Daily intake of 4 to 7 μg dietary vitamin B-12 is associated with steady concentrations of vitamin B-12–related biomarkers in a healthy young population[1–4]

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ABSTRACT

Background: Studies have questioned whether the current Recommended Dietary Allowance (RDA) of 2.4 μg vitamin B-12/d is adequate.

Objective: We examined the association between dietary vitamin B-12 intake and biomarkers of vitamin B-12 status.

Design: Dietary vitamin B-12 intake was estimated, and biomarkers of vitamin B-12 status were measured, in healthy men and women (n = 299; age range: 18–50 y) who were recruited from a Florida community. The National Cancer Institute Diet History Questionnaire was used. Plasma cobalamin, total transcobalamin, holotranscobalamin, methylmalonic acid (MMA), total homocysteine (tHcy), and autoantibodies against intrinsic factor (IF) and *Helicobacter pylori* were analyzed in blood samples.

Results: Antibodies to *H. pylori* were detected in 12% of subjects (35/299), and negative results for IF antibodies were obtained for all subjects. The intake of vitamin B-12 correlated significantly with cobalamin, holo-transcobalamin, MMA, and tHcy. Subjects were divided into quintiles on the basis of their dietary vitamin B-12 intake (range: 0.42–22.7 μg/d), and biomarkers of vitamin B-12 status were plotted against estimated dietary vitamin B-12 intake. All biomarkers appeared to level off at a daily dietary vitamin B-12 intake between 4.2 and 7.0 μg.

Conclusion: In persons with normal absorption, our data indicate that an intake of 4–7 μg vitamin B-12/d is associated with an adequate vitamin B-12 status, which suggests that the current RDA of 2.4 μg vitamin B-12/d might be inadequate for optimal biomarker status even in a healthy population between 18 and 50 y of age. *Am J Clin Nutr* 2010;91:571–7.

INTRODUCTION

Vitamin B-12 is an essential water-soluble vitamin present in foods of animal origin such as fish, shellfish, meat, and dairy products (1). It is essential for neurologic function and for the production of cells such as red blood cells (RBCs). A vitamin B-12 deficiency may lead to anemia and neurologic symptoms ranging from subtle symptoms to severe spinal cord degeneration and nerve damage, and even dementia (2–4).

Low (<148 pmol/L) or borderline (<185 pmol/L) concentrations of serum cobalamin are highly prevalent among the elderly (8.7% and 15–17%, respectively) (5–7) and in younger populations (8.2% and 16.3%, respectively) as shown in the Framingham Offspring Study (8). Thus, 2 main etiologic factors may play a role: inadequate dietary vitamin B-12 intake (9–11) and/or vitamin B-12 malabsorption caused by gastrointestinal malfunction (12, 13).

The Recommended Dietary Allowance (RDA) of vitamin B-12 for adults in the United States is 2.4 μg/d, which denotes the average daily intake that is sufficient to meet the nutrient requirement of ≈98% of healthy individuals (14, 15). In Western diets, the dietary intake of vitamin B-12 is usually higher than the current RDA of 2.4 μg/d (1, 15, 16). Thus, it is assumed that a dietary deficiency of vitamin B-12 is rare. However, data from the Framingham Offspring Study (8) suggest that suboptimal vitamin B-12 status may occur at intakes exceeding the RDA, which leads to the concern of whether the current vitamin B-12 RDA is adequate to promote a normal vitamin status.

The relation between dietary intake and vitamin B-12 status, which is based on biomarkers of vitamin B-12 deficiency, was investigated in only a few studies (7, 8, 17–20), primarily in elderly populations. In 2006 Bor et al (19) showed that vitamin B-12 biomarkers were correlated with vitamin B-12 intake in 98 Danish postmenopausal women. An intake of 6 μg vitamin B-12/d appeared to be sufficient to normalize all vitamin B-12–related variables, which suggested that this dose was more adequate than the current RDA of 2.4 μg vitamin B-12/d. However, the study was limited because it represented a relatively small sample of postmenopausal women.

In the present study, we have continued our inquiry into the relation between dietary vitamin intake and RDA by determining

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biomarkers of vitamin B-12 deficiency in a cohort of 299 healthy adult men and women aged 18–50 y.

SUBJECTS AND METHODS

Subjects

Healthy adult men and women study participants (n = 299) were recruited from the Alachua County, FL, community including university students, faculty, and staff and residents of the surrounding area from 14 October 2004 through 30 August 2005. Subjects were recruited from advertisements and presentations at large group events. Subjects were initially screened by phone and selected for the study on the basis of the following inclusion criteria: *1*) ≥18 and ≤50 y of age, *2*) no change in the consumption of animal-based products over the past ≥3 y, *3*) no vitamin B-12–containing supplement consumption within the past 6 mo, *4*) limited alcohol consumption (<1 drink/d of any kind), *5*) no use of tobacco products, *6*) no chronic use of prescription medications other than oral contraceptive agents, *7*) no history of chronic disease, *8*) no routine blood donations, and *9*) nonpregnant and nonlactating. The ethnicity of the subjects was self-described as white (n = 185), Hispanic (n = 38), Asian (n = 33), Asian Indian (n = 16), black (n = 17), and other (n = 10) (**Table 1**). All subjects signed an informed consent form approved by the University of Florida Institutional Review Board before beginning the study.

Study design and data collection

Subjects were phoned the day before their scheduled study date to remind them to fast overnight (for 8 h). Between 0700 and 0900, eligible subjects reported for their scheduled blood-sample collection followed by a comprehensive information session explaining how to complete the National Cancer Institute Diet History Questionnaire (DHQ) (http://appliedresearch.cancer.gov), which was used to assess dietary intake. The DHQ included questions regarding the subject's diet over the past 12 mo, with an estimate of the frequency of intake and portion size of food items. Subjects completed the DHQ at home, where portion-size measurements and brand names could be checked to improve recall. Subjects returned their completed DHQs by mail, and the questionnaires were checked for completeness by investigators before analysis for nutrient intake.

Blood-sample processing and biochemical analyses

Blood samples were collected in tubes containing EDTA and SST clot activator tubes (Becton, Dickinson, and Co, Franklin Lakes, NJ) for analysis of hematocrit, plasma cobalamin, serum total transcobalamin, holo-transcobalamin, methylmalonic acid (MMA), and total homocysteine (tHcy) concentrations. Tubes containing EDTA were centrifuged at 2000 × g at 4°C for 30 min to obtain plasma for vitamin B-12 analyses, and SST tubes were centrifuged at 650 × g at room temperature for 15 min to obtain serum for total transcobalamin, holo-transcobalamin, MMA, and tHcy determinations. Samples were stored for 6–10 mo at −30° C until analysis.

Plasma cobalamin was analyzed using a commercial method on a Centaur analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY) with a CV of 7%. The serum total transcobalamin and holo-transcobalamin were measured, as previously described, by an enzyme-linked immunosorbent assay (BEP-2000; Dade Behring, Schwalbach, Germany), the analytic imprecision of which was 7% for the total transcobalamin and 8% for the holo-transcobalamin (21, 22). Serum tHcy and MMA concentrations were measured by gas chromatography/mass spectrometry (23, 24) with a CV <10%. Hematocrit values were measured by a capillary tube (BD Worldwide, Franklin Lakes, NJ). The serum and RBC folate concentrations of all subjects were measured

TABLE 1
Demographic and vitamin B-12 intake data of subjects (n = 299)

	All subjects (n = 299)	Men (n = 135)	Women (n = 164)	P^1
Age (y)	23 (18–50)[2]	23 (18–50)	23.5 (18–49)	0.63
25th–75th percentile	21–29	21–29	21–28	—
BMI (kg/m^2)	22.7 (16.5–47.7)	23.7 (17.2–47.7)	21.6 (16.5–41.5)	<0.001
25th–75th percentile	20.7–25.1	21.6–26.2	20.1–24.5	—
Vitamin B-12 intake (μg/d)	4.2 (0.4–22.7)	5.2 (0.4–22.7)	3.9 (0.5–19.4)	0.004
25th–75th percentile	2.4–7.5	2.8–9.4	2.3–6.3	—
Vitamin B-12 intake: fortified foods (μg/d)	0.8 (0–16.6)	0.6 (0–16.6)	0.9 (0–9.5)	0.21
25th–75th percentile	0.4–2.1	0.2–2.0	0.5–2.4	—
Total energy intake (kcal/d)	1833 (605–4914)	2130 (825–4914)	1546 (605–4912)	<0.001
25th–75th percentile	1317–2500	1760–2882	1220–2056	—
Vitamin B-12 intake/total energy intake (μg/1000 kcal)	2.3 (0.4–16.9)	2.3 (0.4–17)	2.3 (0.4–14.2)	0.89
25th–75th percentile	1.5–3.7	1.4–3.7	1.5–3.6	—
Ethnicity/positive for *Helicobacter pylori* immunoglobulin G [*n*/*n* (%)]				
White	185/15 (8)	80/8 (10)	105/7 (7)	—
Hispanic	38/10 (26)	14/5 (36)	24/5 (21)	—
Asian	33/2 (6)	19/1 (5)	14/1 (7)	—
Black	17/3 (18)	9/1 (11)	8/2 (25)	—
Asian Indian	16/4 (25)	6/2 (33)	10/2 (20)	—
Other	10/1 (10)	7/1 (14)	3/0 (0)	—

[1] Student's t test was used (after log transformation) to compare women and men.
[2] Median; range in parentheses (all such values).

with the SimulTRAC-S Radioassay Kit (MP Biomedicals Inc, Orangebury, NY), which is a competitive protein binding assay with a CV of 9%.

Autoantibodies against intrinsic factor (IF) were measured with an enzyme-linked immunosorbent assay by using recombinant human IF with a CV of 11%, as previously described (25). For the detection of immunoglobulin G antibodies to *Helicobacter pylori* in human serum, the IMMULITE 2000 *H. pylori* immunoglobulin G (DPC Diagnostics, Los Angeles, CA), a solid-phase chemiluminescent immunoimetric assay, was used with a CV of 7%. This assay has an analytic sensitivity limit of 0.4 U/mL, with <0.9 U/mL considered to be negative.

Diet analyses

Daily vitamin B-12 intakes were assessed on the basis of data obtained from the DHQ (http://appliedresearch.cancer.gov). The DHQ was previously validated for vitamin B-12 intake in the Eating at America's Table study by Subar et al (26). The DHQ was scanned by Optimal Solutions Corporation (Lynbrook, NY). Once scanned, the Optimal Solutions Corporation sent the dietary data as an ASCII text file to the University of North Carolina (Chapel Hill, NC) where the data were analyzed with the Diet*Calc Analysis program modified for this version of the DHQ (available from the National Cancer Institute at http://www.riskfactor.cancer.gov).

The vitamin B-12 contents of foods included in the DHQ were estimated on the basis of the US Department of Agriculture national nutrient database for standard reference (27). Fortified foods were accounted for in the DHQ, and the vitamin B-12 content of these foods was obtained from the US Department of Agriculture database or product manufacturer.

Statistical methods

Because the data were not normally distributed, all values were log transformed to improve the normality characteristics. If the log-transformed data were not normally distributed, they were log transformed once more. Summary statistics, including the median and ranges (minimum–maximum and 25th–75th percentiles), were calculated for age, body mass index (BMI; in kg/m^2), vitamin B-12 intake, and biochemical variables. Analyses included dividing the subjects into quintiles ranked by their dietary intake. The lowest quintile comprised samples derived from individuals with the lowest vitamin B-12 intake, the second quintile comprised samples derived from the individuals with the second lowest intake, and so on. Values were compared between these 5 quintiles by one-factor analysis of variance. Tukey's method was used for pairwise comparisons. Pearson's correlations were calculated for other data comparisons. Comparisons between groups were made by using Student's *t* test with Welch correction, when it was needed. $P < 0.05$ was considered statistically significant. The relation between vitamin B-12 biomarkers (log transformed) and dietary vitamin B-12 intake (log transformed) was examined by using the multiple linear regression procedure in the SAS program (version 9.2; SAS Institute Inc, Cary, NC), with adjustment for age, sex, total energy intake, and antibodies against *H. pylori.* These analyses were repeated separately for men and women. The data were analyzed with EXCEL (Microsoft, Redmond, WA), PRISM4 (GraphPad Software Inc, El Camino, CA), and SAS (version 9.2; SAS Institute Inc).

RESULTS

Complete dietary and biochemical data were collected for 299 healthy subjects (men, $n = 135$; women, $n = 164$) with a median age of 23 y and a median BMI of 22.7. Key data for the study population are shown in Table 1.

Vitamin B-12 intake and related indexes

Demographic information, *H. pylori* status, and vitamin B-12 intake data for the study population are presented in Table 1. The dietary intake of vitamin B-12 ranged from 0.42 to 22.7 μg/d for all subjects combined. The median vitamin B-12 intake was 5.2 μg/d for men and 3.9 μg/d for women ($P = 0.004$, Student's *t* test). No significant difference in vitamin B-12 intake between men and women was detected when vitamin B-12 intake was adjusted for energy intake. The main sources of vitamin B-12 intake in our population were fortified foods (29%), seafood (flesh fish, shellfish, and shrimp; 24%), dairy and eggs (21%), beef (15%), other (eg, milk-based soups, breads, cakes, and pies; 5.9%), and nonbeef meats (poultry, pork, and mixed meats; 4.7%). On the basis of the intake data reported on the DHQ, 120 subjects were categorized as vegetarians. They received 53% of their vitamin B-12 intake from fortified products.

Data for hematocrit and plasma/serum concentrations of cobalamin, total transcobalamin, holo-transcobalamin, MMA, tHcy, and serum and RBC folate are shown in **Table 2**. Among biochemical variables, only tHcy and hematocrit differed between men and women ($P < 0.001$ for both). Five men (4%) and 8 women (5%) had a hematocrit below the reference interval. RBC folate (except one subject) and serum folate concentrations were normal for all subjects. Dietary intake of vitamin B-12 and vitamin B-12–related indexes did not differ in relation to the ethnic background of subjects (data not shown). Vegetarians ($n = 120$) had significantly lower vitamin B-12 intake and cobalamin and holo-transcobalamin concentrations and significantly higher MMA concentrations than did omnivores ($n = 179$) (data not shown). No significant difference was observed between vegetarians and omnivores concerning tHcy and hematocrit.

IF antibodies and *H. pylori* infection

IF antibodies were not detected in any samples. The prevalence of antibodies against *H. pylori* was 12% (35/299). The highest prevalence was observed in individuals of Hispanic origin 26% (10/38) compared with other ethnic subgroups (chi-square, $P = 0.012$) (Table 1). Lower cobalamin concentrations were detected ($P = 0.011$, Student's *t* test after log transformation) in participants with antibodies against *H. pylori* compared with those without antibodies, but no significant differences in other biomarkers of vitamin B-12 deficiency were detected (data not shown).

Relation between vitamin B-12 intake and biomarkers of vitamin B-12 deficiency

The intake of vitamin B-12 from food sources for the entire cohort ($n = 299$) correlated significantly with cobalamin status

TABLE 2
Vitamin B-12 biomarker and antibody status of subjects (n = 299)[1]

	All subjects (n = 299)	Men (n = 135)	Women (n = 164)	P[2]	Reference interval[3]
Cobalamin (pmol/L)	310 (111–1211)[4]	317 (111–883)	309 (129–1211)	0.91	200–600
25th–75th percentile	250–390	245–384	246–389	—	—
Holo-TC (pmol/L)	49 (8–388)	50 (11–188)	46 (8–388)	0.17	>40
25th–75th percentile	35–69	37–73	33–65	—	—
TC saturation (%)	7 (1–48)	7 (1–29)	7 (1–48)	0.31	>4
25th–75th percentile	5–9	5–9	5–9	—	—
MMA (μmol/L)[5]	0.17 (0.090–1.87)	0.18 (0.1–1.87)	0.17 (0.09–0.91)	0.06	0.07–0.27
25th–75th percentile	0.14–0.24	0.15–0.25	0.13–0.23	—	—
tHcy (μmol/L)[6]	7.1 (3.5–45.7)	7.7 (4.9–45.7)	6.5 (3.5–13)	<0.001	5.4–13.9
25th–75th percentile	6.1–8.4	6.8–8.9	5.6–7.6	—	—
Serum folate (nmol/L)	18.2 (5.2–42.9)	17.9 (5.2–41)	18.9 (6.9–42.9)	0.19	4.0–31.7
25th–75th percentile	15–22.4	14.3–22.1	15.4–22.8	—	—
RBC folate (nmol/L)	804 (180–1700)	805 (284–1700)	795 (180–1655)	0.28	249–1586
25th–75th percentile	628–966	646–976	606–963	—	—
Total TC (pmol/L)	764 (360–1570)	766 (426–1570)	759 (360–1177)	0.34	700–1400
25th–75th percentile	665–859	668–872	656–843	—	—
Hematocrit (%)	42 (28–53)	45 (37–53)	40 (28–50)	<0.001	M: 40–50; F: 35–46
25th–75th percentile	40–45	43–46	38–42	—	—
Antibody against IF (%)	0	0	0	—	—
Antibody against *H. pylori* (%)	12	13	10	—	—

[1] TC, transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine; RBC, red blood cell; IF, intrinsic factor; *H. pylori*, *Helicobacter pylori*.
[2] Student's t test was used (after log transformation) to compare women and men.
[3] Reference intervals used in the laboratory where the analyses were performed.
[4] Median; range in parentheses (all such values).
[5] Data available for 297 subjects.
[6] Data available for 298 subjects.

(r = 0.23, $P < 0.0001$), holo-transcobalamin (r = 0.27, $P < 0.0001$), transcobalamin saturation (r = 0.35, $P < 0.0001$), MMA ($r = -0.19$, P = 0.0008), tHcy ($r = -0.12$, P = 0.04), and hematocrit (r = 0.18, P = 0.0014). Pearson's correlation coefficient, after log transformation, was used to describe the correlation between variables. The relation between total vitamin B-12 intake and biomarkers of vitamin B-12 status was further analyzed after dividing the subjects into quintiles on the basis of their vitamin

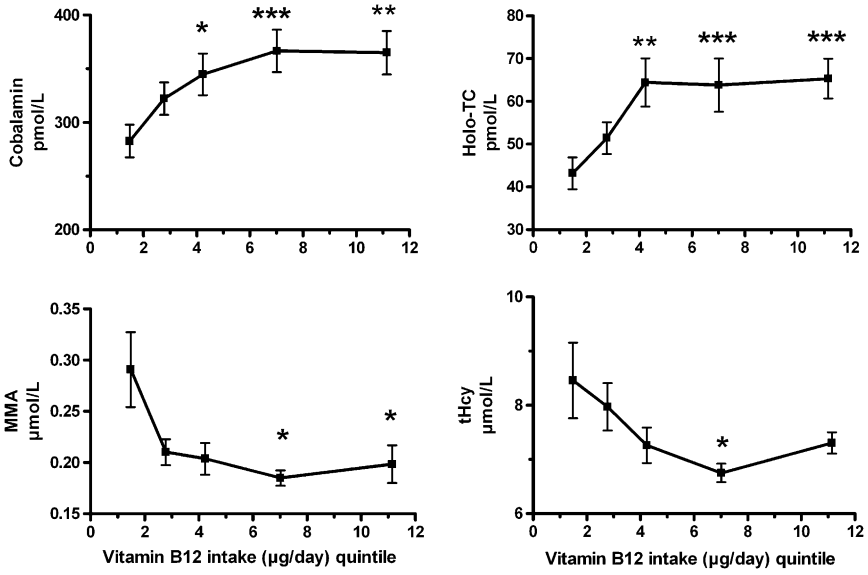

FIGURE 1. Relation between total vitamin B-12 intake and vitamin B-12 biomarkers (n = 299). Mean (±SEM) concentrations are plotted against the median intake for each quintile (each with n = 60, except for the fifth quintile, n = 59). Quintiles cover the following median (range) vitamin B-12 intakes: quintile 1, 1.5 μg (0.4–2.1 μg); quintile 2, 2.8 μg (2.1–3.4 μg); quintile 3, 4.2 μg (3.4–5.3 μg); quintile 4, 7.0 μg (5.4–8.6 μg); and quintile 5, 11.2 μg (8.7–22.7 μg). Concentrations of holo-transcobalamin (Holo-TC; $P < 0.0001$), cobalamin (P = 0.0003), total homocysteine (tHcy; P = 0.017), and methylmalonic acid (MMA; P = 0.009) differed significantly between quintiles for vitamin B-12 intake (one-factor ANOVA, after log transformation). *******Vitamin B-12 intakes in the lowest quintile differed significantly from other quintiles as labeled: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (all by Tukey's multiple-comparisons test).

B-12 intake (**Figure 1**). Each biomarker displayed a characteristic curve with a significant increase (cobalamin and holotranscobalamin) or decrease (MMA and tHcy) with higher intakes of dietary vitamin B-12. Interestingly, all curves appeared to level off at a median daily intake between 4.2 and 7.0 μg (ie, third and fourth quintiles, respectively) (Figure 1). The results were not affected when demographic data, such as sex, energy intake, and antibody against *H. pylori* (data not shown), were controlled for. Women had significantly lower tHcy and hematocrit values compared with men. MMA and tHcy concentrations were age dependent, with a mean increase in MMA and tHcy with a factor of 1.35 and 1.17 μmol/L, respectively, from age 25 to 45 y. No significant differences in hematocrit values with each successive quintile of vitamin B-12 intake were detected for men or women. The mean, median, and range of biomarkers were calculated for each quintile (*see* Supplemental Table 1 under "Supplemental data" in the online issue).

DISCUSSION

We investigated dietary vitamin B-12 intake and its relation with biomarkers of vitamin B-12 status in 299 healthy adults. The median vitamin B-12 intake was 5.2 μg/d for men and 3.9 μg/d for women. All vitamin B-12 biomarkers leveled off at an intake of 4–7 μg/d. These results are in agreement with the median dietary vitamin B-12 intake in the United States of ≈5 μg/d for men and ≈3.5 μg/d for women (15).

Except for a study by Howard et al (17), other studies (7, 8, 17–20) showed correlations between total vitamin B-12 intake and serum cobalamin, metabolite concentrations, or both (**Table 3**). In the current study, the study by Bor et al (19), and in 3 other large studies that examined vitamin B-12 intake and status (Table 3)—the Hordaland Homocysteine Study performed in Norway (20), the Framingham Offspring Study (8), and a study performed in a Hispanic population (7)—plasma cobalamin leveled off at vitamin B-12 intakes between 4 and 10 μg/d, a range that is considerably higher than the current RDA of 2.4 μg vitamin B-12/d.

The reported difference in the suggested daily vitamin B-12 intake between the studies listed in Table 3 may have been explained by differences in the age groups of the populations included and/or the dietary assessment methods used to estimate vitamin B-12 intake. Except for the current study, none of the studies investigated a purely younger population group (median age: 23 y) that was presumably not yet influenced by an age-related decline in the absorption of vitamin B-12. None of the individuals included in our study had autoantibodies against IF, and only 12% of the individuals had antibodies against *H. pylori*, reducing the possibility of a decreased bioavailability of vitamin B-12 from food (28, 29). A decreased concentration of cobalamin was described in patients harboring antibodies against *H. pylori* (29). In our study, none of the other markers of vitamin B-12 status were influenced by the presence of antibodies against *H. pylori*. A likely explanation for this observation is

TABLE 3
Overview of studies (2000–2009) showing a correlation between dietary vitamin B-12 intake and suggested daily intake recommendations on the basis of intakes associated with saturation of vitamin B-12[1]

	This study	Vogiatzoglou et al, 2009 (20)	Bor et al, 2006 (19)	Kwan et al, 2002 (7)	Tucker et al, 2000 (8)
Subjects					
Origin	American (mix)	Hordaland Homocysteine Study (Norway)	Danish	Hispanic	Framingham offspring
n	299	5937	98	449	2999
Age (y)	18–50	47–74	41–75	60–93	26–83
Sex	Mixed	Mixed	Women	Not mentioned	Mixed
Dietary intake					
Method	DHQ	FFQ	7-d diet record	FFQ	FFQ
Supplement users	Not included	Included	Included	Included	Included
Biomarkers					
Cobalamin	+	+	+	+	+
Holo-TC	+	−	+	−	−
MMA	+	+	+	−	−
tHcy	+	+	+	−	−
Additional variables					
IF antibody	+	−	−	−	−
Antibodies against *H. pylori*	+	−	−	−	−
Major result	All vitamin B-12–related biomarkers correlate with vitamin B-12 intake	Plasma cobalamin correlates with vitamin B-12 intake	All vitamin B-12–related biomarkers correlate with vitamin B-12 intake	Plasma cobalamin correlates with vitamin B-12 intake	Plasma cobalamin correlates with vitamin B-12 intake
Suggested intake recommendation (μg vitamin B-12/d)	4–7	6–10	6	7	10

[1] Holo-TC, holo-transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine; IF, intrinsic factor; *H. pylori*, *Helicobacter pylori*; DHQ, Diet History Questionnaire; FFQ, food-frequency questionnaire; +, corresponding parameter is analyzed in the corresponding study; −, corresponding parameter is not analyzed in the corresponding study.

that the participants obtained part of their vitamin B-12 in a nonprotein bound form from vitamin B-12–fortified foods.

In the absence of a universal gold standard for estimating dietary intake, we used a food-frequency questionnaire (National Cancer Institute DHQ) to assess dietary intake over the previous 12 mo. This analysis was subject to the limitations associated with self-reported dietary data and problems of recall (30). The latter limitation was likely less of a problem in the current study because it was confined to healthy persons with normal cognition. The assessment of vitamin B-12 intake over time is particularly important because vitamin B-12 status may be maintained for a long period of time after the initiation of inadequate dietary vitamin B-12 intake (31). Therefore, any short-term review of intake is unlikely to reliably reflect vitamin B-12 nutrition and may be misleading.

Quantitative estimates of nutrition intakes presented as reference values, such as the RDA and Estimated Average Requirement (EAR), are used for planning and assessing diets for healthy people. The process for setting the RDA depends on the ability to establish an EAR, the nutrient intake value that is estimated to meet the requirements of 50% of healthy individuals (14). If the EAR is normally distributed for the population, the RDA is calculated on the basis of the EAR plus twice the SD of the EAR. A CV of 10% is used if the SD cannot be determined, which is the case for most nutrients, including vitamin B-12 (32, 33).

Three approaches were considered for deriving the EAR and RDA for adults for vitamin B-12 (15) as follows: *1*) the determination of the amount of cobalamin needed to maintain an adequate hematologic status and serum cobalamin concentrations in persons with pernicious anemia or with very low intakes, *2*) the use of daily vitamin B-12 turnover to estimate the amount of this vitamin needed to maintain body stores at a specified concentration, and *3*) the estimation of the dietary vitamin B-12 intake by healthy adults that corresponds to adequate serum cobalamin and MMA concentrations.

The current RDA value of vitamin B-12 intake is primarily based on a 1958 study conducted by Darby et al (34) in which approximately one-half of the subjects (4 of 7) with pernicious anemia achieved and maintained maximum erythropoiesis on long-term follow-up [evaluated by hematocrit and mean corpuscular volume (MCV) values] with intramuscular administration of vitamin B-12 (median: 1.4 μg/d). A physiologic average requirement of 1.0 μg vitamin B-12/d was measured after adjustment for the extra loss of vitamin B-12 by subjects with pernicious anemia. After adjustment for incomplete absorption of vitamin B-12 from food (50%), an EAR of 2.0 μg vitamin B-12/d was established. The current RDA of 2.4 μg vitamin B-12/d was derived by multiplying the EAR by 1.2 (2 $\times$ a CV of 10%). Although this approach may have merit, the data used to develop the current RDA came from a small number of subjects, with the use of hematocrit and MCV values as the only endpoints, and did not take into account that many patients with vitamin B-12 deficiency only develop neurologic symptoms (2).

The RDA, by definition, is designed to provide dietary guidance for healthy individuals (14, 32, 33). An important consideration is whether the recommended intake is adequate to prevent the biochemical disturbances that were recently recognized to be widespread and sometimes associated with subtle but important clinical disorders (2, 8), rather than preventing overt disease as used in the design for establishing RDA (15).

In the present study the RDA was estimated as the dietary vitamin B-12 intake in a healthy population above which there was no further change in biomarkers of vitamin B-12 status. This approach was suggested by the Food and Nutrition Board (35). It can be argued that the determination of the RDA should be performed in a general population and not in an ideal, nonexisting population. Thus, our findings may have been criticized as being relevant for only young, healthy people and not for middle-aged and elderly people in whom low vitamin B-12 status is a concern. However, an RDA determined in a young population would be expected to be lower than that which would be adequate for an elderly population, and because of that, an intake of 4–7 μg vitamin B-12/d, as suggested by our data obtained in a young adult population, is likely to be a low estimate for an RDA covering the general population. This view is in agreement with the findings from the study by Bor et al (19), the Hordaland Homocysteine Study (20), the study performed in a Hispanic population (7), and the Framingham Offspring Study (8) conducted in middle-aged and elderly populations, in which a vitamin B-12 intake between 6 and 10 μg/d was shown to normalize vitamin B-12 status.

Our research suggested that the vitamin B-12 intake associated with normal biomarker status was likely to be somewhat higher than the amount needed to prevent deficiency disease. A missing piece of information is the amount of vitamin B-12 intake associated with observable health benefits beyond the prevention of signs and symptoms of deficiency. Randomized trials in high-risk, healthy individuals would be useful in resolving this matter.

In conclusion, this study provides evidence that, for healthy adults between the ages of 18 and 50 y, a dietary vitamin B-12 intake of 4–7 μg/d is associated with normal vitamin B-12 status as judged from measurements of cobalamin, holo-transcobalamin, tHcy, and MMA. Our findings, together with those of previous studies (7, 8, 19, 20), suggest that the current RDA of 2.4 μg vitamin B-12/d may be inadequate for optimal biomarker status. Intervention trials with appropriate clinical endpoints in older populations should be performed to find out whether the RDA needs to be changed.

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The authors' responsibilities were as follows—MVB: participated in analysis of transcobalamin-related variables, performed statistical analyses, interpreted data, and wrote the first draft of manuscript; KMvC-R: assisted in protocol planning, supervised and participated in subject recruitment, processed blood and diet analyses, and approved the final manuscript; GPAK: assisted with all aspects of study planning, designed dietary intake assessment including revision of the DHQ, participated in subject recruitment, and assisted in revision of the final manuscript; SPS: planned and performed MMA and tHcy analyses and approved the final manuscript; RHA: planned and performed MMA and tHcy analyses and approved the final manuscript; DRM: assisted in subject recruitment and blood processing and was responsible for managing laboratory operations in Florida; LBB: was responsible for protocol planning, supervised all aspects of the study, participated in writing, and approved the final manuscript; and EN: participated in study planning, performed transcobalamin-related measures, supervised and helped interpret data, participated in writing, and approved the final manuscript. SPS, RHA, and the University of Colorado hold patents on the use of assays for total homocysteine and other metabolites to diagnose vitamin B-12 and folate deficiencies, and a company was formed at the University of Colorado to perform such assays. None of the other authors had a financial conflict of interest.

REFERENCES

1. Andres E, Loukili NH, Noel E, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ 2004;171:251–9.
2. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med 1988;318:1720–8.
3. Lindenbaum J, Rosenberg IH, Wilson PW, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 1994;60:2–11.
4. Savage D, Gangaidzo I, Lindenbaum J, et al. Vitamin B12 deficiency is the primary cause of megaloblastic anaemia in Zimbabwe. Br J Haematol 1994;86:844–50.
5. Wolters M, Strohle A, Hahn A. Cobalamin: a critical vitamin in the elderly. Prev Med 2004;39:1256–66.
6. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients. J Am Geriatr Soc 1992;40: 1197–204.
7. Kwan LL, Bermudez OI, Tucker KL. Low vitamin B-12 intake and status are more prevalent in Hispanic older adults of Caribbean origin than in neighborhood-matched non-Hispanic whites. J Nutr 2002;132: 2059–64.
8. Tucker KL, Rich S, Rosenberg I, et al. Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. Am J Clin Nutr 2000;71:514–22.
9. Narayanan MN, Dawson DW, Lewis MJ. Dietary deficiency of vitamin B12 is associated with low serum cobalamin levels in non-vegetarians. Eur J Haematol 1991;47:115–8.
10. Larsson CL, Johansson GK. Dietary intake and nutritional status of young vegans and omnivores in Sweden. Am J Clin Nutr 2002;76: 100–6.
11. Alaimo K, McDowell MA, Briefel RR, et al. Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988-91. Adv Data 1994;258:1–28.
12. Clarke R, Grimley EJ, Schneede J, et al. Vitamin B12 and folate deficiency in later life. Age Ageing 2004;33:34–41.
13. Hanger HC, Sainsbury R, Gilchrist NL, Beard ME, Duncan JM. A community study of vitamin B12 and folate levels in the elderly. J Am Geriatr Soc 1991;39:1155–9.
14. King JC, Vorster HH, Tome DG. Nutrient intake valuea (NIVs): recommended terminology and framework for the derivation of values. Food Nutr Bull 2007;28(suppl):s16–26.
15. Institute of Medicine. Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Acadamy Press, 1998.
16. Russell RM, Suter PM. Vitamin requirements of elderly people: an update. Am J Clin Nutr 1993;58:4–14.
17. Howard JM, Azen C, Jacobsen DW, Green R, Carmel R. Dietary intake of cobalamin in elderly people who have abnormal serum cobalamin, methylmalonic acid and homocysteine levels. Eur J Clin Nutr 1998;52: 582–7.
18. van Asselt DZ, de Groot LC, van Staveren WA, et al. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. Am J Clin Nutr 1998;68:328–34.
19. Bor MV, Lydeking-Olsen E, Møller J, Nexø E. A daily intake of approximately 6 micrograms vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. Am J Clin Nutr 2006;83:52–8.
20. Vogiatzoglou A, Smith AD, Nurk E, et al. Dietary sources of vitamin B-12 and their association with plasma vitamin B-12 concentrations in the general population: the Hordaland Homocysteine Study. Am J Clin Nutr 2009;89:1078–87.
21. Nexo E, Christensen AL, Petersen TE, Fedosov SN. Measurement of transcobalamin by ELISA. Clin Chem 2000;46:1643–9.
22. Nexo E, Christensen AL, Hvas AM, Petersen TE, Fedosov SN. Quantification of holotranscobalamin, a marker of vitamin B12 deficiency. Clin Chem 2002;48:561–2.
23. Stabler SP, Lindenbaum J, Savage DG, Allen RH. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. Blood 1993;81:3404–13.
24. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Elevation of 2-methylcitric acid I and II levels in serum, urine, and cerebrospinal fluid of patients with cobalamin deficiency. Metabolism 1993;42:978–88.
25. Nexo E, Nooroya B, Hvas AM, Christensen AL, Waters H. Autoantibodies against intrinsic factor (IF) measured with an ELISA using recombinant human IF as both catching and detecting reagent. Clin Chem Lab Med 2005;43:351–6.
26. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol 2001; 154:1089–99.
27. US Department of Agriculture. USDA national nutrient database for standard reference, release 17: Nutrient Data Laboratory homepage. 2004. Available from: http://www.nal.usda.gov/fnic/foodcomp (cited 1 October 2004).
28. Carmel R, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and food-cobalamin malabsorption. Dig Dis Sci 1994;39:309–14.
29. Kaptan K, Beyan C, Ural AU, et al. *Helicobacter pylori*: is it a novel causative agent in vitamin B12 deficiency. Arch Intern Med 2000;160: 1349–53.
30. Hankin JH. Development of a diet history questionnaire for studies of older persons. Am J Clin Nutr 1989;50(5 suppl):1121–7; discussion 1231-5.
31. Herbert V. Staging vitamin B-12 (cobalamin) status in vegetarians. Am J Clin Nutr 1994;59:1213S–22S.
32. Yates AA. Using criteria to establish nutrient intake values (NIVs). Food Nutr Bull 2007;28(suppl):s38–50.
33. King JC, Garza C. Excutive summary. Food Nutr Bull 2007;28(suppl): S3–12.
34. Darby WJ, Bridgforth EB, le Brocquy J, et al. Vitamin B12 requirement of adult man. Am J Med 1958;25:726–32.
35. Food and Nutrition Board. How should the recommended dietary allowances be revised? Washington, DC: National Academy Press, 1994.