

Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine¹⁻³

David M Kerins, Mark J Koury, Antonieta Capdevila, Sarvadaman Rana, and Conrad Wagner

ABSTRACT

Background: Although plasma total homocysteine has been identified as an independent risk factor for vascular disease in a multitude of studies, there is a considerable overlap in values between patients at risk and control subjects. The difference in values can be used to distinguish statistically between the 2 groups, provided each group is large enough; however, discriminating between individual patients at risk and control subjects is difficult.

Objective: We investigated whether the precursor of homocysteine, S-adenosylhomocysteine, is a more sensitive indicator of risk.

Design: We measured plasma total homocysteine, S-adenosylhomocysteine, S-adenosylmethionine, creatinine, folate, and vitamin B-12 in 30 patients with proven cardiovascular disease and 29 age- and sex-matched control subjects.

Results: The homocysteine values (\pm SD) were 12.8 ± 4.9 (95% CI: 11.0, 14.7) μ mol/L for patients and 11.0 ± 3.2 (9.8, 12.2) μ mol/L for control subjects. The S-adenosylhomocysteine values were 40.0 ± 20.6 (32.3, 47.7) nmol/L for patients and 27.0 ± 6.7 (24.5, 30.0) nmol/L for control subjects ($P = 0.0021$). The S-adenosylmethionine values were 121.8 ± 42.9 (105.8, 137.8) nmol/L for patients and 103.9 ± 21.8 (95.6, 112.2) nmol/L for control subjects ($P = 0.0493$). The creatinine values were 110 ± 27 (97, 120) μ mol/L for patients and 97 ± 9 (80, 100) μ mol/L for control subjects ($P = 0.0025$). Values for folate and vitamin B-12 did not differ significantly between groups.

Conclusions: Plasma S-adenosylhomocysteine appears to be a much more sensitive indicator of the difference between patients with cardiovascular disease and control subjects than is homocysteine. Both plasma total homocysteine and S-adenosylhomocysteine are significantly correlated with plasma creatinine in patients. *Am J Clin Nutr* 2001;74:723-9.

KEY WORDS Homocysteine, S-adenosylhomocysteine, cardiovascular disease, plasma, S-adenosylmethionine, folate, vitamin B-12

INTRODUCTION

Elevated plasma total homocysteine (tHcy) was identified as an independent risk factor for vascular disease (1). The association between abnormal homocysteine metabolism and cardiovascular disease was first reported by Wilcken and Wilcken in 1976 (2). Since then, as pointed out by Ueland et al (3), there have

been >120 articles reporting on case-control studies, almost all of which support the role of homocysteine as a risk factor for vascular disease. The relation between hyperhomocysteinemia and vascular disease is somewhat controversial, however. One group (4) suggested that elevated plasma tHcy is an effect, rather than a cause, of atherosclerotic disease. They propose that an early decline in renal function, which is common in atherosclerosis, causes the elevated plasma tHcy. A second group (3) argued that hyperhomocysteinemia is truly a proximate risk factor that provokes the acute event and strongly interacts with traditional risk factors such as hypertension and smoking. Scott (5) rejected the idea that hyperhomocysteinemia is an effect of early renal disease and suggested that, if plasma tHcy is only a marker for vascular disease, hyperhomocysteinemia may reflect changes in the intracellular concentration of homocysteine or S-adenosylhomocysteine (SAH). In any event, the use of plasma tHcy as an indication of risk of vascular disease is widespread. The use of these homocysteine measurements, however, as a prognostic indicator of an individual's risk of developing vascular disease is not possible unless the elevation in homocysteine concentration is quite marked. This limitation results from the overlap in values for patients with vascular disease and control subjects; this overlap is so great that many individuals are needed for the differences between the 2 groups to be significant.

Plasma tHcy was also shown to be correlated with serum creatinine, which is an indicator of renal function (6). The increased concentrations of plasma homocysteine observed with age were also attributed to the decline in kidney function that occurs with age (7, 8).

SAH is the metabolic precursor of all homocysteine produced in the body. It is formed as a product of methyltransferase reactions involving S-adenosylmethionine (SAM) as the methyl

¹From the Departments of Biochemistry and Medicine, Vanderbilt University School of Medicine, Nashville, TN, and the Department of Veterans Affairs Medical Center, Nashville, TN.

²Supported in part by US Public Health Service grants DK15289 and DK54859 (to CW) and CA82986 (to MJK) and by Merit Review awards (to CW and MJK) from the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

³Address reprint requests to C Wagner, Department of Biochemistry, Vanderbilt University School of Medicine, 620 Light Hall, Nashville, TN 37232-0146. E-mail: conrad.wagner@vanderbilt.edu.

Received October 11, 2000.

Accepted for publication January 31, 2001.

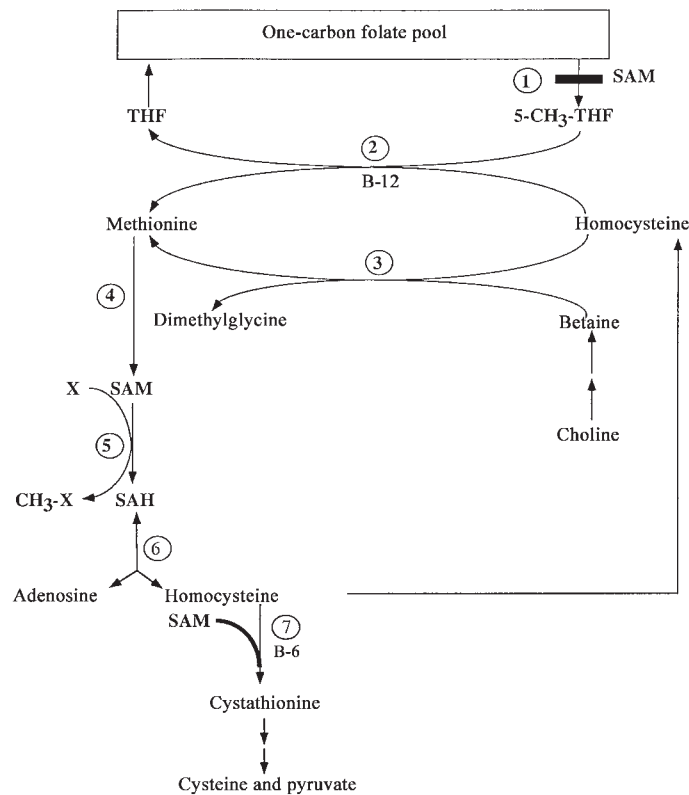


FIGURE 1. Folates and methyl group metabolism. The circled numbers refer to the following enzymes: 1, methylenetetrahydrofolate (THF) reductase; 2, 5-methyltetrahydrofolate–homocysteine *S*-methyltransferase; 3, betaine–homocysteine *S*-methyltransferase; 4, methionine adenosyltransferase; 5, a variety of methyltransferase reactions; 6, adenosylhomocysteinase; 7, cystathionine- β -synthase. The heavy bar in reaction 1 denotes allosteric inhibition by *S*-adenosylmethionine (SAM). The heavy arrow in reaction 7 denotes activation by SAM. SAH, *S*-adenosylhomocysteine. Adapted from reference 9.

donor. As shown in **Figure 1**, reaction 5 refers to a large number of individual enzymes. They carry out reactions in which the methyl group of SAM is transferred to an acceptor identified as X, with the formation of methyl-X and SAH. These reactions include the methylation of small molecules, such as the formation of epinephrine, phosphatidylcholine, and creatine, as well as the methylation of macromolecules including proteins, RNA, and DNA. All of these methylations have profound physiologic consequences. SAH is a potent product inhibitor of the methyltransferases shown in reaction 5 (10), and it has been suggested that the effect of homocysteine on vascular endothelial function is due to the production of SAH from homocysteine (11) as a result of the action of the enzyme adenosylhomocysteinase, or SAH hydrolase (Figure 1, reaction 6).

For these reasons it seemed important to measure plasma SAH to determine whether it might be more informative than plasma tHcy regarding the risk of vascular disease. We now report the results of a study in which we measured homocysteine, SAH, SAM, and creatinine in the plasma of a group of patients with proven cardiovascular disease and in that of a group of control subjects who had no clinical incidence of cardiovascular disease.

SUBJECTS AND METHODS

Subjects

The study was approved by the Institutional Review Boards of the Department of Medical Affairs Medical Center, Nashville,

TN, and Vanderbilt University. Thirty patients with cardiovascular disease were recruited from the patients and staff of the Veterans Affairs Medical Center, Nashville. Most of the patients were male (28 male, 2 female). Fifteen patients had sustained a prior myocardial infarction. All infarcts were >1 y old. All patients had documented coronary artery disease, with the performance of a prior coronary artery bypass graft procedure in 16 patients and a percutaneous coronary intervention in 5 patients. As expected for a cohort of patients with known coronary artery disease, all patients received either aspirin ($n = 26$) or warfarin ($n = 4$). Twenty-four patients were treated with a β -hydroxy- β -methylglutaryl coenzyme A reductase inhibitor, 14 with an angiotensin-converting enzyme inhibitor, and 13 with a β -adrenergic blocker. The control subjects were drawn from the faculty and staff of Vanderbilt University School of Medicine and from the staff of the Veterans Affairs Medical Center. They had no clinical manifestations of coronary artery disease and had not undergone coronary arteriography. A few patients and control subjects were taking multivitamin supplements.

Analytic methods

Nonfasting blood was collected in EDTA-coated tubes. Within 1 h of collection, plasma was separated by centrifugation at $400 \times g$ for 10 min at 23°C . There is no increase in homocysteine resulting from leakage from erythrocytes during this period (12). Plasma tHcy was measured by the Abbott IMx fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL), which has been shown to be equivalent to the

TABLE 1Characteristics and values of the study populations¹

	Patients (n = 28 M, 2 F)	Control subjects (n = 27 M, 2 F)
Age (y)	64.4 ± 8.4 (43–77)	61.2 ± 7.5 (49–75)
Homocysteine (μmol/L)	12.8 ± 4.9 (6–27)	11.0 ± 3.2 (7–20)
S-Adenosylhomocysteine (nmol/L)	40.0 ± 20.6 (12–92)	27.0 ± 6.7 (15–45) ²
S-Adenosylmethionine (nmol/L)	121.8 ± 42.9 (58–241)	103.9 ± 21.8 (71–166) ³
Folate (nmol/L)	34.9 ± 14.1 (15.9–95.2)	32.4 ± 11.1 (11.3–77.1)
Vitamin B-12 (pmol/L)	292 ± 122 (76.8–543)	310 ± 148 (117–778)
Creatinine (μmol/L) ⁴	110 ± 27 (62–119)	97 ± 9 (62–115) ²

¹ $\bar{x} \pm SD$; range in parentheses.^{2,3}Significantly different from patients: ² $P < 0.01$, ³ $P < 0.05$.⁴Values for only 29 patients and 27 control subjects.

traditional HPLC assay for homocysteine (13). Plasma SAM and SAH were measured by a fluorescence HPLC method (14). Plasma vitamin B-12 was measured by the Abbott IMx microparticle intrinsic factor assay, and plasma folate was measured by the Abbott IMx ion capture method with use of folate binding protein. Creatinine was measured by the Jaffe rate method as carried out with use of the Beckman Coulter Synchron system (Beckman Coulter, Brea, CA). The statistical program SUPERANOVA (version 1.11; Abacus Concepts, Inc, Berkeley, CA) was used to perform statistical analysis. Patient and control groups were compared by using a one-factor ANOVA, and significance was determined by Fisher's protected least-significant-difference method. Differences were considered significant at $P < 0.05$.

RESULTS

A comparison of clinical and laboratory findings between the group of patients with cardiovascular disease and the matched control subjects is shown in **Table 1**. Although the mean age of the patient group was 3.2 y higher than that of the control group, this difference was not significant. With respect to the measured indexes, SAH and creatinine concentrations were different between the 2 groups, and these differences were highly significant. There also was a significant difference in the SAM values. There was no significant difference between the homocysteine, folate, and vitamin B-12 concentrations of the patients and control subjects. Plasma tHcy concentrations correlated significantly with plasma SAH concentrations (**Figure 2**). The correlation was stronger in the patients, probably because the range of values for the control subjects was much narrower.

Because the patients were taking a variety of medications for their disease, we evaluated the effects of those drugs that might be expected to affect kidney function—angiotensin-converting enzyme inhibitors, β -adrenergic blockers, and diuretics—on the concentrations of creatinine, homocysteine, and SAH. We found no significant effect of medication (**Table 2**).

The distribution of plasma tHcy and SAH concentrations in the 2 groups is compared directly in **Figure 3**. The tHcy values for the patients (95% CI: 11.0, 14.7 μmol/L) and the control subjects (95% CI: 9.8, 12.2 μmol/L) showed considerable overlap; only 3 patient values fell outside of the range for the control subjects. The SAH values were more widely scattered, and 9 patient values fell outside of the range for the control subjects. The 95% CIs for SAH did not overlap between the patients (32.3, 47.7 nmol/L) and control subjects (24.9, 30.7 nmol/L).

Almost every study has shown a highly significant correlation between the concentrations of homocysteine and creatinine (4). We found this also. As shown in **Figure 4A**, there was a strong correlation between patient plasma creatinine and both plasma tHcy and SAH. In fact, the correlation was stronger with respect to SAH. The weaker correlation in the control group (**Figure 4B**) was probably because all the control subjects had normal creatinine concentrations and presumably normal kidney function.

DISCUSSION

The evidence for an association between elevated plasma tHcy and an increased risk of vascular disease comes from numerous retrospective case-control studies. A meta-analysis published in 1995 evaluated all available published studies and concluded that hyperhomocysteinemia is an independent risk factor for vascular disease (15). A subsequent critical review of the epidemiologic evidence found that the association between homocysteine and vascular disease was strong and biologically plausible (16). The data from prospective studies are weaker and do not necessarily indicate a causal relation (16). In the Physician's Health Study (17) involving 14916 subjects, 271 men who subsequently developed a myocardial infarction were compared with an equal number of matched control subjects. The mean homocysteine concentration of the subjects who developed a myocardial infarction was 11.4 ± 4.0 μmol/L compared with 10.5 ± 2.8 μmol/L for the control group ($P = 0.03$). It was concluded that moderately high concentrations of plasma tHcy are associated with a risk of vascular disease, independent of other risk factors. In a prospective study relating the risk of stroke in middle-aged men to plasma homocysteine, the mean values for tHcy were 13.7 μmol/L (95% CI: 12.7, 14.8 μmol/L) for case subjects and 11.9 μmol/L (95% CI: 11.3, 12.6 μmol/L) for control subjects ($P = 0.004$) (18). This study included 107 case subjects and 114 control subjects. In another prospective study involving 122 case subjects and 478 control subjects, the mean tHcy concentration for the case subjects was 12.7 ± 4.7 μmol/L compared with 11.3 ± 3.7 μmol/L for the control subjects ($P = 0.002$) (19). Note that the values for homocysteine in that study are almost exactly the same as those we obtained in the present study. The difference in homocysteine values between the patients and control subjects in our small sample was not significant, but the difference in plasma SAH values was. Examination of the frequency distribution of values in these larger, earlier studies shows a major overlap in the data, with only a

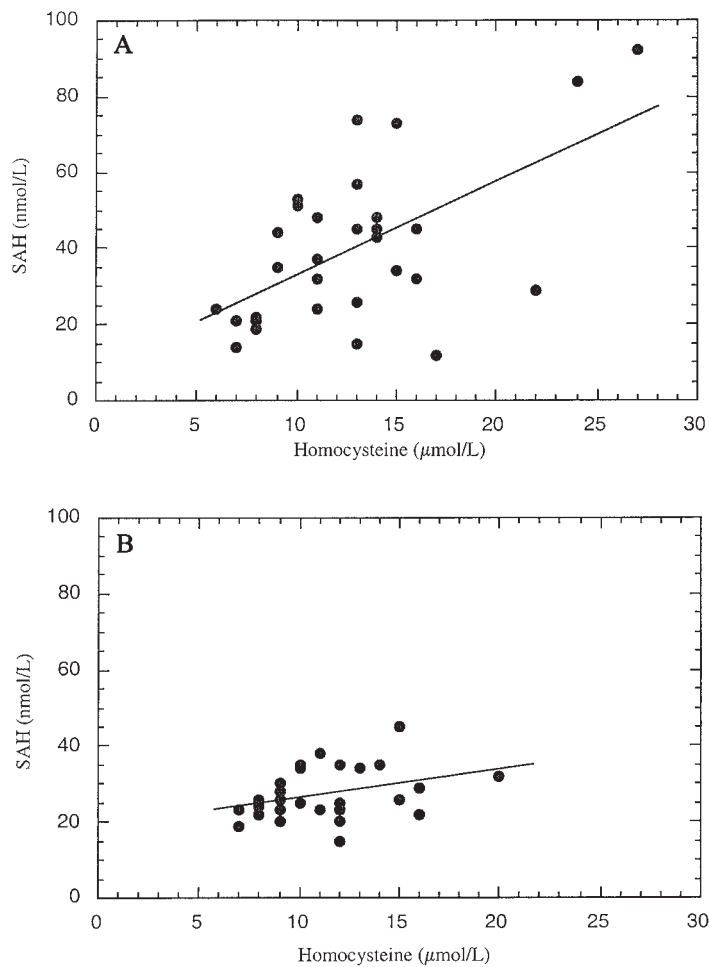


FIGURE 2. Correlation between plasma homocysteine and plasma S-adenosylhomocysteine (SAH) in patients (A; $r = 0.58$, $P = 0.0008$, $n = 30$) and control subjects (B; $r = 0.37$, $P = 0.049$, $n = 29$).

slight upward shift in the values for the case subjects. In these studies it was impossible to distinguish individuals who were clearly at risk from those who were not.

Our data show, for the first time, that plasma SAH is a more sensitive marker of clinical cardiovascular disease than is plasma tHcy. Only a few studies have reported human plasma SAH values. Fowler's group described the appearance of SAH in the plasma after an oral load of SAM (20) and after an oral load of methionine (21). They also showed that patients with end-stage renal disease had higher plasma SAH and SAM, as well as homocysteine, than did healthy control subjects (22). We believe that plasma SAH concentrations reflect tissue SAH concentrations. There are no studies describing how SAH exits cells, and

we believe that plasma SAH simply reflects tissue leakage. The correlation between plasma tHcy and SAH shown in Figure 2 was also shown recently by Yi et al (23). The significance of this observation relates to the hypothesis that SAH may be a key component in the pathophysiology of the homocysteine–vascular disease axis. Although no generally accepted hypothesis directly links homocysteine to damage in the vascular endothelium, several studies showed that increased tissue SAH concentrations have profound physiologic consequences. Elevated SAH enhanced killing of L929-APO-1 (FAS) cells by anti-APO-1 (24). In several tumor cell lines, elevated SAH inhibited isoprenylated cysteine carboxylation and enhanced tumor necrosis factor α cytotoxicity (25). In human breast carcinoma MCF7

TABLE 2

Effect of medication on patient values¹

	ACE inhibitors		β -Blockers		Diuretics	
	Yes ($n = 14$)	No ($n = 16$)	Yes ($n = 13$)	No ($n = 17$)	Yes ($n = 6$)	No ($n = 24$)
Creatinine ($\mu\text{mol/L}$) ²	120 \pm 27	110 \pm 27 [15]	110 \pm 18 [12]	120 \pm 35	120 \pm 18	110 \pm 27 [23]
Homocysteine ($\mu\text{mol/L}$)	14.0 \pm 5.5	11.8 \pm 4.3	11.6 \pm 3.3	13.8 \pm 5.8	14.7 \pm 4.1	12.4 \pm 5.1
S-Adenosylhomocysteine (nmol/L)	45.6 \pm 18.0	35.0 \pm 17.5	36.4 \pm 16.1	42.6 \pm 23.6	46.5 \pm 16.1	38.3 \pm 21.5

¹ $\bar{x} \pm \text{SEM}$. ACE, angiotensin-converting enzyme. There were no significant effects of medication.

²Creatinine data were unavailable for one patient. n is given in brackets.

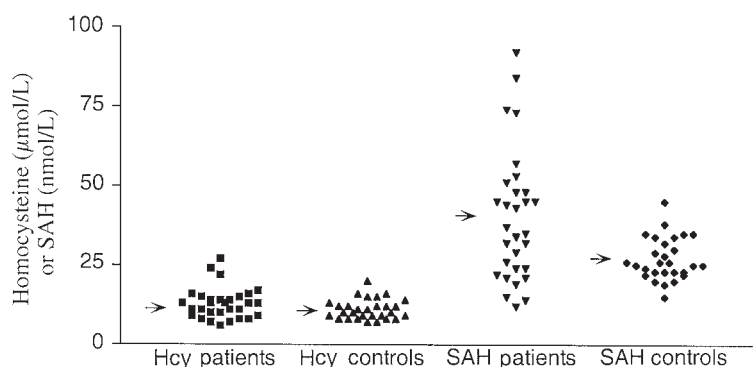


FIGURE 3. Distribution of values for plasma homocysteine and plasma *S*-adenosylhomocysteine (SAH) in patients ($n = 30$) and control subjects ($n = 29$). Arrows indicate the mean values.

cells, elevated SAH concentrations potentiated tumor necrosis factor α -induced cytotoxicity by a ceramide-independent mechanism (26). In pancreatic acinar cells, elevated SAH inhibited cholecystokinin-mediated amylase secretion (27), and an elevation of SAH in the brain causes an imbalance in the ratio of SAM

to SAH and is believed to be the cause of the neuropathy in vitamin B-12 deficiency (28).

SAH is the metabolic precursor of homocysteine in a reaction catalyzed by SAH hydrolase (Figure 1, enzyme 6). The equilibrium of this reaction favors the formation of SAH from

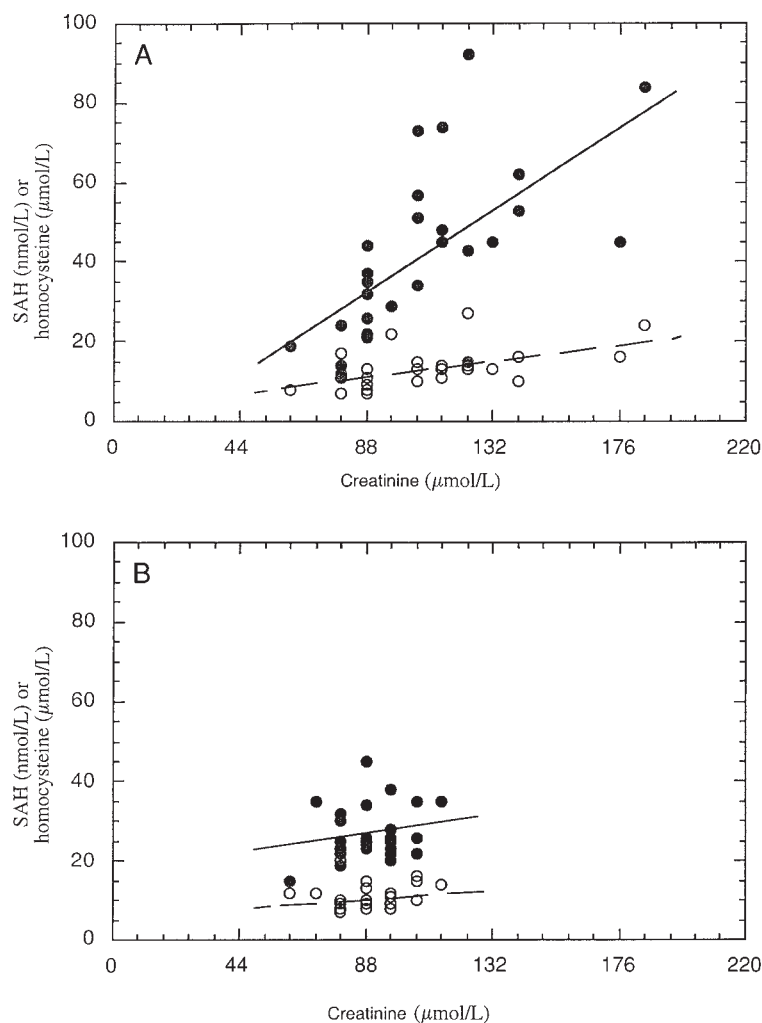



FIGURE 4. Correlation between plasma creatinine and plasma homocysteine (○) or plasma *S*-adenosylhomocysteine (SAH; ●) in patients (A; homocysteine: $r = 0.63$, $P = 0.0021$; SAH: $r = 0.55$, $P = 0.0007$; $n = 30$) and control subjects (B; homocysteine: $r = 0.20$; SAH: $r = 0.20$; $n = 29$).

homocysteine. In tissues, the reaction proceeds in the direction of homocysteine formation because adenosine, the other product of the reaction, is removed by the action of adenosine deaminase. In this regard, it has been shown that as little as 10 μmol homocysteine/L, but not cysteine, markedly increases the intracellular SAH concentration, decreases the methylation of the carboxy-terminal cysteine of p21^{ras}, and inhibits the growth of vascular endothelial cells (29). Defects in the enzymes that utilize homocysteine or deficiencies of the vitamins involved in their catalytic action will cause homocysteine concentrations to increase, and SAH concentrations presumably increase as a result of the reversal of SAH hydrolase. These metabolic defects are associated with homocystinuria or hyperhomocysteinemia and vascular disease (30).

The evidence that hyperhomocysteinemia causes vascular disease is indirect. It has been proposed (31) that it involves endothelial dysfunction followed by platelet activation and thrombus formation. Most of these studies used concentrations of homocysteine that were far greater than those normally found in plasma. Less than 1% of tHcy in human plasma is present as free homocysteine. About 30% is present as disulfides with homocysteine or cysteine, while the largest amount by far ($\approx 70\%$) is bound to albumin via a disulfide bond (32). There is no information about whether SAH in plasma is free or protein bound. SAH has no free sulfhydryl group, however, so it is unable to form adducts in the same way as homocysteine.

Circulating creatinine is a common indicator of kidney function, and renal function is a strong determinant of the concentration of plasma tHcy (33). The metabolic reasons for this are not clearly understood (34). Therefore, it is noteworthy that both plasma SAH and creatinine are significantly different between the patients and the control groups whereas plasma total homocysteine is not. Those factors that may elevate plasma homocysteine as a result of diminished kidney function appear to have a much greater effect on SAH than on homocysteine.

Note that the concentrations of homocysteine in plasma are in micromoles per liter whereas the concentrations of SAM and SAH are in nanomoles per liter. This may be why the changes in SAH are proportionately greater than the changes in homocysteine. Conditions (eg, diminished kidney function) that result in higher concentrations of homocysteine will have a smaller effect on the homocysteine concentration than on the SAH concentration because the plasma pool of homocysteine is ≈ 500 times as great as the pool of SAH, and changes in this much smaller SAH pool are more easily detected. The increase in SAM concentrations is significant. SAH is a known inhibitor of methyltransferase reactions. The elevation in the SAH concentrations could thus cause SAM to accumulate secondarily. If so, then one would not expect the increase in SAM concentrations to be as marked as the increase in SAH concentrations. We suggest that further studies are needed to strengthen the conclusion that measurement of plasma SAH may be more discriminating than measurement of plasma tHcy in studies of vascular disease. 

We are grateful to Barbara Roberts and Amanda Hodges for specimen collection.

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