Is it time for vitamin B-12 fortification? What are the questions?[1–4]

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ABSTRACT

Since the introduction of folic acid fortification of flour 10 y ago, an initiative to consider fortifying flour with vitamin B-12 has gained momentum in the United States. The impetus for this move stems from several considerations, including some evidence that a proportion of neural tube defect pregnancies may be the result of vitamin B-12 rather than folate deficiency. However, no interventional trials have taken place to show the efficacy of vitamin B-12 supplementation or fortification in the primary prevention or recurrence of neural tube defect pregnancies, as was the case with folic acid. Other reasons put forward for the institution of vitamin B-12 fortification include the high prevalence of vitamin B-12 deficiency in certain demographic groups, including the elderly and the young in some countries. Much of this deficiency, however, is subclinical and not associated with manifest morbidity. Moreover, individuals affected by the most severe cases of vitamin B-12 deficiency that are associated with morbidity would not benefit from the concentrations of vitamin B-12 fortification that are practical or that are being considered, because such individuals suffer from malabsorption of vitamin B-12 rather than from an inadequacy of intake of the vitamin. In addition to the well-recognized complications of vitamin B-12 deficiency, such as macrocytic anemia and neurological complications affecting sensory and motor function, more subtle effects have also been described, including osteopenia, neurocognitive impairment, and increased vascular disease risk associated with elevated homocysteine. This analysis focuses on the research questions that are pertinent to the consideration of whether or not to introduce mandatory vitamin B-12 fortification in the United States. *Am J Clin Nutr* 2009;89(suppl):712S–6S.

INTRODUCTION

Mandatory fortification of flour with folic acid was introduced first in the United States and then in Canada ≈10 y ago to reduce the incidence of neural tube defect pregnancies. This public health measure has clearly been efficacious on the basis of estimates of a 20–50% reduction of neural tube defect pregnancies and births (1, 2). Other possible consequences of folic acid fortification are now emerging, some of which are potentially beneficial and others perhaps undesirable. The folate status of the entire US population has shifted. Folate deficiency has been almost completely eradicated, and the entire distribution of serum and red blood cell folate concentrations has shifted to states of sufficiency and even excess (3–5). Homocysteine concentrations have been lowered and the prevalence of hyperhomocysteinemia, a risk factor for vascular disease, has been markedly reduced (6). In the folic acid fortification era, vitamin B-12 deficiency has emerged as the most common modifiable risk factor for hyperhomocysteinemia (7). Some data suggest that a decline in stroke mortality in the United States and Canada coincides with the introduction of folic acid fortification in those countries (8). On the other hand, reduced natural killer cell cytotoxicity, which is important in the host innate immune response to infection and malignancy, is decreased in association with elevated concentrations of unmetabolized folic acid, which occurs with the long-term consumption of folic-acid-fortified foods and supplements (9). Recent observations report an association between the introduction of folic acid fortification and an observed increased incidence of colonic neoplasia (10, 11). Folate may also play a beneficial role in cancer because it is required for a critical DNA repair pathway involving DNA synthesis and repair (12). This apparently paradoxical effect may relate to the timing of folate administration in relation to the stage of the cancer (13). The role of folic acid or folate intake as a risk factor in several cancers remains unsettled at this time (14). Another possible consequence of increased folic acid intake is an alteration in epigenetic programming in utero (15, 16). As a consequence, some countries that have not instituted folic acid fortification but are now considering doing so are carefully weighing the potential risks against the benefits.

At the same time, an initiative is underway in the United States to consider instituting mandatory vitamin B-12 fortification of flour, driven in part by data that indicate that a fraction of neural tube defect pregnancies are associated with low vitamin B-12 (17). There are, however, some fundamental differences between folic acid and vitamin B-12 with respect to mandatory fortification of the food supply with either of these nutrients. First, no interventional trials have taken place to show the efficacy of vitamin B-12 supplementation or fortification in the primary prevention or recurrence of neural tube defect pregnancies, which was the case with folic acid. Second, although there is a high prevalence of

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[2] Presented at the symposium "Is It Time for Mandatory Vitamin B-12 Fortification in Flour?" held at Experimental Biology 2008, San Diego, CA, 8 April 2008.

[3] Supported by NIH grants HL083276, DK077563, and CA116409 and by USDA grant 00-35200-9073 and DOD grant BC063550.

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First published online January 13, 2009; doi: 10.3945/ajcn.2008.26947E.

vitamin B-12 deficiency among the elderly and even in children in some countries, much of this deficiency is subclinical and not associated with manifest morbidity. Moreover, individuals affected by the most severe cases of vitamin B-12 deficiency that are associated with morbidity suffer from malabsorption of vitamin B-12 rather than from an inadequacy of intake of the vitamin and would not benefit from the concentrations of vitamin B-12 fortification that are being considered. In light of these various considerations, it appeared timely to address the issues that are germane to the question ''Is it time for mandatory vitamin B-12 fortification in flour?'' in a breakthrough symposium at the Experimental Biology meetings held on 8 April 2008. Four speakers addressed key issues pertaining to the prevalence of vitamin B-12 deficiency, vitamin B-12 and cognition in the elderly, folate–vitamin B-12 interactions, and vitamin B-12 and neural tube defects. Their presentations appear in this supplement, along with this summary and overview of the unanswered and pressing research questions that should be addressed before embarking on another fortification program, this time with vitamin B-12.

SCOPE OF THE QUESTIONS AND SUBDOMAINS

The efficacy of vitamin B-12 fortification is critically dependent on several physiologic factors involving the multifaceted process of vitamin B-12 absorption. This process involves highly specific protein ligands that select, protect, tightly embrace, and escort vitamin B-12 through its long journey through the threatening milieu of the gastrointestinal tract to present it to its dedicated portal of entry in the terminal ileum (18). The quota of vitamin B-12 that is admitted through this intricate pathway is restricted. Is this solely because the cargo is precious, or might it also be dangerous?

The scope of the questions to be addressed in relation to vitamin B-12 fortification is shown in **Table 1** in the form of a hierarchical algorithm. The first basic question relates to an examination of the data at hand and whether or not there is sufficient information on which to base a decision. There is either enough information at hand to make a clear choice on the basis of a risk-benefit analysis or there is not. In the latter case, a subset of questions then needs to be addressed concerning the critical missing data and how to best obtain this information. If the decision is to fortify, then a series of questions needs to be addressed regarding the amount and form of vitamin B-12 as well as the type of food vehicle and stability considerations. Importantly, decisions should be made regarding mechanisms of monitoring that should be put in place. Likewise, if the decision is to not fortify, then the subsidiary question should be addressed regarding what continuing monitoring should be carried out in case evidence accumulates to necessitate reexamination of the central question.

TABLE 1
Scope and question hierarchy relating to vitamin B-12 fortification

Is there enough information about vitamin B-12 fortification to make an informed decision (recommendation)?
If ''yes'' (sufficient information):
Do benefits outweigh risks? Then fortify.
Do risks outweigh benefits? Then do not fortify.
If ''no'' (insufficient information):
What research questions need to be addressed before revisiting the question?
If the decision is to fortify
How much vitamin B-12 should be used?
What should the food vehicle be?
What are the stability considerations?
What monitors should be put in place?
If the decision is to not fortify
What additional information is required before reconsidering the question?

In addition to the general question domains, there are several subdomains that can be conveniently considered under several categories: *1*) nutritional, *2*) population-based and epidemiologic, *3*) neurocognitive, *4*) cancer risk and other possible toxicity, *5*) causes and correction of vitamin B-12 deficiency, *6*) assessment of deficiency, and *7*) miscellaneous. The relevant questions in each category are summarized in **Tables 2** and **3** and are considered briefly below under the appropriate subheadings.

Nutritional questions

For the several nutritional questions listed in Table 2, partial answers are available and have been reviewed extensively elsewhere (18, 19). Absent from the current available data is detailed information on what the optimal amount and plasma concentrations of vitamin B-12 are for healthy aging, and much of the currently available data stems from assumptions regarding the efficiency of vitamin B-12 absorption, including enterohepatic recirculation in older individuals (19). Although reviewed and revised less than a decade ago (19), the question arises as to whether the daily recommended intake, the recommended daily allowance, and the safe upper limit for vitamin B-12 need to be reassessed.

It is not clear why vitamin B-12 absorption is normally so tightly regulated. Has the mechanism evolved only because the nutrient supply is limited, or is it perhaps harmful in excess? With respect to iron and other trace minerals, the absorptive mechanism is finely tuned to body needs and excessive absorption and accumulation is undesirable. In this regard, new information is emerging concerning the possible generation of vitamin B-12 analogs in the gastrointestinal tract (20). A key unanswered question relating to vitamin B-12 fortification is whether a sizable segment of the target population for vitamin B-12 fortification (the elderly) will benefit from the proposed concentrations of fortification. In the accompanying article in this issue by Allen (21), the question of target groups is addressed. A major problem with simple dietary fortification with vitamin B-12 is that, when the normal physiologic mechanism for absorption is abrogated, only the mechanism for vitamin B-12 absorption by passive diffusion is possible. This mechanism is only $\approx$1% efficient so that large amounts of vitamin B-12, on the order of $\geq$200 μg, would be required to satisfy the recommended daily allowance of 2.4 μg (19).

Population-based and epidemiologic questions

Population-based and epidemiologic questions are also listed in Table 2. Three linked questions that relate to population-wide effects pertain to the following consideration: It is not known whether the undiagnosed subclinical or preclinical vitamin B-12 deficiency that has been reported in the population would be ameliorated by fortification, or what concentration of fortification would be required. Although there is evidence that low oral doses of vitamin B-12 raise the mean serum concentration of vitamin B-12 in the elderly with food vitamin B-12 malabsorption (22), there

TABLE 2
Questions relating to vitamin B-12 fortification in the subdomains of nutrition, epidemiology, neurocognition, and cancer[1]

Nutritional questions
• What is normal and what constitutes nutritional adequacy?
• What concentration of vitamin B-12 is optimal for healthy aging?
• Why is the normal vitamin B-12 absorptive process so tightly regulated?
• What are the biological ramifications of altering the nutritional (vitamin B-12) status of a population?
• Might excessive amounts of vitamin B-12 lead to the generation of unwanted analogs of the vitamin?
• Should the DRI, RDA, and UL for vitamin B-12 be reassessed?
• How is an appropriate amount of vitamin B-12 for fortification best determined? Does one size fit all?
• Does concomitant intake of food limit vitamin B-12 absorption?
• What is the target population for vitamin B-12 fortification and will proposed concentrations of fortification have any effect?
Population-based and epidemiologic questions
• What evidence is there of undiagnosed subclinical or preclinical vitamin B-12 deficiency in the population that would be ameliorated by fortification and what concentration would be required?
• How often does subclinical vitamin B-12 deficiency progress to clinical deficiency?
• What real evidence is there of possible adverse effects of low vitamin B-12 status?
• Would improved vitamin B-12 nutritional status further reduce the incidence of neural tube defects?
• Are we lulled to complacency by the apparent absence of evidence of toxicity of vitamin B-12?
Neurocognitive questions
• With respect to the neurocognitive effects of vitamin B-12 deficiency, is there a threshold or is the effect continuous?
• Is there a risk of cognitive impairment within the normal range of vitamin B-12 and its markers?
• Is raised homocysteine the only or predominant cause of cognitive impairment in vitamin B-12 deficiency?
• Is there another metabolite toxicity?
• Is there deficiency of a downstream product?
• Are there possible deleterious associations of high vitamin B-12 concentrations with impaired neurocognition?
Cancer and other possible toxicity questions
• Is the current lack of evidence of adverse effects of supplemental vitamin B-12 cause for complacency?
• Are there long-term effects of the intake of the (unphysiological) cyanocobalamin form of vitamin B-12?
• Is there a dual (and paradoxical) reciprocal role of vitamin B-12 in the prevention or progression of cancer similar to what has been proposed for folate?
• Are reports of adverse effects of vitamin B-12 in patients with leukemias or other cancers significant?
• Are there cancer-protective effects of vitamin B-12?
• Is there a risk of disruption of intestinal microflora and increased susceptibility to infection with supplemental vitamin B-12?

[1] DRI, Dietary Reference Intake; RDA, Recommended Daily Allowance; UL, upper limit.

are a substantial number of individuals within this demographic group who have undiagnosed pernicious anemia (23) and it is unlikely that they would benefit from such low concentrations of vitamin B-12 added to fortify the food supply. Another major unanswered question is how often subclinical vitamin B-12 deficiency progresses to clinical deficiency. Apart from individuals with evolving (preclinical) pernicious anemia, who transition through a subclinical state, such progression may be extremely rare. Associated with this consideration is the question of what real evidence is there of possible adverse effects of low measurements of vitamin B-12 status?

Neurocognitive questions

The neurognitive issues are addressed in the accompanying article in this issue by Smith and Refsum (24) and the key questions are summarized in Table 2. With respect to the questions of whether or not neurocognitive effects of vitamin B-12 deficiency display a threshold relation or whether there is a continuous effect, the data presented by Smith and Refsum and their previously published work fairly convincingly show that the latter is the case (25). This has been confirmed by others (26). From these data it is apparent that there a risk of cognitive impairment within the normal range of vitamin B-12 and its markers. However, this association does not prove a cause-effect relation, and the question needs further investigation, ideally through controlled interventional studies.

Also, because homocysteine is a risk factor for neurocognitive decline and Alzheimer's disease and vitamin B-12 status has become the primary nutritional determinant of hyperhomocysteinemia (7), the vitamin B-12 effect on cognition may be mediated in part through homocysteine. A theoretical alternative explanation for the association of low vitamin B-12 with impaired neurocognition is that there is some other metabolite that accumulates in vitamin B-12 deficiency and leads to neurological toxicity.

Equally worthy of consideration is the possibility that there may be deficiency of a downstream product from a vitamin B-12–dependent pathway. An example of such a candidate molecule is *S*-adenosylmethionine (18). There is also some evidence to suggest an association between high vitamin B-12 and impaired neurocognition (27).

Cancer and other possible toxicity questions

Possible deleterious effects of increased intake of vitamin B-12 are also summarized in Table 2. The current lack of evidence of adverse effects of supplemental vitamin B-12 should be no cause for complacency, because long-term administration and accumulation of vitamin B-12 has not been systematically studied and reports of safety are based on absence of obvious harmful effects in patients who have been receiving long-term treatment with vitamin B-12 by injection or orally. Cyanocobalamin is not the physiologic form of the vitamin, and there is evidence that the

TABLE 3
Questions relating to causes, correction, and assessment of vitamin B-12 deficiency relating to fortification

Causes and correction of deficiency: questions
• What are the respective contributions of pernicious anemia, food vitamin B-12 malabsorption, and dietary insufficiency to the prevalence of vitamin B-12 deficiency in the elderly?
• Is the absorption of crystalline vitamin B-12 completely normal in food vitamin B-12 malabsorption?
• Are all causes of "simple" atrophic gastritis equal with respect to vitamin B-12 malabsorption?
• What is the role of *Helicobacter pylori?*
Assessment of deficiency: questions
• What is the best way to assess vitamin B-12 status and to monitor response?
• Is there a single best marker for vitamin B-12 deficiency? If not, what is the best combination of markers?
• Which combination of nutrient concentrations (vitamin B-12/folate) is best? Which is worst? Does it matter?
• Why does high folate (with low vitamin B-12) result in high homocysteine, high methylmalonate, and low holotranscobalamin concentrations?
• Will addition of vitamin B-12 alleviate the potentially harmful effects of excess folic acid? Will addition of vitamin B-12 aggravate some harmful effects?
• Will addition of vitamin B-12 enhance any beneficial effects of folic acid?

distribution of vitamin B-12 forms in the plasma changes in patients who have been treated with cyanocobalamin (18). This question requires further study. Regarding a possible role for vitamin B-12 in either the promotion or the prevention of cancer, there are biochemical and metabolic reasons to hypothesize that vitamin B-12 might have effects similar to those of folate with respect to cancer risk and prevention. Both vitamin B-12 and folate are involved in the metabolic pathways responsible for de novo thymidine synthesis and repair and for the generation of *S*-adenosylmethinine that is required for DNA cytosine methylation (12). Consequently, in the same way that folate may prevent initiation of cancer through DNA repair, vitamin B-12 may play a similar role. On the other hand, with an established tumor, folate (and vitamin B-12) may promote the growth of rapidly proliferating cells. Indeed, the efficacy of methotrexate and other antifols depends on the interference of folate (and vitamin B-12)-dependent nucleotide synthesis, and vitamin B-12 analogs have been proposed for use as anticancer agents (18). Additionally, the epigenetic regulation of DNA through methylation can be influenced by folate (and vitamin B-12) availability and hence may affect the expression of critical oncogenes or tumor suppressor genes (13). There are reports in the literature that vitamin B-12 deficiency may be associated with an increased risk for certain cancers, including breast cancer (28). On the other hand, it is possible that established cancers may progress more rapidly if vitamin B-12 is supplied (29). This possible connection between vitamin B-12 and cancer risk is clearly a subject that requires a more thorough and systematic investigation.

Another question that requires careful examination is whether vitamin B-12 can affect the growth and proliferation of pathogenic microorganisms. It is well established that bacterial overgrowth in the small intestine can result in vitamin B-12 deficiency through competition with the host for dietary and enterohepatic vitamin B-12 (12). Putatively, microorganisms supplied with abundant vitamin B-12 might proliferate more rapidly and might influence the severity of such an infection. Whether this occurs elsewhere in the body is not known, but it is noteworthy that granules in neutrophils and their precursors contain haptocorrin, which might play a role in the antimicrobial function of these cells at sites of infection (12).

CAUSES AND CORRECTION OF DEFICIENCY

The questions that pertain to the causes and correction of vitamin B-12 deficiency are summarized in Table 3. All of these questions require further study. Little is known about the respective contributions of pernicious anemia, food vitamin B-12 malabsorption, and dietary insufficiency to the prevalence of vitamin B-12 deficiency in the elderly. Also, it is widely assumed but not known whether the absorption of crystalline vitamin B-12 is completely normal in the condition of food vitamin B-12 malabsorption. There is mounting evidence that *Helicobacter pylori* plays a role in diseases that lead ultimately to vitamin B-12 malabsorption and deficiency, from chronic atrophic gastritis (12) to possibly even pernicious anemia (30). The extent to which widespread infestation with this organism may contribute to vitamin B-12 deficiency among populations requires further study.

Several questions are posed with respect to the assessment of vitamin B-12 deficiency, and these are also summarized in Table 3. There has been considerable discussion concerning the best way to assess vitamin B-12 status and to monitor response to vitamin B-12 administration (18), but increasing evidence is emerging that measurement of holotranscobalamin concentrations may provide the single best measure of vitamin B-12 status (31). Another set of questions is focused on the recent observations that high folate in association with low vitamin B-12 appears to result in the highest homocysteine and methylmalonate concentrations (32). This topic is dealt with extensively in the accompanying article in this issue by Selhub et al (33). The observations made by Selhub raise the question of whether the addition of vitamin B-12 as a fortifier will alleviate the potentially harmful effects of excess folic acid. On the other hand, it should also be considered that the addition of vitamin B-12 could either aggravate harmful effects or enhance beneficial effects of folic acid through the intricate interrelationships between folate and vitamin B-12.

MISCELLANEOUS QUESTIONS

There are other miscellaneous questions that have not yet been addressed under the headings above. There is mounting evidence that genetic polymorphisms in vitamin B-12–related genes may play a role in determining vitamin B-12 status as well as susceptibility to vitamin B-12 deficiency (34). It is possible that evolution and selective pressure have favored the emergence of polymorphisms and that this might have led to adaptive change. Could vitamin B-12 fortification disrupt this adaptation? And what consequences would that have on health? (Other articles in this supplement to the Journal include references 21, 24, 33 and 35.)

The author had no potential conflicts of interest to declare.

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