Patients on antiepileptic drugs (AEDs) frequently have low serum folate and high plasma total homocysteine (tHcy) (1–4). This metabolic disturbance has been implicated in an increased rate of cardiovascular disease, fetal malformations, dementia, and neuropsychiatric symptoms among patients on AEDs (5–7). Homocysteine metabolism is dependent on four B vitamins as cofactors: The methylation of homocysteine to methionine requires folate and vitamin B_{12}, and the irreversible transsulfuration to cysteine requires vitamin B_{6} (8). The recycling of folate cofactors is dependent on vitamin B_{12} and B_{6}, and vitamin B_{6} is necessary for activating vitamin B_{12} to pyridoxal 5′-phosphate (PLP) (9). Vitamin B_{2} and B_{6} status thus may have an impact on tHcy concentrations in patients taking AEDs, but few studies have reported on this matter (3, 4, 10, 11). The methionine loading test detects individuals with impaired homocysteine metabolism not detected by fasting tHcy concentrations alone and is often considered primarily a test of the transsulfuration pathway (1, 8, 12). We therefore determined the fasting and 6 h post-methionine loading (postload) plasma concentrations of thiols and B vitamins in patients taking AEDs.

We recruited 101 patients with symptomatic, cryptogenic, or primary generalized epilepsy from our outpatient clinic. None of them had epilepsy secondary to ischemic stroke or other conditions considered to be associated with high tHcy, and none were on prescribed vitamin supplements. We recruited 101 controls among blood donors and hospital employees. All participants gave written informed consent before entering the study, and The Regional Ethics Committee (University of Bergen, Norway) approved the study. The number of male patients and controls was identical (n = 53), as was the mean (SD) age [37.9 (15.0) and 37.3 (10.3) years for patients and controls, respectively; difference not significant]. There were 34 and 30 smokers among patients and controls, respectively (difference not significant). The patients had been on AEDs for 13 (11) years, and their medication had been unchanged the last 6 months before inclusion. Of the 101 patients, 43 were on carbamazepine monotherapy (group 1) and 24 were on valproate monotherapy (group 2); 21 received phenytoin, phenobarbital, and/or primidone (group 3); and 13 were on various combinations of the five AEDs (group 4). Twenty-four patients reported supplementing their diet with over-the-counter multivitamins: 10 in group 1, 3 in group 2, 7 in group 3, and 4 in group 4. The controls were not stratified for intake of over-the-counter multivitamins. Seven patients and eight controls were homozygous (TT genotype) for the C677T mutation of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, whereas 34 patients and 42 controls were heterozygous (CT genotype; difference not significant) (1, 2, 13).

Fasting blood samples were collected in the morning after an overnight fast. Thereafter, the participants were given l-methionine (100 mg/kg of body weight) and a standardized breakfast (13). A postload blood sample was collected 6 h later. The vials were immediately cooled on ice, protected from daylight, centrifuged at 2000×g for 10 min at 4 °C, and stored at −70 °C until analysis. The plasma concentrations of tHcy, cysteine, and cysteinylglycine were determined by HPLC (14). For the determination of vitamin B_{2} status, plasma concentrations of riboflavin and flavin nucleotides (FNs; i.e., the combined concentration of the coenzymes FAD and flavin mononucleotide) were determined by a modified HPLC method; CVs were <6% and <4%, respectively (15). Plasma PLP and pyridoxic acid were determined by HPLC (16). The serum concentrations of folate and cobalamin were determined by immunoassay (Access; Beckman Instruments). Plasma concentrations of thiols and vitamins did not follow a gaussian distribution as raw data; they therefore were log-transformed before calculations and are presented as geometric means with 95% confidence intervals. Before logistic regression analysis, continuous variables were divided into five groups corresponding to the quintiles. Statistical significance was set at P < 0.05.

In the total sample, both fasting and postload, the mean plasma concentration of riboflavin (i.e., the precursor of FNs) was similar in patients and controls (Table 1). However, analysis of patient (sub)groups and controls showed that fasting riboflavin concentrations were borderline lower in the patients in groups 1 and 3 (Table 1). When the patients on inducer AEDs (groups 1 and 3; n = 64) were analyzed as a single group and compared with controls, they had a low mean fasting riboflavin concentration (4.8 nmol/L; 95% confidence interval, 3.9–5.8 nmol/L; P = 0.02). Logistic regression analysis of patients and controls revealed that fasting riboflavin concentrations below the 20th percentile were more likely to
Table 1. Fasting and post-methionine loading concentrations of plasma thiols and B vitamins in patients on AEDs and in matched healthy controls.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 101)</th>
<th>All patients (n = 101)</th>
<th>Group 1 (CBZ)(^b) (n = 43)</th>
<th>Group 2 (VPA) (n = 24)</th>
<th>Group 3 (PHT, PB, PRD) (n = 21)</th>
<th>Group 4 (combinations)(^c) (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting FNs, nmol/L</td>
<td>44.6 (42.5–46.9)</td>
<td>49.0 (46.7–51.4)(^d)</td>
<td>48.3 (44.7–52.2)</td>
<td>48.9 (43.4–55.1)</td>
<td>46.8 (42.8–51.1)</td>
<td>55.3 (49.4–61.9)(^i)</td>
</tr>
<tr>
<td>Fasting riboflavin, nmol/L</td>
<td>6.3 (5.5–7.3)</td>
<td>5.5 (4.6–6.5)(^e)</td>
<td>5.0 (3.9–6.4)(^h)</td>
<td>7.1 (4.8–10.5)</td>
<td>4.3 (3.0–6.3)(^j)(^l)</td>
<td>6.7 (4.3–10.4)</td>
</tr>
<tr>
<td>FN/Riboflavin ratio</td>
<td>8.8 (9.3–11.8)</td>
<td>12.2 (10.1–14.4)(^d)</td>
<td>13.5 (9.7–17.4)(^i)</td>
<td>8.4 (6.5–10.3)</td>
<td>15.6 (9.4–21.8)(^l)</td>
<td>9.8 (6.6–10.3)</td>
</tr>
<tr>
<td>Fasting PLP, nmol/L</td>
<td>47.5 (43.0–52.6)</td>
<td>39.7 (36.4–43.3)(^d)</td>
<td>41.7 (35.6–48.9)(^j)^(^n)</td>
<td>39.4 (33.7–45.8)</td>
<td>35.8 (29.6–43.3)(^l)</td>
<td>40.6 (32.3–51.2)</td>
</tr>
<tr>
<td>PML PLP, nmol/L</td>
<td>52.6 (48.0–57.5)</td>
<td>40.0 (36.1–44.3)(^f)</td>
<td>45.8 (38.1–55.1)(^i)^(^n)</td>
<td>37.5 (32.5–43.3)(^l)</td>
<td>36.0 (27.9–46.2)(^i)</td>
<td>36.2 (27.3–48.2)(^l)</td>
</tr>
<tr>
<td>Fasting PA, nmol/L</td>
<td>14.6 (12.4–17.3)</td>
<td>10.6 (8.8–12.9)(^d)</td>
<td>10.4 (7.5–14.3)(^l)</td>
<td>10.5 (7.7–14.3)</td>
<td>11.8 (7.3–19.2)(^l)</td>
<td>10.0 (5.3–18.9)</td>
</tr>
<tr>
<td>PML PA, nmol/L</td>
<td>13.8 (12.6–15.2)</td>
<td>10.6 (10.6–15.0)(^d)</td>
<td>15.3 (11.5–20.5)(^l)</td>
<td>8.8 (6.5–11.9)</td>
<td>10.3 (7.3–16.0)</td>
<td>18.1 (11.4–28.8)</td>
</tr>
<tr>
<td>Fasting S-FA, nmol/L</td>
<td>11.0 (10.4–11.7)</td>
<td>9.8 (8.8–10.8)(^d)</td>
<td>8.1 (6.9–9.5)(^l)</td>
<td>11.1 (9.4–13.2)</td>
<td>11.8 (9.0–15.6)(^m)</td>
<td>10.2 (8.5–12.2)</td>
</tr>
<tr>
<td>PML S-FA, nmol/L</td>
<td>9.5 (9.0–10.0)</td>
<td>8.3 (7.6–9.2)(^d)</td>
<td>7.0 (6.1–8.0)(^l)</td>
<td>9.7 (8.1–11.6)</td>
<td>9.8 (7.4–13.1)(^l)</td>
<td>8.7 (7.3–10.4)</td>
</tr>
<tr>
<td>Fasting S-B(_{12}), pmol/L</td>
<td>279 (262–296)</td>
<td>294 (272–319)(^g)</td>
<td>261 (234–291)(^l)</td>
<td>387 (343–437)(^l)</td>
<td>271 (216–339)</td>
<td>301 (234–389)</td>
</tr>
<tr>
<td>Fasting tHcy, μmol/L</td>
<td>8.8 (8.3–9.3)</td>
<td>10.3 (9.5–11.2)(^d)</td>
<td>10.9 (9.4–12.7)(^l)</td>
<td>9.0 (8.1–10.0)</td>
<td>10.8 (8.9–13.2)(^l)</td>
<td>10.5 (8.5–13.0)</td>
</tr>
<tr>
<td>PML tHcy, μmol/L</td>
<td>28.0 (26.4–29.6)</td>
<td>39.2 (36.2–42.4)(^h)</td>
<td>41.8 (36.3–48.1)(^l)</td>
<td>31.7 (28.4–35.4)</td>
<td>46.3 (39.4–55.1)(^l)</td>
<td>35.8 (29.7–43.3)(^l)</td>
</tr>
<tr>
<td>ΔtHcy, μmol/L</td>
<td>18.7 (17.5–20.1)</td>
<td>27.7 (25.3–30.5)(^l)</td>
<td>29.1 (24.5–34.5)(^l)</td>
<td>22.3 (19.3–25.7)</td>
<td>34.8 (28.8–42.2)(^l)</td>
<td>24.8 (20.1–30.7)(^l)</td>
</tr>
<tr>
<td>Fasting Cys, μmol/L</td>
<td>223 (218–228)</td>
<td>243 (231–256)(^d)</td>
<td>231 (208–257)(^l)</td>
<td>240 (226–255)</td>
<td>253 (237–270)(^l)</td>
<td>277 (256–298)</td>
</tr>
<tr>
<td>PML Cys, μmol/L</td>
<td>214 (208–219)</td>
<td>223 (225–241)(^h)</td>
<td>228 (215–242)(^l)</td>
<td>231 (217–245)</td>
<td>229 (209–250)(^l)</td>
<td>261 (237–286)</td>
</tr>
</tbody>
</table>

\(^a\) Concentrations are geometric means (confidence intervals), except for the FN/riboflavin concentration ratios, which are arithmetic means. Because 6 h post-methionine loading concentrations of vitamin B\(_{12}\) and serum vitamin B\(_{12}\) were not different from fasting concentrations, they are not presented. Fasting concentrations are in the morning after an overnight fast. Post-methionine loading (PML) is 6 h postloading. Some subgroup data on tHcy, folate, and vitamin B\(_{12}\) have been published previously (1, 2, 13).

\(^b\) CBZ, carbamazepine; VPA, valproate; PHF, phenytoin; PB, phenobarbital; PRD, primidone; PML, postmethionine loading; PA, pyridoxic acid; S, serum; FA, folate.

\(^c\) Group 4 received various combinations of carbamazepine, valproate, phenytoin, phenobarbital, and primidone.

\(^d\) Two-sample t-test of patients vs controls: \(P \geq 0.01\); \(^h\) not significant (\(P \geq 0.05\)); \(^g\) benign only after exclusion of 24 patients on multivitamins.

\(^i\) Univariate ANOVA (with the post hoc Fisher projected least significant difference test) of the subgroup of patients vs the controls: \(^h\) not significant (\(P > 0.05\)); \(^j\) not significant (\(P > 0.05\)) only after exclusion of 24 patients on multivitamins.

\(^k\) Univariate ANOVA (with Fisher projected least significant difference test) of actual (sub)group of patients vs the controls: \(^l\) not significant (\(P > 0.05\)); \(^m\) not significant (\(P > 0.05\)) only after exclusion of 24 patients on multivitamins.

\(^o\) After exclusion of 10 patients on multivitamins, the fasting mean PLP concentration in group 1 changed to 39.3 (34.6–44.6) nmol/L.

\(^p\) After exclusion of 10 patients on multivitamins, the postload mean concentration of PLP in group 1 changed to 42.3 (36.0–49.7) nmol/L.

\(^q\) ΔtHcy, mean increment in tHcy concentrations from fasting to PML.
represent patients on inducer AEDs (odds ratio, 3.5; \( P = 0.01 \)). Riboflavin concentrations did not change postmethionine loading. The patients had higher fasting plasma concentrations of FNs (the coenzyme forms of vitamin B\(_2\)) than did the controls (Table 1). The fasting FN/riboflavin concentration ratio was increased in patients on inducer AEDs (groups 1 and 3; \( P = 0.003 \) and 0.001, respectively; Table 1). However, patients on valproate (group 2) had FN/riboflavin ratios that did not differ from those of the controls and were significantly lower than the ratio for group 1 and 3 patients (\( P = 0.02 \) and 0.005, respectively). These findings persisted after we excluded the 24 patients on over-the-counter multivitamins. Multiple regression analysis of the total patient sample (n = 101) revealed a negative correlation between plasma riboflavin and tHcy only when adjusting for age and gender (\( r = -0.22; \ P = 0.03 \)), and this correlation disappeared when the other vitamins were introduced into the model. Furthermore, in the patients, fasting plasma riboflavin concentrations were positively correlated with circulating concentrations of folate (\( r = 0.28; \ P = 0.006 \)) and PLP (\( r = 0.30; \ P = 0.003 \); Fig. 1). In the controls, plasma riboflavin was inversely correlated with tHcy (\( P = 0.02 \)) and had a borderline positive relationship with PLP (\( P = 0.08 \)) but not folate.

The present study demonstrates that inducer AEDs (groups 1 and 3) influence the FN/riboflavin concentration ratio and have associated low plasma concentrations of riboflavin. Low plasma concentrations of riboflavin may indicate low vitamin B\(_2\) status (9, 17). FNs are the active coenzyme forms of vitamin B\(_2\), and their major constituent, FAD, is usually tightly regulated and minimally influenced by dietary intake (17). However, Hamajima et al. (18) reported that phenobarbital injections in rodents would induce increased activity of FN-synthesizing enzymes in the liver and thus cause high concentrations of FNs and low concentrations of riboflavin. Previously, Krause et al. (10) reported high erythrocyte glutathione reductase activation coefficients (an indicator of riboflavin deficiency) in patients on carbamazepine, phenytoin, and primidone, but not valproate. Likewise, Lewis et al. (11) reported low urinary excretion of riboflavin in children treated with anticonvulsants. Thus, our results are in agreement with previous findings. Inducer AEDs induce increased enzyme activity in the liver, with increased demand for cofactors. Thus, the low plasma concentrations of riboflavin may be caused by increased liver demand with a shift in body pools and may possibly represent a nondietary riboflavin deficiency (18).

The patients had lower fasting and postload plasma concentrations of PLP (the active form of vitamin B\(_6\)) than did the controls. There was no evidence of differences between patient (sub)groups in this respect (Table 1). In response to methionine loading, PLP concentrations increased in the controls (\( P < 0.0001 \)), but not in the patients. Thus, the difference between patients and controls was more evident postload (Table 1). Logistic regression analysis of the total sample (n = 202) revealed that postload PLP concentrations below the 40th percentile were more likely to represent patients than controls (odds ratio, 5.0; \( P < 0.005 \)). Furthermore, the patients had lower fasting concentrations of pyridoxic acid (an inactive degradation
product of vitamin B₆; Table 1) than the controls. The pyridoxic acid concentrations did not change postload. In a stepwise regression model of the patient sample (n = 101), the strongest independent predictors of fasting tHcy were fasting concentrations of folate (r = −0.42) and PLP (r = −0.16; P < 0.0001). This finding persisted after adjustment for age, gender, use of tobacco, and the presence of the C677T mutation of MTHFR. The strongest independent predictors of postload tHcy concentrations were postload folate (r = −0.29) and PLP (r = −0.17; P < 0.0001). In the control sample, folate was negatively correlated with tHcy (P < 0.001), whereas PLP concentrations were not.

It thus appears that all five AEDs are associated with low plasma concentrations of PLP. Low PLP concentrations may contribute to abnormal homocysteine metabolism. The mechanism behind the postload increment in PLP concentrations in the healthy controls is quite unclear, but it may be related to the increased activity of the transsulfuration pathway in response to methionine loading.

The concentrations of tHcy, cysteine, folate, and cobalamin in all groups are given in Table 1 (1, 2, 13). The concentrations of cysteinylglycine were similar in patients and controls.

In our study, patients on inductor AEDs (groups 1 and 3) had high plasma FN/riboflavin concentration ratios associated with low riboflavin concentrations, which may indicate a functional deficiency or merely a shift in body pools. Riboflavin concentrations were positively correlated with serum folate and PLP concentrations and were weakly correlated with tHcy concentrations. It therefore seems reasonable to ensure an adequate intake of riboflavin in patients on inductor AEDs.

Patients on AEDs had low plasma PLP concentrations, and both fasting and postload PLP concentrations were negatively correlated with tHcy. Patients on AEDs apparently have a decreased efficiency of the transsulfuration pathway, and the low coenzyme concentrations (PLP) may be of relevance in this context. Supplementation with pyridoxine hydrochloride may therefore contribute to improved homocysteine metabolism in patients on AEDs (7). Currently, many patients on AEDs receive supplements containing high doses of folic acid, which may have convulsant effects (19). Because low vitamin B₆ is associated with a lowered seizure threshold (20, 21), pyridoxine supplementation may possibly be beneficial for seizure control in patients on AEDs.

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References

CYP2D6 Poor Metabolizer Status Can Be Ruled Out by a Single Genotyping Assay for the –1584G Promoter Polymorphism, Andrea Gaedigk,1 Darren L. Ryder,1 L. DiAnne Bradford,1 and J. Steven Leeder1 (1 Division of Developmental Pharmacology and Medical Toxicology, The Children’s Mercy Hospital and Clinics, Kansas City, MO 64108; 2 Department of Psychiatry and Medicine, Morehouse School of Medicine, Atlanta, GA 30310; 3 address correspondence to this author at: The Children’s Mercy Hospital, Division of Clinical Pharmacology, 2401 Gillham Rd., Kansas City, MO 64108; fax 816-855-1958, e-mail agaedigk@cmh.edu)

Accurate prediction of CYP2D6 phenotype from genotype data is important for many clinically relevant drugs.