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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 μm, 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 μmol/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 μmol/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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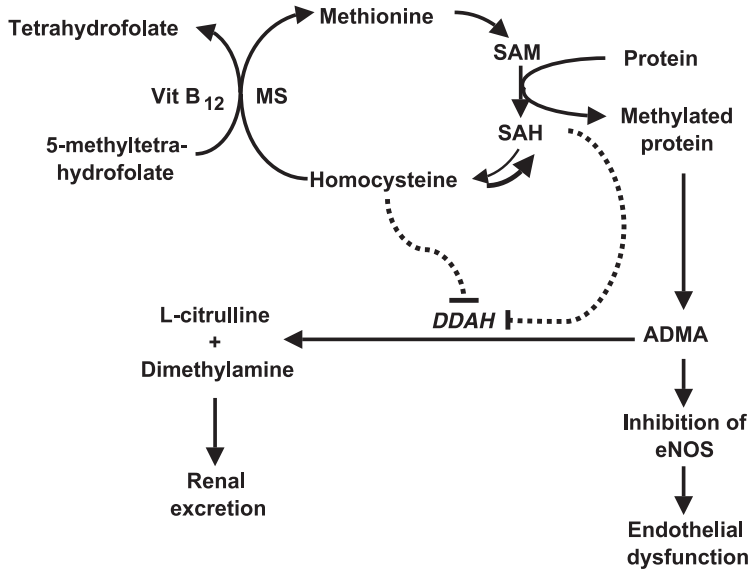

Fig. 1. Putative interrelationship between methionine/homocysteine metabolism, methylation status, ADMA formation, and endothelial function. Homocysteine is formed from methionine via the intermediates SAM and SAH. Homocysteine can be remethylated to methionine via the enzyme methionine synthase with 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 (μg/L)	10.4 ± 3.5
Creatinine (μmol/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

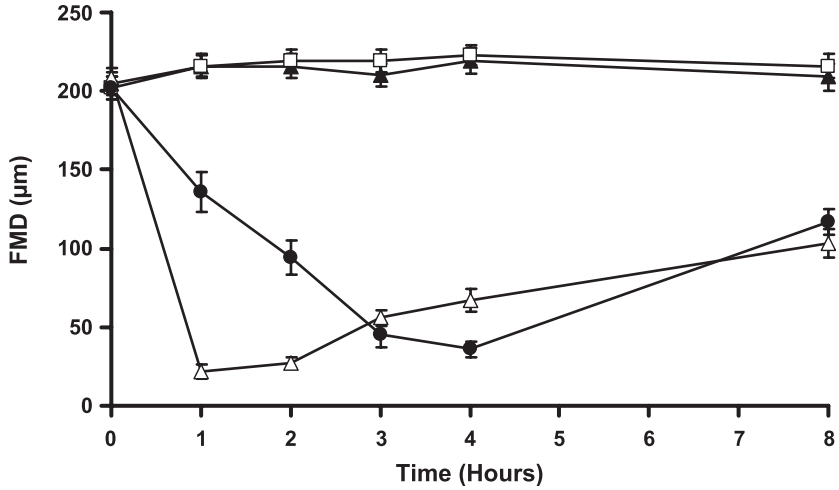

Fig. 2. Effects of methionine (●), homocysteine (△), NAC (□), and placebo (▲) on vascular endothelial function (FMD). Data are presented as mean ± SEM.

2.2. Study design

The study was a randomized, double-blind, placebo-controlled crossover design. All volunteers gave written informed consent and the protocol was approved by the Local Research Ethics Committee. After the initial screening visit, randomized subjects attended on 4 separate visits separated by at least 2 weeks. On each visit, venous blood was drawn from an indwelling cannula sited in an antecubital vein. Reduced homocysteine (0.01 g/kg), methionine (0.1 g/kg), NAC (0.1 g/kg), and placebo were administered orally in a randomized order. Endothelial function was assessed noninvasively by FMD of the brachial artery, an endothelium-dependent NO-mediated phenomenon. Endothelium-independent dilatation was assessed by measurement of brachial artery dilatation after 400 μg sublingual nitrate. Heart rate and blood pressure were measured by an automated monitor (Hewlett Packard, Berkshire, UK) applied to the contralateral arm to endothelial function assessment.

2.3. Study protocol

At each visit, venous blood was collected into vacutainers. Samples were separated by centrifugation within 30 minutes and the serum/plasma stored at −70°C until analysis. Vascular studies were performed by a single experienced operator (SD) in a temperature-controlled room (21°C-24°C) at the same time of day on subjects fasted overnight. At each visit, FMD was measured at baseline before administration of each agent and subsequently at each hour for 4 hours and again at 8 hours after the orally administered agent. Endothelium-independent dilatation was assessed after 400 μg sublingual glyceryl trinitrate (GTN) at baseline, 4, and 8 hours.

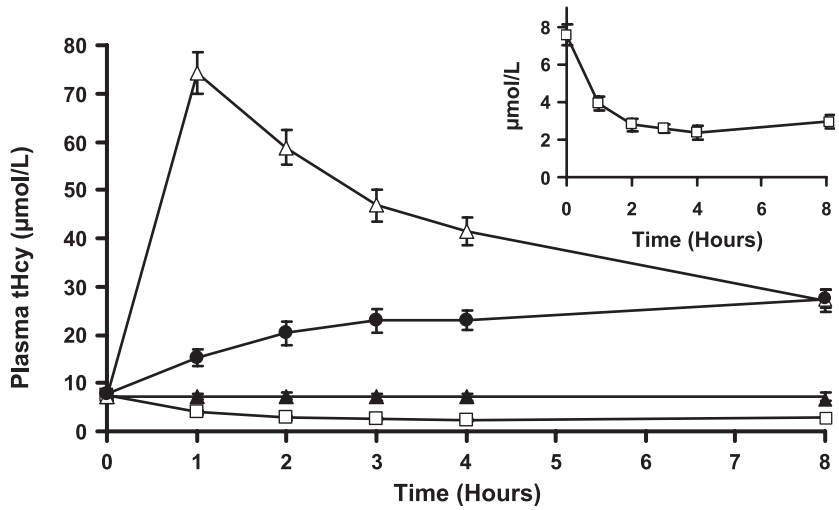

Fig. 3. Changes in plasma tHcy after methionine (●), homocysteine (△), NAC (□), and placebo (▲). Inset shows the tHcy lowering effect of NAC. Data are presented as mean ± SEM.

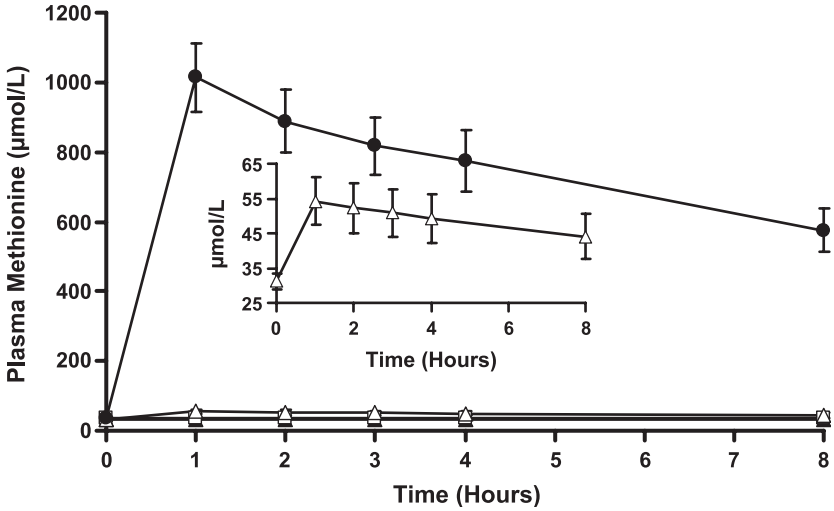

Fig. 4. Plasma concentrations of methionine after the administration of methionine (●), homocysteine (△), NAC (□) and placebo (▲). Inset shows the moderate elevation of plasma methionine after oral homocysteine. Data are presented as mean ± SEM.

2.4. Noninvasive measurement of endothelial function (FMD)

Flow-mediated dilatation was measured using high-resolution ultrasound and wall tracking, as previously described [21,22], in response to increased flow in the brachial artery induced by release of a cuff placed at the wrist inflated for 5 minutes at 250 mm Hg. Flow-mediated dilatation was taken as the greatest absolute increase in vessel end diastolic diameter (EDD) during the first 3 minutes post cuff release. Endothelium-independent dilatation in response to GTN 400 μg was measured after return of the vessel diameter to baseline and reported as the greatest absolute increase in EDD. Blood flow was calculated as the product of the Doppler time velocity integral, heart rate, and brachial artery diameter measured by wall tracking at that time.

2.5. Preparation of oral agents

Reduced L-homocysteine was prepared from L-homocysteine thiolactone (Sigma Chemicals, Poole, UK). The measured quantity of L-homocysteine thiolactone was hydrolyzed with 5 mL of 5 mol/L sodium hydroxide (Sigma Chemicals). After 5 minutes and gentle agitation the mixture was neutralized with 5 mL of 5 mol/L hydrochloric acid (Sigma Chemicals) until a pH of 4 to 5 was achieved. The freshly prepared mixture was administered within 5 minutes of preparation. *N*-Acetylcysteine was administered in the form of Parvolex (Evans Medical, Surrey, UK), an aqueous solution containing 20% wt/vol NAC. Methionine (Scientific Hospital Supplies, Liverpool, UK) in the form of powder was administered after dissolving in water. Orange-flavored cordial was added to each agent to mask the flavor and preserve blinding.

2.6. Biochemical assays

Lipids, glucose, and creatinine were assayed by standard methods on an Aeroset analyzer (Abbott Diagnostics, Berkshire, UK). Serum B12 and folate were measured by competitive protein binding assays on an Elecys 2010 analyzer (Roche Diagnostics, East Sussex, UK). Plasma total homocysteine, cysteine (tCys), cysteinylglycine (CysGly),

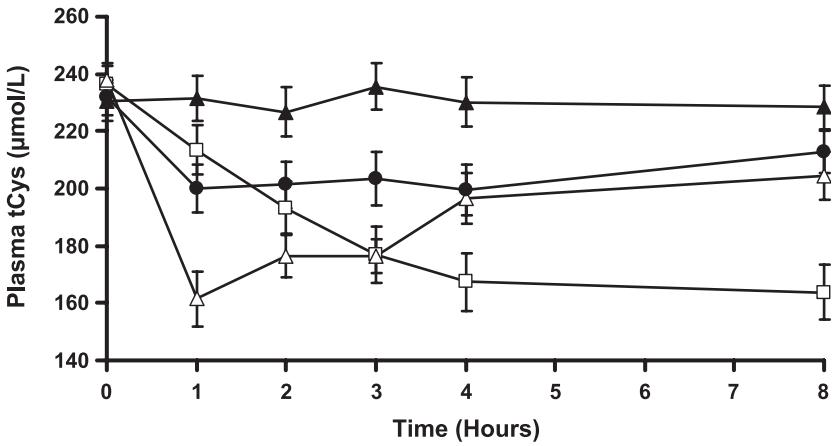

Fig. 5. Plasma tCys concentrations after administration of methionine (●), homocysteine (△), NAC (□), and placebo (▲). Data are presented as mean ± SEM.

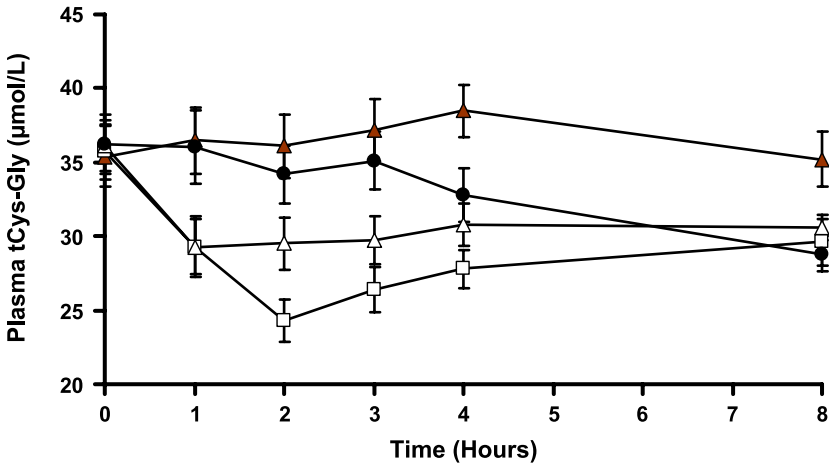

Fig. 6. Plasma tCysGly concentrations after administration of methionine (●), homocysteine (△), NAC (□), and placebo (▲). Data are presented as mean ± SEM.

and NAC were measured by high-performance liquid chromatography (HPLC) with fluorometric detection after reduction, deproteinization, and derivatization with the fluorophore 7-fluorobenzo-2-oxa-1,3,-diazole-4-sulfonic acid (SBDF) [23]. Plasma methionine was assayed by HPLC with fluorometric detection after deproteinization and derivatization with *o*-phthaldehyde [24]. Cystathionine was measured by stable-isotope-dilution gas chromatography mass spectometry (GC-MS) [25]. Plasma SAM and SAH were measured by stable-isotope-dilution liquid chromatography tandem mass spectometry (LC-MS/MS) [26]. Plasma concentrations of ADMA, symmetric dimethylarginine (SDMA), and L-arginine were determined by HPLC with precolumn derivatization with *o*-phthaldehyde with fluorescence detection as previously described [13]. Samples were analyzed in batches ensuring that all samples from each individual were assayed together to reduce any variation.

2.7. Statistical analysis

Data are expressed as mean (SEM), unless stated otherwise. Data were analyzed by parametric 3-way analysis of variance with post hoc comparison between time points and groups. Right-skew data (homocysteine, methionine, cystathionine, SAM, and SAH) were log-transformed before testing. All analyses were performed using SPSS for Windows version 10.0.5 (SPSS, Chicago, Ill) and the SPLUS software package (Insightful, Seattle, Wash).

3. Results

3.1. Baseline characteristics

The study group comprised 9 men and 1 woman aged 29.1 ± 3.9 years. Baseline characteristics are shown in Table 1.

3.2. Effects on FMD and vascular measurements

Flow-mediated dilatation was markedly decreased after homocysteine and methionine loading, which was significant at all time points after loading compared with placebo ($P < .001$) (Fig. 2). However, the time course of FMD change with these 2 agents differed. After homocysteine, FMD reached a

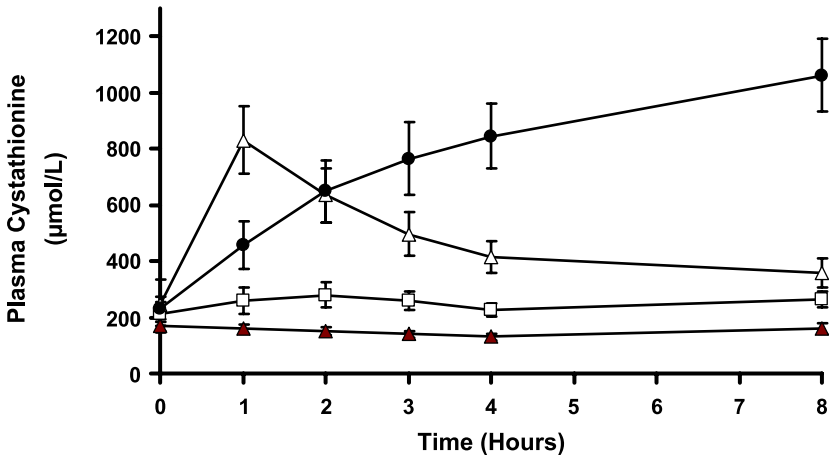

Fig. 7. Plasma cystathionine concentrations after administration of methionine (●), homocysteine (△), NAC (□), and placebo (▲). Data are presented as mean ± SEM.

nadir at 1 hour whereas FMD decreased to a minimum at 4 hours post methionine. Flow-mediated dilatation was not altered after NAC at any time point compared with placebo. Heart rate, blood pressure, baseline brachial artery EDD, peak hyperemic flow, and GTN response did not differ significantly after any agent.

3.3. Effects on biochemical parameters

3.3.1. Plasma homocysteine, cysteine, cysteinylglycine, methionine, and cystathionine

There were no differences in tHcy concentrations between the groups at baseline (Fig. 3). Plasma tHcy was significantly increased at all time points after homocysteine and methionine administration compared with placebo ($P < .001$). Comparing the homocysteine and methionine loading groups, plasma tHcy concentrations were significantly higher after homocysteine loading at hours 1 to 4 ($P < .001$), but were not different at 8 hours ($P = .84$). After NAC, tHcy was significantly lower compared with placebo at all time points decreasing by approximately 70% at 4 hours compared with baseline (7.6 ± 0.6 to 2.4 ± 0.4 μmol/L, $P < .001$) (Fig. 3 inset).

Plasma methionine was grossly elevated at all time points after the oral methionine load compared with placebo ($P < .001$) (Fig. 4). After homocysteine loading, plasma methionine concentrations were significantly increased at all time points ($P < .001$) (Fig. 4 inset). No significant change in plasma methionine was seen after NAC loading. Plasma tCys was significantly decreased after homocysteine loading at all time points ($P < .05$) compared with placebo (Fig. 5). After methionine loading, plasma tCys was significantly ($P < .001$) reduced during the initial 4 hours post load returning toward baseline at 8 hours ($P =$ NS). After NAC, a steady and significant fall in plasma tCys was observed ($P < .001$).

Plasma tCysGly was significantly reduced after both homocysteine and NAC administration compared with placebo ($P < .001$) (Fig. 6). Methionine resulted in a significant reduction in tCysGly at 4 to 8 hours ($P < .001$). Plasma cystathionine was grossly elevated after the methionine load and peaked at 8 hours (Fig. 7). After oral homocysteine, cystathionine was increased significantly at all time points compared with placebo ($P < .001$) and reached a maximum at 1 hour. Plasma cystathionine was increased after NAC at all time points compared with placebo ($P < .001$). Plasma NAC concentrations were undetectable in all subjects at baseline. *N*-Acetylcysteine concentrations were significantly increased at all time points after oral loading with NAC ($P < .001$). Plasma NAC concentration was maximal at 1 hour (132 ± 21 μMol/L) and had significantly ($P < .001$) decreased at 8 hours (67 ± 16 μmol/L).

3.3.2. Plasma ADMA, SDMA, and arginine

Plasma concentrations of ADMA, SDMA, and arginine were not significantly altered after any intervention (Table 2).

Table 2
Plasma ADMA, SDMA, and arginine concentrations in subjects after the different experimental interventions

Intervention	Baseline	4 h	8 h
ADMA (μmol/L)			
Control	0.52 ± 0.049	0.55 ± 0.078	0.53 ± 0.060
Homocysteine	0.59 ± 0.045	0.55 ± 0.050	0.54 ± 0.048
Methionine	0.55 ± 0.037	0.62 ± 0.058	0.59 ± 0.068
NAC	0.58 ± 0.077	0.51 ± 0.035	0.52 ± 0.033
SDMA (μmol/L)			
Control	0.44 ± 0.037	0.54 ± 0.073	0.50 ± 0.066
Homocysteine	0.45 ± 0.049	0.54 ± 0.048	0.46 ± 0.052
Methionine	0.48 ± 0.050	0.51 ± 0.063	0.51 ± 0.051
NAC	0.44 ± 0.059	0.46 ± 0.056	0.42 ± 0.044
Arginine (μmol/L)			
Control	39.4 ± 3.5	37.6 ± 3.2	38.4 ± 2.8
Homocysteine	45.1 ± 4.1	44.4 ± 3.5	46.5 ± 4.5
Methionine	40.5 ± 4.4	43.8 ± 4.4	40.0 ± 3.5
NAC	44.4 ± 4.8	41.3 ± 4.2	43.9 ± 4.4

3.3.3. Plasma SAM and SAH

There were no differences in SAM and SAH concentrations between the 4 groups at baseline (Fig. 8a and b). Plasma SAM was markedly elevated after methionine at all time points after loading compared with placebo ($P < .001$). After homocysteine loading, SAM concentrations were modestly increased at 1 to 3 hours compared with placebo ($P < .05$). Plasma SAH concentrations were significantly elevated at all time points after the methionine load compared with placebo ($P < .001$). After the homocysteine load, plasma SAH concentrations peaked at 1 hour and were increased at 1 to 8 hours compared with placebo ($P < .02$). The peak SAH values were very similar for both homocysteine and methionine loads. The SAM/SAH ratio was markedly increased after methionine administration ($P < .001$) (Fig. 8c). In contrast, the homocysteine load resulted in a reduction in SAM/SAH ratio at 1 to 2 hours ($P < .01$), but returned to baseline values at 3 to 8 hours. SAM and SAH concentrations and SAM/SAH ratio were not altered after NAC administration.

4. Discussion

In the present study we confirmed previous findings that both oral methionine and homocysteine loading impairs endothelial function in healthy human subjects. In contrast to previous findings we have demonstrated that methionine loading does not result in an elevation of plasma ADMA and furthermore demonstrated that oral homocysteine loading did not increase ADMA formation either in healthy human beings. There was no association between endothelial function and methylation status as assessed by plasma SAM and SAH. Another finding was that despite a marked reduction in plasma tHcy (hypohomocysteinemia) after administration of NAC, no improvement in FMD or a reduction in ADMA was observed. These results give further insight as to the role of different metabolites in the methionine/homocysteine pathway on endothelial function.

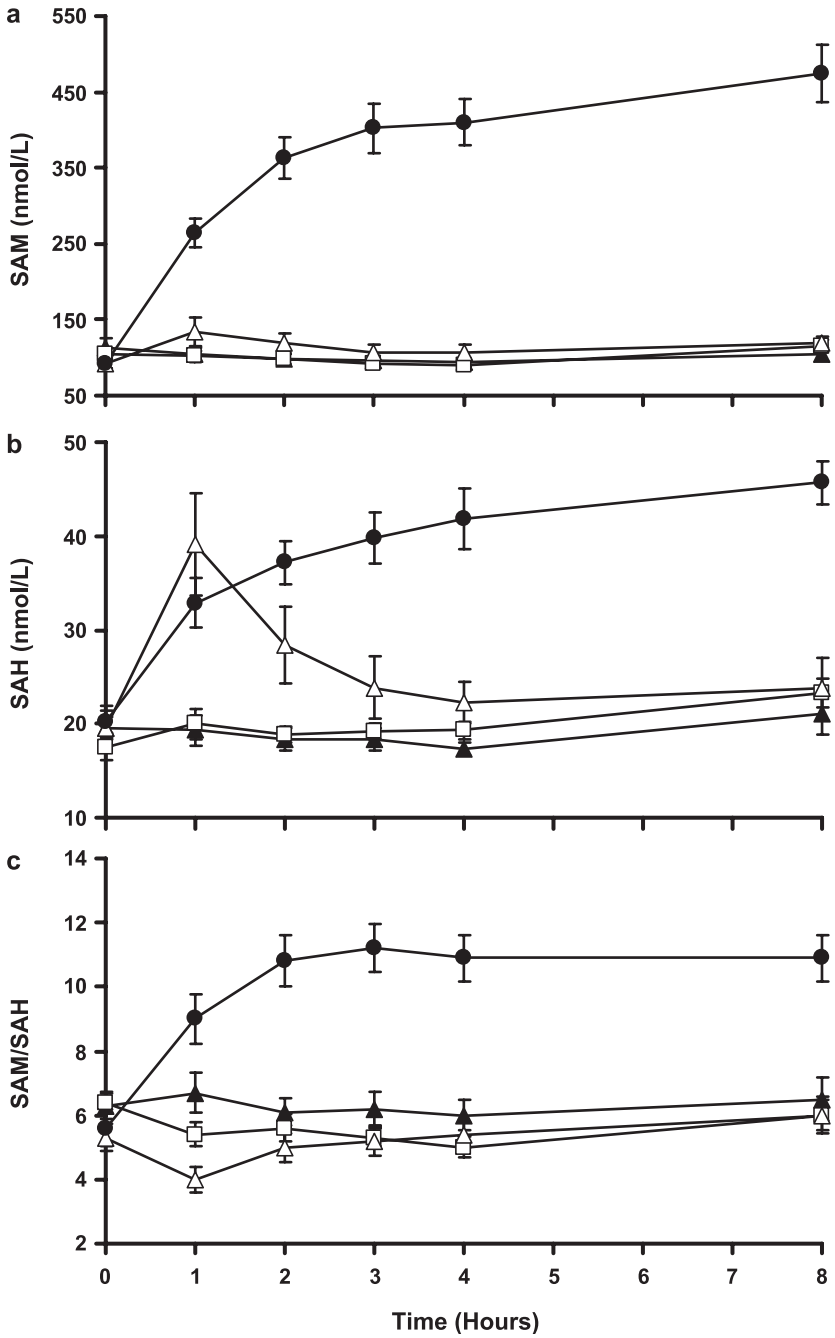

Fig. 8. Effect of methionine (●), homocysteine (△), NAC (□), and placebo (▲) on plasma SAM, SAH, and SAM/SAH ratios. Data are presented as mean ± SEM.

An elevation in plasma ADMA concentration has been implicated as a cause of endothelial dysfunction in patients with cardiovascular disease [27,28]. However, the role of increased ADMA formation, as a cause of endothelial dysfunction after experimental hyperhomocysteinemia in human beings, is equivocal. Two previous studies both found a positive association between endothelial dysfunction and ADMA formation after methionine loading [13,14]. In contrast, we found that methionine loading caused endothelial dysfunction independently of increasing plasma ADMA concentrations. Our findings confirm that of Wanby and colleagues [15], although this study did not assess endothelial function. In addition, we observed no increase in plasma ADMA formation after the homocysteine load despite higher concentrations of tHcy being achieved compared with the methionine load at 4 hours (41.5 ± 2.8 vs 23.1 ± 2.0 μmol/L); both these oral interventions resulted in impairment of endothelial function, an observation confirmed by other investigators [12,29]. This study is the first to assess ADMA formation

after an oral homocysteine load. These findings provide further evidence that experimental hyperhomocysteinemia may not cause endothelial dysfunction mediated by ADMA inhibiting NO production.

If plasma tHcy modulates ADMA, then lowering of tHcy should reduce ADMA concentrations. However, our study demonstrated that lowering tHcy after NAC administration did not result in a reduction of plasma ADMA concentrations. Previous studies have assessed the impact of tHcy lowering on plasma ADMA concentrations using folic acid and other B vitamins. Elevations of plasma ADMA observed in subjects with hyperhomocysteinemia were lowered after folic acid therapy, with an associated reduction in tHcy [30]. In contrast, another study reported that folic acid, vitamins B_6 and B_{12} lowered plasma tHcy in hyperhomocysteinemic (tHcy >15 μmol/L) subjects with cardiovascular disease; however, no effect was exerted on the elevated plasma ADMA concentrations [31].

So, what explanation can be given regarding the apparent discrepancies observed in ADMA concentrations after a methionine load? Firstly, all studies used the standardized dose of methionine (100 mg/kg body weight). Different analytical methodology may explain these differences. However, in the study by Wanby and colleagues [15], duplicate samples were measured independently using different assays and comparison of results confirmed their initial findings. Furthermore, the measurements of plasma ADMA in the present investigation were made by Boger and colleagues. One possible explanation could be the difference in the baseline ADMA concentrations in these studies. The baseline concentrations obtained in our study (0.56 ± 0.03 μmol/L) were comparable to those of Wanby et al (0.66 ± 0.03) [15]. These results are significantly lower than those of Boger et al (1.39 ± 0.2) [13] and Stuhlinger et al (1.0 ± 0.2 μmol/L) [14]; both these studies showed an increase in ADMA formation. Therefore, the basal ADMA concentrations in a study population may reflect the underlying vascular disease, methylation, and the renal status of the group. However, methylation status was not investigated in association with ADMA in the previous studies. Our findings, therefore, do not support the hypothesis that increased concentrations of tHcy after a methionine load or even a homocysteine load result in the impairment in endothelial function via increased concentrations of ADMA. However, our data do not preclude the fact that chronic hyperhomocysteinemia may be a cause of increased ADMA formation in patients with cardiovascular disease. Further investigation is needed in larger numbers of healthy and diseased subjects to assess the impact of vascular damage, methylation status, and its association with ADMA formation.

S-Adenosylmethionine is the most important physiological methyl donor that is involved in numerous methyltransferase reactions, involving DNA, RNA, neurotransmitters, phospholipids, and proteins. The product of these methylation processes is SAH, which is an inhibitor of these reactions. The SAM/SAH ratio has been termed the *methylation index*. Recent data demonstrated a significant correlation between plasma and lymphocyte SAM/SAH ratios, indicating that plasma SAM/SAH may be used as marker of intracellular methylation status [32]. However, both SAM and SAH plasma concentrations are strongly influenced by renal status [26]. The SAM/SAH ratio is low in renal disease which may explain the alterations in plasma and erythrocyte concentrations of SAM and SAH, which have been demonstrated in patients with cardiovascular disease [33-36]. No study has previously investigated the role of alterations in SAM and SAH concentrations as a cause of endothelial dysfunction induced by experimental hyperhomocysteinemia. In this study consisting of healthy subjects, we found that plasma SAM and SAH paralleled the increases in tHcy and decrease in FMD found after homocysteine and methionine loading. However, the SAM/SAH ratio decreased with impairment of FMD after homocysteine loading and the reverse occurred after methionine loading.

The strongest causal relationship between hyperhomocysteinemia and thrombotic vascular disease is seen in patients with cystathionine β-synthase (CβS) deficiency (classic homocystinuria). Methionine loading is widely used to model this clinical situation yet our data show that there are major differences in metabolites between the model and the human disease. The peak methionine concentrations after loading are similar to untreated patients with CβS, yet the peak SAM we found of approximately 400 nmol/L is only 25% as high as previously reported in CβS deficiency [37]. Likewise, the peak concentration of SAH approximately 40 nmol/L is less than 10% of the concentrations observed in CβS deficiency [37]. The SAM/SAH ratio is usually low in CβS deficiency and resembles the results we obtained in the homocysteine loading rather than the high ratio we found with methionine loading. The hyperhomocysteinemia seen in renal failure, like CβS deficiency causes a low SAM/SAH ratio, and our data suggest that homocysteine loading may be a better model of these 2 situations. Further work is needed to look at the long-term effects of disturbances in SAM and SAH concentrations and their role in endothelial function and development of cardiovascular disease, as recent evidence has shown that plasma SAH is a more sensitive indicator of cardiovascular disease than plasma tHcy [36], although renal status was not investigated in this report.

Plasma concentrations of tHcy are reduced by oral administration of NAC, which displaces them from protein binding sites forming NAC-homocysteine mixed disulfides, which is water soluble and readily excreted into the urine [20]. If homocysteine is causally related to atherosclerosis it is plausible that this decrease may be associated with an improvement in endothelial function and hence reduced cardiovascular risk. However, despite a 70% reduction in tHcy after the NAC load, we failed to show any significant increase in FMD. This would suggest that homocysteine

does not exert significant oxidant stress and does not impair endothelial function at these concentrations. A possible explanation for the lack of enhancement may be that endothelial function in healthy subjects cannot be improved. However, previous studies have confirmed that endothelial function in healthy subjects can be improved with ACE inhibitors and exercise [38,39].

5. Conclusions

Our findings confirm that acute experimental hyperhomocysteinemia resulting from oral methionine and homocysteine loading results in impairment in endothelial dysfunction. However, ADMA concentrations do not appear to be associated with the mechanism of this effect. Lowering plasma tHcy to "subnormal" concentrations with NAC does not enhance endothelial function. Methylation status as assessed by SAM and SAH also does not appear to be a determinant of endothelial function in these experimental settings.

Acknowledgments

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