Dietary vitamin B-6 restriction does not alter rates of homocysteine remethylation or synthesis in healthy young women and men1–4

Steven R Davis, Jennifer B Scheer, Eoin P Quinlivan, Bonnie S Coats, Peter W Stacpoole, and Jesse F Gregory III

ABSTRACT

Background: The effects of vitamin B-6 status on steady-state kinetics of homocysteine metabolism in humans are unclear.

Objective: The objective was to determine the effects of dietary vitamin B-6 restriction on the rates of homocysteine remethylation and synthesis in healthy humans.

Design: Primed, constant infusions of [15C5]methionine, [3-13C]serine, and [2H3]leucine were conducted in healthy female (n = 5) and male (n = 4) volunteers (20–30 y) before and after 4 wk of dietary vitamin B-6 restriction (<0.5 mg vitamin B-6/d) to establish whether vitamin B-6 status affects steady-state kinetics of homocysteine metabolism in the absence of concurrent methionine intake. Effects of dietary vitamin B-6 restriction on vitamin B-6 status, plasma amino acid concentrations, and the rates of reactions of homocysteine metabolism were assessed.

Results: Dietary vitamin B-6 restriction significantly reduced plasma pyridoxal 5′-phosphate (PLP) concentrations (55.1 ± 8.3 compared with 22.6 ± 1.3 mmol/L; P = 0.004), significantly increased plasma glycine concentrations (230 ± 14 compared with 296 ± 15; P = 0.008), and significantly reduced basal (43%; P < 0.001) and PLP-stimulated (35%; P = 0.004) lymphocyte serine hydroxymethyltransferase activities in vitro. However, the in vivo fluxes of leucine, methionine, and serine; the rates of homocysteine synthesis and remethylation (total and vitamin B-6–dependent); and the concentrations of homocysteine, methionine, and serine in plasma were not significantly affected by dietary vitamin B-6 restriction.


KEY WORDS Vitamin B-6, homocysteine, human, methionine cycle, one-carbon cycle, pyridoxal 5′-phosphate, pyridoxine, remethylation, serine, transmethylation

INTRODUCTION

Elevated plasma total homocysteine concentration is an independent risk factor for vascular diseases (1, 2). As a result, significant attention was given to identifying the determinants of plasma total homocysteine concentration. Deficiencies of several nutrients required in one-carbon metabolism, including vitamin B-6, are recognized as causes of hyperhomocysteinemia (3). The metabolism of homocysteine through remethylation (to methionine) and transsulfuration (to cysteine and α-ketobutyrate) involves enzymes that require pyridoxal 5′-phosphate (PLP), which is the active coenzyme form of vitamin B-6 (4).

One-carbon units used for remethylation of homocysteine to methionine are derived from formate, glycine, serine, histidine, and betaine. We recently reported that the three-carbon of serine is the primary one-carbon unit used for homocysteine remethylation in humans (5). That result confirmed the importance of the PLP-dependent enzyme serine hydroxymethyltransferase for homocysteine remethylation. Serine hydroxymethyltransferase catalyzes the transfer of the three-carbon of serine to tetrahydrofolate to produce 5,10-methylene-tetrahydrofolate. This compound is reduced by methylenetetrahydrofolate reductase to produce 5-methyl-tetrahydrofolate, which is used explicitly for remethylation of homocysteine by methionine synthase. As a result, vitamin B-6 deficiency might impair homocysteine remethylation if it reduces serine hydroxymethyltransferase activity.

The sensitivity of serine hydroxymethyltransferase activity and homocysteine metabolism to vitamin B-6 status in rodents (6) suggests that vitamin B-6 deficiency also might impair homocysteine remethylation in humans. Arguing against this idea are the results of many studies that found little or no connection between vitamin B-6 status and plasma total homocysteine concentration in humans in the postabsorptive state (7, 8), during which remethylation represents a greater proportion of homocysteine metabolism than in the fed state (9). A stronger relation exists between vitamin B-6 status and postmethionine load plasma total homocysteine concentration, which is thought to reflect the importance of vitamin B-6 status on transsulfuration flux (4).

1 From the Food Science & Human Nutrition Department, Institute of Food and Agricultural Sciences (SRD, JBS, EPQ, JFG), the Division of Endocrinology and Metabolism, Department of Medicine (PWS, BSC), and the Department of Biochemistry and Molecular Biology, College of Medicine (PWS), University of Florida, Gainesville.

2 Supported by grants no. DK 56274 (JFG) and GCRC M01-RR00082 from the NIH and by grant no. 00-35200-9113 (JFG) from the USDA–National Research Initiative.

3 This paper is Florida Agricultural Experiment Station Journal Series No. R-10537.

4 Address reprint requests to IF Gregory III, Food Science & Human Nutrition Department, PO Box 110370, Gainesville, FL 32611-0370. E-mail: jfgy@ufl.edu.

5 Received September 20, 2004.

Accepted for publication November 8, 2004.
The results of our preliminary investigation of the effects of dietary vitamin B-6 restriction on the kinetics of human homocysteine metabolism, which used a different tracer protocol, were inconclusive (7). In the present study we tested the effects of dietary vitamin B-6 restriction on the kinetics of homocysteine synthesis and remethylation in healthy young men and women with the use of an improved multitracer approach (5).

SUBJECTS AND METHODS

Materials

1-[5,5,5-3H]L-Leucine, 1-[3-13C]serine, 1-[3,3,3-2H]cysteine, and 1-[13C5]methionine were purchased from Cambridge Isotopes Laboratories (Woburn, MA). Tracer solutions were prepared in isotonic saline, filter sterilized, and analyzed to assure lack of pyrogenicity and microbial contamination. Pyrogenicity was determined by a commercial laboratory (Focus Technologies, Cypress, CA) that used the Limulus amebocyte lysate assay.

Human subjects

Subjects were healthy, nonsmoking 20–30-y-old men and nonpregnant women who did not use medications (including oral contraceptives) that might interfere with vitamin B-6 metabolism and who agreed to abstain from alcohol use during the study period. To limit genetic variability in this study, subjects were selected for the C/C variant of the methylenetetrahydrofolate reductase 677C T polymorphism as determined by a polymerase chain reaction and restriction fragment length polymorphism procedure (10). At the time of screening, subjects met the inclusion criteria for vitamin status (plasma PLP > 30 nmol/L, plasma folate > 7 nmol/L, and plasma vitamin B-12 > 150 pmol/L) and plasma homocysteine (<13 μmol/L). A medical history, physical examination, and clinical blood chemistry screening were used to confirm general health, including renal function. Informed consent was obtained from all subjects. The University of Florida Institutional Review Board and the General Clinical Research Center (GCRC) Scientific Advisory Committee approved this protocol. Of the 11 subjects enrolled (5 women and 6 men), 9 (5 women and 4 men) completed the entire protocol.

Vitamin B-6–restricted diet

All meals were prepared by the Bionutrition Unit of the GCRC. Nutritionally adequate meals of controlled protein content were consumed by subjects for 2 d before the first infusion. On the day after the first infusion, subjects began consuming the vitamin B-6–deficient diet (<0.5 mg vitamin B-6/d) for 4 wk, which included weekends and holidays (11). Subjects consumed breakfast in the GCRC, were given a take-out lunch to eat at their convenience, and returned to the GCRC to consume their evening meal. Vitamin and mineral inadequacies of the study diets (other than vitamin B-6) were compensated for by custom supplements administered daily to the subjects. Compliance with the dietary regimen was monitored by weekly measurement of plasma PLP concentration (12).

Analytic methods

Plasma PLP concentration was measured as the semicarbazone-derivative by reverse-phase HPLC with fluorescence detection (12). The concentrations of folate and vitamin B-12 in plasma were measured with a commercial competitive radiobinding assay (radioassay; Quantaphase II B12/Folate radiobinding assay; Bio-Rad, Hercules, CA). Plasma total homocysteine concentration was measured as the ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate derivative by reverse-phase HPLC with fluorescence detection (13).

Methionine concentrations in plasma were measured by isotope dilution gas chromatography–mass spectrometry (GC-MS). All plasma samples and calibration standards contained 30 μmol/L [3-13C3]methionine. Amino acids from these samples and standards were isolated, esterified, and derivatized, then separated and analyzed by GCMS as described later for measurement of stable-isotope enrichments. A standard curve was derived from the ratios of the abundances of unlabeled-to-labeled amino acid isotomers in the standard solutions by using a mass-to-charge ratio of 367:372. All samples were assayed in a single run (intra-assay %CV = 3.0).

Concentrations of serine and glycine in plasma were measured as the danyl-derivatives by reverse-phase HPLC with fluorescence detection (λex 250 nm and λem = 470 nm) by using a modification of a previously published method (14). All samples and calibration standards contained 50 μmol/L norleucine as an internal standard. All samples were analyzed within the same run, and intra-assay CVs were 6.3% for serine and 3.4% for glycine.

Lymphocytes were isolated from whole blood by centrifugation (800g, 4 °C, 10 min) through a commercial density gradient separation medium (ICN Biochemical, Aurora, OH). The resulting cell pellets were stored at −80 °C until analyses were performed. Serine hydroxymethyltransferase activities of the cell pellets were measured in vitro as the rate of exchange of the pro-2S proton of glycine with hydrogen atoms of water by the method of Kim et al (15). Activities are presented in units of fmol glycine (h × g protein). PLP-stimulated serine hydroxymethyltransferase activities of each sample were measured similarly except that in those assays the reaction buffer contained 300 μmol PLP/L. The serine hydroxymethyltransferase stimulation index, which is a potential functional measurement of vitamin B-6 deficiency, was calculated as serine hydroxymethyltransferase activity measured in the presence of added PLP divided by serine hydroxymethyltransferase activity measured in the absence of added PLP.

Infusion protocol

Subjects were admitted to the GCRC on the evening before each infusion protocol and consumed no food between 2030 and initiation of the infusion. On the morning of the infusion an angiocatheter was inserted in the antecubital vein of each arm: 1 angiocatheter was used for the tracer infusion and 1 for blood collection. Blood samples were taken before infusion to measure concentrations of PLP, homocysteine, folate, and vitamin B-12 in plasma as well as for measurement of background isotopic enrichment of amino acids. Infusions were initiated at ~0830 with a 5-min, 20-mL priming dose that delivered 9.26 μmol [3-13C3]serine/kg, 1.62 μmol [13C3]methionine/kg, 1.00 μmol [3-13C5]methionine/kg, and 1.87 μmol [13C5]L-leucine/kg. The 9-h constant infusion followed immediately after the priming dose and delivered 20 mL infusion solution/h that contained 9.26 μmol [3-13C3]serine/kg, 1.62 μmol [13C3]methionine/kg, 1.00 μmol
[\textsuperscript{2}H\textsubscript{2}]cysteine/kg, and 1.87 \textmu mol [\textsuperscript{2}H\textsubscript{3}]leucine/kg. Data derived from the [\textsuperscript{2}H\textsubscript{2}]cysteine infusion will be presented in a separate manuscript. Heparinized blood samples were taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 7.5, and 9 h of the infusion. These samples were placed on ice until centrifugation (1500g, 4 °C, 10 min), after which the resulting plasma was stored at −80 °C until analysis. Subjects were kept from a catabolic state during the infusion by hourly consumption of a protein-free formulation containing 1.25 kcal · kg\textsuperscript{−1} · h\textsuperscript{−1}).

**GC-MS analysis of amino acid isotopic enrichments**

Plasma-free amino acids were isolated, derivatized, and analyzed as previously described (5). The N-heptfluorobutyl-n-propyl ester derivatives were dried and solubilized in ethyl acetate and stored at −20 °C until analysis. Isotopic enrichment was determined by negative chemical ionization–mass spectrometry with the use of a Finnigan-Thermoquest Voyager GC-MS and a 30-m poly (5% diphenyl and 95% dimethylsiloxane) fused silica capillary column (Equity 5; Supelco, Bellefonte, PA) as previously described (5). The abundance of specific ions was determined by selected ion monitoring at the following mass-to-charge ratios: 519:520 (serine), 349:352 (leucine), 549:553 (homocysteine). Isotopic enrichments are expressed as molar ratios of labeled to nonlabeled isotopomers after correction for the natural abundance of stable isotopes (9).

**Kinetic analyses**

The [\textsuperscript{13}C\textsubscript{5}]methionine and [3-\textsuperscript{13}C]serine tracer paradigms were described in detail previously (5). Briefly, one 13C-atom is lost when [\textsuperscript{13}C\textsubscript{4}]methionine is used in the methionine cycle for S-adenosylmethionine–dependent methyltransferase reactions (Figure 1). Subsequent remethylation of the resulting [\textsuperscript{13}C\textsubscript{4}]homocysteine with unlabeled methyl groups generates [\textsuperscript{13}C\textsubscript{5}]methionine. Total remethylation is calculated from the plasma plateau enrichments of these 3 species with the use of equations modified from previous tracer models (9, 16). Use of the 13C-labeled carbon of the [3-\textsuperscript{13}C]serine tracer for remethylation by methionine synthase generates [\textsuperscript{13}C\textsubscript{5}]methionine. Vitamin B\textsubscript{6}–dependent remethylation that used the three-carbon of serine was calculated from the plateau enrichments of these 2 species with the use of equations adapted from a radiotracer model used in rat liver (17).

**Flux calculations.** A detailed description of the calculations of kinetic parameters has been published (5, 18). Plateau enrichments (Ep) for infused amino acid tracers were calculated as the mean enrichments for the 5 h to 9 h time points. Plateau enrichments of labeled products were determined by fitting enrichment data to single exponential curves defined by the equation E = E\textsubscript{f} (1 − e\textsuperscript{−kt}), whereby E\textsubscript{f} is the enrichment at infinity (ie, plateau enrichment). Steady-state kinetics of amino acid tracer enrichments were calculated with the use of standard equations, including corrections for overestimation of intracellular enrichments from plasma enrichment data (9).

The flux of an amino acid is the rate of appearance of that amino acid from endogenous production (de novo synthesis and protein breakdown) and the tracer infusion, and it is calculated from the Ep of the corresponding tracer. Specifically, the flux of leucine (Q\textsubscript{Leu}) in the plasma pool was calculated from the [\textsuperscript{2}H\textsubscript{3}]leucine infusion rate (I\textsubscript{Leu}), the [\textsuperscript{2}H\textsubscript{3}]leucine tracer enrichment (E\textsubscript{Leu}), and the [\textsuperscript{2}H\textsubscript{3}]leucine plateau enrichment (E\textsubscript{pLeu}) as:

\[
Q_{\text{Leu}} = \frac{I_{\text{Leu}}}{E_{\text{pLeu}}} \times \left[ \frac{E_{\text{Leu}}}{E_{\text{pLeu}}} - 1 \right] \tag{1}
\]

Serine flux (Q\textsubscript{Ser}) in the plasma pool was calculated similarly after correcting the observed plasma [3-\textsuperscript{13}C]serine enrichment by multiplying by a correction factor of 0.4 (5). Methionine-related fluxes were calculated with the use of approaches analogous to those of Storch et al (9) and MacCoss et al (16). The flux of the carboxyl moiety of methionine is termed Q\textsubscript{C}. Methyl group flux (Q\textsubscript{Me}) is comprised of Q\textsubscript{C} and methionine produced from homocysteine remethylation (RM) (ie, Q\textsubscript{M} = Q\textsubscript{C} + RM). In the kinetic model of Storch et al (9), the plasma methionine tracer enrichment (identified here as E\textsubscript{pMet M + s}) is corrected to better estimate intracellular [\textsuperscript{13}C\textsubscript{5}]methionine enrichment by multiplying that value by a correction factor of 0.8. In the kinetic model of MacCoss et al (16) the intracellular methionine tracer enrichment is estimated with the use of plasma homocysteine enrichment (E\textsubscript{pHcy M + s}) as a surrogate measure. Q\textsubscript{C} is calculated from the sum of the plateau enrichments of the infused [\textsuperscript{13}C\textsubscript{5}]methionine and remethylated [\textsuperscript{13}C\textsubscript{5}]methionine tracers.
The percentage of serine three-carbon flux used for remethylation (RM) from serine can be determined directly from vitamin B-6–dependent RM of appearance of $^{13}$C-labeled methyl groups into methionine (vitamin B-6–dependent RM) is calculated by using the rate of transfer of methyl groups from serine to methionine:

$$Q_{\text{Ser for RM}} = \frac{\text{EpMet M}}{\text{EpSer M}}$$

Total homocysteine remethylation flux (RM) can be calculated as the difference between $Q_{\text{M}}$ and $Q_{\text{C}}$ as follows:

$$\text{RM} = \text{Q}_{\text{M}} - \text{Q}_{\text{C}}$$

The rate of transfer of methyl groups from serine to methionine (vitamin B-6–dependent RM) is calculated by using the rate of appearance of $^{13}$C-labeled methyl groups into methionine ($Q_{\text{Met M} + 4}$) and $\text{EpSer M} + 1$:

$$Q_{\text{Met M} + 4} = Q_{\text{C}} \times \text{EpMet M} + 1$$

Vitamin B-6–dependent RM = $Q_{\text{Met M} + 1}/\text{EpSer M} + 1$

The percentage of remethylation from serine (%RM from serine) can be determined directly from vitamin B-6–dependent RM and RM:

$$\%\text{RM from serine} = \frac{(\text{vitamin B-6–dependent RM/RM}) \times 100}{(\text{vitamin B-6–dependent RM/RM}) \times 100}$$

Relative fluxes. Relative fluxes are rates of appearance of amino acids that are adjusted for protein breakdown. They are calculated by dividing the amino acid fluxes of interest of each subject by the leucine flux for that subject. These equations are analogous to those used in assessing proline synthesis (19) and provide estimates of the synthesis of methionine from homocysteine and of serine from glycine and 3-phosphohydroxyypyruvate (20).

Fractional synthesis rates. The fractional synthesis rate (FSR) indicates the fraction of a product (eg, homocysteine) that is produced from its precursor (eg, methionine) per unit of time. These rates were calculated from the initial rates of synthesis by using early points (0.5–2 h) from the linear portion of the enrichment curves of $[^{13}\text{C}_4]$homocysteine and $[^{13}\text{C}_1]$methionine as follows (5, 18):

$$\text{FSR}_{\text{Hcy}} = \frac{\text{initial rate of Hcy}_{\text{M} + 4} \text{enrichment/EpMet M} + 4 \text{ or EpHcy M + 4}}{\text{EpHcy M + 4}}$$

$$\text{FSR}_{\text{Met M} + 1} = \frac{\text{initial rate of Met}_{\text{M} + 1} \text{enrichment/EpSer M} + 1}{\text{EpSer M} + 1}$$

The absolute synthesis rates of homocysteine and methionine were calculated as the products of their respective plasma concentrations and fractional synthesis rates. These equations are analogous to those used in assessing glutathione synthesis (21).

Results were analyzed statistically with the use of SigmaStat 3.0 (SPSS Inc, Rochester, MN). Significant differences between baseline and after the vitamin B-6–restricted state were assessed by paired t test. Data were also analyzed by two-factor analysis of variance to look for interactions involving sex. Data are reported as means and standard errors, and differences were considered statistically different at $P < 0.05$. 

### RESULTS

Concentrations of PLP in plasma indicate that all subjects had adequate vitamin B-6 status at baseline (Table 1). Consumption of the diet deficient in vitamin B-6 significantly reduced plasma PLP concentrations by 59%. Plasma PLP concentrations after dietary vitamin B-6 restriction (16–29 nmol/L) covered a range from deficient (<20 nmol/L) to marginal vitamin B-6 status (<30 nmol/L). Plasma folate concentrations were normal in all subjects at baseline (36.5 ± 2.3 nmol/L) and were not significantly different after the dietary vitamin B-6–restriction protocol (38.5 ± 1.6 nmol/L). Plasma vitamin B-12 concentrations also were adequate in all subjects at baseline (269 ± 28 pmol/L) and increased modestly (15–25%; $P = 0.004$) after the vitamin B-6–restriction protocol (330 ± 47 pmol/L).

Mean basal lymphocyte serine hydroxymethyltransferase activity, which was measured without added PLP, was reduced by 43% after dietary vitamin B-6 restriction (Table 1). Similarly, mean stimulated serine hydroxymethyltransferase activity, which was measured in the presence of exogenous PLP and reflects total enzyme abundance, was 35% lower after dietary

### TABLE 1

Plasma pyridoxal 5'-phosphate (PLP) concentrations and lymphocyte serine hydroxymethyltransferase (SHMT) activities measured in vitro at baseline and after 4 wk of dietary vitamin B-6 restriction (< 0.5 mg/d)

<table>
<thead>
<tr>
<th>SHMT activity</th>
<th>Plasma PLP</th>
<th>Basal</th>
<th>PLP-stimulated</th>
<th>Stimulation index $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/L</td>
<td>fnol glycine/(h × g protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>55.1 ± 8.3</td>
<td>105.8 ± 9.6</td>
<td>153.8 ± 14.7</td>
<td>1.48 ± 0.11</td>
</tr>
<tr>
<td>Restricted</td>
<td>22.6 ± 1.3</td>
<td>60.6 ± 11.4</td>
<td>100.0 ± 13.7</td>
<td>1.95 ± 0.26</td>
</tr>
</tbody>
</table>

$^1$ All values are $\bar{x}$ ± SEM; $n = 9$. Data were analyzed by paired $t$ test.

$^2$ PLP-stimulated activity/basal activity.

$^3$ Significantly different from baseline: $^3P = 0.004$, $^4P < 0.001$. 

...
vitamin B-6 restriction. Despite these differences, the 31% increase of the lymphocyte serine hydroxymethyltransferase activity stimulation index (stimulated activity and basal activity) caused by dietary vitamin B-6 restriction was not statistically significant ($P > 0.071$).

Concentrations of total homocysteine, serine, and methionine from fasting plasma samples were not significantly affected by dietary vitamin B-6 restriction (Table 2). However, mean plasma glycine concentration was 29% greater after vitamin B-6 restriction than at baseline.

The enrichment of infused tracers rapidly reached plateau in the plasma compartment ($0.5–1$ h), and no significant effects of dietary vitamin B-6 restriction on the shape of the enrichment curves were detected (Figure 2). The plateau enrichments of $\text{[13C}_5\text{]}$methionine ($\sim 7–8\%$), $\text{[13C}_1\text{]}$serine ($\sim 8\%$), and $\text{[2H}_3\text{]}$leucine ($\sim 2.3\%$) were similar to previous reports (5, 18) and were not significantly affected by dietary vitamin B-6 restriction (data not shown). Plateau enrichments for $\text{[13C}_4\text{]}$homocysteine ($\sim 6–7\%$) and remethylated methionine species ($\text{[13C}_4\text{]}$methionine: $\sim 2\%$; $\text{[13C}_1\text{]}$methionine: $\sim 1\%$), which were calculated by fitting the enrichment data (Figure 3) to single exponential curves, also were similar to our previous reports (5, 18) and not significantly affected by dietary vitamin B-6 restriction (data not shown).

Leucine, serine, and methionine fluxes and relative serine fluxes (Table 3) were similar to previous reports (5, 9, 16, 18, 22) and were not significantly affected by dietary vitamin B-6 restriction. Relative methionine flux was modestly (7%) but significantly ($P = 0.029$) greater after dietary vitamin B-6 restriction.

The $Q_M$, $Q_C$, and the homocysteine remethylation rates (both RM and vitamin B-6–dependent RM) derived by using calculations analogous to those proposed by Storch et al (9) and MacCoss et al (16) were not significantly different from each other; therefore, data from only the calculations based on the MacCoss approach are presented here (Table 3 and Table 4). No significant differences of $Q_M$, $Q_C$, or RM as a result of dietary vitamin B-6 restriction were found. Similar to previous reports (5, 18), RM accounted for $\sim 26–29\%$ of the $Q_M$ (ie, $26–29\%$ of total methionine appearing in plasma was derived from RM). Neither the rate of vitamin B-6–dependent RM nor the percentage of RM from serine was affected by dietary vitamin B-6 restriction (Table 4). Further, no differences in the percentage of $Q_{Ser}$ for RM were found ($2.2 \pm 0.1\%$ at baseline compared with $2.4 \pm 0.2\%$ after dietary vitamin B-6 restriction).

Neither the fractional nor absolute rates of homocysteine synthesis differed significantly as a result of dietary vitamin B-6 restriction (Table 5). The fractional and absolute rates of methionine synthesis from serine (measured from $\text{[13C}_1\text{]}$methionine enrichment) also were not significantly affected by dietary vitamin B-6 restriction.

All data were analyzed for differences due to sex by the two-sample $t$ test and by two-factor analysis of variance. No significant differences were found for any static or kinetic measurements between men and women ($P > 0.05$).
DISCUSSION

Plasma PLP concentrations that were low (~30 nmol/L), but still above the current cutoff for normal vitamin B-6 status (20 nmol/L), were associated with increased risks of vascular diseases and stroke in epidemiologic studies (23, 24). Although some results from animal studies suggest that the increased disease risk is mediated by hyperhomocysteinemia (25), the association between vitamin B-6 status and vascular diseases and stroke in humans appears to be independent of fasting plasma total homocysteine concentration (23, 24, 26–28). These findings are consistent with the weak association between vitamin B-6 status and fasting plasma total homocysteine concentration in otherwise healthy populations (7, 8). Combined, these data argue that the increased risks of vascular disease and stroke associated with marginal vitamin B-6 status (20–30 nmol/L) in humans are not mediated by effects on vitamin B-6–dependent pathways of homocysteine remethylation. However, the effects of vitamin B-6 deficiency on rates of homocysteine remethylation and synthesis in humans are uncertain.

Despite the importance of serine hydroxymethyltransferase activity to homocysteine metabolism in humans (5), the results of this investigation argue against any effect of marginal vitamin B-6 status (plasma PLP ~15–30 nmol/L) on homocysteine remethylation or synthesis in the absence of dietary methionine intake. The rates of homocysteine remethylation and synthesis (Tables 4 and 5) and the concentrations of serine, methionine, and homocysteine in plasma (Table 2) were not significantly affected by dietary vitamin B-6 restriction. This finding was true despite basal and PLP-stimulated activities of lymphocyte serine hydroxymethyltransferase in vitro being significantly reduced by dietary vitamin B-6 restriction in this study (Table 1). The lack of effect of low vitamin B-6 status on these reactions of homocysteine metabolism is consistent with the weak relation between vitamin B-6 status and fasting plasma total homocysteine concentration in otherwise healthy populations (7). Combined, these data suggest that serine hydroxymethyltransferase activity is present in excess of that needed for homocysteine remethylation.

![FIGURE 3. Enrichment curves for amino acids synthesized from the infused tracers. Plasma enrichments of \[^{13}C\]\methionine derived from the \[^{3}-^{13}C\]\serine tracer and of \[^{13}C\]homocysteine and \[^{13}C\]\methionine derived from the \[^{13}C\]\methionine tracer at baseline (○) and after 4 wk of dietary vitamin B-6 restriction (△; <0.5 mg/d). Data for individual points are \(\bar{x} \pm \text{SEM}; n = 9\). Calculated plateau enrichments were analyzed by paired \(t\) test.](https://academic.oup.com/ajcn/article-abstract/81/3/648/4649023)

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>(Q_{\text{Leu}})</th>
<th>(Q_{\text{Ser}})</th>
<th>(Q_{\text{Ser}}/Q_{\text{Leu}})</th>
<th>(Q_{M})</th>
<th>(Q_{M}/Q_{\text{Leu}})</th>
<th>(Q_{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>80.0 ± 3.3</td>
<td>271 ± 15</td>
<td>3.4 ± 0.1</td>
<td>24.3 ± 1.1</td>
<td>0.32 ± 0.01</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Restricted</td>
<td>80.9 ± 4.1</td>
<td>281 ± 15</td>
<td>3.5 ± 0.1</td>
<td>24.4 ± 1.0</td>
<td>0.34 ± 0.01(^2)</td>
<td>18.0 ± 0.6</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm \text{SEM}; n = 9\). Data were analyzed by paired \(t\) test.

\(^2\) Significantly different from baseline, \(P = 0.029\).

### TABLE 4

Homocysteine remethylation (RM) at baseline and after 4 wk of dietary vitamin B-6 restriction (< 0.5 mg/d)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B-6--dependent RM</th>
<th>Total RM</th>
<th>Percentage RM from serine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu\text{mol/(kg × h)})</td>
<td>(\mu\text{mol/(kg × h)})</td>
<td>%</td>
</tr>
<tr>
<td>Baseline</td>
<td>5.87 ± 0.28</td>
<td>7.04 ± 0.54</td>
<td>90 ± 12</td>
</tr>
<tr>
<td>Restricted</td>
<td>6.76 ± 0.59</td>
<td>6.40 ± 0.47</td>
<td>109 ± 12</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} \pm \text{SEM}; n = 9\). Data were analyzed by paired \(t\) test. RM indexes measured after vitamin B-6 restriction were not significantly different from those measured at baseline.
Further study is needed to determine whether remethylation is impaired by vitamin B-6 deficiency when methionine is consumed, at which time the rate of homocysteine remethylation is significantly elevated.

SRD was responsible for study coordination, subject screening, data collection and analysis, and primary preparation of manuscript. JBS was responsible for data collection and analysis. EPQ was responsible for data collection and analysis. BSC was responsible for study coordination and subject recruitment and screening. PWS was a coinvestigator and was responsible for experimental design, clinical oversight, and manuscript preparation. JFG was the principal investigator and was responsible for experimental design, oversight of data collection, and manuscript preparation. None of the authors had a personal or financial interest in this research or a conflict of interest of any kind.

**REFERENCES**


