Pros and Cons of Increasing Folic Acid and Vitamin B\textsubscript{12} Intake by Fortification

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Abstract

There is no doubt that folic acid fortification can be effective for reducing the incidence of neural tube defects. The degree of efficacy depends on both the level of folate depletion and other, yet to be fully characterized, genetic and/or environmental factors. This article summarizes briefly data on neural tube defect reduction and other benefits of folic acid fortification as these have been reviewed in more detail elsewhere. More attention is drawn to questions that have been raised about the possible adverse effects of folic acid fortification including the incidence of colorectal cancer and immune function. The main question addressed here is whether folic acid fortification can exacerbate the adverse effects of vitamin B12 deficiency. Most analyses of this question have been conducted in wealthier countries based on data from elderly populations – which have the highest prevalence of vitamin B12 deficiency. However, of potentially greater concern is the increasingly common practice of folic acid fortification in developing countries, where folate status is probably often adequate even prior to fortification, and vitamin B12 depletion or deficiency is common. To add to this information, data from a group of Chilean elderly with a range of vitamin B12 status and exposed to high levels of folic acid fortification will be presented.

Introduction

Flour fortification with folic acid has the primary goal of preventing neural tube defects (NTDs) by improving the periconceptional folate status of women at risk of delivering an infant with these abnormalities. At least 52 countries are currently mandating flour fortification with folic acid [1], although none of them are in Europe where there are stronger concerns about the safety of folic acid fortification and/or its likely effectiveness in regions where industry is already
voluntarily fortifying foods, such as cereals, with the vitamin. After mandatory or voluntary fortification was instigated in different locations, reports began to appear of associations between the timing of fortification, and/or folate status, and a range of positive and negative health outcomes. Mandatory fortification with vitamin B₁₂ is much less common, although the prevalence of deficiency is probably much higher. There are numerous documented and potential adverse effects of this nutrient deficiency that could justify fortification policies. Dual fortification with both vitamins may make sense based on their metabolic interactions and concern about the potential (as yet unproven) for folic acid fortification to exacerbate signs and symptoms of vitamin B₁₂ deficiency.

Folic Acid Fortification

Advantages
Benefits of folic acid fortification have been documented in a number of countries. For example, in the United States where fortification of flour became mandatory in 1998, the incidence of NTDs has been reduced by 19–40% [2–4]. In addition, the prevalence of folate deficiency has fallen from ≈20–25% to ≈1% [5–7]. Hyperhomocysteinemia has been reduced from 17 to 9% [5], which has been associated with reduction in mortality from stroke in the US and Canada [8], although it is not clear that this has had any effect on risk of CVD [9]. High plasma homocysteine in pregnant women is associated with an increased risk of other adverse pregnancy outcomes [10], lower birthweight of the infant [11] and in rats, impaired glucose regulation in the offspring [12], but these associations have not been tested in randomized controlled trials. In Canada, the prevalence of open NTDs fell by 48%, from 1.13 to 0.58 per 1,000 pregnancies within a few years of the start of fortification [13]. There was also a significant, 6%/year fall in the birth prevalence of severe congenital heart defects [14].

Potential for Adverse Effects
Concern has been raised about potential adverse effects of folic acid fortification, as reviewed elsewhere [15, 16]. Folic acid is a substrate for the synthesis of thymidine which is incorporated into DNA, and in folate deficiency there is less thymidine available and more uracil is incorporated into DNA, which can cause breaks in the DNA base sequence. In animal models, this increases the risk of cancer initiation. However, after cancer has been initiated, there may actually be a reduction in proliferation when folate is restricted, which is the basis of treatment with anti-folate drugs such as methotrexate. Thus, fortification with folic acid can theoretically prevent cancer initiation in depleted individuals but stimulate proliferation of preexisting tumors. This hypothesis is supported by an increase in colorectal cancer rates in the United States since folic acid addition to foods commenced [17] and a similar situation in Chile [18]. Although
improvements in ability to detect and diagnose colon cancer were ruled out by the investigators as the explanation for the increase in colon cancer, it is clear that more research is needed before causality can be proven. Moreover, increased mortality from colon cancer has not been demonstrated.

Questions have also been raised about whether high folic acid intakes reduce natural killer (NK) cell cytotoxicity, which is an important component of immune function. Postmenopausal women in the US whose diet was low in folate (<233 μg/day) had better NK cell cytotoxicity if they took folic acid supplements, but those who had a folate-rich diet and in addition took >400 μg/day as a supplement had poorer NK cell cytotoxicity [19]. This association was supported by the cytotoxicity being inversely related to levels of unmetabolized folic acid in plasma; the latter increase with folic acid intake.

**Potential for High Folate Status to Exacerbate Vitamin B₁₂ Deficiency**

Evidence has been accumulating to suggest that high folic acid intakes might exacerbate the adverse effects of vitamin B₁₂ deficiency. Concerns were first raised in the 1940s and 1950s about the possibility that folic acid supplementation might exacerbate both the hematological and the neurological symptoms of vitamin B₁₂ deficiency. These concerns arose during clinical examination of patients with megaloblastic anemia who were treated with folic acid because they were mistakenly diagnosed with folate deficiency, when in fact they were B₁₂ deficient. The folic acid ameliorated the anemia for a few years but the neurological symptoms of vitamin B₁₂ deficiency were reportedly exacerbated or appeared suddenly when folic acid supplementation was started [20, 21]. Higher doses of folic acid, given for a longer time, were most likely to produce these effects, and of 38 cases of vitamin B₁₂ deficiency treated with <1 mg folic acid, only 6 had neurological deterioration, and those cases had been treated longer [21]. Thus, 1 mg/day is generally accepted as being the upper limit for folic acid intake.

Morris et al. [22] analyzed data from the 1999–2002 National Health and Nutrition Examination Survey (NHANES) to investigate whether individuals with higher serum folate concentrations had poorer vitamin B₁₂ status based on biochemical markers. Values were available for 1,459 persons after excluding those with evidence of renal disease, alcoholism, history of stroke, or liver, thyroid or coronary artery disease. Their analyses revealed that having both vitamin B₁₂ deficiency (serum B₁₂ <148 pmol/l or serum methylmalonic acid, MMA >210 μmol/l) and high serum folate (>59 nmol/l, the 80th percentile of the population) increased the risk of both anemia and cognitive impairment (assessed with the Digit Symbol Coding subtest of the Wechsler Adult Intelligence Scale III, which assesses processing speed and memory). The risk of anemia and cognitive impairment was around three times higher in the low-B₁₂, high-folate group compared to those with serum folate ≤59 nmol/l. On the other hand, high serum folate protected against cognitive impairment in those with adequate B₁₂ status.
The same team later performed similar analyses using data from NHANES conducted in 1991–1994 and from 1999 to 2002, this time adding values for total homocysteine (tHcy) and MMA [23]. In that analysis, they found that persons with vitamin B₁₂ deficiency – defined as low serum vitamin B₁₂ (<148 pmol/l) – in combination with high serum folate (>32.6 nmol/l) had significantly higher plasma tHcy and serum MMA than those with B₁₂ deficiency and lower folate status. In the low serum B₁₂ group, tHcy and MMA increased when serum folate started to rise above 20 nmol/l. Subsequent analyses explored additional relationships among data from the 1999–2002 NHANES [24]. Low B₁₂ status was defined as serum B₁₂ <148 pmol/l or serum MMA >210 nmol/l. Compared to subjects with plasma folate ≤59 nmol/l and normal B₁₂ status, the odds ratios of having anemia and impaired cognitive function respectively were 2.1 and 1.7 with low B₁₂ and normal folate, and 4.9 and 5.0 for low B₁₂ and high folate (>59 nmol/l). With increasing serum folate, in participants with serum B₁₂ <148 pmol/l there was again a surprising increase in homocysteine and MMA, whereas there was the opposite trend for those with serum B₁₂ ≥148 pmol/l. These results suggest that the coenzyme functions of vitamin B₁₂ are more impaired at high serum folate concentrations in people with poor vitamin B₁₂ status.

In California, we found a similar pattern in elderly Latinos (participants in the Sacramento Area Latino Study on Aging) [25]. In this analysis, vitamin B₁₂ deficiency was again defined as <148 pmol/l, while high serum folate was defined as >45.3 μmol/l, a cutoff selected because it was the upper limit of accuracy for the assay. The group with B₁₂ deficiency and high serum folate had a significantly higher plasma tHcy and MMA, and lower holoTC and holoTC:B₁₂ ratio, than groups with B₁₂ deficiency and high folate, or adequate B₁₂ status and normal or high folate. However, neither cognitive function (3MSE and delayed recall) nor depressive symptoms were different across the four groups. In contrast, low serum folate was associated with dementia and impaired cognitive function in the same population [26].

In contrast to these reports, a study from Oxford, UK, found that older individuals with a holoTC concentration in the lowest tertile had a 1.8-fold higher risk of cognitive impairment, while for the lower tertile of plasma folate, it was 1.55-fold higher [27]. However, there was no evidence that high folate status (serum folate >30 or >60 nmol/l) increased the risk of anemia or cognitive impairment.

Possible Explanations for the Observed Adverse Associations with Folate Status

For adverse effects of high folic acid intakes to be plausible, there needs to be a plausible explanation for the observed associations. Several such explanations have been offered. Certainly folate and vitamin B₁₂ metabolism are closely linked, and both 5-methyl-tetrahydrofolate and B₁₂ are cofactors for the synthesis of methionine. Thus, an abnormal folate cycle or vitamin B₁₂ deficiency can
have similar effects on both hematopoiesis and the nervous system. In vitamin B\textsubscript{12} deficiency plasma folate increases because there is a block in the utilization of methyl folate; this can lead to megaloblastic anemia and neurological problems. In addition, the apparent associations between vitamin B\textsubscript{12} deficiency and high serum folate may to some extent be explained by this phenomenon rather than high folate causing B\textsubscript{12} depletion. Some investigators have hypothesized that the increase in circulating folic acid, which rises progressively with intakes of folic acid in supplements or fortification [28], may have adverse effects on immune function or the rate of oxidation of forms of methionine synthase [23]. An alternative interpretation is that individuals with high serum folate are those taking multivitamin supplements containing folic acid, in addition to fortified products [29]. Such supplements usually contain vitamin B\textsubscript{12}, so individuals with low serum B\textsubscript{12} but high folate may have problems absorbing vitamin B\textsubscript{12}, i.e. they may have pernicious anemia, and ensuing long-term negative effects on hematology or neurological function. Clearly, all of these mechanisms remain to be confirmed, logically with the inclusion of both animal models and clinical trials.

*Estimating the Appropriate Level of Folic Acid Addition to Flour*

Because of the low occurrence of NTDs (0.8/1,000 in the US to 14/1,000 in China, for example), it has been pointed out that relatively few NTDs are prevented relative to the number of people exposed to folic acid fortification [15]. For example, it has been estimated that increasing folic acid intake in the UK may prevent up to 162 NTDs/year in a population of 60 million people. Increasing intake by 0.2 mg/day would prevent 49 NTDs per year in Australia and 11 in New Zealand [30] out of a population of ≈27 million people. In the UK, were flour to be fortified with 300 μg/100 g, 77–162 NTDs/year would be prevented, but 370,000–780,000 people exposed to excess folic acid per NTD prevented [15]. Thus, it is important to ensure that the level of exposure of the general public to folic acid is not excessive as a result of fortification.

The underlying metabolic and genetic defects that cause NTDs are not completely understood, but it is apparent that low maternal folate status interacts with genetic and environmental risk factors to increase risk [31, 32]. Thus, folic acid fortification is more effective at reducing NTDs in population groups with poor folate status. The folate status of populations varies widely, and tends to be lower in industrialized countries than in poorer regions of the world where there is a relatively high intake of green leafy vegetables and legumes, which are good sources of the vitamin [33]. Countries such as Chile, the United States, Canada, and China had a low intake and generally poor folate status before fortification, and thus would be expected to benefit more from fortification. Moreover, it is clear that the reduction in NTDs after fortification was greatest in areas with the highest rates of NTD and poorer folate status (e.g. Newfoundland compared to Quebec and Manitoba in Canada, Northern China compared to the South, New England compared to the rest of the US) [34]. The question then arises about
whether it is reasonable to expect similar rates of NTD reduction in developing countries, where folate status might be adequate before fortification. For example, it has been estimated that serum folate needs to be 16 nmol/l for maximum prevention of NTDs, and that this can be achieved with a folic acid intake of 279 μg/day, assuming no other dietary intake [35]. It is rational to obtain data on folate status and intake in populations with a low intake of animal source foods (ASF; and/or vitamin B₁₂ deficiency) prior to assuming that folic acid fortification will have sufficient benefit to outweigh any potential harm.

**Vitamin B₁₂ Fortification**

There are reasons why vitamin B₁₂ fortification should be considered, with or without folic acid fortification, especially for the elderly and in populations with a low intake of ASF.

**Prevalence of Deficiency**

It is now apparent that the prevalence of vitamin B₁₂ deficiency (serum B₁₂ <148 pmol/l or <200 pg/ml) and depletion (serum B₁₂ ≥148 to <221 pmol/l) is high in people of all ages in populations with a low intake of ASF, since these are the only natural source of the vitamin. Based on 2005 data from the World Health Organization, the prevalence of B₁₂ deficiency in adults was highest in India (50%) and was about 5–30% in most other countries [36]. The prevalence of vitamin B₁₂ depletion is likely to be at least as high as that of deficiency; in studies in Latin America, for example, prevalence of serum B₁₂ <148 pmol/l is about 15–20%, while over 20% are depleted. This prevalence also applies to a nationally representative sample in Mexico [37]. In all developing country studies where prevalence was reported, serum vitamin B₁₂ concentrations were correlated with intake of the vitamin, or intake of ASF.

Vitamin B₁₂ deficiency has several well-documented adverse effects and is associated with many others. The severe deficiency that occurs in pernicious anemia, strict vegetarianism, and occasionally in elderly with malabsorption of the vitamin from food due to gastric atrophy can result in megaloblastic anemia. However, populations chronically deficient or depleted in the vitamin due to low ASF intake are much less likely to suffer from anemia as a consequence [38], nor does supplementation with B₁₂ affect any hematological symptoms. Vitamin B₁₂ status was a predictor of NTDs in Ireland where flour is not fortified with folic acid [39], and Canada after folic acid fortification of flour was introduced [40]. Neurological disorders including loss of memory, cognitive impairment [41] and rate of brain volume loss with aging [42, 43] and the severity of white matter lesions [44], and depression [Jones et al., unpubl. data] are all associated with poor vitamin B₁₂ status. The results of supplementation trials are not yet definitive for vitamin B₁₂ alone, but homocysteine lowering as a result of
supplementation with vitamin B\textsubscript{12} (0.5 mg/day), vitamin B\textsubscript{6} and folic acid significantly reduced the rate of brain volume loss with aging [44]. A potentially major but poorly documented problem is the low concentration of the vitamin in breast milk produced by mothers who are deficient or depleted in the vitamin [Deegan et al., unpubl. data], which is a concern since infants breastfed by deficient mothers have an increased risk of developmental delays, which may be permanent [45], including delayed motor development [46]. Poor vitamin B\textsubscript{12} status has also been implicated in bone loss [47] possibly as a result of hyperhomocysteinemia, although randomized controlled supplementation trials are needed for confirmation.

\textit{Vitamin B\textsubscript{12} Fortification}

Based on the high prevalence of vitamin B\textsubscript{12} deficiency and depletion, and the confirmed and potential adverse effects of deficiency, it has been recommended that flour be fortified with 2 μg vitamin B\textsubscript{12}/100 g flour in the form of cyanocobalamin in developing countries [48]. This level of addition is restricted by the relatively high cost of the vitamin; in fortification, it is recommended that the total cost of fortificants should not exceed 2% of the total cost of the product. Unfortunately, there are no large-scale fortification trials of the efficacy of this dose. We have conducted a small-scale pilot study of the bioavailability of the labeled vitamin from fortified bread rolls given to healthy adult volunteers, and found absorption from a 0.8 μg dose to be approximately 50%. The efficiency of absorption of the vitamin decreases substantially as intake increases, to about 30% from 5 μg and 5% with 20 μg; above about 25 μg only 1% will be absorbed, by passive transport [49]. There are no known adverse effects of high intakes of vitamin B\textsubscript{12} in supplements, or when taken intramuscularly.

Thus, it may be prudent policy to include both folic acid, at a reasonable level, and vitamin B\textsubscript{12} in flour fortification programs for developing countries. Many elderly are also likely to benefit in countries where ASF intake is higher, since vitamin B\textsubscript{12} deficiency is more common in this group presumably due to gastric atrophy and poor absorption of the vitamin from food. Clearly, trials of dual fortification should be conducted.

\textbf{References}

23 Selhub J, Morris MS, Jacques PF: In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci USA 2007;104:19995–20000.