

Low vitamin B-12 concentrations in patients without anemia: the effect of folic acid fortification of grain¹⁻³

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ABSTRACT

Background: In some patients with vitamin B-12 deficiency mistakenly treated with folic acid, anemia resolved but neurologic complications became worse (masking). Fortification of enriched cereal grains with folic acid has raised concerns that people who consume large quantities of cereal grains, particularly the elderly, may be at increased risk of masking. It is unclear, however, what proportion of people with low vitamin B-12 concentrations do not have anemia and whether the proportion is increasing.

Objective: We investigated whether fortification has increased the proportion of patients with low vitamin B-12 but without anemia.

Design: We reviewed the laboratory results of every patient for whom a vitamin B-12 concentration was measured at the Veterans Affairs Medical Center in Washington, DC, between 1992 and 2000. Those with a low vitamin B-12 concentration (<258 pmol/L) had their hematocrits and mean cell volumes checked. The proportion without anemia was examined by year before, during, and after folic acid fortification began.

Results: There were 1573 subjects with a low vitamin B-12 concentration. The proportion without anemia did not increase significantly from the prefortification period (39.2%) to the period of optional fortification (45.5%) and the postfortification period (37.6%). These findings did not change when the analysis was limited to patients aged >60 y or when a more conservative definition of low vitamin B-12 (<150 pmol/L) was used.

Conclusions: Despite evidence that folic acid exposure has increased dramatically since food fortification began, this population showed no evidence of an increase in low vitamin B-12 concentrations without anemia. If confirmed, these results would indicate that food fortification has not caused a major increase in masking of vitamin B-12 deficiency. *Am J Clin Nutr* 2003;77:1474-7.

KEY WORDS Pernicious anemia, vitamin B-12, cobalamin, folate, folic acid, vitamin B-12 deficiency, masking of pernicious anemia

INTRODUCTION

Before the role of vitamin B-12 in causing megaloblastic anemia was appreciated, vitamin B-12-deficient patients were often treated with folic acid. In many cases the anemia improved initially; however, the associated neurologic damage progressed. This phenomenon, known as masking of vitamin B-12 deficiency, has never been studied systematically for the obvious reason that patients would never knowingly be given the wrong treatment,

particularly when the neurologic damage associated with vitamin B-12 deficiency is often irreversible (1). Therefore, our knowledge of how frequently masking occurs and of the lowest dose of folic acid that produces masking is limited. In fact, little is known about how often patients with low vitamin B-12 have symptoms of neurologic damage without anemia in the absence of folic acid therapy. For an excellent review of this subject, see Savage and Lindenbaum (2).

The decision by the US Food and Drug Administration (USFDA) to fortify enriched cereal grains with folic acid (3) to prevent neural tube defects has stimulated interest in masking of vitamin B-12 deficiency for several reasons. First, the elderly have the greatest risk of low vitamin B-12 concentrations and consume high amounts of cereal grain products (4, 5). Second, although the USFDA chose a fortification level that they believed would limit exposure to <1000 µg folic acid/d (a level most experts consider safe) in almost everybody, there is evidence that the actual fortification level in foods is considerably higher (6).

Anemia provides an important clue to the diagnosis of a low vitamin B-12 concentration, particularly in the elderly, in whom some of the neurologic signs, eg, confusion, paresthesias, and dementia, are seen in many other conditions. Therefore, data on the rate of low vitamin B-12 concentrations occurring without anemia are urgently needed given uncertain folic acid exposures and the lack of good data on maximal safe exposures to folic acid. This study was designed to identify patients with low vitamin B-12 before and after most grain products were fortified with folic acid to determine whether the proportion of cases occurring without anemia increased after fortification.

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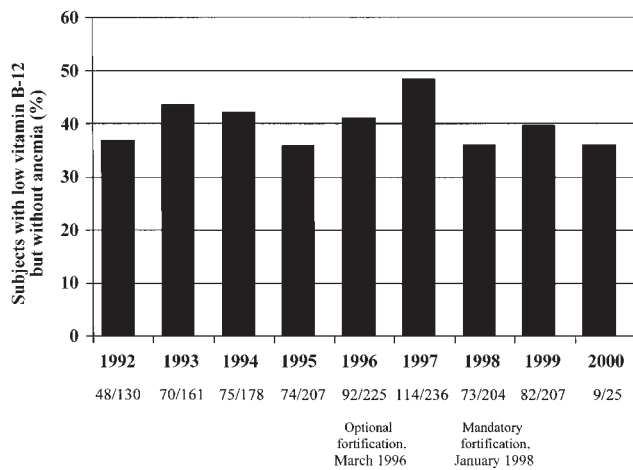


FIGURE 1. Percentages of subjects with low vitamin B-12 concentrations (<258 pmol/L) but without anemia before, during, and after fortification of enriched cereal grains with folic acid. The fractions under the year labels on the x axis indicate the number of subjects without anemia/the number of subjects with a low vitamin B-12 concentration. The percentage of subjects without anemia was not significantly different between the 3 time periods (ie, before, during, and after fortification).

SUBJECTS AND METHODS

All patients for whom a vitamin B-12 concentration was measured at the Veterans Affairs Medical Center in Washington, DC, between 1 January 1992 and 1 March 2000 ($n = 18\ 106$) were identified from laboratory records. To eliminate results that may have been obtained to monitor previously diagnosed patients, only the patients' first vitamin B-12 measurement that was below the normal range was included in the analysis. The laboratory performed all vitamin B-12 assays for the hospital, geriatric clinic, and nursing home over the entire period covered by the study. Vitamin B-12 concentrations were customarily measured in all patients who had neuropathy, dementia, or anemia. This was considered appropriate medical practice, although the actual management by individual physicians may have varied. In addition, the geriatric service screened patients yearly for a low vitamin B-12 concentration, and many primary care physicians on staff screened new patients. Most patients at high risk of folate deficiency, eg, alcohol abusers, were treated prophylactically with folic acid; thus, detection of folate deficiency anemia on the basis of a low folate concentration was uncommon. Red cell folate measurements were obtained in subjects with macrocytic anemia, but low concentrations were uncommon. In all, 99 patients were tested for red cell folate over the study period, and only 2 had a low concentration (<130 ng/mL). The population served by the Medical Center did not change in any major way over the course of the study, ie, no outside hospitals or other facilities began using the laboratory, and no facilities that had been using the laboratory ceased to do so.

For the purpose of this study, vitamin B-12 concentrations <258 pmol/L were considered low because this concentration has been shown to correlate well with vitamin B-12 deficiency as defined by an elevated methylmalonic acid (MMA) concentration (7). The normal range of vitamin B-12 concentrations was the same throughout the study period. The laboratory performing MMA testing changed during the study. Therefore, each laboratory's definition of

an abnormal value was used for the samples that they tested. Testing of antibodies to intrinsic factor was not performed. Hematocrit values of <38.6 and mean cell volumes (MCVs) of >96.7 fL were defined as abnormal. Hematocrit and MCV values had to be obtained ≤ 14 calendar days before the vitamin B-12 value because it is likely that the values reflected the subject's hematologic status immediately before diagnosis. The Chair of the Institutional Review Board of the National Institute of Child Health and Human Development granted this study an exemption from review. Institutional Review Board approval was waived by the Veterans Affairs Medical Center.

Vitamin B-12 concentrations were measured by using competitive protein binding (Abbott IMX initially and then Abbott AxSym; Abbott Laboratories, Abbott Park, IL). MMA was measured by using gas chromatography (Quest Diagnostics, Baltimore).

The association of the proportion of subjects without anemia with age (<60, 60–74, or >74 y) and time period (pre-, peri-, or postfortification) was assessed with the use of multiple logistic regression (8). The 2 independent variables were treated as continuous with 3 equally spaced intervals. The analysis was repeated without the perfortification group to obtain a direct comparison between the pre- and postfortification periods. Odds ratios and 95% CIs were calculated. Kendall's τ was used to evaluate correlations between vitamin B-12 concentrations and hematocrit values or MCVs. The analysis was performed by using SAS (9).

RESULTS

There were 1785 subjects who had a vitamin B-12 concentration <258 pmol/L, and of those, 1573 had hematocrit and MCV measurements performed within the required time frame. The subjects were largely older: their median age was 67 y (interquartile range: 53–75 y). The median age of the subjects did not change significantly over the course of the study, so the expected proportion of subjects with low vitamin B-12 but without anemia was not influenced by a change in the age distribution of the participants (7). Data on race and sex were available for a subset of the subjects. The percentages of African Americans, whites, and others in the study population were 69%, 24%, and 7%, respectively. The percentages of men and women in the study population were 96.1% and 3.9%, respectively.

Fortification was required by 1 January 1998; however, the industry had the option of fortifying anytime after 5 March 1996. Therefore, an increase in the proportion of subjects with low vitamin B-12 concentrations but without anemia could have occurred shortly after fortification began, as soon as folic acid affected red cells maturing in the bone marrow. Of the 1573 subjects with a low vitamin B-12 concentration, the proportion without anemia was 39.2% (275 of 702) before fortification, 45.5% (198 of 435) during the period when fortification was being implemented, and 37.6% (164 of 436) after fortification was completely implemented. The proportion did not change significantly ($P = 0.96$) over the 3 time periods (age-adjusted odds ratio: 1.00; 95% CI: 0.88, 1.13). The data are shown in **Figure 1**.

Because the elderly are at greater risk of having a low vitamin B-12 concentration and consume large amounts of cereal grains (4, 5), we examined the subjects by age group (ie, <60, 60–74, and >74 y) (**Figure 2**). Again, there was no significant increase in the proportion of subjects who had a low vitamin B-12 concentration but normal hematocrit and MCV values over the period when fortification was introduced. The proportion of subjects without

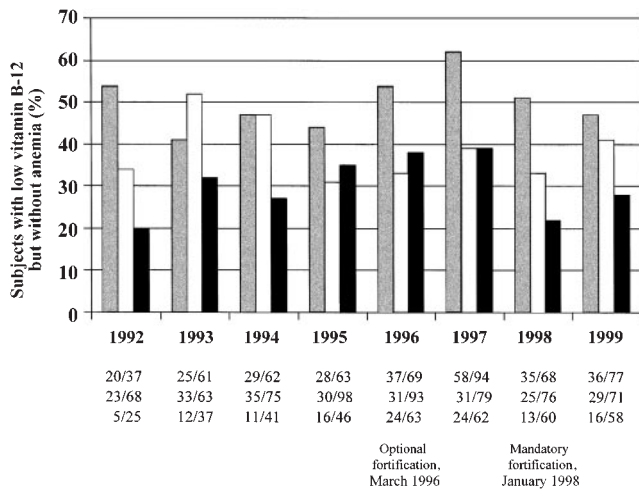


FIGURE 2. Percentages of subjects aged <60 (■), 60–74 (□), and >74 (■) y with low vitamin B-12 concentrations (<258 pmol/L) but without anemia before, during, and after fortification of enriched cereal grains with folic acid. The fractions under the year labels on the x axis indicate, from top to bottom, the number of subjects without anemia/the number of subjects with a low vitamin B-12 concentration among the subjects aged <60, 60–74, and >74 y. The percentage of subjects without anemia was not significantly different between the 3 time periods (ie, before, during, and after fortification). The percentage of subjects without anemia was significantly higher among those aged <60 y than among those in the other 2 age groups ($P < 0.0001$, logistic regression).

anemia was actually higher in the subjects aged <60 y (50.5%, 268 of 531) than in those aged 60–74 y (38.0%, 237 of 623) or >74 y (30.9%, 121 of 392) ($P < 0.0001$, logistic regression).

Because the amount of folic acid in food during the optional fortification period is uncertain, we repeated the analysis by comparing the period before fortification began with that after fortification was complete in order to compare the effects of no fortification of grain with full fortification. Again, there was no significant difference between the proportions of subjects without anemia in the post- and prefortification periods (age-adjusted odds ratio: 0.95; 95% CI: 0.74, 1.22; $P = 0.67$).

The results were unchanged when we changed the cutoff for a low vitamin B-12 concentration to 150 pmol/L. When we examined the group for whom MMA concentrations were measured ($n = 260$) and were high ($n = 123$), the results were unchanged. The proportion without anemia was 30% (9 of 30) before fortification, 32.6% (14 of 43) during the fortification period, and 26% (13 of 50) after fortification. When the 3 age groups were examined separately, the results were also unchanged.

Masking would produce vitamin B-12 abnormalities without anemia, and patients without anemia would be less likely to have vitamin B-12 measurements performed. Therefore, we looked for a drop in the number of subjects who had a complete blood count and vitamin B-12 measurement after fortification. There was none. We also looked for a change in the proportion of subjects with a very low (<75 pmol/L) vitamin B-12 concentration after fortification. Over the pre- and prefortification periods, the proportion of subjects with very low vitamin B-12 concentrations ranged from 0.5% to 2.45%. In 1999, after fortification was complete, the percentage was 1.45%.

To determine whether those who had the lowest vitamin B-12 concentrations were more likely than others to have lower

hematocrit values or higher MCVs, we examined the correlation between vitamin B-12 and both hematocrit and MCV. Vitamin B-12 was significantly correlated with both hematocrit (positive association; $P = 0.0008$) and MCV (negative association; $P = 0.006$), but the correlation coefficients were low ($r = 0.06$ and -0.05 , respectively).

The subjects with an elevated MMA concentration had a significantly ($P = 0.04$) lower mean (\pm SD) vitamin B-12 concentration than did those with a normal MMA concentration (175.3 ± 47.9 compared with 186.7 ± 40.5 pmol/L). The subjects with an elevated MMA concentration were also significantly older (70.0 ± 12.0 compared with 63.6 ± 13.3 y, $P < 0.0001$). Of the 260 subjects who had both a vitamin B-12 concentration <258 pmol/L and MMA measurements, 123 (47%) had an elevated MMA concentration, a known metabolic abnormality in vitamin B-12 function.

DISCUSSION

Our data show that among subjects cared for at a large Veterans Affairs facility, the proportion with low vitamin B-12 concentrations but without anemia has not changed significantly since fortification of enriched cereal grains was mandated by the USFDA. The elderly, a higher risk group because of their higher consumption of cereal grain and higher rates of low vitamin B-12, also showed no increase in low vitamin B-12 without anemia. These data suggest that the amount of folic acid currently being added to food is not causing a major increase in masking of vitamin B-12 deficiency anemia.

It has been shown that the actual amount of folic acid being added to food is $\geq 50\%$ more than that called for (140 $\mu\text{g}/100$ g grain) in the USFDA regulations (6). Thus, it is likely that American consumers are exposed to higher amounts than the USFDA originally estimated. Furthermore, it is likely that many consumers are exposed to >1000 μg folic acid/d, the amount the USFDA considers the safe upper limit (6). It has been shown that blood folate concentrations have risen dramatically since fortification began (10, 11). Therefore, it is particularly important to determine rates of low vitamin B-12 without anemia. Our results provide important reassurance that high folic acid exposure has not resulted in an increase in the proportion of patients, including elderly patients, with a low vitamin B-12 concentration but with normal hematocrit and MCV values.


Almost one-half of the subjects (40.5%) in our population were not anemic at the initial diagnosis of low vitamin B-12. Although this fraction is lower than that reported in elderly populations (7, 12, 13), it illustrates the need to consider a diagnosis of vitamin B-12 deficiency in elderly patients with neurologic signs and symptoms suggesting such a diagnosis regardless of their hematologic status. Our data also show that the subjects aged <60 y were much more likely than were the older subjects to have a low vitamin B-12 concentration without anemia ($P < 0.0001$). This is a concern because clinicians may be less likely to consider a diagnosis of vitamin B-12 deficiency in younger patients and could therefore miss the diagnosis if anemia is absent.

This study has some limitations. The population seeking care at the study facility could have changed over the course of the study. There were, however, no major changes in the catchment area, the type of patients seen, or the facilities served. Similarly, patterns of ordering vitamin B-12 tests could have changed; however, the suggested practice patterns for staff did not change. Although the

geriatric service screened patients yearly, other services did not. Therefore, it is very likely that some patients with a low vitamin B-12 concentration were not identified for study. We did not have access to detailed neurologic data on the subjects. In a sample of patients, we looked at diagnostic codes for neurologic conditions from the ninth revision of the *International Classification of Diseases* (14) but determined that the diagnoses were not specific enough to differentiate between vitamin B-12-related neurologic problems and other common neurologic problems in older populations. Our diagnoses were based only on vitamin B-12 concentrations for most of the subjects; however, the results were unchanged when we repeated the analysis on the subset of subjects who had an elevated MMA concentration. Similarly, making the diagnosis of low vitamin B-12 more stringent by lowering the vitamin B-12 concentration required to qualify to 150 pmol/L did not change the results.

Our subjects were mainly African American and thus were not a representative sample of the US population. Compared with other ethnic groups, African Americans may be more susceptible to pernicious anemia and less likely to have a low vitamin B-12 concentration. Therefore, the African American population would be at high risk for masking and thus would be a good choice for investigation.

The major strengths of the present study include the large number of subjects available for investigation and the stable referral pattern. In addition, the laboratory methods were consistent throughout the study period, reducing the chance that changes in laboratory results could occur for technical reasons.

In conclusion, this study showed that subjects with a low vitamin B-12 concentration were no more likely to be without anemia after food fortification with folic acid began than they were before fortification began. Masking of vitamin B-12 deficiency, ie, deficiency without anemia because of folic acid, does not appear to be increasing as a result of food fortification with folic acid, despite the current high exposures. Diagnosing vitamin B-12 deficiency in the absence of anemia can be very difficult because it depends on the recognition of sometimes subtle neurologic abnormalities. Therefore, it is encouraging that the proportion of patients with low vitamin B-12 concentrations but without anemia has not increased since fortification of grain with folic acid began. 

JLM was the primary designer of the study, oversaw the conduct of the study, and was the main author of the manuscript. IVK assisted in the design of the study and worked on the collection, editing, and analysis of data. MRC was responsible for data editing and analysis and assisted in preparing the man-

uscript. JAZ and REW searched the database, extracted relevant data, and assisted in the study design and analysis. CC conducted the statistical analysis and assisted in interpreting and writing the results. DRD worked on the study design, initial data collection, and analysis of the data. None of the authors had any conflict of interest.

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