Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities[1–3]

Ralph Carmel, Ralph Green, Donald W Jacobsen, Karsten Rasmussen, Mircea Florea, and Colleen Azen

ABSTRACT

Background: Low cobalamin concentrations and mild hyperhomocysteinemia are common in the elderly but ethnic differences have not been defined.

Objective: Our objective was to determine the demographic characteristics of cobalamin deficiency in the elderly and its role in their hyperhomocysteinemia.

Design: We measured serum cobalamin, total homocysteine (Hcys), and methylmalonic acid (MMA) concentrations in 725 subjects >60 y old, and folate concentrations in 520 subjects.

Results: After exclusion of subjects taking cobalamin supplements or with renal insufficiency, high prevalences of low cobalamin (11.8%), high MMA (16.6%), and high Hcys (26.1%) concentrations were seen. Most cobalamin concentrations <140 pmol/L appeared to reflect deficiency because 78.3% of them were accompanied by abnormal metabolites. Subjects with cobalamin concentrations of 140–258 pmol/L had significantly fewer metabolic abnormalities. A low cobalamin concentration and renal insufficiency were the strongest predictors of abnormal Hcys concentrations. Elderly men had higher Hcys concentrations than did women ($P = 0.0001$). Whites and Latin Americans had lower cobalamin concentrations than did blacks and Asian Americans ($P < 0.005$). Whites also had higher Hcys concentrations than all the other groups ($P < 0.05$). When included in the analysis, renal insufficiency in subjects was associated with 23.8% of all high Hcys and 25.5% of all high MMA concentrations; most with renal insufficiency were Asian American and black men.

Conclusions: Mild cobalamin deficiency is most common in elderly white men and least common in black and Asian American women. Hyperhomocysteinemia, which is most strongly associated with low cobalamin concentrations, is also most common in elderly whites, whereas that associated with renal insufficiency is more common in blacks and Asian Americans. Ethnic differences in cobalamin deficiency and the Hcys patterns associated with it or with renal insufficiency warrant consideration in supplementation strategies. Extending suspicion of deficiency to persons with cobalamin concentrations of 140–258 pmol/L appears to provide more disadvantages than advantages. *Am J Clin Nutr* 1999; 70:904–10.

INTRODUCTION

The observation that serum cobalamin concentrations are frequently low in the elderly (1–3) has assumed renewed importance in recent years because of several converging developments. One development was the recognition that low cobalamin concentrations, even when unaccompanied by classical signs of cobalamin deficiency such as megaloblastic anemia, often represent a mild, preclinical deficiency state (3–6). It was previously thought that most low concentrations in the elderly were meaningless and perhaps artifactual.

Another development was the epidemiologic association of hyperhomocysteinemia with vascular disease (7, 8). The resulting interest in hyperhomocysteinemia included its association with cobalamin deficiency (9). Homocysteine concentrations are affected by several factors (10). These include vitamin deficiency (cobalamin, folate, or pyridoxine), enzyme defects, and renal insufficiency (or, perhaps more accurately, creatinine changes).

Finally, the drive to increase folate intake in the general population has stimulated interest in the prevalence of unrecognized cobalamin deficiency. The concern is that high folate intake may adversely affect people with unrecognized cobalamin deficiency.

Therefore, a considerable impetus exists to better define cobalamin status and its relation to homocysteine concentrations in the elderly, who constitute a population at special risk. As part of our examination of cobalamin status in community-dwelling elderly volunteers in Los Angeles, we studied the demographic aspects of cobalamin concentration and its related metabolites. In particular, we focused on the question of ethnic group influences. Ethnic differences in cobalamin concentrations have been noted in the general population (11) but the metabolic relevance, if any, of the differences has not been addressed—especially in the elderly. Not all low cobalamin concentrations represent true deficiency, nor do all normal concentrations indicate metabolic sufficiency. In addition, intriguing observations have suggested that homocysteine metabolism differs between blacks and whites (12, 13). Although many surveys of metabolism in the elderly have appeared recently, ethnic variability has been given little attention and has not been examined by integrating the vitamin and metabolite findings.

SUBJECTS AND METHODS

Community-dwelling elderly people were studied at several sites in Los Angeles: an apartment complex for retirees, 3 social-educational clubs (1 of primarily white, 1 of primarily black, and 1 of primarily Latin American members), and an outpatient clinic of the Department of Veterans Affairs. All persons >60 y old attending regular functions at these sites (ie, functions that were unrelated to the survey) were invited to participate, and more than half of the subjects approached at each site did so. The study, which was approved by Institutional Review Boards at both the University of Southern California and the Department of Veterans Affairs Outpatient Clinic, was completed before the Food and Drug Administration (FDA)–mandated folate fortification of the American diet began.

Venous blood samples and answers to a questionnaire were obtained from 725 volunteers. In most cases, serum was separated within 1–2 h from blood samples kept on ice in the field. The questionnaire solicited demographic data and information about vitamin supplement use, cobalamin treatment, and gastrointestinal symptoms or illnesses. Ethnicity was determined by each person's self-identification. All whites were of European extraction. Blacks were either American-born or from the Caribbean, and Latin Americans were born in the United States, Mexico, or Central America. The Asian Americans were of Chinese, Korean, Vietnamese, Japanese, or Filipino origin; their data were pooled after comparison of the 2 largest subgroups, 25 Chinese and 24 Filipinos, showed no significant differences in results.

Serum cobalamin was measured by radiodilution assay using pure intrinsic factor as the binding protein (14); the reference range is 140–750 pmol/L. Frozen aliquots of the sera were shipped on dry ice to the collaborating laboratories for metabolite assays. Total homocysteine was determined at the Cleveland Clinic by HPLC (15); the reference range for serum homocysteine is 3.9–16.8 μmol/L for women and 8.6–17.1 μmol/L for men. Methylmalonic acid (MMA) was assayed in the first 2 y of the study at the Cleveland Clinic by a modification (16) of the method of Rasmussen (17); the reference range is 79–376 nmol/L. In the final year of the survey, MMA was

determined at Aarhus University Hospital, Aarhus, Denmark, by the original method of Rasmussen (17); the reference range is 50–370 nmol/L (18). Serum folate was assayed by the Quanta-Phase II radioassay (Bio-Rad Laboratories, Hercules, CA); the reference range is 5.7–45.3 nmol/L.

Serum from all subjects found to have abnormal metabolite concentrations (homocysteine or MMA) and also from randomly chosen subjects with normal metabolite concentrations was assayed for creatinine. Creatinine concentrations ≥133 μmol/L in men or ≥115 μmol/L in women were considered suggestive of renal insufficiency.

All data were analyzed after exclusion of the 70 subjects who gave a history of recent use of cobalamin supplements and the 64 subjects who were found to have elevated serum creatinine concentrations because renal insufficiency causes artifactual elevation of serum MMA (19) and serum homocysteine (10, 20, 21) concentrations. However, because the effect of renal insufficiency on homocysteine concentrations is complex and because hyperhomocysteinemia has thrombotic associations regardless of vitamin status (7, 10, 22), the data were also analyzed after reinclusion of the 64 subjects with renal insufficiency.

Before statistical analysis, all continuous variables were subjected to logarithmic transformation to better fit a normal distribution. Statistical tests were performed on the transformed variables, with results reported as antilogs. Comparisons among group means were made by using two-sample t tests and one-way or multifactor analyses of variance, with Bonferroni adjustment for multiple comparisons. Associations between continuous variables were examined by using Spearman rank correlation. Categorical variables were analyzed by chi-square or Fisher's exact test. Multiple logistic regression was used to evaluate significant independent predictors of abnormal results. Probability values <0.05 were considered statistically significant. All analyses were done by using SAS computer software (version 6; SAS Institute Inc, Cary, NC).

All subjects whose results were considered abnormal were invited for further evaluation. The results of follow-up clinical, neurologic, and absorptive testing of patients who agreed to undergo further investigation, which are tangential to the goals of the present report, are reported elsewhere. This includes the prevalence of unsuspected pernicious anemia in the study population (23) and an analysis of dietary intake of cobalamin (24).

RESULTS

Cobalamin

Subnormal cobalamin concentrations (<140 pmol/L) were found in 70 of the 581 subjects (11.8%) after the previously mentioned exclusions. Most of these 70 subjects appeared to be truly cobalamin deficient, because 55.1% of them also had abnormal MMA concentrations and 78.3% had abnormal MMA or homocysteine concentrations, or both (**Table 1**). Only 2 of the subjects with cobalamin concentrations <140 pmol/L also had low folate concentrations, which, in one case appeared to be responsible for the low serum cobalamin concentration.

Because of suggestions that cobalamin concentrations in the lower part of the normal range, ie, 140–258 pmol/L, often indicate cobalamin deficiency (5, 25), we also analyzed the data from subjects with cobalamin concentrations in this range (Table 1). Only 12.4% of them had high MMA concentrations, which is significantly lower than the 55.1% frequency in

TABLE 1
Prevalence of coexisting abnormal metabolite and folate concentrations in elderly subjects with low, low-normal, and high-normal cobalamin concentrations[1]

	Low, <140 pmol/L (n = 70)	Low-normal, 140–258 pmol/L (n = 228)	High-normal, >258 pmol/L (n = 293)
High metabolite concentrations			
MMA	38/69 (55.1)	28/226 (12.4)	31/288 (10.8)
Homocysteine	44/69 (63.8)	65/226 (28.8)	45/288 (15.3)
Both MMA and homocysteine	28/69 (40.6)	13/226 (5.8)	6/288 (2.1)
MMA, homocysteine, or both	54/69 (78.3)	80/226 (35.4)	69/288 (24.0)
Low serum folate concentrations (<5.7 nmol/L)	2/66 (3.0)[2]	10/192 (5.2)	7/224 (3.1)

[1]Percentage in parentheses. Discrepancies between numbers of subjects in the groups and those for individual metabolite results are because 8 subjects did not have MMA measurements done. We included in the analyses only the 583 subjects who were not only not taking cobalamin supplements and did not have elevated creatinine concentrations but who also had all metabolite determinations done. MMA, methylmalonic acid.

[2]One of the 2 subjects with low concentrations of both cobalamin and folate was deemed to be folate deficient (with a secondarily depressed cobalamin concentration) because she had an elevated homocysteine concentration but a normal MMA concentration. The second subject had high concentrations of both metabolites, suggesting deficiency of both vitamins, but refused further evaluation.

subjects with cobalamin concentrations <140 pmol/L (P = 0.0001) and not significantly different from the 10.8% frequency in subjects with cobalamin concentrations >258 pmol/L. Hyperhomocysteinemia, too, was less frequent in those with cobalamin concentrations of 140–258 pmol/L than in those with cobalamin concentrations <140 pmol/L (P = 0.001), although it was also significantly more frequent than in those with cobalamin concentrations >258 pmol/L.

Significant differences were seen among the 4 broad ethnic groupings (**Table 2**). Whites and Latin Americans had significantly lower cobalamin concentrations than did blacks or Asian Americans (P = 0.005). They also tended to have higher prevalences of cobalamin concentrations <140 pmol/L, although this difference was not significant. Whites and Latin Americans also had higher prevalences of cobalamin concentrations of 140–258 pmol/L than did blacks and Asian Americans, who comprised only 25% of the subjects in that group but 49% of those with concentrations >258 pmol/L (P = 0.001). A comparison of cobalamin concentrations <140 pmol/L between subjects who probably had true cobalamin deficiency (because they had accompanying metabolite abnormalities) and those who probably did not (because metabolite abnormalities were absent) showed similar ethnic distributions in the 2 groups.

Men tended to have lower cobalamin concentrations than did women and tended to have subnormal concentrations (<140 pmol/L) more frequently, but the differences were not significant (Table 2). Analysis of variance showed no interaction between the ethnic and sex differences, indicating that the differences were independent of each other. Stratification by decades showed no age trend in serum cobalamin concentrations, nor did the concentrations correlate with age.

Total homocysteine

Elevated homocysteine concentrations were found in 154 of the 591 subjects (26.1%). Homocysteine concentrations correlated inversely with serum cobalamin (r = –0.34, P = 0.0001) and folate (r = –0.29, P = 0.0001) concentrations. Analysis by a stepwise logistic regression model showed that a cobalamin concentration <140 pmol/L was the best predictor for hyperhomocysteinemia; a low serum folate concentration and male sex were the 2 next best predictors.

Nevertheless, only 41.2% of the subjects with hyperhomocysteinemia and normal renal function had either subnormal cobalamin concentrations or elevated MMA concentrations to indicate cobalamin

TABLE 2
Ethnic and sex comparisons of cobalamin concentrations in the elderly

	Geometric mean	95% Range	Percentage with subnormal concentrations (<140 pmol/L)
	pmol/L	*pmol/L*	*%*
Ethnic group[1]			
Latin Americans (n = 134)	221	81–556	11.9
Whites (n = 237)	232	61–675	14.8
Blacks (n = 151)	280[2]	65–887	9.3
Asian-Americans (n = 68)	305[2]	89–763	7.4
Sex[3]			
Men (n = 345)	240	53–623	13.9
Women (n = 246)	262	90–747	8.9

[1]Ethnic information was not available for 1 of the 591 subjects. There were no significant differences in percentages of subnormal concentrations among the ethnic groups.

[2]Significantly different from whites and Latin Americans, P ≤ 0.005 (ANOVA with Bonferroni correction).

[3]The sex differences in cobalamin concentrations and percentages of subnormal concentrations were not significant, P = 0.09 and 0.07, respectively (ANOVA with Bonferroni correction).

TABLE 3
Prevalence of coexisting metabolite and vitamin abnormalities in elderly subjects with elevated serum total homocysteine concentrations not associated with renal insufficiency[1]

Abnormality	Prevalence
Cobalamin (<140 pmol/L)	44/154 (28.6)
MMA (>376 or 370 nmol/L)[2]	47/153 (30.7)
Both cobalamin and MMA	28/153 (18.3)
Cobalamin, MMA, or both	63/153 (41.2)
Folate (<5.7 nmol/L)	10/138 (7.2)

[1]Percentage in parentheses. MMA, methylmalonic acid. Among those patients with elevated homocysteine concentrations, MMA was not measured in 1 patient, and folate was not measured in 16 patients.

[2]Depending on whether the assay was done in Cleveland or Aarhus, Denmark, respectively.

deficiency as its likely explanation and only 7.2% had low serum folate concentrations (**Table 3**). Thus, approximately half of the patients with hyperhomocysteinemia did not have vitamin or metabolite results supporting either cobalamin or folate deficiency as its explanation; vitamin B-6 concentrations, thyroid function, and activity of enzymes of homocysteine metabolism were not determined.

The homocysteine data were also analyzed after reinclusion of the 64 subjects with renal insufficiency, and elevated creatinine concentrations were associated with 48 of the resulting 202 hyperhomocysteinemic subjects (23.8%). Multiple logistic regression analysis showed renal insufficiency to be the strongest predictor of hyperhomocysteinemia now, with cobalamin concentrations <140 pmol/L being the second strongest predictor.

Significant ethnic differences were seen (**Table 4**). Whites had the highest mean homocysteine concentrations ($P \leq 0.05$). The trends for prevalence of hyperhomocysteinemia were similar. Although the differences among the 4 ethnic groups were not significant, hyperhomocysteinemia was significantly more common in whites than in the other 3 groups combined.

Men had significantly higher homocysteine concentrations than women and a higher percentage of abnormal concentrations (Table 4). Homocysteine concentrations also correlated weakly with age ($r = 0.09$, $P = 0.03$). Analysis of variance showed a significant interaction between the age and sex differences ($P = 0.02$). However, the ethnic differences were independent of both age and sex differences. The ethnic differences were more pronounced in

women ($P = 0.006$) on reinclusion of the 64 subjects with renal insufficiency, perhaps because Asian American and black men with hyperhomocysteinemia predominated in those 64 subjects.

Methylmalonic acid

Elevated MMA concentrations were seen in 97 of the 583 tested subjects (16.6%); 8 subjects did not have MMA measurements. Fifty-seven of the 97 abnormal MMA concentrations (58.8%) were accompanied by cobalamin concentrations <140 pmol/L, hyperhomocysteinemia, or both (**Table 5**). MMA concentrations correlated with homocysteine concentrations ($r = 0.24$ for the 132 MMA assays in Denmark, $P = 0.007$; $r = 0.19$ for the 451 in Cleveland, $P = 0.0001$). MMA concentrations also correlated inversely with cobalamin concentrations ($r = -0.22$, $P = 0.01$ and $r = -0.30$, $P = 0.0001$ for MMA assays in Denmark and Cleveland, respectively) and not with folate concentrations.

The ethnic trends resembled those for cobalamin and homocysteine. Whites (19.5%) and Latin Americans (19.6%) had the highest frequency of abnormal MMA concentrations and blacks (10.9%) and Asian Americans (13.6%) had the lowest. However, the differences were not significant nor were significant sex differences seen. The sole demographic factor that showed statistical significance was age. MMA concentrations correlated with age ($P = 0.01$ for the sera assayed in Denmark and $P = 0.003$ for those in Cleveland).

Renal insufficiency

Creatinine was measured in 236 subjects, including all those with abnormal serum metabolite concentrations, to gauge its contribution to such abnormalities. Multivariate logistic regression and multifactor analysis of variance showed that, compared with the rest of the study population, subjects with renal insufficiency were more often male ($P = 0.002$), older ($P = 0.001$), and Asian American or black rather than Latin American or white ($P = 0.04$). When all metabolic data were reanalyzed after inclusion of the 64 subjects with renal insufficiency, 48 of the resulting 202 abnormal homocysteine concentrations (23.8%) and 36 of the 141 abnormal MMA concentrations with sufficient data for analysis (25.5%) were associated with renal insufficiency.

Cobalamin supplement use

The 70 subjects who were taking cobalamin supplements (9.7% of the entire study population) did not differ significantly

TABLE 4
Ethnic and sex comparisons of homocysteine concentrations in elderly subjects (excluding subjects with renal insufficiency)

	Geometric mean	95% Range	Percentage with abnormal concentrations[1]
	μmol/L	μmol/L	%
Ethnic group			
Latin Americans (n = 134)	13.8	7.2–26.5	24.6
Whites (n = 237)	14.8[2]	7.8–28.4	31.2[3]
Blacks (n = 151)	13.8	7.3–26.1	22.5
Asian-Americans (n = 68)	12.8	7.0–23.5	19.1
Sex			
Men (n = 345)	14.9[4]	8.0–27.8	30.1[5]
Women (n = 246)	13.0	6.8–24.8	20.3

[1]Abnormal results defined as homocysteine concentrations >17.1 μmol/L for men and >16.8 μmol/L for women.

[2]Significantly different from other ethnic groups, $P < 0.05$ (ANOVA with Bonferroni correction).

[3]There were no significant differences between the 4 groups ($P = 0.11$) but the percentage of whites with abnormal concentrations was significantly different from that of all other ethnic groups combined, $P = 0.04$ (ANOVA with Bonferroni correction).

[4,5]Significantly different from women (ANOVA with Bonferroni correction): [4]$P = 0.0001$, [5]$P = 0.008$.

TABLE 5
Prevalence of coexisting metabolite and cobalamin abnormalities in elderly subjects with elevated serum methylmalonic acid concentrations not associated with renal insufficiency[1]

Abnormality	Prevalence
Cobalamin (<140 pmol/L)	38/97 (39.2)
Homocysteine (>17.1 μmol/L for men, >16.8 μmol/L for women)	47/97 (48.5)
Both cobalamin and homocysteine	28/97 (28.9)
Cobalamin, homocysteine, or both	57/97 (58.8)

[1]Percentage in parentheses.

from the 655 nonusers in age, sex, or ethnic group distribution. Not surprisingly, they had higher serum cobalamin concentrations ($P < 0.0001$) and lower homocysteine ($P = 0.008$) and MMA ($P = 0.01$ for sera assayed in Cleveland) concentrations. Despite the use of supplements, 13 of the 70 subjects (18.6%) nevertheless still had abnormal homocysteine, MMA, or cobalamin concentrations, or a combination of these.

Folate

Because it was not a primary focus of the study and blood sample volume was limited, serum folate was measured in only 520 of the subjects. These 520 subjects turned out to be younger, more often black, and less often Asian American, and had abnormal cobalamin and metabolite concentrations more often than did the subjects who were not tested. Despite overrepresentation of several characteristics associated with folate deficiency (eg, metabolite abnormality and ethnicity), only 22 of the folate concentrations were found to be <5.7 nmol/L (4.2%). Twelve of these 22 low concentrations were associated with hyperhomocysteinemia, which was the strongest factor associated with a low folate concentration by regression model analysis.

In contrast with the cobalamin and homocysteine findings, Latin Americans and blacks had the highest rates of folate abnormality; the ethnic differences were significant in women (15.4% of Latin American women had low folate concentrations compared with 8.3% of black women and 0% of white or Asian American women, $P = 0.001$) but not in men. No significant difference in folate concentrations was seen between the sexes. Folate concentrations tended to rise with age ($P = 0.02$), but the ethnic differences were independent of age by analysis of variance.

DISCUSSION

The existence of ethnic differences in cobalamin concentrations (11) prompted our examination of whether these ethnic differences also exist in the elderly and include metabolic evidence of cobalamin deficiency because not all low cobalamin concentrations indicate deficiency. We also examined whether hyperhomocysteinemia is subject to ethnic variation comparable with its previously noted age and sex differences (22, 26–29).

Before we address the demographic findings, it is worthwhile to note the metabolic and related findings themselves. The 11.8% prevalence of subnormal cobalamin concentrations (<140 pmol/L) confirms that low concentrations are common in the elderly (3), although the prevalence may be even higher in Europe (30), where the population is largely white. In support of results in previous reports (4–6, 30), most of the low cobalamin concentrations indeed

reflected metabolic evidence of mild deficiency: 55.1% of them were accompanied by abnormal MMA concentrations and 78.3% by abnormal MMA concentrations, homocysteine concentrations, or both. Because low cobalamin concentrations can occur in the absence of deficiency, the term "cobalamin deficiency" should probably be reserved for those cases with accompanying clinical or metabolic evidence supporting the diagnosis.

Some investigators have suggested that cobalamin concentrations as high as 258 pmol/L should be viewed as potentially abnormal (5, 25). However, our data showed metabolite abnormalities to be much less frequent in the elderly subjects with cobalamin concentrations in the 140–258-pmol/L range than in those with subnormal cobalamin concentrations; their MMA concentrations were also no worse than those of subjects with cobalamin concentrations >258 pmol/L. These findings suggest that the benefit of identifying a few more mildly deficient subjects by extending suspicion of deficiency well into the current normal cobalamin concentration range is outweighed by the huge number of subjects caught in that net whose status is normal.

As noted consistently by others (9, 27, 30, 31), hyperhomocysteinemia was extremely common in the elderly. It occurred in 26.1% of all our subjects without renal insufficiency. Nearly half of these elevated homocysteine concentrations were associated with low cobalamin or folate concentrations. Moreover, homocysteine concentrations correlated inversely with cobalamin and folate concentrations overall ($P = 0.001$ for each), as also noted by others (26, 32). Renal insufficiency appeared to be a common cause of hyperhomocysteinemia also; if data from those with renal insufficiency were reincluded in the analysis, it accounted for 23.8% of all high homocysteine concentrations.

Cobalamin concentrations <140 pmol/L were the strongest predictors of abnormal homocysteine concentrations in our survey and were second to renal insufficiency if subjects with abnormal creatinine concentrations were included; low folate concentrations were third. Previous reports have varied in their order of rankings of predictors of hyperhomocysteinemia, but all have listed cobalamin, folate, and creatinine concentrations along with age at or near the top (32–34).

Nevertheless, some high homocysteine concentrations remained unexplained. Several of them were associated with cobalamin concentrations of 140–258 pmol/L, although most were not accompanied by high MMA concentrations and therefore probably did not represent cobalamin deficiency; renal insufficiency has already been mentioned as a cause of hyperhomocysteinemia. It seems unlikely that vitamin B-6 deficiency was a common factor (35), but we did not measure vitamin B-6 concentrations. Other possibilities include folate deficiency despite normal serum folate concentrations, enzymatic defects affecting remethylation or transsulfuration of homocysteine, hypothyroidism (9), and artifacts.

High MMA concentrations were less frequent than was hyperhomocysteinemia. The 16.6% frequency (20.5% if one includes the artifactually high MMA concentrations associated with renal insufficiency) agrees with previous reports (25). MMA concentrations correlated with homocysteine concentrations and, inversely, with cobalamin concentrations but not with folate concentrations. However, 41.2% of abnormal MMA concentrations were not associated with any other markers of cobalamin deficiency.

The complication that even mild renal insufficiency introduces in interpreting serum metabolite concentrations bears emphasis. Although not every abnormality associated with a high creatinine concentration may be attributable solely to it,

renal insufficiency must be considered before attributing the abnormal metabolite concentration, especially one not accompanied by an abnormal vitamin concentration, to vitamin deficiency. This is particularly true in the elderly.

Another modifying influence in surveys is vitamin supplement use. Seventy subjects (9.7%) admitted to taking cobalamin supplements, without which the prevalence of deficiency might have been even higher. Nevertheless, 18.6% of these supplement users still had biochemical evidence of possible cobalamin deficiency. Although lower than the 37.2% prevalence of abnormality in those not taking cobalamin supplements, this still high prevalence of cobalamin deficiency underscores the fact that oral cobalamin supplement use does not always eliminate the deficiency in the elderly. As suggested previously, malabsorption may explain why supplement use reduced the prevalence of deficiency only by half (24, 30). Half of elderly cobalamin-deficient patients may have some form of malabsorption, most often that of food-bound cobalamin (3).

Demographic findings

Elderly men were more likely to have hyperhomocysteinemia than were elderly women, as seen in many previous studies (22, 28, 29, 31). They also had nonsignificantly lower cobalamin concentrations than women. Although not significant in our study, a sex difference in cobalamin concentrations was noted previously in elderly (31) but not in younger (36) adults. The continued sex difference in homocysteine concentrations into old age also suggests that menopause does not end the lesser female predisposition to hyperhomocysteinemia (32).

It is not surprising, thus, that elderly men had more frequent cobalamin or metabolite abnormalities or both overall than did women (42.1% compared with 31.0%, for men and women respectively; $P = 0.007$). This suggests that cobalamin deficiency is more common in elderly men. Because women have a greater susceptibility to pernicious anemia (23), defined as cobalamin malabsorption due to lack of gastric intrinsic factor, other underlying causes of cobalamin deficiency must predominate in elderly men to account for this difference.

A major demographic finding of our study concerns ethnic variations. Ethnicity has rarely been examined in regard to metabolite status. European surveys have included quite homogeneous populations and most North American studies have focused on white subjects. A lower prevalence of both hyper-homocysteinemia and cobalamin deficiency in blacks than in whites seems intuitive given both their lower prevalence of thermolabile methylene tetrahydrofolate reductase (37) and their higher cobalamin concentrations (11). The first indication of ethnic differences in homocysteine status came from South Africa. Black adults had lower homocysteine concentrations and lacked the rightward skew seen in whites' values (13). Detailed study of a subset of young subjects suggested that blacks metabolized homocysteine more efficiently than did whites (12). The blacks also did not improve metabolically after vitamin therapy, whereas the whites did. On the other hand, study of surplus samples from the third National Health and Nutrition Examination Survey (NHANES III) in the United States (29) showed no difference in homocysteine concentrations between whites and blacks although it found other minor ethnic differences among women.

We found that homocysteine concentrations not related to renal insufficiency were significantly higher in whites than in blacks, Latin Americans, and especially Asian Americans (who, to our knowledge, had not been studied previously). The ethnic

differences were independent of the differences between the sexes. These data tend to support the South African findings.

Moreover, the results from our sample of elderly community dwellers may not be incompatible with the NHANES III findings (29), which were extensive but incorporated neither vitamin nor renal function analyses. That report suggested that whites, at least white women, tend to have higher homocysteine concentrations than other ethnic groups. Our data, too, tended to show greater ethnic differences among the women than among the men, although men more frequently had abnormalities than did women. Moreover, the absence of ethnic differences among men and the general absence of differences between whites and blacks in the NHANES III study may be attributable in part to the fact that creatinine concentrations were not taken into account. We found that whites had higher homocysteine concentrations unrelated to renal insufficiency (eg, due to cobalamin deficiency) than did Asian Americans, blacks, and Latin Americans, whereas homocysteine concentrations associated with high creatinine concentrations showed an almost exactly opposite ethnic pattern.

Demographic studies of MMA concentrations have been rare (38) and we know of no ethnic analyses. The only significant demographic feature we showed was a correlation with age. Ethnic trends in MMA concentrations resembled those seen for cobalamin and homocysteine, but they were not significant.

Folate status was not the primary target of our survey and folate was not measured in all subjects, but the findings were of interest for their contrast with cobalamin data. Not only were low folate concentrations infrequent in the elderly (4.2%), as also found by others (25), but the ethnic patterns were virtually opposite those seen for cobalamin and homocysteine. Folate concentrations <5.7 nmol/L were most common in Latin Americans and blacks, although the ethnic differences were only significant in women ($P = 0.001$). Even though folate concentrations, unlike cobalamin, are strongly affected by dietary intake (24), the explanation for the ethnic differences in folate concentrations is uncertain. Blacks had the lowest folate intake but Latin Americans, with their even poorer folate status, had above average intakes (R Carmel, J Howard, unpublished observations, 1996).

Our data suggest that when one focuses on associations other than abnormal renal status, elderly whites—and especially elderly white men—are at greatest risk not only for hyperhomocysteinemia but also for mild cobalamin deficiency. Moreover, the abnormalities are closely interrelated. On the other hand, elderly blacks and Asian Americans—especially women—are at lower risk. The lower cobalamin concentrations in whites than in blacks thus appear to represent real differences in metabolic status because no ethnic differences were seen between subjects with cobalamin concentrations <140 pmol/L that were accompanied by metabolite abnormalities and those that were not. This suggests that separate reference ranges for serum cobalamin concentrations for different ethnic groups are not appropriate.

Our data also show that the ethnic pattern of hyperhomocysteinemia that is associated with high creatinine concentrations differs from that of homocysteine results not associated with renal insufficiency in the elderly and is most common in Asian American and black men. Therefore, ethnic differences should be considered in formulating public health and other policies. The ethnic differences we found in homocysteine concentrations, in cobalamin deficiency, and in the association of hyperhomocysteinemia with renal insufficiency, along with the reported greater efficiency of homocysteine metabolism in blacks (12), suggest

that plans for increased folate supplementation should be more precisely targeted. Studies will need to determine whether responses to vitamin supplementation differ not only among the different ethnic groups but also among the different causes of hyperhomocysteinemia. Moreover, concurrent cobalamin supplementation may be particularly necessary for elderly whites, given their higher prevalence of mild cobalamin deficiency.

REFERENCES

1. Boger WP, Wright LD, Strickland SC, Gylfe JS, Ciminera JL. Vitamin B$_{12}$: correlation of serum concentrations and age. Proc Soc Exp Biol Med 1955;89:375–8.
2. Chow BF, Wood R, Horonick A, Okuda K. Agewise variation of vitamin B$_{12}$ serum levels. J Gerontol 1956;11:142–6.
3. Carmel R. Cobalamin, the stomach, and aging. Am J Clin Nutr 1997;66:750–9.
4. Carmel R, Sinow RM, Karnaze DS. Atypical cobalamin deficiency: subtle biochemical evidence of deficiency is commonly demonstrable in patients without megaloblastic anemia and is often associated with protein-bound cobalamin malabsorption. J Lab Clin Med 1987; 109:454–63.
5. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients. J Am Geriatr Soc 1992; 40:1197–204.
6. Carmel R, Gott PS, Waters CH, et al. The frequently low cobalamin levels in dementia usually signify treatable metabolic, neurologic and electrophysiologic abnormalities. Eur J Haematol 1995;54:245–53.
7. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–28.
8. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease; probable benefits of increasing folate intakes. JAMA 1995;274:1049–57.
9. Green R. Metabolite assays in cobalamin and folate deficiency. Bailliere Clin Haematol 1995;8:533–66.
10. Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. J Lab Clin Med 1989;114:473–501.
11. Carmel R. Ethnic and racial factors in cobalamin metabolism and its disorders. Semin Hematol 1999;36:88–100.
12. Ubbink JB, Vermaak WJH, Delport R, van der Merwe A, Becker PJ, Potgieter H. Effective homocysteine metabolism may protect South African blacks against coronary heart disease. Am J Clin Nutr 1995;62:802–8.
13. Ubbink JB, Delport R, Vermaak WJH. Plasma homocysteine concentrations in a population with low coronary disease prevalence. J Nutr 1996;126:1254S–7S.
14. Carmel R, Karnaze DS, Weiner JM. Neurologic abnormalities in cobalamin deficiency are associated with higher cobalamin "analogue" values than are hematologic abnormalities. J Lab Clin Med 1988;111:57–62.
15. Jacobsen DW, Gatautis VJ, Green R, et al. Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 1994;40:873–81.
16. Carmel R, Rasmussen K, Jacobsen DW, Green R. Comparison of deoxyuridine suppression test with serum levels of methylmalonic acid and homocysteine in mild cobalamin deficiency. Br J Haematol 1996;93:311–8.
17. Rasmussen K. Solid-phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable-isotope-dilution method. Clin Chem 1989;35:260–4.
18. Rasmussen K, Möller J, Östergaard K, Kristensen MÖ, Jensen J. Methylmalonic acid concentrations in serum of normal subjects: biological variability and effect of oral L-isoleucine loads before and after intramuscular administration of cobalamin. Clin Chem 1990;36:1295–9.
19. Rasmussen K, Vyberg B, Pedersen KO, Bröchner-Mortensen J. Methylmalonic acid in renal insufficiency: evidence of accumulation and implications for diagnosis of cobalamin deficiency. Clin Chem 1990;36:1523–4.
20. Wilcken DEL, Dudman NPD, Tyrrell PA, Robertson MR. Folic acid lowers plasma homocysteine in chronic renal insufficiency: possible implications for prevention of vascular disease. Metabolism 1988; 37:697–701.
21. Soria C, Chadefaux B, Coudé M, Gaillard O, Kamoun P. Concentrations of total homocysteine in plasma in chronic renal failure. Clin Chem 1990;36:2137–8.
22. Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland homocysteine study. JAMA 1995;274:1526–33.
23. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. Arch Intern Med 1996;156:1097–100.
24. Howard JM, Azen C, Jacobsen DW, Green R, Carmel R. Dietary intake of cobalamin in elderly people who have abnormal serum cobalamin, methylmalonic acid and homocysteine levels. Eur J Clin Nutr 1998;52:582–7.
25. Lindenbaum J, Rosenberg IH, Wilson PWF, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 1994;60:2–11.
26. Andersson A, Brattstrom L, Israelsson B, Isaksson A, Hamfeld A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. Eur J Clin Invest 1992;22:79–87.
27. Joosten E, van den Berg A, Riezler R, et al. Metabolic evidence that deficiencies of vitamin B-12, folate, and vitamin B-6 occur commonly in elderly people. Am J Clin Nutr 1993;58:468–76.
28. Clarke R, Woodhouse P, Ulvik A, et al. Variability and determinants of total homocysteine concentrations in plasma in an elderly population. Clin Chem 1998;44:102–7.
29. Jacques PF, Rosenberg IH, Rogers G, et al. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999;69:482–9.
30. van Asselt DZB, de Groot LCPGM, van Staveren WA, et al. Role of cobalamin intake and atrophic gastritis in mild cobalamin deficiency in older Dutch subjects. Am J Clin Nutr 1998;68:328–34.
31. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and input as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
32. Brattstrom L, Lindgren A, Israelsson B, Andersson A, Hultberg B. Homocysteine and cysteine: determinants of plasma levels in middle-aged and elderly subjects. J Intern Med 1994;236:633–41.
33. Koehler KN, Romero LJ, Stauber PN, et al. Vitamin supplementation and other variables affecting serum homocysteine and methylmalonic acid concentrations in elderly men and women. J Am Coll Nutr 1996; 15:364–76.
34. Lussier-Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest J Jr. Plasma total homocysteine in healthy subjects: sex-specific relation with biological traits. Am J Clin Nutr 1996;64:587–93.
35. Miller JW, Ribaya-Mercado JD, Russell RM, et al. Effect of vitamin B-6 deficiency on fasting plasma homocysteine concentrations. Am J Clin Nutr 1992;55:1154–60.
36. Saxena S, Carmel R. Racial differences in vitamin B$_{12}$ levels in the United States. Am J Clin Pathol 1987;88:95–7.
37. McAndrew PE, Brandt JT, Pearl DK, Prior TW. The incidence of the gene for thermolabile methylene tetrahydrofolate reductase in African Americans. Thromb Res 1996;83:195–8.
38. Rasmussen K, Möller J, Lyngbak M, Holm Pedersen AM, Dybkjaer L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. Clin Chem 1996;42:630–6.