

Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring Study¹⁻³

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ABSTRACT

Background: Low vitamin B-12 status is prevalent among the elderly, but few studies have examined the association between vitamin B-12 status and intake.

Objective: We hypothesized that vitamin B-12 concentrations vary according to intake source.

Design: Plasma concentrations and dietary intakes were assessed cross-sectionally for 2999 subjects in the Framingham Offspring Study. The prevalence of vitamin B-12 concentrations <148, 185, and 258 pmol/L was examined by age group (26–49, 50–64, and 65–83 y), supplement use, and the following food intake sources: fortified breakfast cereal, dairy products, and meat.

Results: Thirty-nine percent of subjects had plasma vitamin B-12 concentrations <258 pmol/L, 17% had concentrations <185 pmol/L, and 9% had concentrations <148 pmol/L, with little difference between age groups. Supplement users were significantly less likely than non-supplement-users to have concentrations <185 pmol/L (8% compared with 20%, respectively). Among non-supplement-users, there were significant differences between those who consumed fortified cereal >4 times/wk (12%) and those who consumed no fortified cereal (23%) and between those in the highest and those in the lowest tertile of dairy intake (13% compared with 24%, respectively), but no significant differences by meat tertile. Regression of plasma vitamin B-12 on log of intake, by source, yielded significant slopes for each contributor adjusted for the others. For the total group, $b = 40.6$ for vitamin B-12 from vitamin supplements. Among non-supplement-users, $b = 56.4$ for dairy products, 35.2 for cereal, and 16.7 for meat. Only the meat slope differed significantly from the others.

Conclusions: In contrast with previous reports, plasma vitamin B-12 concentrations were associated with vitamin B-12 intake. Use of supplements, fortified cereal, and milk appears to protect against lower concentrations. Further research is needed to investigate possible differences in bioavailability. *Am J Clin Nutr* 2000;71:514–22.

KEY WORDS Vitamin B-12, cobalamin, vitamin supplements, breakfast cereal, dairy products, Framingham Offspring Study, elderly

INTRODUCTION

Vitamin B-12 deficiency is associated with both megaloblastic anemia and neurologic manifestations, including vibratory

sensory loss, ataxia, paresthesia, and cognitive and mood changes. The observation of vitamin B-12-related neurologic signs and symptoms in patients with vitamin B-12 concentrations within the range formerly considered low normal—and without associated anemia or macrocytosis—makes the identification of vitamin B-12 deficiency all the more important (1–4). There is also some concern that the recent fortification of the food supply with folic acid may augment the risk of undiagnosed progressive neurologic damage as a result of unidentified vitamin B-12 deficiency, which can be masked by the partial hematologic response to folate. In addition to potentially masking the anemia associated with vitamin B-12 deficiency, exposure to high concentrations of folic acid may even precipitate or worsen the progression of vitamin B-12-related neurologic effects. Contrary to expectations, some investigators found that the severity of vitamin B-12-related neurologic symptoms was greater when subjects had no anemia (4, 5).

There is also evidence that vitamin B-12 deficiency among the elderly may be more prevalent than previously thought (6–8). Loss of stomach acidity with aging, resulting from type B atrophic gastritis, has been implicated in impaired vitamin B-12 status (9). Atrophic gastritis may affect up to 40% of elders and is associated with impaired absorption of protein-bound vitamin B-12; however, unbound vitamin B-12 found in vitamin supplements is often better absorbed (10, 11). These observations contributed to the recently released dietary reference intakes by the Food and Nutrition Board (12). The new recommended intake of vitamin B-12 for adults was increased to 2.4 $\mu\text{g}/\text{d}$ from a 1989 recommended dietary allowance of 2.0 $\mu\text{g}/\text{d}$ (13), with the added recommendation for those older than 50 y that most of this come from supplements or fortified foods. A recent study of 173 elders found no association between dietary intake and

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serum cobalamin status (14). The investigators concluded that “the high frequency of mildly abnormal cobalamin status in the elderly cannot be attributed to poor intake of cobalamin” and that “nondietary explanations...must always be sought.” A recent study of 105 Dutch elders concluded that severe atrophic gastritis explained only 25% of the cases of low vitamin B-12 status (8).

Most studies of vitamin B-12 have focused on elders. In this study, we estimated the prevalence of plasma vitamin B-12 concentrations below specified cutoffs for 2999 subjects in the Framingham Offspring Study aged 26–83 y old, and explored associations between vitamin B-12 intake and plasma concentrations. We hypothesized that the prevalence of low concentrations would increase with age and that intake from supplements and fortified breakfast cereal would be more protective of vitamin B-12 concentrations than would intake from other food sources.

SUBJECTS AND METHODS

Subjects

Subjects in this study were members of the Framingham Offspring Cohort. These are the children (and their spouses) of the original Framingham Study Cohort, a prospective study that began in 1948 to examine risk factors for heart disease (15). The offspring study began with 5135 participants in 1971 and subjects are examined every 4 y. There were 3799 participants in the fifth examination cycle, which covered 1991–1995. Plasma vitamin B-12 concentrations and valid food-frequency questionnaires were both available for the 2999 subjects (1564 women and 1435 men) included in this analysis. Nine hundred eighty-four of these subjects were aged 26–49 y, 1460 were aged 50–64 y, and 555 were aged ≥ 65 y. The protocol for this study was approved by the Institutional Review Board for Human Research at Boston University.

Plasma vitamin B-12 concentrations

Blood was drawn from subjects during the fifth examination cycle and was stored at -80°C . Plasma vitamin B-12 concentrations were measured by using the Biorad Quantaphase II radioassay (Hercules, CA). Pooled plasma was used for quality control. There is currently no clearly accepted cutoff for vitamin B-12 deficiency. A commonly used clinical cutoff for low vitamin B-12 status is 148 pmol/L (200 pg/mL) (16). However, there is evidence that the sensitivity of this clinical cutoff is poor and that many individuals with what was previously labeled low-normal status have clinical symptoms (2, 3). Lindenbaum et al (2) found responsive symptoms in individuals with plasma concentrations as high as 258 pmol/L (350 pg/mL). We therefore used 3 descriptive cutoffs: 148 pmol/L (the current clinical cutoff), 258 pmol/L (a point at which individuals may be at risk of deficiency, although further testing is needed), and 185 pmol/L (250 pg/mL; an intermediate point). In the absence of additional metabolic or clinical indicators, 185 pmol/L may be the closest estimate of the prevalence of vitamin B-12 deficiency, although some truly deficient individuals may have higher concentrations and some individuals with lower concentrations may not actually be deficient. Individuals with concentrations < 185 pmol/L have been shown to be significantly more likely to have elevated methylmalonic acid concentrations, a metabolic indicator of vitamin B-12 deficiency, than those with concentrations above this cutoff (6).

Dietary intake

Usual dietary intake was assessed during the fifth examination by using a semiquantitative, 126-item food-frequency questionnaire (17, 18). The questionnaires were mailed to the subjects before the examination and the subjects were asked to complete them and bring them to their appointments. The questionnaire also included questions about the use of vitamin supplements and the type of breakfast cereal most frequently consumed. The forms were processed at Harvard University to obtain total nutrient intake and food contributions to nutrient intake. This food-frequency questionnaire has been validated for many nutrients and in several populations (17–19). The correlation between vitamin B-12 intake from the questionnaire and multiple diet records has been reported to be 0.56 (17); that with plasma vitamin B-12 concentrations has been reported to be 0.35 (19). Questionnaires resulting in energy intakes < 2.51 MJ/d (600 kcal/d) or > 16.74 – 17.57 MJ/d (4000–4200 kcal/d) for women and men, respectively, or with ≥ 12 food items left blank (a total of 151 of 3150 forms) were considered invalid and excluded from further analysis.

To calculate vitamin B-12 intake from individual food sources, we analyzed the food contributions to total vitamin B-12 intake for each subject. Total intake was divided into vitamin B-12 intake from supplements; breakfast cereal; meat, poultry, and fish; dairy sources; and all other foods.

Statistical analysis

All statistical analyses were performed with SAS (version 6.12; SAS Institute Inc, Cary, NC). The prevalences of plasma vitamin B-12 concentrations below the 3 cutoffs were estimated. Major food sources were identified and ranked for subjects above and below the 185- and 148-pmol/L cutoffs. Prevalence was estimated for men and women separately and for subjects by age category (26–49, 50–64, and 65–83 y). The effects of supplement use, breakfast cereal use, and intake (by tertiles) of vitamin B-12 from meat, poultry, and fish or dairy sources were considered. Tests for significant differences in mean intake and cobalamin concentration across age group, sex, and intake source categories were completed with the general linear models procedure in SAS. Similarly, significance testing across groups for differences in prevalence estimates were done with logistic regression.

Mean (\pm SE) plasma vitamin B-12 concentrations were estimated for each decile of vitamin B-12 intake, first for total intake for the entire sample and then for total intake for non-supplement-users only. These least-squares means were obtained by using the general linear models procedure in SAS, with adjustment for age, sex, alcohol use, and total energy intake. These were also repeated for men and women separately and for subjects by age group. Mean plasma vitamin B-12 concentrations per intake decile were plotted against the median intake (in μg vitamin B-12/d) for each corresponding intake decile group. Similar plots were made with the mean plasma vitamin B-12 concentration associated with the median of each quintile of intake from specific sources, including supplements, breakfast cereal, and other foods. Further breakdowns were plotted for non-supplement-users for breakfast cereal, meat, and dairy foods. For these source-specific quintile analyses, the same set of adjustment variables was used, with the addition of vitamin B-12 intake from sources other than the one being examined. Tests for trend were made by regressing the plasma

concentration on the log of intake, both overall and by source, with the set of adjustment variables described above.

To compare the association between vitamin B-12 intake patterns and concentrations more directly, we also grouped individuals into patterns derived from cluster analysis by using the FASTCLUS procedure in SAS. Food groups that contributed vitamin B-12 were entered into the analysis as percentages of total individual vitamin B-12 intake. The cluster procedure assigns individuals to predetermined numbers of clusters in a manner that maximizes the difference across groups for the included variables. This allows more direct examination of differential intake for different groups of individuals, without the need for statistical adjustment for other sources, and therefore provides information on the potential effect of different intake patterns.

RESULTS

Vitamin B-12 concentrations

The subjects' mean vitamin B-12 intake ($\mu\text{g}/\text{d}$) and plasma concentrations and the percentage of subjects with concentrations <258 pmol/L (350 pg/mL), <185 pmol/L (250 pg/mL), and <148 pmol/L (200 pg/mL) are presented in **Table 1**. The mean plasma vitamin B-12 concentration for these 2999 adults was 329 pmol/L. Although there was a significant linear trend toward lower concentrations with increasing age ($P < 0.05$ with linear regression), the categorical tests for differences across age groups were not significant. Thirty-nine percent of subjects had plasma vitamin B-12 concentrations <258 pmol/L, 17% had concentrations <185 pmol/L, and 9% had concentrations <148 pmol/L. About 16% of 26–49-y-olds and of 50–64-y-olds had vitamin B-12 concentrations <185 pmol/L, compared with 17% of 65–83-y-olds (NS). Similarly, differences by age or sex were not significant for the proportions of subjects with vitamin B-12 concentrations below the other 2 cutoffs.

A comparison of major intake sources of vitamin B-12 by plasma vitamin B-12 status ($<$ compared with ≥ 185 and 148 pmol/L) showed significant differences in intake patterns (**Table 2**). Patterns were similar for categorization across both cutoffs. Although all groups got more of their vitamin B-12 from meat than from any other source, those with lower vitamin B-12 concentrations obtained significantly more of their vitamin B-12 intake from meat than did those with higher concentrations. The group with vitamin B-12 concentrations below either cutoff also

obtained significantly more of their intake from fish, soups, sandwiches, poultry, and pizza than did the group with higher plasma concentrations, who obtained significantly more from supplements, milk, and breakfast cereal.

Significant differences in prevalence were also seen with different specific intake sources (**Table 3**). Twenty-eight percent of the subjects took supplements containing vitamin B-12. Among these supplement users, the prevalence of vitamin B-12 concentrations <185 pmol/L (and <148 pmol/L, respectively) was 8% (4%), compared with 20% (10%) among non-supplement-users ($P < 0.0001$ for both comparisons). Similar numbers of subjects in each age group used supplements of similar average amounts, and further examination of prevalence patterns by supplement use within an age group did not show consistent differences from the total group (data not shown).

Another source of added vitamin B-12 to the diet was fortified breakfast cereal. Among non-supplement-users, those consuming cereal >4 times/wk, at least some of which was fortified with vitamin B-12, had significantly higher plasma vitamin B-12 concentrations than did those who ate no cereal (Table 3). In the total group, 44% of subjects reported consuming some type of breakfast cereal containing vitamin B-12. The 41% of subjects who neither took supplements nor consumed fortified breakfast cereal had the highest prevalence of vitamin B-12 concentrations <185 pmol/L: 23%. In contrast, only 12% of non-supplement-users who consumed cereal >4 times/wk and 17% who consumed cereal sometimes, but <4 times/wk, had vitamin B-12 concentrations below this cutoff. Cereal consumers also were significantly less likely to have plasma concentrations <148 pmol/L than were those who ate no cereal.

Vitamin intake from other foods was also associated with plasma concentrations in non-supplement-users, but different patterns were seen with dairy and meat (including meat, poultry, and fish) sources. The group in the highest tertile of vitamin B-12 intake from dairy foods consumed about twice the average total vitamin B-12 as did the group in the lowest tertile, with consistent differences in plasma concentrations and proportions of subjects with vitamin B-12 concentrations <185 and <148 pmol/L (Table 3). In contrast, the group in the highest tertile of vitamin B-12 intake from meat consumed almost 3 times the vitamin B-12 as did the group in the lowest tertile, yet the differences in plasma concentrations across tertiles were lower than for other sources and the proportions of subjects with vitamin B-12 concentrations below the cutoffs did not differ significantly.

TABLE 1

Subjects' mean vitamin B-12 intake and plasma concentration and percentage of subjects with plasma concentrations below defined cutoffs, by sex and age¹

	Vitamin B-12 intake	Plasma concentration	Percentage < 258 pmol/L	Percentage < 185 pmol/L	Percentage < 148 pmol/L
	μg	pmol/L^2	%	%	%
All subjects ($n = 2999$)	8.7 ± 0.3^3	329.5 ± 3.1	38.5	16.5	8.6
Men ($n = 1435$)	9.1 ± 0.4	326.5 ± 4.3	37.8	16.0	8.2
Women ($n = 1564$)	8.4 ± 0.3	332.3 ± 4.5	39.1	16.9	9.0
Aged 26–49 y ($n = 984$)	8.9 ± 0.3	336.0 ± 5.5	36.7	16.3	8.3
Aged 50–64 y ($n = 1460$)	8.7 ± 0.3	328.0 ± 4.5	39.0	16.4	8.8
Aged 65–83 y ($n = 555$)	8.5 ± 0.4	321.9 ± 7.0	40.2	17.1	8.7

¹There were no statistically significant differences by age or sex.

²To convert from pmol/L to pg/mL, multiply by 1.3514.

³ $\bar{x} \pm \text{SE}$.

TABLE 2

Sources of vitamin B-12 intake by plasma vitamin B-12 status

Food	≥185	<185	≥148	<148
	pmol/L	pmol/L	pmol/L	pmol/L
Meat (%) ¹	17.7 ²	21.1	18.0 ³	20.9
Supplements (%) ¹	16.1 ²	6.7	15.2 ³	6.9
Milk (%) ¹	11.8 ²	10.6	11.8 ³	10.0
Fish (%) ¹	11.4 ²	13.8	11.7	13.0
Soups (%) ¹	7.9 ²	9.8	8.1 ³	10.0
Cereal (%) ¹	7.0 ²	5.6	6.9 ³	5.0
Liver (%) ¹	5.1	4.0	4.9	5.2
Sandwiches (%) ¹	4.6 ²	5.8	4.8	5.1
Poultry (%) ¹	2.9 ²	3.7	3.0 ³	3.6
Pizza (%) ¹	2.7 ²	3.5	2.8 ³	3.8
Total vitamin B-12 intake (μg)	9.2 ²	6.3	9.0 ³	5.7

¹Percentage contribution to total intake. Average actual intake can be calculated by multiplying each percentage by the total group intake.

²Significantly different from <185 pmol/L, $P \leq 0.05$ (t test).

³Significantly different from <148 pmol/L, $P \leq 0.05$ (t test).

Association between intake and plasma concentrations of vitamin B-12

The apparent protective effect of supplements, cereal, or both may have been due to 2 factors. First, these additional sources may have increased the total intake of vitamin B-12; second, the form of vitamin B-12 in supplements and in cereal, and perhaps in dairy foods, may be more bioavailable than the vitamin B-12 in other foods. The relation between vitamin B-12 intake from all sources and plasma vitamin B-12 is presented in **Figure 1** for the total group and for the non-supplement-users. The mean plasma value for each decile group of vitamin B-12 intake was plotted against the mean intake for each respective decile group. The curve for the total group shows a clear and strong increase in plasma vitamin B-12 concentration with

greater intake through ≈ 10 μg/d, at which point the curve appears to level off. A comparison of mean plasma concentrations between those consuming 3–9 μg vitamin B-12/d from supplements and those consuming 10–30 μg/d from supplements was not significant, consistent with the leveling of the association seen in **Figure 1**.

Regression of plasma vitamin B-12 on the log of vitamin B-12 intake (transformed to improve linearity), with adjustment for age, sex, alcohol use, and total energy intake, yielded a β coefficient of 65.5 pmol/L per unit log vitamin B-12 intake ($P < 0.0001$). This can be interpreted as an increase in plasma vitamin B-12 concentration of 45.4 pmol/L for each doubling of vitamin B-12 intake. Age was negatively associated with plasma vitamin B-12 concentrations in this model ($P < 0.05$). In addition, alcohol intake was also negatively associated with plasma vitamin B-12 concentrations ($P < 0.01$).

The curve for non-supplement-users in **Figure 1** also shows a clear increase in plasma concentrations with greater intake, again through ≈ 10 μg vitamin B-12/d, after which the curve levels off. The coefficient from the regression of the plasma concentrations on the log of vitamin B-12 intake remained highly significant in this group of non-supplement-users [49.1 μmol/L per unit log vitamin B-12 intake ($P = 0.0001$), or a change of 34 pmol/L for each doubling of intake].

Because we expected to see a decrease in absorption of vitamin B-12 from food with age, we examined the relation shown in **Figure 1** for each of the 3 age groups. In contrast with our expectations, we found only a trend toward lower associations between intake and plasma concentrations with age. There were no significant differences in these associations by age for either the total group or for the non-supplement-users. These associations for non-supplement-users are shown in **Figure 2**.

Shown in **Figures 3** and **4** are plots of the mean plasma vitamin B-12 concentrations of subjects in each quintile of intake from particular sources. This analysis was performed to examine whether there were differences in the association between

TABLE 3Subjects' mean vitamin B-12 intake and plasma concentration and percentage of subjects with plasma concentrations below defined cutoffs, by intake source¹

	Vitamin B-12 intake	Plasma concentration	Percentage <185 pmol/L	Percentage <148 pmol/L
	μg	pmol/L ²	%	%
Supplement users ($n = 841$)	15.3 ± 0.6 ^{3,4}	392.8 ± 6.6 ⁴	8.1 ⁴	3.9 ⁴
Non-supplement-users ($n = 2156$)	6.2 ± 0.2	304.8 ± 3.4	19.7	10.4
Cereal consumption				
>4 times/wk ($n = 302$)	7.9 ± 0.2 ⁵	354.4 ± 10.0 ⁴	12.3 ⁴	5.6 ⁵
>0 to 4 times/wk ($n = 635$)	6.7 ± 0.5	306.9 ± 5.9	17.3 ⁶	8.0 ⁵
None ($n = 1219$)	5.5 ± 0.3	276.5 ± 3.1	22.9	12.9
Dairy				
Upper tertile ($n = 718$)	8.2 ± 0.7 ⁷	339.1 ± 6.4 ⁷	13.4 ⁷	6.8 ⁷
Middle tertile ($n = 721$)	5.7 ± 0.2	300.0 ± 5.5 ⁸	21.2	10.4
Lowest tertile ($n = 719$)	4.5 ± 0.1	291.5 ± 5.2	24.5	14.1
Meat				
Upper tertile ($n = 718$)	10.1 ± 0.7 ⁷	316.0 ± 6.3 ⁸	17.4	8.5
Middle tertile ($n = 722$)	4.8 ± 0.1	305.3 ± 5.6	20.4	11.4
Lowest tertile ($n = 718$)	3.6 ± 0.1	293.2 ± 5.5	21.5	11.4

¹Cereal data were missing for 2 subjects. Numerical mean values presented are unadjusted and tests were assessed with Bonferroni adjustment for multiple comparisons.

²To convert from pmol/L to pg/mL, multiply by 1.3514.

³ $\bar{x} \pm SE$.

⁴⁻⁶Significantly different from no cereal consumed (adjusted for age and sex): ⁴ $P < 0.001$, ⁵ $P < 0.01$, ⁶ $P < 0.05$.

^{7,8}Significantly different from lowest tertile (adjusted for age and sex): ⁷ $P < 0.001$, ⁸ $P < 0.05$.

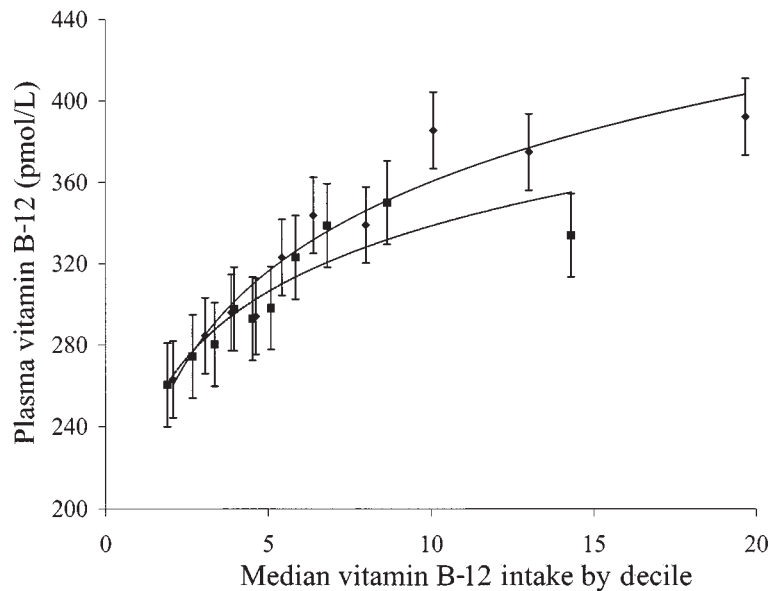


FIGURE 1. Relation between vitamin B-12 intake and plasma concentrations for the total population (◆) and for non-supplement-users (■). Mean (\pm SE) plasma concentrations, adjusted for age and sex, are plotted against the median intake for each intake decile group.

plasma concentrations and intake that suggested possible differences in bioavailability by source. The plots for the total group include vitamin B-12 intake from supplements, breakfast cereal, and other foods (Figure 3). The relation between plasma concentrations and intake appeared strongest for supplements and cereal and weaker for other foods. Linear regression of plasma vitamin B-12 concentrations on the log of intake from each of these 3 sources simultaneously, with adjustment for age, sex, body mass index, smoking, alcohol use, and total energy intake

resulted in significant coefficients for supplements ($\beta = 40.6$, $P < 0.0001$), cereal ($\beta = 34.1$, $P < 0.0001$), and other food sources ($\beta = 27.0$, $P = 0.0002$). These can be interpreted as increases in plasma concentrations of 28.1, 23.6, and 18.7 pmol/L, respectively, for each doubling of vitamin B-12 intake. A comparison of these slopes did not show significant differences.

Among non-supplement-users, plots were included for intake from breakfast cereal, meat, and dairy sources (Figure 4). These show that the relation between plasma concentrations and intake

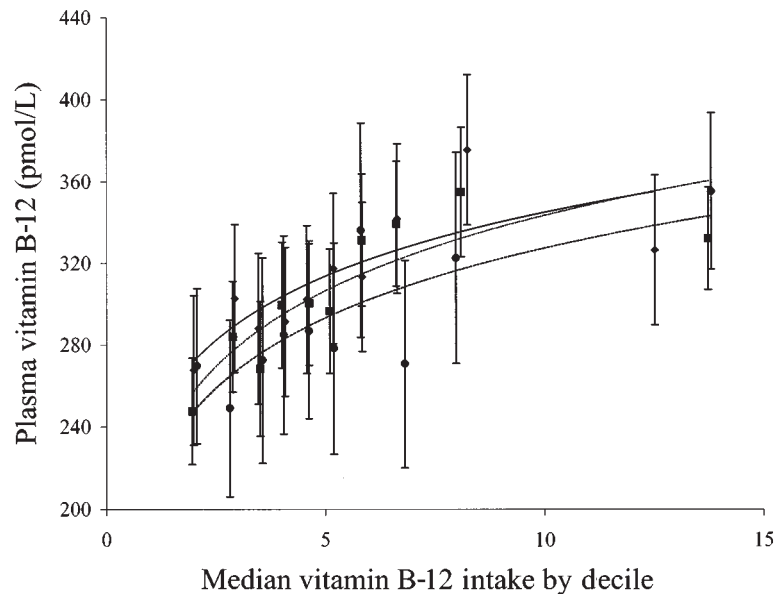


FIGURE 2. Relation between vitamin B-12 intake and plasma concentrations for non-supplement-users, by age group: ◆, 26–49 y; ■, 50–64 y; and ●, ≥ 65 y. Mean (\pm SE) plasma concentrations, adjusted for age and sex, are plotted against the median intake for each intake decile group.

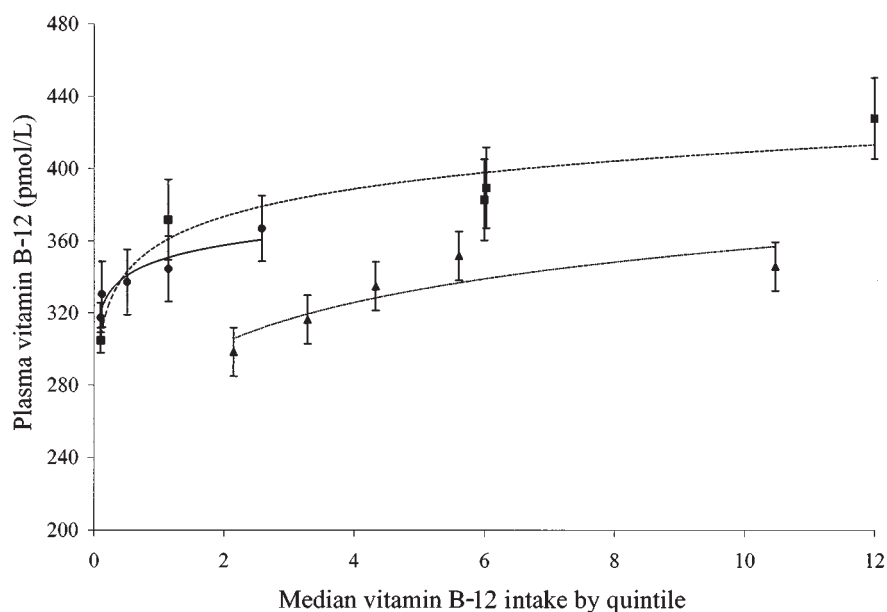


FIGURE 3. Relation between vitamin B-12 intake and plasma concentrations by intake source: ●, breakfast cereal; ■, supplements; and ▲, other foods. Mean (\pm SE) plasma concentrations, adjusted for age, sex, and other sources of vitamin B-12, are plotted against the median intake for each intake quintile group.

from dairy products closely resembled that between plasma concentrations and the added vitamin B-12 in cereal, whereas the relation for meat appeared to be weaker. Regression coefficients for the log of each intake source were significant after adjustment for all other sources and for age, sex, alcohol use, and total energy intake. The coefficients were 35.2 for cereal, 56.4 for

dairy (each $P = 0.0001$), and 16.7 for meat, poultry, and fish ($P < 0.05$) sources, showing increases in plasma vitamin B-12 concentration of 24.4, 39.1, and 11.6 pmol/L, respectively, for each doubling in vitamin B-12 intake. The slopes for cereal and dairy sources did not differ significantly, but that for meat was significantly lower than the others.

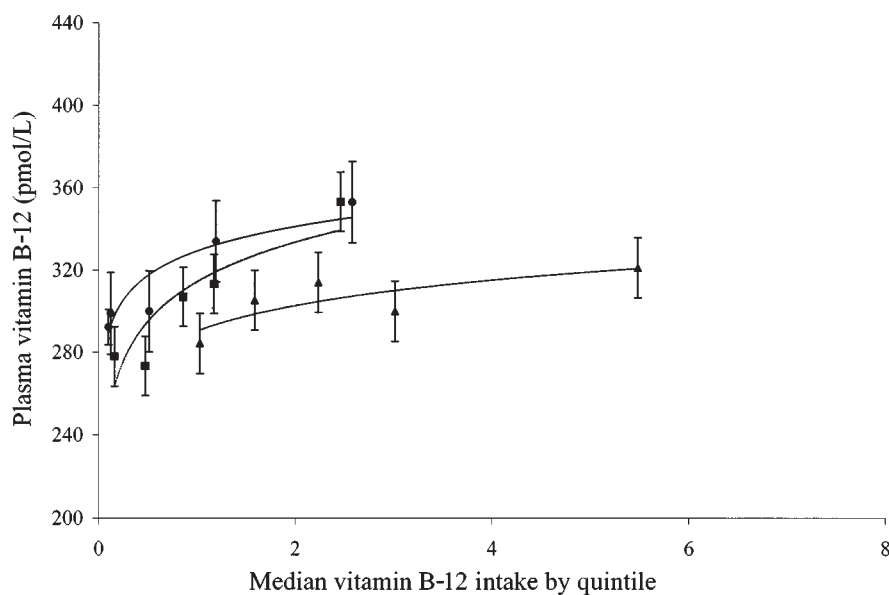


FIGURE 4. Relation between vitamin B-12 intake and plasma concentrations among non-supplement-users by intake source: ●, breakfast cereal; ■, dairy foods; and ▲, meat, poultry, and fish. Mean (\pm SE) plasma concentrations, adjusted for age, sex, and other sources of vitamin B-12, are plotted against the median intake for each intake quintile group.

Finally, the results of the cluster analysis are presented in **Table 4**. The solution with 6 groups led to the clearest separation of vitamin B-12 sources, resulting in groups of subjects with the following predominant sources of vitamin B-12: group 1, supplements; group 2, meat; group 3, milk; group 4, cereal; group 5, meat and soups; and group 6, fish. Plasma concentrations were significantly lower in the meat group than in the cereal and milk groups, despite similar average vitamin B-12 intakes in these 3 groups. Subjects in all food intake groups were significantly more likely to have plasma vitamin B-12 concentrations <185 pmol/L than were subjects in the supplement group, with odds ratios ranging from 1.6 for the milk group to 2.4 for the meat group. The meat group was the only group that differed significantly from the supplement group for likelihood of plasma vitamin B-12 concentrations <148 pmol/L (odds ratio = 2.0; 95% CI: 1.2, 3.3).

DISCUSSION

These results have several important implications. More than 8% of these generally healthy adults had plasma vitamin B-12 concentrations <148 pmol/L; 16% had concentrations <185 pmol/L and 39% had concentrations <258 pmol/L. Because persons with cobalamin concentrations as high as 258 pmol/L have been shown to be at some risk for neurologic signs and symptoms of vitamin B-12 deficiency and for hyperhomocysteinemia (1–4, 6), these prevalences raise concerns about the need for improved diagnosis and treatment of vitamin B-12 deficiency in the general adult population.

High prevalences of vitamin B-12 deficiency were documented previously in elders. Using a combination of serum concentrations and functional metabolite measures, Lindenbaum et al (6) reported a 12% prevalence of vitamin B-12 deficiency among elderly participants in the Framingham Study (the parents of those examined here), although only 5.3% had serum concentrations below the clinical cutoff of 148 pmol/L. A recent study of Dutch elders found 25% with plasma cobalamin concentrations <150 pmol/L (8). When a higher cutoff (<260 pmol/L) was

used in conjunction with methylmalonic acid concentrations (>0.32 $\mu\text{mol/L}$), 24% of the Dutch elders were found to be at least mildly deficient.

We had hypothesized that vitamin B-12 concentrations would vary by age group on the basis of previous observations that absorption tends to decline with age (11). Although we did see a significant negative linear association between plasma concentrations and age, the prevalence of vitamin B-12 concentrations did not differ significantly by age group in this population for any of the cutoffs examined. This result differs from an earlier finding that the Framingham elders (aged 67–96 y) were twice as likely as young control subjects to have serum cobalamin concentrations <258 pmol/L (6). Similarly, vitamin B-12 concentrations <185 pmol/L were reported for 6% of adults aged 20–59 y and for 11% of those aged ≥ 60 y in recent analyses from the third National Health and Nutrition Examination Survey (20). It is possible that, despite our finding of similar prevalences of plasma vitamin B-12 concentrations below specified cutoffs across age groups, there may be more functional consequences at older ages. Joosten et al (7) also reported similar serum vitamin B-12 distributions for young and older adults, but reported that older subjects had higher concentrations of homocysteine and methylmalonic acid—functional indicators of vitamin B-12 status.

In contrast with most earlier studies, we showed that vitamin B-12 concentrations were significantly associated with total vitamin B-12 intake in analyses that both included and excluded supplement users. One recent study compared the dietary intakes of 95 elders with low cobalamin status with those of 78 elders with normal cobalamin status and found no significant difference in intake by group and no correlation between total intake and either cobalamin or metabolite concentrations (14). van Asselt et al (8) found that plasma cobalamin correlated with total cobalamin intake ($r = 0.36$) in Dutch elders, but that mean intakes did not differ significantly by cobalamin status group.

In addition to a correlation between total dietary intake and plasma vitamin B-12 concentrations, we saw striking differences in the prevalence of vitamin B-12 concentrations below the

TABLE 4

Subjects' mean vitamin B-12 intake and plasma concentration and percentage of subjects with plasma concentrations below defined cutoffs, by intake pattern group¹

Intake pattern	Vitamin B-12 intake <i>μg</i>	Plasma concentration <i>pmol/L</i>	Percentage < 185 pmol/L [OR (95% CI)] ²	Percentage < 148 pmol/L [OR (95% CI)] ²
	<i>μg</i>	<i>pmol/L</i>	%	%
Supplements 61%, meat 11% ($n = 648$)	16.3 \pm 0.8 ^{d,3}	398.1 \pm 7.8 ^c	7.9 [1.0]	4.2 [1.0]
Meat 57% ($n = 740$)	8.6 \pm 0.3 ^c	304.1 \pm 5.9 ^a	19.6 ⁴ [2.4 (1.7, 3.4)]	11.4 ⁵ [2.0 (1.2, 3.3)]
Milk 38%, meat 21%, fish 10% ($n = 361$)	7.0 \pm 1.3 ^c	336.4 \pm 8.2 ^b	15.8 ⁶ [1.6 (1.1, 2.5)]	6.9 [1.0 (0.5, 1.8)]
Cereal 37%, meat 17%, milk 12% ($n = 316$)	6.9 \pm 0.2 ^{b,c}	348.2 \pm 9.9 ^b	16.1 ⁵ [1.8 (1.2, 2.8)]	5.7 [0.9 (0.5, 1.7)]
Meat 25%, soups 24%, fish 12%, milk 10% ($n = 592$)	5.4 \pm 0.1 ^{a,b}	291.9 \pm 5.8 ^a	20.3 ⁴ [2.2 (1.5, 3.3)]	10.6 [1.6 (0.9, 2.6)]
Fish 35%, meat 21%, other dairy 10% ($n = 342$)	3.9 \pm 0.1 ^a	294.9 \pm 8.1 ^a	20.5 ⁵ [2.1 (1.4, 3.3)]	12.0 [1.6 (0.9, 2.8)]

¹Major percentage sources of vitamin B-12 intake, separated by cluster analysis; foods listed contributed >10% of intake. Means within a column with different superscript letters are significantly different, $P < 0.05$ (after adjustment for age, sex, total energy intake, and, for blood concentrations, total vitamin B-12 intake). Numerical mean values presented are unadjusted.

²Odds ratio and 95% CIs, comparing each group to the high supplement intake group, adjusted for age, sex, total energy intake, and total vitamin B-12 intake.

³ $\bar{x} \pm \text{SE}$.

⁴⁻⁶Significantly different from high supplement intake group: ⁴ $P < 0.0001$, ⁵ $P < 0.01$, ⁶ $P < 0.05$.

cutoffs by predominant intake source. The prevalence of vitamin B-12 concentrations below each of the cutoffs among non-supplement-users was approximately twice that among supplement users. These findings support the protective effect of vitamin B-12 supplements that was reported previously (8, 21). It is also important to note that among supplement users, those consuming ≈ 6 μg from supplements (the usual dose found in multivitamins) did not have significantly lower plasma concentrations than did those consuming 10–30 μg , suggesting that the standard dose is probably adequate for the general healthy adult population.


We also found that consumption of vitamin-fortified breakfast cereal was strongly related to plasma vitamin B-12 concentrations, with evidence of a dose response. Non-supplement-users who consumed cereal >4 times/wk were half as likely to have vitamin B-12 concentrations below each of the cutoffs as were those who consumed neither supplements nor cereal. These results lend support to the recent recommendation by the National Academy of Sciences that adults aged >50 y obtain most of the recommended intake of vitamin B-12 from supplements or fortified foods, but also raises questions about whether younger adults should consider the addition of these sources to their diet as well.

The plots of vitamin B-12 concentrations by intake quintiles for specific sources were adjusted for the total remaining vitamin B-12 intake from other sources. These plots suggested that the vitamin B-12 in supplements, fortified breakfast cereal, and dairy products may be more efficiently absorbed than the vitamin B-12 in meat, poultry, and fish sources. At least one other study found a stronger association between dairy foods and vitamin B-12 status than between other sources and vitamin B-12 status (22). In that study, 51% of vegetarian adults (aged 21–70 y) had cobalamin concentrations <148 pmol/L and concentrations were significantly greater ($P < 0.01$) when dairy foods, but not when eggs or seafood, were included in the diet (22). Meat, poultry, and seafood are rich sources of vitamin B-12 but unlike most dairy foods are consumed after cooking, which subjects the vitamin to possible heat degradation and loss. More research is needed to determine the bioavailability of vitamin B-12 from specific foods.

This analysis is subject to the limitations associated with self-reported dietary data. Imprecision in dietary reporting may be greater for most foods than for supplement use or for consumption of breakfast cereal. The effect of this error is to flatten slopes and make detection of associations more difficult. However, we have no evidence that meat intake was less accurately reported in this study than was intake of dairy products or breakfast cereal. A further statistical concern is the artifactual effect of statistical adjustment on the y axis placement of the least-squares means, which may have distorted the visual appearance of differences across the intake sources as presented in Figures 3 and 4. In this case, however, adjusted means did not differ greatly from crude means. Finally, although the slopes and statistical comparisons across slopes were valid, the range of intake differed for differing sources and the placement in that range may have affected the slope. These statistical limitations are not present in the cluster comparison, however, and the consistency between results obtained by cluster groupings and regression analysis supports our conclusions.

The high prevalence of relatively low plasma vitamin B-12 concentrations among younger adults has not been reported previously. Measurement error is not a likely explanation for this find-

ing given the strong association between dietary intake and plasma concentration seen in these data. Further confirmation of the prevalence of low vitamin B-12 status and exploration of its consequences for younger adults is needed. Additional factors that are hypothesized to contribute to poor vitamin B-12 status should be further explored; these include *Helicobacter pylori* infection, *Giardia lamblia* infection, exposure to nitrous oxide during surgery (23, 24), and chronic use of medications that suppress gastric acidity or otherwise affect absorption, including cimetidine, metformin, potassium chloride, and cholestyramine (25).

Studies that compared diagnosed signs and symptoms of vitamin B-12 deficiency with blood indicators of deficiency have shown that individual presentation can vary greatly (2–4). On the basis of these reports, several scientists have called for better diagnostic criteria for vitamin B-12 deficiency. The data presented here clearly suggest that inadequate intake is an important contributor to low vitamin B-12 concentrations and that the vitamin B-12 in meat, the major source of vitamin B-12 for most individuals, may be less available than the vitamin B-12 in dairy products. Further work is needed to both better identify those persons with vitamin B-12 deficiency and to clarify the role of contributing causal factors to this apparently prevalent subclinical condition. 

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