Folic acid treatment increases homocysteine remethylation and methionine transmethylation in healthy subjects

Frank STAM†, Yvo M. SMULDERS†, Coen VAN GULDENER§, Cornelis JAKOBS‡, Coen D. A. STEHOUWER∥∥ and Kees DE MEER‡

†Department of Internal Medicine, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands, †Institute for Cardiovascular Research, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands, ‡Department of Clinical Chemistry, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands, §Department of Internal Medicine, Amphia Hospital, Langendijk, PO Box 90517, 4800 RL Breda, The Netherlands, ||Department of Internal Medicine, Academic Hospital Maastricht, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands, and ¶Cardiovascular Research Institute Maastricht, Maastricht University, PO Box 5800, 6202 AZ Maastricht, The Netherlands

ABSTRACT

Folic acid treatment decreases plasma total homocysteine concentrations in healthy subjects, but the effects on homocysteine metabolism are unknown. In the present study, we investigated the effect of 3 weeks of oral treatment with 5 mg of folic acid on one-carbon flux rates in 12 healthy subjects, using in vivo stable isotope methods. In addition, we determined the effect of folic acid on blood concentrations of amino acids which may have regulatory roles in homocysteine metabolism, i.e. homocysteine, AdoMet (S-adenosylmethionine), AdoHcy (S-adenosylhomocysteine), serine and glycine. Primed, continuous infusions with [3H3-methyl-1-13C]methionine were used to determine flux rates of methionine transmethylation, homocysteine remethylation and homocysteine trans-sulphuration. Metabolic homocysteine clearance was defined as the ratio of trans-sulphuration and plasma homocysteine level. Folic acid treatment increased the homocysteine remethylation rate by 59 % [95 % CI (confidence interval), 13–97 %; \( P = 0.02 \)] and methionine transmethylation rate by 20 % (95 % CI, 3–41 %; \( P = 0.03 \)). Plasma total homocysteine concentration (−18 %; 95 % CI, −28 to −9 %; \( P < 0.01 \)) and the serine/glycine ratio (−20 %; 95 % CI, −63 to −6 %; \( P < 0.01 \)) decreased significantly, and the AdoMet/AdoHcy ratio (11 %; 95 % CI, 1–20 %; \( P = 0.02 \)) increased significantly. Changes in one-carbon flux rates did not correlate significantly with changes in plasma concentration of these amino acids. In conclusion, folic acid treatment lowered plasma homocysteine concentration and increased whole-body remethylation and transmethylation flux in healthy subjects.

INTRODUCTION

Homocysteinaemia is a continuous independent risk factor for cardiovascular disease [1]. Blood concentrations of folic acid and pyridoxine (vitamin B6) and of cobalamin (vitamin B12) are determinants of homocysteinaemia, even in the normal range of the plasma homocysteine concentrations [2,3]. In turn, the nutritional status for folic acid and pyridoxine (vitamin B6) is inversely associated with the risk of coronary heart disease [4,5].

Homocysteine metabolism is complex (Figure 1), with a central role for folic acid, a compound name for a range

Key words: glycine, folic acid, homocysteine, remethylation, S-adenosylmethionine, S-adenosylhomocysteine, serine.

Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; APE, atom percentage excess; CI, confidence interval; CV, coefficient of variation; FFM, fat-free mass; MPE, mole percentage enrichment.

Correspondence: Dr Frank Stam, Department of Internal Medicine, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands (email f.stam@vumc.nl).

© 2005 The Biochemical Society
Figure 1 An overview of homocysteine metabolism

A concise overview of homocysteine metabolism, with open arrows indicating metabolic fluxes, ovals indicating enzymes and italics indicating vitamins. Homocysteine is the transmethylation (TM) product of the essential sulphur-containing amino acid methionine, with AdoMet and AdoHcy as intermediates. Homocysteine can be either remethylated to methionine or degraded by trans-sulphuration (TS). In the folic-acid-dependent remethylation reaction, which is catalysed by methionine synthase (MS) and uses vitamin B₁₂ as a cofactor, 5-methyltetrahydrofolate (5-MTHF) donates a methyl group to homocysteine. Subsequently, tetrahydrofolate (THF) receives a methylene group from the serine/glycine couple, a reaction that uses vitamin B₆ as a co-factor. Tetrahydrofolate can also be generated by reduction of supplemented synthetic folic acid to dihydrofolate (DHF) and subsequently to THF. Next, 5,10-methylenetetrahydrofolate (5,10-MeTHF) is reduced to 5-MTHF, requiring the enzyme methylenetetrahydrofolate reductase (MTHFR). Another folic-acid-independent remethylation reaction, which is quantitatively unimportant in humans in physiological conditions [6], uses betaine as a methyl group donor, generating dimethylglycine (DMG). In the irreversible catabolic (trans-sulphuration) pathway, the rate-limiting reaction is catalysed by cystathionine β-synthase (CBS) and requires the active form of vitamin B₆ as a cofactor.

of biochemical forms of this B vitamin. Conceivably, the steady-state plasma homocysteine concentration is only partly determined by the rate of homocysteine remethylation. Rather, remethylation, transmethylation and trans-sulphuration act in concert on homocysteine homoeostasis. Remethylation, transmethylation and trans-sulphuration have been thought to be regulated not only by the concentrations of the B vitamins involved and the (genetically determined) activity of specific enzymes, but also by the concentration of intermediate metabolites in homocysteine metabolism. For example, single enzyme kinetic studies have provided evidence that AdoMet (5-adenosylmethionine) stimulates homocysteine trans-sulphuration by activating cystathionine β-synthase and inhibits methionine remethylation by inhibiting methylenetetrahydrofolate reductase [6]. In contrast, AdoHcy (5-adenosylhomocysteine) stimulates both homocysteine trans-sulphuration and remethylation by activating cystathionine β-synthase and methionine synthase [6]. The ratio of AdoMet to AdoHcy determines the activity of the methyltransferases involved in methionine transmethylation [6].

Folic acid (pteroylglutamic acid) has been the cornerstone of most homocysteine-lowering regimens [7], and has been shown to lower plasma homocysteine even in healthy folic-acid-replete normohomocysteinaemic subjects [8]. However, its homocysteine-lowering mechanism is not entirely clear. By conversion into 5-methyltetrahydrofolate, folic acid is thought to stimulate remethylation. However, it is unknown how exactly the homocysteine-lowering effect of folic acid is quantitatively related to folic-acid-induced changes in whole-body remethylation. It is arguable that this may not be a simple linear relationship, because plasma total homocysteine concentrations are an indirect reflection of actual homocysteine concentrations in a wide array of different intracellular compartments. In addition, increased intracellular AdoMet concentrations, in response to folic-acid-stimulated remethylation, may stimulate homocysteine trans-sulphuration and inhibit homocysteine remethylation [9].

The aim of the present study was to quantify folic-acid-induced changes in whole-body one-carbon flux rates in healthy subjects, using stable isotope methods [10,11]. In addition, we determined the effect of folic acid on plasma concentrations of homocysteine, AdoMet, AdoHcy, serine and glycine, which may have regulatory roles in homocysteine metabolism.
Table 1  Baseline characteristics
Values of continuous variables are presented as medians (range). CC denotes wild-type; CT, heterozygous for C677T; TT, homozygous for C677T; −/−, wild-type; −/+ heterozygous for 84ins68; +/+ homozygous for 84ins68; AA, wild-type; AG, heterozygous for A2756G; GG, homozygous for A2756G.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>6/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29 (19–64)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22 (19–30)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65 (57–108)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>52 (40–90)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>89 (62–105)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
</tr>
<tr>
<td>5,10-Methylenetetrahydrofolate reductase</td>
<td>8 CC, 4 CT and 0 TT</td>
</tr>
<tr>
<td>Cystathionine β-synthase</td>
<td>9 −/−, 3 −/+ and 0 +/+</td>
</tr>
<tr>
<td>Methionine synthase</td>
<td>10 AA, 1 AG and 1 GG</td>
</tr>
</tbody>
</table>

**METHODS**

**Subjects**

Twelve healthy adults (five active smokers) were studied. Baseline characteristics are given in Table 1. Subjects who used vitamin supplements or drank more than two units of alcohol a day were not included. None of the participants used any medication.

The study protocol was approved by the Ethics Committee of the VU University Medical Center, and all participants gave their written informed consent.

**Experimental protocol**

Subjects were instructed to use oral supplements of folic acid for 21 days (one tablet containing 5 mg of folic acid daily at 18:00 hours). Stable isotope infusion was performed at baseline and after 3 weeks of folic acid treatment, where the last folic acid tablet was taken the day before the second measurement. The study protocol concerning the stable isotope infusion has been described in detail previously [10]. Briefly, the participants were kept on a stable protein diet of 1.0 g•kg⁻¹ of body weight•day⁻¹ for 3 days prior to the experiment. All subjects remained fasting and recumbent during the test. At 08:00 hours, two intravenous catheters were placed in a dorsal hand vein, one for infusion of substances and one, in the contralateral hand, for sampling. Arterialized blood samples were drawn from the dorsal hand vein after the hand was inserted in a heated box [12]. Blood was collected in heparinized glass tubes, immediately placed on ice and centrifuged for 10 min at 1000 g at −4 °C within 15 min. Plasma was stored at −30 °C until analysed. Samples of end-tidal expired breath air were collected in a 15 ml Venoject® tube by instructing the subjects to exhale through a straw. During the last 3 s of expiration, the straw was withdrawn from the tube, which was immediately closed by the investigator. After baseline samples were taken, a priming bolus of 5.9 µmol NaH¹³CO₃ (99 % [¹³C]; ARC Laboratories, Apeldoorn, The Netherlands) was administered, followed by a primed (3.5 µmol/kg of body weight) constant infusion of L-[¹³H₃]-methyl-¹-¹³C]methionine (95 % doubly labelled; 99 % [¹-¹³C]; 99 % [¹H₃]; Isotec, Miamisburg, OH, U.S.A.) at a rate of 2.2 µmol•kg⁻¹•h⁻¹ for 5 h. Plateau-enrichment concentrations were calculated as the mean of the final five 20-min interval samples of the infusion period. Body weight was measured on a balance scale (accuracy 50 g), and four skinfolds were measured using a calliper (Holtain; accuracy 0.1 mm). FFM (fat-free mass) was calculated from skinfold measurements according to the method of Durnin and Womersley [13].

**Laboratory analyses**

Plasma total (free plus protein bound) homocysteine was measured with the use of a microparticle enzyme immunoassay method based on fluorescence polarization (IMX analyser; Abbott, Chicago, IL, U.S.A.). Intra- and inter-assay CVs (coefficients of variation) were 2.1 and 5.1 % respectively. Serine (inter-assay CV, 3 %) and glycine (inter-assay CV, 3 %) analyses were performed by HPLC after precolumn derivatization with orthophthalaldehyde. AdoMet and AdoHcy were measured in plasma and whole blood using stable-isotope dilution tandem MS [14]. The intra-assay CVs for AdoMet and AdoHcy were 4.2 and 4.0 % respectively, and the inter-assay CVs for AdoMet and AdoHcy were 7.6 % and 5.9 % respectively. Serum creatinine was measured by means of a modified Jaffé method. Serum folic acid (intra- and inter-assay CVs, 4 and 5 % respectively) and vitamin B₁₂ (intra- and inter-assay CVs, 4 and 5 % respectively) were measured by radioassay (ICN, Costa Mesa, CA, U.S.A.), and serum vitamin B₉ (inter-assay CV 7 %) with the use of fluorescence HPLC [15]. The methionine concentration in the infusate was measured with an amino acid analyser equipped with a high-pressure analytical column packed with Ultrapac 8 resin (Pharmacia Biotech, Cambridge, U.K.). Isotopic enrichment of methionine in plasma was measured in the acetyl-3,5-bis(trifluoromethyl)benzyl derivative with the use of GC–MS, as described previously [16]. Enrichments, expressed in MPE (mole percentage enrichment), were calculated on the basis of the relative abundance of the (m + 0), (m + 1) and (m + 4) methionine species [17], and calibration curves obtained from weighed amounts of tracer (m + 1 and m + 4) and tracee methionine were used to correct for minor instrument variation [16]. The [¹³C]-enrichment of CO₂ in breath samples was measured on a dual-inlet isotope ratio mass spectrometer (VG OPTIMA; Fisons Instruments, Middlewich, Cheshire, U.K.) and expressed in APE (atom percentage excess).
Gene polymorphisms
The polymorphisms of 5,10-methylenetetrahydrofolate reductase (C677T transition), cystathionine β-synthase (844ins68 variant) and methionine synthase (A2756G transition) were assessed in DNA obtained from the buffy coat of EDTA-collected blood as described by Tsai et al. [18].

Calculations
L-[2H3-methyl-1-13C]methionine was used as a tracer, according to the method described by Storch et al. [17]. This stable isotope has a molecular mass of m + 4 relative to natural methionine (m). The 2H3-methyl label is removed from methionine during transmethylation and thus [2H3-methyl-1-13C]methionine is converted into [1-13C]homocysteine. Remethylation will result in the generation of m + 1 methionine, because the [13C] atom of the carboxyl moiety of homocysteine remains intact. In contrast, during trans-sulphuration, the carboxyl moiety of [1-13C]homocysteine loses its [13C] atom. When α-ketobutyrate is oxidized in the Krebs cycle, the label ultimately appears as 13CO2 in breath air. The m + 4 methionine tracer is diluted by methionine entering the pool via the diet, from homocysteine folic-acid-dependent remethylation and by free methionine entering from protein breakdown in the tissues. In the steady state, the rate of appearance of methionine from these sources equals the rate of disappearance (i.e. protein synthesis and transmethylation). In this model, it is assumed that the intracellular and intravascular compartments are in rapid and complete isotopic equilibrium.

From the plasma enrichments of methionine, the whole-body methionine/methyl rate of appearance and disappearance (Qm) and methionine/carboxyl rate of appearance and disappearance (Qc) are calculated as follows:

\[ Q_m = I \times \left( E_a / E_4 - 1 \right) \]
\[ Q_c = I \times \left[ E_a / (E_1 + E_4) - 1 \right] \]

where I is the tracer infusion rate, \( E_a \) is the enrichment of the tracer in the infusate, and \( E_1 \) and \( E_4 \) are the plasma plateau enrichments of \( m + 1 \) and \( m + 4 \) methionine respectively.

As the rate of appearance equals the rate of disappearance, it follows for \( Q_m \):

\[ Q_m = \text{appearance} = D + B + \text{RM} \]
\[ = \text{disappearance} = S + \text{TM} \]

and for \( Q_c \):

\[ Q_c = \text{appearance} = D + B = \text{disappearance} = S + \text{TS} \]

where D is methionine intake via the diet (which is zero during the infusion protocol), B is methionine release from protein breakdown, RM is homocysteine remethylation, S is methionine incorporation in protein synthesis, TM is methionine transmethylation, and TS is homocysteine trans-sulphuration. It follows that:

\[ \text{RM} = Q_m - Q_c. \]

The trans-sulphuration rate is calculated from 13CO2 excretion in breath air (\( V^{13} \text{CO}_2 \)) as follows:

\[ \text{TS} = V^{13} \text{CO}_2 \times \left( 1 / [\text{13C}] \text{methionine enrichment in plasma} \right. \]
\[ \left. - 1 / [\text{13C}] \text{methionine enrichment in tracer infusate} \right) \]

Note that we made the assumption that, in healthy subjects, the transamination pathway is negligible [19]. However, if the transamination pathway is significant, 13CO2 from the \([13C]\)carboxyl-labelled tracer would arise via AdoMet-independent pathways, leading to overestimation of trans-sulphuration and transmethylation in our model. Nevertheless, the estimate of total methionine oxidation would not be affected.

As methionine is the only precursor of homocysteine, homocysteine disappearance (RM + TS) equals homocysteine appearance (TM), thus TM = RM + TS.

The flux rates for remethylation, transmethylation and trans-sulphuration were expressed as µmol·h⁻¹·kg⁻¹ body weight and µmol·h⁻¹·kg⁻¹ of FFM. Because the metabolic homocysteine clearance by trans-sulphuration reflects homocysteine disposal capacity in the steady state [20], we also calculated homocysteine clearance by trans-sulphuration (expressed in l/h) with the formula: [TS (as expressed per kg of FFM) × FFM]/total plasma homocysteine.

Statistical methods
Data were analysed using SPSS version 11.5.0 (SPSS, Chicago, IL, U.S.A.). CIs (confidence intervals) were calculated by CIA version 2.0.0 (Medical Statistics Computing, Southampton, U.K.). The plateau isotopic-enrichment levels were analysed by visual inspection and ANOVA. Values are expressed as medians (range). Differences between medians are expressed as relative changes from baseline in percentages with 95% CIs. Differences between values before and after treatment were tested with the Wilcoxon signed-ranks test. Differences between subgroups were tested with the Mann–Whitney test. Pearson’s test was used for correlation analyses. For calculation of correlations with flux rates, the flux rates were expressed per kg of FFM. A P value < 0.05 was considered to reflect statistical significance.

RESULTS
All 12 subjects completed the study protocol. Plateau plasma methionine and breath air 13CO2 enrichments...
Table 2  Effects of folic acid treatment (5 mg/day) on vitamins and amino acids involved in methionine and homocysteine metabolism in 12 healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>At baseline</th>
<th>After folic acid</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folic acid (nmol/l)</td>
<td>14.6 (7.3–18.7)</td>
<td>42.0 (14.1–180.0)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Serum vitamin B6 (nmol/l)</td>
<td>29 (8–58)</td>
<td>24 (12–55)</td>
<td>0.92</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/l)</td>
<td>223 (175–332)</td>
<td>215 (168–424)</td>
<td>0.48</td>
</tr>
<tr>
<td>Plasma total homocysteine (µmol/l)</td>
<td>7.4 (5.6–10.6)</td>
<td>6.4 (4.5–8.2)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Plasma serine (µmol/l)</td>
<td>90 (75–112)</td>
<td>88 (71–120)</td>
<td>0.06</td>
</tr>
<tr>
<td>Plasma glycine (µmol/l)</td>
<td>184 (52–241)</td>
<td>199 (74–279)</td>
<td>0.03</td>
</tr>
<tr>
<td>Plasma serine/glycine ratio</td>
<td>0.51 (0.37–1.73)</td>
<td>0.47 (0.32–1.20)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AdoMet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In plasma (nmol/l)</td>
<td>88 (61–112)</td>
<td>89 (73–120)</td>
<td>0.70</td>
</tr>
<tr>
<td>In whole blood (nmol/l)</td>
<td>1580 (1095–1865)</td>
<td>1483 (984–1910)</td>
<td>0.25</td>
</tr>
<tr>
<td>AdoHcy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In plasma (nmol/l)</td>
<td>12 (9–17)</td>
<td>11 (8–18)</td>
<td>0.28</td>
</tr>
<tr>
<td>In whole blood (nmol/l)</td>
<td>62 (47–88)</td>
<td>53 (39–97)</td>
<td>0.29</td>
</tr>
<tr>
<td>AdoMet/AdoHcy ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In plasma</td>
<td>6.8 (5.7–10.4)</td>
<td>7.6 (5.9–10.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>In whole blood</td>
<td>25.1 (13.9–39.7)</td>
<td>25.0 (18.7–38.1)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

were obtained in all individuals. Plateau was reached after 220 min. Conditions for MS measurements were similar before and after folic acid treatment (isotopic enrichment ranged between 1–2 MPE for [1-13C]methionine and between 5–10 MPE for [3H3-methyl-1-13C]methionine, and breath air 13CO2 was > 0.002 APE in each subject).

The effects of treatment with folic acid on blood concentrations of vitamins and amino acids are shown in Table 2. In summary, all subjects showed an increase in the serum folic acid concentration (284 %; 95 % CI, 136–629 %). Compared with baseline, plasma total homocysteine decreased by 18 % (95 % CI, −28 to −9 %). The ratio of AdoMet and AdoHcy in plasma increased significantly by 11 % (95 % CI, 1–20 %), which was not the case in whole blood. Changes in plasma and whole-blood concentrations of AdoMet and AdoHcy separately did not reach significance. In plasma, glycine increased significantly by 10 % (95 % CI, 2–22 %) and serine showed a decrease that did not reach the level of significance (−7 %; 95 % CI, −12 to +1 %). The serine/glycine ratio decreased significantly by 20 % (95 % CI, −63 to −6 %).

After folic acid treatment, there was a significant increase in (for FFM corrected) remethylation rate (59 %; 95 % CI, 13–97 %) and transmethylation rate (20 %; 95 % CI, 3–41 %). Changes in trans-sulphuration rate (−9 %; 95 % CI, −27 to +11 %) and metabolic homocysteine clearance by trans-sulphuration (8 %; 95 % CI, −18 to + 35 %) were not significant (Table 3). There were no significant differences in changes in flux rates and homocysteine clearance between men and women, and smokers and non-smokers (results not shown).

Figure 2 shows the effect of folic acid supplementation on remethylation and transmethylation rates of subjects. The two subjects with the highest baseline remethylation rates showed decreases in both remethylation rate as well as transmethylation rate, yet exhibited a decrease in plasma total homocysteine. These two subjects were homozygous wild-type for the C677T transition of the methylenetetrahydrofolate reductase gene, the 844ins68 variant of the cystathionine β-synthase gene, as well as the A2756G transition of the methionine synthase gene, and had baseline concentrations of folic acid and increases of folic acid concentrations that were comparable with the concentrations in the other ten subjects (results not shown).

Changes in remethylation rate did not correlate significantly with changes in plasma total homocysteine concentration (r = −0.38; 95 % CI, −0.74 to + 0.14; P = 0.22). No significant correlations could be demonstrated between changes in any of the one-carbon fluxes and changes in blood concentrations of total homocysteine, AdoMet, AdoHcy, serine, glycine or B vitamins (results not shown).

**DISCUSSION**

The results of the present study show that, in healthy subjects, treatment with folic acid increased rates of whole-body homocysteine remethylation and methionine transmethylation and decreased plasma total homocysteine concentration. Folic acid supplementation did not significantly change homocysteine trans-sulphuration or metabolic homocysteine clearance. In addition,
Table 3 Effects of folic acid treatment (5 mg/day) on one-carbon flux rates of methionine and homocysteine metabolism in 12 healthy subjects in the postabsorptive state

Values are presented as medians (range). BW, body weight. P values were determined using the Wilcoxon signed-ranks test.

<table>
<thead>
<tr>
<th>Flux Type</th>
<th>At baseline</th>
<th>After folic acid</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remethylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressd in BW</td>
<td>4.0 (2.5–7.0)</td>
<td>6.3 (3.8–10.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Expressd in FFM</td>
<td>4.6 (3.4–10.1)</td>
<td>7.6 (4.7–12.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Transmethylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressd in BW</td>
<td>7.1 (4.0–9.8)</td>
<td>8.3 (3.9–13.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>Expressd in FFM</td>
<td>9.3 (5.5–14.2)</td>
<td>10.1 (5.3–16.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Trans-sulphuration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressd in BW</td>
<td>2.9 (1.5–4.6)</td>
<td>2.3 (1.7–5.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>Expressd in FFM</td>
<td>4.0 (2.1–5.5)</td>
<td>3.1 (2.1–6.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Homocysteine clearance by trans-sulphuration</td>
<td>0.41 (0.20–0.75)</td>
<td>0.35 (0.25–0.98)</td>
<td>0.48</td>
</tr>
<tr>
<td>Methionine methyl flux</td>
<td>22.1 (17.0–34.1)</td>
<td>21.8 (18.2–29.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Methionine carboxyl flux</td>
<td>27.2 (23.3–49.2)</td>
<td>28.5 (22.4–35.7)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Figure 2 Individual changes in remethylation and transmethylation after treatment with folic acid (5 mg/day)

The present study shows that folic acid increased the plasma AdoMet/AdoHcy ratio and decreased the serine/glycine ratio.

The 18% decrease in plasma homocysteine concentration after folic acid treatment was comparable with the 16% decrease which was found in the meta-analysis of the Homocysteine Lowering Trialists’ Collaboration in the subgroup of patients with baseline plasma homocysteine concentrations <8.9 μmol/l [7].

We did not find a significant relationship between changes of plasma homocysteine concentration and remethylation rate. Moreover, two subjects demonstrated a decrease in the plasma homocysteine concentration as well as in the remethylation rate. In one subject, this might be explained by the simultaneous increase of homocysteine clearance, which might have accounted for the decrease in plasma homocysteine concentration. In the other subject, a statistical, rather than a biological, explanation for the combined decrease in remethylation rate and plasma homocysteine concentration seems more likely (regression to the mean). In this regard, it should be stressed that our present study was not designed (and thus not powered) to examine relationships between the one-carbon fluxes and plasma concentrations of the various compounds, but rather to examine their mean changes after folate supplementation.

It is possible that in the initial phase of folic acid treatment, an increase in homocysteine remethylation rate lowered the plasma homocysteine concentration (for example by intracellular storage and/or insertion of methionine in proteins). A secondary increase in methionine transmethylation rate could balance the increased remethylation rate at a lower plasma homocysteine concentration. The trans-sulphuration rate was unchanged after folic acid treatment. In weight-maintaining adults, the trans-sulphuration rate reflects oxidation of methionine from dietary intake, because methionine is the only precursor of homocysteine, and trans-sulphuration is the only way of homocysteine disposal (methionine transamination is negligible in normal subjects [19]). In the design of our present study, protein intake was kept constant, and it is thus not surprising that trans-sulphuration
rate was not affected by folic acid treatment. However, it has to be kept in mind that our results reflect the status of methionine and homocysteine metabolism reached after 3 weeks of folic acid treatment, which, inherent to the stable isotope model, do not necessarily reflect previous (possibly transient) changes in flux rates and metabolite concentrations.

Dose–response effects of supplemented folic acid on folic acid and homocysteine concentrations in blood have been shown to flatten at intakes of 400 µg/day [21,22], a dose that could be reached with food fortification [23]. However, the quantitative effects of folic acid supplementation on methyl flux rates are unknown. Therefore it is not clear whether a similar threshold exists for changes in methyl flux rates. Thus we applied a high dose of folic acid with the intention to stimulate remethylation flux maximally. It is not known whether a lower folic acid dose (e.g. 400 µg) induces similar changes in one-carbon fluxes as in our present study. Therefore studies on homocysteine metabolism at the internationally recommended daily folic acid intake of 200 µg [24,25] to 400 µg [26] are warranted.

In our present study, methionine transmethylation was increased by folic acid supplementation. An increase in transmethylation may play a crucial role in any beneficial effect of folic acid intervention. There are indications that hypomethylation, as reflected by DNA methylation status, is important in the pathogenesis of atherosclerosis [27,28]. In addition, patients with manifest vascular disease had low plasma AdoMet/AdoHcy ratios in conjunction with DNA hypomethylation [29]. Hypomethylation can be reversed by folic acid treatment, as demonstrated in end-stage renal disease patients by Ingrosso et al. [30]. However, our present study suggests that changes in transmethylation rate may not accurately be reflected by changes in plasma homocysteine concentration in healthy subjects. Thus the degree of homocysteine concentration reduction may not be a good predictor of clinical benefit of folic-acid-based interventions [31].

Folic acid therapy lowered the serine/glycine ratio in the present study. Serine is, by conversion into glycine, a major donor of one-carbon units used in the folic-acid-dependent remethylation of homocysteine to methionine [32]. The observed decrease in the serine/glycine ratio is consistent with the use of serine as a one-carbon donor [33]. Glycine itself is needed for several biosynthetic pathways, including creatine, porphyrins, purines, bile acids and glutathione [34]. It can be hypothesized that the folic-acid-induced changes in the serine/glycine ratio might have affected these biosynthetic pathways, but the exact implications of these findings are unknown.

Taken together, our present data show that oral folic acid administration lowers plasma homocysteine concentrations and increases whole-body remethylation and transmethylation fluxes.

ACKNOWLEDGMENTS

This study was supported financially by the Dutch Kidney Foundation. We thank Desirée Smith and Wim Kulik for technical assistance.

REFERENCES


Received 12 October 2004; accepted 12 January 2005
Published as Immediate Publication 12 January 2005, DOI 10.1042/CS20040295