NTD risk (22–24).

With respect to NTDs, the contribution of low vitamin B-12 status was unclear, because folate insufficiency seemed to be the larger contributing factor (22, 25, 26). A number of studies have now found a significant increase in the odds ratio for NTDs in mothers with poor vitamin B-12 status (Table 1). Comparisons between studies are difficult when there are differences in sample sizes, selection of cases and controls, and the methods of measuring vitamin B-12 status. Some studies measured serum vitamin B-12 concentrations, others measured methylmalonic acid (MMA), and a few measured holo transcobalamin (holoTC) concentrations. Only one small case-control study was reported in which the investigators found a nonsignificant associated risk of NTD in the presence of a low holoTC [crude odds ratio (OR):

---

**Table 1**

Summary of published case-control studies evaluating the odds ratios for risk of neural tube defects (NTDs) in association with indicators of maternal vitamin B-12 status

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of NTD cases</th>
<th>No. of non-NTD controls</th>
<th>Serum-plasma analyte</th>
<th>Comparison of abnormal and normal cutoffs</th>
<th>Odds ratio (95% CI) for NTD cases compared with controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirke et al (27)</td>
<td>81</td>
<td>247</td>
<td>Vitamin B-12 and folate</td>
<td>Lower quartile compared with upper quartile, both analytes</td>
<td>5.4 (1.2, 25.2)</td>
</tr>
<tr>
<td>Molloy et al (28)</td>
<td>32</td>
<td>384</td>
<td>Vitamin B-12</td>
<td>≤185 pmol/L compared with &gt;185 pmol/L</td>
<td>0.9 (0.4, 1.9)</td>
</tr>
<tr>
<td>van der Put et al (29)</td>
<td>60</td>
<td>94</td>
<td>Vitamin B-12</td>
<td>≤5th centile compared with &gt;95th centile</td>
<td>3.9 (1.3, 11.9)</td>
</tr>
<tr>
<td>Groenen et al (30)</td>
<td>44</td>
<td>83</td>
<td>Vitamin B-12</td>
<td>≤10th centile compared with &gt;10th centile</td>
<td>3.5 (1.3, 8.9)</td>
</tr>
<tr>
<td>Suarez et al (31)</td>
<td>157</td>
<td>186</td>
<td>Vitamin B-12</td>
<td>Lower quintile compared with upper quintile</td>
<td>2.6 (1.2–5.4)</td>
</tr>
<tr>
<td>Wilson et al (32)</td>
<td>58</td>
<td>89</td>
<td>Vitamin B-12</td>
<td>Lower quartile compared with ≥second quartile</td>
<td>2.1 (0.86, 5.2)</td>
</tr>
<tr>
<td>Adams et al (33)</td>
<td>33</td>
<td>132</td>
<td>MMA</td>
<td>≥900th centile compared with &lt;10th centile</td>
<td>13.3 (2.7, 65.5)</td>
</tr>
<tr>
<td>Afman et al (34)</td>
<td>46</td>
<td>73</td>
<td>Vitamin B-12</td>
<td>Lower quartile compared with ≥upper quartile HoloTC</td>
<td>1.8 (0.6, 5.2)</td>
</tr>
<tr>
<td>Ray et al (22)</td>
<td>89</td>
<td>434</td>
<td>HoloTC</td>
<td>Lower quartile compared with ≥upper quartile</td>
<td>2.9 (0.9, 9.2)</td>
</tr>
</tbody>
</table>

1 MTRR, methionine synthase reductase 66A→G polymorphism; MMA, methylmalonic acid; holoTC, holotranscobalamin.
2.9; 95% CI: 0.9, 9.2) (34). No other study adjusted for maternal socioeconomic status or ethnicity. Only 2 studies controlled for maternal folate status (31, 33). In the latter study, comprising 33 cases and 132 controls, the adjusted OR for NTD in relation to poor vitamin B-12 status was 13.3 (95% CI: 2.7, 65.5), based on the presence of a maternal serum methylmalonic acid concentration >90th centile (33), the latter being another sensitive functional indicator of vitamin B-12 deficiency in pregnancy (37). In another study, the combination of a low vitamin B-12 and a genetic polymorphism of maternal methionine synthase reductase, MTRR 66A>G, was associated with an OR for NTD of 4.8 (95% CI: 1.5, 15.8) (32). In general, although there appeared to be a consistent association between maternal vitamin B-12 insufficiency and NTD risk, only a limited number of studies examined maternal vitamin B-12 status with methods of sufficient sensitivity, or controlled for influential maternal risk factors, such as folate status (Table 1).

At the beginning of the FA fortification program, concern was widespread that there might be potential masking of vitamin B-12 deficiency. This was offset to some extent by the fact that measurement of serum vitamin B-12 has become relatively routine. Some uncertainty remains, however, about which measurements and cutoffs are best to determine vitamin B-12 deficiency, because the biochemical profile varies among persons.

In view of these concerns, we completed a large case-control study examining the relation between low maternal vitamin B-12 status and NTD risk (Table 1)—the first to evaluate this risk in a population largely exposed to FA-fortified food. We used a clinically relevant measure of vitamin B-12 (serum holoTC), which is the fraction of total circulating vitamin B-12 bound to transcobalamin II, and the fraction thus available to tissues through cellular transcobalamin II receptors. When we restricted our analysis to the period after FA fortification, the associated OR of NTD in the presence of a holoTC at the lowest quartile was 3.2 (95% CI: 0.94, 11.0). These data also suggested that the population-attributable risk percentage for NTD in the presence of low vitamin B-12 status was as much as 35%. The latter is underscored by recent data that as many as 1 in 20 women in Ontario may be deficient in vitamin B-12 during the period of closure of the embryonic neural tube (22, 24, 26).

CONCLUSION: THE NEED FOR A RANDOMIZED CONTROLLED TRIAL

We suggest there is an urgent need for a multicenter randomized controlled trial to compare the combination of periconceptional vitamin B-12 and FA against FA alone for the prevention of NTDs. Such a trial might be optimally performed in an area with a high prevalence of vitamin B-12 deficiency, such as India, where vegetarianism is a main cause of low vitamin B-12 status and where the incidence of NTDs may be among the highest worldwide (29, 32). (Other articles in this supplement to the Journal include references 35–38.)

The authors’ responsibilities were as follows—MDT: coordinated the sample collection for the B-12 study (22) and the primary author of this review; DECC: was senior coinvestigator under the PSI Foundation grant supporting the B-12 study and contributed to the content of this review; and JGR: was senior coinvestigator for the PSI Foundation grant that supported many of the publications from our group cited herein. None of the authors had a conflict of interest to declare in relation to this review.

REFERENCES


34. Afman LA, Van Der Put NM, Thomas CM, et al. Reduced vitamin B\textsubscript{12} binding by transcobalamin II increases the risk of neural tube defects. QJM 2001;94:159–66.

35. Allen LH. How common is vitamin B\textsubscript{12} deficiency? Am J Clin Nutr 2009;89(suppl):693S–6S.

36. Green R. Is it time for vitamin B\textsubscript{12} fortification? What are the questions? Am J Clin Nutr 2009;89(suppl):712S–6S.


38. Selhub J, Morris MS, Jacques PF, Rosenberg IH. Folate–vitamin B\textsubscript{12} interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B\textsubscript{12} deficiency. Am J Clin Nutr 2009;89(suppl):702S–6S.