Effect of methionine loading on 5-methyltetrahydrofolate, S-adenosylmethionine and S-adenosylhomocysteine in plasma of healthy humans

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(Received 16 November 1995/29 January 1996; accepted 19 March 1996)

INTRODUCTION

The essential protein amino acid methionine is converted to S-adenosylmethionine by methionine adenosyltransferase (EC 2.5.1.6). S-adenosylmethionine is the methyl donor in many important transmethylation reactions that lead to the formation of the very short-lived S-adenosylhomocysteine [1]. Enzymic hydrolysis yields homocysteine, which can be catabolized via the irreversible trans-sulphuration pathway, the first step in which is catalysed by the pyridoxal phosphate-dependent enzyme cystathionine $\beta$-synthase (CS; EC 4.2.1.22). Alternatively, it can be remethylated to methionine by 5-methyltetrahydrofolate–homocysteine methyltransferase (methionine synthase, MS; EC 2.1.1.13), which requires vitamin B$_{12}$, or by betaine–homocysteine methyltransferase (EC 2.1.1.5; Fig. 1). Regulation of these pathways depends on many factors [2–5], but a vital role of S-adenosylmethionine in the co-ordinate control of remethylation and trans-sulphuration seems evident from studies in uitro [6, 7]. S-Adenosylmethionine acts as an allosteric inhibitor of 5,10-methylenetetrahydrofolate reductase (MTHFR; EC 1.1.1.68), which is crucial for 5-methyltetrahydrofolate synthesis [6] and as an activator of CS [7] at micromolar concentrations.

Disturbances of methionine metabolism are associated with a variety of disease states. For example, inborn errors due to CS, MTHFR and MS deficiencies lead to hyperhomocystinaemia and severe disease, including ocular, skeletal, neurological and vascular pathology [1, 8, 9]. Furthermore, elevated plasma homocysteine levels, either in the fasting state or after methionine loading, have been found in a significant proportion of patients with coronary artery [10–13], peripheral arterial occlusive [14, 15] or cerebral vascular disease [15–17], pointing to mild hyperhomocystinaemia as an independent risk factor for arteriosclerotic disease. The exact cause of this is so far unknown, but moderate deficiencies of MTHFR [18–20] and CS [21] and nutritional deficiencies of vitamin B$_{12}$ and folate [22, 23] have been implicated.

Key words: S-adenosylhomocysteine, S-adenosylmethionine, amino acids, blood, homocysteine, human, metabolism, 5-methyltetrahydrofolate.

Abbreviations: CS, cystathionine $\beta$-synthase; MS, methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase; SBDF, 7-fluorobeno-2-oxo-1,3-diazole-4-sulphonic acid.

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Administration of methionine followed by measurement of sulphur amino acids in blood and urine has been used to study homozygous and heterozygous CS deficiency [24–27]. Furthermore, Boers et al. [28] reported post-methionine load increases in homocysteine similar to those in obligate heterozygotes for CS deficiency in 36% of 25 patients with peripheral and 28% of 25 patients with cerebrovascular disease. Several studies have confirmed this abnormality in patients with different forms of vascular disease [21, 28–30].

It is thought that methionine loading mainly stresses catabolism through homocysteine transsulphuration [31]. However, Clarke et al. [21] showed an inverse relation between vitamin B12 or folate and post-load homocysteine in patients with different forms of vascular disease. On the other hand, in previous studies by Brattström et al. [30] and Andersson et al. [32], no correlation between post-load homocysteine and vitamin B12, folate or pyridoxal phosphate was found in patients with vascular disease. Previous studies on the relationship between folate and homocysteine have all used measurement of the total blood concentrations of the vitamin rather than the methyl form, which specifically participate in the remethylation reaction.

Until now methionine loading studies have concentrated on changes in sulphur amino acid levels [33, 34], and little is known about the influence of methionine on levels of the numerous other metabolites and cofactors in humans (Fig. 1).

In this study the effect of oral methionine on plasma levels of key compounds involved in the transmethylation pathway were studied over 24 h. In particular, and in addition to the often measured homocysteine, we determined the levels of 5-methyltetrahydrofolate, the form of folate active in remethylation of homocysteine, and the intermediate methionine metabolites S-adenosylmethionine and S-adenosylhomocysteine. Additionally, the heat-stable activity of MTHFR in lymphocytes was assessed.

**MATERIALS AND METHODS**

L-Methionine was obtained from SHS Clinical Nutrition (Heilbronn, Germany). Tri-n-butylphosphine, N,N-dimethylformamide, 7-fluorobenzo-2-oxo-1,3-diazole-4-sulphonic acid (SBDF) and 2-mercaptoethylamine (cysteamine) were purchased from Sigma Chemicals (Buchs, Switzerland). L-Homocysteine, 5-methyltetrahydrofolate and chloroacetalddehyde were from Fluka Chemicals (Buchs, Switzerland). S-Adenosylmethionine was obtained from Boehringer Mannheim (Switzerland). [5,14C]Methyltetrahydrofolic acid barium salt was from Amersham Life Science (Zurich, Switzerland).
Methionine metabolism

Table I. Maximum effect of methionine administration on metabolites and co-substrates of methionine metabolism. Shown are baseline values, peak values in concentration and as percentage change of baseline values, and the time of maximum change (t_max) after methionine loading in all subjects (n = 12), after methionine loading in subjects II, III and XII and without previous methionine administration in subjects II, III and XII (control subjects). Values are means ± SD (range). P-values (baseline versus peak concentrations) are based on the Wilcoxon signed-rank test.

<table>
<thead>
<tr>
<th>Homocysteine (µmol/l)</th>
<th>Baseline value</th>
<th>Peak value (concentration)</th>
<th>Peak value as % of baseline value</th>
<th>P-value</th>
<th>t_max (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading (all)</td>
<td>8.0 ± 1.3 (6.1-9.7)</td>
<td>32.6 ± 10.3 (19.0-49.9)</td>
<td>408 ± 95 (203-546)</td>
<td>&lt;0.001</td>
<td>7.2 ± 2.9 (2-12)</td>
</tr>
<tr>
<td>Loading (II, III, XII)</td>
<td>8.5 ± 0.7 (7.7-9.4)</td>
<td>26.7 ± 6.6 (19.0-35.1)</td>
<td>320 ± 68.6 (103-416)</td>
<td>0.3 ± 4.0 (0-17)</td>
<td></td>
</tr>
<tr>
<td>Control (II, III, XII)</td>
<td>7.9 ± 1.2 (6.2-8.9)</td>
<td>8.8 ± 0.6 (8.1-9.5)</td>
<td>113 ± 12.2 (103-130)</td>
<td>&lt;0.001</td>
<td>11.6 ± 6.7 (2-24)</td>
</tr>
<tr>
<td>S-Methyltetrahydrofolate (nmol/l)</td>
<td>Loading (all)</td>
<td>23.2 ± 7.2 (15.9-41.4)</td>
<td>13.1 ± 2.9 (10.2-16.9)</td>
<td>58.9 ± 16.0 (40.9-100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Loading (II, III, XII)</td>
<td>29.9 ± 0.6 (26.1-27.5)</td>
<td>12.8 ± 2.1 (11.2-15.8)</td>
<td>47.8 ± 9.0 (41.3-60.5)</td>
<td>8.0 ± 4.0 (0-12)</td>
<td></td>
</tr>
<tr>
<td>Control (II, III, XII)</td>
<td>28.3 ± 0.7 (27.3-28.9)</td>
<td>27.4 ± 0.2 (27.2-27.7)</td>
<td>96.9 ± 2.4 (94.1-100)</td>
<td>8.0 ± 4.0 (0-12)</td>
<td></td>
</tr>
<tr>
<td>S-Adenosylmethionine (nmol/l)</td>
<td>Loading (all)</td>
<td>37.9 ± 25.0 (19.8-116.0)</td>
<td>240.3 ± 109.2 (69.1-408.9)</td>
<td>729 ± 325 (324-1247)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Loading (II, III, XII)</td>
<td>25.0 ± 4.8 (21.4-30.4)</td>
<td>235.5 ± 121.6 (69.1-356.9)</td>
<td>897 ± 497 (324-1195)</td>
<td>8.0 ± 4.0 (0-12)</td>
<td></td>
</tr>
<tr>
<td>Control (II, III, XII)</td>
<td>36.3 ± 10.0 (19.3-40.6)</td>
<td>39.6 ± 30.5 (19.2-74.7)</td>
<td>137 ± 36 (96-184)</td>
<td>8.0 ± 4.0 (0-12)</td>
<td></td>
</tr>
<tr>
<td>Ratio S-adenosylmethionine / S-adenosylhomocysteine</td>
<td>Loading (all)</td>
<td>1.2 ± 0.7</td>
<td>11.7 ± 11.6 (1.8-35.7)</td>
<td>&lt;0.001</td>
<td>8.0 ± 4.0 (0-12)</td>
</tr>
<tr>
<td>Loading (II, III, XII)</td>
<td>0.9 ± 0.3 (0.7-1.2)</td>
<td>5.4 ± 1.2 (3.8-6.9)</td>
<td>137 ± 36 (96-184)</td>
<td>8.0 ± 4.0 (0-12)</td>
<td></td>
</tr>
<tr>
<td>Control (II, III, XII)</td>
<td>1.1 ± 1.0 (0.4-2.3)</td>
<td>1.6 ± 0.8 (0.7-2.5)</td>
<td>137 ± 36 (96-184)</td>
<td>8.0 ± 4.0 (0-12)</td>
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All other chemicals were of the highest purity commercially available.

Subjects

Twelve healthy subjects (six women, six men; age 25-47; 48-87 kg) participated in the study after giving written informed consent. All had fasting plasma total homocysteine concentrations (Table I) within our own healthy population normal range (2.2-13.2 µmol/l, n = 50). The normal range was defined by the 95% tolerance interval (with α = 0.05), i.e. the interval that contains at least 95% of the population with a probability of 1 - α. These intervals were calculated using the tolerance factor obtained from scientific tables [35]. Biochemical and haematological parameters were determined in each subject to exclude haematological disorders as well as abnormal liver and kidney function. None of these subjects had any family history of premature vascular disease. The protocol for this study was approved by the ethics committee of the University Hospital Basel.

Methionine loading and sample preparation

Methionine was administered at a dose of 0.1 g/kg body weight in 200 ml of orange juice after 12 h fasting. Blood was collected immediately before and 2, 4, 6, 9, 12 and 24 h after methionine intake. All subjects received a standardized diet containing only foods low in methionine and folate from the evening before until 24 h after methionine intake. As controls, three of the subjects repeated this procedure 3 months later but without methionine intake. EDTA blood samples for homocysteine, S-methyltetrahydrofolate, S-adenosylmethionine and S-adenosylhomocysteine determination were placed on ice after collection and processed within half an hour. Plasma for S-methyltetrahydrofolate measurement, processed under light protection, was mixed with ascorbic acid (10 mg/ml). For S-adenosylmethionine and S-adenosylhomocysteine measurements, plasma was deproteinized immediately by adding 0.625 ml of a 10% perchloric acid solution to 1 ml of plasma with thorough mixing. For lymphocytes blood samples were kept at room temperature until isolation of the cells. All samples were stored at -70°C until analysis.

HPLC determination of homocysteine, S-methyltetrahydrofolate, S-adenosylmethionine and S-adenosylhomocysteine in plasma

HPLC separation was performed with a JA-980 Jasco pump, a Uniflow four-channel degasser and a Jasco AS-950 or Merck 655A-40 autosampler, both with cooling. Compounds were detected with a Linear Instruments LC 304 fluorescence detector. The entire system was controlled and data handling was carried out by an AXXIOM software package series 747/MK2 running on an IBM personal computer.

Plasma total homocysteine was measured as previously described by Vester et al. [36] with the following modifications. Cysteamine was used as internal standard. Compounds were separated on a 4.6 x 250 mm Nucleosil 120 C18 (5 µm) column with a 4.6 x 20 mm guard column filled with the same packing material. Isocratic elution was performed at a flow rate of 1 ml/min with 0.1 mol/l potassium dihydrogen phosphate containing 4.5% acetonitrile.
adjusted to pH 2.1 with 85% o-phosphoric acid. A three-point calibration curve was obtained using standards added to pooled plasma with each assay batch. The interassay coefficient of variation was 6.1% (n = 20), the intra-assay coefficient of variation was 4.5% (n = 10) and the detection limit was 1 μmol/l in plasma (signal-to-noise-ratio ≥ 5).

Plasma 5-methyltetrahydrofolate analysis was performed by the procedure of Leeming et al. [37] with fluorescence detection. Samples were handled under light protection and kept on ice. Briefly, frozen plasma samples containing 10 mg/ml ascorbic acid were thawed, then 300 μl was thoroughly mixed with 100 μl of freshly prepared 10% perchloric acid containing 1% ascorbic acid, centrifuged and analysed immediately. Samples were thawed only once to avoid degradation of 5-methyltetrahydrofolate. Deproteinized samples were stable for 5 h at 4°C. A 100-μl aliquot was injected on the same type of column as described for homocysteine. 5-Methyltetrahydrofolate was eluted isocratically with a 0.033 mol/l o-phosphoric acid buffer containing 8% acetonitrile, adjusted with solid sodium hydroxide to pH 2.33, at a flow rate of 0.75 ml/min. Standard solutions for the calibration curve were prepared in 0.5% ascorbic acid solution and stored in aliquots at -20°C. The interassay coefficient of variation was 7.1% (n = 12), the intra-assay coefficient of variation was 4.6% (n = 10) and the detection limit was 1 nmol/l in plasma.

S-Adenosylmethionine and S-adenosylhomocysteine was measured according to Weir et al. [38] with slight modifications. Deproteinized samples were thawed and centrifuged (3000g, 4°C, 5 min) and 200 μl of supernatant was derivatized with 50 μl of chloroacetaldehyde at a pH between 3.5 and 4 adjusted with 3 mol/l sodium acetate. After incubation for 8 h at 39°C, samples were cooled on ice and frozen at -20°C until analysis, which was performed within 24 h. Separation was performed on a 4.6 x 250 mm Lichrosorb RP-8 (5 μm) column. Compounds were eluted isocratically at a flow rate of 1 ml/min with 0.1 mol/l sodium acetate containing 4.2% acetonitrile adjusted to pH 4.5 with acetic acid. Peaks were detected by fluorescence. A three-point calibration curve was obtained using standards added to pooled plasma with each assay batch. The interassay coefficient of variation was 8.2% (n = 20), the intra-assay coefficient of variation was 9.1% (n = 10) and the detection limit for S-adenosylmethionine was 15 nmol/l and for S-adenosylhomocysteine 8 nmol/l in plasma.

Methylenetetrahydrofolate reductase activity assay

Lymphocyte isolation. Lymphocytes were isolated at room temperature from whole-blood samples as previously reported [39].

Assay of methylenetetrahydrofolate reductase activity. The enzyme was assayed according to the method of Rosenblatt and Erbe [40] with the following modifications. [5,14C]Methylenetetrahydrofolate was pretreated with dimedone and extracted with toluene to reduce the blank values. The final assay volume was 100 μl and extraction of the reaction product was performed with 1 ml of toluene. Activity was measured without (specific activity) and after heating at 42°C for 5 min (heat-stable activity). These conditions for determining heat lability were selected after investigations in control cells at temperatures between 42 and 46°C for different times.

Statistical analysis and calculations

The significance of the change of a particular analyte over 24 h was tested by the Friedman test (non-parametric analysis of variance). The difference between the values before and after loading was compared by Wilcoxon signed-rank test and the difference between sexes by Mann-Whitney U-test. The relationship between variables was tested by the use of a linear regression analysis. P-values < 0.05 were considered significant. All tests were performed by the software package Statview (Abacus Concepts) [41]. Unless indicated otherwise, values are expressed as means ± SD.

The elimination rate constant was obtained by linear regression of at least the last three data points of the log-transformed concentration-time curve. The half-life (t) was calculated by dividing 0.693 by the elimination rate constant [42]. The area under the curve over 24 h [AUC(t0–24)] was calculated by the trapezoidal rule method.

RESULTS

The mean concentrations of total homocysteine, S-adenosylmethionine, S-adenosylhomocysteine and 5-methyltetrahydrofolate in plasma after methionine loading in 12 healthy subjects and in three control subjects (without methionine administration) are depicted in Fig. 2. Table 1 summarizes the mean values of preload concentrations, the highest or lowest values measured within the 24-h post-load period (peak value), expressed as both concentration and percentage of the baseline value, and the time of the maximum change. These values are also shown separately for the three control subjects (II, III, XII), who were studied twice (with and without methionine administration). For homocysteine, 5-methyltetrahydrofolate and S-adenosylmethionine changes over a 24-h period were all statistically significant (P < 0.001, Friedman test), whereas S-adenosylhomocysteine concentrations (mean value at time 0: 40.9 ± 16.6 nmol/l) did not change significantly. All subjects showed an increase in S-adenosylmethionine and S-adenosylhomocysteine of 408% and 729% respectively, and the average decrease in S-
Methionine metabolism

Fig. 3. Concentrations of total homocysteine (tHcy, ○), S-adenosylmethionine (SAM, □), S-adenosylhomocysteine (SAH, ■) and S-methyltetrahydrofolate (MeTHF, ▲) in plasma after methionine loading over a period of 24h in subjects XI (a) and VIII (b).

Fig. 2. Concentrations of total homocysteine (a), S-adenosylmethionine (b), S-adenosylhomocysteine (c) and S-methyltetrahydrofolate (d) in plasma after methionine loading in 12 healthy subjects (closed symbols) and without methionine administration in three control subjects (open symbols) over a period of 24h. Results are expressed as means ± SEM.

Methyltetrahydrofolate of 59%, were highly statistically significant (Table I). Mean $t_{\text{max}}$ values were similar for homocysteine (7.2h) and S-adenosylmethionine (7.6h) but clearly longer for 5-methyltetrahydrofolate (11.6h), but interindividual variability was large, as reflected by the relatively wide standard deviations. However, homocysteine increased in all subjects within 2h, reaching on average 86% of its maximum concentration ($C_{\text{max}}$) with considerable variations in $t_{\text{max}}$, as found in other studies [26, 32]. The half-life of homocysteine after methionine loading in these subjects was $11.2 \pm 2.5$ h (range 8.9–15.7 h). For S-adenosylmethionine, the half-life differed more than for homocysteine between the individual subjects, ranging from 2.7 to 17.0 h with a mean of $7.1 \pm 3.9$ h. Figure 3 shows the individual values of each parameter at all times in one subject (XI) with a typical response (Fig. 3a) and one (VIII) with an exceptional response (Fig. 3b) to methionine loading. The latter subject showed no decrease in 5-methyltetrahydrofolate at all during the 24-h period although a large increase in homocysteine was observed. In addition, the only marked increase in S-adenosylhomocysteine after loading was observed in this subject, increasing from $11.0 \text{nmol/l}$ to $44.2 \text{nmol/l}$ after 9h. There were no significant differences between women and men for preload values, maximum or minimum values or change from preload values. Also, no correlation was found between age and these values.

There was a significant correlation between the mean concentrations of homocysteine and S-adenosylmethionine ($r^2 = 0.92$, $P < 0.001$) but not
Fig. 4. Time-dependent relation between mean concentrations of S-methyltetrahydrofolate and (a) S-adenosylmethionine and (b) homocysteine after methionine loading over a time period of 24 h. The data points are connected by lines according to increasing time after loading. Point '0' indicates preload values and '24' indicates the value 24 h after methionine intake.

between homocysteine and S-methyltetrahydrofolate or between S-adenosylmethionine and S-methyltetrahydrofolate. However, a time-dependent relation between S-methyltetrahydrofolate and homocysteine (Fig. 4a) and S-adenosylmethionine (Fig. 4b) was observed. The response of S-methyltetrahydrofolate to both homocysteine and S-adenosylmethionine is characterized by a hysteresis (clockwise) curve, which demonstrates clearly that the maximum decrease in S-methyltetrahydrofolate occurs at a time when the concentrations of homocysteine and S-adenosylmethionine are already returning to normal. Baseline concentrations of each parameter were compared with both the degree of maximum change and the change in the AUC(t0–24) (related to baseline) of the same parameter as well as of the other compounds in the individual subjects. This revealed a significant positive correlation between the concentration at time 0 and the maximum percentage change ($r^2 = 0.57$, $P < 0.05$) and the AUCs ($r^2 = 0.80$, $P < 0.001$) for S-methyltetrahydrofolate only.

Measurement of methylenetetrahydrofolate reductase activity in lymphocytes before and after heating revealed levels of thermostable enzyme ranging from 29% to 58% (45.6 ± 7.8, mean ± SD) of total activity. These values compare with a range of 27–51% (37.6 ± 5.6%) in controls reported by Kang et al. [43] and suggest that none of these subjects had the mutation leading to increased thermolability.

**DISCUSSION**

This study set out to investigate the effects of methionine loading on S-adenosylhomocysteine, S-adenosylmethionine and S-methyltetrahydrofolate and their relation to homocysteine in normal subjects.

That the 12 subjects handled methionine normally was suggested by the finding of normal fasting homocysteine values, which increased to a mean of $32.6 ± 10.3 \mu mol/l$, which compares with the mean of $35 ± 6 \mu mol/l$ found by Mansoor et al. [33]. However, this does not completely exclude the homozygous state for CS deficiency in all individuals as some overlap of post-methionine homocysteine levels between control subjects and obligate heterozygotes has been reported [27]. The variable time to peak values in different subjects in our study emphasizes individual variation in such metabolic studies in humans. It helps to explain the better discrimination of obligate heterozygotes from control subjects observed when several post-load samples were analysed than in simplified tests using a single post-load measurement. Such variable peak level times have important implications both for the studies in vascular disease patients and for investigations of metabolic inter-relationships as in this study.

The major conclusions from this study are that methionine loading in normal human subjects leads to simultaneous increases in homocysteine and S-adenosylmethionine, without significant changes in S-adenosylhomocysteine, and to a later fall of S-methyltetrahydrofolate. These changes must be consequent to methionine loading and do not simply reflect circadian variations, as demonstrated by the lack of such changes in three control subjects who received no methionine. The increase in S-adenosylmethionine probably reflects liver metabolism of methionine and increases in S-adenosylmethionine concentration as seen in rats injected with a single dose of methionine [44]. This is supported by studies in mammals, which showed that adaptation to an excess of methionine occurs in liver as only the liver-specific isoenzyme of methionine adenosyltransferase can adapt immediately to changes in methionine concentrations [45]. The absence of a change in S-adenosylhomocysteine concentration after methionine loading despite the large increase in homocysteine is surprising. It was shown by Hoffman et al. [46] that in isolated rat liver the administration of homocysteine results in an accumulation of S-adenosylhomocysteine if homocysteine is not removed immediately. However, Guttormsen et al. [47] reported no change in S-adenosylhomocysteine concentration after homocysteine loading, which resulted in similar concentrations of homocysteine to those found after meth-
ionine loading. If the lack of increase in S-adenosylhomocysteine in plasma does indeed reflect tissue levels, this could be explained simply by a transient increase in homocysteine to concentrations below those required to inhibit S-adenosylhomocysteine hydrolase [46] and by a fully active trans-sulphuration pathway owing to normal enzyme activities in normal subjects.

The decrease in 5-methyltetrahydrofolate found in this study could result from an increase in homocysteine levels, which may result in an enhanced turnover of the remethylation reaction, thereby depleting the co-substrate 5-methyltetrahydrofolate. Alternatively, elevated S-adenosylmethionine could lead to allosteric inhibition of MTHFR, as shown previously in vitro [6], at the concentrations of S-adenosylmethionine obtained in rat liver by injection of similar doses of methionine [44]. It is possible that the two effects may operate in combination. The significant positive correlation between fasting level and both the maximum change and the AUC(0–24) in 5-methyltetrahydrofolate indicates smaller decreases in 5-methyltetrahydrofolate in subjects with lower baseline values. This suggests that the extent of remethylation in the presence of homocysteine excess may also depend on the availability of 5-methyltetrahydrofolate. The one exceptional subject (VIII) who showed no decrease in 5-methyltetrahydrofolate but the highest post-load level of S-adenosylhomocysteine may reflect the extreme of normal variation. However, these findings could also result from a disturbed remethylation pathway, preventing normal conversion of 5-methyltetrahydrofolate to methionine, and subsequent stress of the trans-sulphuration pathway reflected by increased S-adenosylhomocysteine. However, this subject exhibited 36% heat-stable activity of MTHFR, which is well above the range of 2.5–4.5 SDs below the mean value, which was defined by Kang et al. [43] as indicating the thermolabile MTHFR mutation.

An additional finding in this study was that the S-adenosylmethionine/S-adenosylhomocysteine ratio in fasting human plasma, at 1.2, is lower than that reported for erythrocytes (3.3) [48] and cerebrospinal fluid (6.9) [38], indicating variation of this ratio between different tissues and compartments. Previous workers have shown that this ratio and not S-adenosylmethionine alone is a critical factor in the influence of methyltransferases [49].

The finding that methionine loading leads to decreases in 5-methyltetrahydrofolate subsequent to increases in homocysteine and S-adenosylmethionine indicates that a change in either homocysteine or S-adenosylmethionine may cause reductions in 5-methyltetrahydrofolate. This must be considered in evaluating the relationship between folate and homocysteine in vascular disease. The metabolic relationships illustrated in this study should be evaluated in the search for pathogenetic mechanisms of mild hyperhomocysteinaemia and vascular disease.

ACKNOWLEDGMENTS

This work was supported by grants from the Swiss National Science Foundation (grant no. 3200-039439.93), the Treubel Foundation and the Sandoz Foundation, Basel, Switzerland.

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