

# Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects<sup>1-3</sup>

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## ABSTRACT

**Background:** The reason for the high prevalence of mild cobalamin (vitamin B-12) deficiency in the elderly is poorly understood.

**Objective:** We aimed to determine the reason for this high prevalence.

**Design:** We examined cobalamin intake, the presence and severity of atrophic gastritis, the presence of *Helicobacter pylori* infection, and plasma cobalamin and methylmalonic acid (MMA) concentrations in 105 healthy, free-living, older subjects aged 74–80 y.

**Results:** Mild cobalamin deficiency, ie, low to low-normal plasma cobalamin concentrations (<260 pmol/L) and elevated plasma MMA concentrations (>0.32  $\mu$ mol/L), were found in 23.8% of subjects; 25.7% of subjects were not cobalamin deficient (plasma cobalamin  $\geq$ 260 pmol/L and plasma MMA  $\leq$ 0.32  $\mu$ mol/L). Six subjects (5.8%), including 1 with mild cobalamin deficiency, had dietary cobalamin intakes below the Dutch recommended dietary intake of 2.5  $\mu$ g/d. Mildly cobalamin-deficient subjects had lower total (diet plus supplements) cobalamin intakes (median: 4.9  $\mu$ g/d; 25th and 75th percentiles: 3.9, 6.4) than did non-cobalamin-deficient subjects (median: 6.3  $\mu$ g/d; 25th and 75th percentiles: 5.4, 7.9) ( $P = 0.0336$ ), mainly because of less frequent use of cobalamin supplements (8% compared with 29.6%;  $\chi^2 = 3.9$ ,  $P = 0.048$ ). Atrophic gastritis was found in 32.4% of the total study group: mild to moderate in 19.6% and severe in 12.7%. The prevalence of severe atrophic gastritis, but not mild-to-moderate atrophic gastritis, was higher in mildly cobalamin-deficient subjects (25%) than in non-cobalamin-deficient subjects (3.7%) ( $\chi^2 = 4.6$ ,  $P = 0.032$ ). The prevalence of immunoglobulin G antibodies to *H. pylori* was similar in mildly cobalamin-deficient subjects (54.2%) and in non-cobalamin-deficient subjects (44.4%) ( $\chi^2 = 0.5$ ,  $P = 0.5$ ).

**Conclusions:** The high prevalence of mild cobalamin deficiency in healthy, free-living, older Dutch subjects could be explained by inadequate cobalamin intake or severe atrophic gastritis in only 28% of the study population. Other mechanisms explaining mild cobalamin deficiency in older people must be sought. *Am J Clin Nutr* 1998;68:328–34.

**KEY WORDS** Cobalamin deficiency, elderly persons, dietary cobalamin intake, atrophic gastritis, *Helicobacter pylori*, methylmalonic acid, homocysteine

See corresponding editorial on page 222.

## INTRODUCTION

Cobalamin (vitamin B-12) deficiency causes elevated methylmalonic acid (MMA) concentrations in blood because cobalamin is an essential cofactor in the enzymatic conversion of methylmalonyl-CoA to succinyl-CoA. The only other important reason for elevated blood MMA concentrations seems to be impaired renal function. Studies have shown that elevated blood MMA concentrations are common in the elderly, with reported prevalences ranging from 39% to 44% in Europe (1, 2) and prevalences of 15% in the United States (3). Most of these persons with elevated MMA concentrations have low to low-normal plasma cobalamin concentrations in the absence of overt anemia or neurologic disease, a condition known as mild or subtle cobalamin deficiency (4). The reported prevalence of these mild cobalamin deficiencies in older persons ranges from 12% to 15% (3, 5). Because of the metabolic evidence of cobalamin deficiency in older subjects with low-normal plasma cobalamin concentrations (3, 5) and the fact that intramuscular cobalamin supplementation normalizes elevated plasma metabolite concentrations, which is considered to support the presence of tissue cobalamin deficiency (1, 5), it has been proposed that the plasma cobalamin cutoff for diagnosing deficiency be raised from 160 to 260 pmol/L (6, 7). Although there is evidence that elevated plasma MMA concentrations are clinically relevant in subjects with apparently asymptomatic, low plasma cobalamin concentrations (8, 9) and even in some patients with normal cobalamin concentrations and anemia (10), the clinical significance of elevated metabolites in subjects with low-normal plasma cobalamin concentrations in the absence of overt anemia or neurologic disease is still unclear.

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The reason for the high prevalence of mild cobalamin deficiency in older persons is poorly understood. But 2 etiologic factors may play a role: dietary cobalamin deficiency and cobalamin malabsorption resulting from atrophic gastritis. Dietary cobalamin deficiency is considered to be a major etiologic factor by some researchers for 3 reasons. First, some patients with unexplained low cobalamin concentrations have inadequate dietary cobalamin intakes; second, most patients with low cobalamin concentrations have normal results on the Schilling test; and third, sometimes plasma cobalamin concentrations increase spontaneously during hospitalization (11–13). Reported cobalamin intake from both diet and supplements in free-living elderly persons in the United States, however, meets recommended allowances (14, 15). The relation between dietary intake and mild cobalamin deficiency has never been investigated in older subjects.

Cobalamin malabsorption due to atrophic gastritis, which is supposed to be common in older people, is considered to be the most important cause of cobalamin deficiency (16–18). Although pernicious anemia is found in only 1–1.9% of older people (3, 19), moderate gastric atrophy without inadequate intrinsic factor secretion can cause cobalamin malabsorption from food (20) because gastric acid and pepsin are needed to liberate protein-bound cobalamin in food (21). In addition, bacterial overgrowth because of hypochlorhydria could play a role (22). *Helicobacter pylori* infection induces chronic gastritis in infected subjects and is related to the progression of gastritis to atrophic gastritis (23). Severe cobalamin malabsorption from food has been associated with *H. pylori* infection (24). Measurement of plasma pepsinogens A and C and immunoglobulin (Ig) G antibodies to *H. pylori* makes it possible to determine the prevalence and severity of atrophic gastritis and the presence of *H. pylori* infection in a non-invasive manner in large groups (18, 25). Measurement of plasma pepsinogens and IgG antibodies to *H. pylori* might provide further insight into the etiology of mild cobalamin deficiency. To determine the reason for the high prevalence of mild cobalamin deficiency in elderly persons, we studied cobalamin intake, the presence and severity of atrophic gastritis, and the presence of *H. pylori* infection in a cohort of free-living, apparently healthy elderly persons ( $n = 105$ ) from the general population.

## SUBJECTS AND METHODS

### Study population

Of the 238 apparently healthy, free-living Dutch elderly persons who participated in the SENECA (a Study in Europe on Nutrition and the Elderly, a Concerted Action) baseline study in 1988 (26), 120 participated in the 1993 follow-up because 38 had died, 69 refused further participation, and 11 persons could not be located. We report data for 105 subjects who participated in the follow-up and in whom plasma cobalamin and MMA concentrations were measured.

### Control subjects

Healthy volunteers were recruited from the general population for the determination of plasma MMA reference values. The following exclusion criteria were applied: impaired renal function (serum creatinine  $>120 \mu\text{mol/L}$ ), vegetarianism, liver disease, alcoholism, gastrointestinal disease or surgery, chronic diarrhea, unexplained weight loss, anemia, cobalamin or folate deficiency

in the past, and treatment with cobalamin, folate, antacids, antibiotics, chloral hydrate, vitamin C, anticonvulsants, metformin, potassium salt, or cholestyramine. Twenty-five volunteers with apparently normal cobalamin status (median age: 74 y; range: 66–87 y; plasma cobalamin:  $\geq 160 \text{ pmol/L}$ ) were selected. The study protocol was approved by the Committee for Experimental Research with Humans of the University Hospital Nijmegen. The Medical Ethical Committee of the Department of Human Nutrition, Wageningen Agricultural University, approved the SENECA Study. Written informed consent was obtained from all participants.

### Protocol

Blood was drawn by antecubital venipuncture after subjects had fasted overnight. Blood was collected into EDTA-containing tubes for measurement of plasma cobalamin, MMA, total homocysteine (tHcy), and pepsinogen I (PGI) and II (PGII). Serum was obtained for measurement of creatinine.

The procedure for the modified dietary history is described in detail in SENECA's manual of operation (27). It is characterized mainly by the use of a checklist of foods, which was based on the meal pattern in the Netherlands. Usual food intake was questioned, with the past month as a reference period. Portion sizes were checked by weighing or by using standardized household measures. Trained investigators obtained food-consumption data and information on the use of vitamin supplements. The recorded foods were coded and analyzed for nutrient composition with the 1992 release of the computerized version of the Dutch Nutrient Table (28). Because the Dutch Nutrient Table contained no information on the cobalamin content of foods, this information was obtained from food tables from the United States, United Kingdom, Germany, and Sweden.

### Laboratory techniques

Plasma cobalamin and plasma folate concentrations were measured with the Dualcount Solid Phase Boil assay (Diagnostic Products Corporation, Los Angeles), which is based on competitive radioisotope-binding techniques. The within-assay CV was  $<5\%$  and the between-assay CV was  $<10\%$ . Plasma MMA concentrations were measured by stable-isotope-dilution capillary gas chromatography–mass spectrometry (29). The within-assay CV was 2.3% and the between-assay CV was 6.8%. Plasma MMA concentrations  $>0.32 \mu\text{mol/L}$  (95th percentile in control subjects) were considered elevated. Plasma tHcy concentrations were measured by automated HPLC (30), with both a within-assay and a between-assay CV  $<5\%$ . Plasma tHcy concentrations  $>19.9 \mu\text{mol/L}$  (95th percentile in control subjects) were considered elevated. Plasma PGI and PGII concentrations were measured by radioimmunoassay (31). Mild-to-moderate atrophic gastritis was defined as a ratio of PGI to PGII (PGI:PGII)  $<1.6$  combined with a PGI concentration  $\geq 17 \mu\text{g/L}$  (32). Severe atrophic gastritis or gastric atrophy were defined as a PGI:PGII  $<1.6$  combined with a PGI concentration  $<17 \mu\text{g/L}$  (32). IgG antibodies to *H. pylori* were measured with a modified enzyme-linked immunosorbent assay (33).

### Statistical analysis

Results are presented as medians with 25th and 75th percentiles. The 95th percentile value in the reference group, determined by a nonparametric method with 90% reliability, was used to determine the upper decision limit of plasma MMA. The



**TABLE 1**Biochemical, dietary, and clinical characteristics of 3 groups of older subjects with different cobalamin deficiency states<sup>1</sup>

	Mildly cobalamin deficient ( <i>n</i> = 25)	Possibly cobalamin deficient ( <i>n</i> = 53)	Not cobalamin deficient ( <i>n</i> = 27)
Plasma cobalamin (pmol/L)	140 (105, 200) <sup>2,3</sup> [37–240]	210 (160, 270) <sup>2</sup> [77–860]	310 (290, 430) [260–780]
Plasma MMA (μmol/L)	0.43 (0.38, 0.68) <sup>2,3</sup> [0.33–18.2]	0.26 (0.20, 0.37) <sup>2</sup> [0.14–1.52]	0.22 (0.19, 0.26) [0.15–0.32]
Plasma tHcy (μmol/L)	17.9 (14.7, 20.9) <sup>2</sup> [9.7–59.2]	16.0 (13.3, 19.3) [10.2–51.4]	15.4 (12.9, 17.4) [9.4–28.2]
Plasma folate (nmol/L)	11.5 (10.3, 15.8) [4.9–22.0]	13.0 (10.5, 16.0) [5.9–47.0]	14.0 (11.0, 18.0) [7.4–52.0]
Dietary cobalamin intake (μg/d)	4.9 (3.9, 6.3) [2.1–16.9]	5.0 (4.1, 6.8) [2.0–13.6]	5.8 (4.3, 6.7) [0.5–9.3]
Total cobalamin intake (μg/d) <sup>4</sup>	4.9 (3.9, 6.4) <sup>2</sup> [2.1–16.9]	5.1 (4.1, 7.0) [2.0–32.0]	6.3 (5.4, 7.9) [0.5–29.0]
PGI:PGII	2.1 (0.8, 2.9) [0.3–3.8]	2.1 (1.4, 3.0) [0.3–4.8]	2.6 (1.7, 3.0) [0.4–4.1]
PGI (μg/L)	40.5 (11.8, 68.8) [3.0–204.0]	40.0 (29.0, 68.0) [4.0–672.0]	43.0 (31.0, 67.0) [6.0–130.0]

<sup>1</sup>Median (25th percentile, 75 percentile); range in brackets. MMA, methylmalonic acid; tHcy, total homocysteine; PGI, pepsinogen I; PGII, pepsinogen II.<sup>2</sup>Significantly different from not cobalamin deficient, *P* < 0.05 (Mann-Whitney *U* test).<sup>3</sup>Significantly different from possibly cobalamin deficient, *P* < 0.05 (Mann-Whitney *U* test).<sup>4</sup>Total cobalamin intake is dietary intake plus supplements.

Mann-Whitney *U* test and the Kruskal-Wallis test were used to compare 2 or several, respectively, continuous variables of unpaired samples. The chi-square test was used to assess the association between categorical variables. The Spearman correlation coefficient was used to describe the correlation between continuous variables. A *P* value ≤ 0.05 was considered statistically significant. The SPSS for WINDOWS program (release 6.1; SPSS Inc, Chicago) was used for statistical analysis.

## RESULTS

### Subjects

The study group of 105 subjects comprised 59 women and 46 men, with a median age of 76 y (range: 74–80 y). Eighteen (17.1%) subjects considered their health to be very good, 60 (57.1%) considered it to be good, 23 (21.9%) considered it to be fair, only 3 (2.9%) considered it to be poor, and 1 did not know. Fifty-nine (56.2%) subjects reported having ≥ 1 of the following chronic diseases: ischemic heart disease (13%), hypertension (12%), respiratory problems (10%), inflammatory bowel disease (9%), diabetes (8%), arthritis (7%), and others (25%). One subject had had a partial gastrectomy: he had low-normal plasma cobalamin concentrations (200 pmol/L) but normal plasma MMA concentrations. Two subjects had been diagnosed previously with cobalamin deficiency: both had normal plasma cobalamin concentrations and plasma MMA concentrations and only 1 used oral cobalamin supplements. One subject with Crohn disease had a low plasma cobalamin concentration (81 pmol/L) with an elevated plasma MMA concentration (0.71 μmol/L). None of the subjects reported having renal disease, atrophic gastritis, pernicious anemia, or ileal resection. Scores on the Mini-Mental State Examination (34) below the cutoff of 24, which is indicative of dementia, were found in 22% of subjects and scores on the 15-item Geriatric Depression Scale (35) above the cutoff of 5, which is indicative of depression, were found in 4.8% of subjects (5/104).

### Plasma cobalamin and MMA concentrations

The median plasma cobalamin concentration was 230 pmol/L (percentiles: 110, 430). Plasma cobalamin was low (≤ 150 pmol/L) in 24.8% (26/105) and low-normal (160–260 pmol/L) in 35.3% (37/105) of subjects. The median plasma MMA concentration was 0.28 μmol/L (percentiles: 0.18, 0.62). Plasma MMA was elevated (> 0.32 μmol/L) in 38.1% (40/105) of subjects, of

whom 37.5% (15/40) had low, 25.0% (10/40) had low-normal, and 37.5% (14/40) had normal plasma cobalamin concentrations. Nine of the 40 subjects with elevated plasma MMA concentrations had increased serum creatinine concentrations (> 110 μmol/L in men and > 90 μmol/L in women).

Subjects with elevated plasma MMA concentrations and low to low-normal plasma cobalamin concentrations (< 260 pmol/L) were defined as mildly cobalamin deficient (23.8%, 25/105) (Table 1). Subjects with normal plasma MMA and normal plasma cobalamin concentrations (≥ 260 pmol/L) were defined as not cobalamin deficient (25.7%, 27/105). The remaining subjects, who had either elevated plasma MMA concentrations (14.3%, 15/105) or low-normal plasma cobalamin concentrations (36.2%, 38/105), were defined as possibly cobalamin deficient (50.5%, 53/105). This group of possibly cobalamin-deficient subjects consisted of subjects with elevated plasma MMA and increased serum creatinine concentrations (*n* = 4), subjects with elevated MMA and normal cobalamin concentrations (*n* = 11), and subjects with normal MMA but low cobalamin concentrations (*n* = 38).

Mildly cobalamin-deficient subjects had significantly lower plasma cobalamin and significantly higher plasma MMA and tHcy concentrations than non-cobalamin-deficient subjects (Table 1). Possibly cobalamin-deficient subjects had significantly lower plasma cobalamin and significantly higher plasma MMA concentrations, but not higher plasma tHcy concentrations (*P* = 0.3, Mann-Whitney *U* test). Plasma folate concentrations did not differ between the three groups (*P* = 0.2, Kruskal-Wallis test).

Plasma MMA concentrations were not correlated with serum creatinine and only weakly correlated with plasma cobalamin (Table 2). Plasma tHcy was not correlated with plasma MMA and only weakly correlated with serum creatinine.

### Hematologic findings

Anemia (defined according the World Health Organization criteria: hemoglobin concentration < 8.1 mmol/L for men and < 7.4 mmol/L for women) was present in 8.7% of subjects (9/104): 2 were mildly cobalamin deficient, 4 were possibly cobalamin deficient, and 2 were not cobalamin deficient. Of the 4 anemic subjects with possible cobalamin deficiency, only 1 had elevated plasma MMA concentrations; the other 3 had isolated low-normal plasma cobalamin concentrations. Macrocytosis (mean cell volume ≥ 100 fL) was found in 1.9% of subjects (2/104). One subject had macrocytic anemia with very low plasma cobalamin concentrations (37 pmol/L) and elevated plasma MMA concentrations (18.2 μmol/L).

TABLE 2

Spearman correlation coefficients between metabolites and other variables in healthy elderly subjects<sup>1</sup>

	Cobalamin		MMA		tHcy	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Plasma cobalamin	—		-0.19	0.046	-0.28	0.004
Plasma folate	0.21	0.034	-0.17	0.083	-0.42	< 0.001
Total cobalamin intake	0.36	< 0.0001	-0.01	0.9	-0.17	0.09
PGI:PGII	0.21	0.035	-0.09	0.4	-0.14	0.1
PGI	0.10	0.3	-0.18	0.064	0.18	0.071
Serum creatinine	0.04	0.7	-0.09	0.4	0.30	0.002
Age	-0.11	0.3	-0.03	0.7	0.10	0.3
Plasma tHcy	-0.28	0.004	0.16	0.093	—	

<sup>1</sup>MMA, methylmalonic acid; PGI, pepsinogen I; PGII, pepsinogen II; tHcy, total homocysteine.

### Dietary cobalamin intake

None of the subjects were vegetarian. Dietary cobalamin intake was correlated with intake of animal protein ( $r = 0.66$ ,  $P \leq 0.0001$ ). The median dietary cobalamin intake was 5.0  $\mu\text{g}/\text{d}$  (percentiles: 4.1, 6.6) for women and 5.8  $\mu\text{g}/\text{d}$  (percentiles: 4.2, 7.0) for men ( $P = 0.1948$ , Mann-Whitney  $U$  test). Dietary cobalamin intake did not differ among the 3 groups (Table 1). Six of 103 subjects (5.8%)—1 mildly cobalamin deficient, 4 possibly cobalamin deficient, and 1 not cobalamin deficient—had intakes below the Dutch recommended dietary allowance of 2.5  $\mu\text{g}/\text{d}$  (36). The subject with mild cobalamin deficiency had a low plasma cobalamin concentration. These numbers did not change after correction for supplement use.

Fifteen (14.3%) subjects took oral supplements containing cobalamin. Supplements contained a median amount of 2  $\mu\text{g}$  cobalamin (range: 0.5–25.0  $\mu\text{g}$ ). The median total cobalamin intake (diet plus supplements) was 5.3  $\mu\text{g}/\text{d}$  (percentiles: 4.1, 6.7) in women and 5.9  $\mu\text{g}/\text{d}$  (percentiles: 4.4, 7.8) in men ( $P = 0.2$ , Mann-Whitney  $U$  test). More non-cobalamin-deficient subjects (29.6%) than mildly cobalamin-deficient subjects (8.0%) ( $\chi^2 = 3.9$ ,  $P = 0.048$ ) or possibly cobalamin-deficient subjects (9.8%) ( $\chi^2 = 5.0$ ,  $P = 0.0254$ ) used supplements (Table 3). As a result, the total cobalamin intake by non-cobalamin-deficient subjects was higher than that by mildly cobalamin-deficient subjects ( $P = 0.0336$ , Mann-Whitney  $U$  test) and possibly cobalamin-deficient subjects ( $P = 0.0725$ , Mann-Whitney  $U$  test) (Table 1).

Those taking supplements had higher total cobalamin intakes [7.8  $\mu\text{g}/\text{d}$  (percentiles: 4.7, 30.2)] and higher cobalamin concentrations [300 pmol/L (percentiles: 130, 705)] than those not taking supplements [5.2  $\mu\text{g}/\text{d}$  (percentiles: 2.8, 8.4),  $P = 0.0001$  (Mann-Whitney  $U$  test), and 220 pmol/L (percentiles: 110, 385),  $P = 0.0307$  (Mann-Whitney  $U$  test), respectively]. However, subjects taking supplements had plas-

ma MMA concentrations [0.26  $\mu\text{mol}/\text{L}$  (percentiles: 0.19, 0.30)] similar to those not taking supplements [0.29  $\mu\text{mol}/\text{L}$  (percentiles: 0.21, 0.41),  $P = 0.2$  (Mann-Whitney  $U$  test)]. Total cobalamin intake correlated with plasma cobalamin but not with plasma MMA or plasma tHcy (Table 2), independent of renal function or presence and severity of atrophic gastritis.

### Atrophic gastritis and *Helicobacter pylori* infection

Atrophic gastritis was found in 32.4% of subjects (33/102), mild to moderate in 19.6% (20/102) and severe in 12.7% (13/102). PGI:PGII, PGI concentrations, and the prevalence of atrophic gastritis did not differ among the 3 cobalamin-deficiency groups (Tables 1 and 3). However, the prevalence of severe atrophic gastritis was higher in the mildly cobalamin-deficient group (Table 3). All but 1 of the subjects with severe atrophic gastritis in the mildly cobalamin-deficient group had low plasma cobalamin concentrations. PGI:PGII was correlated with plasma cobalamin and the PGI concentration was correlated weakly with plasma MMA (Table 2).

The prevalence of IgG antibodies to *H. pylori* was 58.4% (59/101) in the total study group, 78.8% (26/33) in the group with atrophic gastritis and 48.5% (33/68) in the group without atrophic gastritis ( $\chi^2 = 8.4$ ,  $P = 0.0038$ ). Antibodies to *H. pylori* were present in only 2 of 6 subjects with severe atrophic gastritis in the mildly cobalamin-deficient group compared with 5 of 6 subjects with severe atrophic gastritis in the possibly cobalamin-deficient group. The prevalence of antibodies against *H. pylori* was higher in the possibly cobalamin-deficient group than in the non-cobalamin-deficient group ( $P = 0.044$ , Mann-Whitney  $U$  test; Table 3) because of the high number of subjects with low to low-normal plasma cobalamin concentrations and IgG antibodies to *H. pylori* (69.4%) in the possibly cobalamin-deficient group.

TABLE 3

Number of elderly subjects who used cobalamin supplements, had atrophic gastritis, or had immunoglobulin (Ig) G antibodies to *Helicobacter pylori*

	Mildly cobalamin deficient ( $n = 25$ )	Possibly cobalamin deficient ( $n = 53$ )	Not cobalamin deficient ( $n = 27$ )
	<i>n</i> (%)		
Supplement users	2/25 (8.0) <sup>1</sup>	5/51 (9.8) <sup>1</sup>	8/27 (29.6)
Mild-to-moderate atrophic gastritis	4/24 (16.7)	11/51 (21.6)	5/27 (18.5)
Severe atrophic gastritis	6/24 (25.0) <sup>1</sup>	6/51 (11.8)	1/27 (3.7)
IgG antibodies to <i>Helicobacter pylori</i>	13/24 (54.2)	34/50 (68.0) <sup>1</sup>	12/27 (44.4)

<sup>1</sup>Significantly different from not cobalamin deficient,  $P < 0.05$  (chi-square).

## DISCUSSION

Mild cobalamin deficiency was common,  $\geq 23.8\%$ , in this cohort of free-living Dutch elderly persons from the general population. These subjects had low to low-normal plasma cobalamin concentrations and elevated plasma MMA concentrations; only 2 subjects had anemia or macrocytosis. The elevated plasma MMA concentrations were not considered to have resulted from renal dysfunction because serum creatinine concentrations were normal in most subjects and plasma MMA concentrations were not correlated with serum creatinine. A cause for the mild cobalamin deficiency was determined in only 8 of 25 cases: severe atrophic gastritis in 6, inadequate cobalamin intake in 1, and Crohn disease in 1. It seems noteworthy that 7 of these 8 subjects had low plasma cobalamin concentrations ( $\leq 150$  pmol/L).

The prevalence of mild cobalamin deficiency in Dutch elderly persons is even higher than the prevalence of 12–15% in the United States (3, 5). There are several possible explanations for this. First, the difference may be explained in part by the higher reported prevalences of elevated plasma MMA concentrations in Europeans (1, 2, and the present study). There is no universally accepted upper limit for plasma MMA, with reported figures ranging from 0.25 to 0.38  $\mu\text{mol/L}$  (1, 3, 37). This variation probably reflects differences in analytic methods, statistical analysis, and the composition of control populations. We chose our control subjects from the same age group as the study subjects. Some (38) found plasma MMA concentrations to increase with age whereas others did not (39). The reason for an age-related increase is unclear but seems unrelated to cobalamin status (38) and appears to be related to the age-related decline in renal function. Therefore, a comparison of the metabolite results from elderly subjects with reference values from younger adults could be misleading and erroneous. We tried to exclude control subjects with high MMA concentrations due to cobalamin deficiency by including only those subjects with apparently normal cobalamin status as defined by our criteria and by a normal plasma cobalamin concentration. Our MMA cutoff approached the reported upper limit of 0.29  $\mu\text{mol/L}$  in healthy subjects after cobalamin supplementation (38). Second, the high prevalence of mild cobalamin deficiency could be related to poorer cobalamin intake or higher prevalence of atrophic gastritis in older Europeans, the subjects of this study, than in older Americans.

This is the first time data have been presented on the dietary cobalamin intake of older Europeans. The method used for assessing the diet, ie, the modified dietary history method, showed good agreement with the weighed diet record and with other evaluation criteria (40). The Dutch Nutrient Table contained little information on the cobalamin content of foods. Therefore, we completed the table with data from food data banks from other countries. Thus, our intake data are rough estimates and the results should be interpreted with caution. Dietary cobalamin intake was correlated with animal protein intake and plasma cobalamin concentrations. Because of the lack of data on cobalamin concentrations in foods, the interpretation of cobalamin intake results was also hampered in other studies (15).


The median total (diet plus supplement) cobalamin intake by free-living, older Dutch subjects was comparable with that by free-living, older American subjects (14, 15). There was, however, a difference in how this total intake was achieved. More free-living, older American subjects use cobalamin supplements: 30–40% (3, 14, 15) compared with 14% in the present

study. Moreover, the median cobalamin content of these supplements is greater in the United States:  $\approx 6$   $\mu\text{g}$  (3, 14) compared with 2  $\mu\text{g}$  in the Netherlands. In addition, another source of crystalline cobalamin in the United States, but not in the Netherlands, is fortified cereals. A higher intake of crystalline cobalamin by older American subjects might protect some of them against cobalamin deficiency. In the older Framingham population, subjects taking supplements less often had reduced cobalamin and elevated plasma MMA concentrations compared with those not taking supplements (3). We could not attribute the high prevalence of cobalamin deficiency in our elderly subjects to inadequate cobalamin intake because only 5.8% had cobalamin intakes below the Dutch recommended dietary intake of 2.5  $\mu\text{g/d}$  (36). Furthermore, in both the Framingham study (3) and the present study, the relation between plasma MMA and cobalamin intake was poor.

We used the PGI:PGII to determine the presence of atrophic gastritis and the PGI concentration to determine the severity of atrophic gastritis. The prevalence of atrophic gastritis was 32.4%, mild to moderate in 19.6% and severe in 12.7% of subjects. This prevalence of atrophic gastritis is consistent with the prevalence of 31.5% reported for free-living and institutionalized elderly persons in the greater Boston area (18) but much higher than the recently reported prevalence of 9% for independently living elderly subjects in Kansas City (41). In both of these studies, the prevalence of atrophic gastritis was indicated by the serum pepsinogen ratio. The prevalence of severe atrophic gastritis in older Dutch subjects was higher than the 8.1% reported by Krasinski et al (18). This difference might explain part of the difference in the prevalence of mild cobalamin deficiency between the Netherlands and the United States.

We could relate atrophic gastritis only in its severe form to mild cobalamin deficiency. Indeed, we showed previously that absorption of protein-bound cobalamin is not impaired in healthy subjects with mild-to-moderate atrophic gastritis (42) and we showed that cobalamin malabsorption in older cobalamin-deficient patients is related to severe atrophic gastritis but not to mild-to-moderate atrophic gastritis (unpublished observations, 1997). In addition, Krasinski et al (18) found low plasma cobalamin concentrations in only 8.5% of older subjects with mild-to-moderate atrophic gastritis but in 53.3% of subjects with severe atrophic gastritis. Older subjects with mild-to-moderate atrophic gastritis, however, may be at risk for developing cobalamin deficiency when their atrophic gastritis progresses.

IgG antibodies to *H. pylori* infection were found in 56% of the older Dutch subjects. The prevalence of *H. pylori* infection, therefore, seems comparable with that in older American subjects (43). Although we found *H. pylori* infection to be related to atrophic gastritis, which supports its role in the pathogenesis of gastric atrophy, we could not relate mild cobalamin deficiency to this infection. However, at the stage of total gastric atrophy, as in pernicious anemia, infected subjects become helicobacter negative (23) as a result of the virtual absence of acid production, which inhibits survival of *H. pylori*. Indeed, no antibodies to *H. pylori* could be found in most subjects with severe atrophic gastritis in the group with mild cobalamin deficiency. This resulted in a lower prevalence of antibodies to *H. pylori* in this group. *H. pylori* infection was associated only with low to low-normal plasma cobalamin concentrations in subjects with normal plasma MMA concentrations.

The etiology of mild cobalamin deficiency in older subjects is unclear. Inadequate dietary cobalamin intake or the presence of severe atrophic gastritis can only partly explain the high prevalence of mild cobalamin deficiency in older persons. Other mechanisms by which mild cobalamin deficiency arises need to be sought. One possibility is the proposed alteration in the binding site for cobalamin on transcobalamin II (44, 45) or an alteration in cellular cobalamin metabolism. Nevertheless, in light of the high prevalence of mild cobalamin deficiency, its possible neuropsychiatric consequences (46, 47), and possible increased risk of cardiovascular diseases through elevated homocysteine concentrations (48), the possible benefit of cobalamin supplementation must be investigated. On the basis of our data it seems that an increase in the recommended dietary intake would not suffice for treating mild cobalamin deficiency. Whether oral cobalamin supplementation would be sufficient for treating mild cobalamin deficiency cannot be inferred from this study. 

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