Vitamin B-6 Deficiency Suppresses the Hepatic Transsulfuration Pathway but Increases Glutathione Concentration in Rats Fed AIN-76A or AIN-93G Diets

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Abstract

The transsulfuration pathway, which aids in regulating homocysteine concentration and mediates cysteine synthesis, may be sensitive to vitamin B-6 status because cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) require pyridoxal 5’-phosphate (PLP). To assess relations between vitamin B-6 and transsulfuration, we evaluated the effects of dietary pyridoxine (PN) on the hepatic concentration of relevant metabolites and in vitro activity of CBS and CGL. Growing rats were fed AIN-93G or AIN-76A–based diets that ranged from adequate to deficient in vitamin B-6 (2, 1, 0.5, 0.1, or 0 mg of PN/kg diet, n = 5). This design allowed assessment of the effects of supplemental methionine (AIN-76A) vs. cysteine (AIN-93G) in common research diets over a range of vitamin B-6 levels. CBS activity, assayed in the presence or absence of added S-adenosylmethionine, was independent of diet type and PN level. CGL activity was independent of diet type but proportional to dietary PN. Rats fed deficient (0 and 0.1 mg PN/kg) diets exhibited only ~30% of the CGL activity of those fed the 2 mg PN/kg diets. Hepatic cystathionine increased from 20 to 30 nmol/g for the 1–2 mg PN/kg diets to ~85 nmol/g for the 0 mg PN/kg diet; however, cysteine was reduced only in B-6–deficient rats consuming the AIN-93G diet (means of 30–40 nmol/g for adequate to 11.6 nmol/g for 0 mg PN/kg AIN-76A diet). In spite of these effects, hepatic glutathione concentration increased in vitamin B-6 deficiency. These results suggest that vitamin B-6–dependent changes in transsulfuration do not limit hepatic glutathione production.

Introduction

Epidemiologic studies showed that low vitamin B-6 nutritional status is associated with increased risk of cardiovascular disease (1–6), venous thrombosis (7), stroke (6,8,9), and possibly colon cancer (10), but the mechanism(s) responsible remain unclear. Unlike similar associations with low folate status, the enhanced risk of vascular disease associated with low vitamin B-6 status is not necessarily dependent on elevated plasma homocysteine (7,8). We reported recently that marginal vitamin B-6 deficiency reduces serine hydroxymethyltransferase (SHMT) activity in rat liver (11) and human lymphocytes (12), which suggests one mechanism by which the regulation of 1-carbon units may be altered. We also showed that marginal vitamin B-6 deficiency yields increased fasting glycine in rat liver and human plasma (11,12), and an increase in plasma cystathionine and glutathione concentration, but no change in the tracer-derived in vivo rate of total and vitamin B-6–dependent homocysteine remethylation and no change in cysteine flux in humans under conditions of minimal amino acid intake (12,13). The extent to which vitamin B-6 deficiency affects 1-carbon metabolism and transsulfuration flux under conditions of greater amino acid intake (e.g., postprandial conditions) is unclear and under investigation.

The transsulfuration pathway, which contributes to homocysteine regulation and provides cysteine synthesis, consists of sequential reactions catalyzed by cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) (14). CBS catalyzes the condensation of homocysteine and serine to form cystathionine in a reaction that is subject to positive allosteric regulation by S-adenosylmethionine (SAM), whereas CGL catalyzes the cleavage of cystathionine to yield α-ketobutyrate, ammonia, and cysteine. Because both CBS and CGL require pyridoxal 5’-phosphate (PLP) as a coenzyme (15–18), inadequate vitamin B-6 status might lead to impaired regulation of cellular homocysteine concentration and reduced synthesis of cysteine. In addition, a reduction in cellular SAM concentration caused by vitamin B-6 deficiency (19) could cause reduced in vivo activity of CBS and, hence, reduced flux through the transsulfuration pathway.

Previous studies showed effects of vitamin B-6 deficiency on aspects of the transsulfuration process (15–20–26). However, such studies involved mainly comparisons of pronounced...
vitamin B-6–deficient vs. adequate or supplemented states and provided little information about states of marginal-to-moderate deficiency that are particularly relevant to human nutrition (27). Although vitamin B-6 deficiency in rats was reported to yield a significant reduction in hepatic CBS activity (15), others found little reduction in CBS activity in perhaps less severe deficiency states (20, 24). Sturman et al. (20) compared the activity of CGL and CBS in rats administered a high pyridoxine (PN) diet (50 mg PN/kg diet) with that of deficient rats administered no dietary PN. The deficient diet reduced liver CBS activity, but caused a much greater reduction in CGL activity. Sato et al. (21) confirmed these findings and showed that turnover of CGL increases in vitamin B-6 deficiency due to increased proteolytic turnover of apo-CGL.

The study reported here was conducted to assess the dependence of the hepatic activities of CBS and CGL and the in vivo concentration of the products cystathionine, cysteine, and glutathione on vitamin B-6 nutritional status ranging from adequacy through deficiency. By evaluating basal diets supplemented with either methionine or cysteine (AIN-76A vs. AIN-93G, respectively), this study yielded initial information about the effect of dietary methionine and cysteine intakes on the functional effect of vitamin B-6 deficiency on transsulfuration. In addition, we tested the hypothesis that vitamin B-6 deficiency would yield lower concentrations of hepatic glutathione because of lower production of its precursor cysteine.

Materials and Methods

Materials. Pyridoxine HCl, pyridoxal 5’-phosphate, dl-homocysteine, l-serine, 5-adenosylmethionine, dl-propargylglycine, glutathione, and other chemical reagents were obtained from Sigma Chemical. 3H-cystathionine was purchased from C/D/N Isotopes.

Animals and diets. We report here the results of further analyses of tissue fractions collected in a previous study of weaning male rats (Hsd:Sprague-Dawley; Harlan Laboratories) weighing ~50 g conducted to investigate the effects of relations between diet composition and vitamin B-6 content on intestinal β-glucosidases (28) and hepatic SHMT activities (11). Rats were housed in hanging wire-mesh stainless steel cages and maintained at a constant temperature with a 12-h light:dark cycle. All procedures for animal care and treatment were approved by the University of Florida Institutional Animal Care and Use Committee.

As described previously (28), the study used 2 basal rodent diet formulations (AIN-76A and AIN-93G) modified only with respect to vitamin B-6 content (Dyets). The AIN-76A diet contains by weight 20% casein and 0.30% dl-methionine (29), whereas the AIN-93G diet contains 20% casein and 0.30% l-cysteine (30). Rats (n = 50) were divided into 10 groups, n = 5, to receive AIN-76A or AIN-93G containing 2, 1, 0.5, 0.1, or 0 mg/kg PN added as PN-HCl. Food intake and rat weight were monitored daily, and the rats had free access to food and water. Starting on the evening of d 35, food was withheld for 12 h; then the rats were anesthetized using Halothane (Alocarbon Laboratories) inhalation and exsanguinated by cardiac puncture the following morning. The livers were removed, and cooled on ice.

In preparation for enzyme activity assays, a portion of each liver was homogenized (Polytron, Brinkmann Instruments) in 10 volumes of ice-cold 0.01 mol/L potassium phosphate buffer (pH 7.5) with 0.1 mol/L potassium chloride and 0.5 mmol/L EDTA (26) containing a protease inhibitor cocktail (Sigma P8340) added just before use. After centrifugation at 200,000 × g for 30 min at 4°C, portions of the supernatant were maintained at ~80°C until analyzed. The remainder of each liver was maintained at ~80°C until use in chemical analyses. All laboratory procedures were performed under low fluorescent light to minimize the photochemical degradation of vitamin B-6. The enzymatic analyses and quantitative analyses of amino acids and aminothiols were conducted concurrently with those regarding SHMT reported previously (11).

Cystathionine concentrations were measured ~1 y later, after validation of the assay.

Analytical methods. PLP was measured using reverse-phase fluorometric HPLC of the semicarbazone derivative according to the method of Ubbink et al. (31) with minor modifications. Protein concentration was determined using the Markwell protein assay (32) with bovine serum albumin as the standard protein. The concentration of hepatic total cysteine, homocysteine, and glutathione (free and disulfide forms) was determined by fluorometric HPLC (33). Cystathionine in liver was determined as the heptafluorobutyryl n-propyl ester derivative using GC-MS in negative chemical ionization mode with selected-ion monitoring for quantification of endogenous cystathionine using [3H]cystathionine as an internal standard (13).

Cystathionine β-lyase (CGL) activity. CGL activity was determined by a modification of the method of Heinonen (37). Reaction mixtures were composed of 100 mmol/L tris-hydroxymethylaminomethane (pH 8.4) and 8 mmol/L l-cystathionine in a total volume of 50 μL. Each liver extract was assayed both with and without the inclusion of 0.5 mmol/L SAM in the reaction mixture. The product, cystathionine, was quantified by HPLC (36). Preliminary experiments verified that product formation was linearly related to incubation time and enzyme concentration under these conditions. Activity was expressed as micromoles of cystathionine produced per minute per milligram of protein.

Cystathionine γ-lyase (CGL) activity. CGL activity was determined by a modified method of the method of Heinonen (37). Reaction mixtures were composed of 100 mmol/L tris-hydroxymethylaminomethane (pH 8.4) and 8 mmol/L l-cystathionine in a total volume of 50 μL. Each liver extract was assayed both with and without added PLP (apo-CGL + holocystathionine) and the ratio of activities with and without added PLP (apo-CGL + holocystathionine) indicated the fraction of total CGL that was in the apo-enzyme form (21). Samples assayed without exogenous PLP represented basal activity. Reactions were started by the addition of 5 μL of liver extract, incubated at 37°C for 10 min, and then stopped by incubation in a boiling water bath for 3 min. Cysteine concentrations in the resulting supernatants were measured by HPLC (33). Activities were expressed as micromoles of cysteine produced per milligram of protein per minute. Product formation was linear with incubation time and enzyme concentration. The concentration of cystathionine was varied from 0 to 8 mmol/L in a preliminary study conducted to evaluate the K_m of rat liver CGL for cystathionine under these conditions.

Statistical analysis. The data were evaluated using 2-way ANOVA, with diet type (AIN-76A or AIN-93G) and PN concentration as main factors, with multiple comparisons using the Holm-Sidak method and Sigma Stat 3.0 software (SPSS). Data were transformed by log10 conversion as necessary to improve normality, equalize variance, or both. The results are reported as least-squares means and pooled SEM. The P-values designating the significance of differences among treatments reported in the tables are derived from 2-way ANOVA. For the statistical analyses for CBS and CGL (Table 2), the data are presented as the least-square mean for each level of dietary PN because the main effect of diet type and the diet × PN interaction were not significant in the 2-way ANOVA. Relations between selected variables, including Michaelis-Menten analysis for the determination of the cystathionine K_m were evaluated by regression analysis using Microsoft Excel or SAAM II. Differences with P < 0.05 were considered to be significant.
Results

Effects of dietary pyridoxine on nutritional status. Liver PLP concentration increased in proportion to dietary PN concentration (P < 0.001), which confirms that the dietary treatment affected vitamin B-6 status and coenzyme concentration at the tissue level (Table 1). Plasma PLP and weight gain in this study, which also reflect vitamin B-6 nutritional status, were reported previously in other papers derived from this study (11,28). The appearance of dermatological abnormalities (acrodynia) in the rats consuming the 0 mg PN/kg diets confirmed the existence of overt vitamin B-6 deficiency associated with that dietary level (28).

Hepatic cystathionine β-synthase activity. Neither diet type (AIN-76A or AIN-93G) nor dietary PN concentration affected liver CBS activity either with or without the addition of SAM for allosteric activation (Table 2). In this analysis, exogenous SAM increased CBS activity >2-fold, and there was no significant effect of diet type or dietary PN concentration (Table 2). In addition, there was no relation between basal or SAM-stimulated CBS activity and liver PLP concentration (data not shown). In view of the large dilution in tissue preparation and CBS assay, endogenous SAM would have little effect on this in vitro allosteric stimulation.

Hepatic cystathionine γ-lyase activity. Basal and PLP-stimulated CGL activity were significantly and positively related to liver CBS activity either with or without the addition of SAM for allosteric activation (Table 2). In this analysis, exogenous SAM increased CBS activity >2-fold, and there was no significant effect of diet type or dietary PN concentration (Table 2). In addition, there was no relation between basal or SAM-stimulated CBS activity and liver PLP concentration (data not shown). In view of the large dilution in tissue preparation and CBS assay, endogenous SAM would have little effect on this in vitro allosteric stimulation.

In regression analysis of pooled data for all treatment groups, CGL activity was correlated linearly with liver PLP concentration without the in vitro addition of PLP (r² = 0.446, P < 0.001, data not shown) and with the addition of PLP (r² = 0.326, P < 0.001; data not shown). A PLP stimulation index calculated as the ratio of PLP-stimulated CGL activity:basal CGL activity ranged from 1.37 for 2 mg PN/kg to 2.09 for 0 mg PN/kg diet (effect of dietary PN significant, P < 0.001). However, the relation between PLP stimulation and dietary PN content was distinctly nonlinear, with only the 2 most deficient diets yielding significantly greater PLP stimulation than the 3 higher PN diets. Thus, the proportion of CGL protein in apo-enzyme form, which is reflected by the degree of stimulation, was not a linear function of dietary PN concentration.

TABLE 1 Liver PLP concentration of rats fed either AIN-76A or AIN-93G diets containing variable amounts of PN for 5 wk

<table>
<thead>
<tr>
<th>Diet PN, mg/kg</th>
<th>AIN-76A</th>
<th>AIN-93G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/g</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>49.9a</td>
<td>50.5a</td>
</tr>
<tr>
<td>1</td>
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</tr>
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<td>0</td>
<td>37.4a</td>
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</tr>
<tr>
<td>Pooled SEM</td>
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<td>1.70</td>
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2-way ANOVA P-values

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>PN</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diet × PN</td>
<td>0.026</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5, except AIN-76A for which the 1 mg/kg group was n = 4. Means in a column without a common letter differ, P < 0.05.

2 Different from AIN-76A, P < 0.05. Adapted from Ref. (11).

TABLE 2 In vitro activity of CBS and CGL in liver of rats fed basal diets (AIN 76A or AIN 93G) containing varying concentrations of PN for 5 wk

<table>
<thead>
<tr>
<th>Diet PN, mg/kg</th>
<th>Addition to assay</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cystathionine β-synthase⁵</td>
<td>Cystathionine γ-lyase⁵</td>
</tr>
<tr>
<td></td>
<td>−SAM</td>
<td>+SAM</td>
</tr>
<tr>
<td>2</td>
<td>38.4</td>
<td>148</td>
</tr>
<tr>
<td>1</td>
<td>39.1</td>
<td>131</td>
</tr>
<tr>
<td>0.5</td>
<td>40.4</td>
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</tr>
<tr>
<td>0.1</td>
<td>30.1</td>
<td>104</td>
</tr>
<tr>
<td>0</td>
<td>36.1</td>
<td>136</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>6.56</td>
<td>15.6</td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, n = 10, except AIN-76A for which the 1 mg/kg group was n = 4. Means in a column without a common letter differ, P < 0.05.

2 CBS assays were conducted without and with the addition of SAM. Values are means and SEM, n = 10 (except for 1 mg/kg, n = 9) with results for both diets pooled because effects of diet (AIN-76A vs. 93G) were not significant. Two-way ANOVA results: −SAM (diet P = 0.101, PN P = 0.590, interaction P = 0.271); +SAM (diet P = 0.780, PN P = 0.239, interaction P = 0.578); ratio (diet P = 0.522, PN P = 0.817, interaction P = 0.795).

3 CGL assays were conducted without and with the addition of PLP. Values are means and SEM, n = 10 (except for 1 mg/kg, n = 9) with results for both diets pooled because effects of diet (AIN-76A vs. 93G) were not significant. Two-way ANOVA results: −PLP (diet P = 0.370, PN P = 0.001, interaction P = 0.211); +PLP (diet P = 0.527, PN P < 0.001, interaction P = 0.066); ratio (diet P = 0.496, PN P < 0.015, interaction P = 0.630).

In regression analysis of pooled data for all treatment groups, CGL activity was correlated linearly with liver PLP concentration without the in vitro addition of PLP (r² = 0.446, P < 0.001, data not shown) and with the addition of PLP (r² = 0.326, P < 0.001; data not shown). A PLP stimulation index calculated as the ratio of PLP-stimulated CGL activity:basal CGL activity ranged from 1.37 for 2 mg PN/kg to 2.09 for 0 mg PN/kg diet. However, the relation between PLP stimulation and dietary PN content was distinctly nonlinear, with only the 2 most deficient diets yielding significantly greater PLP stimulation than the 3 higher PN diets. Thus, the proportion of CGL protein in apo-enzyme form, which is reflected by the degree of stimulation, was not a linear function of dietary PN concentration.

Hepatic methionine, homocysteine, cystathionine, cysteine and glutathione concentrations. Rats that consumed the AIN-76A diet had significantly higher hepatic methionine concentration than those consuming the AIN-93G diet (P = 0.008; Table 3), but there was no significant effect of dietary PN concentration (P = 0.663) and no significant interaction (P = 0.301).

Hepatic total homocysteine concentration was affected by both diet type (P = 0.006) and dietary PN concentration
Liver cysteine was influenced significantly by diet type ($P = 0.026$) and dietary PN concentration ($P < 0.001$), with a significant interaction between diet type and PN concentration ($P = 0.016$). Hepatic cysteine declined during vitamin B6 deficiency most notably in rats fed the methionine-supplemented AIN-76A diet. For example, hepatic cysteine was 41.4 and 11.6 nmol/g in rats fed AIN-76A diets containing 2.0 and 0 mg PN/kg.

Liver glutathione concentration was not affected by diet type but in contrast with its precursor, cysteine, increased significantly with decreasing dietary PN ($P = 0.009$; Table 3). This overall significant inverse relation between liver glutathione concentration and dietary PN was largely attributable to the response of groups fed the AIN-93G diet.

**Discussion**

This paper extends our investigations of the effects of inadequate vitamin B-6 nutritional status on 1-carbon metabolism (11–13,24,38) by reporting information concerning the dependence of hepatic transsulfuration on dietary vitamin B-6 concentration and vitamin B-6 status. The experimental design also permitted an evaluation of the effects of supplemental dietary methionine vs. cysteine in 2 common research diets that would be considered adequate in protein and sulfur amino acid content (30). The AIN-76A formulation provided 0.30 g added dl-methionine, 0.52 g l-methionine from casein, 0.82 g total methionine, and 0.08 g total l-cysteine from casein/100 g of diet. In contrast, the AIN-93G provided 0.52 g l-methionine from casein, 0.30 g added l-cysteine, 0.08 g l-cysteine from casein, and 0.38 g total l-cysteine/100 g diet. The results of this study illustrate how the selection of the basal diet formulation can influence conclusions in studies involving the metabolism of sulfur amino acids and related nutritional variables.

All of the concentrations of dietary PN used (2–2 mg PN/kg diet) were less than the NRC recommendation of 7 mg PN/kg diet (39); however, many previous studies showed that vitamin B6 adequacy occurs in growing rats consuming between 1 and 2 mg PN/kg diet [see, for example (40)]. An important objective of this study was to evaluate the response in the intermediate range of vitamin B-6 deficiency, which is most relevant to human vitamin B-6 deficiency. The extent to which liver PLP declined in growing rats fed the various levels of dietary PN is consistent with the AIN-93G diet.
with previous studies (e.g., 24,40) and reflects the existence of slow turnover pools of vitamin B-6.

The potential suppression of transsulfuration during vitamin B-6 deficiency has long been recognized, but the physiological implications have not been fully determined. The methionine load test constitutes an evaluation of the metabolic capacity of the transsulfuration pathway to handle the load of substrate (homocysteine) derived from the large dose of methionine. The capacity to metabolize this methionine load is suppressed as a function of vitamin B-6 deficiency (e.g., 22,41). In spite of the diagnostic usefulness of the methionine load test, previous research has not clarified fully whether such changes in transsulfuration capacity predict changes in actual metabolic flux through the pathway during mild vitamin B-6 deficiency.

Vitamin B-6 deficiency can cause elevated homocysteine concentration in tissue and plasma (e.g., 24,41,42), although this effect is less pronounced than that of folate deficiency (41). In this study, liver homocysteine exhibited an unexpected decline with decreasing vitamin B-6 status. This result is inconsistent with our previous findings using a similar dietary protocol (24) and may be a function of food intake and, thus, methionine intake, that was not controlled for in this study. In spite of the 12-h period of food withdrawal before the rats were killed, it is likely that methionine and homocysteine concentrations were affected by short-term consumption. In addition, the data for liver homocysteine in this study are subject to some uncertainty due to potential action of SAH hydrolase during tissue fractionation (43). The reductive treatment used in this homocysteine assay (33) presumably yields accurate measurement of total homocysteine and accurately reflects relative differences among dietary groups.

Several studies showed that the concentration of the transsulfuration intermediate, cystathionine, in the plasma of humans (13,22) and rats (23) is particularly sensitive to inadequate vitamin B-6 status. The present study provides, to our knowledge, the first study of the dose-response relation of vitamin B-6 with hepatic CBS and CGL activities and metabolite concentrations. This study complements that of Stabler et al. (23) who reported the effects of graded levels of dietary PN on plasma concentrations of cystathionine and other components of 1-carbon metabolism in rats. Taken together, these studies clearly show that plasma and liver cystathionine concentration is a very sensitive biomarker of vitamin B-6 insufficiency ranging from slightly suboptimal to frankly deficient levels of vitamin B-6 status. In addition, our data strongly suggest that the sensitivity of hepatic cystathionine concentration to vitamin B-6 deficiency is related primarily to the resulting depletion of the holo-enzyme form of hepatic CGL coupled with the previously shown (21) reduction in the total CGL pool due to the faster turnover of apo-CGL.

Although an influence of vitamin B-6 status on in vivo activity of hepatic CBS is plausible, as reported by Finkelstein and Chalmers (15), our results suggest that hepatic CBS activity was largely independent of both dietary PN concentration and hepatic PLP concentration. This lack of influence of vitamin B-6 status on CBS activity was consistent with observations by Sturman et al. (20). Under the conditions of this study, vitamin B-6 deficiency may have affected the extent to which CBS undergoes activation by SAM (19), which may not have been detected under the conditions of CBS assays in this study. Our previous data suggest that the primary effect of vitamin B-6 deficiency on transsulfuration is on the CGL reaction, which was impaired even under conditions in which hepatic SAM was only slightly reduced (24). The affinity constants for binding PLP by CBS are of a magnitude similar to that reported for CGL (16–18); thus, the greater reduction of CGL cannot be explained simply on the basis of coenzyme affinity. The greater sensitivity of CGL than CBS to vitamin B-6 depletion may reflect differences in the rate of turnover of these proteins.

The mechanisms by which vitamin B-6 deficiency affects glutathione concentration remain unclear. Under conditions in which the supply of cysteine, whether from dietary sources or synthesis, limits glutathione synthesis, one would predict lower hepatic glutathione in vitamin B-6 deficiency. However, if vitamin B-6 deficiency is associated with an inflammatory state (44,45) or oxidative stress (45–47) that may trigger enhanced glutathione synthesis, then hepatic glutathione concentration would parallel the extent of deficiency as seen here. In this study, there was a highly significant (P = 0.009) inverse effect of dietary PN concentration on glutathione concentration. Even the group fed 0 mg PN/kg of the AIN-76A diet, which exhibited a large reduction in hepatic cysteine, maintained hepatic glutathione, which is contrary to our initial hypothesis that vitamin B-6 deficiency would depress glutathione production. Various forms of oxidative stress were shown to activate CBS (48–50). Such an effect in vitamin B-6 deficiency may have contributed to the elevated cystathionine and glutathione observed in this study, but cysteine concentrations do not support such an interpretation. It also is noteworthy that, in the absence of other pathological conditions, marginal vitamin B-6 deficiency in humans does not cause an increase in C-reactive protein, a common marker of systemic inflammation (13).

This study involved only the measurement of steady-state concentrations of various substrates and metabolites and cannot predict in vivo rates of reactions. Our recent study of marginal vitamin B-6 deficiency in humans showed a large increase in the cystathionine pool but no change in flux of the cysteine pool (13). Because the method did not allow measurement of the flux through the CGL reaction, overall transsulfuration flux could not be determined with that tracer protocol. Thus, the net effect of vitamin B-6 deficiency on the transsulfuration pathway in vivo remains unclear. Under the in vitro conditions of the CGL assay, we observed a K_m for cystathionine of 2.1 ± 0.2 mmol/L, which is consistent with the reported K_m of 3 mmol/L for purified CGL (51). If hepatic CBS behaved similarly in vivo, then the reaction would be only half-saturated at a liver cystathionine concentration of ~2.1 μmol/g. In view of the fact that liver cystathionine concentrations ranged from ~20–90 mmol/g, one would conclude that the in vivo CGL reaction rate would be almost first order with respect to cystathionine concentration. Thus, elevated hepatic cystathionine concentration would yield a higher rate of the CGL reaction, which would tend to offset losses of CGL activity due to vitamin B-6 deficiency. This reasoning may explain our observation that hepatic cysteine is maintained under most conditions of vitamin B-6 deficiency. It also may explain why cysteine flux was unchanged in vitamin B-6 deficiency in our recent tracer study in humans (13). The mechanism and implications of the increases in liver glutathione (in this study) and plasma glutathione in humans (13) in proportion to vitamin B-6 deficiency are under investigation. The effects of vitamin B-6 deficiency on hepatic glutathione reported in previous rodent studies are inconsistent (25,47).

This study extends our understanding of the metabolic changes associated with intermediate levels of vitamin B-6 deficiency. We recently reported in an accompanying paper regarding this study that cytosolic and mitochondrial forms of hepatic SHMT undergo substantial reductions in activity (11) that are similar to those shown here for CGL over this range of vitamin B-6 status, with comparable reductions also seen in
human lymphocyte SHMT (12) in marginal vitamin B-6 deficiency. Thus, the related processes of 1-carbon metabolism and transsulfuration potentially can be impaired in marginal vitamin B-6 deficiency, particularly under conditions of dietary or metabolic increases in substrate loads or genetic alterations in enzyme activities. Our recent studies of marginal vitamin B-6 deficiency in humans showed elevated plasma glycine (12) and cystathionine (13) at levels of vitamin B-6 status considered adequate as defined in calculating the current RDA value (27), as reflected by plasma PLP ≥20 nmol/L. Thus, the relations among vitamin B-6-dependent metabolic processes, human health, and dietary recommendations require further clarification.

**Literature Cited**


