Deficiencies of folate and vitamin B₆ exert distinct effects on homocysteine, serine, and methionine kinetics

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Cuskelly, Geraldine J., Peter W. Stacpoole, Jerry Williamson, Thomas G. Baumgartner, and Jesse F. Gregory III. Deficiencies of folate and vitamin B₆ exert distinct effects on homocysteine, serine, and methionine kinetics. Am J Physiol Endocrinol Metab 281: E1182–E1190, 2001.—Folate and vitamin B₆ act in generating methyl groups for homocysteine remethylation, but the kinetic effects of folate or vitamin B₆ deficiency are not known. We used an intravenous primed, constant infusion of stable isotope-labeled serine, methionine, and leucine to investigate one-carbon metabolism in healthy control (n = 5), folate-deficient (n = 4), and vitamin B₆-deficient (n = 5) human subjects. The plasma homocysteine concentration in folate-deficient subjects [15.9 ± 2.1 (SD) μmol/l] was approximately two times that of control (7.4 ± 1.7 μmol/l) and vitamin B₆-deficient (7.7 ± 2.1 μmol/l) subjects. The rate of methionine synthesis by homocysteine remethylation was depressed (P = 0.027) in folate deficiency but not in vitamin B₆ deficiency. For all subjects, the homocysteine remethylation rate was not significantly associated with plasma homocysteine concentration (r = −0.44, P = 0.12). The fractional synthesis rate of homocysteine from methionine was positively correlated with plasma homocysteine concentration (r = 0.60, P = 0.031), and a model incorporating both homocysteine remethylation and synthesis rates closely predicted plasma homocysteine levels (r = 0.85, P = 0.0015). Rates of homocysteine remethylation and serine synthesis were inversely correlated (r = −0.89, P < 0.001). These studies demonstrate distinctly different metabolic consequences of vitamin B₆ and folate deficiencies.

MILD ELEVATION OF PLASMA HOMOCYSTEINE is an independent risk factor for cardiovascular disease, peripheral arterial occlusive disease, stroke, and venous thrombosis (1, 2, 5, 12, 29). For example, Boushey et al. (1) calculated in a meta-analysis that the odds ratio for coronary artery disease in males for each 5 μmol/l increase in plasma homocysteine is 1.6. However, it is unclear whether homocysteine is directly involved in the pathogenesis of these vascular disorders or whether elevated homocysteine is a marker for other pathogenic events. This controversy has been addressed in recent reviews (4, 34, 44).

Steady-state concentrations of homocysteine in tissues and plasma are a function of the rates of homocysteine formation, remethylation, catabolism, and renal excretion (29, 35). Plasma homocysteine concentration is modulated by vitamin nutritional status for folate, vitamin B₆, and vitamin B₁₂ (3, 6, 20, 36). Plasma homocysteine concentration is influenced by the intake of these vitamins from dietary sources and nutritional supplements, and supplementation can at least partially normalize elevations in plasma homocysteine. It is not currently known whether reduction of plasma homocysteine by vitamin supplementation reduces the risk associated with elevated homocysteine. However, it is clear that homocysteine metabolism is altered by deficiency of these vitamins. Folate and vitamin B₆ insufficiency states have been associated with the risk and incidence of vascular disease, and a significant inverse relationship is reported to exist between the risk of fatal coronary heart disease and nutritional status for folate, vitamin B₆, or both (24, 30–32, 45–46). In the largest study to date, Rimm et al. (30) reported that risk of coronary heart disease in ~80,000 nurses declined with increasing intake of folate and of vitamin B₆.

Both folate and vitamin B₆ play essential functional roles in the metabolism of homocysteine. The remethylation of homocysteine to form methionine occurs primarily by transfer of a methyl group from 5-methyltetrahydrofolate (5-methyl-THF), a reaction catalyzed by methionine synthase, and, to a lesser extent, from betaine, catalyzed by betaine-homocysteine methyltransferase (10, 47). Serine and glycine, which undergo interconversion through serine hydroxymethyltransferase (SHMT)-catalyzed reactions, are major sources of one-carbon units for essential cellular functions.
including the conversion of homocysteine to methionine and for the synthesis of purines and thymidylate (8, 47). Inadequate folate nutrition raises fasting plasma homocysteine concentration by reducing the cellular concentration of folate coenzymes necessary for the acquisition of one-carbon units and their enzymatic reduction to methyl groups (35, 47). We have reported in vivo kinetic evidence, using [2,3,3-2H]serine as a tracer, that serine is processed in human one-carbon metabolism through pathways involving both cytosolic and mitochondrial forms of SHMT to yield labeled methyl groups (as 5-methyl-THF) available for remethylation of homocysteine (14).

Vitamin B6 deficiency elevates plasma homocysteine (23, 36–38), although the effect is less pronounced than that of folate deficiency on fasting plasma homocysteine concentration (22). Vitamin B6 can alter homocysteine metabolism by reducing the activity of SHMT and by suppressing homocysteine catabolism (35, 39, 43). Both cytosolic and mitochondrial forms of SHMT require pyridoxal 5'-phosphate (PLP) as a coenzyme for catalysis of the reversible transfer of the three-carbon of serine to THF to form 5,10-methylene-THF and glycine. In the transsulfuration pathway of homocysteine catabolism, both cystathionine β-synthase and γ-cystathionase require PLP.

The affinity of SHMT for its coenzyme, PLP, is rather weak (dissociation constant \(27 \mu M\); see Ref. 18), which suggests that in vivo SHMT activity would be sensitive to mild vitamin B6 deficiency without clinically overt signs of deficiency, as may occur in humans (13). Substantial reductions in hepatic SHMT activity and in vivo rate of homocysteine remethylation have been observed in vitamin B6-deficient rats (21). Because of complex regulatory interactions, such as the inhibitory effect of S-adenosylmethionine (SAM) on methyl group formation and the stimulation by SAM of cystathionine β-synthase (47), the full effects of a vitamin B6 deficiency may not be predictable solely on the basis of coenzyme availability. γ-Cystathionase, the second enzyme in the transsulfuration pathway, appears to be more susceptible to coenzyme depletion than is cystathionine β-synthase (21, 39).

Stable isotopically labeled amino acids have enabled determination of the basic kinetics of methionine, homocysteine, and cysteine in humans, as reviewed by Young et al. (51). However, little information is available regarding the integration of serine and other carbon sources in one-carbon metabolism. Radiolabeled serine (3-14C) has been used to investigate the overall kinetics of one-carbon acquisition and labeling (via 5-methyl-THF) of the methionine methyl group pool in rats (33), and analogous studies have been conducted in cell culture (25). The protocol reported here is an extension of these procedures, in which we use stable isotope-labeled serine, methionine, and leucine tracers to simultaneously examine several aspects of one-carbon metabolism and transsulfuration reactions that influence homocysteine levels. In this study, we obtained data on the kinetics of human one-carbon metabolism in young adults who were in adequate nutritional status or who were deficient in folate or vitamin B6. We measured the rates of 1) appearance of serine, methionine, and leucine, 2) remethylation of homocysteine to form methionine, 3) synthesis of homocysteine from methionine via transmethylolation, and 4) acquisition of carbon units from serine and their use in homocysteine remethylation in these subjects in the basal state and during each nutritional perturbation.

**METHODS**

**Materials**

L-[5,5,5-2H]leucine and L-[2,3,3-2H]serine were purchased from Cambridge Isotope Laboratories (Woburn, MA), and [1-13C]methionine was purchased from Isotec (Miamisburg, OH). Before each infusion procedure, these tracers were dissolved in isotonic saline solution, sterilized by filtration, and analyzed to verify sterility and lack of pyrogenicity. Pyrogenicity assays were performed by a commercial clinical laboratory using a quantitative kinetic chromogenic limulus amebocyte lysate procedure.

**Human Subjects**

Subjects were healthy men and women (age 20–30 yr) who were nonsmokers, not taking oral contraceptives or medications known to interfere with folate or B6 metabolism, and not taking vitamin supplements. The protocol was approved by the University of Florida Institutional Review Board, and all subjects gave written informed consent to participate. The subjects were in good health as determined by a medical questionnaire, physical examination, and a clinical biochemistry screen. Nutritional status was determined initially and was monitored during dietary treatments by measuring the plasma levels of folate (41), PLP (42), and total homocysteine (28). DNA from whole blood was also analyzed to determine the genotype with respect to the common C677T polymorphism (cytosine → thymine) at base pair 677 of methylenetetrahydrofolate reductase (MTHFR; see Ref. 11).

**Dietary Treatments**

Subjects were randomly assigned to control (adequately nourished), folate-deficient, or vitamin B6-deficient groups with approximately balanced distribution by gender. To induce a moderate state of folate depletion, four subjects were fed a folate-deficient diet (50 μg total folate/day) for 6 wk (27). Another five subjects consumed a vitamin B6-deficient diet (0.3 mg vitamin B6/day) for 4 wk (7). A third group consumed their habitual self-selected diet, and each subject was verified biochemically to be in a state of nutritional adequacy (control group). In groups receiving deficient diets, custom multivitamin-mineral supplements that provided the 1989 Recommended Dietary Allowance (26) were administered daily to maintain adequate nutritional status for nutrients other than those intentionally depleted. The control subjects were fed a standardized, weight-maintaining diet for 3 days before the infusion, with the sole purpose of avoiding extremes in dietary intake, especially with respect to protein.

**Infusion Protocol**

Subjects were admitted to the University of Florida General Clinical Research Center at Shands Hospital on the evening before infusion and consumed no food after 8:30 PM until the procedure was started the following morning. At 7:30 AM, a 21-gauge needle was inserted in a vein on each
arm, one for infusion and one for blood sampling. The study began at 8:30 AM after subjects had been fasted for 12 h. At 8:20 AM, a fasting blood sample was taken. At 8:30 AM, each subject received a 20-ml priming dose of 9.26, 1.91, and 0.25 μmol/kg of [3H3]serine, [3H3]leucine, and [1-13C]methionine, respectively, in saline over 5 min. Immediately afterward, a constant infusion of the same tracer solution was begun and was continued at 20 ml/h for 9 h. Blood samples were taken at time 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7.5, and 9 h. Hourly during the infusion, subjects were given a low-protein liquid formula to provide a source of energy from carbohydrate and fat (14).

**Determination of Isotopic Labeling of Plasma Amino Acids**

Free amino acids were isolated and purified from plasma by cation-exchange chromatography (19). A 0.2-ml aliquot of plasma was acidified with an equal volume of 2 mol/l acetic acid. Disposable cation-exchange columns were prepared with 1 ml of AG-50W-X8 cation-exchange resin, H+ form (Bio-Rad Laboratories, Hercules, CA). The resin was washed two times with 1 mol/l HCl, and the acidified plasma was applied. After two washes with distilled, deionized water, the amino acids were eluted from the columns with 3 ml of 3 mol/l ammonium hydroxide and then evaporated to dryness under a stream of N2 gas at ~50°C. Because the isolation of plasma amino acids did not involve reduction of disulfide bonds, this analysis would be specific for free forms of cysteine and homocysteine. N-heptafluorobutyryl n-propyl ester derivatives of leucine, serine, cysteine, homocysteine, and methionine were made in a two-step derivatization procedure under anhydrous conditions (19). The samples were esterified with a solution of n-propanol and acetyl chloride (5:1 vol/vol) at 110°C for 1 h and then evaporated to complete dryness under nitrogen. Samples were then reacted with heptafluorobutyric anhydride at 60°C for 1 h. Ethanethiol was added to the reaction mixture in the esterification and derivatization steps to increase the yield of cysteine and homocysteine (15). The samples were dissolved in ethyl acetate, transferred to autosampler vials, and stored at ~20°C until analyzed.

Isotopic enrichment was determined by electron-capture negative chemical-ionization gas chromatography-mass spectrometry (GC-MS) with methane as reagent gas (42), using a Finnigan-Thermoquest Voyager instrument (San Jose, CA). The analysis was conducted by selected ion monitoring at the following mass-to-charge ratios for the analytes of interest: serine, 519–522; leucine, 349–352; homocysteine, 549–550; cysteine, 535–538; and methionine, 367–370. Enrichment values are expressed as molar ratios of labeled/nonlabeled isotopomers after correction for the natural abundance of stable isotopes. These computations were conducted essentially as described by Storch et al. (40).

**Kinetic Analysis and Interpretation of Data**

Isotopic equilibrium was assessed initially by visual inspection, and plateau enrichments were calculated on the basis of mean values for plateau points. The isotopic enrichment data are presented as the group mean at each time point of infusion. Kinetic variables were calculated for each subject using standard procedures (48), as follows.

**Rate of appearance of serine, leucine, and methionine in the plasma pool.** The rate of appearance (Ra) of serine, leucine, and methionine in the plasma pool was calculated as

\[
Ra = \frac{\text{tracer infusion rate}}{\text{plasma plateau enrichment of [1-13C]serine}}
\]

As has been observed in previous studies in rats and humans (14, 21), the infused [3H3]serine tracer undergoes in vivo changes of labeling because of the reversible passage of serine through SHMT-catalyzed reactions and, potentially, through reversible oxidation and reduction of a labeled carbon unit via 5,10-methyltetrahydrofolate dehydrogenase before SHMT-catalyzed coupling to unlabeled glycine. Consequently, 3H and 2H forms of serine appear in plasma in addition to the predominant [3H3]serine from the infusion. For calculation of Ra, we used the plateau enrichment of the infused species, [3H3]serine, as the denominator (14).

**Relative in vivo synthesis rates for methionine and serine.** Determining the rate of synthesis of methionine and serine required a means of adjusting the Ra values obtained above by the rate at which methionine and serine appear from the turnover of body proteins. Based on the assumption that such proteolysis would release serine and methionine in direct proportion to the release of leucine, we determined the relative in vivo rates of synthesis of methionine and serine by dividing each subject’s Ra value for serine and methionine by the Ra value for leucine (i.e., Ra_Met/Ra_Leu and Ra_Ser/Ra_Leu). Because leucine appeared solely from protein turnover in this protocol, the ratio served as a way to normalize for variation in serine and methionine appearance to compensate for differences in protein turnover. Thus this ratio allowed us to determine relative rates of in vivo synthesis of methionine, by homocysteine remethylation, and of serine, via SHMT and from transamination of 3-phosphohydroxyypyruvate. This procedure is analogous to that used previously for assessing proline synthesis (17) and avoids assumptions regarding absolute rates of release of amino acids from turnover of body proteins (48).

**Cytosolic fractional synthesis rate of methionine from serine.** The cytosolic fractional synthesis rate (FSR) of methionine from serine was calculated as

\[
\text{FSR (methionine from serine)} = \frac{\text{slope} \times [\text{2H}]\text{methionine}}{\text{plasma plateau enrichment of [3H3]serine}}
\]

where slope is the initial rate of labeling. The cytosolic fractional synthesis rate (methionine from serine) measures the net rate of remethylation and reflects the net carbon flux from serine directly through cytosolic (nonmitochondrial) metabolism involving reactions catalyzed by cytosolic SHMT, MTHFR, and methionine synthase (14).

**Fractional synthesis rate of homocysteine from methionine.** The fractional synthesis rate of homocysteine from methionine was calculated as

\[
\text{FSR (homocysteine from methionine)} = \frac{\text{slope} \times [1-13C]\text{homocysteine}}{\text{plateau enrichment of [1-13C]methionine}}
\]

This measurement reflects the net rate of conversion of methionine to homocysteine via synthesis of SAM, participation in transmethylation reactions, and hydrolysis of the resulting S-adenosylhomocysteine, as determined from [1-13C]methionine and [1-13C]homocysteine enrichment data. We recognize that, in this protocol, the measurement of [1-13C]methionine is confounded partially by the metabolic formation of [methyl-2H3]methionine formed by homocysteine remethylation that occurs with one-carbon units derived mainly from mitochondrial metabolism of [3H3]serine (14). Because of their identical masses, [1-13C]methionine and [methyl-2H3]methionine cannot be differentiated by GC-MS. However, because the rate of infusion of [1-13C]methionine greatly exceeds the extent of metabolic formation of [methyl-2H3]methionine, this does not seriously bias measurements of Ra_Met and FSR (homocysteine from methionine) in this study.
Statistical Analysis

Data were analyzed by one-way ANOVA with multiple comparisons by the Student-Newman-Keuls method (13). Data are presented as means ± SD. Multiple regression and Pearson product moment correlation analyses were conducted on plasma homocysteine and kinetic values from all subjects to evaluate the strength of any relationships among these variables (13). Differences were considered significant at \( P < 0.05 \).

RESULTS

Nutritional Status of Subjects

The dietary treatments were effective in inducing moderate deficiencies of folate and vitamin B6. Subjects consuming the low-folate diet exhibited a change in plasma folate from 23.0 ± 5.8 to 8.2 ± 3.8 nmol/l. Those consuming the low-B6 diet had a reduction in plasma PLP concentration from 40.7 ± 10.0 to 12.3 ± 4.0 nmol/l over the course of dietary treatment. The mean fasting plasma total homocysteine concentration of the folate-deficient group at the end of the depletion period (15.9 ± 2.1 \( \mu \)mol/l) was approximately two times that of the control group (7.4 ± 1.7 \( \mu \)mol/l) and of the vitamin B6-deficient group (7.7 ± 2.1 \( \mu \)mol/l; \( P < 0.05 \)). In contrast, there was no significant difference in plasma homocysteine concentration between the control group and the vitamin B6-deficient group at the end of dietary depletion.

Plasma Leucine, Methionine, and Serine Turnover Kinetics

The typical labeling curves for the various plasma amino acids examined in this study are presented in Fig. 1, which graphically presents data for the control group and shows the rate and extent of labeling. Table 1 shows the plateau enrichments for plasma leucine, methionine, serine, and homocysteine and steady-state Ra Met, Ra Ser, and Ra Leu for all treatment groups. No significant labeling of cysteine was detected. Ra Leu did not change significantly with diet treatment. However, the tendency toward higher Ra Leu and greater between-subject variability in folate deficiency suggests that a faster protein turnover occurred during folate deficiency in some subjects. No significant differences were observed in Ra Met values among diet groups, although the mean for the folate-deficient group was 27% lower than for the control group. The Ra Ser (Table 2) did not change significantly with diet treatment, although group means and between-subject variability increased substantially in folate and B6 deficiency. This greater variability during folate and vitamin B6 deficiencies is shown clearly in Fig. 2, which presents Ra data for individual subjects.

Methionine, Serine, and Homocysteine Synthesis Kinetics

The “relative synthesis rates” for methionine (Ra Met/Ra Leu) and for serine (Ra Ser/Ra Leu) are shown in Table 2. The rate of methionine synthesis (by remethylation of homocysteine) of the folate-deficient group was significantly less than the control group (by 39%, \( P = 0.027 \)), although there was no significant difference between the vitamin B6-deficient and control groups. No significant effects of diet were observed on the relative synthesis rate (Ra Ser/Ra Leu) of serine (\( P = 0.27 \)), although a substantial increase of the mean and significantly greater within-group variability were observed in fo-

Table 1. Plateau isotopic enrichment of stable isotope-labeled amino acids during steady-state primed, constant infusion of controls, folate-deficient subjects, and vitamin B6-deficient subjects with \([\text{2H}_3]\text{serine}, [\text{3H}_2]\text{leucine}, \text{and} [\text{1-13C}]\text{methionine}

<table>
<thead>
<tr>
<th>Amino Acid Species</th>
<th>Control ((n = 5))</th>
<th>Folate Deficient ((n = 4))</th>
<th>Vitamin B6 Deficient ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{2H}_3]\text{serine})</td>
<td>0.0509 ± 0.0095</td>
<td>0.0304 ± 0.0207</td>
<td>0.0554 ± 0.2097</td>
</tr>
<tr>
<td>([\text{2H}_2]\text{serine})</td>
<td>0.0187 ± 0.0061</td>
<td>0.0100 ± 0.0063</td>
<td>0.0129 ± 0.0042</td>
</tr>
<tr>
<td>([\text{3H}]\text{leucine})</td>
<td>0.0186 ± 0.0036</td>
<td>0.0120 ± 0.0093</td>
<td>0.0135 ± 0.0089</td>
</tr>
<tr>
<td>([\text{1-13C}]\text{methionine})</td>
<td>0.0184 ± 0.0010</td>
<td>0.0150 ± 0.0046</td>
<td>0.0172 ± 0.0037</td>
</tr>
<tr>
<td>([\text{1-13C}]\text{homocysteine})</td>
<td>0.0061 ± 0.0072</td>
<td>0.1111 ± 0.0543</td>
<td>0.0713 ± 0.0159</td>
</tr>
<tr>
<td>([\text{1-13C}]\text{methionine})</td>
<td>0.0026 ± 0.0009</td>
<td>0.0026 ± 0.0006</td>
<td>0.0036 ± 0.0011</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \), no. of subjects. Isotopic enrichment is expressed as the molar tracer/tracee ratio after correction for natural abundance.

Fig. 1. Time course of isotopic labeling of plasma amino acids during primed, constant infusion with \([\text{2H}_3]\text{serine}, [\text{3H}_2]\text{leucine}, \text{and} [\text{1-13C}]\text{methionine}. Values shown are means ± SD for control subjects. Results for folate-deficient and vitamin B6-deficient subjects were qualitatively similar.
late deficiency and vitamin B₆ deficiency. These findings suggest that serine synthesis is increased during these deficiency states.

The \( \frac{R_{a \text{Met}}}{R_{a \text{Leu}}} \) reflects the total rate of methionine synthesis by homocysteine remethylation (i.e., from methyl groups formed from both mitochondrial and cytosolic one-carbon metabolism). In addition, measuring the rate of production of \([\text{methyl-}^{2}\text{H}_2]\text{methionine}\) allows us to estimate the aggregate rates of the cytosolic acquisition of one-carbon units from infused \(\text{[^{2}H}_3]\text{serine}\), their conversion to methyl groups, and their use in homocysteine remethylation (14). The fractional synthesis rates for homocysteine (Table 2). No significant differences were observed among controls (0.046 ± 0.008 h⁻¹), folate-deficient subjects (0.069 ± 0.022 h⁻¹), or vitamin B₆-deficient subjects (0.041 ± 0.006 h⁻¹).

**Distribution of Serine Isotopomers**

The action of SHMT and 5,10-methylenetetrahydrofolate dehydrogenase on the infused \(\text{[^{2}H}_3]\text{serine}\) tracer formed \(\text{[^{2}H}_1]\text{serine}\) and \(\text{[^{2}H}_2]\text{serine}\) during the infusion (Fig. 1 and Table 2). There was no significant effect of dietary treatment on the relative concentrations of these serine isotopomers, so all data were pooled in calculating the percentage of distributions (\(\text{[^{2}H}_1, 21.0 ± 2.9\%} \), \(\text{[^{2}H}_2, 19.5 ± 2.2\%} \), and \(\text{[^{2}H}_3, 59.5 ± 3.0\%} \)).

**Relationships Among Kinetic Variables and Plasma Homocysteine**

When the data for all subjects in all three dietary treatment groups were pooled, we found that the relative rate of homocysteine remethylation (\( \frac{R_{a \text{Met}}}{R_{a \text{Leu}}} \)) was negatively correlated with that of serine production (\( \frac{R_{a \text{Ser}}}{R_{a \text{Leu}}} \); \( r = -0.89, P < 0.001 \); Fig. 3). Plasma total homocysteine concentration was positively correlated with the fractional synthesis rate of homocysteine from methionine (\( r = 0.64, P = 0.033 \); Fig. 4). Nonsignificant trends were observed between plasma

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**Table 2. Kinetic analysis of amino acid turnover and synthesis in adequately nourished controls, folate-deficient subjects, and vitamin B₆-deficient subjects calculated from steady-state primed, constant infusion with \(\text{[^{2}H}_3]\text{serine}\), \(\text{[^{2}H}_3]\text{leucine}\), and \([1-13\text{C}]\text{methionine}\)**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control ((n = 5))</th>
<th>Folate Deficient ((n = 4))</th>
<th>Vitamin B₆ Deficient ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_{a \text{Ser}} ), ( \mu\text{mol-kg}^{-1}\cdot\text{h}^{-1} )</td>
<td>188 ± 44</td>
<td>504 ± 444</td>
<td>274 ± 278</td>
</tr>
<tr>
<td>( R_{a \text{Leu}} ), ( \mu\text{mol-kg}^{-1}\cdot\text{h}^{-1} )</td>
<td>111 ± 18</td>
<td>137 ± 42</td>
<td>117 ± 31</td>
</tr>
<tr>
<td>Relative rate of serine synthesis, ( \frac{R_{a \text{Ser}}}{R_{a \text{Leu}}} )</td>
<td>1.69 ± 0.12</td>
<td>3.48 ± 2.31</td>
<td>2.07 ± 1.42</td>
</tr>
<tr>
<td>Cytosolic FSR (Met from Ser), h⁻¹</td>
<td>0.0180 ± 0.0064</td>
<td>0.0157 ± 0.0157</td>
<td>0.0388 ± 0.053</td>
</tr>
<tr>
<td>FSR (homocysteine from Met), h⁻¹</td>
<td>0.0456 ± 0.017</td>
<td>0.0686 ± 0.0437</td>
<td>0.0410 ± 0.0128</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \), no. of subjects. \( R_a \), rate of appearance; FSR, fractional synthesis rate. Values followed by different superscripts are significantly different, \( P < 0.05 \).
homocysteine concentration and the relative rate of homocysteine remethylation ($R_{a\ Met}/R_{a\ Leu}$; $r = -0.44$, $P = 0.12$) and between plasma homocysteine and the cytosolic fractional synthesis rate of methionine from serine ($r = -0.49$, $P = 0.087$; Fig. 4).

We used multiple regression analysis to determine the degree to which plasma homocysteine concentration is predicted by the relative rate of homocysteine remethylation ($R_{a\ Met}/R_{a\ Leu}$) and the fractional synthesis rate of homocysteine production by transmethylation from SAM. The multiple regression equation

$$\text{Plasma homocysteine (}$\mu\text{mol/l}$) = 14.6 - (46.7 \times R_{a\ met}/R_{a\ leu}) + [100.1 \times \text{FSR(homocysteine from Met)}]$$

was highly significant ($r = 0.85$, $P = 0.0015$) and showed that the combined effects of homocysteine remethylation rate and synthesis rate were highly predictive of fasting homocysteine concentration, more so than any single kinetic factor.

**Effects of MTHFR C677T Polymorphism**

The C677T MTHFR genotypes of the subjects, determined after the conclusion of the study, were as follows, with cytosine as C and thymine as T: control (2 C/C, 3 C/T), folate deficient (2 C/T, 2 T/T), and vitamin B6 deficient (2 C/C, 3 C/T). Within the limited power of this study, there were no discernible trends or effects of the genotype. Particularly noteworthy was the folate-deficient group, in which by chance both T/T subjects were assigned. The folate-deficient subjects exhibited a wide range of relative rates of methionine synthesis by homocysteine remethylation. On a population basis, it would be expected that the T/T genotype is associated with lower rates of homocysteine remethylation than in C/C or C/T, especially during folate deficiency (4, 11, 29, 34–35, 44). However, the two T/T subjects in the folate-deficient group actually showed higher rates of homocysteine remethylation than did the two C/T subjects in this group, as shown in Fig. 3.

Fig. 3. Reciprocal relationship between the relative rate of serine synthesis ($R_{a\ Ser}/R_{a\ Leu}$) and methionine synthesis from homocysteine remethylation ($R_{a\ Met}/R_{a\ Leu}$). Each data point constitutes results from an individual subject. The genotype for the methylenetetrahydrofolate reductase C677T polymorphism is designated for folate-deficient subjects.

Fig. 4. Correlation between plasma total homocysteine concentration and the fractional synthesis rate of homocysteine (A), the relative rate of methionine synthesis (B), and the fractional synthesis rate of cytosolic methionine synthesis from serine-derived methyl groups (C). Each data point constitutes results from an individual subject.
We modified a recently reported (14) primed, constant infusion protocol to investigate the influence of dietary deficiency of folate or vitamin B₆ on in vivo human one-carbon metabolic reactions and on the kinetics of several aspects of methionine and homocysteine metabolism. Although the number of subjects was small, the statistical power was sufficient to detect significant effects of folate deficiency and relationships among plasma homocysteine concentrations and the kinetic variables. The data should be useful in designing additional research to further clarify the nutritional and genetic control of homocysteine and one-carbon metabolism in humans. The subjects in folate- and vitamin B₆-deficient groups achieved levels of deficiency that are equivalent to those encountered from nutritionally inadequate diets. The depletion of functional tissue folate pools was indicated by the change of plasma folate from 23.0 ± 5.8 to 8.2 ± 3.8 nmol/l and by the final mean homocysteine concentration (15.9 ± 2.1 μmol/l) that is clearly in the range associated with increased risk of vascular disease (1, 29). Although the mean plasma folate concentration of depleted subjects was greater than the generally used cutoff of 6 nmol/l (16), the fact that plasma homocysteine concentration greatly exceeded that of the control confirms that a functional deficiency of folate was induced in tissues. Changes in plasma PLP concentration in the vitamin B₆-deficient group (40.7 ± 10.0 nmol/l at start of the study, 12.3 ± 4.0 nmol/l after dietary treatment) demonstrate that depletion of vitamin B₆ occurred. The mean plasma PLP of 12.3 nmol/l is substantially less than the general cutoff value of 20 nmol/l (16); however, this was not associated with significant elevation in plasma homocysteine concentration. Moderate depletion of vitamin B₆ has little effect on fasting homocysteine concentrations (23), and the measurement of the increase in plasma homocysteine after a methionine load, which was not performed in this study, serves as a common functional test for effects of vitamin B₆ status on homocysteine catabolism (22).

To our knowledge, this is the first kinetic investigation of the metabolic effects of folate and vitamin B₆ deficiencies in humans. Ra Met/Ra Leu in control subjects [111 ± 18 (SD) μmol·kg⁻¹·h⁻¹] is similar to that observed previously by us for a single adequately nourished subject (14) and is slightly higher than that reported by others (94 ± 3.6 (SE) μmol·kg⁻¹·h⁻¹; see Ref. 44). The concurrent use of leucine, serine, and methionine tracers allowed us to measure several indexes of homocysteine and one-carbon metabolism but provides relative, rather than absolute, rates of homocysteine remethylation. Thus it lacks many of the quantitative aspects of that devised by Storch et al. (40) for thoroughly investigating methionine metabolism. However, our method generates information regarding the role of serine in homocysteine remethylation and thus yields data that cannot be obtained otherwise.

Schalinske and Steele (33a) reported data from a radioisotopic study of one-carbon metabolism in rats which indicated that folate deficiency caused a two-fold increase in the carbon flux in folate-dependent catabolism of histidine to CO₂. This observation is contrary to the expected effect of folate deficiency. Compared with the results of this study in rats, the results of the present study in which folate deficiency caused a reduction in the relative rate of homocysteine remethylation suggest that folate-dependent homocysteine remethylation may be more sensitive to nutritional deficiency than is histidine catabolism. In addition, these comparative results suggest different regulation of these two folate-dependent pathways during folate deficiency.

Perhaps the most important observation of this study is the large reduction in the rate of methionine synthesis in folate deficiency. This indicates that a substantial impairment of folate-dependent remethylation of homocysteine occurs at the level of folate depletion present in our subjects. Compared with the control group, the folate-deficient subjects exhibited ~115% greater plasma homocysteine concentration. This was concurrent with a 37% difference between the control and folate-deficient groups in the overall remethylation rate. The fact that plasma homocysteine concentration increased to a greater extent than the homocysteine remethylation rate was reduced is consistent with the current understanding that the remethylation rate is but one of several mechanisms by which plasma homocysteine concentration is controlled. This is further shown by the fact that we found only a nonsignificant trend toward an inverse relationship between fasting plasma homocysteine concentration and the relative rate of homocysteine remethylation (Ra Met/Ra Leu; r = 0.44, P = 0.12). However, the close relationship between our kinetic measures of both homocysteine remethylation and synthesis rates and plasma homocysteine concentration shown by multiple regression (r = 0.85, P = 0.0015) indicates that most of the variation in fasting plasma homocysteine can be explained by the combination of these processes under the conditions of this study. Conversely, these findings show that plasma homocysteine concentration is highly sensitive to changes in both the rate of folate-dependent remethylation and transmethylation reactions and that the betaine-dependent homocysteine remethylation process (10) does not compensate for the effects of folate deficiency under the conditions of this study. The lack of a discernible effect of vitamin B₆ deficiency on the overall rate of homocysteine remethylation is consistent with the belief that marginal deficiency of vitamin B₆ has little effect on homocysteine metabolism unless the pathways are challenged with excess substrate, as in a methionine load test (35). The lack of an effect of the vitamin B₆ deficiency on homocysteine metabolism in this study is in contrast to our recent study in rats that showed major reductions in both remethylation and transulfuration kinetics in vitamin B₆ deficiency (21). This apparent discrepancy may be due to the more severe degree of vitamin B₆ deficiency induced.
in the animals along with the use of a flooding-dose technique. It is likely that vitamin B₆ deficiency also indirectly affects homocysteine remethylation by serine-derived one-carbon units because of the substantial roles of PLP-dependent serine dehydratase and serine-pyruvate/alanine-glyoxylate aminotransferase in serine homoeostasis (49, 50). The trend in the vitamin B₆-deficient group toward an increased rate of homocysteine remethylation via cytosolic one-carbon metabolism observed in this study may illustrate such an effect.

Several other findings of this study shed new light on human one-carbon and homocysteine metabolism. First, the significant correlation between plasma homocysteine concentration and the fractional synthesis rate of homocysteine from methionine indicates that, under the conditions we employed, homocysteine formation through the transmethylation cycle is actually increased. This is unexpected in view of known regulatory mechanisms by which reduced availability of SAM, as would be expected to occur under conditions of high plasma homocysteine, would enhance the production of additional 5-methyl-THF (47). Perhaps such increases in the rate of homocysteine synthesis are accompanied by equal or greater increases in the rate of transsulfuration. Unfortunately, no labeling of plasma cysteine was detected during the time course of these infusions, which precluded an assessment of dietary effects on transsulfuration kinetics. A similar lack of measurable labeling of plasma cysteine during primed, constant infusion of a serine tracer in humans has been reported previously (9), but we have observed labeling of plasma and hepatic cysteine after an intraperitoneal flooding dose of serine in rats (21).

The second interesting relationship found in this study is the reciprocity between the rates of serine synthesis and methionine synthesis. Serine synthesis from glycine and the remethylation of homocysteine are two processes that represent alternative uses of the one-carbon group 5,10-methylenetetrahydrofolate. Our observation suggests that, under conditions of a reduced rate of methyl group generation, the one-carbon unit of 5,10-methylenetetrahydrofolate is directed toward serine synthesis. This may constitute a means of conserving one-carbon units for subsequent use in cellular metabolism. Finally, we also found a negative trend between plasma homocysteine and the cytosolic fractional synthesis rate of methionine from serine. This observation, if confirmed in a study of greater statistical power, is consistent with our previous interpretation of observations in rats and in a preliminary human investigation that both cytosolic and mitochondrial pathways are operative in generating one-carbon units for use in homocysteine remethylation (23, 31). Finally, the observation of increased variability of kinetic data during both folate deficiency and vitamin B₆ deficiency suggests that future studies of nutritional effects should involve kinetic analysis before and after nutritional intervention in each human subject.

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