

## S-Adenosylhomocysteine—a better indicator of vascular disease than homocysteine?<sup>1–3</sup>

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### ABSTRACT

It is widely accepted that elevated plasma total homocysteine is an independent risk factor for vascular disease. The relation is believed to be causal, but there is no generally accepted mechanism for the pathophysiology involved. The metabolic precursor of homocysteine in all tissues is *S*-adenosylhomocysteine (AdoHcy). AdoHcy is present in normal human plasma at concentrations approximately 1-500th of those of homocysteine, a fact that presents difficulties in measurement. The requirement for specialized equipment, complicated time-consuming methodology, or both is a reason that measurement of plasma AdoHcy has not generally been carried out in large studies. A recently published rapid immunoassay for AdoHcy in human plasma should make measurement of this important metabolite available for general use. Advantages of the measurement of plasma AdoHcy include 1) a smaller overlap of values between control subjects and patients, and thus the possibility of observing significant differences in fewer samples, 2) an accepted mechanism of metabolic activity as an inhibitor of all *S*-adenosylmethionine-mediated methyltransferases, and 3) evidence (from recent studies) that a higher plasma concentration of AdoHcy is a more sensitive indicator of vascular disease than is a higher plasma concentration of homocysteine. *Am J Clin Nutr* 2007;86:1581–5.

**KEY WORDS** *S*-Adenosylhomocysteine, *S*-adenosylmethionine, homocysteine, vascular disease, methionine, risk factors, plasma

### INTRODUCTION

*S*-Adenosylhomocysteine (AdoHcy) is the immediate precursor of all of the homocysteine produced in the body. The reaction is catalyzed by *S*-adenosylhomocysteine hydrolase and is reversible with the equilibrium favoring formation of AdoHcy. In vivo, the reaction is driven in the direction of homocysteine formation by the action of the enzyme adenosine deaminase, which converts the second product of the *S*-adenosylhomocysteine hydrolase reaction, adenosine, to inosine (1). Homocysteine is a branch point in the metabolism of methionine. In one direction, it can be remethylated either by the vitamin B-12–dependent enzyme system, methionine synthase, or it can accept a methyl group from betaine to regenerate methionine. In a second direction, homocysteine can be degraded by the transsulfuration pathway by conversion to cystathionine with the use of the enzyme cystathionine- $\beta$ -synthase (2).

### EVIDENCE FOR HOMOCYSTEINE'S INVOLVEMENT IN VASCULAR DISEASE

The initial study by McCully (3) showed that homocystinuria resulted in massive thromboses and generalized vascular damage. The associated elevation in plasma homocysteine accompanying the homocystinuria in such cases can range from 150 to 500  $\mu\text{mol/L}$  (normal:  $\approx 10 \mu\text{mol/L}$ ). However, it was not until Wilcken and Wilcken (4) examined patients with and without cardiovascular disease (CVD) that it was suggested that a moderate increase in plasma homocysteine was associated with vascular disease. Since that time, thousands of journal articles have been published on the relation between plasma or serum concentrations of homocysteine and vascular disease. Wilcken and Wilcken showed that  $\approx 28\%$  of patients with coronary heart disease had an abnormal methionine load test, which indicated a lower ability to metabolize methionine. An oral methionine load stresses the systems metabolizing methionine and results in a higher and more prolonged increase in plasma methionine in patients with CVD than in control subjects. An abnormal methionine load test was shown by Clarke et al (5) to be an independent risk factor for coronary, peripheral, and cerebral vascular disease. An elevation of plasma homocysteine in patients with vascular disease was also observed in those without a methionine load (6). Many subsequent studies have provided support for this conclusion. The meta-analysis of 27 studies by Boushey et al (7) concluded that there was a strong association of elevated plasma homocysteine with coronary, cerebrovascular, and peripheral vascular disease and that as much as 10% of the coronary artery disease in the United States could be attributed to high plasma concentrations of homocysteine. A survey of articles published between 1966 and 1998 analyzed results from 30 prospective or retrospective studies (8) and found a stronger association in the retrospective than in the prospective studies. The common polymorphism in the methylenetetrahydrofolate reductase gene (677C $\rightarrow$ T) found in  $\approx 10\%$  of the population is associated with

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higher plasma homocysteine concentrations in persons with below-normal folate concentrations (9). Another meta-analysis of 72 studies investigated the effect of mutations in this gene on homocysteine concentrations and vascular disease (10). The authors of the meta-analysis concluded from these genetic studies as well as from prospective studies showing a highly significant association between plasma homocysteine and a variety of vascular diseases that this association was causal, at least with respect to CVD (10).

There is also a dietary component. Many studies have provided evidence that, because folate, vitamin B-12, and vitamin B-6 are cofactors in the metabolic disposition of homocysteine, elevated plasma homocysteine is inversely correlated with plasma concentrations of these vitamins (11) (12). In the absence of vitamin B-12 deficiency, elevated plasma homocysteine concentrations are most responsive to folate supplementation and can be returned to normal (13) by that treatment.

### WHAT IS THE MECHANISM OF THE VASCULAR DAMAGE?

There is no generally accepted mechanism for the pathophysiology of elevated plasma homocysteine as a cause of vascular disease. Various mechanisms for the toxic action of homocysteine include a change in the redox status of the tissues with production of reactive oxygen species (14); an inhibition of anticoagulation mechanisms mediated by the vascular endothelium (15); antiplatelet effects related to the reaction of elevated homocysteine with nitric oxide to form *S*-nitrosomethionine (16, 17); a direct effect of homocysteine on vascular endothelial (18) or smooth muscle cells (19); the formation of homocysteine thiolactone that modifies endothelial proteins (20); and the induction of programmed death of endothelial cells (21). In most cases, these actions have been indicated by effects caused by the addition of homocysteine to cells in culture. The principal problem with most of these studies has been the use of concentrations of homocysteine far higher (50–1000  $\mu\text{mol/L}$ ) than those present in plasma to show these effects. Rarely have any effects been shown with concentrations of homocysteine as low as 10  $\mu\text{mol/L}$ . Homocysteine has a free sulfhydryl group and is oxidized with a second homocysteine molecule to form the disulfide, homocystine, and also with cysteine to form a mixed disulfide. In human plasma, most homocysteine exists in disulfide linkage to cysteine in albumin. For this reason, it has been the standard practice to measure total homocysteine (tHcy) that is produced after the reduction of the bound homocysteine. The normal concentration of tHcy in human males is  $\approx 10 \mu\text{mol/L}$ . However, as was pointed out by Jacobsen (22), the amount of free homocysteine in human plasma is  $< 1\%$  ( $< 0.1 \mu\text{mol/L}$ ). Therefore, although high plasma concentrations of homocysteine are associated with vascular disease, it has been difficult to show that they are the proximal cause of the damage.

### COULD *S*-ADENOSYLHOMOCYSTEINE BE A CAUSE OF THE VASCULAR DAMAGE?

An alternative possible cause of the pathophysiology associated with hyperhomocysteinemia is AdoHcy. This compound is the precursor of all of the homocysteine in tissues. Except for methyl transfer from betaine and from methylcobalamin in the

methionine synthase reaction, AdoHcy is the product of all methylation reactions that involve *S*-adenosylmethionine (AdoMet) as the methyl donor. There are  $\approx 50$  reactions that carry out methyl transfer in cells. AdoHcy is well known as a potent inhibitor of most, if not all, methyltransferases (23). Increased concentrations of AdoHcy in tissues are usually accompanied by decreased concentrations of AdoMet. The use of the ratio of AdoMet to AdoHcy as an indicator of the methylating capacity of the cell was first suggested by Cantoni et al (24), and this ratio has been referred to as the "methylation index" (25). However, in certain situations, the elevation of AdoHcy appears to be a better indication of the inhibition of methylation than does the ratio of AdoMet to AdoHcy (26, 27). Methylation is significant in epigenetic regulation of protein expression via DNA and histone methylation. The inhibition of these AdoMet-mediated processes by AdoHcy is a proven mechanism for metabolic alteration. Because the conversion of AdoHcy to homocysteine is reversible, with the equilibrium favoring the formation of AdoHcy, increases in plasma homocysteine are accompanied by an elevation of AdoHcy in most cases. Measurement of plasma AdoHcy has not been carried out in most studies, mostly because the concentration of AdoHcy in plasma is  $\approx 1/500$ th that of plasma tHcy, and complicated methods are needed to measure AdoHcy. Most of these methods are cumbersome and time-consuming, or they involve specialized equipment (28, 29).

### IS THERE ANY ADVANTAGE TO THE MEASUREMENT OF *S*-ADENOSYLHOMOCYSTEINE RATHER THAN HOMOCYSTEINE?

Relatively few studies have directly compared plasma homocysteine and plasma AdoHcy as indicators of vascular disease, probably because of the complex methods involved in the measurement of plasma AdoHcy in large studies. Several small studies have shown that measurement of plasma AdoHcy is a better indicator of the risk of vascular disease than is measurement of plasma tHcy. Loehrer et al (30) first showed that, when compared with control subjects, patients with end-stage renal disease had 44-fold greater plasma AdoHcy but only 5-fold greater plasma homocysteine concentrations. Both measurements were significantly ( $P < 0.001$ ) different, but the authors drew no conclusions about which measurement was more sensitive. In a study published in 2001 comparing patients with proven CVD and matched controls, there was a significant difference in the plasma AdoHcy concentrations between the patients and controls but no significant difference in the homocysteine concentrations (31). This inability to discriminate between patients with CVD and controls by using homocysteine concentrations was probably due to the small numbers of patients ( $n = 30$ ) and control subjects ( $n = 29$ ) in the study. This insensitivity illustrates one of the major problems of using plasma homocysteine as an indicator of the risk of vascular disease. Because there is a large overlap in values between patients and control subjects, large numbers of subjects are needed to show a relation between high homocysteine concentrations and vascular disease. This makes it impossible to predict that any one person with moderately elevated plasma homocysteine is at greater risk than any other person with the same plasma homocysteine concentration. An association of high homocysteine concentrations with low renal function has been noted many times. There is evidence for significant metabolism of methionine by the kidney (32). Plasma homocysteine is

highly elevated in patients with renal disease—to concentrations that are generally higher than those in patients with CVD. Contrary to its effect in other patients with hyperhomocysteinemia, supplementation with folic acid in those with renal disease lowers, but does not normalize, plasma homocysteine (33, 34). A study comparing adult renal disease patients with control subjects showed that plasma AdoHcy was a significantly more sensitive test of renal insufficiency than was homocysteine (35). In the studies comparing plasma homocysteine and plasma AdoHcy in patients who were selected because they had renal disease (35) and in patients who were selected because they had CVD (31), the values for plasma AdoHcy in both patients and control subjects overlapped much less than those for homocysteine. Two studies have noted that both plasma AdoHcy and homocysteine are elevated in patients with kidney disease (30, 35). Adults with kidney disease generally are older and have other diseases that are known to be associated with elevated plasma homocysteine (eg, hypertension, diabetes, and CVD), which makes it difficult to determine the primary reason for the elevated homocysteine. To determine whether decreased renal function was the reason for the elevated homocysteine, a group of children who had only renal disease were studied (36). In that group of patients, there was no statistical correlation between glomerular filtration rate and plasma tHcy, but there was a strong correlation with plasma AdoHcy. The study suggested that a reduction in the ability to metabolize or excrete AdoHcy (or both) is a primary event in renal disease. This change is probably a function of the fact that AdoHcy is readily excreted in the urine (37), whereas homocysteine is not (38). A significant correlation between elevated plasma homocysteine and serum creatinine has been noted in many previous studies of the association of homocysteine with CVD (39), which raises the question of whether decreased renal function and kidney disease due to the involvement of renal vessels with the vascular component of CVD may have been the underlying reason for the elevated plasma homocysteine in some of those earlier studies.

Although it may be expected that plasma AdoHcy and homocysteine values would tend to change in the same way, that is not always the case, as shown above for the children with renal disease only (36). In a particularly revealing study, Becker et al (40) showed that, in contrast to the plasma homocysteine concentration, the plasma AdoHcy concentration was not associated with folate concentrations. As pointed out by Becker et al, if AdoHcy is the actual toxic agent rather than homocysteine, then the use of folic acid supplementation to reduce plasma homocysteine concentrations will do nothing to reduce the incidence of vascular disease. This possibility is noteworthy in view of recent epidemiologic studies showing that folate supplementation did not reduce the risk of vascular disease, although plasma homocysteine was reduced (41, 42). It should be noted, however, that these studies were secondary prevention trials and that any effect of folate in reducing risk may have taken place at a time before supplementation was begun. Whether reduction of plasma homocysteine concentrations by B vitamin supplementation can reduce the incidence of vascular disease is under investigation in current clinical trials (41, 42). In a review of several large clinical trials, Clarke et al (43) carried out a meta-analysis of 4 trials that have been completed. It was concluded that there were no beneficial effects of B vitamin supplementation on either coronary

heart disease or stroke. An additional 8 large studies are underway; together, these trials may have the statistical power to answer this question (43).

#### MEASUREMENT OF PLASMA HOMOCYSTEINE AND S-ADENOSYLHOMOCYSTEINE

Many methods for the measurement of homocysteine in human plasma have been published. Because homocysteine contains a free thiol group and because it can form disulfide linkages with another molecule of homocysteine, with free cysteine, or with cysteine residues in proteins, only a small amount of the tHcy in plasma is free. Jacobsen has estimated that <1% is the free thiol (22). For this reason, plasma or serum must first be treated with a reducing agent to obtain the total amount of homocysteine. The normal concentration of tHcy is  $\approx 10 \mu\text{mol/L}$ , and analytic methods usually involve a reduction step that is followed first by derivatization to a form more easily detected and then by separation with the use of HPLC (39). An immunoassay was developed to detect the homocysteine in plasma after reduction (44). Measurement of AdoHcy in plasma presents a greater challenge, because its concentrations are  $\approx 1/500$ th of those of homocysteine in normal plasma— $\approx 20 \text{ nmol/L}$ . Indeed, the existence of AdoHcy in plasma was unexpected until Lohrer et al (45) devised the first sensitive method for its measurement. This method depended on the formation of the fluorescent 1,*N*<sup>6</sup>-etheno derivative of the adenosine portion of AdoHcy and then on separation by HPLC. This method measured AdoMet as well as AdoHcy in plasma; the reaction took a long time, although the results for measurement of AdoHcy compared favorably with those of other methods. More recently, Castro et al (46) were able to shorten the derivatization time from 8 to 4 h, and they could detect as little as 2.5 nmol AdoHcy/L in plasma. Their method uses a single HPLC column but requires the use of 1.0 mL plasma, an amount that may be difficult to obtain from small children. A method for measuring AdoHcy (and AdoMet) by using a very sensitive reaction with naphthalene dicarboxaldehyde to produce a fluorescent derivative was developed, but it too was cumbersome, requiring 2 HPLC separations in addition to the derivatization step (47).

Several other methods have used highly specialized equipment such as tandem mass spectrometry (29, 37, 48) and coulometric electrochemical detection (28) to obtain greater sensitivity. In hindsight, it would seem useful to have measured plasma AdoHcy as well as homocysteine and the relation of AdoHcy concentrations in response to folate and other B vitamins. The reason for not having done so is that the existing methods were not suitable for epidemiologic studies. Recently, a simple, rapid immunoassay developed for the measurement of AdoHcy in human plasma promises to be useful in such studies (49). No significant change was seen in concentrations of AdoHcy in plasma or serum samples that had been frozen at  $-80^\circ\text{C}$  and then thawed and kept for 2 h at  $4^\circ\text{C}$  (37). We have seen no change in values for plasma AdoHcy kept for  $\geq 4$  y at  $-80^\circ\text{C}$  (C Wagner, unpublished data, 2007).

With regard to the stability of AdoHcy in freshly drawn plasma, we have noted, when using a method that measures both AdoMet and AdoHcy (47), that, when fresh plasma is kept at room temperature, there is little or no loss of either AdoMet or AdoHcy for 5 h. However, when frozen plasma is thawed and kept at room temperature for 5 h, there is a rapid loss of AdoMet



but a very slight increase in AdoHcy. The amount of change varied from subject to subject. We ascribe these findings to some sort of activation of an enzyme in plasma, because it can be prevented by the addition of HgCl<sub>2</sub> (C Wagner, unpublished data, 2005). We do not know whether the changes described above are due to the conversion of AdoMet to AdoHcy by a methyltransferase that is activated in frozen plasma. We believe that such changes are unlikely if the samples are kept on ice while being thawed and before analysis. The results described by Capdevila et al (49) for normal values obtained by this immunoassay are comparable to normal values published for several other, more complicated methods. If *S*-adenosylhomocysteine hydrolase is present and active in human plasma, there is a possibility that free AdoHcy in plasma could react with plasma adenosine to change AdoHcy concentrations; however, we are unaware of any reports of such activity in human plasma.

Elevated homocysteine has been implicated as a risk factor in numerous neurologic disorders (50–52), and a recent study showed that concentrations of homocysteine, AdoHcy, and AdoMet in plasma and cerebrospinal fluid are correlated (53). It would seem useful to determine whether cerebrospinal fluid measurements of AdoHcy are more informative than those of homocysteine in neurologic disorders such as dementia and Alzheimer disease.

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