Elevated serum *S*-adenosylhomocysteine in cobalamin-deficient elderly and response to treatment[1–3]

Sally P Stabler, Robert H Allen, Evi T Dolce, and Mary Ann Johnson

ABSTRACT

Background: *S*-Adenosylmethionine (SAM)–dependent methylation reactions produce *S*-adenosylhomocysteine (SAH), the precursor of homocysteine, which has been associated with adverse events when it is elevated.

Objective: We studied a cohort of elderly with a high prevalence of cobalamin deficiency to determine whether SAH, SAM, or their ratio was abnormal; whether they correlated with other markers of vitamin deficiency; and whether they changed with cobalamin therapy.

Design: A convenience sample of elderly attending nutrition centers was enrolled for baseline demographic, biochemical, and nutritional assessments. Methylmalonic acid (MMA), total homocysteine, and other metabolites were measured by using gas chromatography–mass spectrometry. Serum SAM and SAH were measured by using stable-isotope-dilution liquid chromatography–mass spectrometry. Subjects found to have elevated serum MMA were treated with oral cyanocobalamin tablets (1000 μg/d) for 3 mo. Subjects with normal MMA were randomly assigned to 1 of 3 dosage groups: 0, 25, or 100 μg cyanocobalamin/d.

Results: The 149 elderly subjects had a mean age of 76.3 y; 81% were female, and 30% were African American. Serum MMA concentrations were elevated in 30% and SAH concentrations were elevated in 64% of the cohort. Those with elevated MMA concentrations had higher SAH and SAM concentrations. High-dose oral cobalamin lowered SAH, MMA, and total homocysteine concentrations significantly, although subjects with creatinine concentrations >109 umol/L had higher posttreatment SAH than did those with lower creatinine.

Conclusions: Elevated serum SAH concentrations are common in elderly and are strongly influenced by both renal status and cobalamin deficiency. These elevated concentrations can be lowered with high-dose oral cobalamin therapy. *Am J Clin Nutr* 2006;84:1422–9.

KEY WORDS Methylmalonic acid, vitamin B-12, total homocysteine, folate, *S*-adenosylmethionine

INTRODUCTION

Hyperhomocysteinemia is associated with adverse events such as mortality (1, 2), cardiovascular events (1, 3), stroke (3, 4), and cognitive disorders (5, 6), including Alzheimer disease (7). The underlying pathophysiology of the deleterious effects of hyperhomocysteinemia is not clear. It is possible that elevated total homocysteine (tHcy) is simply a marker of underlying vitamin deficiency or that the compound itself actually causes abnormal methylation status or other toxicity (8).

Homocysteine is methylated to form methionine, which is the precursor of *S*-adenosylmethionine (SAM), the physiologic methylator of creatine, phospholipids, neurotransmitters, and methylated DNA (9). *S*-Adenosylhomocysteine (SAH) is a product of these transmethylation reactions, which can be hydrolyzed to form homocysteine and adenosine (9). SAH is an inhibitor of many transmethylation reactions (10), and it is thought that maintenance of the appropriate ratio of SAM to SAH methylation (SAM:SAH) is important for health (11). Several studies have explored differences in SAM and SAH concentrations between healthy subjects and subjects with Alzheimer disease; results have been mixed (12, 13).

The SAM-dependent methylations are largely intracellular reactions, and it is not clear whether an assay of SAM and SAH in serum or plasma will reflect the intracellular status of methionine metabolism. Studies in normal volunteers have shown that most serum SAM and a large proportion of SAH are cleared by the kidneys (12). Therefore, subjects with impaired renal function will have build-up of these compounds in the blood (14–17), which may explain the relation between elevated SAH and vascular disease that was described elsewhere (17–20). However, it was also shown that, in inborn errors of metabolism, hypermethioninemia, or both (21, 22) and in severe cobalamin deficiency (23), elevated serum SAM or SAH concentrations change in response to methionine restriction or vitamin treatments. Thus, to some extent, the serum values probably do reflect intracellular methionine metabolism. To determine whether elevated values for SAH correlated with vitamin-related variables or poor cognition, we studied the determinants of serum SAM and SAH and their relations with hyperhomocysteinemia, renal insufficiency,

[1] From the Department of Medicine, University of Colorado Health Sciences Center, Denver, CO (RHA and SPS), and the Department of Foods and Nutrition, University of Georgia, Athens, GA (MAJ and ETD).

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[3] Address reprint requests to SP Stabler, Box B170, 4200 East 9th Avenue, Denver, CO 80262. E-mail: sally.stabler@UCHSC.edu.

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impaired cognition, and other clinical characteristics in an ambulatory elderly population from rural Georgia. Evidence of cobalamin deficiency, renal insufficiency, or both was found in a large proportion of the subjects. Oral cobalamin at various doses was given to assess the changes in SAH and other metabolites in response to treatment. An elderly cohort was chosen to assess these relations because of the likelihood of finding persons with hyperhomocysteinemia due to vitamin deficiency, renal insufficiency, or both and of finding persons with impaired cognition so that relations could be explored.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from the Older Americans Act Nutrition Program at 6 senior centers in northeast Georgia, by sending a letter describing the study to the directors of the centers in Clarke, Barrow, Morgan, Jackson, Franklin, and Newton counties (24). The directors and other employees of the centers recruited clients of the centers for the study. All interviews and procedures were performed at the senior centers.

Baseline measurements of demographic, biochemical, and clinical variables were taken for all subjects, who were then treated with oral cobalamin supplements or placebo for 3 mo. Measurements were repeated after treatment. Subjects were asked about their multivitamin use at the baseline assessment. Participants were not excluded for taking nutritional supplements, including those supplements containing cobalamin. The only exclusion criterion was medical treatment for cobalamin deficiency by injection or high-dose oral administration. At baseline, 149 participants were enrolled in the study.

Written informed consent was obtained from each participant. The institutional review boards on human subjects of the Georgia Department of Human Resources, the University of Georgia, the University of Colorado, and the Athens Community Council on Aging approved the questionnaires and procedures used in this study.

Baseline assessment

Participants were not asked to fast before blood collection because of their advanced age and possible frailty. Venous blood samples were stored on ice for 1–4 h before separation of serum for assays of SAM, SAH, total homocysteine (tHcy), methylmalonic acid (MMA), cystathionine, and methylcitric acid. The serum was frozen at −20 °C and sent, frozen on dry ice, by overnight delivery to the University of Colorado for analysis of the metabolites. Serum MMA, tHcy, methylcitric acid, and cystathionine concentrations were measured by using stable-isotope-dilution capillary gas chromatography–mass spectrometry (25, 26). SAM and SAH concentrations were measured by using stable-isotope-dilution liquid chromatography–mass spectrometry as described previously (14). Despite immediate chilling of samples, some assays resulted in uninterpretable chromatography for SAM, and values were available for a lower number of samples, which is indicated in the tables as applicable. The previously determined normal ranges were 73–271 nmol/L for MMA (25), 5.4–13.9 μmol/L for tHcy, 44–342 nmol/L for cystathionine (27), and 60–228 nmol/L for methylcitric acid (25). Normal ranges, determined in 48 healthy controls aged 22–59 y, were 71–168 nmol/L for SAM, 8–26 nmol/L for SAH, and 4.4–12.4 for SAM:SAH (14).

Automated complete blood counts and serum creatinine concentrations were assayed by a local clinical laboratory (SmithKline-Beecham Clinical Laboratories, Atlanta, GA). Sera for folate and cobalamin analyses were frozen at −70 °C in cryogenic vials with minimal airspace and sent by overnight delivery for analysis with a radioassay (Quantaphase II Vitamin B-12/Folate Radioassay; Bio-Rad, Richmond, CA) at the Centers for Disease Control and Prevention (Atlanta, GA) (28). Serum pepsinogen 1 was measured with the use of a kit (SORIN/Bio-medica kit P2560; INCSTAR Corporation, Stillwater, MN). Serum pepsinogen 1 was used as an indirect index of atrophic gastritis (29). Participants were considered to have mild atrophic gastritis if their pepsinogen 1 concentrations were between 10 and 60 μg/L and to have severe atrophic gastritis if their pepsinogen 1 concentrations were <10 μg/L (29).

The Orientation-Memory-Concentration (OMC) test (30), which is a brief cognitive measurement tool, was administered to all participants at baseline and after treatment. This instrument has 6 questions: the current month, year, and time of day; phrases to be repeated; counting backwards; and naming the months of the year in reverse order. A score of ≤8 indicated normal cognition or minimal impairment, 9–19 indicated moderate impairment, and ≥20 indicated severe impairment. The correlations of MMA and homocysteine with detailed neurocognitive screening were previously reported in a subgroup of the current cohort (31). The OMC test had shown a correlation of poor scores with elevated MMA and tHcy in another cohort from a Georgia senior center (24). Thus we hypothesized that elevated SAH concentrations or abnormal SAM:SAH may also correlate with poor cognition.

Treatment phase

Tablets of identical appearance containing 0, 25, 100, or 1000 μg cyanocobalamin were used (Sunstar Pharmaceutical Inc, Elgin, IL). After baseline testing, the participants were assigned to a treatment group. Those with serum MMA concentrations >271 nmol/L ($n = 45$) were offered daily high-dose oral cobalamin in 1000-μg tablets. For reasons of subject safety and to ensure treatment efficacy, the phlebotomist and the research coordinator ensured that participants with elevated MMA were aware of their potential for cobalamin deficiency. Other researchers and staff did not have access to information about the MMA status of the participants. In addition, the physicians of the participants with elevated MMA were notified in writing by the phlebotomist and research coordinator. Therefore, the intervention in those with elevated MMA was neither randomized nor blinded.

The 104 subjects with MMA ≤271 nmol/L were invited to participate in a randomized, double-blind, placebo-controlled trial comparing 3 doses of cobalamin (0, 25, and 100 μg/d). After ≈3 mo of supplementation, 132 participants completed a follow-up examination involving the same methods for phlebotomy and cognitive screening as were used at baseline. Compliance was monitored by calculating pill counts and, for the high-dose groups, by evaluating the follow-up serum cobalamin concentration.

The group taking 0 and the group taking 1000 μg cobalamin/d continued the treatment for another 6 mo and underwent a second

TABLE 1
Baseline variables in the elderly subjects[1]

Variable	Mean ± SD	Geometric mean	Range	Subjects with values above or below cutoffs
				%
Age (y)	76.3 ± 7.6	75.9	58–97	
Creatinine (μmol/L)	99 ± 44	94	54–418	
>109 μmol/L				20.9
SAM (nmol/L)	118 ± 61	108	42–511	
>168 nmol/L				10.1
<71 nmol/L				10.1
SAH (nmol/L)	35 ± 22	30	9–157	
>26 nmol/L				64.4
SAM:SAH	4.0 ± 2.0	3.6	0.9–9.6	
<4.4				68.2
MMA (nmol/L)	272 ± 213	238	85–1972	
>271 nmol/L				30.2
tHcy (μmol/L)	10.7 ± 4.2	10.1	5.1–39.8	
>13.9 μmol/L				13.4
Cystathionine (nmol/L)	258 ± 128	234	89–968	
Methylcitric acid (nmol/L)	188 ± 66	178	58–453	
Cobalamin (pmol/L)	365 ± 161	329	74–996	
<258 pmol/L[2]				26.2
Folate (nmol/L)	46.3 ± 27.7	39.4	8.8–163	
<13.6 nmol/L[2]				2.0
Pepsinogen (μg/L)	99 ± 71	77	9–550	
OMC test score	6.2 ± 6.7	0	0–28	

[1] OMC, orientation-memory-concentration. $n = 149$ for all variables except $n = 148$ for creatinine, *S*-adenosylmethionine (SAM), and the ratio of SAM to *S*-adenosylhomocysteine (SAH) (SAM:SAH), and $n = 147$ for pepsinogen. The cutoffs shown are the upper 97.5 or lower 2.5 percentile for 48 normal subjects as previously determined (14), except MMA and tHcy, which were determined previously in 60 subjects (27).

[2] The cutoffs shown are determined from many previous studies.

round of cognitive testing ≈9 mo after the initiation of supplementation.

Statistical analysis

Statistical analysis was performed by using SAS software (version 8.2; SAS Institute, Cary, NC) and SPSS software (version 13.0; SPSS Inc, Chicago, IL). $P < 0.05$ was considered significant. Data were log transformed to approximate normal distributions when necessary. Levene's test for equality of variance was employed, and the *t* value for unequal variance was used when appropriate. For baseline values, cross-sectional analyses including *t* tests for continuous variables and chi-square analyses for dichotomous variables were used to examine differences between subjects with and subjects without elevated MMA. Multiple stepwise regression analyses were used to evaluate the independent effects of variables on SAM, SAH, SAM:SAH, tHcy, and OMC test score. The independent variables were age, sex, race, creatinine, MMA, tHcy, cystathionine, methylcitric acid, folate, cobalamin, pepsinogen, OMC test score, SAM, SAH, and SAM:SAH. However, SAM and SAH were not used in models for SAM:SAH, and vice versa. Paired *t* tests were used to analyze posttreatment changes in the subjects receiving the 1000-μg/d dose. The pretreatment and posttreatment values for the randomly assigned dose groups (ie, 0, 25, or 100 μg) were evaluated by using analysis of variance, and changes were compared by using general linear models and least-significant-difference tests.

RESULTS

Baseline variables

One hundred forty-nine elderly subjects entered into the study, of whom 81% were female and 30% were African American. The mean age and biochemical variables are shown in **Table 1**. Although the mean serum creatinine concentration was normal, values ranged to 418 umol/L. This cohort appeared to have high folate status because the mean serum folate concentration was 46.3 nmol/L and the lowest value was 8.8 nmol/L. The proportions of the subjects who had biochemical values either higher or lower than the 95% CIs that we had found in younger populations are also shown. As expected, higher creatinine, MMA, and tHcy and lower cobalamin were found in a significantly greater percentage of subjects in the current study than in a younger population. However, the most striking change was that the SAH value was higher than our previously determined upper limit in 64% of the subjects, whereas the SAM value was high in only 10% or low in only 10%.

When the patients were divided into 2 groups—younger and older than the mean age of 76 y—no significant difference in the mean creatinine, cobalamin, folate, SAH, SAM, or SAM:SAH values was found (data not shown). In contrast, MMA (325 compared with 225 nmol/L; $P = 0.003$) and tHcy (11.4 compared with 9.9 umol/L; $P = 0.024$) were significantly higher in the older subjects than in the previous younger group. Methylcitric acid and cystathionine (data not shown) were also higher in the

TABLE 2
Pearson's correlation coefficient between variables[1]

	Creatinine	SAM	SAH	SAM:SAH	MMA	tHcy	Cystathionine	Methylcitric acid	Cobalamin	Folate	Pepsinogen 1	OMC
Age	0.17[2]	0.09	0.13	−0.002	0.26[3]	0.23[2]	0.20[2]	0.30[3]	0.03	0.09	−0.04	0.27[3]
Creatinine		0.60[3]	0.77[3]	−0.19[2]	0.18[2]	0.67[3]	0.60[3]	0.60[3]	0.08	0.05	0.45[3]	0.08
SAM			0.61[3]	0.20[2]	0.28[3]	0.41[3]	0.58[3]	0.52[3]	0.08	0.25[3]	0.34[3]	−0.08
SAH				0.51[3]	0.22[2]	0.59[3]	0.52[3]	0.67[3]	−0.01	0.59[2]	0.34[3]	0.03
SAM:SAH					0.01	−0.13	0.02	−0.22[2]	0.15	0.09	−0.11	−0.05
MMA						0.49[3]	0.36[3]	0.51[3]	−0.32[3]	0.14[4]	0.02	0.20[2]
tHcy							0.57[3]	0.64[3]	−0.24[2]	−0.20[2]	0.18[2]	0.18[2]
Cystathionine								0.68[3]	0.04	−0.13	0.41[2]	0.14[4]
Methylcitric acid									−0.07	−0.08	0.33[3]	0.05
Cobalamin										0.36[3]	0.15	0.10
Folate											−0.02	−0.11
Pepsinogen 1												−0.13

[1] SAM, *S*-adenosylmethionine; SAH, *S*-adenosylthomocysteine; SAM:SAH, ratio of SAM to SAH; MMA, methylmalonic acid; tHcy, total homocysteine; OMC, orientation-memory-concentration test.
[2] $P < 0.05$.
[3] $P < 0.001$.
[4] $P < 0.1$.

older subjects than in the younger subjects. The female subjects had lower creatinine than did the males (91 and 123 umol/L, respectively; $P = 0.018$), whereas tHcy was higher (13.0 and 10.1 umol/L, respectively; $P = 0.035$) and SAH trended higher (44 and 33 nmol/L, respectively; $P = 0.073$) in the male subjects. Compared with the white subjects in the current study, the African American subjects had lower serum folate (37.0 and 50.4 nmol/L, respectively; $P = 0.003$) and had a trend toward higher serum cobalamin (402 and 349 pmol/L, respectively; $P = 0.067$). Serum MMA was lower (207 compared with 301 nmol/L; $P = 0.001$), and serum SAM concentrations were much lower (98 compared with 126 nmol/L; $P = 0.001$) in the African American than in the white subjects, respectively. The OMC test score was significantly higher (indicating poorer cognition) in the African Americans (9.0) than in the whites (5.0; $P = 0.003$). Multivitamin use in 40% of the cohort resulted in the expected increase in serum folate and cobalamin and decreases in MMA, methylcitric acid, tHcy, and cystathionine (data not shown). The OMC test score was also lower (indicating better cognition)—5.0 compared with 7.4 ($P = 0.031$)—in those who took multivitamins than in those who did not. However, SAM, SAH, and SAM:SAH did not differ between those ingesting and those not ingesting multivitamins.

Correlations between variables

The univariate correlations between variables are shown in **Table 2**. Strong correlations were seen between creatinine, SAH, SAM, tHcy, methylcitric acid, and cystathionine. SAH was strongly correlated with tHcy, cystathionine, and methylcitric acid and more weakly correlated with MMA. However, after adjustment for serum creatinine, SAH was correlated only with SAM, SAM:SAH, and methylcitric acid, although it trended toward correlation with homocysteine (data not shown). SAM was strongly correlated with creatinine, methylcitric acid, tHcy, cystathionine, and SAH. After adjustment for creatinine, the correlation with MMA, methylcitric acid, cystathionine, and SAH remained significant (data not shown). Serum folate was correlated with SAM and SAH, and this correlation remained after adjustment for creatinine. Serum cobalamin was not correlated with SAM, SAH, or SAM:SAH unless adjusted for creatinine, in which case cobalamin was correlated with SAM:SAH ratio. Results of a stepwise ordinary least-squares regression for a number of dependent variables are shown in **Table 3**. SAM and SAH were mutually predictive. It is interesting that creatinine was a strong predictor of SAH but not of SAM. Methylcitric acid independently affected SAH and tHcy. The OMC test score was predicted by MMA but not by SAM, SAH, SAM:SAH, or tHcy.

Subjects with elevated methylmalonic acid

Of the 149 subjects, 45 subjects had elevated baseline MMA concentrations—>271 nmol/L. The variables in these subjects and in those without elevated MMA were compared as shown in **Table 4**. The serum cobalamin was lower, and age, creatinine, tHcy, SAM, and SAH were higher in those with elevated MMA than in those without. The other cobalamin-related metabolites—cystathionine and methylcitric acid—were also higher in the subjects with elevated MMA than in those with normal MMA. Of the 45 subjects with elevated MMA, 18 had serum cobalamin concentrations < 258 pmol/L and therefore met a definition of metabolic cobalamin deficiency that we used previously (24). SAM, SAH, SAM:SAH, and tHcy did not differ significantly between these 18 subjects with lower cobalamin values and the subjects with higher cobalamin values. Serum folate was significantly lower and serum creatinine trended lower in those with lower cobalamin than in those with higher cobalamin. The data indicate that the group with elevated MMA included subjects with renal failure–associated elevated MMA, cobalamin deficiency–associated elevated MMA, or (probably) both.

Effects of oral cobalamin treatment on metabolites

The subjects with high baseline MMA were offered daily high-dose (1000 μg) oral cyanocobalamin tablets. Forty subjects accepted the treatment, but 1 was dropped from analysis because of admitted noncompliance. By pill count, the mean compliance in the elevated MMA group was 91%, although pill count data

TABLE 3
Ordinary least-squares regression for biochemical variables in 149 elderly subjects[1]

Dependent variables	Adjusted R^2	Independent variables	β	SE β	P
SAH	0.68	Creatinine	23.28	2.99	<0.001
		Methylcitric acid	0.119	0.022	<0.001
		SAM	0.069	0.022	0.002
		Cystathionine	−0.027	0.012	0.024
SAM	0.56	Cystathionine	0.193	0.033	<0.001
		SAH	1.04	0.186	<0.001
		Folate	1.45	0.285	<0.001
		OMC	−1.277	0.52	0.015
		MMA	0.034	0.017	0.046
SAM:SAH	0.09	Methylcitric acid	−0.013	0.003	<0.001
		Cystathionine	0.005	0.002	0.0034
tHcy	0.65	Creatinine	5.333	0.595	<0.001
		MMA	0.004	0.001	<0.001
		Folate	0.049	0.018	0.009
		Pepsinogen	−0.001	0.003	0.010
		Methylcitric acid	0.012	0.005	0.010
		Cobalamin	−0.002	0.001	0.025
OMC	0.17	Race	4.542	1.120	<0.001
		MMA	0.006	0.002	0.012
		Age	0.193	0.070	0.006

[1] SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; OMC, orientation-memory-concentration test; SAM:SAH, ratio of SAM to SAH; MMA, methylmalonic acid; tHcy, total homocysteine.
[2] Included demographics and all baseline metabolites except that SAM:SAH was not used for SAM or SAH, and SAM and SAH were not used for SAM:SAH.

were adequate in only 25 subjects. Ninety-two subjects with normal serum MMA were randomly assigned to 1 of 3 oral cyanocobalamin dosage groups—0, 25, or 100 μg/d for 3 mo. Judgment of compliance in these subjects was dependent on the subject's history and pill count; compliance averaged 93%, 89%, and 101% for the 0-, 25-, and 100-μg doses, respectively. The baseline variables in these 3 randomized dose groups were analyzed for differences by one-way analysis of variance. The only significant difference was that mean ± SD SAH was higher in the group receiving 25 μg than in the placebo group: 33 ± 14 compared with 25 ± 9 nmol/L ($P = 0.049$). Changes in these variables were compared for these doses by using general linear models and least-significance tests; only changes in MMA and tHcy were statistically significant. In the placebo group, MMA increased by 10 nmol/L ($P < 0.01$) and tHcy decreased by 0.6 umol/L ($P < 0.01$ compared with placebo) in the group receiving the 100-μg/d dose. Mean and posttreatment values in the 100- and 1000-μg/d groups are shown in **Table 5**. Those taking 100 μg cobalamin/d had a significant rise in mean serum cobalamin and folate and a significant decrease in tHcy. In contrast, the highest dose (1000 μg/d) raised serum cobalamin markedly and reduced tHcy by 24%, MMA by 45%, and SAH by 14%; however, SAM:SAH did not change significantly. The group with elevated MMA included subjects in whom cobalamin deficiency was likely and subjects who had chronic renal insufficiency with associated increased MMA.

TABLE 4
Comparison of subjects with or without baseline methylmalonic acid (MMA) >271 nmol/L[1]

	Elevated MMA		
	Yes ($n = 45$)	No ($n = 104$)	P^2
Age (y)	79.2 ± 6.5[3]	75.0 ± 7.7	0.002
Creatinine (μmol/L)	122 ± 68	88 ± 18	0.003
SAM (nmol/L)	152 ± 80	103 ± 43	<0.001
SAH (nmol/L)	48 ± 30	29 ± 13	<0.001
SAM:SAH	3.6 ± 1.6	4.2 ± 2.1	0.074
MMA (nmol/L)	450 ± 320	196 ± 45	
tHcy (μmol/L)	13.8 ± 5.8	9.3 ± 2.3	<0.001
Cystathionine (nmol/L)	336 ± 166	224 ± 88	<0.001
Cobalamin (pmol/L)	312 ± 166	387 ± 154	0.009
Folate (nmol/L)	44.3 ± 32.7	47.2 ± 24.6	0.540
OMC score	8.2 ± 8.1	5.4 ± 5.8	0.043
Methylcitric acid (nmol/L)	242 ± 80	165 ± 43	<0.001

[1] SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; SAM:SAH, ratio of SAM to SAH; tHcy, total homocysteine; OMC, orientation-memory-concentration test.
[2] *t* Test after testing also for equality of the variances.
[3] $\bar{x}$ ± SD (all such values).

The subjects with high MMA were thus divided into 2 groups: those with serum creatinine concentrations >109 umol/L, designated as the high creatinine group ($n = 21$), and those whose creatinine was <109 μmol/L ($n = 24$). The baseline metabolites in those with high and those with low creatinine are shown in **Table 6**. Compared with the values in those with low creatinine, SAH was almost doubled in those with higher creatinine, and SAM was also markedly increased, but SAM:SAH did not differ significantly. After 3 mo of treatment, the SAH value fell in both groups but remained significantly higher in those with high creatinine than in those with low creatinine (46 and 29 nmol/L, respectively), as did tHcy (11.4 and 8.3 umol/L, respectively), whereas serum MMA concentrations did not differ significantly between the 2 groups. Although the posttreatment values for SAH and homocysteine were higher in those with high creatinine, the change from pretreatment to posttreatment did not differ

TABLE 5
Values before and after oral cobalamin treatment for 3 mo[1]

	Oral cobalamin dose			
	100 μg/d (n = 32)		1000 μg/d (n = 39)	
	Before	After	Before	After
SAH (nmol/L)	28 ± 15	28 ± 14	43 ± 20	37 ± 19[2]
SAM (nmol/L)[3]	95 ± 27	113 ± 36	153 ± 65	159 ± 62
SAM:SAH	4.9 ± 2.2	5.3 ± 3.3	4.1 ± 1.7	5.1 ± 2.5
MMA (nmol/L)	199 ± 37	187 ± 52	434 ± 285	240 ± 76[4]
tHcy (μmol/L)	9.0 ± 2.0	8.2 ± 2.2[5]	12.7 ± 3.6	9.6 ± 3.0[4]
Cobalamin (pmol/L)	364 ± 123	424 ± 147[5]	312 ± 171	831 ± 559[4]
Folate (nmol/L)	47.0 ± 23.6	55.8 ± 30.6[2]	41.3 ± 28.1	54.2 ± 48.1

[1] All values are $\bar{x}$ ± SD. SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SAM:SAH, ratio of SAM to SAH; MMA, methylmalonic acid; tHey, total homocysteine. Subjects receiving 1000 μg cobalamin had serum MMA concentrations >271 nmol/L and were not randomly assigned. Therefore, values are not directly compared between the 2 dose groups in the table.

[2,4,5] Before and after values were significantly different in each subject in the dose group (paired t test): [2]$P < 0.05$, [4]$P < 0.001$, [5]$P < 0.01$.

[3] n = 22 for the 100-μg dose; n = 27 for the 1000-μg dose.

significantly, as shown in Table 6. The mean OMC test scores did not change with treatment in any group either at 3 mo or when treatment was extended to 9 mo in the placebo and 1000-μg dose group (data not shown).

DISCUSSION

Although many studies show that elevated tHcy is a risk factor for adverse events (1–8), it is not clear whether high homocysteine is deleterious in itself or is a marker of impaired vitamin or methylation status (8). One prominent theory is that, in cobalamin or folate deficiency, SAM synthesis may be deficient, which could lead to impaired methylation reactions. Increased SAH also could be important because SAH is an inhibitor of transmethylation reactions (11). Most SAM-dependent methylation reactions are intracellular, as is methionine metabolism in general, which poses practical problems in clinical research. Therefore, much interest exists around the questions of whether plasma concentrations of SAM or SAH or SAM:SAH would predict deleterious effects of tissue hyperhomocysteinemia and whether they would correlate with abnormal methylation status. Previous studies in healthy controls showed that the mean fractional excretions of SAM and SAH, as compared with the excretion of creatinine, are very high: 93% for SAM and 39% for SAH (14). Thus, we were not surprised to find that renal status had a marked influence on the serum concentrations of SAM and SAH in this cohort of elderly. The influence of serum creatinine on these values is stronger than that of vitamin status. This situation may make the diagnostic use of these metabolites less useful than that of tHcy for predicting adverse events that are independent of the well-known greater risk of vascular disease in chronic renal disease.

We studied a mostly female elderly cohort, who in general had very high folate status. The mean tHcy in these elderly was remarkably low at 10.7 umol/L, which is lower than or equivalent

TABLE 6
Effect of serum creatinine concentrations >109 μmol/L on baseline and post–cobalamin treatment metabolites in subjects with elevated methylmalonic acid (MMA)[1]

	High baseline creatinine			High posttreatment creatinine		Difference between high pre- and posttreatment creatinine[2]		
	No	Yes	P[3]	No	Yes	No	Yes	P[3]
n	24	21		22	17	22	17	
Age (y)	78 ± 6.3	81 ± 6.6	0.17					
Creatinine (μmol/L)	87 ± 14	162 ± 81						
SAH (nmol/L)[4]	36 ± 15	63 ± 36	0.003	29 ± 11	46 ± 23	−7 ± 14	−6 ± 18	0.834
SAM (nmol/L)[5]	119 ± 33	189 ± 101	0.005	146 ± 43	176 ± 80	21 ± 51	−13 ± 59	0.117
SAM:SAH[6]	3.9 ± 1.8	3.4 ± 1.3	0.285	5.8 ± 2.9	4.1 ± 1.5	1.6 ± 4.0	0.2 ± 1.8	0.246
MMA (nmol/L)	443 ± 348	458 ± 294	0.858	227 ± 61	256 ± 92	−230 ± 363	−149 ± 142	0.389
tHcy (μmol/L)	11.4 ± 3.5	16.4 ± 6.7	0.003	8.3 ± 2.0	11.4 ± 3.3	−3.3 ± 2.8	−2.8 ± 3.8	0.616
Cobalamin (pmol/L)	300 ± 180	326 ± 152	0.608	756 ± 380	926 ± 731	458 ± 336	596 ± 682	0.414
Folate (nmol/L)	47.4 ± 37.2	40.5 ± 27.0	0.495	48.6 ± 43.5	73.4 ± 53.6	5.5 ± 43.1	22.7 ± 42.4	0.221

[1] SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SAM:SAH, ratio of SAM to SAH; tHcy, total homocysteine.

[2] Calculated individually for each subject.

[3] t Test.

[4] Posttreatment SAH: n = 21 without high creatinine, n = 16 with high creatinine.

[5] Posttreatment SAM: n = 16 without high creatinine, n = 12 with high creatinine.

[6] Posttreatment SAM:SAH: n = 15 without high creatinine, n = 11 with high creatinine.

to values seen in younger subjects in unfortified populations (32, 33). Despite this relatively low tHcy, the mean SAH concentration was above the range we previously measured in 64% of the subjects. Multivariate analysis in our cohort showed that only creatinine, SAM, cystathionine, and methylcitric acid were independent predictors of SAH, but together they accounted for most of the variability. Because creatine, the precursor of creatinine, is a product of the SAM-to-SAH reaction, it is not surprising that a strong correlation may be found, although creatinine was not an independent predictor of SAM. Methylcitric acid is formed in excess in persons with severe cobalamin deficiency; however, methylcitric acid concentrations are also highly dependent on renal excretion (25), which may explain the relation with SAH in our cohort. It is surprising that tHcy was not a predictor of the SAH value in the regression analysis. Serum cobalamin was not a predictor of SAM or SAH, and serum folate predicted only SAM in the regression model. Other investigations reported similar data (15, 16, 18).

Elevated MMA is frequently found in elderly subjects, and it predicts elevated tHcy. Both metabolites readily decrease with cobalamin therapy, which suggests that depleted vitamin status rather than age is the variable more likely to cause elevated values (24). The subjects with elevated MMA in the current study had the expected higher concentrations of creatinine, tHcy, cystathionine, and methylcitric acid, as well as higher SAM and SAH. We chose to treat all subjects with elevated MMA with high-dose oral cobalamin tablets. As expected, the serum MMA and tHcy fell markedly in these subjects after treatment. In addition, after treatment with high-dose oral cobalamin, serum SAH fell significantly in both groups of subjects: those with high and those with low creatinine. Improved cobalamin status apparently lowers the serum SAH value, possibly as a result of greater tissue methionine synthase activity after treatment. We previously showed that cobalamin-deficient subjects with megaloblastic anemia also had decreases in serum SAH with cobalamin treatment (23). Thus, it appears from our data that the serum SAH concentration is mainly influenced by renal status, but that an effect of cobalamin status is also seen, which is easier to appreciate when renal status is normal. These findings may affect the interpretation of studies of the role of SAH abnormalities in vascular disease, because impaired renal status is extremely common in such populations and is associated with such cardiovascular disease risk factors as hypertension, diabetes, male sex, and age. One can expect that the plasma SAH will be high in such persons with those traits or conditions (15–20).

Impaired methionine synthesis, and therefore deficient synthesis of SAM with a decrease in transmethylation reactions, is a potential underlying cause of pathophysiologic conditions in cobalamin or folate deficiency. However, we previously showed that even severely cobalamin-deficient subjects rarely have low serum methionine (34), and we did not find that SAM was low in subjects with severe cobalamin deficiency anemia (23). The current investigation showed similar findings, in that the SAM was significantly higher in those subjects with elevated MMA than in those without elevated MMA, and no change was seen in SAM after treatment with 1000 μg cobalamin/d. The elevated SAM in the high-dose group may have been due to the impaired renal status of many of those with elevated MMA. Multivitamin use did not affect SAM:SAH or the SAM concentration. However, in multivariate analysis, the serum SAM concentration was modestly affected by folate. In addition, the African American subjects had lower SAM than did the white subjects, but, because the African Americans also had lower serum folate values, this relation may not be completely independent. SAM:SAH was not affected by cobalamin treatment, although it trended lower in subjects with elevated MMA at baseline.

Hyperhomocysteinemia may be a risk factor for cognitive defects and even for the future diagnosis of Alzheimer disease (6, 7). We also previously showed that depression (35) and cognition (24, 31) are related to cobalamin status. We used a screening instrument to test cognition in the current study and found that age, MMA, and race influenced the score, but tHcy, SAM, and SAH did not. Therefore, our data do not provide any documentation of the concept that abnormal methylation ratios play a role in cognitive disorders in elderly—a finding that is similar to that of a recent report of spinal fluid, SAM, and SAH values in patients with dementia (13). Tissue SAM and SAH values may be more revealing but usually will not be available. We also did not find an improvement in cognition score after treatment with high-dose cobalamin, despite the marked lowering of MMA and tHcy in those subjects. However, the measure for cognition reported here may not be sensitive enough to allow detection of changes (31).

Post vivo changes in SAH and SAM in both unseparated and separated blood are significant and seem to be preventable with proper handling. We attempted to overcome these difficulties by placing the phlebotomized blood on ice and preparing serum under cold conditions, followed by continuous freezing of samples until they were assayed. Despite these precautions, some SAM values were uninterpretable because of poor chromatography peaks (*see* Subjects and Methods). It is possible that artifactual increases in SAH due to less-than-optimal sample handling could explain some of the SAH elevations we saw in this cohort of elderly. If that were the case, however, we would not expect the significant associations seen with other variables (that are not affected by sample handling) or the significant decrease in SAH after cobalamin treatment. Future epidemiologic studies must address these concerns.

In conclusion, we found that the most significant determinants of serum SAH in a cohort of elderly are the serum SAM and creatinine concentrations. Yet, some influence is also due to poor cobalamin status because high-dose cobalamin tablets in subjects both with and without elevated creatinine lowered SAH significantly but much less than it lowered elevated MMA or tHcy. It seems likely that serum SAM and SAH will be less useful than the other cobalamin-dependent metabolites in the diagnosis of deficiency and in correlations with clinical syndromes.

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MAJ and ETD were responsible for the design and performance of the clinical aspects of the study; SS and RA were responsible for the development and performance of blood assay procedures; all authors contributed to the writing and editing of the manuscript. Two of the authors (SPS and RHA) and the University of Colorado hold patents on the use of assays for total homocysteine and other metabolites to diagnose vitamin B-12 and folate deficiencies, and a company has been formed at the University of Colorado to perform such assays. The other authors had no personal or financial conflict of interest.

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