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# Relationship between *S*-adenosylmethionine, *S*-adenosylhomocysteine, asymmetric dimethylarginine, and endothelial function in healthy human subjects during experimental hyper- and hypohomocysteinemia

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**Abstract**

Experimental hyperhomocysteinemia after an oral methionine or homocysteine load is associated with impaired nitric oxide–dependent vasodilatation in healthy human beings. However, it remains unproven that this effect is mediated by elevations in plasma homocysteine. There is evidence that an increase in plasma homocysteine may increase the formation of asymmetric dimethylarginine (ADMA), an inhibitor of nitric oxide synthase. The methyl groups within ADMA are derived from the conversion of *S*-adenosylmethionine to *S*-adenosylhomocysteine intermediates in the methionine/homocysteine pathway. No previous study has assessed the role of methylation status, its impact on ADMA formation, and their association with endothelial function in healthy human beings. In a randomized, placebo-controlled, crossover study, 10 healthy subjects (mean age, 29.1 ± 3.9 years) were administered an oral dose of methionine (0.1 g/kg), L-homocysteine (0.01 g/kg), *N*-acetylcysteine (NAC) (0.1 g/kg), or placebo. Endothelial function as assessed by flow-mediated dilatation (FMD) of the brachial artery was impaired after both the methionine and homocysteine load compared with placebo at 4 hours (36 ± 15, 67 ± 23 vs 219 ± 26 $\mu$m, respectively, $P < .001$). *N*-Acetylcysteine had no effect on flow-mediated dilatation. Plasma total homocysteine was significantly elevated at 4 hours after methionine (23.1 ± 6.2) and homocysteine (41.5 ± 8.9) loading, but significantly reduced after NAC 2.4 ± 0.6 vs 7.1 ± 2.1 $\mu$mol/L in the placebo ($P < .001$). Plasma *S*-adenosylmethionine/*S*-adenosylhomocysteine ratio was significantly ($P < .001$) increased at 4 hours after methionine (10.9 ± 0.7) compared with homocysteine (5.4 ± 0.4), NAC (5.0 ± 0.3), and placebo (6.0 ± 0.5). Plasma ADMA concentrations were not altered by any intervention. Our results suggest that endothelial dysfunction due to methionine or homocysteine loading is not associated with an increase in plasma ADMA or a disruption in methylation status.


## 1. Introduction

Homocysteine, a thiol containing amino acid derived from dietary methionine, is associated with an increased risk of coronary heart disease (CHD). Plasma total homocysteine (tHcy) has been confirmed in a number of studies to hold a graded relationship with CHD risk, with no threshold level [1]. In a recent meta-analysis it has been inferred that a 3 $\mu$mol/L reduction of tHcy would be expected to reduce CHD risk by 16% [2]. However, it remains controversial as to whether the increased risk is mediated directly by homocysteine or whether it may simply be acting as a marker for another metabolite.

Acute increases in plasma tHcy after oral methionine and homocysteine loading are associated with impairment of vascular endothelial function, as assessed by flow-mediated dilatation (FMD) of the brachial artery [3-6]. It has been argued that homocysteine exerts its damaging effects on the endothelium by the generation of reactive oxygen species. This view gained support from the observation that administration of the antioxidant vitamin C prevented/

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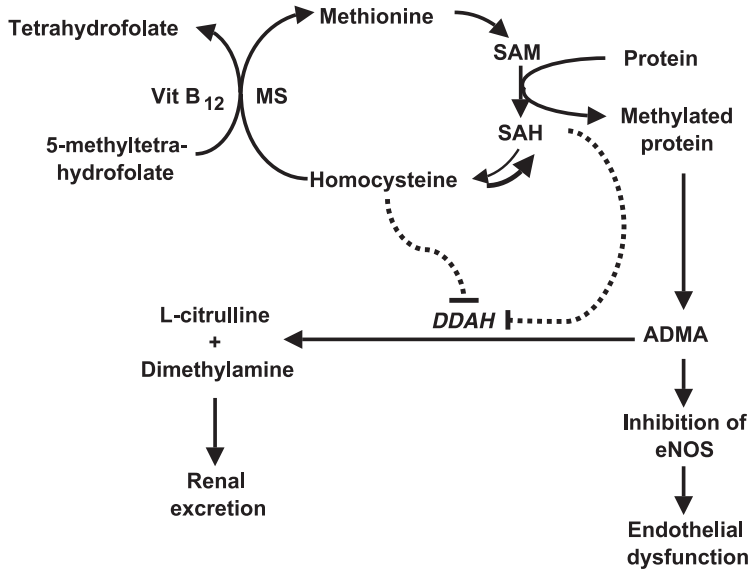


Fig. 1. Putative interrelationship between methionine/homocysteine metabolism, methylation status, ADMA formation, and endothelial function. Homocysteine is formed from methionine via the intermediates SAM and SAH. Homocysteine can be remethylated to methionine via the enzyme methionine synthase with 5-methyltetrahydrofolate and vitamin $B_{12}$ as cofactors. Asymmetric dimethylarginine is formed by the methylation of arginine residues within proteins by the enzyme protein-arginine methyltransferase. Asymmetric dimethylarginine undergoes enzymatic degradation by dimethylarginine dimethyl-aminohydrolase (DDAH). Therefore, inhibition of DDAH by homocysteine and potentially SAH may result in the increased formation of ADMA and thereby inhibition of endothelial function.

reversed endothelial dysfunction in subjects with hyperhomocysteinemia after an oral methionine load [7-9]. However, the protective role of vitamin C is most likely due to the stabilization and increased production of the endothelial nitric oxide synthase (eNOS) cofactor tetrahydrobiopterin [10,11] and not free radical scavenging. Moreover, the time course of maximal impairment of endothelial function does not reflect closely the time course of the different plasma forms of homocysteine [12], suggesting that the mechanism for this observed impairment of FMD may not be mediated by homocysteine itself.

There is evidence that acute increases in plasma tHcy after a methionine load may increase the formation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS and although some investigators have reported that methionine loading in human beings may increase ADMA formation [13,14], others have not [15]. In addition, incubation of endothelial cells in vitro with homocysteine or methionine resulted in an increase in ADMA export and a reduction in activity of eNOS and dimethylarginine-dimethylaminohydrolase, the enzyme involved in ADMA degradation [16]. Dimethylarginines result from the degradation of methylated proteins. The methyl groups contained within ADMA are derived from the *S*-adenosylmethionine (SAM) to *S*-adenosylhomocysteine (SAH) reaction [17] (Fig. 1).

We sought to investigate the relationship between vascular endothelial function and concentrations of ADMA and the methylation intermediates SAM and SAH after methionine, homocysteine, and *N*-acetylcysteine (NAC) loading studies. Methionine and homocysteine administration results in acute hyperhomocysteinemia [18,19] and NAC results in hypohomocysteinemia [20].

## 2. Materials and methods

### 2.1. Subjects

Ten healthy subjects were recruited who were free from medications and from factors associated with endothelial dysfunction, namely, hyperlipidemia (total cholesterol > 6.5 mmol/L), hypertension (blood pressure >145/85 mm Hg), diabetes mellitus, family history of premature coronary disease (age <60 years), and smoking. Volunteers were also excluded if they were taking vitamin supplements (including folic acid and other B vitamins).

Table 1
Clinical and biochemical characteristics of study subjects

| Parameters | Mean ± SD |
|---|---|
| Age (y) | 29.1 ± 3.9 |
| Male/female | 9/1 |
| Body mass index | 26.6 ± 4.9 |
| $B_{12}$ (ng/L) | 398 ± 100 |
| Folate ($\mu$g/L) | 10.4 ± 3.5 |
| Creatinine ($\mu$mol/L) | 90.2 ± 5.8 |
| Triglycerides (mmol/L) | 0.94 ± 0.47 |
| Cholesterol (mmol/L) | 4.63 ± 1.02 |
| High-density lipoprotein (mmol/L) | 1.24 ± 0.21 |
| Low-density lipoprotein (mmol/L) | 2.95 ± 0.84 |
| Systolic BP (mm Hg) | 124 ± 7 |
| Diastolic BP (mm Hg) | 70 ± 7 |