Prevalence of cobalamin deficiency in the Framingham elderly population[1–3]

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ABSTRACT To determine whether the increased prevalence of low serum cobalamin concentrations in elderly people represents true deficiency, serum concentrations of cobalamin and folate and of metabolites that are sensitive indicators of cobalamin deficiency were measured in 548 surviving members of the original Framingham Study cohort. Serum cobalamin concentrations < 258 pmol/L were found in 222 subjects (40.5%) compared with 17.9% of younger control subjects ($P < 0.001$). Serum methylmalonic acid and total homocysteine concentrations were markedly elevated in association with cobalamin values < 258 pmol/L in 11.3% and 5.7%, respectively, of the cohort. Both metabolites were increased in 3.8% of the cohort, associated with significantly lower erythrocyte counts and higher mean cell volumes. Serum metabolites correlated best with serum cobalamin values, even when subnormal determinations were excluded. The prevalence of cobalamin deficiency was ≥ 12% in a large sample of free-living elderly Americans. Many elderly people with "normal" serum vitamin concentrations are metabolically deficient in cobalamin or folate. *Am J Clin Nutr* 1994;60:2–11.

KEY WORDS Cobalamin deficiency, folate deficiency, methylmalonic acid, homocysteine, vitamin B-12, Framingham Study, elderly people

Introduction

Over more than three decades, many investigators have reported a strikingly increased prevalence of low serum cobalamin (Cbl; vitamin B-12) concentrations in elderly people (1–16), although a minority have disputed this finding (11, 17–19). Some have regarded the decreased Cbl values in geriatric populations as a laboratory phenomenon of little or no clinical significance or as the result of bias in patient selection. The majority of elderly individuals with low serum concentrations have not been anemic nor have they developed megaloblastic anemia after follow-up (4, 15). On the other hand, Cbl deficiency may cause serious neuropsychiatric damage, including impairment in cognitive function, in the absence of anemia (20–22). Furthermore, homocysteine (Hcys) and its derivatives accumulate in Cbl deficiency (23, 24), and elevated serum Hcys concentrations are increasingly recognized as a major risk factor for cerebral, coronary, and peripheral vascular disease (25–27). Therefore it is important to determine whether true deficiency of Cbl is a significant problem in elderly people, especially because it is easily treatable and its effects are reversible if detected in time.

Serum concentrations of methylmalonic acid (MMA) and total Hcys have proven to be highly sensitive indicators of tissue deficiency of cobalamin (21, 23, 28–32). The concentration of one or both metabolites was found to be markedly elevated (> 3 SD above the mean in normal control subjects) in 99.8% of a consecutive series of > 400 patients with clinically proven Cbl deficiency (32). Furthermore, ≥ 5% of patients with clear-cut hematologic or neurologic disorders (or both) caused by lack of the vitamin have serum Cbl values in the lower end of the normal range, even though circulating metabolite concentrations are unequivocally elevated (31). Increases in serum metabolites are also frequently seen before the development of low Cbl values in patients with pernicious anemia in remission, when maintenance vitamin B-12 injections are not given for several months (31). Although MMA accumulates in Cbl but not folate deficiency, Hcys concentrations are also increased in folate deficiency (23, 30, 32, 33), and the combined measurement of both metabolites facilitates the differentiation of these disorders (32, 34).

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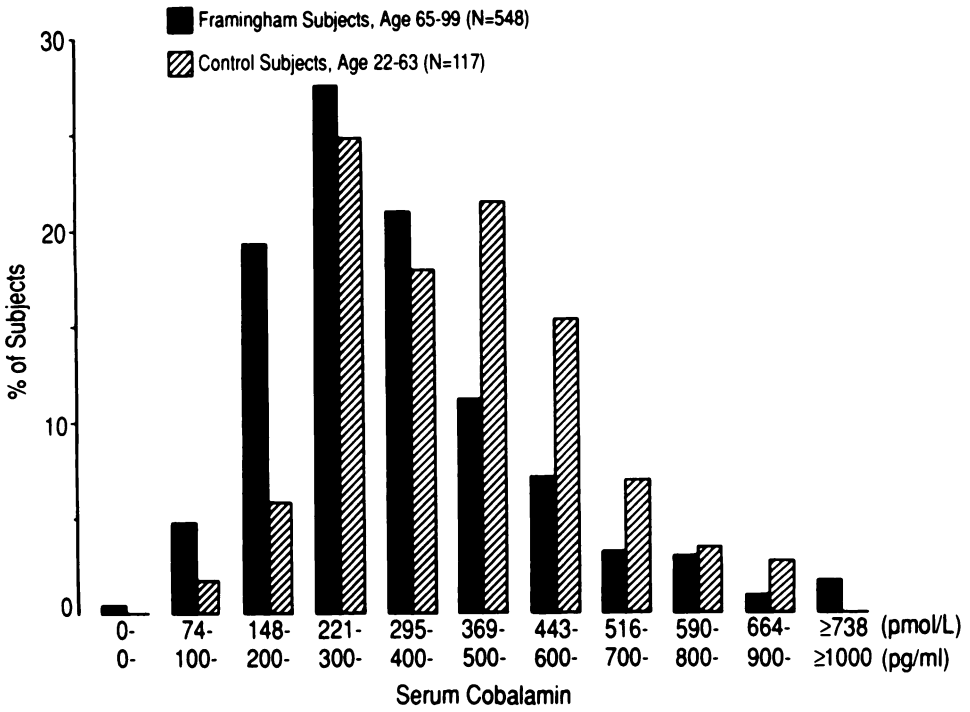

FIG 1. Distribution of serum cobalamin concentrations in 548 elderly subjects from the Framingham Heart Study and 117 younger control subjects.

We therefore decided to assess the problem of whether there is a significant increased prevalence of cobalamin deficiency in elderly people by measuring both vitamins and both metabolites in a large sample of a well-defined ambulatory population—the surviving members of the original Framingham Heart Study.

Subjects and methods

The study was approved by the Human Investigations Review Committee at the New England Medical Center.

The Framingham study, an epidemiologic investigation of heart disease, was established in Framingham, MA, between 1948 and 1950 (35). The original cohort consisted of 5209 subjects 29–63 y of age. The surviving members of this cohort have been examined every 2 y. In 1988–89 ≈1200 survivors participated in the 20th examination. Serum was available for assay of vitamin and metabolite concentrations from 548 of the first 567 people to report for that examination. Healthy young medical personnel 22–63 y of age employed by Columbia University served as control subjects for the serum vitamin assays. There were 117 control subjects (59 females, 58 males; median age 30 y) for the Cbl assay and 59 (30 females, 29 males; median age 30 y) for the folate assay. Fifty healthy blood donors (25 males, 25 females) from Denver, ranging in age from 18 to 65 y (median 35 y), served as control subjects for the metabolite assays, which were performed in Denver (30).

Blood was obtained from the Framingham subjects at 1300 h and maintained in the refrigerated state for 18–24 h, followed by centrifugation for 30 min at 2500 × *g* at 4 °C and separation of serum, which was stored at −20 °C. Blood specimens were obtained from the younger control subjects between 0900 and 1300 h and centrifuged for 30 min at 2500 × *g* at 4 °C after 1 h at room temperature; the serum was stored at −20 °C. Andersson et al (36) reported modest and essentially equivalent increments in plasma Hcys concentrations when whole blood was centrifuged after 24 h of refrigeration or after 1 h at room temperature. Separate aliquots of frozen sera were thawed once and assayed for vitamin or metabolite concentrations 2–21 mo after collection. Metabolite concentrations are stable in serum maintained at −20 °C for many years (23, 28, 32).

Serum Cbl and folate concentrations were determined by simultaneous radioassays using purified intrinsic factor and milk folate binder, respectively (Quantaphase; Bio-Rad Laboratories, Richmond, CA). Serum MMA and total Hcys were measured by modifications (30, 37) of techniques using capillary gas chromatography and mass spectrometry (38, 39). An automated complete blood count including platelet count and mean cell volume (MCV) was performed as well as measurements of serum electrolytes, blood urea nitrogen (BUN), creatinine, transaminases, alkaline phosphatase, lactic dehydrogenase, albumin, total protein, calcium, phosphate, and magnesium. Creatinine clearance was calculated by a formula including serum creatinine, age, and body weight (40). Serum antibodies to intrinsic factor (41) were measured when additional serum was available from subjects with laboratory tests suggestive of Cbl deficiency.

Information regarding vitamin supplementation was available from a questionnaire (42) mailed to all of the subjects that was returned by 401 members (73.2%) of the cohort. The number of years of formal education and the stated average amount of daily alcohol consumption was available for the entire cohort.

Bivariate associations between variables were tested by calculating Pearson linear-regression coefficients of correlation. Log-transformed data were used for serum MMA, Hcys, Cbl, folate, creatinine, and BUN because the distribution of each of these variables was skewed toward higher values. For multivari-

ate analysis of factors predicting serum MMA and Hcys, a stepwise multiple linear-regression model (*BMDP2R* system) was used (43) to select the most strongly related predictor variables and to calculate a multiple unadjusted R^2, with an F of 4 for entry or removal.

Results

Elderly population

The 548 subjects included 348 women and 200 men aged 67–96 y ($\bar{x}$ ± 1 SD 77.4 ± 6.2, median 77.5 y). All were white. Results of automated biochemical screening tests were normal in most subjects. Elevations of serum transaminases were found in 21 of the 548, which were modest (< 200 U/L) in all but two instances. The serum creatinine was increased (> 124 μmol/L in men, > 106 μmol/L in women) in 47 (8.5%) of the 548 subjects, > 177 μmol/L (2.0 mg/dL) in 9, and > 265 μmol/L in 3. The calculated creatinine clearance was ≥ 80 mL/min (a value often used clinically as a lower limit of normal in younger patients) in only 15.4% of the cohort and was < 60 mL/min in 51.3%.

Cobalamin deficiency

The mean (±SD) serum Cbl concentration in the 548 members of the Framingham elderly cohort (315 ± 145 pmol/L, 427 ± 196 pg/mL) was lower than that of the 117 healthy control subjects < 65 y of age (375 ± 124 pmol/L, 508 ± 168 pg/mL; $P < 0.001$). In the elderly group, 29 subjects (5.3%) had Cbl values < 148 pmol/L (200 pg/mL), the manufacturer's stated lower limit of normal for the radioassay used, in contrast with the 1.7% for the control subjects ($X^2 = 2.8$, $P > 0.05$). Serum concentrations < 258 pmol/L (< 350 pg/mL) were found in 222 (40.5%) of the elderly subjects compared with 21 (17.9%) of the control subjects ($X^2 = 21.2$, $P < 0.001$). The distribution of Cbl concentrations was generally shifted toward lower values in the elderly cohort (**Fig 1**).

The serum MMA concentration was increased (> 376 nmol/L, or 3 SD above the control mean) in 62 of the 222 elderly subjects with serum Cbl concentrations < 258 pmol/L (**Fig 2**). These 62 subjects constituted 11.3% of the entire cohort of 548 individuals. Unless otherwise indicated, the terms increased and elevated will be used throughout this article to denote values for MMA and/or Hcys > 3 SD above the mean in normal control subjects 18–65 y of age. In the clinical assessment of patients with megaloblastic anemias, we have found that values 3 SD above the mean provide greater specificity in the diagnosis of the vitamin deficiency states, despite the sacrifice of some sensitivity (32).

The serum total Hcys value was elevated (> 21.3 μmol/L, 3 SD above the control mean) in 31 of the 222 elderly subjects with Cbl concentrations < 258 pmol/L (5.7% of the entire cohort; Fig 2). In 72 subjects with a Cbl value < 258 pmol/L (13.1% of the cohort), either the MMA and/or Hcys concentration was increased and in 21 (3.8%) both values were elevated.

The two highest values for both MMA and Hcys were in the two persons in whom serum Cbl was < 74 pmol/L (< 100 pg/mL) (Fig 2). In subjects with elevated metabolite concentrations, the degree of elevation was similar in those with serum Cbl values between 74 and 147 pmol/L as in those with values in the range 148–258 pmol/L (Fig 2).

Folate deficiency

The mean serum folate concentration in the 548 elderly subjects did not differ from that of the control subjects < 65 y of age [22.0 nmol/L (9.7 ± 8.3 ng/mL) vs 17.4 nmol/L (7.7 ± 3.4 ng/mL), respectively; $P > 0.05$]. In 16 (2.9%) of the 548 elderly subjects serum folate was < 5.9 nmol/L (2.6 ng/mL), the manufacturer's stated lower limit of normal for the radioassay, in

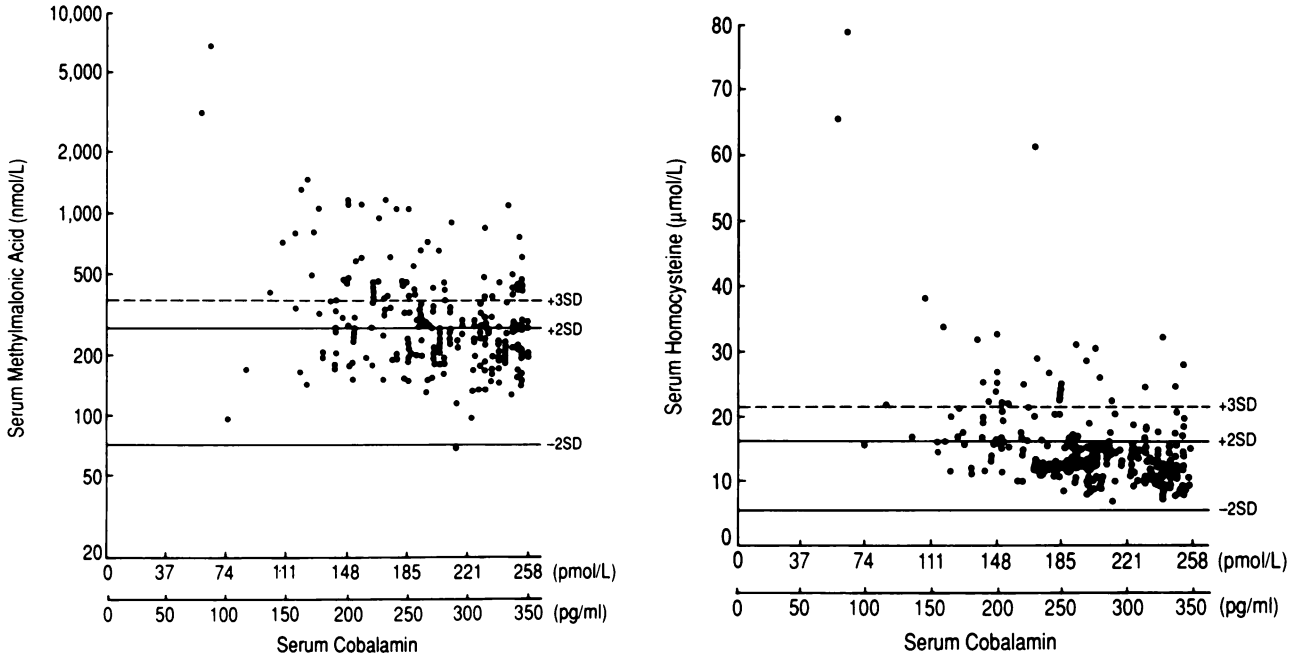

FIG 2. Serum methylmalonic acid and total homocysteine concentrations in elderly Framingham subjects with serum cobalamin concentrations < 258 pmol/L (< 350 pg/mL).

contrast to 1 (1.7%) of the 59 control subjects, ($P > 0.05$). Serum concentrations < 11.3 nmol/L (< 5.0 ng/mL) were found in 129 (23.5%) of the elderly and 14 (23.7%) of the control subjects ($P > 0.05$).

Serum Hcys was elevated in 19 of the 129 elderly subjects with a serum folate concentration < 11.3 nmol/L (3.5% of the entire cohort). However, in 17 of these 19 subjects, serum Cbl was also < 258 pmol/L, accompanied in 10 instances by an elevation of serum MMA.

There was a tendency for low serum concentrations of the two vitamins to be associated with each other. Serum folate was < 11.3 nmol/L in 82 (36.9%) of the 222 subjects with a Cbl concentration < 258 pmol/L, in contrast to 47 (14.4%) of 326 subjects with a Cbl concentration ≥ 258 pmol/L ($X^2 = 37.2$, $P < 0.001$). Serum Cbl was < 258 pmol/L in 82 (63.6%) of the 129 subjects with a serum folate < 11.3 nmol/L. In the entire cohort, serum Cbl correlated with serum folate ($r = 0.48$ for the log of each serum concentration, $P < 0.001$). Neither the folate nor Cbl concentration correlated with the hematocrit, MCV, stated amount of alcohol intake, or number of years of education.

Serum methylmalonic acid

Serum MMA was elevated in 82 (15.0%) of the 548 elderly subjects. Members of the cohort with increased MMA values were older and had significantly lower Cbl and folate concentrations and higher serum creatinine values (**Table 1**). In **Figure 3** the percent of subjects at various intervals of serum Cbl concentrations who had MMA elevations is shown, as compared with the percentage of the entire cohort with an increased MMA concentration (15%), as indicated by the interrupted line. Serum MMA concentrations were increased in > 15% of subjects with serum Cbl values < 258 pmol/L and in < 10% of those with values ≥ 258 pmol/L. Serum Cbl was < 258 pmol/L in 62 (75.6%) of the 82 subjects with MMA elevations; in 10 of these 62, serum creatinine was above normal.

Serum Cbl was ≥ 258 pmol/L in 20 subjects with an elevated MMA. Serum creatinine was increased in 10 of them and the creatinine clearance was < 60 mL/min in 7 of the 10 in whom serum creatinine was normal. In the remaining three subjects, serum Cbl varied from 273 to 365 pmol/L and the creatinine clearance from 64 to 75 mL/min.

The most striking MMA elevations were noted in the subjects with serum Cbl concentrations < 258 pmol/L. In 23 of the 62 persons with Cbl values below this concentration and with a high metabolite concentration, serum MMA was in the range 662–6820 nmol/L, whereas the highest MMA value in the 20 subjects with serum Cbl ≥ 258 pmol/L was 638 nmol/L ($X^2 = 8.6$, $P < 0.005$).

Serum total homocysteine

Serum Hcys was elevated in 39 (7.1%) of the 548 members of the elderly cohort. Subjects with increased Hcys values were older and had significantly lower serum Cbl and folate and higher serum creatinine concentrations than those without increased values (Table 1). These differences were also significant when male and female members of the cohort were analyzed separately, except that the mean creatinine values in males did not differ (data not shown).

Serum Cbl was < 258 pmol/L in 31 (79.5%) of the 39 subjects with Hcys elevations and was associated with an increased MMA concentration in 21. Nineteen (48.7%) of the 39 subjects with elevated Hcys values had serum folate concentrations < 11.3 nmol/L; however, 17 of the 19 also had Cbl concentrations < 258 pmol/L.

Sixteen (41.0%) of the 39 subjects with elevated Hcys values had increased serum creatinine concentrations. Six of the eight subjects with Hcys elevations who had Cbl concentrations > 258 pmol/L had increases in serum creatinine and folate values ≥ 11.3 nmol/L. In the remaining two subjects the folate concentration was < 11.3 nmol/L and creatinine was normal.

The combination of a serum folate concentration < 11.3 nmol/L and a normal MMA was seen in 9 of the 39 persons with Hcys elevations (1.6% of the entire cohort). In seven of the nine serum Cbl was < 258 pmol/L and in two of the seven serum creatinine was elevated as well. Each of the 39 subjects with an increased Hcys concentration had a low or low-normal Cbl or folate concentration, an increased creatinine concentration, or some combination of these findings.

Elevations of both metabolites

Serum concentrations of both MMA and Hcys were elevated in 25 (4.6%) of the 548 elderly subjects. Serum Cbl was < 258 pmol/L in 21 of the 25; 7 of the 21 also had increased serum creatinine. Serum creatinine was also high in each of the four individuals with elevations of both metabolites and a serum Cbl value ≥ 258 pmol/L. Serum folate was < 11.3 nmol/L in 10 of the 25 subjects in whom both MMA and Hcys were increased; serum Cbl was < 258 pmol/L in each of them.

TABLE 1
Serum cobalamin, folate, and creatinine concentrations in elderly subjects with and without markedly elevated metabolite concentrations[1]

	Serum MMA		Serum Hcys	
	>3 SD ($n = 82$)	≤3 SD ($n = 456$)	>3 SD ($n = 39$)	≤3 SD ($n = 509$)
Age (y)	80 ± 7[2]	77 ± 6	81 ± 8[2]	77 ± 6
Serum cobalamin (pmol/L)	217 ± 83[2]	332 ± 146	197 ± 77[2]	325 ± 145
Serum folate (nmol/L)	18.1 ± 12.5[3]	22.7 ± 19.5	12.7 ± 8.2[2]	22.9 ± 19.0
Serum creatinine (μmol/L)	106 ± 53[2]	88 ± 27	115 ± 35[2]	88 ± 27

[1] $\bar{x}$ ± SD. MMA, methylamonic acid; Hcys, total homocysteine; >3 SD, 3 SDs above the mean in normal control subjects aged 18–65 y. To convert cobalamin values to pg/mL, multiply by 1.355. To convert folate values to ng/mL, multiply by 0.44. To convert creatinine values to mg/dL, multiply by 0.011.

[2,3] Significantly different from ≤3 SD for same metabolite: [2] $P < 0.001$, [3] $P < 0.05$.

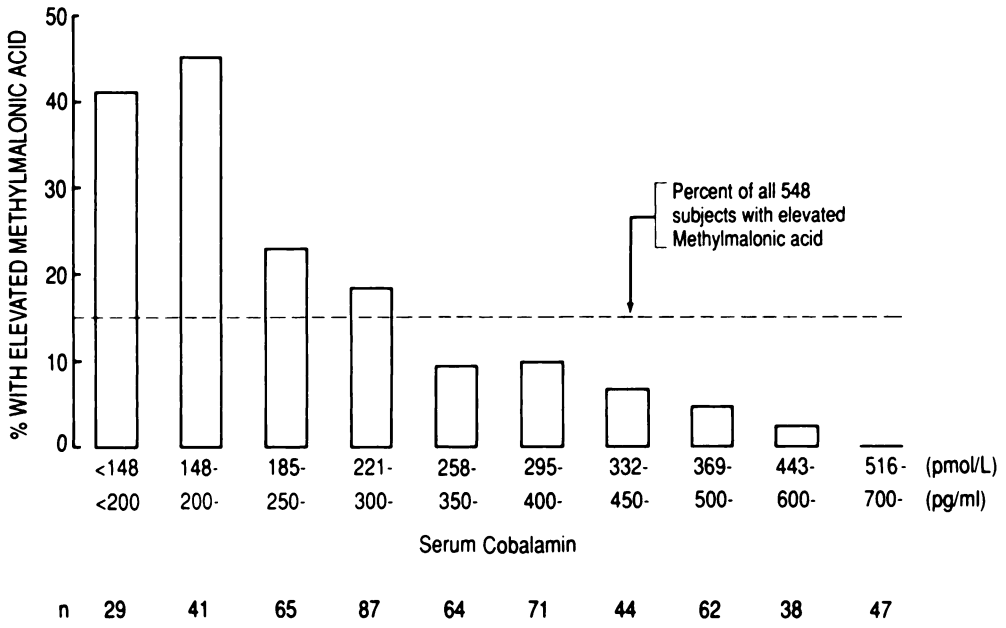

FIG 3. Percent of subjects with elevated serum methylmalonic acid concentrations (> 3 SD above the mean in younger normal control subjects) in 548 members of the Framingham elderly population with various concentrations of serum cobalamin. n = number of subjects.

Serum vitamin and metabolite abnormalities

The findings in the 74 members of the cohort with elevated concentrations of one or both metabolites associated with decreased values for one or both vitamins are summarized in **Table 2.** In 44 subjects (8.0% of the entire cohort) represented on the first two lines of Table 2, serum Cbl was < 258 pmol/L and the folate concentration was normal, accompanied by increases in one or both metabolite values. These subjects were likely to have isolated deficiency of Cbl. In 21 additional subjects (line 3, Table 2), lack of Cbl was also highly probable, but an associated folate deficiency could not be excluded; in seven subjects there may have been a deficiency of one or the other, or of both. Serum creatinine was elevated in a minority of subjects in each of these categories. In only two subjects (0.4% of the cohort) were the findings clearly indicative of isolated folate deficiency.

Because we defined an elevated serum metabolite concentration as a value > 3 SD above the mean in a group of younger blood donors, we considered the possibility that most of the elevations were not due to Cbl deficiency but merely indicated an upward shift in the normal range of concentrations for MMA and Hcys in elderly people. We therefore examined a subset of the Framingham cohort with unequivocally normal serum Cbl concentrations in whom frank renal insufficiency was absent. There were 125 subjects with a cobalamin value ≥ 369 pmol/L (500 pg/mL), associated with a normal serum creatinine and a BUN < 10.7 nmol/L (< 30 mg/dL). Most of those in this subset that returned the questionnaire reported taking Cbl supplements. The highest MMA and Hcys concentrations in the 125 subjects were 364 nmol/L and 18.0 μmol/L, respectively, values < 3 SD above the mean in our younger control group.

Correlations with serum metabolites

As indicated by bivariate (ie, so-called "univariate") correlation coefficients unadjusted for confounding variables (**Table**

TABLE 2
Interpretation of findings in elderly subjects with elevated metabolite determinations and decreased serum concentrations of cobalamin (Cbl), folate, or both vitamins[1]

Deficiency state	Cbl (<258 pmol/L)	Folate (<11.3 nmol/L)	↑ MMA	↑ Hcys	Number with ↑ creatinine
Cbl (n = 41)	+	−	+	±[2]	5
Cbl (n = 3)	+	−	−	+	1
Cbl ± folate (n = 21)	+	+	+	±[3]	5
Cbl and/or folate (n = 7)	+	+	−	+	2
Folate (n = 2)	−	+	−	+	0

[1] MMA, methylmalonic acid; Hcys, total homocysteine; ↑ creatinine, serum concentration > 106 μmol/L (> 1.2 mg/dL) in females, > 124 μmol/L (> 1.4 mg/dL) in males; ↑ MMA, > 376 nmol/L; ↑ Hcys > 21.3 μmol/L. A Cbl concentration of 258 pmol/L = 350 pg/mL; a folate concentration of 11.3 nmol/L = 5.0 ng/mL.
[2] 11 of 41 had an ↑ Hcys.
[3] 10 of 21 had an ↑ Hcys.

3), the serum MMA concentration correlated most strongly with serum Cbl and also with various measures of renal function as well as with age. Only a weak correlation was apparent between MMA and serum folate. If the 29 subjects with Cbl values < 148 pmol/L were excluded from the analysis, the correlation between serum Cbl and MMA was still good ($r = -0.37$, $P < 0.001$), but if the 222 persons with Cbl values < 258 pmol/L (< 350 pg/mL) were excluded, the correlation was much weaker ($r = -0.12$).

Serum Hcys correlated best with serum Cbl, folate, and creatinine and also correlated with serum uric acid, BUN, and age (Table 3). The correlation of serum Cbl and Hcys remained strong ($r = -0.41$) when subjects with Cbl concentrations < 148 pmol/L were excluded, and remained significant ($r = -0.28$, $P < 0.001$) even when those with concentrations < 258 pmol/L were excluded, as did the correlation between serum folate and Hcys ($r = -0.39$). When the 16 subjects with folate values < 5.9 nmol/L were excluded, the correlation between folate and Hcys values was only slightly weakened ($r = -0.37$). Even when the 129 subjects with folate concentrations < 11.3 nmol/L were excluded, it was lessened but still apparent ($r = -0.28$). On the other hand, removal of these 129 subjects had little effect on the correlation between Cbl and Hcys concentrations ($r = -0.47$).

MMA and Hcys concentrations correlated strongly with each other, but neither metabolite correlated with the number of years of schooling or the amount of alcohol consumed. Multivariate stepwise-logistic-regression analysis was performed to assess the combined effects on each of the metabolite concentrations of the variables listed in Table 3. The serum Cbl concentration was found to be the strongest independent predictor of both MMA and Hcys concentrations (data not shown).

Hematologic findings

Blood counts were available from 501 of the 548 subjects. Anemia (hematocrit < 0.40 in men, < 0.35 in women) was present in 59 subjects (11.8%). In only eight subjects was the hematocrit < 0.30. The anemia was typically normocytic (MCV 80–100 fL) and in 20 was associated with an elevation of serum creatinine. In two anemic individuals the MCV was > 100 fL and in four it was < 80 fL. In an additional eight subjects the MCV was > 100 fL in the absence of anemia.

In 3 of the 10 subjects with an MCV > 100 fL, Cbl was < 258 pmol/L and MMA was increased (**Table 4;** subjects 1, 2, and 4) in the absence of anemia. Subjects 1 and 2 also had elevated Hcys values and decreased or low-normal folate concentrations. Cbl deficiency (with or without associated folate depletion) may have been responsible for the macrocytosis in these subjects. In an additional subject (Table 4; subject 3) with a high MCV, mild anemia, low serum folate, and a high Hcys concentration, folate deficiency may have been the cause of the macrocytic anemia. In 13 other subjects (Table 4; subjects 5–17), MCVs in the upper end of the normal range (95–99 fL) were associated with low or low-normal Cbl concentrations and elevations of one or both metabolites. It is possible that some of them had early hematologic abnormalities due to Cbl deficiency.

Other than the subject with macrocytic anemia and laboratory data consistent with folate deficiency (Table 4; subject 3), there were 13 persons with serum folate values < 11.3 nmol/L who were anemic; Hcys was elevated in only 3 of them. In each of the three Cbl was < 258 pmol/L, serum creatinine was elevated, and the MCV was normal or low.

The mean MCV and hematocrit of subjects with Cbl values < 258 pmol/L or with folate values < 11.3 nmol/L did not differ from those of subjects with higher values. However, the mean MCV of the subset of persons with a serum Cbl < 258 pmol/L and elevations of both metabolites (95.4 ± 8.9 fL, $n = 17$) was higher than that of the remaining subjects in the cohort (91.0 ± 5.4 fL, $n = 484$; $P < 0.005$), and the mean red blood cell count was lower (4.19 ± 0.66 vs 4.50 ± 0.54 × 10^6 cells/L, respectively; $P < 0.025$), although hematocrit values did not differ (0.396 ± 0.052 vs 0.409 ± 0.048, respectively; $P > 0.05$).

Antibodies to intrinsic factor

Serum was available for testing for antibodies to intrinsic factor from 58 of the 72 subjects with a Cbl concentration < 258 pmol/L and an elevation of MMA and/or Hcys, including 19 with elevations of both metabolites, and 9 with Cbl concentrations < 148 pmol/L in the absence of such elevations. Antibodies to intrinsic factor were found in 1 of the 67 subjects (a 79-y-old man with a hematocrit of 0.42, an MCV of 93 fL, serum Cbl of 118 pmol/L, MMA of 1301 nmol/L, and Hcys of 33.8 μmol/L).

Vitamin supplements

Of the 401 subjects responding to the questionnaire, 115 (28.7%) and 81 (20.2%) reported taking oral vitamin supplements containing Cbl and folate, respectively. The median daily doses were 6 μg Cbl and 400 μg folic acid. Nineteen (11.9%) of 160 subjects with Cbl values < 258 pmol/L reported taking supplements containing Cbl, in contrast with 96 (39.8%) of 241 with higher values ($X^2 = 52$, $P < 0.001$). Cbl supplements were taken by 8 (14.2%) of 56 subjects with MMA elevations, in contrast with 107 (31.0%) of 345 without elevations ($P < 0.02$). Three of 10 subjects who had high MMA concentrations and Cbl values < 148 pmol/L reported ingesting Cbl supplements. Supplements containing folic acid were taken by 65 (41.9%) of 155 persons with serum folate concentrations > 20 nmol/L, compared with 16 (6.5%) of 246 with lower folate values ($P < 0.001$).

After log transformation of the data, the reported intake of the respective vitamins correlated with serum vitamin concentrations ($r = 0.39$ for cobalamin and 0.43 for folate); the correlations with serum metabolite concentrations, however, were not impressive (for Cbl intake with MMA, $r = -0.15$ and with Hcys, $r = -0.26$; for folic acid intake and Hcys, $r = -0.25$).

Discussion

Two major findings were demonstrated by this study: *1*) a strikingly high prevalence of metabolically significant Cbl deficiency was found in the large, well-defined ambulatory elderly population of the Framingham community, and *2*) the majority of subjects with metabolic evidence of deficiency had serum Cbl concentrations within the conventionally defined normal range. Of the 548 members of the cohort, 65 (11.9%) had elevations of one or both metabolites and a serum Cbl concentration < 258 pmol/L (Table 2), findings strongly suggestive of Cbl deficiency (21, 23, 28–32). An additional seven subjects (1.3%) may have been deficient in Cbl (Table 2, line 4). The prevalence of deficiency may have been even greater, because an additional 35 subjects (6.4%) with serum Cbl concentrations < 258 pmol/L had MMA values between 2 and 3 SD above the mean in young control subjects in association with normal serum creatinine con-

TABLE 3
Pearson correlation coefficients (r) between serum metabolites (MMA and Hcys) and other variables in elderly subjects[1]

	MMA	Hcys
Serum cobalamin	−0.46	−0.50
Serum folate	−0.12	−0.42
Serum creatinine	0.30	0.38
Creatinine clearance	−0.30	−0.28
Serum urea nitrogen	0.24	0.25
Serum uric acid	0.17	0.33
Age	0.25	0.27
Serum Hcys	0.64	—

[1] P values for all correlations were < 0.001, except for the correlation between serum folate and MMA ($P < 0.01$). For all variables except age, creatinine clearance, and uric acid, data were log transformed before analysis. MMA, methylmalonic acid; Hcys, total homocysteine.

centrations, and a few subjects with serum Cbl values ≥ 258 pmol/L may have been deficient (16, 31).

The impressively high prevalence of metabolic evidence of lack of Cbl in a substantial cross-section of the Framingham elderly community is consistent with the findings of four recently reported series of consecutively studied geriatric outpatients seeking care in clinic or private office settings in whom serum metabolites were measured (16, 44–46). As in many previous reports, the hematologic changes typical of megaloblastic anemia (47) were absent in the great majority of subjects with evidence of deficiency, although modest abnormalities were demonstrable in a relatively small subset.

We chose a serum Cbl concentration of 258 pmol/L (350 pg/mL) as a cutoff point for suspecting Cbl deficiency, based on the overrepresentation of high serum MMA values in members of the cohort with Cbl concentrations below this number (**Fig 3**). Also, the degree of elevation of metabolite concentrations in subjects with Cbl determinations of 74–147 pmol/L was similar to that seen in subjects with values of 148–258 pmol/L (Fig 2). Indeed, the correlation between serum Cbl and each of the metabolite concentrations remained robust even when members of the cohort with serum vitamin values < 148 pmol/L were excluded from the analysis. Other investigators have also noted a negative correlation between serum Cbl values within the normal range and serum Hcys (25, 48–51).

There is other evidence to indicate that a conventional lower limit of normal for serum Cbl, as determined by subtracting 2 SDs from the mean of a young control population, will fail to detect a significant number of patients who are depleted of the vitamin. At least 5% of patients with unequivocal clinical evidence of Cbl were found to have serum Cbl determinations in the range 160–350 pmol/L, as measured by a precise and accurate modern radioassay, even though metabolite concentrations were increased (31). Also, patients with pernicious anemia receiving infrequent maintenance treatment doses, whose Cbl status was borderline or who showed minimal hematologic evidence of deficiency, often had serum Cbl values of 148–370 pmol/L even though one or both metabolite concentrations were clearly increased (31). Furthermore, in a recent study of ambulatory geriatric clinic patients who were screened for evidence of deficiency, all subjects found to have increased concentrations of one or both metabolites and serum Cbl determinations between 148 and 221 pmol/L responded to treatment with vitamin B-12

TABLE 4
Subjects in the Framingham elderly cohort with increased or high normal MCVs associated with elevated serum metabolite concentrations[1]

Subject number	Age (and sex)	MCV	Hematocrit	Cbl	Serum concentrations			
					Folate	MMA	Hcys	Creatinine
	y	*fL*		*pmol/L*	*nmol/L*	*nmol/L*	*μmol/L*	*μmol/L*
1	80 (M)	115	0.45	210	8.8	919	22.2	106
2	85 (F)	113	0.43	170	4.5	1172	61.1	124
3	81 (M)	103	0.34	302	4.3	149	27.6	88
4	81 (F)	101	0.40	232	52.0	857	17.7	106
5	73 (F)	100	0.42	144	9.7	470	13.5	80
6	91 (F)	99	0.39	162	16.1	439	24.8	124
7	80 (F)	99	0.43	188	26.1	400	12.9	80
8	71 (F)	98	0.47	199	9.7	432	28.3	62
9	71 (M)	98	0.44	136	7.7	369	31.4	80
10	80 (M)	97	0.41	55	22.7	3145	65.4	115
11	73 (F)	97	0.43	203	14.3	233	30.1	71
12	88 (F)	97	0.25	251	16.3	439	25.6	71
13	88 (F)	96	0.44	207	10.0	421	25.7	106
14	77 (F)	96	0.41	255	19.5	461	15.8	71
15	74 (M)	95	0.36	63	22.7	6820	77.5	88
16	86 (M)	95	0.39	148	11.1	1080	32.6	177
17	77 (F)	95	0.40	255	19.7	610	14.6	80
Normal value[2]		80–100	females ≥ 0.35, males ≥ 0.40	148–665	5.9–29.5	53–376	4.1–21.3	females ≤ 106, males ≤ 124

[1] MCV, mean cell volume; Cbl, cobalamin; MMA, methylmalonic acid; Hcys, total homocysteine. To convert Cbl values to pg/mL, multiply by 1.355. To convert folate values to ng/mL, multiply by 0.44. To convert creatinine values to mg/dL, multiply by 0.011.
[2] Normal ranges for MMA and Hcys indicate mean ± 3 SD.

with complete correction or a marked fall in the metabolite elevations (16). Similar findings have been reported in a study of elderly volunteers (52).

An increased prevalence of folate deficiency has also been reported in some elderly populations (53). Although depletion of folate appeared to be less common than that of Cbl in the Framingham cohort, folate deficiency may have caused or contributed to elevations in serum Hcys concentrations in some subjects. The importance of folate deficiency in the cohort was more difficult to evaluate because the majority of subjects with serum folate determinations < 11.3 nmol/L and high Hcys values also had Cbl concentrations < 258 pmol/L and MMA elevations, consistent with the presence of Cbl deficiency. Nonetheless, serum folate correlated inversely with Hcys, independent of serum Cbl. The relationship between folate status and the Hcys concentration was evident within the normal range, as has been reported by others for both plasma (51) and red cell folate values (25, 48–50, 54). Thus, as with serum Cbl, folate concentrations within the lower part of the normal range do not exclude deficiency. In addition, there are many reports of patients with frankly megaloblastic bone marrow changes due to folate deficiency despite normal serum or erythrocyte folate values (32, 55–61).

Our observations also indicate that moderate renal dysfunction in the absence of frank kidney failure may influence serum metabolite concentrations, although not as strongly as the vitamin deficiency states. The only significant limitation to the high specificity of serum MMA for Cbl deficiency in the diagnosis of megaloblastic anemias has been the presence of renal dysfunction (28, 32, 62). In the members of the Framingham cohort with increased MMA values, the higher the serum Cbl concentration, the more likely it was that renal dysfunction was present, although the metabolite elevations solely attributable to this factor were relatively modest. A somewhat stronger relationship was noted between serum creatinine and Hcys concentrations, which was also noted by others (26, 48–50, 63–65), although factors other than decreased renal function may contribute to this association, because it has been estimated that much of the Hcys produced is generated when methyl groups are utilized to form creatine-creatinine (50, 66). The correlation between serum Hcys and uric acid concentrations, which has been observed previously (26, 64, 67), is not well understood (64).

The reason for the high prevalence of Cbl deficiency in elderly people has not been established. Our finding that only 1 of 67 members of the Framingham cohort with evidence of Cbl deficiency had serum antibodies to intrinsic factor suggests that underlying pernicious anemia was not usually the cause, because such antibodies are found in more than half of patients with this disorder (68). Indeed, standard Schilling tests have been normal in the majority of elderly subjects with low serum Cbl determinations in the absence of megaloblastic anemia (7, 18, 69–71). Some workers have concluded from this finding, in addition to a tendency of low serum vitamin values to normalize after patients are hospitalized, that dietary Cbl deficiency is the major etiologic factor (7, 10). The association of low folate and Cbl concentrations with each other in our subjects could reflect dietary inadequacy. However, gastric atrophy, not advanced enough to cause pernicious anemia but sufficient to impair the secretion of acid and pepsin, which are needed to liberate Cbl from food, has been shown to be present in a substantial fraction of elderly patients with low serum Cbl values and normal Schilling tests (69, 71–73). In others, however, tests of gastric function or of food Cbl absorption have been normal (69, 71, 73). Gastric and intestinal bacterial overgrowth may contribute to Cbl malabsorption (69, 74). Clearly, further study is needed to unravel the mechanisms underlying the high prevalence of Cbl deficiency in elderly people.

In conclusion, in the elderly survivors of the original Framingham study metabolic evidence of Cbl deficiency was extremely common, of the order of 1 in 8 or 1 in 5 subjects. Folate deficiency was also seen but was less frequent. Metabolite elevations indicative of these deficiency states were often encountered in persons with normal serum values of the respective vitamins and in the absence of macrocytosis or anemia. The clinical importance of these findings remains to be explored.

Cbl deficiency, even in the absence of anemia, may have deleterious effects on the nervous system (20–22). An elevated serum Hcys concentration, which occurs in both Cbl and folate deficiency, is now a recognized risk factor for cerebral, coronary, and peripheral vascular disease (25–27, 75). Although most work has focused on subjects with premature arterial disease, several studies have implicated Hcys in elderly patients with these disorders (49, 64, 67, 76). The relationship of Cbl and folate deficiency to vascular complications in the Framingham geriatric cohort is currently under study.

Because the vitamin deficiencies seen in elderly people are easily treated and preventable, therapeutic interventions (75, 77, 78) should be rigorously evaluated. Indeed, the negative correlations between the intake of the vitamins and their serum concentrations indicate that these deficiencies can, at least in part, be prevented by oral supplementation in this population, although the dose of Cbl administered may have to be much larger than that usually given in routine multivitamin preparations. Some of the subjects with MMA elevations and low or low-normal Cbl values reported taking Cbl supplements at doses ≤ 10 μg/d. Patients with Cbl malabsorption often require daily oral supplements of 500–1000 μg to maintain normal serum concentrations of the vitamin (79).

Widespread supplementation with pharmacologic doses of folic acid has been proposed as an "innocuous" means of preventing vascular disease (78). Our findings suggest that at least in elderly people, generous doses of vitamin B-12 should be given simultaneously to prevent inappropriate mistreatment of Cbl deficiency with folic acid. The effects of such supplementation on thrombotic complications as well as neuropsychiatric function in elderly people should be the subject of a well-designed and controlled prospective investigation.

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