

Disturbed ratio of erythrocyte and plasma *S*-adenosylmethionine/*S*-adenosylhomocysteine in peripheral arterial occlusive disease

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Abstract

Altered homocysteine metabolism associated with peripheral arterial occlusive disease (PAOD) may lead to impairment of vital methylation reactions through accumulation of *S*-adenosylhomocysteine (AdoHcy) as well as through alteration of the ratio *S*-adenosylmethionine (AdoMet)/AdoHcy. We determined AdoMet, AdoHcy, their ratio, and homocysteine in plasma as well as AdoMet, AdoHcy, and their ratio in erythrocytes of 61 patients with PAOD (age 49–93) and 50 healthy controls (age 41–87). Geometric mean values of plasma homocysteine, AdoMet, and AdoHcy were significantly increased in patients compared with controls (15.5 vs. 10.4 $\mu\text{mol/l}^{**}$; 107 vs. 52.3* nmol/l ; 55.0 vs. 23.1** nmol/l , respectively; * $P < 0.01$, ** $P < 0.001$), while the ratio of AdoMet/AdoHcy was decreased in patients (1.92 vs. 2.52*). In erythrocytes patients exhibited increased levels of AdoHcy compared with controls (309 vs. 205 nmol/l^{**}) whereas AdoMet (3351 vs. 3732 nmol/l^*) and the ratio of AdoMet/AdoHcy (11.8 vs. 19.1**) were decreased. The odds ratio (OR) for developing PAOD with decreased AdoMet/AdoHcy ratio after adjustment for kidney function was significant for erythrocyte levels ≤ 14.2 (OR, 7.1 (6.9–7.2, 95% CI). In addition, hematocrit levels were found to be significantly decreased in patients versus controls (0.35 vs. 0.42 $1/l^{**}$) and were significantly correlated with the ratio of AdoMet/AdoHcy in erythrocytes of the patients. Since the ratio of AdoMet/AdoHcy is closely linked with the activity of numerous enzymatic methylation reactions, these results suggest that methylation may be impaired in these patients. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

In the last two decades several retrospective and prospective studies have shown that mild hyperhomo-

cysteinemia is associated with peripheral arterial occlusive (PAOD), coronary artery, and cerebrovascular disease, as well as with venous thrombosis independent of other risk factors such as smoking, arterial hypertension, hypercholesterolemia, and diabetes mellitus [1–12]. The pathophysiological implications of hyperhomocysteinemia, however, are not fully understood. Homocysteine is produced during methionine metabolism via the adenosylated compounds *S*-adenosylmethionine (AdoMet) and *S*-adenosylhomocysteine (AdoHcy) [13]. The main physiological function of this metabolic pathway (Fig. 1) is probably the maintenance of adequate amounts of AdoMet, which not only plays

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a key role in the enzymatic regulation of homocysteine metabolising reactions, but is also the main methyl donor in numerous enzymatic *trans*-methylation reactions, which lead to the formation of AdoHcy [14]. AdoHcy acts as a competitive inhibitor of these AdoMet-dependent *trans*-methylation reactions and its affinity to most methyltransferases is greater than the affinity of the substrate AdoMet [15]. Increases in AdoHcy concentration relative to AdoMet will therefore result in inhibition of *trans*-methylation reactions. Indeed, the ratio of AdoMet/AdoHcy is crucial in the regulation of enzymatic *trans*-methylation reactions [15,16] and a decrease of the ratio may inhibit *trans*-methylation reactions in various tissues [16] thereby potentially affecting biosynthesis of a wide range of endogenous compounds such as proteins, hormones, phospholipids, neurotransmitters, DNA and RNA [17] (Fig. 1). Even though thermodynamics favour the biosynthesis of AdoHcy, AdoHcy normally remains low in vivo provided that homocysteine is removed immediately [15]. Hence, elevations of homocysteine levels may lead to an increase in AdoHcy and therefore to the potentially detrimental alterations of the ratio AdoMet/AdoHcy described above.

Indeed, in a previous study [18], we detected low whole blood AdoMet concentrations in patients with coronary artery disease, pointing to possible alterations of methylation reactions in these patients compared with healthy controls. Moreover, recent studies showed a decreased ratio of AdoMet/AdoHcy in erythrocytes [19,20] and in plasma [21] of end-stage renal failure patients in whom the incidence of moderate hyperhomocysteinemia is high as is the risk for vascular disease. So far no information about AdoMet/AdoHcy ratios is

available in patients with PAOD. The aim of this study was to investigate AdoMet and AdoHcy in erythrocytes and plasma as a metabolic consequence of altered homocysteine metabolism or of related factors such as renal function in patients with PAOD.

2. Subjects and methods

2.1. Patients and control subjects

Sixty one patients (aged 49–93; 32 women and 29 men, Table 1) with PAOD were investigated at the University Hospital in Basel (between 1994 and 1997) after giving informed consent. Six patients had claudicatio (Fontaine, stage II), and 59 suffered from critical limb ischemia (stage III/IV). Thirteen patients had diagnosed chronic renal insufficiency, 24 patients had diabetes; 27 were hypertensive; 13 were current, 16 previous and 20 were non-smokers. Twenty-six patients had a history of one or more vascular events other than PAOD (in total 24 coronary artery and five cerebrovascular events). Fifty healthy control subjects (aged 41–87; 28 women and 22 men, three smokers, Table 1) were randomly selected within the same city. At least 4 h after the last food intake, blood samples for the determination of homocysteine and its metabolites, hematological parameters and for routine clinical chemistry (Hitachi 911 Automatic Analyser) were drawn.

2.2. Sample preparation

Ethylene diamine tetra acetic acid (EDTA) blood samples for homocysteine, 5-methyltetrahydrofolate (MeTHF), AdoMet, and AdoHcy were placed on ice after collection and processed within half an hour. Plasma for MeTHF measurement, processed under light protection, was added to ascorbic acid (10 mg/ml). For AdoMet and AdoHcy, samples were deproteinised immediately by adding 0.625 ml of a 10% perchloric acid solution to 1 ml of plasma or an equal amount of 5% perchloric acid solution to whole blood, respectively, followed by thorough mixing. All samples were stored at -70°C until analysis [18,22].

2.3. High performance liquid chromatography (HPLC) determination of homocysteine, MeTHF (in plasma), AdoMet and AdoHcy (in plasma and erythrocytes)

Plasma samples for total homocysteine, MeTHF, AdoMet, and AdoHcy determination in plasma were processed and subjected to reversed phase chromatography with fluorescence detection as previously described [22], with the following modifications of the HPLC conditions for AdoHcy and AdoMet. Etheno-derivatives of both metabolites were analysed sep-

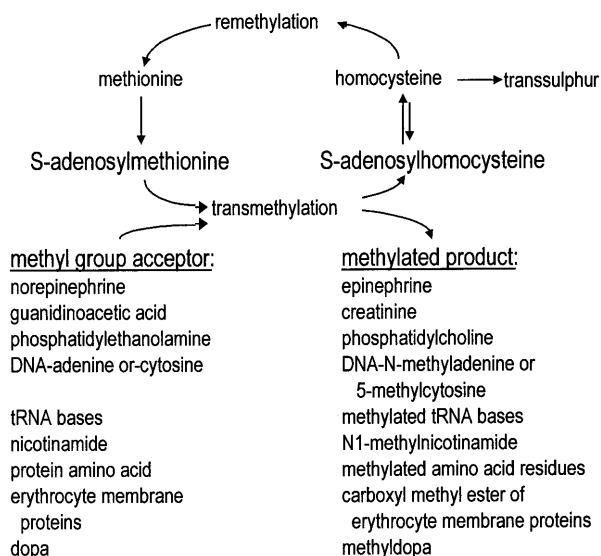


Fig. 1. Important AdoMet-dependent *trans*-methylation reactions in mammals [17].

Table 1
Biochemical, haematological and demographic parameters of patients with PAOD ($n = 61$) and healthy control subjects ($n = 50$)^a

	Patients	Controls	P-values
Age (year)	74 (59–90)	67 (43–83)	<0.01
BMI	23.3 (16.3–33.7)	23.9 (18.8–30.2)	NS
Estimated creatinine clearance (ml/min)	56.4 (11.8–131)	79.2 (53.9–134)	<0.001
<i>Plasma values</i>			
ALAT (U/l)	16.0 (6.1–54)	14.5 (7.1–46)	NS
Haemoglobin (g/l)	113 (80–152)	140 (132–170)	<0.001
Hematocrit (l/l)	0.35 (0.27–0.49)	0.42 (0.37–0.48)	<0.001
Cholesterol	4.6 (3.0–6.9)	5.5 (4.1–7.3)	<0.001
Triglycerides (mmol/l)	1.9 (0.84–4.13)	1.6 (0.69–4.47)	NS
Total homocysteine (μmol/l)	15.5 (8.1–35.5)	10.4 (6.2–16.4)	<0.001
AdoHcy (nmol/l)	55.0 (15.0–257)	23.1 (11.8–39.4)	<0.001
AdoMet (nmol/l)	107 (54.5–285)	52.3 (34.8–79.5)	<0.001
AdoMet/AdoHcy	1.92 (1.09–5.25)	2.52 (1.25–44.8)	<0.01
MeTHF (nmol/l)	16.2 (3.6–69)	19.7 (11.3–71)	0.06
Vitamin B ₁₂ (pmol/l)	387 (218–1107)	330 (231–463)	0.06
<i>Erythrocyte values</i>			
AdoHcy (nmol/l)	309 (157–4219)	205 (128–328)	<0.001
AdoMet (nmol/l)	3351 (485–4352)	3732 (2701–5344)	<0.01
AdoMet/AdoHcy	11.8 (0.69–22.3)	19.1 (9.73–34.2)	<0.001

^a Values are expressed as median (95% range). Note that the values of AdoMet/AdoHcy ratios are the median ratios of individual subjects, not ratios of the median concentrations of the whole group. The estimated creatinine clearance was calculated using the following equation [$150 - \text{age (year)}] \times \text{weight (kg)} \times 0.9$ for females, $\times 1.1$ for males/serum creatinine ($\mu\text{mol/l}$) [23].

rately on a 4.0×200 mm Hypersil RP-18 ($3 \mu\text{m}$) column with a guard column 4.0×20 mm filled with the same packing material. Etheno-AdoHcy was eluted at a flow rate of 0.8 ml/min with a 0.1 mol/l sodium acetate buffer containing 4.3% acetonitrile adjusted to pH 4.45 with acetic acid. Separation of the Etheno-AdoMet was performed at a flow rate of 0.7 ml/min with the same buffer containing 10 mmol/l heptanesulfonic acid and 3.95% acetonitrile. After 35 min the column was flushed with 100% acetonitrile for 10 min followed by equilibration at initial conditions for 10 min. The retention time for AdoHcy was 10–12 min, and for AdoMet 29–31 min. Standards in the range 15–1000 nmol/l for AdoMet and 5–500 nmol/l for AdoHcy were prepared in 0.4 mol/l perchloric acid and aliquots stored at -20°C . The inter-assay coefficients of variation (CV) for AdoMet and AdoHcy were 8.2 and 9.1%, respectively ($n = 15$); the intra-assay CVs 9.8 and 9.9%, respectively ($n = 15$), and the detection limits 10 and 5 nmol/l, respectively, with a signal to noise ratio ≥ 5 .

AdoMet and AdoHcy in erythrocytes were determined in deproteinised whole blood samples after thawing and centrifuging for 3 min at $4500 \times g$. The supernatant was filtered through a $0.45 \mu\text{m}$ HV Milipore filter and analysed immediately by a slightly modified method of Perna et al. [20]. Briefly 100 μl of sample was injected on a 4.6×250 mm Zorbax C-8 column. Compounds were detected with a Milton Roy Spectro Monitor 3100 UV detector at 254 nm. The

column was equilibrated with a 50 mmol/l $\text{NaH}_2\text{PO}_4/10$ mmol/l heptanesulfonic acid buffer containing 5% acetonitrile adjusted to pH 3.21. The compounds were eluted at a flow rate of 1.5 ml/min with a linear gradient increasing from 5 to 20% acetonitrile over 20 min, and then with 20% acetonitrile for a further 10 min. The retention time for AdoHcy was 14 min, for AdoMet 17 min. Repeated analysis in 20 selected samples confirmed stability for at least 10 h at a temperature below 10°C .

Standards in the range 0.25–5 $\mu\text{mol/l}$ for AdoMet and 0.1–15 $\mu\text{mol/l}$ for AdoHcy were prepared in 0.4 mol/l perchloric acid and aliquots stored at -20°C . The inter-assay CV for AdoMet and AdoHcy were 6.8 and 9.7%, respectively ($n = 10$); the intra-assay CVs 4.6 and 7.6%, respectively ($n = 10$), and the detection limits 0.1 and 0.05 $\mu\text{mol/l}$, respectively. The recovery of AdoMet and AdoHcy determined by adding trace amounts of both compounds to whole blood prior to deproteinisation was 98 ± 2.6 and $97 \pm 2.9\%$ (mean \pm S.D.), respectively. Erythrocyte concentrations were calculated by multiplying the difference of plasma and whole blood values by 100 divided by hematocrit.

2.4. Vitamin B₁₂ determination

Total vitamin B₁₂ determination was performed by a dual competitive binding assay using the Dualcount Solid Phase No Boil radioassay from Diagnostic Products Corporation (Los Angeles, CA, USA).

3. Statistical analysis

All patients and control subjects were included in the computations regardless of renal function, which was considered by adjustment for estimated creatinine clearance [23] in the statistical analysis. Unpaired comparisons were tested by the Mann–Whitney *U*-test. The relationship between pairs of variables was tested by linear regression analysis and/or multiple regression analysis. Adjustments for age, gender, estimated creatinine clearance and/or homocysteine as well as the influence of diseases, gender or smoking on methionine metabolites were tested by multifactorial analysis (ANOVA). *P*-values < 0.05 were considered significant. These tests were performed with the software package Student SYSTAT 1.0 for windows (SYSTAT Inc., Evanston, IL, USA). Logistic regression analysis (odds ratio (OR)) was performed using the PROC PROBIT procedure with the SAS software package. Unless indicated otherwise values are expressed as the medians (95% range).

4. Results

Table 1 shows the biochemical, hematological and demographic parameters of patients and control subjects. In plasma, AdoMet and AdoHcy levels were both significantly higher and the ratio significantly lower in patients compared with the control group. In erythrocytes of patients AdoHcy was also markedly elevated, while AdoMet was significantly decreased, resulting in

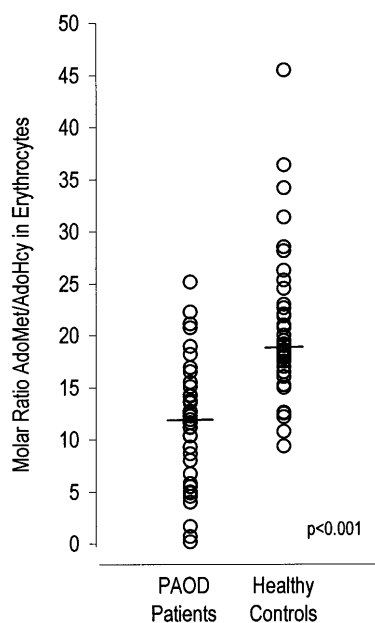


Fig. 2. AdoMet/AdoHcy ratio in erythrocytes of 61 patients with POAD and 50 healthy controls. Horizontal lines indicate mean values.

an even greater decrease of this ratio in erythrocytes of PAOD patients compared with controls (Fig. 2).

Homocysteine concentrations were above 13.5 $\mu\text{mol/l}$ in 11 (21%) of the control subjects, which is the upper limit of our own healthy control population normal range [18]. This limit, however, was determined in a younger control group with subjects aged 20–70 years. The 95th percentile in the present control group was 15.8 $\mu\text{mol/l}$. In patients mean total plasma homocysteine concentrations were significantly elevated compared with healthy subjects. Thirty-nine (64%) patients had homocysteine levels above 13.5 $\mu\text{mol/l}$ and 29 (47.5%) above 15.8 $\mu\text{mol/l}$ (95th percentile of the control subjects).

Bivariate linear regression analysis revealed no relationship between the adenosylated metabolites and homocysteine in patients or controls, whereas renal function (expressed as estimated creatinine clearance) was correlated with plasma AdoMet ($R = -0.4$, $P < 0.01$, in patients), plasma AdoHcy ($R = -0.5$, $P < 0.001$, in patients) and homocysteine ($R = -0.34$, $P < 0.01$ and $R = -0.39$, $P < 0.01$, in patients and controls, respectively). Multiple regression analysis was performed to further evaluate the relationship of plasma AdoHcy and/or homocysteine with AdoMet. This revealed a strong influence of AdoHcy on AdoMet ($R = 0.836$, $P < 0.001$), but no correlation with homocysteine ($R = 0.03$, $P = \text{NS}$).

Patients (42%) had impaired renal function (creatinine clearance < 50 ml/min). After multifactorial ANOVA with estimated creatinine clearance, age, and gender as covariates, the differences between patients and controls of the standardised mean values of homocysteine and the adenosylated metabolites remained significant, but not the difference in the ratio of AdoMet/AdoHcy in plasma, which was mainly determined by renal function (standardised mean values (\pm S.E.M.), 2.42 ± 0.2 and 2.60 ± 0.2 for patients and controls, respectively, $P = \text{NS}$, and $P = 0.02$ for the influence of renal function in the model). Further inclusion of homocysteine in the above model had no additional influence on the difference of the adenosylated metabolites between the two groups.

The OR for cases and control subjects per quartile increase of the AdoMet/AdoHcy ratios in erythrocytes and plasma, after adjustment for the estimated creatinine clearance, showed a high prevalence of PAOD within the lowest quartile of the ratio for erythrocytes (≤ 14.2), but not for plasma values (Table 2).

No relationship between the vitamins MeTHF or vitamin B₁₂ and homocysteine or between the vitamins and the adenosylated metabolites was observed.

Gender differences were evident for body mass index (BMI) in patients (21.6 (16.1–32.4) kg/m² and 25.4 (19.4–33.7), for women and men, respectively, $P < 0.05$) and for plasma total homocysteine in patients

Table 2
OR for patients to develop PAOD by quartile decrease of the ratio of AdoMet/AdoHcy in plasma and in erythrocytes (quartile 1 vs. quartile 4) after adjustment for the estimated creatinine clearance

Quartile ^a	Ratio in erythrocytes ^b	Ratio in plasma	
		Adjusted OR ^c (95% CI ^d)	Adjusted OR ^c (95% CI ^d)
1	≤14.2	7.07 (6.92, 7.22)	≤1.86
2	14.2–19.1	2.06 (–1.90, 2.23)	1.87–2.52
3	≥19.2	1	2.53–3.3
4			≥3.31

^a Based on the control values only.

^b Based on control values but the last two quartiles were made into one as there were no patients in the last quartile.

^c Odds ratio adjusted for the estimated creatinine clearance by means of a logistic model.

^d Confidence interval.

(15.3 (7.9–27.2) $\mu\text{mol/l}$ and 18.3 (8.7–40.3), for women and men, respectively, $P < 0.001$). The gender difference in homocysteine persisted also when renal function or BMI was considered.

In addition no influence of smoking, diabetes mellitus or arterial hypertension on any of the measured metabolites was observed in the two groups.

Hematocrit and haemoglobin levels were significantly decreased in patients compared with the controls (Table 1). No difference was found for other haematological parameters such as MCH, MCHC, MCV (values not shown). After multifactorial ANOVA analysis with the ratio of AdoMet/AdoHcy in erythrocytes and the estimated creatinine clearance as covariates the difference of the standardised mean values of hematocrit and haemoglobin between patients and controls was less strong but still significant ($P < 0.05$ for both parameters), mainly influenced by the AdoMet/AdoHcy ratio in erythrocytes ($P < 0.05$). Linear regression analysis in the whole patient group revealed a significant correlation between hematocrit and AdoMet/AdoHcy ratio in erythrocytes ($R = 0.421$, $P = 0.01$, Fig. 3), which was absent in the control group. This correlation remained significant when adjusted for the estimated creatinine clearance. No correlation between the haematological parameters and any of the plasma parameters measured was found.

5. Discussion

This study on homocysteine and related metabolites such as AdoMet and AdoHcy determined in different compartments (plasma, erythrocyte) of PAOD patients revealed a number of notable findings.

First, in accordance with other studies [8,24–26] a substantial proportion of our PAOD patients showed elevated plasma homocysteine, one of whose suggested toxic effects is disturbed endothelial function [27]. The percentage of patients with homocysteine levels above

the 95th percentile of the control group was higher than in previous reports [8,25], probably because patients with impaired kidney function, a strong predictor of homocysteine concentrations [28,29] and frequent in PAOD [30], were included in our study and because patients were on average older than in many previous studies.

Second, in erythrocytes the ratio of AdoMet/AdoHcy was markedly reduced in the patient group with more minor changes in the concentrations of these metabolites. Our control values are somewhat higher than those of AdoMet, $2.7 \pm 0.1 \mu\text{mol/l}$ and AdoHcy, 0.77 ± 0.09 , resulting in a much lower ratio of 3.5 reported by Perna et al. [31]. This may be due to the determination of the metabolites in whole blood and plasma separately and calculation of the erythrocyte concentrations based on hematocrit levels in our study, as well as rapid deproteinisation thereby minimising any possible ex vivo losses. Such losses could result in a shift of the value of the AdoMet/AdoHcy ratio. The

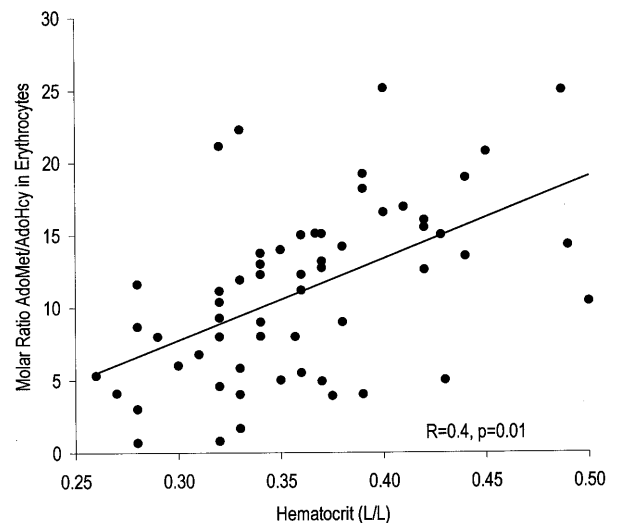


Fig. 3. Relationship between the molar ratio of AdoMet/AdoHcy in erythrocytes and hematocrit in patients ($n = 61$) with PAOD.

lower ratios in patients could be a consequence of increased extracellular levels of homocysteine which can freely enter erythrocytes [19] and be converted to AdoHcy due to the thermodynamics of the AdoHcy hydrolase reaction [15], whereas remethylation of homocysteine to methionine does not occur in erythrocytes [19,31] preventing de novo synthesis of AdoMet. Alternatively an accelerated requirement of AdoMet for *trans*-methylation reactions e.g. methylation of erythrocyte membrane proteins [19], could lead to increased formation of AdoHcy, which subsequently leads to a release of the resulting homocysteine into plasma as demonstrated by Dudman and co-workers 1998 [32], and, due to the lack of remethylation in red cells, to a depletion of AdoMet. In both cases the ratio of AdoMet/AdoHcy in erythrocytes may be expected to decrease to a much larger extent than in plasma as indeed found in this study.

The ratio of AdoMet/AdoHcy and the concentration of AdoHcy are reported to be crucial in the regulation of *trans*-methylation reactions, for example as reported in brain [16]. Thus the decreased ratios in erythrocytes, which are independent of kidney function and the association between PAOD and low AdoMet/AdoHcy values demonstrated by the OR may point to disturbed *trans*-methylation reactions in vascular disease patients. Indeed disturbed erythrocyte membrane protein methyl esterification, a *trans*-methylation reaction, was associated with increased intra-cellular AdoHcy levels and a decreased AdoMet/AdoHcy ratio [20], and suggested to cause hemolysis and anaemia in patients with chronic renal failure. This observation is supported by the correlation between hematocrit and the AdoMet/AdoHcy ratio in our patients, which remained when estimated creatinine clearance was included as a co-variate in the statistical analysis. Whether the hematocrit and the AdoMet/AdoHcy ratio are causally linked requires further studies focussing on occurrence of anaemia, which has also been observed in cardiovascular disease patients [33] and including additional red cell parameters such as reticulocyte counts.

Third, plasma levels of both AdoMet and AdoHcy were increased in the patient group with relatively higher increases of the latter resulting in a reduced ratio of AdoMet/AdoHcy. The compartmental origin of plasma AdoMet and AdoHcy is not fully understood but increases of these compounds, seen post methionine loading in control subjects [22], and evidence of AdoHcy export in tissue culture [34] suggests plasma levels of these two compounds may reflect tissue metabolism. Regression analysis was performed in attempting to explain these changes in plasma levels of AdoMet and AdoHcy. Although these levels correlate with each other, possibly reflecting inhibition of enzymatic AdoMet dependent *trans*-methylation reactions [35], they do not correlate with homocysteine, even though

the thermodynamics of the AdoHcy hydrolase reaction favour the synthesis of AdoHcy [15] when homocysteine accumulates.

The lack of a statistical correlation between homocysteine and AdoMet or AdoHcy may reflect the mainly intra-cellular nature of the adenosyl derivatives whereas homocysteine is readily exported into the extracellular compartment. Also regulation of AdoMet and AdoHcy is multifactorial and other factors than the accumulation of homocysteine will influence AdoMet and AdoHcy levels and their ratio in plasma. For example there was a striking influence of kidney function on the plasma ratios of AdoMet/AdoHcy as demonstrated by the multifactorial ANOVA as well as by the OR in plasma after adjustment for renal dysfunction. In contrast to the AdoMet/AdoHcy ratios in erythrocytes which were related to PAOD independently from kidney function, decreased plasma ratios seem not to be directly related to the disease itself, but are rather predicted by the estimated creatinine clearance. These findings confirm the modulation of methionine metabolism by renal function, which has been shown to be a good predictor for homocysteine especially when kidney function is impaired [28,29] as in a substantial proportion of our patients.

Fourth, mean MeTHF values were slightly lower in our patients compared with controls, consistent with our previous findings in patients with coronary artery disease (5.2–54.8 and 8.5–74.7 nmol/l in patients and controls, respectively; $P < 0.05$ [18]). However, there was no statistically significant correlation between homocysteine and folate (measured as MeTHF) in the present study, in contrast to our [18], and another previous study [36] the latter measuring total folate. This may be due to the higher MeTHF in the present study, with MeTHF levels below the 5th percentile (11 nmol/l) of our own healthy population range in only 16% of our patients, since there is evidence that this association is mainly found when folate levels are low [37,38].

In conclusion, the findings in this and previous studies [18,19,21] point to possible patho-physiological changes of AdoMet and AdoHcy concentrations especially in erythrocytes of PAOD and of other cardiovascular disease patients. Whether these changes reflect or lead to disturbances of any of the many vitally important *trans*-methylation reactions (Fig. 1) [17] requires further investigation. For example the correlation between hematocrit and the AdoMet/AdoHcy ratio may point to a clinically relevant disturbance of erythropoiesis or erythrocyte survival. It needs to be established whether homocysteine lowering therapy e.g. with folic acid [39–41] in vascular disease patients will also normalise AdoMet and AdoHcy concentrations. Since methylation reactions may be influenced by the AdoMet/AdoHcy ratio as well as absolute AdoMet

concentrations, combined treatment with vitamin B₆, which stimulates *trans*-sulphuration and should lower AdoHcy, together with folate, which will increase the AdoMet pool, may be more effective than either vitamin given singly.

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