Racial differences in prevalence of cobalamin and folate deficiencies in disabled elderly women[1–3]

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See corresponding editorial on page 789.

ABSTRACT

Background: Many previous investigations of cobalamin and folate status were performed in white populations.

Objective: Our objective was to determine whether there are racial differences in the prevalence of cobalamin and folate deficiency.

Design: The study was a cross-sectional comparison of baseline serum cobalamin, folate, methylmalonic acid (MMA), total homocysteine (tHcy), and creatinine concentrations, complete blood count, and vitamin supplementation in 550 white and 212 African American subjects from a cohort of physically disabled older women.

Results: The mean (±SD) serum MMA concentration was significantly higher in whites than in African Americans: 284 ± 229 compared with 218 ± 158 nmol/L ($P = 0.0001$). tHcy concentration was higher in African Americans than in whites: 12.4 ± 7.0 compared with 10.9 ± 4.6 μmol/L ($P = 0.001$). Serum cobalamin was lower in whites ($P = 0.0002$). Cobalamin deficiency (serum cobalamin <258 pmol/L and MMA >271 nmol/L) was more frequent in the white women (19% compared with 8%; $P < 0.0003$). Folate deficiency (serum folate <11.4 nmol/L, tHcy >13.9 μmol/L, and MMA <271 nmol/L) was more prevalent in African Americans than in whites (5% compared with 2%; $P = 0.01$). Multivitamin use was associated with lower tHcy but not with MMA concentrations. Regression models showed that age >85 y, African American race, serum creatinine >90 μmol/L, and high MMA concentration were all significantly correlated with higher tHcy. Creatinine >90 μmol/L, white race, and folate concentration were positively associated with MMA concentration.

Conclusions: Cobalamin deficiency with elevated serum MMA concentration is more prevalent in elderly white than in African American women and elevated serum tHcy and folate deficiency are more prevalent in elderly African American than in white women. *Am J Clin Nutr* 1999;70:911–19.

KEY WORDS Race, methylmalonic acid, homocysteine, cobalamin, folate, women, elderly, multivitamin use

INTRODUCTION

Virtually all patients with clinical abnormalities due to cobalamin (vitamin B-12) deficiency that are correctable by cobalamin therapy have elevated serum methylmalonic acid (MMA) (1–5) or total homocysteine (tHcy) (1–3, 6) concentrations, or both, even when serum cobalamin concentrations are in the normal range (3, 7). Homocysteine concentration is elevated in most folate-deficient patients with megaloblastic anemia (1, 8) despite serum folate concentrations in the low-normal range in ≈25% of them (1). Previous studies showed that elevated concentrations of MMA and tHcy associated with low or low-normal serum cobalamin values are highly prevalent (10–20%) in elderly cohorts (9–14). After vitamin treatment, the elevated MMA and tHcy concentrations fall into the normal ranges seen in younger individuals (9, 15). The response to cobalamin therapy alone or combined with folic acid and vitamin B-6 suggests that many older people have unrecognized cobalamin, folate, vitamin B-6, or combined deficiencies. Most of the subjects studied previously were white Americans or Europeans. Therefore, it is not known whether elderly African Americans have a similar prevalence of cobalamin or other vitamin deficiencies. Previous investigations found that pernicious anemia is as prevalent in African Americans as it is in white Americans (1, 16–19). However, most elderly cobalamin-deficient subjects do not appear to have pernicious anemia (9, 10). Information on the underlying prevalence of cobalamin and folate deficiency in racial and ethnic subgroups of the population would be useful because the recently implemented food folate fortification program in the United States will

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have universal effects (20). Also, the Food and Nutrition Board recently recommended that elderly people obtain their cobalamin from a synthetic source because of the underlying risk of malabsorption of food cobalamin (21).

An elevated concentration of tHcy (hyperhomocysteinemia) is recognized as an independent risk factor for vascular disease (22). Serum tHcy concentrations correlate inversely with serum cobalamin and folate concentrations and directly with creatinine concentration and age (23). Previous investigations in mostly white populations showed that most elderly subjects with hyperhomocysteinemia also have elevated MMA concentrations due to cobalamin deficiency and, in some cases, renal insufficiency (9, 10, 24). Our objective was to determine whether there are racial differences in the prevalence of cobalamin and folate deficiency.

SUBJECTS AND METHODS

The data for these analyses are from the baseline assessment of the Women's Health and Aging Study (WHAS; 25), an epidemiologic study of the causes and course of disability in a representative sample of physically disabled older women without severe dementia living in the community. The sampling strategy and study eligibility criteria were described in detail previously (25). Briefly, an age-stratified random sample of 6521 community-dwelling women aged ≥65 y, with oversampling of those aged ≥85 y, was selected from the Health Care Financing Administration's Medicare enrollment file for 12 contiguous zip code areas in Baltimore. After we excluded those who had died, were institutionalized, or had moved from the area, 5316 women were eligible for screening; 4137 participated in the screening and 3841 (982 African Americans and 2859 whites or other) finished the interview themselves. Of these, 1409 women (367 African Americans and 1042 whites or other) met the criteria for study eligibility, scoring ≥18 on the Mini-Mental State Examination and reporting difficulty in performing daily tasks in ≥2 domains of functioning. Among those eligible, 1002 (284 African Americans, 713 whites, and 5 others) participated in the full baseline interview and then underwent a comprehensive examination 1–2 wk later in their homes. Women self-reported their race; those who reported races other than white or African American were combined with whites.

At the time of the examination, participants were invited to give a blood sample, which was collected by a phlebotomist at a third home visit. This report uses data from 762 women (212 African Americans and 550 whites) for whom blood test results were available for all relevant measures. Compared with women who provided blood samples, those refusing to provide them were significantly older (81.1 y compared with 77.5 y) and more disabled in self-care tasks, but were not different in terms of race or educational status.

At the baseline interview, participants were requested to present all multivitamin and vitamin medication containers. Interviewers recorded vitamin names, forms, strengths, prescribed dosage, and whether the vitamins were prescribed or over-the-counter. Information was also sought on vitamins for which containers were not available. Chronic disease status was ascertained for 17 conditions by using standardized algorithms that work with information from the interview, examination, medications, radiographs, laboratory tests, physicians' reports, and medical records. The Joint Committee on Clinical Investigation approved the investigation at the Johns Hopkins University School of Medicine, Baltimore.

Assays of metabolites

MMA, tHcy, cystathionine, 2-methylcitric acid, and total cysteine (26–29) were assayed as described previously by stable-isotope dilution and capillary gas chromatography–mass spectrometry with selected ion monitoring. Serum samples were collected and coded in Baltimore and the frozen serum was shipped to the University of Colorado Health Sciences Center for metabolite assays. The normal ranges for the serum metabolites had been determined previously for 60 normal blood donors (30 male) aged 18–65 y and were defined as 2 SD above and below the mean after log normalization to correct for skewness of the data (28, 29). The normal ranges were as follows: tHcy, 5.4–13.9 μmol/L; MMA, 73–271 nmol/L; cystathionine, 44–342 nmol/L; total 2-methylcitric acid, 60–228 nmol/L; and total cysteine, 203–369 μmol/L. The previous normal range for tHcy was 5.4–16.2 μmol/L and was determined in serum samples from 60 normal blood donors (30 male) aged 18–65 y. Blood was allowed to clot for 1–4 h (with an even distribution and a mean of 2.4 h). The current normal range was determined in a second group of 60 similar normal blood donors but blood was clotted for only 30 min before separation by centrifugation. The higher previous normal range was due to release of homocysteine by blood cells at room temperature before separation of the serum.

Serum folate, cobalamin, and pyridoxal phosphate tests were performed by Quest Diagnostics, Teterboro, NJ. Serum cobalamin and folate were measured by competitive protein binding assays using intrinsic factor and folate binding protein by the method of Ciba-Corning Diagnostics Corporation, Medfield, MA.

Serum creatinine was assayed by using sodium picrate with the Ciba-Corning creatinine procedure. The normal range is 50–100 μmol/L (0.6–1.1 mg/dL) for females. Creatinine clearance was estimated by using the formula of Cockcroft and Gault (30): creatinine clearance (mL/s) = weight (kg) × [140 − age (y)] /[72 × serum creatinine (mg/dL)] × 0.85 × 0.01667 (factor adjustment for Système International units).

Definitions of vitamin deficiency

Previous investigations showed that serum vitamin concentrations alone are too nonspecific and insensitive to be used to assign the diagnosis of deficiency (7, 9, 10). We showed previously that >95% of subjects with cobalamin-deficient megaloblastic anemia or neurologic abnormalities have elevated MMA and tHcy concentrations and folate-deficient subjects with megaloblastic anemia have elevated tHcy concentration only (1). Cystathionine concentrations are elevated in many subjects with clinically proven cobalamin or folate deficiency (28) and in animals with dietary vitamin B-6 deficiency (31). 2-Methylcitric acid concentrations are often elevated in patients with severe cobalamin deficiency, but are also elevated commonly by poor renal function (29). The serum 2-methylcitric acid value is higher than the MMA value in most subjects with elevated serum creatinine (29). In contrast, the MMA concentration is always higher than the 2-methylcitric acid concentration in clinically proven cobalamin deficiency (29).

The study population was expected to have individuals with vitamin deficiency or renal insufficiency or both. Therefore, we developed definitions of vitamin deficiency based on our previous studies of clinically documented deficiency or renal insufficiency cited above. Cobalamin deficiency was defined as serum

TABLE 1
Demographics, chronic diseases, and multivitamin use of the study population[1]

	Total (n = 762)	Whites (n = 550)	African Americans (n = 212)
Age (%)			
65–74 y	47	45[2]	55
75–84 y	40	41	37
≥85 y	13	14	8
Education (%)			
0–11 y	64	58[3]	78
≥12 y	36	42	22
Income (%)			
$0–7999	36	27[3]	60
$8000–24 999	46	51	34
≥$25 000	18	22	6
Multivitamin use (%)	19	22[4]	12
Number of chronic diseases	4.4	4.5[4]	4.0

[1] All results are based on weighted data to adjust for the age-stratified sampling design.

[2,3] Significantly different from African Americans (chi-square test): [2] $P < 0.01$, [3] $P < 0.001$.

[4] Significantly different from African Americans, $P < 0.01$ (t test).

cobalamin <258 pmol/L with an MMA concentration >271 nmol/L and greater than the total methylcitric acid concentration. For some analyses the cobalamin-deficient subjects were divided into "high cutoff" and "low cutoff" groups. Cobalamin-deficient subjects in the high-cutoff group had cobalamin and MMA values defined above. Cobalamin-deficient subjects in the low cutoff group had a serum cobalamin concentration <148 pmol/L and an MMA concentration >271 nmol/L and that was greater than the total methylcitric acid concentration. Because both cobalamin and folate deficiencies cause elevated serum tHcy but only cobalamin deficiency causes elevated MMA, the relative pattern of the 2 metabolites was used in the definition of folate deficiency. Folate deficiency was defined as serum folate <11.4 nmol/L, a tHcy concentration >13.9 μmol/L, and an MMA concentration ≤271 nmol/L. Cobalamin deficiency with possible associated folate deficiency was defined according to the subset of subjects who met the definition of cobalamin deficiency (and therefore had elevated MMA concentrations) and also had a tHcy concentration >13.9 μmol/L and a serum folate concentration <11.4 nmol/L. The cohort was also analyzed by using the clinical laboratory cutoff for low serum folate of 5.7 nmol/L. Vitamin B-6 deficiency was defined as a serum pyridoxal phosphate concentration <14.6 nmol/L, cystathionine >342 nmol/L, and MMA <271 nmol/L.

Renal failure with elevated metabolites was defined as not caused by cobalamin or folate deficiency with serum creatinine ≥120 μmol/L (1.4 mg/dL) and one or more of the following: MMA >271 nmol/L, tHcy >13.9 μmol/L, cystathionine >342 nmol/L, or 2-methylcitric acid >228 nmol/L. The category "no deficiency or renal failure" included all subjects who were not included in the above groups.

Statistical analysis

Differences in categorical variables between African Americans and whites were tested by using the chi-square statistic. Differences in means for continuous variables across 2 categories were evaluated with a t test. For tests of trend for continuous variables across categories of serum creatinine, creatinine categories were classified as ordinal variables that were each used as the independent variable in simple linear regression models. In analyses evaluating the effect of both serum creatinine concentration and multivitamin use on vitamin and metabolite concentrations, the interactions between these 2 variables were tested in linear regression models. In cases in which an interaction was not present, the main effects of lower compared with higher serum creatinine concentration and multivitamin use compared with no use were evaluated by using t tests. For those instances in which an interaction was present, analysis of variance was used to compare the effects of specific combinations of creatinine concentration and multivitamin use. Analysis of variance was used to test differences in means of serum metabolites across multiple categories of cobalamin supplementation. All analyses used weighted data to adjust for the age-stratified sampling design. Finally, multiple linear regression models were used to evaluate the independent effects of several factors on tHcy and MMA concentrations. These variables were log transformed because of their skewed distribution, resulting in nearly normal distributions and a substantially better model fit. The analyses included age as a covariate so they did not use age-weighted data, and results of weighted regression models were virtually identical. Numbers in the tables do not total 762 in some cases because of missing values. The statistical program SAS (version 6.0; SAS Institute, Cary, NC) was used for the analyses.

RESULTS

Demographics of study population

Demographic and other characteristics of the total study population according to race are shown in **Table 1**. The age distribution of the African Americans compared with the whites was significantly different, with fewer African Americans in the group aged ≥85 y. More African American women had <12 y of education than did the white women and the income distribution was lower in the African Americans than in the whites. Multivitamin use was substantial in this group of disabled women, with 19% reporting use in the 2 wk before the examination. There was a significant racial difference in the use of multivitamins. Only 12% of the African Americans compared with 22% of the whites reported use of multivitamins. The white women reported a slightly higher number of chronic diseases than did the African American women.

Prevalence of vitamin deficiency

The mean serum MMA concentration was significantly higher in the whites than in the African Americans (**Table 2**); 37% of the white women had elevated MMA concentrations compared with 21% of the African American women. In contrast, mean tHcy concentrations were higher in African Americans than in whites; concentrations were elevated in 29% of the African Americans compared with 17% of the whites. Cystathionine and total methylcitric acid concentrations were not significantly different. Serum creatinine was significantly higher in the African American than in the white women, with African Americans having twice the rate of elevated creatinine for both the 105 and 120 μmol/L (1.2 and 1.4 mg/dL) cutoffs. However, the calculated creatinine clearance was similar for both groups. Serum cobalamin concentrations were significantly lower in whites than in African Americans

TABLE 2
Serum metabolite and vitamin concentrations by race[1]

	Whites (n = 550)	African Americans (n = 212)	P
Mean age (y)	76.1	74.5	—
Total homocysteine (μmol/L)	10.9 ± 4.6[2]	12.4 ± 7.0	0.001
>13.9 μmol/L (%)	17	29	
Methylmalonic acid (nmol/L)	284 ± 229	218 ± 158	0.0001
>271 nmol/L (%)	37	21	
Cystathionine (nmol/L)	246 ± 185	262 ± 202	0.30
>342 nmol/L (%)	17	19	
Total methylcitric acid (nmol/L)	138 ± 74	145 ± 109	0.30
>228 nmol/L (%)	8	7	
Creatinine (μmol/L)	88.4 ± 21	106 ± 61	0.0001
≥105 μmol/L (%)	15	33	
≥120 μmol/L (%)	6	12	
Creatinine clearance (mL/s)	0.90 ± 0.35	0.90 ± 0.36	0.8
<0.50 mL/s (%)	9	14	
Cobalamin (pmol/L)	345 ± 189	406 ± 209	0.0002
≤148 pmol/L (%)	7	4	
≤258 pmol/L (%)	37	24	
Folate (nmol/L)	31.8 ± 45.4	24.7 ± 48.1	0.3
≤5.7 nmol/L (%)	1	2	
≤11.4 nmol/L (%)	13	18	
Vitamin B-6 (nmol/L)	52.5 ± 74.8	39.6 ± 72.5	0.06
<14.6 nmol/L (%)	16	30	

[1] All results based on weighted data to adjust for the age-stratified sampling design.
[2] $\bar{x}$ ± SD.

and serum folate concentrations were higher. Very few subjects (<1% of whites and 2% of African Americans) in either racial group had a serum folate concentration below the clinical laboratory cutoff of <5.7 nmol/L. More African American than white subjects had low pyridoxal phosphate concentrations.

The prevalence of vitamin deficiency and renal failure with elevated metabolites in whites compared with African Americans is shown in **Table 3**. There was a high prevalence of cobalamin deficiency in whites and it was almost 2.5 times as prevalent as in African Americans. When the low-cutoff definition for cobalamin deficiency was applied, the prevalence of deficiency was lower in both groups but still significantly more prevalent in whites. Folate deficiency (as defined in Methods but specifically excluding subjects with elevated serum MMA) was 2.5 times more prevalent in African Americans than in whites. There was a small percentage of subjects, 3% of whites and 1% of African Americans (P = 0.09), who had low concentrations of both serum cobalamin and folate and had elevated concentrations of both MMA and tHcy (data not shown). These subjects may have had either only cobalamin deficiency or folate deficiency complicated by cobalamin deficiency. A small percentage of subjects with low pyridoxal phosphate concentrations and elevated cystathionine concentrations met our diagnosis of vitamin B-6 deficiency, with no racial difference. Twice as many African Americans as whites met our definition of renal failure with associated elevated metabolites. There was no difference in prevalence between the 2 races in subjects who did not have any of the deficiency syndromes or renal failure.

Clinical and metabolic variables in vitamin-deficient subjects

The cobalamin-deficient subjects (as defined by the high cutoff, 15.8% of the whole cohort) had a mean age of 79.6 y and were 88% white. Their mean (range) MMA and tHcy concentrations were 549 ± 376 (273–2480) nmol/L and 14.9 ± 5.6 (4.7–36.8) μmol/L, respectively. MMA values were >1000 nmol/L in 2% of the entire cohort. Mean cystathionine was also elevated in the cobalamin-deficient subjects: 311 ± 203 (73–1500) nmol/L. The mean (range) serum cobalamin and folate concentrations were 176 ± 50 (76–256) pmol/L and 19.5 ± 13.2 (4.8–99) nmol/L, respectively. Mean (range) hematocrit, mean corpuscular volume (MCV), and serum creatinine [0.39 ± 0.05 (0.26–0.50), 94 ± 8 (65–113) fL, and 90 ± 180 (60–160) μmol/L, or 1.1 ± 2.0 (0.7–1.8) mg/dL], respectively, were not significantly different from the group with no deficiencies.

Folate-deficient subjects (2.8% of the whole cohort) had a mean age of 75.8 y and were 45% white. They had a mean (range) tHcy concentration of 23.2 ± 13.5 (14.0–63.3) μmol/L, a serum folate concentration of 7.7 ± 2.0 (4.3–11) nmol/L, and a mean serum cobalamin concentration 322 ± 363 (340–505) pmol/L. Hematocrit, 0.39 ± 0.06 (0.28–0.50), and MCV, 94 ± 10 (75–112) fL, were not significantly different from the group with no deficiencies. There were only 14 (1.8%) subjects meeting our criteria for vitamin B-6 deficiency: mean (range) cystathionine and pyridoxal phosphate concentrations was 464 ± 178 (349–1030) and 10.9 ± 2.4 (6.5–14) nmol/L, respectively.

In previous studies, most cobalamin-deficient patients who had clinical abnormalities that were corrected with cobalamin therapy had serum MMA concentrations >500 nmol/L (1, 2, 5). We performed a subgroup analysis of subjects with the highest 5% of serum MMA concentrations (>608 nmol/L; n = 39). Compared with the total cohort, of which 28% were African

TABLE 3
Prevalence of vitamin deficiency and renal failure with elevated metabolite concentrations in whites compared with African Americans[1]

	Whites (n = 550)	African Americans (n = 212)	P
	%	%	
Cobalamin deficiency			
High cutoff (<258 pmol/L with elevated MMA)	19	8	0.0003
Low cutoff (<148 pmol/L with elevated MMA)	5	2	0.04
Folate deficiency	2	5	0.01
Vitamin B-6 deficiency	2	3	0.3
Renal failure with elevated metabolites	6	12	0.01
No deficiency or renal failure	71	74	0.6

[1] All results are based on weighted data to adjust for the age-stratified sampling design.

TABLE 4
Demographic data and mean serum metabolite concentrations according to creatinine concentration after subjects with cobalamin and folate deficiencies were excluded[1]

	Serum creatinine (μmol/L)[2]					
	<90 (<1.0) (*n* = 208)	90–100 (1.0–1.1) (*n* = 232)	105–115 (1.2–1.3) (*n* = 68)	120–170 (1.4–1.9) (*n* = 34)	≥180 (≥2.0) (*n* = 12)	*P* for trend
Percentage white (%)	81	69	51	59	26	
Mean age (y)	74.0	76.0	75.9	78.1	74.6	0.001
tHcy (μmol/L)	8.5	10.5	12.1	15.1	19.7	0.001
MMA (nmol/L)	187	217	232	279	504	0.001
Cystathionine (nmol/L)	163	238	297	386	729	0.001
Methylcitric acid (nmol/L)	101	128	163	214	505	0.001
Total cysteine (μmol/L)	312	337	364	407	408	0.001
Cobalamin (pmol/L)	380	409	415	447	513	0.006
Folate (nmol/L)	27.7	27.5	27.5	30.8	81.0	0.01
Vitamin B-6 (nmol/L)	57.9	47.1	35.2	96.8	72.5	0.6

[1] All results are based on weighted data to adjust for the age-stratified sampling design. MMA, methylmalonic acid; tHcy, total homocysteine.
[2] Creatinine concentration as mg/dL in parentheses.

American, only 15% of the group with the highest 5% of serum MMA were African American—6 compared with 33 subjects—and only 2 of these 6 were cobalamin deficient, although 1 other subject had a missing cobalamin value and a normal serum creatinine concentration and could not be classified. In the other 3 African American subjects, serum creatinine concentrations ranged from 180 to 920 μmol/L (2–10.4 mg/dL) and serum cobalamin concentrations were normal, ranging from 362 to 1600 pmol/L. Low cobalamin concentrations (<258 pmol/L) were found in 27 of 34 (5 missing values) of the subjects with the highest MMA concentrations. Serum creatinine was ≥120 μmol/L (≥1.4 mg/dL) in 4 of the 7 subjects with elevated MMA and normal serum cobalamin concentrations. Cobalamin concentrations were <148 pmol/L in 41% of those with MMA concentrations >608 nmol/L and between 149 and 258 pmol/L in 39%. In the 13 subjects with MMA concentrations >1000 nmol/L (who also had a cobalamin value), only 5 had a cobalamin concentration <148 pmol/L and 7 had cobalamin values between 149 and 258 pmol/L. Serum folate was low (<11.4 nmol/L) in 5 of the 39 subjects with the highest MMA concentrations and tHcy was elevated in 31 of these 39 subjects. Anemia (hemoglobin <120 g/L) was present in 12 of the 39 with the highest 5% of MMA concentrations, but only 4 had an elevated MCV (>100 fL). The most severely cobalamin-deficient subject (an MMA concentration of 2480 nmol/L and a serum cobalamin concentration of 89 pmol/L) actually had microcytic anemia.

In the whole cohort there were 71 subjects with an MCV >100 fL. Of these, 25% met our definition of cobalamin deficiency and 7% met our definition of folate deficiency. Only 13 of the individuals with elevated MCV were also anemic, but 46% of the anemic subjects were cobalamin deficient compared with 20% of those without anemia.

Role of renal insufficiency and multivitamin use in elevated metabolites

Serum creatinine and multivitamin use were significantly different by race (Tables 1 and 2). To explore whether these factors could explain racial differences in vitamin-dependent metabolite concentrations, we first evaluated the relations of creatinine and multivitamin use with the metabolite values. Mean serum metabolite concentrations according to ranges of serum creatinine concentrations, after exclusion of those subjects who met the criteria for cobalamin or folate deficiency, is shown in **Table 4**. As expected, mean age was higher in subjects with higher serum creatinine concentrations. All metabolites and serum folate values were significantly higher in subjects with higher serum creatinine concentrations. tHcy and MMA concentrations were 2–3-fold higher and cystathionine and total methylcitric acid had a more dramatic 4–5-fold relation with higher creatinine concentrations.

Multivitamin use was associated with significantly lower tHcy concentrations (**Table 5**). Mean metabolite values in the subjects taking multivitamins compared with those not taking multivitamins and divided into 2 groups, serum creatinine concentrations ≤105 μmol/L (1.2 mg/dL) and >105 μmol/L, are also shown in Table 5. Interactions between multivitamin use and serum creatinine were tested in separate linear regression models for each metabolite and vitamin concentration. The interaction term was significant only for the models predicting folate and vitamin B-6 concentrations, indicating that the effect of multivitamin use was significantly greater in the subgroup with higher serum creatinine. For example, in the subgroup with lower creatinine, multivitamin use doubled the concentration of serum folate (41.5 compared with 20.9 nmol/L), whereas in the subgroup with higher creatinine, those taking multivitamins had a folate concentration 4 times higher than those not taking multivitamins (79.9 compared with 19.5 nmol/L). For other factors, only the main effects of multivitamin use and creatinine were tested. Overall, multivitamin use was associated with significantly lower tHcy concentrations. The lowest mean tHcy concentration (8.6 ± 2.4 μmol/L) was found in the multivitamin users who had lower serum creatinine. The percentage reduction in tHcy concentration resulting from multivitamin use was similar in those with lower serum creatinine (22%) and those with higher serum creatinine (25%). Multivitamin use was associated with significantly lower concentrations of cystathionine and higher concentrations of serum cobalamin, folate, and vitamin B-6. Significant associations were observed between serum creatinine concentration and tHcy, cystathionine, methylcitric acid, total cysteine, and serum cobalamin concentrations.

TABLE 5
Effect of multivitamin use on serum metabolite and vitamin concentrations in subjects grouped according to lower or higher serum creatinine[1]

	Serum creatinine			
	≤105 μmol/L Multivitamin use		>105 μmol/L Multivitamin use	
	No (n = 466)	Yes (n = 119)	No (n = 118)	Yes (n = 22)
tHcy (μmol/L)[2,3]	11.0 ± 5.1	8.6 ± 2.4	15.8 ± 6.8	11.8 ± 3.8
MMA (nmol/L)	262 ± 219	242 ± 222	319 ± 201	282 ± 128
Cystathionine (nmol/L)[2,3]	235 ± 190	184 ± 88	384 ± 216	317 ± 201
Methylcitric acid (nmol/L)[3]	127 ± 69	123 ± 50	204 ± 104	249 ± 187
Total cysteine (μmol/L)[3]	325 ± 49	333 ± 53	375 ± 62	394 ± 69
Cobalamin (pmol/L)[2,3]	333 ± 167	429 ± 211	344 ± 168	471 ± 301
Folate (nmol/L)	20.9 ± 15.4[4]	41.5 ± 30.8[5]	19.5 ± 12.2[4]	79.9 ± 131
Vitamin B-6 (nmol/L)	42.6 ± 69.6[4]	65.4 ± 66.2	33.6 ± 44.1[4]	102 ± 103

[1] $\bar{x}$ ± SD. tHcy, total homocysteine; MMA, methylmalonic acid. There was a significant interaction between multivitamin use and serum creatinine category for folate and vitamin B-6, $P < 0.05$. All results are based on weighted data to adjust for the age-stratified sampling design. 105 μmol creatinine/L = 1.2 mg/dL.
[2] Significant main effect of multivitamin use, $P < 0.05$.
[3] Significant main effect of serum creatinine category, $P < 0.05$.
[4] Significantly different from respective multivitamin users, $P < 0.05$.
[5] Significantly different from multivitamin users in high serum creatinine category, $P < 0.05$.

The oral dose of cobalamin was determined for 72 subjects ingesting it in vitamin supplements, and serum metabolites were compared with the 602 subjects who were not taking cobalamin-containing supplements. The prevalence of subjects with low-cutoff (<148 pmol/L) and high-cutoff (between 149 and 258 pmol/L) cobalamin concentrations was significantly lower in those using oral cobalamin supplements (chi-square test, $P < 0.05$). None of those taking doses of ≥9 μg had serum cobalamin concentrations <148 pmol/L and only 1 subject had a serum cobalamin concentration <258 pmol/L. However, there were subjects (4.9%) with low cobalamin concentrations and cobalamin concentrations between 149 and 258 pmol/L (24.4%) in the group ingesting 1–6 μg cobalamin.

Role of oral cobalamin supplementation and elevated metabolites

Mean serum metabolites in subjects taking different oral doses of cobalamin are shown in **Table 6**. The lowest MMA concentration was found in the subjects consuming the largest amounts of cobalamin (33–1000 μg) and there was a significant trend in MMA concentration across doses of serum cobalamin. The lowest mean tHcy concentration (8.8 μmol/L) was found in the group ingesting 33–1000 μg cobalamin and the trend across concentrations of cobalamin ingestion was also significant. Only one-third to one-half of the subjects taking the higher doses of cobalamin were also taking folic acid, whereas virtually every subject with the lowest cobalamin dose (≤6 μg) was also taking 400 μg folate. Mean cystathionine concentrations also showed a significant trend with oral cobalamin dose (Table 6).

After multiple linear regression with adjustment for all variables shown in **Table 7**, race remained significantly associated with higher tHcy concentrations, as was age >85 y compared with the youngest subgroup, creatinine concentration >90 μmol/L (l mg/dL), and the higher serum MMA concentrations. Serum cobalamin, serum folate, and multivitamin use were all inversely correlated with the tHcy concentration in all subjects and in those with no deficiencies. In both the total population and those with no deficiencies, African Americans had significantly lower MMA concentrations than whites after adjustment for all other variables in the models. These results are in contrast with the higher tHcy concentrations found for African Americans in those models. tHcy concentration was also associated with MMA concentration in all subjects and in those with no deficiencies. Creatinine >90 μmol/L and cobalamin and folate concentration were associated with MMA concentrations in all subjects, but these associations were not significant in the subgroup with no deficiencies. A substantial amount of the variance in tHcy and MMA was explained by the variables in these models. Other variables tested in the models, including BMI, albumin concentration, education, and income were not independently correlated with tHcy or MMA concentration.

DISCUSSION

We determined that there were racial differences in the prevalence of elevated MMA and tHcy concentrations and in cobalamin and folate deficiencies in a group of elderly disabled women. We found that the mean MMA concentration is higher, the mean cobalamin concentration is lower, and the prevalence of cobalamin deficiency is 2–3 times as common in elderly white than in African American women. Conversely, we found that the mean tHcy concentration is higher, the mean folate concentration is lower, and the prevalence of folate deficiency is 2–3 times as high in elderly disabled African American women as in white women in this cohort. Multivitamin use was higher in the white women and was associated with lower serum tHcy concentrations, but did not explain racial differences in tHcy. The dose of cobalamin in multivitamin preparations (≤6 μg) was not associated with lower serum MMA concentrations or a lower prevalence of cobalamin deficiency. The racial differences we found in the prevalence of cobalamin deficiency are surprising because pernicious anemia was shown to be equally prevalent in African Americans and whites in several studies (16–18). It is likely, however, that most of the cobalamin-deficient subjects in our study did not have pernicious anemia. We did not investigate the cause of deficiency in these subjects but previous investigations in similar elderly cohorts have uncovered

TABLE 6
Serum metabolites in subjects ingesting various doses of cobalamin[1]

	Cobalamin dose				
	33–1000 μg[2] (n = 12)	9–25 μg[3] (n = 14)	≤6 μg[4] (n = 46)	None[5] (n = 602)	P for trend[6]
MMA (nmol/L)	187 ± 74	186 ± 51	249 ± 217	275 ± 220	0.03
tHcy (μmol/L)	8.8 ± 2.2	9.1 ± 1.8	9.1 ± 3.5	11.8 ± 5.8	0.001
Cystathionine (nmol/L)	199 ± 144	170 ± 83	218 ± 169	263 ± 202	0.02

[1] $\bar{x}$ ± SD. All results are based on weighted data to adjust for the age-stratified sampling design. MMA, methylmalonic acid; tHcy, total homocysteine.
[2–5] Folate intake: [2] 5 took none, 6 ≤ 400 μg, 1 > 400 μg; [3] 10 took none, 3 ≤ 400 μg, 1 > 400 μg; [4] 1 took none, 44 took ≤ 400 μg; [5] 598 took none, 4 took > 400 μg.
[6] Adjusted for age and race.

very few cobalamin-deficient subjects with anti-intrinsic factor antibodies (9, 10). They may have had food cobalamin malabsorption and lesser degrees of atrophic gastritis.

Previous studies in Africa and the United States have shown that black people have higher serum cobalamin concentrations than do whites (32–35) and that they may have differences in their cobalamin binding proteins. MMA was not measured in these studies, however. Because we found that MMA concentrations were higher and serum cobalamin concentrations were lower in whites, there may be true biochemical differences in cobalamin status between the races.

Mean tHcy was 12% higher in the elderly African American women than in the white women. The cause of this difference is not known and is not explained by differences in use of multivitamins or in serum creatinine (Table 7). Although serum creatinine concentrations were 20% higher in the African American women, the calculated creatinine clearance was the same as that in the white women because of the higher body mass in the former group. The higher tHcy concentrations in the African Americans in this study were most likely not because of the thermolabile 5,10-methylenetetrahydrofolate reductase polymorphism because it is uncommon in this group (36–38). There are few reported data about tHcy concentrations in whites compared with African Americans. tHcy was 1.2 μmol/L greater in African Americans than in whites in 2 reports (38, 39). Serum folate was lower in African Americans and was related to the incidence of ischemic strokes in participants of the first National Health and Nutrition Examination Survey (NHANES I; 40). However, in 2 other reports, tHcy concentrations were lower in South African black subjects (41) and in African American men (42) than in whites. tHcy concentration was not different in non-Hispanic whites and blacks in a report from NHANES III (43).

The strong relation between tHcy and serum creatinine concentrations that we found in this elderly cohort has implications for studies of the role of tHcy in vascular disease. A recent investigation found that both creatinine and tHcy were risk factors for mortality from cardiovascular disease (44). Intervention studies of vitamin therapy with investigations of both creatinine and tHcy in vascular disease may be able to show whether tHcy is actually an independent causative risk factor or merely a sensitive indicator of ongoing renal damage from occult vascular disease.

TABLE 7
Linear regression model relating demographic characteristics, laboratory values, and multivitamin use to serum ln(tHcy) and ln(MMA)[1]

	ln(tHcy)		ln(MMA)	
Variable	Model 1, all subjects	Model 2, no deficiencies	Model 1, all subjects	Model 2, no deficiencies
		B		
Age				
75–84 vs 65–74 y	0.006	0.029	0.11[2]	0.089[3]
≥85 vs 65–74 y	0.11[2]	0.109[2]	0.12[2]	0.116[3]
African American vs white race	0.099[2]	0.106[2]	−0.33[2]	−0.312[2]
Creatinine				
90–100 vs < 90 μmol/L	0.17[2]	0.153[2]	0.123[2]	0.089[3]
105–115 vs < 90 μmol/L	0.32[2]	0.227[2]	0.178[2]	0.160[3]
≥120 vs < 90 μmol/L	0.49[2]	0.220[2]	0.408[2]	0.008
Cobalamin	−0.0003[2]	−0.0002[2]	−0.0005[2]	−0.00001
Folate	−0.003[2]	−0.003[2]	0.006[2]	0.001
MMA	0.0004[2]	0.0009[2]	NA	NA
tHcy	NA	NA	0.027[2]	0.034[2]
Multivitamin use, yes vs no	−0.10[2]	−0.037	−0.08	−0.098[3]
Intercept	2.1[2]	1.9[2]	5.6[2]	5.2[2]
Adjusted R^2	0.41	0.30	0.32	0.17

[1] MMA, methylmalonic acid; tHcy, total homocysteine; NA, not applicable.
[2] $P < 0.01$.
[3] $P < 0.05$.

We found, similar to past investigations, that multivitamin use was associated with lower serum tHcy concentrations (10, 13), but that it has less of an effect on MMA concentrations. The lowest effective oral dose of cobalamin is not yet known for patients with cobalamin malabsorption, even though it has been suggested that elderly Americans take synthetic cobalamin to satisfy their daily requirement (21). Our previous investigations found that doses of cobalamin ≤10 μg can decrease, but not eliminate, the prevalence of elevated MMA concentrations and cobalamin deficiency (10, 13). Our current data show that there was little difference in serum MMA concentrations between those taking ≤6 μg cobalamin (the standard multivitamin dose) and those taking none. Mean serum MMA was lower in those taking >9 μg cobalamin/d, especially in the group ingesting 33–1000 μg. An interesting new finding was that tHcy concentration was strongly influenced by the oral cobalamin dose (Table 6). The relation remained significant even after adjustment for folate concentration, multivitamin use, and other variables predictive of tHcy concentration (Table 7). This is particularly interesting because 60% of the subjects in the 2 groups taking the highest cobalamin dose were not taking supplemental folic acid and those taking conventional multivitamins, which provide ≤6 μg cobalamin, were taking 400 μg folic acid. It is possible that the role of cobalamin nutriture in lowering tHcy concentrations in the elderly has been overlooked because the multivitamin supplements studied supplied an inadequate cobalamin dose. Further studies are urgently needed to find the effective oral doses of cobalamin that decrease elevated MMA and tHcy concentrations in elderly subjects.

It is possible that our observations about the prevalence of elevated MMA and tHcy concentrations and vitamin deficiency cannot be generalized to the general population because we studied a disabled, elderly female cohort. However, the overall 15.8% prevalence of cobalamin deficiency found in this study corresponds well with results of our previous studies, which found cobalamin deficiency in 12% of 548 male and female subjects in the Framingham cohort (10) and in 14.5% of 152 consecutive geriatric outpatients (mostly male) at a Veterans Administration Hospital (9). We found that 2% of this disabled cohort had strikingly high serum MMA values, >1000 nmol/L, which is similar to the prevalence found in 4 previous cohorts ranging from 1% in healthy elderly (13), 2% in the Framingham cohort (10), 4% in a European elderly cohort (11), to 6% in the Veterans Administration outpatient cohort (9). It seems reasonable to conclude that ≥2% of the white elderly men and women in the United States have biochemical evidence of moderately severe cobalamin deficiency.

Our study sheds some new light on the risks and benefits of the food folate fortification program implemented in January 1998. Our data show that there will probably be a benefit for the African American women in our elderly, disabled cohort because of their greater prevalence of folate deficiency and higher mean tHcy concentrations. These elderly African American women also had a lower intake of multivitamins, so that the involuntary food fortification will be important in increasing their intake. The effects of the folate fortification program on the elderly disabled white women in our cohort are much harder to predict. High MMA concentrations with a concurrent diagnosis of cobalamin deficiency were highly prevalent (in 19% of this cohort). It is unlikely that an increased folate intake in these cobalamin-deficient subjects will mask megaloblastic anemia because there was a very low prevalence of megaloblastic anemia in the deficient subjects, even in those with the highest MMA concentrations. It is also difficult to predict whether mean tHcy will decrease in this subgroup because much of the elevation in tHcy concentrations may be attributed to cobalamin deficiency. Physicians and other health care providers need to be aware of the high prevalence of cobalamin deficiency in populations similar to the ones studied here to detect and treat neurologic complications of cobalamin deficiency.

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