

Oral Cyanocobalamin Supplementation in Older People With Vitamin B₁₂ Deficiency

A Dose-Finding Trial

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Background: Supplementation with high doses of oral cobalamin is as effective as cobalamin administered by intramuscular injection to correct plasma markers of vitamin B₁₂ deficiency, but the effects of lower oral doses of cobalamin on such markers are uncertain.

Methods: We conducted a randomized, parallel-group, double-blind, dose-finding trial to determine the lowest oral dose of cyanocobalamin required to normalize biochemical markers of vitamin B₁₂ deficiency in older people with mild vitamin B₁₂ deficiency, defined as a serum vitamin B₁₂ level of 100 to 300 pmol/L (135-406 pg/mL) and a methylmalonic acid level of 0.26 μmol/L or greater. We assessed the effects of daily oral doses of 2.5, 100, 250, 500, and 1000 μg of cyanocobalamin administered for 16 weeks on biochemical markers of vitamin B₁₂ deficiency in 120 people. The main outcome measure was the dose of oral cyanocobalamin that pro-

duced 80% to 90% of the estimated maximal reduction in the plasma methylmalonic acid concentration.

Results: Supplementation with cyanocobalamin in daily oral doses of 2.5, 100, 250, 500, and 1000 μg was associated with mean reductions in plasma methylmalonic acid concentrations of 16%, 16%, 23%, 33%, and 33%, respectively. Daily doses of 647 to 1032 μg of cyanocobalamin were associated with 80% to 90% of the estimated maximum reduction in the plasma methylmalonic acid concentration.

Conclusion: The lowest dose of oral cyanocobalamin required to normalize mild vitamin B₁₂ deficiency is more than 200 times greater than the recommended dietary allowance, which is approximately 3 μg daily.

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VITAMIN B₁₂ DEFICIENCY, DUE to intrinsic factor deficiency, hypochlorhydria, or food-bound malabsorption, affects mainly older people.¹⁻³ Symptoms of vitamin B₁₂ deficiency include anemia, neuropathy, and neuropsychiatric disorders, but it more commonly leads to nonspecific tiredness or malaise in older people.³⁻⁵ Approximately 20% of the circulating plasma vitamin B₁₂ is transported as holotranscobalamin (holoTC), which can be taken up by all cells, and the remaining 80% is transported as haptocorrin, which is believed not to be metabolically active.^{6,7} In the cell, vitamin B₁₂ acts as a cofactor for methionine synthase, an enzyme that remethylates homocysteine (Hcy) to methionine, and for methylmalonyl-coenzyme A mutase, an enzyme that converts methylmalonyl-coenzyme A to succinyl-coenzyme A. In the setting of vitamin B₁₂ deficiency, methylmalonyl-

coenzyme A is hydrolyzed to methylmalonic acid (MMA). Thus, elevated plasma concentrations of MMA and total Hcy (tHcy) can be used as biochemical markers to aid in the diagnosis of vitamin B₁₂ deficiency and to monitor the response to cobalamin supplementation.^{8,9}

Active absorption of protein-bound vitamin B₁₂ in food is impaired in individuals with vitamin B₁₂ deficiency, but approximately 1% of orally administered crystalline cobalamin is absorbed by passive diffusion.^{3,10} Consequently, vitamin B₁₂ deficiency is usually treated by monthly intramuscular injections of 1000 μg of hydroxycobalamin or cyanocobalamin. However, daily dietary supplementation with 1000 to 2000 μg of cyanocobalamin administered orally has been shown to be as effective as¹¹ or even more effective than¹² cobalamin administered by intramuscular injections to correct biochemical markers of vitamin B₁₂ deficiency. Previous

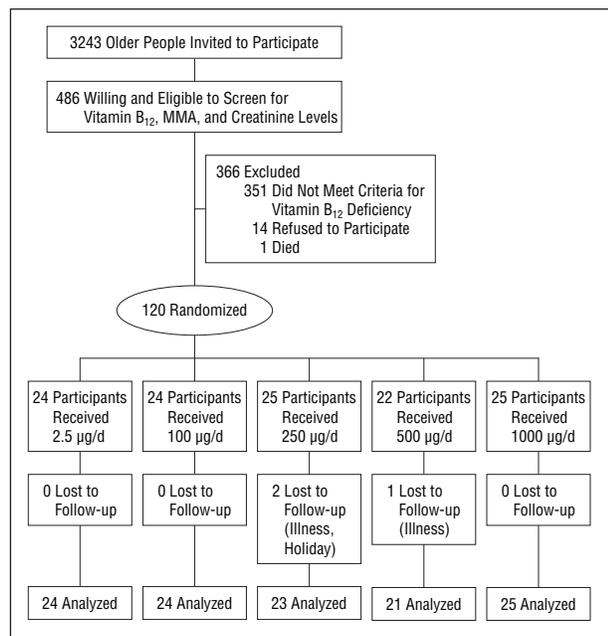


Figure 1. Recruitment procedure and flow of participants through the study. MMA indicates methylmalonic acid.

trials^{13,14} that examined the effects on biochemical markers of vitamin B₁₂ status of daily dietary oral supplements ranging from 10 to 100 µg of cobalamin did not determine the lowest effective dose required to correct vitamin B₁₂ deficiency. A major knowledge gap concerns the lowest dose of oral cobalamin supplementation that will normalize elevated MMA concentrations. The aim of the present trial is to determine the lowest dose of cyanocobalamin that is required for a maximal reduction in MMA concentrations in a randomized, parallel-group, double-blind, controlled, dose-finding study in older people with mild vitamin B₁₂ deficiency. The doses used cover the total spectrum from the recommended dietary allowance to the commonly used dose in cobalamin injections.

METHODS

PARTICIPANTS

Free-living older people (aged ≥70 years) were recruited in the Wageningen area of the Netherlands through a database of individuals who had previously indicated interest in participation in such a trial. Individuals with self-reported anemia, surgery or diseases of the stomach or small intestine, or any life-threatening diseases were excluded, as were individuals who reported current use of multivitamin supplements containing folic acid, cobalamin, or pyridoxine hydrochloride and those currently receiving cobalamin injections. The concomitant use of medications known to affect vitamin B₁₂ absorption (eg, proton pump inhibitors, H₂-antagonists, and metformin) was permitted if the medication had been provided at least 3 months before enrollment and was scheduled to be continued for the duration of the trial. Individuals who fulfilled these criteria were invited to give a blood sample at a screening visit. People were eligible for the trial if their serum vitamin B₁₂ concentration was between 100 and 300 pmol/L (135-406 pg/mL), their plasma

MMA concentration was 0.26 µmol/L or greater, and their serum creatinine concentration was 120 µmol/L or less (≤1.4 mg/dL), the latter reflecting normal kidney function.³ **Figure 1** shows the recruitment procedure and the flow of participants through the phases of the study. The study protocol was approved by the Medical Ethical Committee of Wageningen University, and written informed consent was obtained from all participants before the screening visit.

PROTOCOL

Eligible people who agreed to be enrolled in a 3- to 4-week placebo run-in period before randomization and who had proved to be compliant (>90% intake of capsules) during the run-in period were randomized to receive 16 weeks of treatment in a parallel-group design with daily oral doses of 2.5, 100, 250, 500, or 1000 µg of cyanocobalamin (Figure 1). The doses selected for this study were based on the recommended dietary allowance of the Netherlands, which was 2.5 µg daily at the start of the trial, and 1000 µg, which served as a positive control and is administered in the form of intramuscular injections to treat vitamin B₁₂ deficiency. The 100-, 250-, and 500-µg doses were chosen to provide an optimum dose-response curve. We did not include a placebo in the study design for ethical reasons. Randomization was based on plasma MMA concentration at the screening visit, age, and sex. We used strata to ensure a balanced distribution of participants with respect to MMA concentration (0.26–0.309, 0.31–0.359, and ≥0.36 µmol/L), age (≤75 and >75 years), and sex. All investigators and participants were masked to study treatment.

Assuming a within-person SD for MMA of 0.25 µmol/L for changes in plasma MMA concentrations induced by hydroxycobalamin supplementation,¹⁵ sample size calculations indicated that 17 participants per group provided 80% power to detect an absolute difference of 0.22 µmol/L in MMA concentrations among the treated groups. To control for an estimated dropout rate of 23%,¹⁶ at least 20 participants were to be enrolled in each group.

Cyanocobalamin was administered in capsules that were identical in appearance, smell, and taste in all treatment groups. The mean (SD) measured doses of cyanocobalamin for the capsules intended to contain 2.5, 100, 250, 500, and 1000 µg were 3 (single pooled assessment), 112 (4.7), 270 (3.4), 553 (1.7), and 860 (9.7) µg, respectively.

Participants were asked to maintain their regular diet and to avoid the use of supplements containing B vitamins during the trial. All participants were asked to complete a diary to record their daily intake of capsules, their use of nonstudy medications, and the occurrence of any new illnesses during the trial. No adverse events were reported. Compliance was checked by counting the unused capsules remaining in the capsule dispensers and by verifying pill counts in the participants' diaries. Mean compliance was 98%, and because compliance for each participant was greater than 90%, data for all participants were included in the analyses.

DATA COLLECTION AND ANALYTICAL METHODS

A blood sample was collected at the screening and randomization visits and after 8 and 16 weeks of active treatment. Height and weight were also measured at the randomization visit. Participants were asked to be fasting at the randomization visit but were allowed to eat a light breakfast (without fruit, fruit juices, meat, or eggs) at least 1 hour before attending the screening and follow-up visits. The study was carried out between February 27, 2002, and February 28, 2003. A sample of blood for

the subsequent measurement of MMA (the primary outcome measure) and tHcy and holoTC (secondary outcome measures) concentrations was collected in a 10-mL vacutainer containing EDTA. This blood sample was placed in ice water and was centrifuged at 2600 rpm for 10 minutes at 4°C within 30 minutes of collection. All plasma samples were stored at -80°C before laboratory analyses. Plasma concentrations of MMA and tHcy were determined by gas chromatography-mass spectroscopy after derivatization with methylchloroformate.¹⁷ The plasma concentration of holoTC was measured using the AXIS-Shield radioimmunoassay method.¹⁸ A blood sample was collected in a 5-mL gel tube for measurement of serum vitamin B₁₂ (secondary outcome measure) and creatinine levels. The serum samples for vitamin B₁₂ determination were stored at room temperature in the dark for measurement later that day using the Immulite 2000 cobalamin method (Diagnostic Products Corp, Los Angeles, Calif).¹⁹ In addition, at the randomization visit, a sample of blood was collected in 5-mL evacuated tubes containing EDTA and were stored at room temperature for measurement later that day of hematologic variables (hemoglobin level, hematocrit level, mean cell volume, and hypersegmentation of neutrophils) and plasma folate concentrations.

STATISTICAL ANALYSIS

Baseline concentrations of the biochemical variables were calculated as the average of the measurements recorded at the screening and randomization visits for each individual. The proportional changes in plasma concentrations of MMA, tHcy, and holoTC and in serum concentrations of vitamin B₁₂ were calculated by dividing each participant's absolute change in concentration after 16 weeks of treatment by their concentration at baseline. The lowest dose of oral cyanocobalamin required to achieve a maximum reduction in MMA concentrations was determined using a closed test procedure.²⁰ The Kruskal-Wallis test was used to investigate whether differences in median proportional changes were present among dose groups, and the Mann-Whitney test was used to investigate between which 2 dose groups differences in the median changes occurred. In addition, curve fitting that plots the proportional reductions in MMA concentrations against the incremental doses of cyanocobalamin administered was used to assess the dose-response relationship. The best-fit dose-response curves showed a 1-phase exponential decay estimated by the following non-linear regression equation:

$$\text{Change (\%)} = (\text{Top} - \text{Bottom}) \times \text{Exp}(-k \times \text{Cyanocobalamin Dose}) + \text{Bottom}.$$

This regression equation was used to identify the lowest oral dose of cyanocobalamin required to achieve a maximal reduction in MMA concentrations. This dose was defined as the dose that produces 80% to 90% of the maximum estimated reduction in plasma MMA concentrations. Statistical analyses were conducted using SAS statistical software (SAS Institute Inc, Cary, NC), and curve fitting was performed using GraphPad Prism software (GraphPad Software Inc, San Diego, Calif).

RESULTS

CHARACTERISTICS OF PARTICIPANTS

Selected characteristics of the study participants are given in **Table 1**. At baseline, the study population was, on average, not undernourished since the median body mass index (calculated as weight in kilograms divided by the square of height in meters) was 25.3.²¹ There were no

Table 1. Characteristics of the Study Population at Baseline*

	Value
Descriptive characteristics	
Age, y	80 (7) [70-94]†
Sex, male	44
Use of medication‡	53
Anemia§	12
Macrocytosis	6
Hypersegmentation¶	57
Hypersegmentation#	7
BMI, kg/m ²	25.3 (4.6) [19.7-35.3]
Folate, nmol/L	6.4 (4.8) [1.1-18.8]
Creatinine, μmol/L	88 (20) [53-122]
Biochemical characteristics	
MMA, μmol/L	0.33 (0.16) [0.23-5.16]
tHcy, μmol/L	14.5 (5.7) [7.8-114.0]
holoTC, pmol/L	47 (35) [8-121]
Vitamin B ₁₂ , pmol/L	208 (87) [113-362]

Abbreviations: BMI, body mass index; holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine.

SI conversion factors: To convert creatinine to milligrams per deciliter, divide by 88.4; folate to nanograms per milliliter, divide by 2.266; MMA to milligrams per liter, divide by 8.475; tHcy to milligrams per liter, divide by 7.397; vitamin B₁₂ to picograms per milliliter, divide by 0.738.

*Data are given as median (interquartile range [Q3 - Q1]) [absolute range] or as prevalence.

†One participant was aged 64 years.

‡Use of proton pump inhibitors, histamine₂-antagonists, or metformin.

§Defined as a hemoglobin level less than 13 g/dL (<8.1 mmol/L) in men and less than 12 g/dL (<7.4 mmol/L) in women.

||Defined as a mean corpuscular volume greater than 100 fL.

¶Defined as 5-lobed neutrophils/100 neutrophils.

#Defined as 6-lobed neutrophils/100 neutrophils.

significant differences in the mean concentrations of MMA, tHcy, holoTC, and vitamin B₁₂ between the screening and randomization visits. The median baseline concentrations of serum vitamin B₁₂ and plasma MMA were well matched by treatment groups, indicating that the randomization procedure had been successful. At baseline, serum vitamin B₁₂ concentrations were correlated with plasma holoTC ($\rho = 0.53$; $P < .001$), plasma MMA ($\rho = -0.34$; $P < .001$), and tHcy ($\rho = -0.25$; $P = .006$) concentrations. Plasma holoTC concentrations were correlated with MMA ($\rho = -0.41$) and plasma tHcy ($\rho = -0.38$) concentrations ($P < .001$ for both), whereas plasma tHcy concentrations were correlated with MMA concentrations ($\rho = 0.85$; $P < .001$) but not with folate concentrations ($\rho = -0.01$; $P = .91$).

ABSOLUTE EFFECTS OF DIFFERENT DOSES OF CYANOCOBALAMIN

On average, the absolute decreases in plasma MMA and tHcy concentrations and the absolute increases in plasma vitamin B₁₂ and holoTC concentrations increased with increasing doses of cyanocobalamin (**Table 2**). The reductions in MMA concentrations in all cyanocobalamin-treated groups were significant during the first 8 weeks of treatment and remained stable during the second 8 weeks of treatment. The absolute reduction in MMA concentrations of at least 0.22 μmol/L observed after 8 and

Table 2. Concentrations of MMA, tHcy, holoTC, and Vitamin B₁₂ at 8 and 16 Weeks and Absolute Effects After 8 and 16 Weeks of Cyanocobalamin Supplementation by Intervention Group

	Cyanocobalamin Dose, µg/d*	8 Weeks			16 Weeks		
		Participants, No.	Median (IQR)	Response (95% CI)	Participants, No.	Median (IQR)	Response (95% CI)
MMA, µmol/L	2.5	24	0.29 (0.13)	-0.04 (-0.06 to -0.02)	24	0.28 (0.07)	-0.07 (-0.09 to -0.04)
	100	24	0.29 (0.06)	-0.09 (-0.14 to -0.04)	24	0.30 (0.07)	-0.08 (-0.13 to -0.03)
	250	25	0.25 (0.11)	-0.13 (-0.21 to -0.05)	23	0.28 (0.11)	-0.14 (-0.22 to -0.05)
	500	22	0.25 (0.09)	-0.22 (-0.37 to -0.07)	21	0.26 (0.03)	-0.23 (-0.37 to -0.08)
	1000	25	0.26 (0.04)	-0.31 (-0.69 to 0.07)	25	0.25 (0.04)	-0.34 (-0.74 to 0.06)
tHcy, µmol/L	2.5	24	14.4 (8.8)	-0.7 (-1.4 to -0.1)	24	14.0 (6.5)	-0.9 (-1.7 to -0.1)
	100	24	13.7 (4.0)	-0.5 (-1.1 to -0.1)	24	13.6 (4.5)	-0.7 (-1.5 to 0.1)
	250	25	14.1 (5.3)	-1.0 (-1.7 to -0.3)	23	13.8 (6.1)	-1.4 (-2.4 to -0.4)
	500	22	13.8 (6.0)	-1.9 (-2.9 to -1.0)	21	13.1 (5.4)	-2.4 (-3.4 to -1.5)
	1000	25	11.1 (4.2)	-5.1 (-11.3 to 1.1)	25	10.4 (5.1)	-5.7 (-13.1 to 1.7)
holoTC, pmol/L	2.5	24	67 (43)	8 (2 to 15)	24	63 (40)	8 (3 to 14)
	100	24	70 (43)	26 (16 to 35)	24	77 (38)	28 (19 to 38)
	250	25	85 (40)	40 (29 to 50)	23	94 (67)	48 (36 to 60)
	500	22	97 (39)	49 (39 to 59)	21	106 (48)	60 (49 to 71)
	1000	25	127 (60)	67 (55 to 79)	25	132 (43)	73 (61 to 85)
Vitamin B ₁₂ , pmol/L	2.5	24	269 (81)	70 (29 to 88)	24	290 (119)	64 (20 to 74)
	100	24	300 (98)	108 (72 to 145)	24	279 (184)	129 (74 to 183)
	250	25	327 (158)	128 (100 to 157)	23	347 (188)	153 (111 to 195)
	500	22	372 (47)	182 (141 to 224)	21	404 (293)	212 (149 to 274)
	1000	25	449 (334)	248 (185 to 311)	25	574 (418)	328 (247 to 109)

Abbreviations: CI, confidence interval; holoTC, holotranscobalamin; IQR, interquartile range (Q3 - Q1); MMA, methylmalonic acid; tHcy, total homocysteine. SI conversion factors: To convert MMA to milligrams per liter, divide by 8.475; tHcy to milligrams per liter, divide by 7.397; vitamin B₁₂ to picograms per milliliter, divide by 0.738.

*The treatment groups of 2.5, 100, 250, 500, and 1000 µg of cyanocobalamin contained, on average, 3, 112, 270, 553, and 860 µg of cyanocobalamin, respectively.

16 weeks of supplementation with 500 and 1000 µg of cyanocobalamin indicated that the study had sufficient power to detect differences among the randomly allocated doses of cyanocobalamin. In addition, the absolute effects of cyanocobalamin supplementation on MMA concentrations were assessed using the proportion of the trial population that achieved an MMA concentration below the laboratory reference interval for MMA of 0.26 µmol/L (J.S., oral communication, February 15, 2002). Daily supplementation with 2.5, 100, 250, 500, or 1000 µg of cyanocobalamin resulted in reductions in MMA concentrations to below the reference interval of 0.26 µmol/L in 21%, 38%, 52%, 62%, and 76% of the participants, respectively.

PROPORTIONAL EFFECTS OF DIFFERENT DOSES OF CYANOCOBALAMIN

The determination of the lowest dose of cyanocobalamin associated with the maximum reductions in MMA levels or maximum increases in holoTC levels using the closed test procedure¹⁹ (which defined the optimum dose as that dose that differed significantly from the lower doses but not from the higher doses) concluded that the intended daily dose of 500 µg of cyanocobalamin was the lowest oral dose associated with a maximum reduction in MMA concentrations and a maximum increase in holoTC concentrations. The proportional reductions in MMA concentrations after daily supplementation with 2.5, 100, 250, and 500 µg of cyanocobalamin differed significantly from each other, whereas the proportional re-

ductions in MMA concentrations did not differ significantly from each other after daily supplementation with 500 and 1000 µg of cyanocobalamin ($P = .2$).

The proportional decreases in MMA and tHcy levels and the proportional increases in vitamin B₁₂ and holoTC concentrations observed with incremental doses of cyanocobalamin after 16 weeks of supplementation are shown in **Figure 2**. The mean reduction in plasma MMA concentrations after 16 weeks of supplementation compared with baseline varied from 16% to 33% in the groups receiving 2.5 to 1000 µg of cyanocobalamin per day. The proportional reduction in MMA after 16 weeks of supplementation was calculated using the following formula:

$$25.82 \times \text{Exp}(-0.0018626 \times \text{Cyanocobalamin Dose}) - 39.6.$$

The lowest daily oral dose of cyanocobalamin that resulted in 80% to 90% of the maximum reduction in MMA concentrations varied from 647 to 1032 µg. On average, such doses of cyanocobalamin reduced plasma MMA concentrations by approximately 33%.

COMMENT

The results of this dose-finding trial demonstrate that the lowest oral dose of cyanocobalamin associated with 80% to 90% of the estimated maximum reduction in plasma MMA concentrations in an older population with mild vitamin B₁₂ deficiency varied from 647 to 1032 µg/d, and such doses reduce plasma MMA concentrations by approximately 33%. However, daily doses of 2.5 to 250 µg

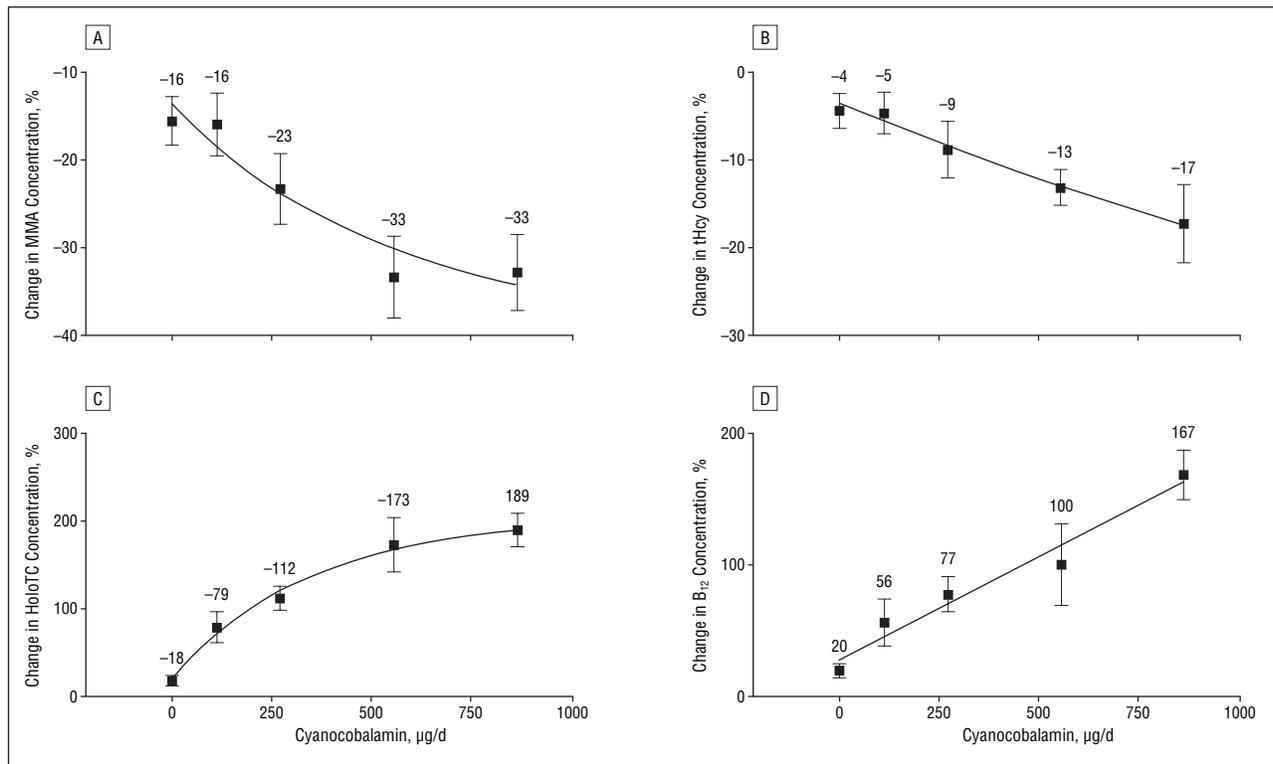


Figure 2. Proportional effects of different doses of cyanocobalamin on mean methylmalonic acid (MMA) (A), total homocysteine (tHcy) (B), holotranscobalamin (holoTC) (C), and vitamin B₁₂ (D) concentrations after 16 weeks of supplementation. Error bars represent SD.

of cyanocobalamin produce statistically significant reductions in MMA concentrations of 16% to 23% in this population. The conclusions of this trial are based primarily on reductions in plasma MMA concentrations because MMA reflects tissue levels of vitamin B₁₂.^{3,9,12}

Comparable proportional increases in concentrations of serum vitamin B₁₂ and plasma holoTC were observed in response to the different doses of cyanocobalamin. The dose-finding curve for holoTC demonstrated that daily oral doses of 527 to 759 µg of cyanocobalamin resulted in 80% to 90% of the estimated maximum increase in holoTC concentrations.

In contrast to the dose-finding curves for MMA and holoTC, the curve for tHcy does not show a plateau effect. This finding may be related to the selection criteria, which did not include tHcy because tHcy is not a specific marker of vitamin B₁₂ status but is also affected by folate status and a variety of lifestyle factors.²² Most likely, tHcy concentrations in these participants are less responsive to cyanocobalamin supplementation. Therefore, we cannot assume that, based on these data, a full dose-response curve can be fitted for tHcy.

The conclusions of this trial may reflect the definition of vitamin B₁₂ deficiency and the variable absorption of vitamin B₁₂ in older people. The diagnosis of vitamin B₁₂ deficiency is complicated by the limitations of current assay techniques because serum vitamin B₁₂ concentrations alone may misclassify a significant proportion of individuals with vitamin B₁₂ deficiency.^{3,9,23} Moreover, there is no consensus about the cutoff points for vitamin B₁₂ deficiency or metabolites to define vitamin B₁₂ deficiency. The present trial enrolled healthy older people with mild vitamin B₁₂ defi-

ciency, defined as serum vitamin B₁₂ levels of 100-300 pmol/L (135 to 406 pg/mL) combined with plasma MMA levels of 0.26 µmol/L or greater in individuals without renal dysfunction. Analysis of a subgroup of participants with more severe vitamin B₁₂ deficiency (using MMA concentrations ≥0.32 µmol/L at baseline, present in 67 participants) resulted in more pronounced changes in MMA, tHcy, holoTC, and vitamin B₁₂ concentrations and confirmed the results of the closed test procedure (data not shown). According to the corresponding dose-finding curves for MMA and holoTC, 830 µg/d would provide 80% of the maximal reduction in MMA levels, and 449 µg/d would provide 80% of the maximal increase in holoTC levels.

Vitamin B₁₂ can be absorbed actively, with a limited capacity of approximately 3 µg per meal in the presence of intrinsic factor and normal functioning of the stomach, pancreas, and terminal ileum. However, the bioavailability of crystalline vitamin B₁₂ is unaffected by the underlying causes of vitamin B₁₂ deficiency, and approximately 1% of crystalline cobalamin (typically used in oral cobalamin supplements) is absorbed by passive absorption.³ This study did not distinguish the extent to which differences in individual responses were due to active as opposed to passive absorption of vitamin B₁₂.

The results of this trial differ from those of Seal et al,¹³ who compared the effects on serum vitamin B₁₂ and tHcy concentrations of oral cyanocobalamin using daily oral doses of 10 to 50 µg or placebo for 4 weeks in 31 older people who had a pretreatment vitamin B₁₂ concentration between 100 and 150 pmol/L (135-203 pg/mL). Seal et al¹³ showed that supplementation with 50 µg/d increased serum vitamin B₁₂ levels but had no significant ef-

fects on tHcy concentrations. Rajan et al¹⁴ compared the effects of sequential daily treatment with 25, 100, and 1000 µg of cyanocobalamin for 6 weeks on serum vitamin B₁₂ and plasma MMA concentrations in 23 older people who had a pretreatment vitamin B₁₂ concentration less than 221 pmol/L (<299 pg/mL) in combination with an MMA concentration greater than 0.27 µmol/L. Rajan et al¹⁴ reported that daily treatment with 25 or 100 µg of cyanocobalamin lowered, but did not normalize, MMA levels and that a daily dose of 1000 µg of cyanocobalamin was required to normalize MMA concentrations.

The results of this trial indicate that the lowest dose of oral cyanocobalamin required to normalize biochemical markers of mild vitamin B₁₂ deficiency in older people with a mild vitamin B₁₂ deficiency is more than 200 times greater than the recommended dietary allowance for vitamin B₁₂ of approximately 3 µg/d. Clinical trials are currently assessing the effects of high doses of oral cobalamin on markers of cognitive function and depression. If such trials can demonstrate that the reported associations of vitamin B₁₂ deficiency with cognitive impairment or depression are causal and reversible by treatment,²⁴ the relevance of correction of vitamin B₁₂ deficiency in older people could be substantial. However, the present trial demonstrates that much higher doses of cyanocobalamin are required to normalize vitamin B₁₂ deficiency than were previously believed.

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