

A daily intake of approximately 6 μg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women¹⁻³

Mustafa Vakur Bor, Eva Lydeking-Olsen, Jan Møller, and Ebba Nexø

ABSTRACT

Background: Recommended daily intakes of vitamin B-12 vary between 2 and 6 μg .

Objective: The objective was to examine the associations between vitamin B-12 intake and markers of vitamin B-12 deficiency.

Design: We studied 98 Danish postmenopausal women aged 41–75 y. Serum cobalamin, transcobalamin (TC) saturated with vitamin B-12 (holo-TC), TC saturation (holo-TC/total TC), methylmalonic acid (MMA), and total homocysteine (tHcy) were measured to assess vitamin B-12 status. Dietary intakes of vitamin B-12 were determined from 7-d weighed food records. Gastric pH measurement and the alkali-challenge test were performed with the use of Heidelberg radiotelemetric capsules.

Results: The total intake of vitamin B-12 ranged from 1.2 to 23.9 $\mu\text{g}/\text{d}$. All vitamin B-12-related variables, except gastric pH, correlated significantly with total vitamin B-12 intake. Those taking supplements (54%) had higher circulating concentrations of cobalamin and TC saturation and lower concentrations of MMA and tHcy than did those not taking supplements. All subjects were divided into quintiles according to their total vitamin B-12 intake. For all the variables analyzed, the curves appeared to level off at a daily vitamin B-12 intake of $\approx 6 \mu\text{g}$; the median (and 25th–75th percentiles) for cobalamin was 380 (270–480) pmol/L, for holo-TC was 119 (92–162) pmol/L, for MMA was 0.12 (0.14–0.17) $\mu\text{mol}/\text{L}$, and for tHcy was 9.75 (8.3–11.40) $\mu\text{mol}/\text{L}$ ($n = 58$).

Conclusion: A daily vitamin B-12 intake of 6 μg appeared to be sufficient to correct all the vitamin B-12-related variables measured in the postmenopausal Danish women in this study. *Am J Clin Nutr* 2006;83:52–8.

KEY WORDS Vitamin status, cobalamin, methylmalonic acid, homocysteine, vitamin B-12 supplements, transcobalamin, holo-transcobalamin, elderly postmenopausal women

INTRODUCTION

Vitamin B-12 is an essential nutrient that must be supplied by dietary meat or dairy products. The Recommended Dietary Allowance (RDA) for vitamin B-12 is 2.4 $\mu\text{g}/\text{d}$ for adults (1). A reduced intake of vitamin B-12 from food, as seen in vegetarians or in impaired intestinal absorption, will induce a negative balance and ultimately lead to severe deficiency when the tissue stores of the vitamin are depleted. The clinical signs of vitamin

B-12 deficiency, including megaloblastic anemia and progressive neurologic diseases, represent late stages of this condition (2–5).

Recent studies indicate that subnormal or borderline serum cobalamin concentrations are highly prevalent in the elderly population (2, 5–10). However, the cause of this observation has not been clearly established. Two etiologic factors may play a role, dietary vitamin B-12 deficiency or vitamin B-12 malabsorption resulting from atrophic gastritis or other gastrointestinal malfunction (2, 5, 11, 12). It is believed that dietary deficiency of vitamin B-12 is extremely rare, except in vegetarians. However, the elderly often have poorer diets than do younger persons (13); therefore, dietary explanations for low serum cobalamin concentrations in this group are often possible. The relation between dietary intake and mild vitamin B-12 deficiency has been investigated in only a few studies, which have had conflicting results (14–17).

For the past 50 y, the measurement of cobalamin has been used as a diagnostic test for vitamin B-12 deficiency (18, 19). Recently, a low holo-transcobalamin (holo-TC) concentration was suggested to be the earliest and most sensitive indicator of a negative vitamin B-12 balance and therefore to be a better indicator of vitamin B-12 status than serum cobalamin (20–22). Methylmalonic acid (MMA) and total homocysteine (tHcy) accumulate in patients with vitamin B-12 deficiency and measurements of the metabolites in blood are also used as sensitive diagnostic tests for functional vitamin deficiency in tissues (5, 18, 19).

¹ From the Department of Clinical Biochemistry, Nørrebrogade Section, Aarhus Hospital (MVB and EN), the Department of Clinical Biochemistry, Aalborg Hospital (MVB), and the Department of Clinical Biochemistry, Skejby Hospital (JM), the University Hospital of Aarhus, Aarhus, Denmark; and the Institute for Optimum Nutrition, Copenhagen, Denmark (EL-O).

² Partly supported by an EU BIOMED project and by grants from The Danish Ministry of Health, Fondation Idella, Glunz and Jensen Foundation, Civilingenieur Frode V Nyegaard and Wife's Foundation, Director E Danielsen and Wife Foundation, and the IMK Foundation. The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

³ Reprints not available. Address correspondence to MV Bor, Department of Clinical Biochemistry, University Hospital of Aarhus, Nørrebrogade 44, DK-8000 Aarhus C, Denmark. E-mail: vakurbor@hotmail.com.

Received February 24, 2005.

Accepted for publication September 6, 2005.

In the present study we examined the relation between vitamin B-12 intake and the current variables used to evaluate vitamin B-12 status in 98 postmenopausal women. In addition, we included the newly introduced markers holo-TC and TC saturation.

SUBJECTS AND METHODS

Subjects

The participants of this substudy were described recently (23). The subjects recruited (in 1998) were screened initially via telephone to ensure that they were ≥ 1 y postmenopausal and were no older than 75 y. Of 290 women screened by telephone, 107 (61%) were eligible to be included in the study. Of this total, 8 did not deliver diet records and 1 was excluded from the study because she was receiving intramuscular vitamin B-12 treatments; this left 98 women for inclusion. Exclusion criteria were drug or alcohol addiction, malignant disease, current steroid treatment, osteomalacia, diabetes, unstable thyroid disease, chronic inflammatory diseases, and active liver or kidney disease.

The protocol and consent forms were approved by the Regional Ethical Committee for Copenhagen and Frederiksberg, Denmark (J no. 02-131/1997); The Danish Medicines Agency (J no. 5312-240-1997); and The Danish Data Protection Agency (J no. 1998-1200-113).

Data collection

Individual information on health, medical, reproductive, and menopausal history and lifestyle were obtained for all subjects by interview, aided by semiquantitative questionnaires. Nutrition and lifestyle history were used to elicit usual intake patterns of food items, special diets, and vitamin and mineral supplement use.

A dietary and lifestyle assessment showed that the median energy intake of the subjects was 7.3 MJ/d. Protein, fat, and carbohydrate accounted for 14.6%, 30.8%, and 50.3% of the energy intake, respectively, and 2.4% of the energy intake was from alcohol. The median alcohol intake was 3 servings/wk (range: 0–23 servings/wk). There were 48.3% past smokers and 20.2% current smokers, which reflected the high prevalence of smoking among Danish women.

Seven-day weighed food records were obtained at baseline after the subjects were provided thorough oral and written instructions and were shown how to use the supplied electronic scale (limit of 2000 g; Soehnle Domino, Murrhardt, Germany) as described previously (23). Briefly, weights were controlled for correct measurement by weighing items of known weight in each round of food recording. Food recording covered all 7 d of the week, and the subjects provided food labels and recipes of mixed dishes to ensure the correct coding of items. Food records were analyzed by using the DANKOST 3000 computerized nutrient database program (Danish Catering Center, Inc, Herlev, Denmark), which is based on the national Danish food-composition table.

These analyses did not include vitamin and mineral supplements. Information on the nutrient content of the vitamin and mineral supplements used by all subjects was obtained from the manufacturers or from the administration database of the Veterinary and Food Administration and recorded. The total intakes reported reflected intakes from the diet and from supplements.

Measurement of gastric pH and the alkali-challenge test were performed with the use of Heidelberg radiotelemetric capsules as

described previously (24, 25). If stomach function is normal and acid is secreted sufficiently in response to the alkali challenge, the pH returns to normal (pH between 1.0 and 2.0) within 20 min. The results of gastric pH measurement were available for 97 patients.

Biochemical analysis

Blood was drawn by standard antecubital venipuncture while the subjects were in a sitting position and in a fasted state. Plasma was separated from the blood cells < 2 h after venipuncture. Serum and plasma were stored frozen at -20°C for subsequent analysis.

Serum total TC and holo-TC were measured by enzyme-linked immunosorbent assay, as recently described, with the use of an automated enzyme-linked immunosorbent assay (BEP-2000; Dade Behring, Schwalbach, Germany), the analytic imprecision of which was 5% for total TC and 8% for holo-TC (26, 27). The reference interval was > 50 pmol/L for holo-TC and > 0.05 for TC saturation.

Plasma MMA concentrations were measured by stable-isotope-dilution capillary gas chromatography–mass spectrometry (28). The analytic imprecision for the method was $< 8\%$, and the reference interval was 0.08–0.28 $\mu\text{mol/L}$ (29). MMA concentrations were available for 93 subjects.

Plasma tHcy was measured with an immunologic method by using an IMx (Abbott, Chicago, IL) instrument, the analytic imprecision of this method was $< 5\%$. The reference interval was 5.8–11.9 $\mu\text{mol/L}$ (29, 30). Serum cobalamin was analyzed by using a chemiluminescent immunometric assay, with a CV of 8% (31). The reference interval was 130–670 pmol/L.

Standard methods were used to determine hematologic values. The reference intervals for females were as follows: 7.4–9.6 mmol/L for blood hemoglobin, 85–100 fL for erythrocyte mean cell volume, and $3.7\text{--}5.5 \times 10^{12}/\text{L}$ for erythrocyte count. Plasma creatinine was measured by the Jaffe method; the reference interval for females was 44–115 $\mu\text{mol/L}$.

Statistical analysis

Because the data were not normally distributed, the results are presented as medians and interquartile ranges. Two nonparametric tests, the Mann-Whitney *U* test and the Kruskal-Wallis test, were used to compare 2 or more continuous variables of unpaired samples. *P* values were corrected with Dunn's multiple comparisons test to adjust for the multiple comparisons. The Spearman correlation coefficient was used to describe the correlation between continuous variables. A *P* value of 0.05 was considered statistically significant. The analysis involved dividing all of the subjects into dietary intake quintiles. The data were analyzed by using EXCEL (Microsoft, Redmond, WA) and PRISM2 (Graph-Pad Software, Inc, El Camino, CA) software.

RESULTS

We recorded the vitamin B-12 intake for 98 postmenopausal women and related the intake to markers of vitamin B-12 deficiency. Subject characteristics are listed in **Table 1**. The age of the participants ranged from 41 to 75 y. Because no correlation was observed between the serum concentration of cobalamin and the age of the subjects, we did not divide the group according to age.

Table 1
Subject characteristics and laboratory values at baseline¹

	Value ² (<i>n</i> = 98)	Reference interval	Recalculated interval ³ (<i>n</i> = 58)
Age (y)	57 (53–64)	—	—
BMI (kg/m ²)	23.1 (21–25.8)	—	—
Blood variables			
Vitamin B-12 (pmol/L)	315 (240–445)	130–670	270–480
holo-TC (pmol/L)	111 (78–143)	>50	92–162
TC saturation	0.14 (0.1–0.19)	>0.05	0.12–0.23
MMA (μmol/L) ⁴	0.15 (0.12–0.19)	0.08–0.28	0.14–0.17
tHcy (μmol/L)	10.4 (8.8–13.0)	5.80–11.90	8.3–11.40
Creatinine (μmol/L)	80 (75–86)	44–115	—
Hemoglobin (mmol/L)	8.6 (8.1–8.8)	7.4–9.6	—
Erythrocyte count (10 ¹² /L)	4.4 (4.2–4.7)	3.7–5.5	—
Mean cell volume (fL)	92 (90–95)	85–100	—

¹ TC, transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

² All values are medians; interquartile ranges in parentheses.

³ Calculated as 25th and 75th percentiles from data obtained from individuals receiving ≥ 6 μg covering the third, fourth, and fifth quintiles of vitamin B-12 intake shown in Figure 1.

⁴ *n* = 93.

Vitamin B-12 intake

The total intake of vitamin B-12 (dietary plus supplemental intakes) ranged from 1.2 to 23.9 μg/d for the 98 subjects. Only 4 of the subjects (4.1%) had an intake below the RDA of 2.4 μg/d. Forty-five of the subjects (46%) did not use vitamin B-12 supplements. The dietary intakes of vitamin B-12 for the supplement users (*n* = 53) and nonusers (*n* = 45) are shown in **Table 2**.

Relation between vitamin B-12 intake and vitamin B-12-related variables

The relations between vitamin B-12 intake and vitamin B-12-related variables are presented for the total group and for the nonsupplement and supplement users (**Table 3**). The total vitamin B-12 intake correlated significantly with all vitamin B-12-related variables, whereas only serum cobalamin and TC saturation were significantly associated with the dietary intake of vitamin B-12 (Table 3).

The influence of total vitamin B-12 intake on the status of vitamin B-12-related variables was further analyzed by ordering the 98 subjects by quintiles from the lowest to the highest intakes of vitamin B-12 (**Figure 1**). The mean vitamin B-12-related variables for each quintile group of vitamin B-12 intake were

Table 2
Vitamin B-12 intakes in 98 menopausal women¹

	Vitamin B-12 intake ² μg/d
Total vitamin B-12 intake (<i>n</i> = 98)	6.0 (4.2–9.4)
Vitamin B-12 intake from food only (<i>n</i> = 45)	4.3 (3.1–8.7)
Total vitamin B-12 intake for supplement users (<i>n</i> = 53)	6.6 (4.9–11.0)
Vitamin B-12 intake from food for supplement users (<i>n</i> = 53)	4.8 (3.5–5.9)
Vitamin B-12 intake from supplements for supplement users (<i>n</i> = 53)	1 (1.0–4.5)

¹ All values are medians; interquartile ranges in parentheses.

² The Recommended Dietary Allowance for vitamin B-12 is 2.4 μg/d for adults.

plotted against the median intake for each respective quintile group. The curve for the total group shows a clear and strong increase or decrease in vitamin B-12-related variables, with an intake up to ≈ 6 μg/d, at which point the curve appears to level off at the following median and 25th and 75th percentiles covering the third, fourth, and fifth quintiles (*n* = 58 subjects who received ≥ 6 μg): 380 (270–480) pmol/L for cobalamin, 119 (92–162) for holo-TC, 0.12 (0.14–0.17) μmol/L for MMA, and 9.75 (8.3–11.40) μmol/L for tHcy.

Cobalamin (*P* = 0.0004), holo-TC (*P* = 0.009), and tHcy (*P* = 0.003)—but not MMA (*P* = 0.06)—concentrations differed significantly between the quintiles for vitamin B-12 intake. The holo-TC and cobalamin concentrations were lower and the tHcy concentrations were higher in the lowest quintile than in the third, fourth, and fifth quintiles (Figure 1).

Vitamin B-12 supplement use

Fifty-three of the subjects (54%) took vitamin supplements regularly, usually as multivitamin preparations. The supplements contained a median amount of 1 μg vitamin B-12 (range: 1–18 μg). Thirty-four of the subjects who used supplements (64%) were taking oral multivitamin supplements containing 1 μg vitamin B-12. Supplement users presented significantly higher values for cobalamin and TC saturation and lower values for tHcy and MMA in serum (**Table 4**). Supplement use did not correlate with the subjects' vitamin B-12 intake from food (*r* = −0.09), which suggested that their use of supplements was not dictated by any dietary need.

Table 3
Correlations (*r*) between total vitamin B-12 intakes (from both diet and supplements) and vitamin-related variables measured in blood samples¹

	Cobalamin		holo-TC		TC saturation		MMA ²		tHcy	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Total vitamin B-12 intake (<i>n</i> = 98)	0.4	<0.0001	0.3	0.02	0.4	<0.0001	−0.3	0.004	−0.29	0.04
Vitamin B-12 intake from diet only (<i>n</i> = 45)	0.3	0.02	NS		0.3	0.03	NS		NS	
Vitamin B-12 intake for supplement users (<i>n</i> = 53)		NS	0.3	0.04	0.3	0.02	NS		NS	

¹ TC, transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine. Spearman correlation coefficients were used to describe the correlation between variables.

² Values were available for 41 nonsupplement users and 52 supplement users.

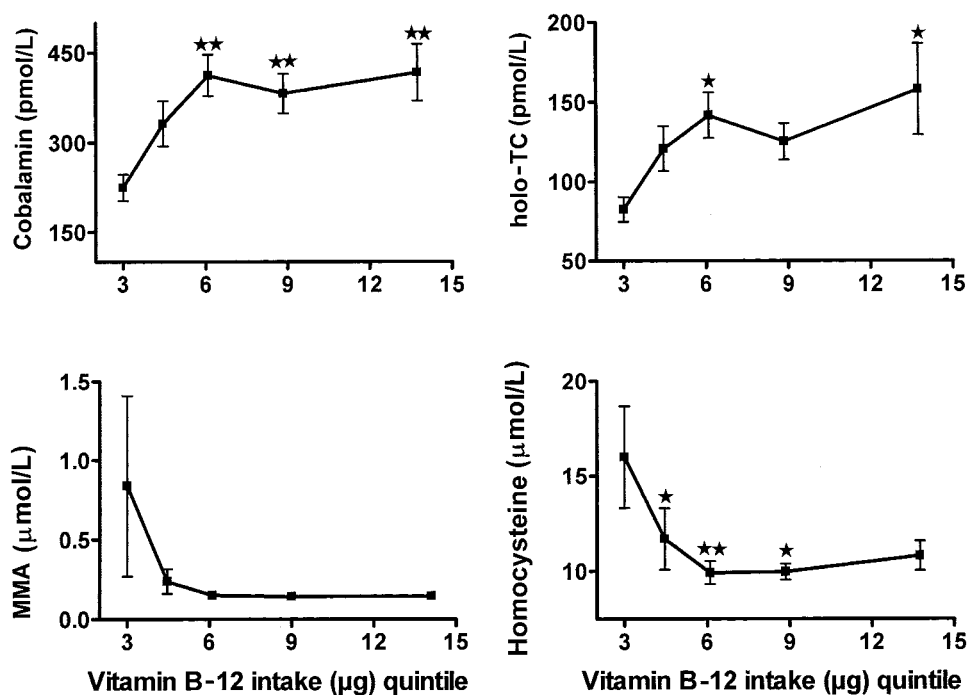


FIGURE 1. Relation between vitamin B-12 intake and concentrations of cobalamin, holo-transcobalamin (holo-TC), methylmalonic acid (MMA), and total homocysteine for all ($n = 98$) subjects. Mean (\pm SEM) concentrations are plotted against the median intake for each quintile ($n = 20$ for all quintiles, except quintile 5, $n = 18$). Concentrations of holo-TC ($P = 0.009$), cobalamin ($P = 0.0004$), and total homocysteine ($P = 0.003$), but not of MMA ($P = 0.06$), differed significantly between quintiles of vitamin B-12 intake (Kruskal-Wallis test). ***Significantly different from the lowest quintile (Dunn's multiple comparisons test): * $P < 0.05$, ** $P < 0.01$.

Relation between gastric pH measurement and vitamin B-12-related variables

Thirty-five of the 97 subjects (36%) had a gastric pH ≥ 3 (pH: 3–5) or a prolonged challenge test (after challenge with bicarbonate, it takes >20 min to reach a pH between 1 and 2; see Subjects and Methods). The vitamin B-12-related variables for these subjects did not differ significantly from those of the subjects who had a normal gastric pH of 1–2 or a normal challenge test ($n = 62$) (data not shown). Furthermore, there was no correlation between gastric pH measurement and vitamin B-12-related variables for the whole population. A comparison of supplement use between the subjects with normal gastric pH

($n = 62$) and with abnormal gastric pH ($n = 35$) supplement showed no significant difference between the 2 groups ($n = 32$ and 22, respectively; $P = 0.3$). The amount of vitamin B-12 consumed as supplements was also not significantly different between the 2 groups ($P = 0.3$).

Laboratory findings of 2 patients presenting macrocytosis

None of the subjects had a hemoglobin concentration <7.5 mmol/L. Macrocytosis (mean cell volume >100 fL) was found in 2 of the 98 subjects. These 2 subjects had very low serum concentrations of cobalamin (37 and 80 pmol/L, respectively), elevated plasma MMA (11 and 1.6 $\mu\text{mol/L}$, respectively), and elevated plasma tHcy (64.4 and 40.5 $\mu\text{mol/L}$, respectively). Total vitamin intake was 3.4 and 4.3 μg , respectively, and the gastric pH was normal (pH: 1 and 2, respectively). The results of their challenge tests were 8 and 21 min, respectively.

Table 4

Vitamin B-12 intake and vitamin B-12-related variables in supplement and nonsupplement users¹

	Nonsupplement user ($n = 45$)	Supplement user ($n = 53$)	P^2
Vitamin B-12 intake (μg)	4.3 (3.1–8.7)	6.6 (4.9–11.0)	0.001
Biochemical variables			
Cobalamin (pmol/L)	280 (209–375)	360 (265–480)	0.006
holo-TC (pmol/L)	107 (75–132)	118 (81–162)	0.11
TC saturation	0.13 (0.08–0.17)	0.15 (0.11–0.21)	0.05
MMA ($\mu\text{mol/L}$) ³	0.16 (0.13–0.21)	0.14 (0.12–0.17)	0.07
tHcy ($\mu\text{mol/L}$)	11.7 (9.2–13.7)	9.6 (7.8–11.0)	0.001

¹ All values are medians; interquartile ranges in parentheses. TC, transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

² The Mann-Whitney U test was used to compare the 2 groups.

³ Values were available for 41 nonsupplement users and 52 supplement users.

DISCUSSION

The relation between dietary intake and vitamin B-12 status is often ignored because the dietary intake of vitamin B-12 is usually far above dietary reference intakes in mixed Western diets. This intake contributes 3–30 μg vitamin B-12/d compared with the minimum recommended daily intake of 2.4 μg (2). However, recent findings in different populations suggest that the relation between vitamin B-12 intakes and vitamin B-12 status be studied further (15–17).

In the present study, we investigated the relation between total vitamin B-12 intake and vitamin B-12 status in 98 postmenopausal Danish women. We showed that vitamin B-12 status, as judged by available laboratory tests, was associated with vitamin

Table 5Overview of recent studies that investigated the relation between dietary intake and vitamin B-12 status¹

	Present study	Howard et al (14)	van Asselt et al (17)	Tucker et al (16)	Kwan et al (15)
Population					
Origin	Danish	American (mix)	Dutch	Framingham Study offspring	Hispanics
<i>n</i>	98	173	105	2999	449
Age (y)	41–75	>60	74–80	26–83	60–93
Sex	Female	Male and female	Not mentioned	Male and female	Not mentioned
Dietary intake method	7-d diet record	FFQ	Diet history	FFQ	FFQ
Markers used for correlation with vitamin B-12 intake					
Cobalamin	+	+	+	+	+
holo-TC	+	–	–	–	–
MMA	+	+	+	–	–
tHcy	+	+	+	–	–
Gastric pH	+	–	–	–	–
PGI and PGII	–	–	+	–	–
Major results	All vitamin B-12–related variables, except gastric pH, correlated with vitamin B-12 intake	None of the markers investigated correlated with vitamin B-12 intake	Plasma cobalamin, but not MMA and tHcy, correlated with vitamin B-12 intake and PGI and PGII	Plasma cobalamin correlated with vitamin B-12 intake	Plasma cobalamin correlated with vitamin B-12 intake
Study conclusion	A daily intake of 6 μg vitamin B-12 appeared to be sufficient to correct all the vitamin B-12–related variables	The high frequency of mildly abnormal cobalamin status in the elderly could not be attributed to poor intake (> 6.7 $\mu\text{g}/\text{d}$)	Inadequate dietary cobalamin intake (<4.9 μg) or the presence of severe gastritis only partly explained the high prevalence of mild cobalamin deficiency in older persons	Inadequate intake (<5.7 μg) was an important contributor to low cobalamin concentrations	The high prevalence of low vitamin B-12 status in Hispanics appeared to be attributed to an inadequate intake (15% of Hispanics consumed <1.6 $\mu\text{g}/\text{d}$)

¹ FFQ, semiquantitative food-frequency questionnaire; TC, transcobalamin; MMA, methylmalonic acid; tHcy, total homocysteine; PG, pepsinogen.

B-12 intake in this population and that the use of supplements appears to protect against concentrations of vitamin B-12–related variables in the deficiency range. A daily vitamin B-12 intake of 6 μg appears to be sufficient to normalize all of the vitamin B-12–related variables, which suggests that this dose might be more adequate for the general adult population than the current RDA of 2.4 μg .

Our study had its limitations. First, the subject group was small. Second, only postmenopausal women were studied. The strength of the study was the method used for collecting data on vitamin B-12 intake, the examination of gastric pH, and the inclusion of 4 markers of vitamin B-12 status.

Most of the surveys relating vitamin B-12 intake to biochemical markers of vitamin B-12 status have relied on the measurement of serum cobalamin only (15, 16). The use of serum cobalamin as a surrogate marker of vitamin B-12 status may lead to an overestimation of the requirement for vitamin B-12 not reflecting the point at which the cells are saturated with the vitamin. Thus, it seems of relevance to include other markers. In our study, we included holo-TC and TC saturation as well as MMA and tHcy. Holo-TC denotes the part of vitamin B-12 that is accessible for the cells of the body, and is considered to be a sensitive marker of vitamin B-12 status (20, 21, 27). The metabolic markers MMA

and tHcy reflect intracellular vitamin B-12 status, and these metabolites increase only after an intracellular deficiency has developed. They are frequently used as gold standards for the assessment of vitamin function (5, 18, 19).

Interestingly, all 4 markers studied indicated that a daily dose of $\geq 6 \mu\text{g}$ vitamin B-12 will ensure a steady concentration of the markers (Figure 1). There seems to be no difference in the amount of vitamin B-12 absorbed from an oral dose of vitamin B-12 ranging from a median intake of 6 to 15 $\mu\text{g}/\text{d}$ on the basis of the concentration of all 4 markers. It should be stressed that it is possible to increase the uptake of vitamin B-12 by administering a pharmacologic dose of the vitamin, which will induce an increase in the circulating concentrations of both vitamin B-12 and holo-TC (22) and a further decrease in the metabolic markers (E Nexø and AM Hvas, unpublished observations, 2002). The most likely explanation is that the passive absorption of $\approx 1\%$ is a significant contribution to the apparently saturated active transport (32).

The relation between dietary intake and vitamin B-12 status has been investigated in different populations, with conflicting results as shown in **Table 5** (14–17). Our results are consistent with those of the Framingham Study and with the study performed in a group of Hispanic persons (15, 16). In these 2 large

studies, plasma cobalamin concentrations were associated with total vitamin B-12 intake; in both studies, plasma cobalamin leveled off at intakes of vitamin B-12 that were considerably higher (≈ 10 and $7 \mu\text{g/d}$, respectively) than the RDA of $2.4 \mu\text{g/d}$. Our study further confirmed the need for a vitamin B-12 intake $>2.4 \mu\text{g/d}$ because not only plasma cobalamin, but also 3 other markers of vitamin B-12 deficiency (holo-TC, MMA, and tHcy concentrations), leveled off at an intake of $\approx 6 \mu\text{g/d}$ or higher. Also, in Dutch elders, van Asselt et al (17) found that total vitamin B-12 intake correlated with plasma vitamin B-12. However, in that study, such a correlation was not found with plasma MMA and tHcy. In contrast with the studies referred to so far, an earlier study by Howard et al (14) found no correlation between total intake and either serum cobalamin or metabolite concentrations. Conflicting results on this issue might be explained by the different dietary profiles or habits of the study populations, because the subjects in the abovementioned studies were of different ethnicities (Table 5).


It has also been speculated that previously observed conflicting results might be due to the application of the different assessment methods of vitamin B-12 intake, because different methods—eg, diet records, 24-h dietary recall, dietary histories, and semiquantitative food-frequency questionnaires—have been used for this purpose, as shown in Table 5 (14–17). In our study, we preferred diet records over the other 3 techniques for 2 reasons. This method does not rely heavily on memory, as do food-frequency questionnaires; but most importantly, this method is believed to give the most valid estimates of an individual's usual intake (33, 34).

On the basis of our data and previously published data, it seems that a vitamin B-12 intake between 6 and $10 \mu\text{g}$ might help correct subnormal vitamin B-12 status (15, 16). A $6\text{-}\mu\text{g}$ intake was previously suggested to be a more desirable dietary goal for vitamin B-12 (35). It is important to note, however, that this amount is considerably greater than the current RDA for vitamin B-12. When establishing the RDA, a major concern is to ensure the minimum of the vitamin required to avoid the risk of developing vitamin deficiency. The current estimates of adequate intake are based on freedom from hematologic and neurologic disorders; it is not known whether recommended intakes are adequate to prevent the biochemical disturbances recently recognized to be more widespread and sometimes associated with more subtle clinical disorders. The RDA for vitamin B-12 is $2.4 \mu\text{g/d}$, but it is debatable whether this recommendation is too low. On the basis of the results of our study and those of 2 large studies (15, 16), it might be concluded that a daily intake of $6\text{--}10 \mu\text{g}$ is required to ensure a steady concentration of circulating cobalamin.

Loss of stomach acidity, resulting from type B atrophic gastritis, has been implicated in impaired vitamin B-12 status (36, 37). We determined the participants' gastric acid secretory ability under conditions simulating ingestion of food by using Heidelberg's pH capsule system. This system was previously shown to offer a convenient and accurate testing method for evaluating gastric function (25). In our study, 64% of the participants were considered normal on the basis of their gastric pH (<3) or the results of a challenge test (ability of gastric reacidification within 20 min after intake of bicarbonate). One-third of our study group (36%) had a moderate increase in pH (pH: 3–5) or a prolonged challenge test, which indicated that these subjects had at least a

moderately decreased gastric function. Interestingly, this decrease did not seem to influence the subjects' ability to absorb vitamin B-12 from the diet. We found no significant difference in vitamin B-12 intakes and vitamin B-12-related variables between subjects with normal gastric pH and those with a higher pH. Thus, on the basis of our data, a mild-to-moderate decrease in gastric acidity does not impair vitamin B-12 absorption. This finding agrees with those of previous studies in older vitamin B-12-deficient patients, in whom vitamin B-12 absorption was found to be impaired only in patients with severe atrophic gastritis and not in patients with mild-to-moderate atrophic gastritis (17, 37, 38).

The median total (diet plus supplement) vitamin B-12 intake in our population was comparable with that of free-living American and Dutch subjects (17, 39). It is of incidental interest to note that nearly one-half of our surveyed subjects used vitamin supplements. Most commercially available multivitamin preparations in Denmark contain small amounts of vitamin B-12 (median content: $1 \mu\text{g}$; range: $1\text{--}20 \mu\text{g}$), compared with $6 \mu\text{g}$ in the United States and $2 \mu\text{g}$ in the Netherlands (17, 39). The subjects in our study who took vitamin B-12 supplements (dose: $1\text{--}18 \mu\text{g/d}$) had significantly higher TC saturation and serum concentrations of vitamin B-12 and lower MMA and tHcy concentrations. However, the supplement users' dietary vitamin B-12 intake was the same as that of the nonusers, which indicated that the supplement users did not initially have a poor intake.

In conclusion, our results, together with those of others, strongly suggest that the RDA of $2.4 \mu\text{g/d}$ should be increased. We showed that a daily dose of $6 \mu\text{g}$ vitamin B-12 ensures a steady concentration of serum cobalamin, holo-TC, and the metabolic markers tHcy and MMA. Nothing is gained by increasing this dose in individuals with a normal gastric acid production or with a moderately decreased ability to acidify the gastric juice. We further suggest that reference intervals for the markers of vitamin B-12 deficiency should be calculated on the basis of data obtained from individuals receiving $\geq 6 \mu\text{g}$ vitamin B-12/d, except for those taking pharmacologic doses. 

The excellent technical assistance of Anna-Lisa Christensen is warmly acknowledged. Special thanks goes to the nutrition students and staff who undertook the diet analysis and coding: Mette Munkholm, Jeanette Joergensen, Karna Riis Larsen, and Louise Fisker.

MVB planned the study, participated in the analysis of the TC-related variables, performed the statistical analyses, interpreted the data, and wrote the article. EL-O wrote the original protocol, supervised and participated in the recruitment of the patients, processed the blood and the 7-d diet records, measured gastric pH, and approved the final manuscript. JM planned and performed the MMA and tHcy analyses and approved the final manuscript. EN planned and performed the TC-related analysis, helped interpret the data, helped write the manuscript, and approved the final manuscript. None of the authors had a financial conflict of interest in relation to this study.

REFERENCES

1. Institute of Medicine. Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B-6, folate, vitamin B-12, pantothenic acid, biotins and choline. Washington, DC: National Academy Press, 1998.
2. Andres E, Loukili NH, Noel E, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. *Can Med Assoc J* 2004;171:251–9.
3. Lindenbaum J, Heaton 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.
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. Clarke R, Grimley EJ, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Aging* 2004;33:34–41.
7. 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.
8. 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.
9. Nilsson-Ehle H, Jagenburg R, Landahl S, Lindstedt S, Svanborg A, Westin J. Serum cobalamins in the elderly: a longitudinal study of a representative population sample from age 70 to 81. *Eur J Haematol* 1991;47:10–6.
10. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc* 1992;40:1197–204.
11. Elsborg L, Lung V, Bastrup-Madsen P. Serum vitamin B12 levels in the aged. *Acta Med Scand* 1976;200:309–14.
12. 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.
13. Ryan AS, Craig LD, Finn SC. Nutrient intakes and dietary patterns of older Americans: a national study. *J Gerontol* 1992;47:M145–50.
14. 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.
15. 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.
16. 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.
17. 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.
18. Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. *Clin Chem* 2000;46:1277–83.
19. Nexø E, Hansen M, Rasmussen K, Lindgren A, Grasbeck R. How to diagnose cobalamin deficiency. *Scand J Clin Lab Invest Suppl* 1994;219:61–76.
20. Hvas AM, Nexø E. Holotranscobalamin as a predictor of vitamin B12 status. *Clin Chem Lab Med* 2003;41:1489–92.
21. Lloyd-Wright Z, Hvas AM, Møller J, Sanders TA, Nexø E. Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. *Clin Chem* 2003;49:2076–8.
22. Nexø E, Hvas AM, Bleie O, et al. Holo-transcobalamin is an early marker of changes in cobalamin homeostasis. A randomized placebo-controlled study. *Clin Chem* 2002;48:1768–71.
23. Lydeking-Olsen E, Beck-Jensen JE, Satchell KD, Holm-Jensen T. Soy milk or progesterone for prevention of bone loss—a 2 year randomized, placebo-controlled trial. *Eur J Nutr* 2004;43:246–57.
24. Noeller HG. The use of a radiotracer capsule for the measurement of gastric pH. *German Med Monthly* 1961;6:3.
25. Russell TL, Berardi RR, Barnett JL, et al. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. *Pharm Res* 1993;10:187–96.
26. Nexø E, Christensen AL, Petersen TE, Fedosov SN. Measurement of transcobalamin by ELISA. *Clin Chem* 2000;46:1643–9.
27. Nexø E, Christensen AL, Hvas AM, Petersen TE, Fedosov SN. Quantification of holo-transcobalamin, a marker of vitamin B12 deficiency. *Clin Chem* 2002;48:561–2.
28. Rasmussen K. Solid-phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable-isotope-dilution method. *Clin Chem* 1989;35:260–4.
29. Rasmussen K, Møller J, Lyngbak M, Pedersen AM, Dybkjaer L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. *Clin Chem* 1996;42:630–6.
30. Hvas AM, Ellegaard J, Nexø E. Vitamin B12 treatment normalizes metabolic markers but has limited clinical effect: a randomized placebo-controlled study. *Clin Chem* 2001;47:1396–404.
31. Babson AL, Olson DR, Palmieri T, Ross AF, Becker DM, Mulqueen PJ. The IMMULITE assay tube: a new approach to heterogeneous ligand assay. *Clin Chem* 1991;37:1521–2.
32. Bor MV, Nexø E, Hvas AM. Holo-transcobalamin concentration and transcobalamin saturation reflect recent vitamin B12 absorption better than does serum vitamin B12. *Clin Chem* 2004;50:1043–9.
33. Nelson M. Assessment of food composition and nutrient intake. In: Margetts BM, Nelson M, eds. *Design concepts in nutritional epidemiology*. London, United Kingdom: Oxford University Press, 1997:123–68.
34. Potosky AL, Block G, Hartman AM. The apparent validity of diet questionnaires is influenced by number of diet-record days used for comparison. *J Am Diet Assoc* 1990;90:810–3.
35. Langseth L, Vanderveen J, Frattali V. The new reference daily intakes: for better or for worse? *Nutr Rev* 1992;50:119–24.
36. Hurwitz A, Brady DA, Schaal SE, Samloff IM, Dedon J, Ruhl CE. Gastric acidity in older adults. *JAMA* 1997;278:659–62.
37. Krasinski SD, Russell RM, Samloff IM, et al. Fundic atrophic gastritis in an elderly population. Effect on hemoglobin and several serum nutritional indicators. *J Am Geriatr Soc* 1986;34:800–6.
38. van Asselt DZ, van den Broek WJ, Lamers CB, Corstens FH, Hoefnagels WH. Free and protein-bound cobalamin absorption in healthy middle-aged and older subjects. *J Am Geriatr Soc* 1996;44:949–53.
39. Garry PJ, Goodwin JS, Hunt WC. Folate and vitamin B12 status in a healthy elderly population. *J Am Geriatr Soc* 1984;32:719–26.

