Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people[1-3]

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ABSTRACT Measurements of the serum concentrations of the metabolites homocysteine, cystathionine, methylmalonic acid, and 2-methylcitric acid, which accumulates when vitamin B-12–, folate–, and vitamin B-6–dependent enzymatic reactions are impaired, should provide a better indication of intracellular deficiency of these vitamins. We measured the serum concentration of these vitamins and the four metabolites in 99 healthy young people, 64 healthy elderly subjects, and 286 elderly hospitalized patients. A low serum vitamin B-12 concentration was found in 6% and 5%, low folate in 5% and 19%, and low vitamin B-6 in 9% and 51%, and one or more metabolites were elevated in 63% and 83% of healthy elderly subjects and elderly hospitalized patients, respectively. These results strongly suggest that the prevalence of tissue deficiencies of vitamin B-12, folate, and vitamin B-6 as demonstrated by the elevated metabolite concentrations is substantially higher than that estimated by measuring concentrations of the vitamins. *Am J Clin Nutr* 1993;58:468–76.

KEY WORDS Vitamin B-12 (cobalamin), folate, vitamin B-6 (pyridoxine), homocysteine, cystathionine, methylmalonic acid, 2-methylcitric acid, elderly adults

Introduction

An increasing prevalence of low serum vitamin B-12 (cobalamin), folate, and vitamin B-6 (pyridoxal phosphate) concentrations with advancing age has been found by many but not all investigators (1–13). Moreover, prevalence estimates for these vitamin deficiencies vary widely depending on the population groups studied. Until now it has been unclear whether this increased prevalence is a normal age-related phenomenon or a true reflection of tissue vitamin deficiency and whether the low serum vitamin concentrations are a reliable indicator of functional intracellular deficiency. With recently developed techniques that use capillary gas chromatography and mass spectrometry (14–16) it is possible to determine the serum concentrations of the metabolites homocysteine (HC), cystathionine, methylmalonic acid (MMA), and 2-methylcitric acid (2-MCA), which are affected by vitamin B-12–, folate–, and vitamin B-6–dependent enzymatic reactions (for a brief summary *see* **Fig 1**) (17).

Vitamin B-12 in the form of 5′-deoxyadenosylcobalamin is an essential cofactor in the enzymatic conversion of methylmalonyl-CoA into succinyl-CoA. The remethylation of HC to methionine catalyzed by methionine synthase requires folate (methyltetrahydrofolate) and vitamin B-12 in the form of methylcobalamin. HC is condensed with serine to form cystathionine, a reaction catalyzed by cystathionine β-synthase, which requires vitamin B-6. Cystathionine is hydrolyzed in another vitamin B-6–dependent reaction to cysteine and α-ketobutyrate. Elevations in serum HC and MMA concentrations are useful clinical tests of functional intracellular deficiency of vitamin B-12 and folate (18–24). Increased serum concentrations of cystathionine are seen in both deficiencies, and 2-MCA is elevated in vitamin B-12 deficiency (25, 26). HC and cystathionine should be elevated in patients with intracellular deficiency of vitamin B-6 but the results are not consistent (27–29).

To evaluate whether there is an increased prevalence of tissue deficiency of vitamins in elderly people we conducted a multicenter epidemiologic study in population groups consisting of healthy young adults, healthy elderly subjects, and elderly hospitalized patients. Serum vitamin B-12, folate, and vitamin B-6 concentrations as well as the serum concentrations of the four related metabolites were measured in all of the subjects.

Methods

Study population

From September 1991 to February 1992, a total of 449 subjects were recruited at the geriatric departments of university and general hospitals in Belgium, Germany, and The Netherlands

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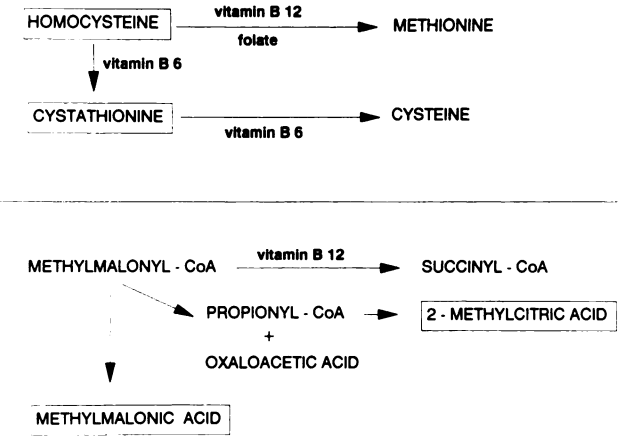

FIG 1. Metabolic pathways involving vitamin B-12, folate, and vitamin B-6 and the metabolites, homocysteine, cystathionine, methylmalonic acid, and 2-methylcitric acid.

and by a general practitioner in The Netherlands. The study population consisted of three groups: *1*) 99 healthy young subjects belonging to the medical and nursing staffs of the participating centers (53 males and 46 females), aged 19–55 y (median 28 y, mean 30 y), who did not suffer from any concomitant disease and used no medication; *2*) 64 healthy elderly people selected by a general practitioner (20 males and 44 females), aged 65–88 y (median 76 y, mean 76 y), living at home independently and able to carry out all normal daily activities. [Exclusion criteria included the presence of cancer, hematological malignancy, gastrointestinal surgery, alcoholism, history of renal insufficiency (serum creatinine > 177 μmol/L), vascular disease, psychiatric and neurological disorders, and concomitant treatment with iron, vitamins, H_2 histamin blockers, antibiotics, corticosteroids, cytotoxic drugs, and anticonvulsant drugs.]; and *3*) 286 hospitalized elderly patients (115 males and 171 females), aged 61–97 y (median 79 y, mean 78 y), with common geriatric diseases, eg, vascular disease, dementia, diabetes mellitus, osteoporosis, and osteoarthritis. Patients with any life-threatening disease were excluded. Informed consent was obtained from all subjects involved in the study. This study was approved by the Freiburger Ethik Kommission, Germany.

Serum vitamin and metabolite concentrations

In all subjects selected for the study, venous blood was obtained in the morning after an overnight fast. The blood was separated within 1 h after collection and the serum was transported in dry ice to the central laboratory. Serum vitamin B-12 and folate were measured by a quantitative radioassay method with purified intrinsic factor and purified folate-binding protein (Vitamin B-12/folate dual RIA kit CT301/CT302; Amersham, UK). Vitamin B-6 was measured by a radioenzymatic assay method: serum is incubated with apoenzyme tyrosine-apodecarboxylase and [^{14}C]tyrosine is added to start the enzymatic reaction, which is stopped with hydrochloric acid. Subsequently, the free [^{14}C]-carbon dioxide is adsorbed by filter paper impregnated with potassium hydroxide. The measured ^{14}C activity is directly proportional to the vitamin B-6 concentration (Laboratory Bioscientia, Germany). Serum metabolites—HC, cystathionine, MMA, and 2-MCA—were assayed by capillary gas chromatography and mass spectrometry (15, 16).

Because renal function can influence serum metabolite concentrations (23, 30), serum creatinine concentrations were measured in all subjects (Jaffe photometric method; Diagnostica Merck, Darmstadt, Germany): normal range 62–124 μmol/L. Creatinine clearance was calculated by using the formula of Cockroft and Gault (31).

Statistical methods

Statistical analysis was done with the SAS statistical package (version 6.03; SAS Institute Inc, Cary, NC). Nonparametric data for two or more groups were tested with the two-sample Wilcoxon rank-sum test (with Bonferroni's correction for the significance level α) and the Kruskall-Wallis test. Reference intervals were calculated by using results of the healthy young subjects. Because the frequency distribution of the values of each index were markedly abnormal, they were transformed to normal distributions by using logarithmic transformation. The sample prevalence p with 95% confidence intervals of low serum vitamin B-12, folate, and vitamin B-6 concentrations was calculated as

$$\left(p \pm 2\sqrt{p\frac{(1-p)}{n}}\right) \times 100$$

where n is the total sample size and p is the number of low serum vitamin concentrations/n; low serum concentration was defined as < mean − 2 SD.

The sample prevalence p with 95% confidence intervals of elevated serum metabolite concentrations was calculated by using the same formula, where p is the number elevated serum metabolite concentrations/n; elevated concentration was defined as > mean + 2 SD. For measurement of correlations between two groups of continuous variables, the Spearman correlation coefficient was used.

Results

The normal ranges for the serum concentrations of the vitamins calculated as the mean ± 2 SD of the values in the 99 healthy subjects 19–55 y of age were 103–406 pmol/L for vitamin B-12, 5.4–16.3 nmol/L for folate, 28.7–162 nmol/L for vitamin B-6, 5.0–13.9 μmol/L for HC, 72–245 nmol/L for cystathionine, 62–247 nmol/L for MMA, and 62–192 nmol/L for 2-MCA. The normal ranges are indicated by the interrupted lines in **Figures 2–8.** The absolute values of the measured serum vitamin concentrations are shown in Figures 2–4. The lowest serum vitamin concentrations were found among the elderly patients. In a few healthy elderly subjects the concentrations were very high, suggestive of concomitant intake of vitamins, but because the subjects had not reported vitamin use to the investigator, all of these subjects were included in the analysis.

The results of the measured serum metabolite concentrations for the three groups studied are shown in Figures 5–8. The serum concentrations of all four metabolites were highest in the elderly patients; the differences between the concentrations of each metabolite were statistically significant ($P < 0.001$) when compared with the healthy young subjects. When compared with the healthy elderly subjects there were also statistically significant differences ($P < 0.001$) except for MMA. In the healthy elderly subjects the serum metabolite concentrations were significantly higher ($P < 0.001$) than in the healthy young subjects. It can be seen from the figures for each metabolite that many of the ele-

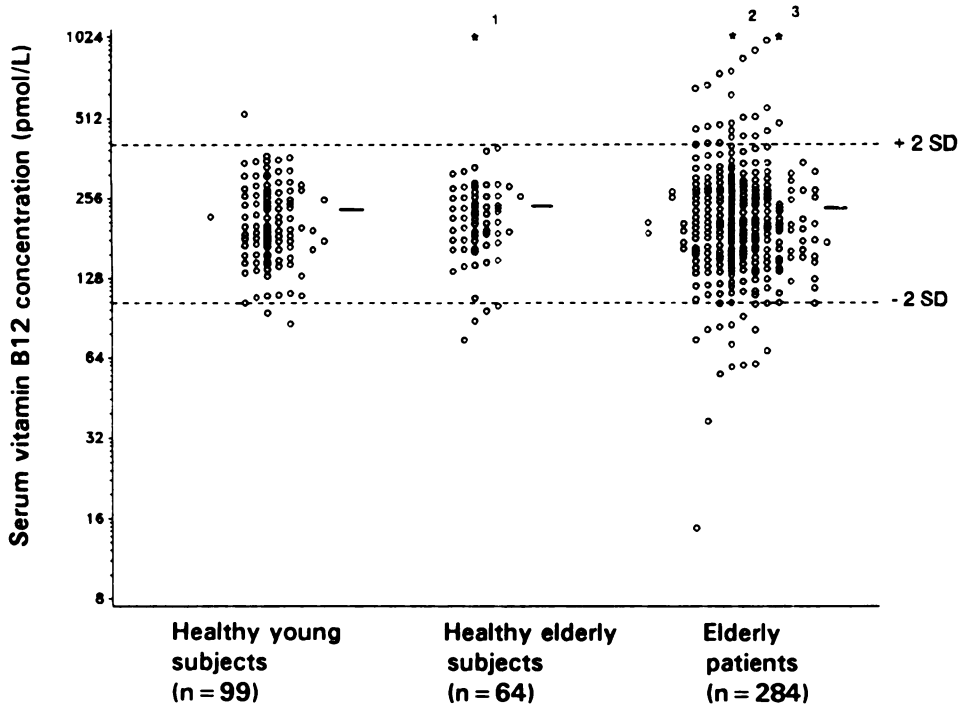

FIG 2. Serum vitamin B-12 concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects (*1 = 1370 pmol/L, *2 = 1135 pmol/L, *3 = 2951 pmol/L). Bold lines represent the geometric mean for each group.

vated serum concentrations in the elderly people were substantial; in some of the elderly patients very high concentrations were found, similar to those seen in florid clinical deficiencies of vitamin B-12 or folate (18–20, 25). In the healthy young subjects there was only a weak though significant inverse correlation between HC and folate ($r = -0.25$, $P = 0.012$) and cystathionine

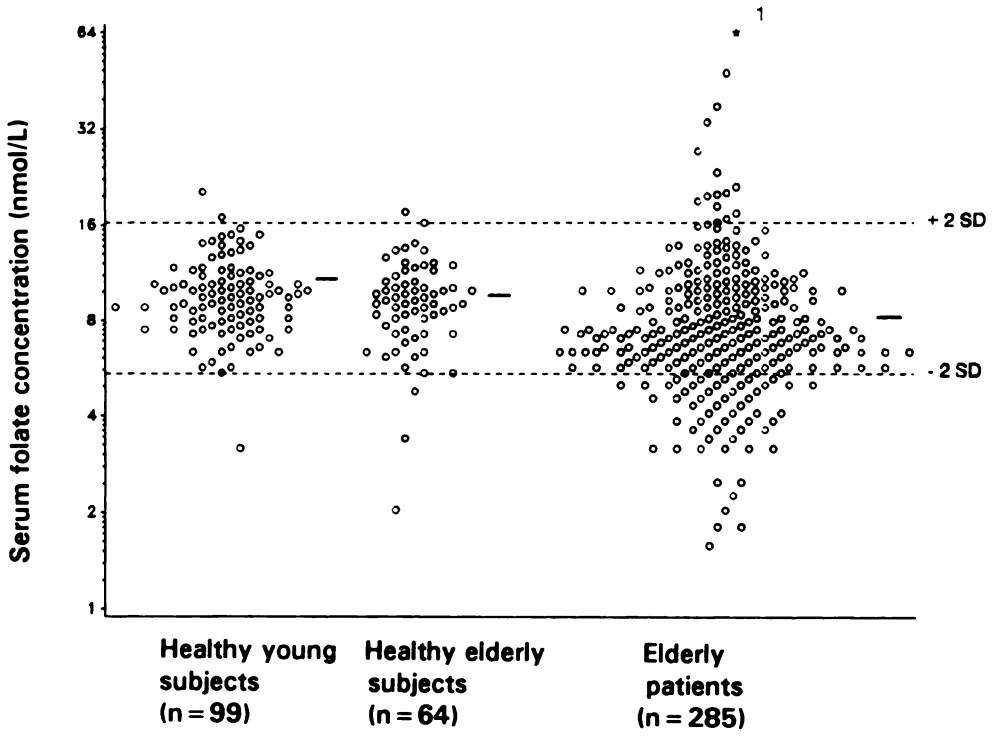

FIG 3. Serum folate concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects (*1 = 86.8 nmol/L). Bold lines represent the geometric mean for each group.

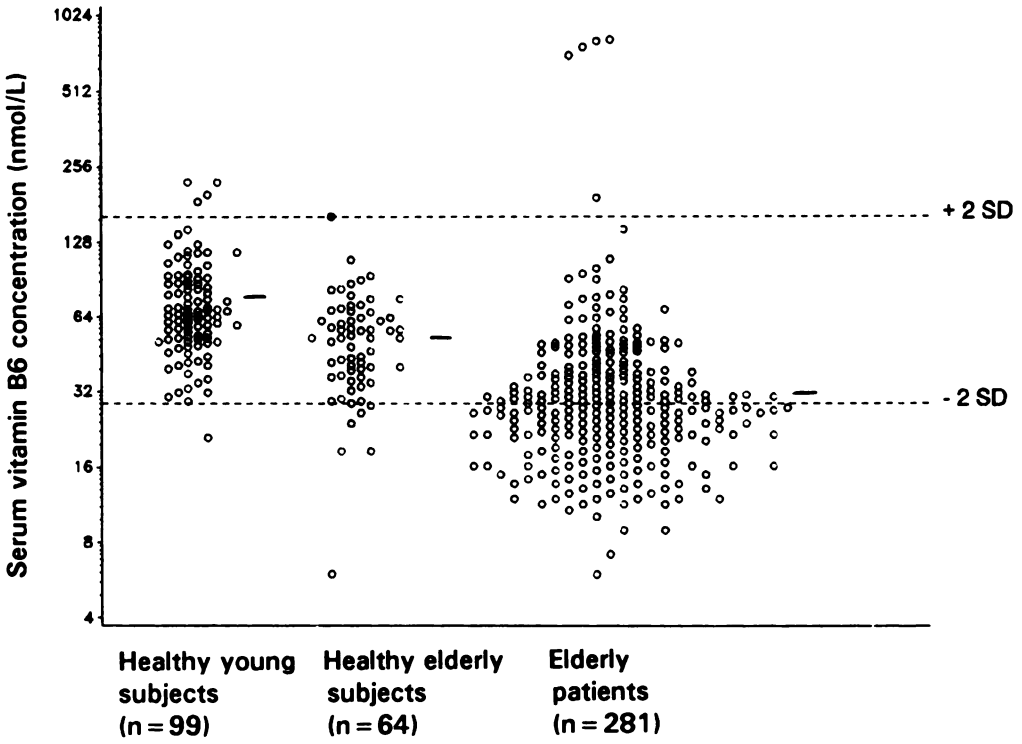

FIG 4. Serum vitamin B-6 concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects. Bold lines represent the geometric mean for each group.

and folate ($r = -0.21$, $P = 0.038$). In the healthy elderly subjects MMA and vitamin B-12 ($r = -0.36$, $P = 0.004$) and HC and folate ($r = -0.38$; $P = 0.0017$) were significantly correlated. In the elderly patients there were also significant correlations between MMA and vitamin B-12 ($r = -0.33$, $P = 0.0001$), HC and vitamin B-12 ($r = -0.27$, $P = 0.0001$), and HC and folate

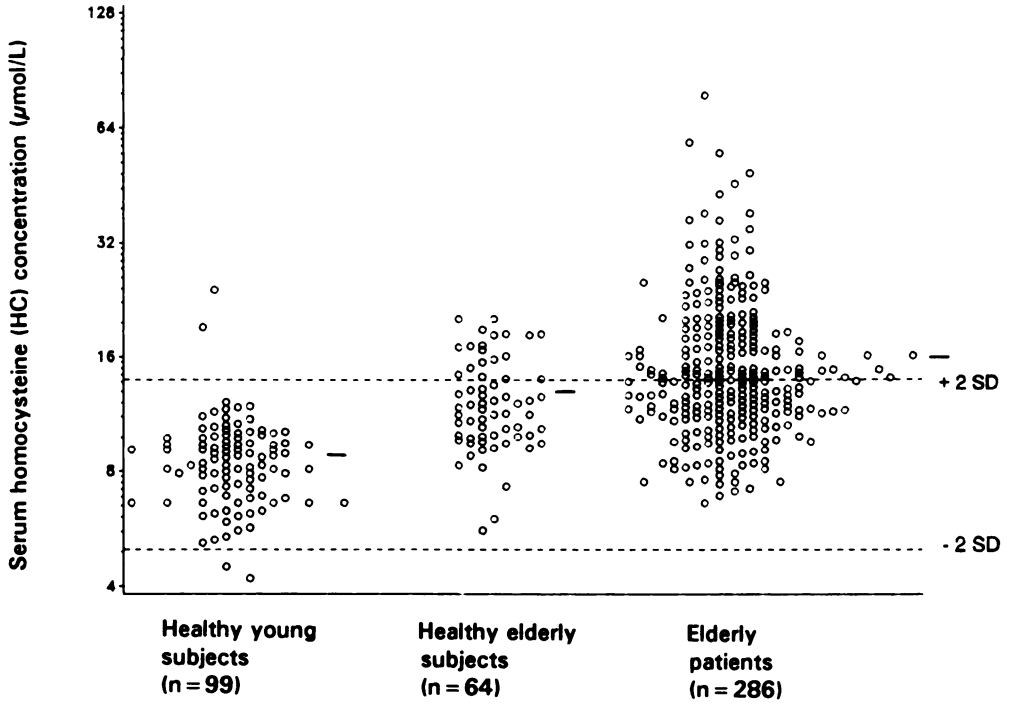

FIG 5. Serum homocysteine (HC) concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects. Bold lines represent the geometric mean for each group.

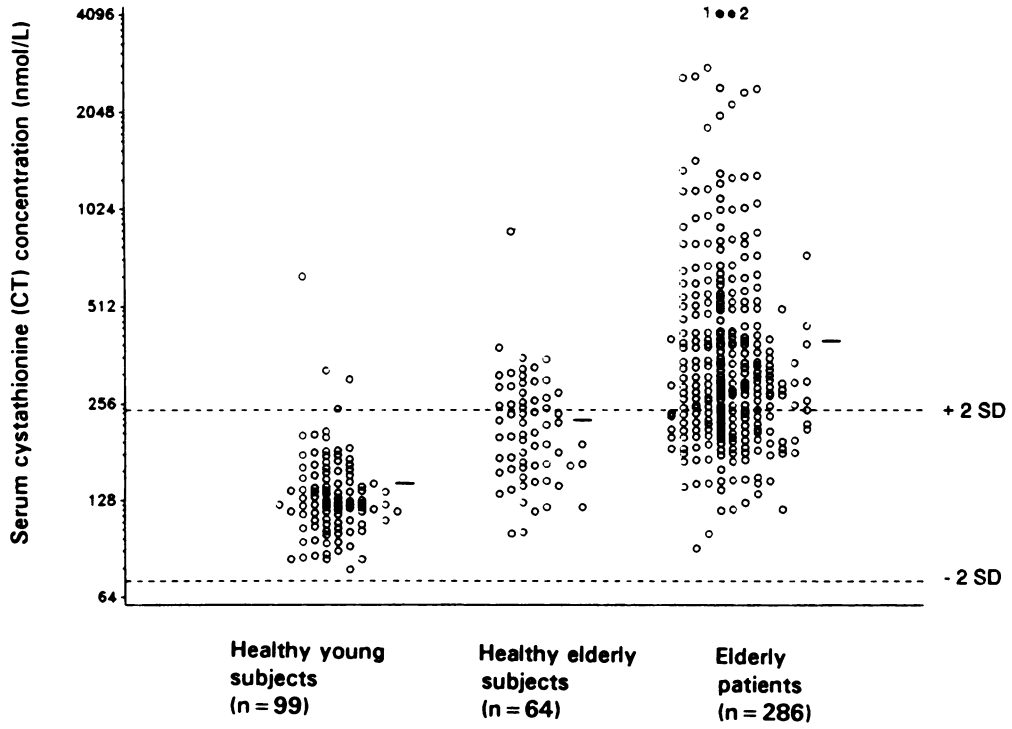

FIG 6. Serum cystathionine concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects (· 1 = 4832 nmol/L, · 2 = 8593 nmol/L). Bold lines represent the geometric mean for each group.

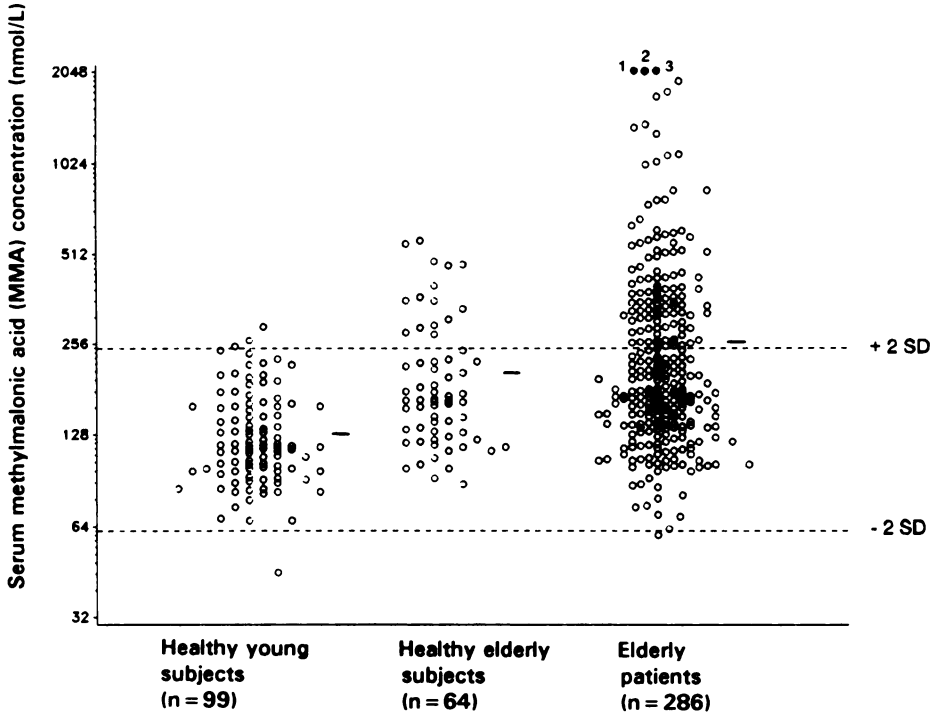

FIG 7. Serum methylmalonic acid (MMA) concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects (· 1 = 2072 nmol/L, · 2 = 8506 nmol/L, · 3 = 11980 nmol/L). Bold lines represent the geometric mean for each group.

($r = -0.38$, $P = 0.0001$). There was a significant but weak correlation between all four metabolites and vitamin B-6: HC: $r = -0.19$, $P = 0.001$; cystathionine: $r = -0.21$, $P = 0.0002$; MMA: $r = -0.12$, $P = 0.04$; and 2-MCA: $r = -0.14$, $P = 0.01$).

Table 1 shows the prevalence of abnormal serum concentrations of the vitamins and the four metabolites in both elderly study groups. Low serum concentrations of at least one vitamin were found in 19% of the healthy elderly group and 60% of the elderly patients. Low serum vitamin B-12 concentrations were found in 4 (6%) and 15 (5%), low folate concentrations in 3 (5%) and 54 (19%), and low vitamin B-6 concentrations in 6 (9%) and 144 (51%) of the healthy elderly subjects and elderly patients, respectively. On the other hand one or more of the serum metabolite concentrations were elevated in 63% of the healthy elderly subjects and in 82% of the elderly patients. HC and cystathionine, which are both related to all three vitamins, were elevated in the serum of 19 (30%) and 146 (51%) and of 23 (36%) and 195 (68%) of healthy elderly subjects and elderly patients, respectively. MMA concentrations were increased in 15 (23%) and 111 (39%) subjects of the two elderly groups and 2-MCA concentrations were elevated in 12 (19%) and 124 (43%) subjects, respectively.

In some subjects serum vitamin concentrations were low although metabolite concentrations were normal. This was observed in 3% of the healthy young subjects, 6% of the healthy elderly subjects, and 8% of the elderly patients (**Table 2**). There were, however, many more subjects who had elevated serum metabolite concentrations even though all three serum vitamin concentrations were normal. This was noted in 50% of the healthy elderly subjects vs 28% of the elderly patients and 13% of the healthy young subjects (Table 2). In at least 50% of these subjects and patients with one or more elevated metabolites and normal vitamin concentrations, the serum vitamin concentrations were well within the normal range: vitamin B-12 concentrations > 200 pmol/L, folate concentrations > 8 nmol/L, and

TABLE 1
Prevalence of abnormal serum concentrations of vitamins and metabolites in the elderly population*

	Healthy elderly subjects (n = 64)	Elderly patients (n = 286)
	%	
Low serum vitamin B-12	6 (0–12) [4]†	5 (3–8) [15]
Low serum folate	5 (0–10) [3]	19 (14–24) [54]
Low serum vitamin B-6	9 (2–17) [6]	51 (45–57) [144]
Low serum concentration of one or more vitamins	19 (9–29) [12]	60 (55–66) [73]
Elevated serum HC	30 (18–41) [19]	51 (45–57) [146]
Elevated serum cystathionine	36 (24–48) [23]	68 (63–74) [195]
Elevated serum MMA	23 (13–34) [15]	39 (33–45) [111]
Elevated serum 2-MCA	19 (9–29) [12]	43 (37–49) [124]
Elevated serum concentration of one or more metabolites	63 (50–75) [40]	82 (77–86) [234]

* HC, homocysteine; MMA, methylmalonic acid; 2-MCA, 2-methylcitric acid.

† 95% confidence interval in parentheses; n in brackets.

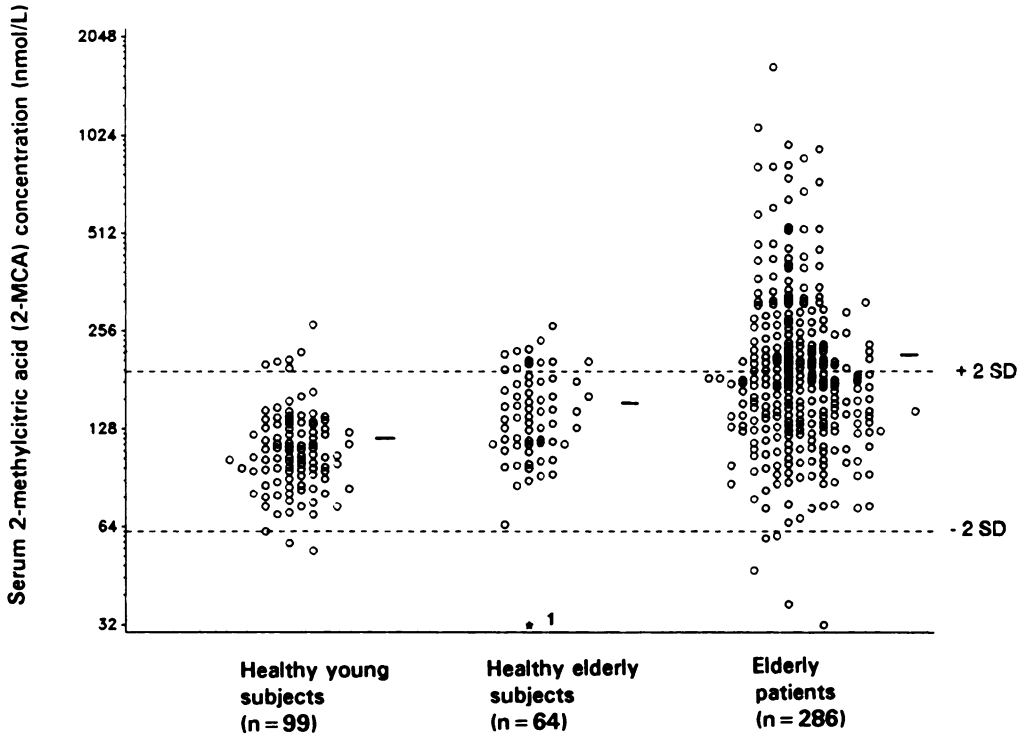

FIG 8. Serum 2-methylcitric acid (2-MCA) concentrations in the three groups of subjects. Horizontal lines represent 2 SD above and below the mean for the healthy young subjects (*1 = 11 nmol/L). Bold lines represent the geometric mean for each group.

TABLE 2
Relation between abnormal serum concentrations of vitamins and metabolites*

	Healthy young subjects (n = 99)	Healthy elderly subjects (n = 64)	Elderly patients (n = 286)
	%		
All four serum metabolite concentrations normal and one or more serum vitamin concentration low	3 [3]†	6 [4]	8 [22]
All four serum metabolite concentrations normal and serum vitamin B-12 concentration low	2 [2]	2 [1]	0 [1]
All four serum metabolite concentrations normal and serum folate concentration low	1 [1]	2 [1]	2 [5]
All four serum metabolite concentrations normal and serum vitamin B-6 concentration low	1 [1]	3 [2]	6 [18]
All three serum vitamin concentrations normal and one or more serum metabolite concentrations elevated	13 [13]	50 [32]	28 [79]
All three serum vitamin concentrations normal and serum HC concentration elevated	2 [2]	23 [15]	15 [44]
All three serum vitamin concentrations normal and serum cystathionine concentration elevated	4 [4]	30 [19]	21 [61]
All three serum vitamin concentrations normal and serum MMA concentration elevated	3 [3]	16 [10]	11 [31]
All three serum vitamin concentrations normal and 2-MCA serum concentration elevated	6 [6]	17 [11]	14 [41]

* HC, homocysteine; MMA, methylmalonic acid; 2-MCA, 2-methylcitric acid.
n in brackets.

vitamin B-6 concentrations > 40 nmol/L were found respectively in 6, 11, and 12 subjects of the healthy young subjects (n = 13, Table 2); in 19, 23, and 23 subjects of the healthy elderly subgroup (n = 32, Table 2); and in 45, 42, and 43 of the elderly patients (n = 79, Table 2).

In the healthy elderly subjects as well as in the elderly patients there were statistically significant but weak correlations between the calculated creatinine clearance and the serum concentrations of the metabolites. In the healthy elderly subjects the creatinine clearance correlated significantly ($P < 0.001$ in each case) with serum HC ($r = -0.40$), MMA ($r = -0.41$), and 2-MCA ($r = -0.44$); no significant correlation was found with cystathionine. In the elderly patients the calculated creatinine clearance correlated significantly ($P < 0.001$ in each case) with the serum concentrations of HC ($r = -0.44$), cystathionine ($r = -0.46$), MMA ($r = -0.43$), and 2-MCA ($r = -0.47$). In the healthy young subjects no correlation was found between creatinine clearance and any of the serum metabolite concentrations.

Discussion

The prevalence of low serum concentrations of vitamin B-12, folate, and vitamin B-6 found in this study corresponds with that reported by others (4, 6, 12). In addition, however, we found that elevations of serum metabolite concentrations consistent with deficiency of one or more of these vitamins were much more frequent in elderly subjects than were low serum concentrations of the vitamins. In 63% of the healthy elderly subjects and in 82% of the elderly patients an increased serum concentration of at least one metabolite related to these three vitamins was demonstrated, which contrasts with the 19% and 60% prevalence of low serum concentration of at least one vitamin in the same groups, respectively. For vitamin B-12 this contrast is most obvious. A low serum vitamin B-12 concentration was seen in 5% and 6% of the healthy elderly subjects and elderly patients, respectively, whereas an elevated serum MMA concentration was present in 23% and 39% of the subjects of the same groups. Furthermore, the individual serum metabolite concentrations are significantly higher in the elderly patients than in the healthy elderly subjects and significantly higher in the healthy young subjects than in the healthy elderly subjects. These findings strongly suggest that there is a higher prevalence of vitamin deficiency than could have been estimated by measuring serum concentrations of the vitamins. That increases in serum metabolite concentrations may indicate tissue deficiency in patients with normal serum vitamin concentrations was apparent in a study of patients with pernicious anemia, who were infrequently treated with vitamin B-12; marked elevations in serum MMA and serum HC values were common even though the serum vitamin B-12 concentration remained normal (14).

By the nature of the design of this cross-sectional population study, it could not be clearly determined which of the vitamin deficiencies was responsible for an elevated concentration of one or more metabolites in a given patient. This will require intensive metabolic and clinical studies in individual subjects before and after therapeutic trials with single vitamins. Alternative explanations of the elevated metabolites in elderly people should be considered, however. There could be an age-related impairment of the enzymatic reactions, which has been suggested for cystathionine β-synthase measured in cultured skin fibroblasts (32). This could explain why there are many subjects with elevated metabolite concentrations despite normal serum vitamin concentrations. Furthermore, in elderly subjects the metabolite concentrations were also correlated with creatinine clearance, in accordance with previous studies (23, 30).

An intervention study is ongoing to evaluate whether these elevated serum concentrations of the metabolites can be lowered by treatment with these vitamins. Preliminary results show that

weekly injections of vitamin B-12, folate, and vitamin B-6 are effective in normalizing elevated metabolite concentrations in virtually every elderly patient (HJ Naurath, A van den Berg, R Riezler, G van Breukeler, RH Allen, unpublished observations, 1992). Other studies describe treatment with folate (33) as effective in lowering plasma HC elevations and treatment with vitamin B-12 normalizes increased serum HC and MMA concentrations (20, 24). It may be debated whether patients with elevated serum metabolite concentrations who lack overt accompanying clinical symptoms should be treated. It has been shown, however, that elevated metabolite concentrations can have functional implications of clinical importance because thrombotic complications of atherosclerosis may be related to elevated serum HC concentrations (23, 34–38) and neuropsychiatric disorders associated with elevated serum HC and MMA concentrations may respond to treatment with vitamin B-12 (20, 24). Taking into account this association with atherosclerotic diseases and the potentially reversible neuropsychiatric disorders, and in view of our findings of a high prevalence of elevated serum metabolite concentrations, we believe that early detection of tissue deficiency of vitamin B-12, folate, and vitamin B-6 by assay of serum metabolites seems justified in elderly people in order to determine which patients might benefit from subsequent vitamin therapy. Long-term follow-up studies of these patients will be necessary to evaluate whether therapy with the appropriate vitamins is effective in terms of slowing, preventing, or reversing atherosclerosis and neuropsychiatric disorders.

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Erratum

E Joosten, A van den Berg, R Riezler, HJ Naurath J Lindenbaum, SP Stabler, RH Allen. Metabolic evidence that deficiencies of vitamin B-12 (cobalamin), folate, and vitamin B-6 occur commonly in elderly people. Am J Clin Nutr 1993;58:468–76. The section of the methods that describes the determination of serum vitamin and metabolite concentrations on page 469 should read (left-hand column, second paragraph, line 7), "Vitamin B-6 was measured by a radioenzymatic assay method: *deproteinized* serum is incubated with apoenzyme tyrosine apodecarboxylase. . . ."