B vitamin status and concentrations of homocysteine and methylmalonic acid in elderly German women[1–3]

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ABSTRACT

Background: Prior investigations found that elderly persons are at higher risk than are younger persons for B vitamin deficiency, which leads to elevated plasma total homocysteine (tHcy) concentrations that are associated with an increased risk for certain diseases such as coronary artery disease. To date, published data have shown decreased vitamin status and elevated tHcy among the elderly.

Objective: We evaluated the dietary intake and the blood status of various B vitamins and tHcy and methylmalonic acid (MMA) concentrations in 178 younger (60–70-y-old) female seniors.

Design: Dietary intake was assessed with a 3-d diet record. Thiamine, riboflavin, and vitamin B-6 activity coefficients of erythrocyte transketolase (EC 2.2.1.1), erythrocyte glutathione reductase (EC 1.6.4.2), and erythrocyte α-aspartic aminotransferase (EC 2.6.1.1) were used as functional indexes for the status of the 3 vitamins, respectively. Concentrations of serum and red blood cell folate, serum cobalamin and MMA, and plasma tHcy were measured.

Results: Indexes of thiamine, pyridoxine, and cobalamin indicated insufficient status in one-third of the women, whereas tHcy and MMA concentrations were elevated in 17.4% and 9.6% of the women, respectively. An association between vitamin intake and vitamin concentration in the blood was found only for folate. The mean tHcy concentration in subjects in the lowest quartile of serum folate concentration was 23% higher than that in subjects in the highest quartile. There was no association between riboflavin and tHcy concentrations. MMA was positively correlated with age and inversely correlated with serum cobalamin concentration.

Conclusions: Even in younger, well-educated, female seniors, the prevalence of low B vitamin status and elevated plasma tHcy concentration is high. Thiamine, pyridoxine, folate, and cobalamin supplementation should be considered. *Am J Clin Nutr* 2003;78:765–72.

KEY WORDS Elderly, women, dietary intake, vitamins, folate, thiamine, riboflavin, vitamin B-6, vitamin B-12, pyridoxine, cobalamin, homocysteine, methylmalonic acid

INTRODUCTION

There is a variety of reasons why elderly persons are at higher risk than are younger persons for poor B vitamin status, including a higher prevalence of drug intake and lower vitamin bioavailability, especially because of atrophic gastritis or gastric hypoacidity (1, 2). An inadequate status of B vitamins is associated with impairments and diseases such as reduced immune (3, 4) and cognitive (5–7) function. Furthermore, epidemiologic data implicated a low folate supply as a risk factor in cardiovascular disease (CVD; 8, 9) and cancer (10–12), presumably because of the role of impaired methylation as a determining factor (2). Hyperhomocysteinemia has been associated with CVD and other age-related diseases (13, 14), but no data exist on whether lowering tHcy concentrations reduces CVD morbidity (8). There is, however, increasing evidence that folic acid protects against CVD disease via mechanisms other than the lowering of plasma homocysteine concentration (15, 16).

As indexes of thiamine, riboflavin, and pyridoxine status, the activity coefficients of erythrocyte transketolase (ETK; EC 2.2.1.1), erythrocyte glutathione reductase (EGR; EC 1.6.4.2), and erythrocyte α-aspartic aminotransferase (α-EAST; EC 2.6.1.1) are useful markers. Activity coefficients of these enzymes increase with vitamin depletion and have been determined as long-term variables of the vitamin status (17–22).

An elevated concentration of plasma total homocysteine (tHcy) can be a sensitive marker for both poor folate and poor cobalamin status (23). An elevated concentration of methylmalonic acid (MMA) is thus the preferred indicator for vitamin B-12 status. It represents a metabolic change that is highly specific to cobalamin deficiency. Even in mild, preclinical cobalamin deficiency, MMA concentrations are elevated (24, 25).

The main determinants of tHcy concentration are intakes and plasma concentrations of folate and vitamin B-12, whereas the results regarding vitamin B-6 are inconsistent (26–28). Riboflavin, in the form of flavin adenine dinucleotide, takes part in homocysteine metabolism as cofactor of methylentetrahydrofolate reductase (EC 1.7.99.5), and studies showed that low dietary intake and low plasma riboflavin concentrations are associated with elevated tHcy (27, 29, 30).

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Sufficient B vitamin intake and status seem to be important and predictive for the risk of certain diseases. Some studies indicate an inadequate B vitamin status as well as increased tHcy and MMA concentrations among the elderly over 65 or 70 y of age (31–33). The aim of our study was to evaluate B vitamin status, including tHcy and MMA concentrations, and to assess associated factors such as dietary intake and sociodemographic factors that might help to predict homocysteine concentrations in healthy younger seniors.

SUBJECTS AND METHODS

Subjects

Women aged 60–70 y (n = 178) were recruited by advertisements in several newspapers in and around the city of Hanover, Germany. Subjects with severe chronic diseases, cancer, or a history of gastrointestinal resection; smokers; and supplement users were excluded. All subjects gave written informed consent. The study was conducted in accordance with the Helsinki Declaration of 1964 as amended in 1983 and 1996.

Diet record, questionnaires, and anthropometric data

A 3-d diet record assessed energy and nutrient intakes of the women. This 3-d diet record was adapted from a validated 7-d diet record (34). The dietary record was reduced to 3 d because we assumed a higher validity if the burden was less. It was slightly modified to adapt the food variety to elderly people. The energy and nutrient values were calculated with FOODOPT software (version 2.65; Albat & Wirsam, Linden, Germany), which is based on the German Food Code and Nutrient Database (BLS II.2) (35). To evaluate the correctness of the documented data, we calculated a ratio of the reported energy intake to the estimated basal metabolic rate on the basis of individual measured body weight. The FAO/WHO/UNO equation for women aged 60–74 y was used to calculate basal metabolic rate (36). The mean ratio of energy intake to basal metabolic rate in our study was 1.51, which was higher than the proposed minimum mean ratio of energy intake to basal metabolic rate of 1.49 for a sample size of 200 with 4 d of food intake data (37).

To assess the calculated nutrient intake of the sample, the intake was compared with the corresponding recommended dietary allowance (RDA) for the particular age and sex group. Because this is the dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy persons, it overestimates the requirements of nearly 50% of a group. Therefore, we also compared the nutrient intake of our sample to the estimated average requirement. This is the nutrient intake value that is estimated to meet the requirements of half the healthy persons in a life-stage and sex group (38).

Anthropometric data were determined on the same day blood samples were drawn. Questionnaires asking for sociodemographic data were sent to the study participants and returned on the day of the blood sampling.

Blood sampling and storage

Blood samples were drawn after an overnight fast and centrifuged at 2665 × g for 10 min at 19 °C. Serum aliquots for vitamin analysis were stored at −4 °C and transported to the laboratory (Department of Clinical Chemistry, University of Giessen, Germany) within 5 h. Serum aliquots for the determination of MMA were stored at −20 °C and transported to the Medical Diagnostic Institute of the City Hospital of Karlsruhe, Germany.

Analytic methods

Serum concentrations of folate, cobalamin, and MMA as well as plasma tHcy were determined. The activity coefficients of α-EAST, ETK, and EGR were measured in blood drawn into heparinized tubes. Red blood cell (RBC) folate was measured in blood from EDTA-coated tubes to evaluate long-term folate status.

An automated chemiluminescence system (ACS:180; Chiron Diagnostics, Fernwald, Germany) was used to measure serum cobalamin and folate concentrations in serum and erythrocytes (39–41). ETK activity was measured before and after stimulation by the addition of thiamine diphosphate (18,19). EGR activity was used for assessing riboflavin status (21). The stimulation of α-EAST by pyridoxal-5′-phosphate (PLP), which increases with vitamin B-6 depletion, has been measured as a variable of pyridoxine status (22).

We measured tHcy by using the IMx assay (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany). This method uses fluorescence polarization immunoassay (FPIA) technology (42).

Serum MMA was measured with the use of gas chromatography–mass spectrometry. For sample preparation, 3 mL methanol was placed on an anion-exchange solid phase extraction column (200 mg; Merck, Darmstadt, Germany), and the column was centrifuged at 15 × g for 2 min at 20° C. The procedure was repeated with 3 mL demineralized water. A 1-mL serum sample was mixed with 1 mL internal standard solution (500 ng ethylmalonic acid/mL in demineralized water) and was placed on the preconditioned column and centrifuged at 300 U/min for 2 min. The column was then washed in the same way twice with 3 mL demineralized water and once with 3 mL methanol. MMA and the internal standard were diluted from the solid phase extraction column in 1 mL of 95:5 (by vol) methanol:formic acid without centrifugation. A 0.5-mL aliquot of the eluate was then evaporated under nitrogen to remove the methanol. To eliminate the less volatile formic acid, the residue was transferred to 100 μL acetonitrile and again evaporated under nitrogen. For derivatization, 100 μL of the silanizing agent (1 mL *N*-methyl-*N*-trimethylsilyltrifluoracetamide + 50 μL trimethylchlorsilane + 2 mL acetonitrile) was added to the vial and heated at 60 °C for 30 min. Derivatized extracts were analyzed on a gas chromatograph (model GC-17A/GCMS-QP 5000; Shimadzu, Duisburg, Germany). Separations were achieved on a fused-silica DB-5MS capillary column (30-m × 0.25-mm ID) coated with a 0.25-μm film (J&W Scientific, Folsom, CA). The gas chromatograph was equipped with a programmable-temperature vaporization injector with quartz line. A 1-μL aliquot was injected; the splitting ratio was 1:23. The temperature program for the programmable-temperature vaporization injector was set at 90 °C for 0.5 min, then at 250 °C/min to 300 °C, followed by a 5-min hold. The helium carrier gas flow rate was 1.1 mL/min. The oven temperature program was set at a 3-min hold at 70 °C, then at 20 °C/min to 110 °C, at 5 °C/min to 140 °C, and at 40 °C/min to 300 °C, followed by a 5-min hold. The mass spectrometer was operated in the selected ion–monitoring mode; detection of MMA and the internal standard was performed by using the mass-to-charge ratio (m/z) of 147. The interface temperature was 300 °C; the electron ionization energy was 2.0 kV. Intraassay CVs were 6.7% and 10.7% with 50 ng/mL (423 nmol/L) and 10 ng/mL

TABLE 1
Defined cutoffs of concentrations and activity coefficients of the variables

Blood variable and reference	Cutoff
Erythrocyte transketolase (19)	≥1.15
Erythrocyte glutathione reductase (21)	>1.34
Erythrocyte α-aspartic aminotransferase (22)	≥1.60
Serum cobalamin, pmol/L (43)	<258
Serum folate, nmol/L (44, 45)	<7
Red blood cell folate, nmol/L (44)	<320
Plasma total homocysteine, μmol/L (49, 50)	>12
Serum methylmalonic acid, nmol/L (54)	>271

(84.7 nmol/L) MMA, respectively. Interassay CVs were 4.7% and 6.6% with 70 ng/mL (593 nmol/L) and 40 ng/mL (339 nmol/L) MMA, respectively.

Cutoff values

Because elevated MMA and homocysteine concentrations have been reported in association with plasma cobalamin < 258 pmol/L (43), this cobalamin value was taken to indicate inadequate status. As a cutoff for adequate RBC folate status, a value of ≥ 320 nmol/L was chosen (44). A serum folate concentration of < 7 nmol/L indicates a negative folate balance (38, 45). Subjects with ETK activity coefficients < 1.15 are considered to be at low risk for thiamine deficiency, whereas those with activity coefficients of 1.15–1.25 or > 1.25 are considered to be at moderate or high risk, respectively (20). A cutoff of ≥ 1.15 was taken to indicate inadequate thiamine status (19). In a study including several hundred apparently healthy subjects aged ≥ 60 y, a cutoff for the EGR activity coefficient of 1.34 was observed to be the upper limit of normality, based on the $\bar{x} \pm 2$ SDs (46). An α-EAST activity coefficient of < 1.6 was proposed as an indicator of adequate vitamin B-6 status (47). Suggested normal tHcy values ranged in the literature from < 14 μmol (48) to 5–13.6 μmol/L (49) and 4.9–11.7 μmol/L (50). Many studies agree that a tHcy concentration of ≤ 10 μmol/L is optimal (51–54). Because tHcy concentrations increase with age (49), we defined a tHcy concentration of ≤ 12 μmol/L as normal. The range of serum MMA values in healthy subjects (± 2 SDs) is 73–271 nmol/L (55). MMA concentrations rise because of an inadequate vitamin B-12 intake or absorption (49). All defined cutoffs are shown in **Table 1**.

Statistical analysis

Data were analyzed by using SPSS software (version 10.0.7; SPSS Inc, Chicago). The nutrient and food intake data of the diet record were also transferred to SPSS for analyzing. Results are shown as means ± SDs. Normal distributions of data were checked by using the Kolmogorov-Smirnov-test. If data were skewed, they were log transformed to normalize them. Bivariate correlations were analyzed using the Pearson correlation coefficient to identify associations among normally distributed variables. Spearman correlation coefficients were calculated in the case of skewed distribution that could not be normalized. To assess for differences in vitamin status between employed and unemployed women or between women living alone and those living in a family, the independent-sample *t* test was used. Analysis of variance (ANOVA), in this case a one-way procedure with trend analysis, was used to test for different educational levels as influencing factors of blood variables. Furthermore, ANOVA (one-way procedure with Scheffe's procedure and Tukey's test for post hoc analysis) was used to test for differences in homocysteine concentrations in quartiles of folate status and for differences in MMA and homocysteine concentrations as quartiles of cobalamin. $P < 0.05$ was considered significant.

TABLE 2
Characteristics of the study population[1]

Variables	Value
Anthropometric (*n* = 178)	
Age (y)	63.2 ± 2.73[2]
Height (m)	1.64 ± 0.06
Weight (kg)	68.7 ± 10.6
BMI (kg/m^2)	25.6 ± 3.77
Dietary intake and EI:BMR (*n* = 174)	
Energy	
(MJ)	8.37 ± 1.84
(kcal)	2000 ± 440
EI:BMR	1.51 ± 0.34
Protein (g)	78.5 ± 20.9 (17% of energy)
Fat (g)	80.4 ± 24.0 (35% of energy)
Carbohydrate (g)	214.0 ± 52.4 (44% of energy)
Alcohol (g)	11.2 ± 12.8 (4% of energy)
Dietary and scioeconomic (*n* = 178)	
Type of diet (*n*)	
Omnivorous	162
Vegetarian	8
Other	8
Meal preparation (*n*)	
By subjects	173
By others	5
Living arrangements (*n*)	
With a family	105
Alone	53
Unspecified	17
Employment (*n*)	
Yes	25
No	153

[1] EI, energy intake; BMR, basal metabolic rate.
[2] $\bar{x} \pm$ SD.

RESULTS

Subject characteristics and dietary intake

The characteristics of the study population as well as the dietary intake of energy and macronutrients are shown in **Table 2**. Whereas the anthropometric data were normally distributed, the age distribution (median: 62.0 y; 5–95 percentiles: 60.0–68.1) was markedly skewed. The study participants were generally better educated than the general German population: 21% had the general qualification for university entrance compared with only 8% of women aged ≥ 60 y in the German population (56).

The intakes of thiamine, riboflavin, vitamins B-6 and B-12, and folate of the 174 women who completed the diet record, as well as the RDAs, the estimated average requirement of vitamins, and the percentage of women who failed to achieve these values, are shown in **Table 3**. Folate intake was seen as the most critical, because more than 80% of the sample did not achieve the RDA value, and the intake of 60% is even below the estimated average requirement. Furthermore, the intakes of thiamine and vitamin B-6 are below the RDA in 29% and 17% of cases, respectively.

TABLE 3
Daily dietary intake, recommended dietary allowance (RDA) and estimated average requirement (EAR) of the B vitamins, and frequency of intakes below the RDA and EAR values in the study population[1]

Nutrient	Daily intake	RDA	Women with vitamin intake below RDA	EAR	Women with vitamin intake below EAR
			n (%)		*n* (%)
Thiamine					
(mg)	1.32 ± 0.36[2]	1.1[3]	51 (29.3)	0.9[3]	13 (7.5)
(mg/MJ)	0.16 ± 0.04				
Riboflavin (mg)	1.77 ± 0.50	1.1[4]	4 (2.3)	0.9[4]	1 (0.6)
Vitamin B-6					
(mg)	1.96 ± 0.53	1.5[3]	29 (16.7)	1.3[3]	8 (4.6)
(μg/g protein)	25.7 ± 6.65				
Vitamin B-12 (μg)	5.08 ± 3.32	2.4[3]	11 (6.3)	2.0[3]	2 (1.1)
Folate equivalents (μg)	318 ± 96.3	400[3]	144 (82.8)	320[3]	104 (59.8)

[1] RDAs and EARs from reference 40.
[2] $\bar{x}$ ± SD.
[3] For women aged ≥51 y.
[4] For women aged 19–70 y.

B vitamin status

Blood values and the percentages of women with concentrations not meeting the defined cutoffs are presented in **Table 4**. The mean homocysteine value was 9.88 μmol/L, and concentrations ranged from 5.3 to 19.1 μmol/L. The concentrations of MMA ranged from 76.2 to 448 nmol/L, and the mean was 175 nmol/L. More than 17% of the sample had tHcy concentrations > 12 μmol/L and serum cobalamin concentrations below the defined cutoff of 258 pmol/L. Furthermore, 37% of the women had both elevated ETK and α-EAST activity coefficients, which indicated a low thiamine and pyridoxine status. The 8 lactoovovegetarians had significantly higher mean MMA values (259 ± 82.5 nmol/L; $P = 0.001$) than did the omnivores, although the 2 groups did not differ significantly in age or cobalamin status.

Relations between metabolic indicators of B vitamin status and other factors

No significant association was found between the intake of different food groups, such as meat or vegetables, and blood variables. Regarding single nutrients, a weak but statistically significant correlation was seen between the intake of folate equivalents and serum folate concentration ($r = 0.20$, $P = 0.009$). No significant correlations were observed between the dietary intake of thiamine, riboflavin, vitamin B-6, or cobalamin and their particular dependent blood variables.

Cobalamin status was slightly but significantly associated with folate status. Pearson correlation coefficients were 0.16 ($P = 0.034$) and 0.228 ($P = 0.003$) for RBC folate and serum folate, respectively. In contrast, the pyridoxine status was inversely correlated to cobalamin ($r = -0.316$, $P < 0.001$). A significant inverse correlation was also found for α-EAST and RBC folate ($r = -0.191$, $P = 0.011$) as well as serum folate ($r = -0.194$, $P = 0.011$). The EGR activity coefficient was not significantly associated with any of the other vitamin variables. No correlations were found between either body mass index (in kg/m^2) or age and any of the blood variables.

The tHcy concentrations were inversely correlated with serum folate ($r = -0.420$, $P < 0.001$) and with RBC folate ($r = -0.323$, $P < 0.001$). An inverse correlation was also observed between serum cobalamin and tHcy ($r = -0.208$, $P = 0.006$). Neither the α-EAST activity coefficient nor the EGR activity coefficient was significantly correlated with plasma tHcy concentrations. Furthermore, there was no significant association between dietary intake of evaluated vitamins, alcohol or protein intake, and tHcy concentrations. There was a significant positive association between tHcy concentrations and age ($r = 0.22$, $P = 0.003$) but not between tHcy concentrations and body mass index. MMA was positively correlated with tHcy ($r = 0.208$, $P = 0.005$).

The mean of tHcy in quartile categories of serum and RBC folate is shown in **Table 5**. Participants who were in the highest quartile for serum folate concentration had a 23% lower mean tHcy concentration than those with serum folate concentrations in the lowest quartile. A similar result can be seen for RBC folate concentrations. The mean tHcy concentration in subjects in the highest quartile of RBC folate was 20% lower than that in

TABLE 4
Activity coefficients of erythrocyte transketolase (ETK), erythrocyte glutathione reductase (EGR), and erythrocyte α-aspartic aminotransferase (α-EAST); concentrations of red blood cell and serum folate, serum cobalamin, serum MMA, and plasma tHcy; and frequency of women with values deviating from the cutoff[1]

Blood variable	Measured value	Women with values not in the normal range
		n (%)
Activity coefficient		
ETK (*n* = 177)	1.14 ± 0.07[2]	65 (36.7)
EGR (*n* = 177)	1.16 ± 0.11	11 (6.2)
α-EAST (*n* = 177)	1.56 ± 0.21	65 (36.7)
Folate (nmol/L)		
Serum (*n* = 172)	20.1 ± 6.67	0
Red blood cell (*n* = 176)	631 ± 185	4 (2.3)
Serum cobalamin (pmol) (*n* = 177)	290 ± 98.1	76 (42.9)
Plasma tHcy (μmol/L) (*n* = 178)	9.88 ± 2.41	31 (17.4)
Serum MMA (nmol/L) (*n* = 177)	175 ± 70.5	17 (9.6)

[1] MMA, methylmalonic acid; tHcy, total homocysteine.
[2] $\bar{x}$ ± SD.

TABLE 5
Plasma total homocysteine (tHcy) by quartile (Q) of serum and red blood cell (RBC) folate[1]

	Q1	Q2	Q3	Q4
tHcy				
By Q of serum folate[2]	11.5 ± 2.6	10.3 ± 2.4	9.1 ± 2.0[3]	8.8 ± 1.7[3,4]
By Q of RBC folate[2]	11.1 ± 2.9	9.9 ± 2.1	9.6 ± 1.9[5]	8.9 ± 2.1

[1] $\bar{x}$ ± SD. For serum folate (n = 172), the quartiles (in nmol/L) were as follows: Q1, ≤15.0; Q2, 15.1 to ≤19.0; Q3, 19.1 to ≤24.0; Q4, >24.0. For RBC folate (n = 176), the quartiles (in nmol/L) were as follows: Q1, ≤513; Q2, 514 to ≤608; Q3, 609 to ≤726; Q4, >727.
[2] Significant difference between quartiles, $P < 0.001$ (one-way ANOVA).
[3,5] Significantly different from Q1 (Scheffe's post hoc test): [3] $P < 0.001$, [5] $P = 0.029$.
[4] Significantly different from Q2, $P = 0.021$ (Scheffe's post hoc test).

subjects in the lowest quartile. Differences between categories were significant for serum and RBC folate.

As expected, a significant inverse correlation was found between serum cobalamin and MMA ($r = -0.25$, $P = 0.001$). The correlation between serum cobalamin and MMA was even higher when only the 76 women with low cobalamin status (cobalamin concentration < 258 pmol/L) were considered ($r = -444$, $P < 0.001$). Of these women, 11.8% had elevated concentrations of serum MMA. There was a significant association between age and MMA concentrations ($r = 0.175$, $P = 0.02$).

As shown in **Table 6**, MMA concentrations did not consistently decline with increasing serum cobalamin. Differences in MMA between the 4 quartiles were significant when the second and the fourth quartiles were compared with the lowest quartile.

In considerations of socioeconomic characteristics, data on different training or educational levels were contradictory. If different levels of education only were assessed, one-way ANOVA showed no significant differences in blood values. However, between women with different levels of vocational training, the ANOVA showed a significant linear trend in thiamine status ($P = 0.017$). The women with the highest education (university degree) had significantly lower thiamine status than did the women with apprenticeship or no vocational training (**Table 7**). Except for homocysteine concentrations, which were significantly ($P = 0.049$) higher in the employed women, blood values did not differ between the women who were employed (n = 21) and those who were not (n = 142). Blood variables did not differ between women who lived alone (n = 53) and those who lived in a family (n = 105).

DISCUSSION

The results show that low thiamine, vitamin B-6, and cobalamin status and elevated tHcy concentrations are highly prevalent among German women aged 60–70 y. The fact that more than one-third of the women in our study displayed an increased ETK activity coefficient reflects their low thiamine status. This result is in accord with results of earlier investigations (57–59). Almost one-half of the participants of a Canadian study sample aged ≥65 y had a low status, but, in contrast to our findings, their thiamine intake was in the upper level of RDA. According to our results, no correlation between dietary thiamine intake and biochemical status was found (58). The high percentage of women with low thiamine intake in our sample, however, agrees with the high prevalence of poor status. The fact that ETK activity is generally lower in older people than in younger people must be taken into consideration. Studies have shown that, between the ages of 18 and 90 y, there is a significant (25%) decline in enzymatic activity, irrespective of sex and disease. This measured decline continues to persist even after the addition of thiamine diphosphate to the blood samples for determination of the activity and is therefore not reflected in the activity coefficient (60).

As previously discussed (61, 62), our data confirm that pyridoxine is a critical vitamin in the elderly. One-third of all participants had an insufficient vitamin B-6 status. In British elderly, low plasma PLP has been observed in combination with an increase in 4-pyridoxic acid, a product of vitamin B-6, in fasting plasma, which is indicative of a higher catabolic rate (61). In a German study, 25% of the seniors aged 65–75 y had decreased vitamin B-6 concentrations (63). In contrast to other studies (61, 62), the current study did not find a correlation between dietary vitamin B-6 intake and α-EAST. This lack of association between intake and status might have been a result of impaired absorption or reduced metabolic conversion to PLP in the elderly due to the lower activity of the enzymes involved.

The mean daily folate intake of our participants was well below the RDA: >80% of our subjects failed to achieve the RDA. In Germany, with few exceptions, cereals are not enriched with folate. As was seen among the elderly in other studies (33, 64), we observed a significant correlation between serum folate concentrations and folate intake. All of the women in our sample reached the cutoff for serum folate concentration, and only 4

TABLE 6
Serum methylmalonic acid (MMA) and plasma total homocysteine (tHcy) by quartile (Q) of serum cobalamin[1]

	Serum cobalamin (pmol/L)			
	Q1 (≤226)	Q2 (227 to ≤269)	Q3 (270 to ≤330)	Q4 (>331)
MMA (nmol/L)[2]	212 ± 79.9	157 ± 56.7[3]	183 ± 73.6	152 ± 54.4[4]
tHcy (μmol/L)[5]	10.6 ± 2.7	10.2 ± 2.7	9.6 ± 2.0	9.2 ± 2.1[6]

[1] $\bar{x}$ ± SD; n = 177.
[2,5] Significant difference between quartiles (one-way ANOVA): [2] $P < 0.001$, [5] $P = 0.042$.
[3,4] Significantly different from Q1 (Scheffe's post hoc test): [3] $P = 0.002$, [4] $P < 0.001$.
[6] Significantly different from Q1, $P = 0.048$ (Tukey's post hoc test).

TABLE 7
Indicators of vitamin status by education or training[1]

Blood variable	No job training (n = 46)	Apprenticeship (n = 75)	University degree (n = 42)
Activity coefficient			
ETK[2]	1.12 ± 0.07	1.13 ± 0.07	1.16 ± 0.07
EGR	1.17 ± 0.11	1.17 ± 0.11	1.14 ± 0.09
α-EAST	1.53 ± 0.19	1.55 ± 0.22	1.59 ± 0.20
Folate			
Serum (nmol/L)	19.9 ± 8.11	20.2 ± 6.27	20.5 ± 5.92
Red blood cell (nmol/L)	596 ± 179	675 ± 193	613 ± 156
Serum cobalamin (pmol/L)	294 ± 122	286 ± 101	295 ± 71.5
Plasma tHcy (μmol/L)	9.81 ± 2.57	10.0 ± 2.31	9.59 ± 2.50
Serum MMA (nmol/L)	176 ± 72.7	177 ± 71.4	180 ± 71.7

[1] $\bar{x}$ ± SD. ETK, erythrocyte transketolase; EGR, erythrocyte glutathione reductase; α-EAST, erythrocyte α-aspartic aminotransferase; tHcy, total homocysteine; MMA, methylmalonic acid.
[2] P for trend = 0.017 (one-way ANOVA).

had RBC folate concentrations below the reference value of 320 nmol/L. This result disagrees with the low dietary intake and might be caused by insufficient capturing of important sources of dietary folate by the 3-d food record. The discrepancy between the low percentage of women who failed to achieve adequate blood folate concentrations and the high percentage of those exceeding the cutoff for tHcy could be due to impaired renal function, which was not assessed in our sample, but which may also lead to hyperhomocysteinemia (65). Another reason for the discrepancy may be that tHcy is a more sensitive indicator of folate status than is the serum or erythrocyte concentration of the vitamin (23).

An insufficient cobalamin status in the elderly is rarely due to low dietary intake. In people aged > 60 y, high prevalence of atrophic gastritis (type A and type B) results in less absorption of cobalamin than that in a younger population (24). This may explain why we, like previous investigators (64), were unable to find an association between vitamin B-12 intake and blood status.

The reported prevalence of subnormal cobalamin concentration in the elderly ranges from 3.0% to 40.5%, depending on the diagnostic criteria used (24). Our cutoff was the same as that of the Framingham study for a population of free-living seniors aged 67–96 y. The prevalence of cobalamin deficiency (42.5%) reported for the Framingham study was almost the same as that in our subjects (43). In a further study, 46% of the elderly sample (aged 65–100 y) reported regular cobalamin supplement intake or fortified food consumption, or both. Nevertheless, 13% of this sample was classified as cobalamin-deficient (serum cobalamin ≤ 221 pmol/L and MMA > 271 nmol/L) (32). Our chosen cutoff of 258 pmol/L might be too high; 220 pmol/L [as suggested by Rajan et al (32)] is likely to be a more adequate cutoff, because the MMA concentrations in subjects in the 2nd quartile of serum cobalamin were not significantly different from those in subjects in the 3rd and 4th quartiles. Carmel et al (66) found elevated MMA in 55.1% of elderly subjects with cobalamin concentrations < 140 pmol/L but in only 12.4% of those with serum cobalamin concentrations of 140–258 pmol/L. Whereas MMA was elevated in only 10% of our subjects, it was elevated in 23% of healthy German seniors aged 65–75 y who were studied by Hermann et al (63); in contrast, serum cobalamin was decreased in only 9% of their sample, which indicated that their defined cutoff of 132 pmol/L was too low (63). In another study, a high prevalence of elevated MMA was also found in elderly Americans aged ≥ 65 y (67). MMA concentrations by quintiles of serum cobalamin were much higher than those in our sample, despite similar ranges of cobalamin concentrations. Subjects in the lowest vitamin B-12 quintile (< 217 pmol/L) in the study by Morris et al (67) had a mean MMA of 327 nmol/L, whereas subjects in the lowest cobalamin quartile in the present study had a mean MMA of only 212 nmol/L.

It has been shown that MMA is elevated in strict vegans, lactoovovegetarians, and subjects who rarely eat animal-derived products (68, 69), but the comparably high mean MMA among the 8 lactoovovegetarians in the current study may indicate that a lactoovovegetarian diet could be a further risk factor for low cobalamin status in the elderly.

Homocysteine concentrations were inversely correlated to serum and RBC folate values in the current study. A strong correlation between folate concentrations and tHcy was also shown in other studies (27, 48). The relatively weak association with vitamin B-12 may have been influenced by the low folate intake in the population in the current study. Specifically, studies show that folic acid supplementation weakens the relation between tHcy and folate concentrations but strengthens the association between tHcy and vitamin B-12 concentrations (70). Thus, a weak relation between tHcy and vitamin B-12 might be expected under conditions of low folate intake.

It appears that the riboflavin intake and status of elderly women are, for the most part, sufficient. Our results are in agreement with those of an earlier study of 270 independently living healthy elderly people (71). The mean EGR activity coefficient of those not taking supplements was 1.16 ± 0.10, and that in only 3 subjects exceeded 1.35. In a Spanish population in another study, 10.6% of women aged 65–90 y failed to meet the defined EGR activity coefficient cutoff of > 1.2 (72). Although we defined a slightly higher cutoff (> 1.34), only 6.2% of the women in our sample had values above the reference. This may be due to the younger age of our sample and may reflect sufficient riboflavin intake. In contrast to other findings (73), we observed no association between riboflavin and vitamin B-6 status. A physiologic interaction exists between the 2 vitamins, because riboflavin is necessary for the conversion of most naturally available vitamin B-6 to PLP. We might not have found this positive association because of the good riboflavin status in nearly all of the women investigated. Hence, riboflavin was probably not a limiting factor in PLP biosynthesis. Differences in blood values based on sociodemographic factors were not consistent and might have been incidental.

In conclusion, our data indicate that low thiamine, pyridoxine, and cobalamin status is highly prevalent in younger female seniors, as hyperhomocysteinemia and hypermethylmalonic acidemia are to a lesser extent. Because our study sample was more highly educated than is the average population, poor status might actually be more prevalent among the general population. Supplementation with the deficient or critical vitamins would probably reduce the risk of resulting problems such as cognitive and neurologic dysfunction and prevent the development of CVD (9, 32). It is therefore important to consider supplementation, especially among risk groups. The use of supplements is more common among the elderly, among women, among subjects with a higher educational level, and among those whose vitamin supply by diet alone is sufficient (74–77). To reduce the prevalence of B vitamin

deficiency and the resulting diseases, the major task ahead is to improve B vitamin intakes, particularly by the use of supplements or fortified foods, in younger seniors.

AH and MW originated and designed the study. SH coordinated the study and organized blood sampling and data collection. MW wrote the paper. None of the authors had any conflicts of interest.

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