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Determinants of hyperhomocysteinemia in patients with chronic liver disease and after orthotopic liver transplantation[1–3]

Anja Bosy-Westphal, Martina Ruschmeyer, Norbert Czech, Gerd Oehler, Holger Hinrichsen, Matthias Plauth, Erich Lotterer, Wolfgang Fleig, and Manfred James Müller

ABSTRACT

Background: Homocysteine metabolism may be impaired in chronic liver disease, possibly contributing to fibrogenesis and disease complications.

Objective: The goal was to investigate the prevalence and determinants of basal and postprandial hyperhomocysteinemia in patients with chronic liver disease and after orthotopic liver transplantation (OLT).

Design: This was a cross-sectional study of 323 patients with chronic liver disease (93 with hepatitis, 8 with fatty liver, 168 with cirrhosis, and 54 after OLT) and 25 healthy control subjects. Portohepatovenous gradients of total homocysteine (tHcy) and methionine and postload methionine and tHcy kinetics before and after 10 d of supplementation with folate plus vitamin B-6 were investigated in subgroups.

Results: Basal hyperhomocysteinemia was observed in all patient groups (34% of patients with hepatitis, 50% with fatty liver, 54% with cirrhosis, and 52% after OLT). It was more frequently seen in patients with elevated plasma creatinine concentrations and at advanced stages of liver disease. Mean plasma folate was normal in patients with liver disease, but vitamin B-12 was elevated in cirrhosis and vitamin B-6 was low after OLT. There were significant negative associations between tHcy and folic acid or vitamin B-12 concentrations in control subjects and in patients with hepatitis and after OLT. No systematic association between portohepatovenous differences in tHcy and methionine concentrations was found. Cirrhosis was accompanied by impaired methionine clearance. After vitamin supplementation, the area under the tHcy curve improved in cirrhosis at nearly unchanged basal tHcy concentrations.

Conclusions: Basal hyperhomocysteinemia is seen in ≈50% of patients with cirrhosis and after OLT. Basal tHcy concentrations do not change significantly after supplementation with folate and vitamin B-6, but postprandial Hcy metabolism improves. *Am J Clin Nutr* 2003;77:1269–77.

KEY WORDS Homocysteine, methionine load, hepatitis, liver cirrhosis, liver transplantation, folic acid, vitamin B-12, vitamin B-6

INTRODUCTION

The liver is central in methionine and homocysteine (Hcy) metabolism. Therefore, disturbances in liver function are likely to affect the metabolism of both methionine and Hcy. Methionine metabolism is impaired in patients with cirrhosis (1, 2). In addition, impairment of postprandial Hcy metabolism was recently reported in a group of patients with chronic liver disease (3). Regarding basal total Hcy (tHcy), the data suggest that patients with alcoholic cirrhosis have higher plasma concentrations than do healthy control subjects (4). Because greater alcohol consumption (5) and alcoholism (6, 7) also increase tHcy concentrations, the effects of hepatitis and cirrhosis by themselves remain unclear. Hyperhomocysteinemia was also observed in patients with nonalcoholic cirrhosis (8–10). tHcy is higher in cirrhosis than in noncirrhotic liver disease (8) and was even normal in a group of patients with chronic hepatitis C (11). Hyperhomocysteinemia was associated with the clinical course of liver disease and was more pronounced at advanced stages of cirrhosis (8). Increased serum tHcy was also observed 4 mo or > 12 mo after orthotopic liver transplantation (OLT; 12, 13). In these patients, the prevalence of hyperhomocysteinemia was 27% (12) and 47% (13), respectively.

Concerning the mechanisms of basal hyperhomocysteinemia in cirrhosis, impaired transsulfuration (8, 9) and remethylation (9, 14) have been proposed. The messenger RNA levels of numerous enzymes involved in methionine and Hcy metabolism [eg, methionine adenosyltransferase (MAT; EC 2.5.1.6), methionine synthetase (6.1.1.10), and cystathionine β-synthase (EC 4.2.1.22)] are reduced in cirrhotic liver (15). Plasma concentrations of the physiologic determinants of Hcy metabolism (ie, folate, vitamin B-12, and vitamin B-6) showed no or only weak associations with basal tHcy concentrations in these patients (4, 9). Plasma concentrations of these vitamins may not reflect tissue stores in cirrhosis because of cellular damage and thus leakage into plasma (16, 17). In OLT patients, tHcy correlated with folate concentrations, but folate explained only 4% of tHcy variability (13). Nevertheless, treatment with folic acid reduced basal tHcy concentrations in 9 of 10 OLT patients (12). As to further determinants of tHcy, increased serum creatinine concentrations were associated with elevated tHcy in cirrhotic (8, 9) and OLT (12, 13) patients.

Thus, in cirrhosis, impaired liver function seems to be accompanied by basal and postprandial hyperhomocysteinemia. It is tempting to speculate that increased tHcy concentrations add to

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[2] Supported by the Else Kröner Fresenius Stiftung.

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Received February 26, 2002.

Accepted for publication October 30, 2002.

TABLE 1
Characterization of the study population[1]

	Age	BMI	Albumin	Quick	Bilirubin	GOT	GPT	Creatinine
	y	*kg/m²*	*g/L*	*%*	*μmol/L*	*U/L*	*U/L*	*μmol/L*
Control subjects (*n* = 12 M, 13 F)	48.6 ± 11.1[a,b]	25.5 ± 3.9[a,b]	—	—	—	—	—	—
Patients								
Hepatitis (*n* = 41 M, 52 F)	45.1 ± 11.8[a]	24.9 ± 4.3[a,b]	43 ± 3[b]	107 ± 19[b]	13.7 ± 6.8[a]	26.1 ± 35.8[a,b]	37.6 ± 49.2	60.7 ± 10.5[a]
Viral disease (*n* = 31 M, 34 F)	45.2 ± 12.8	25.0 ± 4.6	43 ± 3	107 ± 18	13.7 ± 5.1	31.8 ± 41.5	47.2 ± 56.1	61.9 ± 10.5
HCV (*n* = 27 M, 26 F)	45.9 ± 11.9	24.6 ± 4.1	43 ± 3	108 ± 19	13.7 ± 6.8	36.5 ± 44.6	54.3 ± 59.5	61.4 ± 10.8
HBV (*n* = 4 M, 8 F)	42.0 ± 16.3	27.0 ± 6.3	44 ± 3	100 ± 13	13.7 ± 5.1	11.0 ± 4.6	15.4 ± 14.0	64.2 ± 9.5
Autoimmune disease (*n* = 1 M, 5 F)	43.2 ± 10.5	26.9 ± 3.3	41 ± 3	108 ± 4	13.7 ± 5.1	14.3 ± 4.8	18.3 ± 11.1	53.8 ± 8.0
Other (*n* = 1 M, 2 F)	49.0 ± 12.8	20.1 ± 0.9	42 ± 4	95 ± 8	32.5 ± 8.6	16.3 ± 5.1	17.7 ± 6.4	52.4 ± 6.2
In remission (*n* = 8 M, 11 F)	44.5 ± 8.8	24.4 ± 3.6	42 ± 2	108 ± 28	10.3 ± 5.1	11.9 ± 5.4	14.2 ± 7.7	59.8 ± 10.7
Fatty liver (*n* = 4 M, 4 F)	56.5 ± 9.4[a,b]	26.5 ± 6.5[a,b]	42 ± 6[a,b]	115 ± 17[b]	17.1 ± 8.6[a,b]	19.0 ± 12.2[a,b]	23.1 ± 15.7	68.0 ± 20.6[a,b]
Liver cirrhosis (*n* = 93 M, 75 F)	53.1 ± 10.8[b]	25.7 ± 4.8[b]	37 ± 7[a]	86 ± 34[a]	39.3 ± 47.9[b]	33.1 ± 43.4[b]	29.3 ± 34.1	70.0 ± 39.2[a,b]
Child A (*n* = 42 M, 35 F)	52.6 ± 10.5	26.4 ± 4.8	41 ± 5[z]	92 ± 19[y]	20.5 ± 15.4[x]	25.6 ± 20.4[x]	29.1 ± 23.2	67.1 ± 26.8[x]
Child B (*n* = 37 M, 28 F)	53.8 ± 11.7	25.5 ± 4.8	36 ± 6[y]	88 ± 45[y]	30.8 ± 18.8[x]	29.8 ± 23.3[x]	26.7 ± 34.1	62.9 ± 16.7[x]
Child C (*n* = 14 M, 12 F)	53.1 ± 9.3	24.4 ± 4.8	31 ± 7[x]	62 ± 26[x]	111.2 ± 80.4[y]	64.0 ± 94.3[y]	36.4 ± 55.7	97.3 ± 81.3[y]
Alcohol disease (*n* = 47 M, 35 F)	52.4 ± 9.0	25.7 ± 5.6	37 ± 7	84 ± 45	51.3 ± 63.3	28.1 ± 39.9	19.8 ± 16.9	72.4 ± 44.2
Viral disease (*n* = 32 M, 13 F)	56.2 ± 11.8	25.9 ± 3.4	37 ± 9	82 ± 18	25.7 ± 18.8	36.4 ± 25.1	41.7 ± 40.1	76.0 ± 43.8
Biliary disease (*n* = 1 M, 11 F)	50.6 ± 14.5	26.1 ± 4.4	40 ± 4	108 ± 24	23.9 ± 20.5	24.2 ± 16.0	24.0 ± 13.2	58.6 ± 16.2
Autoimmune disease (*n* = 2 M, 8 F)	51.6 ± 13.5	24.7 ± 4.2	33 ± 6	78 ± 14	35.9 ± 22.2	72.4 ±118.8	55.8 ± 86.1	59.5 ± 16.5
Other (*n* = 11 M, 8 F)	51.6 ± 11.2	25.9 ± 4.3	38 ± 5	92 ± 16	30.8 ± 22.2	30.8 ± 21.7	28.1 ± 19.5	58.7 ± 10.6
OLT (*n* = 27 M, 27 F)	50.4 ± 10.9[a,b]	22.7 ± 3.7[a]	40 ± 5[a]	101 ± 23[b]	22.2 ± 13.7[a]	13.7 ± 15.2[a]	20.7 ± 25.4	80.1 ± 27.1[b]

[1] $\bar{x}$ ± SD. Quick, Quick prothrombin test; GOT, glutamic-oxaloacetic transaminase (EC 2.6.1.1); GPT, glutamic-pyruvic transaminase (EC 2.6.1.2); HCV, hepatitis C virus; HBV, hepatitis B virus; OLT, after orthotopic liver transplantation. Test-specific reference ranges are as follows: albumin, 35–50 g/L; Quick, 70–130%; bilirubin, 3.4–17 μmol/L; GOT, <15 U/L; GPT, <22 U/L. For the 5 primary subject groups, values in the same column with different superscript letters (a, b, c) are significanty different, $P < 0.05$, and for the 3 child categories, values in the same column with different superscript letters (x, y, z) are significantly different, $P < 0.05$ (ANOVA with Bonferroni's post hoc test).

oxidant stress and DNA damage (18, 19) and increased hepatocellular apoptosis and proliferation (20) and thus contribute to liver fibrosis (21). In addition, elevated tHcy concentrations may be associated with vascular complications associated with cirrhosis (9) and with atherosclerotic disease seen in liver transplant recipients (13). However, the exact prevalence of hyperhomocysteinemia, detailed analyses of subgroups, and possible determinants of hyperhomocysteinemia are unknown. The present study was designed to investigate *1)* the prevalence of hyperhomocysteinemia and the determinants of basal tHcy concentrations in chronic liver disease, *2)* the portohepatovenous gradients of tHcy and methionine, *3)* post-methionine-load tHcy and methionine kinetics, and *4)* the effect of 10 d of supplementation with folate plus vitamin B-6 on basal and postprandial tHcy and methionine kinetics.

SUBJECTS AND METHODS

Study populations and protocols

The cross-sectional part of the study included 323 patients with chronic liver disease (93 patients with chronic hepatitis, 8 with fatty liver, 168 with liver cirrhosis, and 54 after OLT) and 25 healthy age-matched control subjects with no history of renal or hepatobiliary disease. Clinical characterization was performed as described previously (22). All patients underwent standard clinical and biochemical evaluations, and clinical characteristics are given in **Table 1**. Diagnosis of liver cirrhosis was histologically proven, and subjects were subdivided for severity of liver disease according to the Child-Pugh classification (23). Drug therapy in cirrhotic patients included diuretic treatment (50%), antihypertensives (23%), and antidiabetics (11%). Patients with liver disease of autoimmune etiology received low-dose corticosteroids. For OLT patients, the mean (±SD) time since transplantation was 2 ± 4 y (range: 1 mo to 14 y). Immunosuppressive therapy in these patients included cyclosporine (40 patients, 11 were also treated with azathioprine and 9 with mycophenolate mofetil), tacrolimus (12 patients, 2 were additionally receiving mycophenolate mofetil), azathioprine (2 patients), or mycophenolate mofetil (2 patients). Ninety percent of the OLT patients ($n = 45$) were also taking corticosteroids and 17% were receiving vitamin B supplements ($n = 9$). All patients had dietary recommendations according to their complications (eg, sodium restriction for patients with ascitis and a protein intake of 0.8 g/kg body wt for patients with clinical signs of encephalopathy). Assuming a methionine content of 2% of ingested protein, the estimated methionine intake was ≈16 mg · kg body $wt^{-1} \cdot d^{-1}$. Blood samples were taken after the subjects had fasted overnight.

In a second protocol, portohepatovenous gradients of amino acids were determined in 16 patients with liver cirrhosis. Blood samples of the vena portae and vena hepatica were collected during implantation of a transjugular intrahepatic portosystemic stent shunt (TIPS) for therapy of portal hypertension.

In a third protocol, post-methionine-load tHcy metabolism was investigated in a subgroup of 16 consecutively recruited patients with liver cirrhosis [mean (±SD) age and body mass index (in kg/m^2) for 13 male and 3 female patients: 54 ± 9.6 y and 25.4 ± 5.6, respectively] and in 6 female and 2 male healthy control subjects (age: 27 ± 3.2 y; BMI: 21.0 ± 1.2; group difference in age, $P < 0.01$). Ten patients were classified as having Child A liver disease severity, 4 as having Child B, and 2 as having Child C. For these patients, the mean serum creatinine concentration was

68.0 ± 10.4 μmol/L, which was not significantly different from that for the whole group of 168 cirrhotic patients studied in protocol 1. Exclusion criteria for the methionine load protocol were clinical instability, clinical signs of portosystemic encephalopathy, pregnancy, restriction of dietary protein (< 0.8 g · kg body $wt^{-1} \cdot d^{-1}$), and elevated ammonia and serum creatinine concentrations (ammonia > 55 μmol/L for males and > 48 μmol/L for females; creatinine > 106 μmol/L for males and > 80 μmol/L for females). After the subjects had fasted overnight, venous blood samples were obtained and 0.05 g L-methionine/kg body wt was administered orally. To overcome an unpleasant taste, 37.5 mg L-methionine/kg body wt was dissolved in 200 mL orange juice. Because of impaired fuel homeostasis in cirrhosis (24), the remaining methionine was administered together with a commercial formula diet (Salvipeptid Nephro; Nestlé Clinical Nutrition GmbH, Munich, Germany) containing 4.8 g L-methionine/100 g to avoid a 9-h starvation period in the control subjects and patients with cirrhosis. The orange juice and formula diet were ingested by the subjects within 10 min. An intravenous catheter was placed in an antecubital vein, and its patency was maintained by injection of 2 mL heparin in saline after each blood collection. Samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and 9 h after the methionine load. Observational data obtained in one healthy subject showed that tHcy concentrations returned to baseline within 24 h of the methionine load. In this case, ≈50% of the area under the time-dependent plasma concentration curve of tHcy (AUC_{Hcy}) could be recorded within 9 h after the methionine load. For practical reasons, the duration of the loading test had to be confined to 9 h. During this time, the subjects were allowed to drink the protein-free energy component of the formula diet containing 1928 kJ/100 g.

A subsequent intervention (protocol 4) aimed to improve basal and postrandial tHcy metabolism through the oral administration of 5 mg folic acid (Folsäure Hevert; Hevert-Arzneimittel GmbH, Bad Sobernheim, Germany) and 20 mg vitamin B-6 (Vitamin B-6-Jenapharm; Jenapharm GmbH, Jena, Germany) for 10 d in patients and control subjects. On day 11, the study period was completed with a second methionine load.

The study protocols were approved by the local ethical committee of the Christian-Albrechts University Kiel. All patients and control subjects gave their written informed consent before the study.

Laboratory analyses

Venous blood samples were drawn in EDTA-coated evacuated tubes on ice. Plasma was separated within 30 min and was stored at –40 °C until analyzed. Concentrations of plasma amino acids and the vitamin B-6 vitamer pyridoxal-*P* (PLP) were measured by HPLC and fluorescence detection by the methods of Fermo et al (25) and Kimura et al (26), respectively. tHcy and cysteine comprise bound protein as well as disulfide forms and free thiols. A detailed description of the technical equipment and chromatographic conditions is given elsewhere (9). Plasma tHcy, serine, and cysteine concentrations that were 2 SDs greater than the mean of the healthy control subjects were considered to be elevated. All reagents were purchased from Sigma (Deisenhofen, Germany), except for the cysteine, serine, and methionine standards, which were from Fluka (Deisenhofen, Germany), and the derivatization reagent fluoraldehyde *o*-phthaldialdehyde solution, which was from Pierce (no. 26025; Rockford, IL). Plasma folate and vitamin B-12 were analyzed by using a radioimmunoasay kit (DPC; Biermann diagnostica GmbH, Bad Naunheim, Germany). The normal range for plasma folic acid is 3–17 ng/mL and that for vitamin B-12 is 200–950 pg/mL.

Statistical and pharmacokinetic analyses

All data are expressed as arithmetic means ± SDs. Significant differences between patient groups and between the patients and the healthy control subjects were determined by analysis of variance with Bonferroni's post hoc test. This analysis was also used to compare the Child disease severity groups. Portohepatovenous differences were tested by use of Wilcoxon's signed-rank sum test for related samples. Differences in vitamin, tHcy, and methionine concentrations or in pharmacokinetic variables between patients and control subjects before supplementation were evaluated for significance by standard Student's *t* tests. Corresponding intraindividual differences before versus after vitamin supplementation were tested by paired-sample *t* tests. The difference in response to treatment between patients and control subjects was tested by comparing the means of intraindividual changes before versus after vitamin supplementation for both groups by Student's *t* test. The *r* coefficients of correlation analysis are given as nonparametric Spearman's coefficients. Variables that were significantly correlated with tHcy were included in a multiple stepwise regression analysis in which tHcy was the dependent variable. The explained variance (R^2) was calculated. In all analyses, tests were two-tailed and a probability value < 0.05 was considered statistically significant. Statistical analyses were performed by using SPSS version 6.1 (SPSS Inc, Chicago).

AUCs for methionine were calculated by NCSS (trial version; NCSS Statistical Software, Kaysville, UT) by using the trapezoidal rule. The elimination rate constant (k_e) of methionine was estimated by using the slope of the least-squares regression line on a semilogarithmic plot of the plasma concentration versus time curve at decay. The monoexponential methionine decay reflects first-order elimination kinetics. The elimination half-life for methionine ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2/k_e$. The volume of distribution (V_D) was obtained by using the *y* intercept (C_0) of the least-squares regression line: V_D = administered methionine dose/C_0.

RESULTS

Protocol 1: cross-sectional study

Mean plasma concentrations of tHcy, methionine, cysteine, serine, folic acid, vitamin B-6, and vitamin B-12 and the corresponding prevalences of elevated or low concentrations in both the patients and the control subjects are given in **Table 2**. Basal tHcy concentrations were elevated in all patient groups, rising from hepatitis and fatty liver to OLT and cirrhosis. There was a trend toward higher tHcy concentrations in more severe stages of liver disease that was reflected by *1)* significantly higher tHcy concentrations in cirrhosis than in hepatitis (14.3 and 17.6 μmol/L; $P < 0.001$) and *2)* an increase in tHcy from Child A to Child C in cirrhosis (from 16.4 to 21.3 μmol/L; $P < 0.05$). However, the difference in tHcy between the Child A and Child C groups disappeared after the exclusion of patients with elevated serum creatinine concentrations (15.2 ± 5.2 and 18.2 ± 0.4 μmol/L, respectively; NS). Among the subgroups with distinct etiologies of liver disease (toxic, viral, biliary, or autoimmune), there were no significant differences in mean tHcy concentrations either in hepatitis or in cirrhosis (data not shown).

TABLE 2
Concentrations of plasma amino acids and vitamins and the prevalence of high or low values in 25 healthy control subjects and 323 patients with liver disease[1]

	Homocysteine	Methionine	Cysteine	Serine	Folic acid	Vitamin B-12	Vitamin B-6
	μmol/L (%)	*μmol/L (%)*	*μmol/L (%)*	*μmol/L (%)*	*ng/mL (%)*	*pg/mL (%)*	*pmol/mL (%)*
Control subjects (n = 25)	9.5 ± 2.7^{a} (0)	22.8 ± 9.3^{a} (4)	293 ± 87^{a} (0)	105 ± 9.3^{a} (4)	7.8 ± 2.9 (0)	325 ± 136^{a} (0)	64 ± 54^{b} (12)
Patients							
Hepatitis (n = 93)	14.3 ± 6.1^{a,b} (34)	31.9 ± 7.3^{a,b} (31)	293 ± 72^{a} (1)	163 ± 67 (3)	7.3 ± 3.8 (4)	409 ± 258^{a} (7)	40 ± 37^{a,b} (31)
Fatty liver (n = 8)	15.2 ± 6.1^{a,b,c} (50)	26.1 ± 6.5^{a,b} (50)	320 ± 109^{a,b} (13)	100 ± 21^{a} (0)	9.8 ± 4.3 (0)	447 ± 252^{a,b} (88)	57 ± 50^{a,b} (25)
Liver cirrhosis (n = 168)	17.6 ± 9.9^{c} (54)	42.8 ± 44.5^{b} (49)	340 ± 109^{a} (12)	163 ± 89 (39)	7.6 ± 4.6 (4)	738 ± 611^{b} (20)	53 ± 80^{b} (35)
Child A (n = 77)	16.4 ± 9.6^{x} (48)	31.2 ± 11.0^{x} (34)	317 ± 84^{x} (7)	162 ± 76 (39)	7.5 ± 4.7 (4)	534 ± 384^{x} (5)	51 ± 70 (35)
Child B (n = 65)	17.6 ± 8.7^{x,y} (55)	43.3 ± 31.4^{x} (62)	351 ± 114^{x} (14)	162 ± 85 (40)	7.6 ± 4.4 (6)	724 ± 506^{x} (20)	52 ± 85 (32)
Child C (n = 26)	21.3 ± 12.6^{y} (69)	75.9 ± 93.8^{y} (62)	382 ± 146^{y} (23)	170 ± 122 (39)	9.8 ± 6.5 (0)	1538 ± 918^{y} (62)	58 ± 98 (35)
Serum creatinine normal[2] (n = 149)	16.3 ± 6.8^{c} (50)	43.9 ± 46.9^{b} (50)	333 ± 104^{a} (11)	166 ± 88 (41)	7.4 ± 4.2 (5)	707 ± 582^{b} (17)	54 ± 83^{b} (36)
Serum creatinine elevated[3] (n = 19)	27.9 ± 20.0 (79)	34.1 ± 13.1 (32)	394 ± 137 (21)	137 ± 82 (36)	9.1 ± 7.3 (5)	1029 ± 770 (37)	40 ± 51 (21)
OLT (n = 54)	16.9 ± 5.8^{b,c} (52)	29.7 ± 13.5^{a,b} (13)	390 ± 116^{b} (30)	144 ± 70^{a} (28)	6.7 ± 3.7 (6)	464 ± 256^{a} (6)	30 ± 49^{a} (61)
Serum creatinine normal[2] (n = 36)	15.0 ± 4.0^{b,c} (42)	29.8 ± 15.5^{a,b} (19)	384 ± 114^{b} (28)	156 ± 78^{a} (31)	6.6 ± 3.0 (8)	494 ± 280^{a} (8)	17 ± 13^{a} (67)
Serum creatinine elevated[3] (n = 18)	20.4 ± 7.0 (72)	29.4 ± 11.2 (11)	401 ± 123 (33)	119 ± 46 (22)	6.9 ± 4.8 (0)	397 ± 178 (11)	58 ± 78 (56)

[1] $\bar{x}$ ± SD; prevalence of high or low values in parentheses. High or low values were as follows: homocysteine, >15 μmol/L; methionine, >25 μmol/L; cysteine, >466 μmol/L; serine, >153 μmol/L; folic acid, <3 ng/mL; vitamin B-12, >200 pg/mL; vitamin B-6, <20 pg/mL. OLT, after orthotopic liver transplantation. For the 5 primary subject groups, values in the same column with different superscript letters (a, b, c) are significanty different, $P < 0.05$, and for the 3 Child categories, values in the same column with different superscript letters (x, y, z) are significantly different, $P < 0.05$ (ANOVA with Bonferroni's post hoc test).

[2] Creatinine <80 μmol/L for females and <106 μmol/L for males.

[3] Creatinine >80 μmol/L for females and >106 μmol/L for males.

To examine the effect of renal function on tHcy concentrations, a separate analysis was done for patients with cirrhosis and after OLT with normal and elevated serum creatinine concentrations. Patients with elevated creatinine concentrations had significantly higher tHcy concentrations [27.9 compared with 16.3 μmol/L in cirrhosis ($P < 0.001$) and 20.4 compared with 15.0 μmol/L after OLT ($P < 0.05$)]. Hyperhomocysteinemia was still observed in the cirrhosis and OLT patients after the exclusion of patients with elevated creatinine concentrations (Table 2). Positive correlations between tHcy and creatinine were found in cirrhosis ($r = 0.356$, $P < 0.001$) and after OLT ($r = 0.384$, $P < 0.01$).

Similar to tHcy, cysteine concentrations tended to increase with the severity of liver disease. The highest cysteine concentrations were observed in Child C patients and after OLT (Table 2). There was a positive correlation between tHcy and cysteine concentrations in cirrhosis ($r = 0.273$, $P < 0.001$). The elevation of plasma serine was similar in all patient groups except for fatty liver and OLT patients, for whom normal serine concentrations were observed (Table 2). Vitamin B-12 and methionine concentrations were elevated in cirrhosis. No significant differences in folic acid concentrations were found between the groups. Plasma concentrations of the vitamin B-6 vitamer PLP were reduced in OLT patients (Table 2).

Nine OLT patients were receiving vitamin supplements at the time of the study (7 were taking 2 mg vitamin B-6 and 1 μg vitamin B-12; 2 were taking 50 mg vitamin B-6 and 1 mg vitamin B-12). In the vitamin-supplemented subgroup, mean plasma concentrations of tHcy, vitamin B-12, and vitamin B-6 were 16.8 μmol/L, 523.6 pg/mL, and 100.5 pmol/mL, respectively, compared with 17.2 μmol/L, 444.4 pg/mL, and 17.2 pmol/mL, respectively in the unsupplemented group.

The respective coefficients of correlation for the relation between tHcy and its metabolic cofactors are summarized in **Table 3**. Significant negative associations were observed for tHcy and plasma concentrations of folic acid and vitamin B-12 in healthy control subjects, in patients with hepatitis, and in patients after OLT. By contrast, in cirrhosis, a weak correlation only was found between tHcy and folic acid that disappeared at advanced stages of liver disease (Child B and C). Whereas plasma concentrations of vitamin B-6 showed no association with tHcy, PLP was inversely correlated with alkaline phosphatase in hepatitis ($r = -0.21$, $P < 0.05$), liver cirrhosis ($r = -0.26$, $P < 0.01$), and after OLT ($r = -0.32$, $P < 0.05$) and with fibrinogen

TABLE 3
Spearman's correlation coefficients for relations between total homocysteine and nutritive cofactors in healthy cotrol subjects and in patients with hepatitis or liver cirrhosis and after orthotopic liver transplantation (OLT)

	Folic acid	Vitamin B-12	Vitamin B-6
Control subjects (n = 25)	−0.403[1]	−0.452[1]	NS
Patients			
Hepatitis (n = 93)	−0.371[2]	−0.261[1]	NS
Cirrhosis (n = 163)	−0.177[1]	NS	NS
Child A (n = 77)	−0.365[3]	NS	NS
Child B (n = 65)	NS	NS	NS
Child C (n = 26)	NS	NS	NS
OLT (n = 54)	−0.251[1]	−0.352[3]	NS

[1] $P < 0.05$.

[2] $P < 0.001$.

[3] $P < 0.01$.

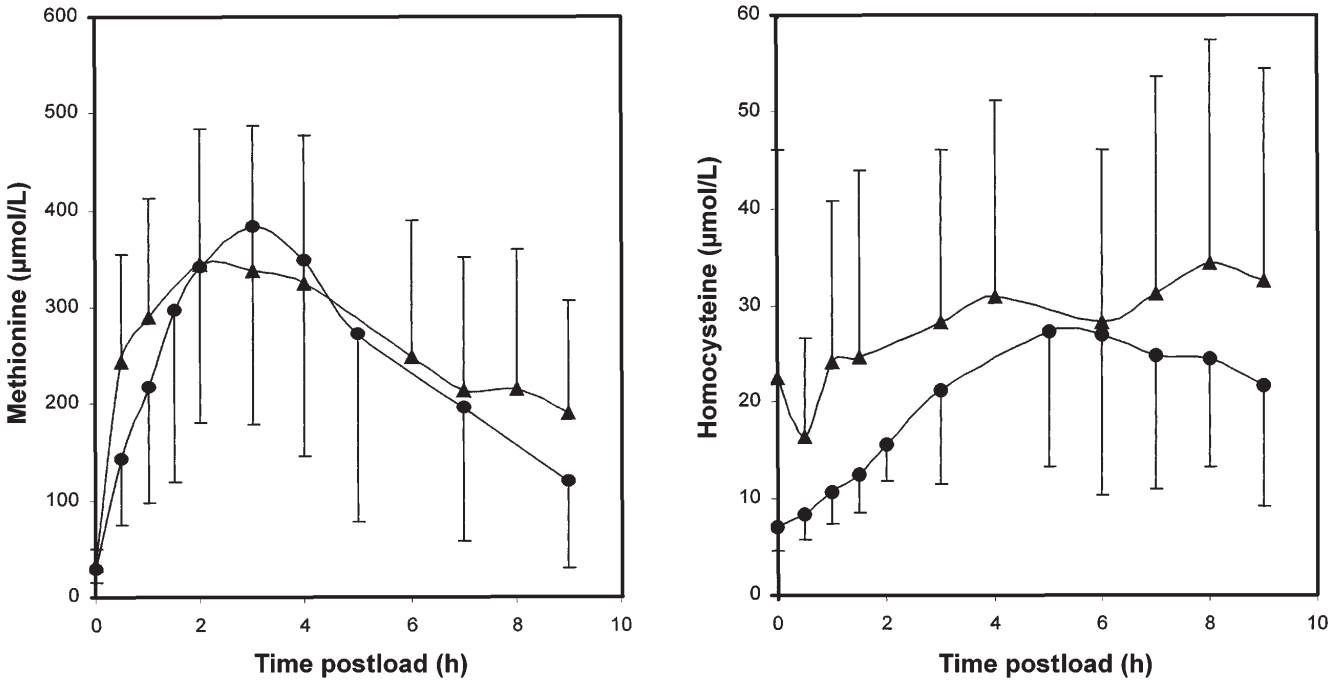

FIGURE 1. Mean (±SD) plasma methionine and homocysteine concentrations after an oral methionine load (50 mg/kg body wt) in 16 patients with liver cirrhosis (▲) and in 8 healthy control subjects (●).

and C-reactive protein after OLT ($r = -0.31$, $P < 0.05$, and $r = -0.34$, $P < 0.01$, respectively).

Clinical and biochemical indexes of liver disease were examined as potential determinants of tHcy concentrations. In this analysis, significant and positive associations were observed for tHcy and fibrinogen ($r = 0.354$, $P < 0.01$ for hepatitis, and $r = 0.281$, $P < 0.01$ for cirrhosis), leukocyte count ($r = 0.323$, $P < 0.001$ for cirrhosis), CRP ($r = 0.217$, $P < 0.05$ for cirrhosis), and creatinine concentrations ($r = 0.356$, $P < 0.001$ for cirrhosis, and $r = 0.384$, $P < 0.01$ after OLT).

A subsequent stepwise multiple regression analysis was performed to analyze the main determinants of tHcy concentrations in the different patient groups. In hepatitis, only fibrinogen entered the prediction model and explained 14% of the variance in plasma tHcy. In cirrhosis, leukocyte count, cysteine, and folic acid were also included in the regression formula. These 4 variables together explained 44% of the variance in plasma tHcy in cirrhosis. The main determinant of plasma tHcy in OLT patients was serum creatinine, which explained 14% of the variance in tHcy.

Protocol 2: portohepatovenous gradients of amino acids

Patients with liver cirrhosis after TIPS implantation showed a great variability in portohepatovenous gradients of plasma amino acids. Mean (±SEM) portohepatovenous differences in tHcy and methionine concentrations across the liver were –0.88 ± 0.39 µmol/L and −6.28 ± 2.99 µmol/L, respectively. The mean (±SEM) fractional gradients of these amino acids as a percentage of their portal concentrations were −8.98 ± 3.07 µmol/L for tHcy and −9.79 ± 8.16 µmol/L for methionine. Twelve of 16 patients had negative portohepatovenous differences in tHcy (9 also had a negative mean methionine difference), reflecting secretion or leakage of Hcy from the liver. In 4 patients, the tHcy gradient was positive (3 also had a positive methionine difference), reflecting an uptake of Hcy by the liver. Methionine gradients were negative in 10 patients and positive in 6 patients. A comparison of patients with negative portohepatovenous gradients for tHcy and methionine concentrations with patients with a positive amino acid difference showed no significant differences in portal concentrations of tHcy, methionine, folic acid, vitamin B-12, or vitamin B-6.

Protocol 3: post-methionine-load homocysteine metabolism

The time courses of plasma methionine and tHcy concentrations during the 9 h after the methionine load are given in **Figure 1**. The kinetic variables for methionine and tHcy for patients and control subjects before and after vitamin supplementation are shown in **Table 4**. Basal plasma concentrations of methionine, peak postload methionine concentrations, and the calculated volume of distribution tended to be higher in patients than in healthy control subjects. However, these differences were not significant. The only significant difference in methionine kinetics was found for k_e, which was significantly lower in patients with cirrhosis, indicating impaired clearance after the methionine load in these patients. In contrast with basal concentrations, postload tHcy concentrations in patients with cirrhosis and normal vitamin B-6 concentrations did not differ significantly from those in healthy control subjects.

There was a significant correlation between basal methionine concentrations and impaired methionine degradation (basal methionine versus methionine $t_{1/2}$: $r = 0.452$, $P < 0.05$; basal methionine versus methionine k_e: $r = -0.518$, $P < 0.05$). Patients with higher basal methionine concentrations had a higher methionine half-life and a lower elimination rate constant for methionine. As shown in **Figure 2**, there was a negative association between the maximum rise in postload plasma tHcy concentrations and the $t_{1/2}$ for plasma methionine in patients and control

TABLE 4
Plasma concentrations of vitamins, methionine, and total homocysteine (tHcy) and kinetic variables of methionine and tHcy after a methionine load before and after supplementation with 5 mg folic acid and 20 mg vitamin B-6 in healthy control subjects and patients with liver cirrhosis[1]

	Before vitamin supplementation		After vitamin supplementation	
	Control subjects (n = 8)	Cirrhosis patients (n = 16)	Control subjects (n = 8)	Cirrhosis patients (n = 16)
Folic acid (ng/mL)	7.7 ± 4.1	7.5 ± 4.9	35.2 ± 22.2[2]	25.2 ± 11.9[3]
Vitamin B-6 (pmol/mL)	83.5 ± 44.7	128.1 ± 167.9	417.5 ± 185.6[4]	434.4 ± 263.0[4]
Vitamin B-12 (pg/mL)	230.6 ± 52.4	505.7 ± 193.0[5]	263.2 ± 168.9	481.3 ± 268.7
Methionine basal (μmol/L)	27.6 ± 13.3	34.1 ± 16.3	37.5 ± 25.3	50.4 ± 27.5[2]
Methionine post load				
C_{max} (μmol/L)	399.1 ± 194.4	428.3 ± 118.2	394.0 ± 210.3	498.5 ± 324.8
t_{max} (h)	2.7 ± 0.8	2.2 ± 0.8	3.0 ± 1.1	2.3 ± 1.7
AUC (μmol·h/L)	2356.3 ± 1896.6	2235.0 ± 819.2	2148.9 ± 1935.3	2431.8 ± 1974.7
C_0 (μmol/L)	711.3 ± 401.6	525.2 ± 123.5	749.5 ± 397.9	669.5 ± 598.9
V_D (L)	32.2 ± 16.2	40.3 ± 8.9	29.5 ± 25.1	49.8 ± 13.5
k_e	0.19 ± 0.05	0.13 ± 0.06[6]	0.21 ± 0.06	0.15 ± 0.05
$t_{1/2}$ (h)	3.94 ± 0.95	6.97 ± 4.10[6]	3.60 ± 1.04	5.42 ± 2.09
Homocysteine basal (μmol/L)	7.1 ± 2.6	22.7 ± 23.5[6]	6.5 ± 2.4	16.5 ± 15.4
Homocysteine postload				
C_{max} (μmol/L)	31.4 ± 14.4)	38.6 ± 23.0	25.3 ± 13.4[2]	33.0 ± 25.7
t_{max} (h)	6.4 ± 1.8	7.0 ± 1.7	6.1 ± 2.2	6.8 ± 1.9
AUC (μmol·h/L)	156.1 ± 95.4	94.8 ± 76.5	136.7 ± 111.4	70.0 ± 51.7[2]

[1] $\bar{x}$ ± SD. C_{max}, peak concentration; t_{max}, time to peak concentration; AUC, area under the time-dependent plasma concentration curve; C_0, y intercept; V_D, volume of distribution; k_e, elimination rate constant; $t_{1/2}$, elimination half-life.
[2–4] Significantly different from before supplementation (paired sample t test): [2] $P < 0.05$, [3] $P < 0.001$, [4] $P < 0.01$.
[5,6] Significantly different from control subjects before supplementation (paired sample t test): [5] $P < 0.01$, [6] $P < 0.05$.

subjects. Subjects with a shorter methionine $t_{1/2}$ had a higher postload tHcy increase than did subjects with an impaired degradation of methionine.

Protocol 4: intervention with folic acid and vitamin B-6

The results of 10 d of oral supplementation with 5 mg folic acid and 20 mg vitamin B-6 in 16 patients with liver cirrhosis and in healthy control subjects are given in **Table 4**. The intervention increased plasma concentrations of folic acid and vitamin B-6 in both patients and control subjects, whereas vitamin B-12 remained unchanged in both groups.

Effects on basal total homocysteine metabolism

After vitamin supplementation, there was a significant increase in basal methionine in cirrhosis (Table 4). Basal tHcy decreased in 5 of 8 control subjects (−14.8 ± 7%) and in 14 of 16 patients with liver cirrhosis (−31.3 ± 19%). However, the mean tHcy concentration in either group was not significantly different after the intervention compared with before. "Responders" and "nonresponders" could not be differentiated on the basis of the data obtained in this study protocol.

Effects on postload total homocysteine metabolism

No significant group differences in response to treatment (postminus presupplementation values of variables of tHcy and methionine kinetics) were found. In cirrhotic patients, vitamin supplementation significantly decreased the AUC_{Hcy}. Shown in **Figure 3** is a linear regression between before and after treatment changes in AUC_{Hcy} and pretreatment AUC_{Hcy} in patients with liver cirrhosis. Reduction of AUC_{Hcy} after supplementation was dependent on pretreatment AUC_{Hcy} in cirrhotic patients but not in control subjects. This effect was more pronounced in cirrhotic patients with higher pretreatment AUC_{Hcy} values.

DISCUSSION

Basal homocysteine metabolism

The major finding of this study was a high prevalence of hyperhomocysteinemia in all patient groups (34% in the patients with hepatits, 54% in those with cirrhosis, and 52% in those after OLT; Table 2). This effect was independent of the etiology of liver

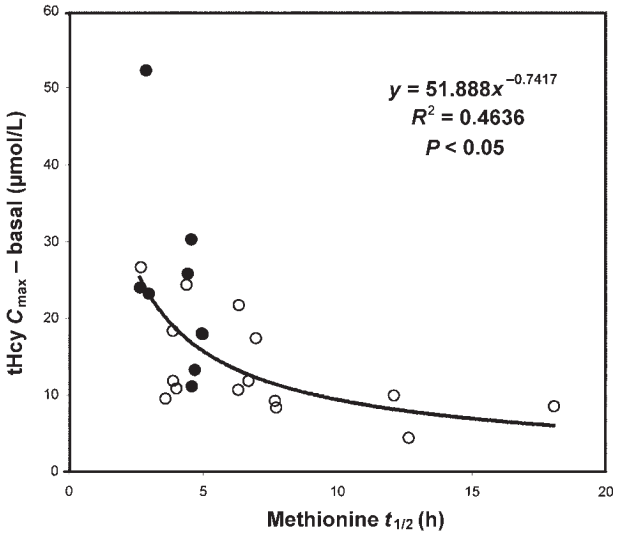

FIGURE 2. Association between the elimination half-life ($t_{1/2}$) for methionine and the maximum rise in total plasma homocysteine concentrations (tHcy) after the methionine load (maximum concentration − basal plasma concentration) in 16 patients with liver cirrhosis (○) and in 8 healthy control subjects (●). C_{max}, peak postload methionine concentration.

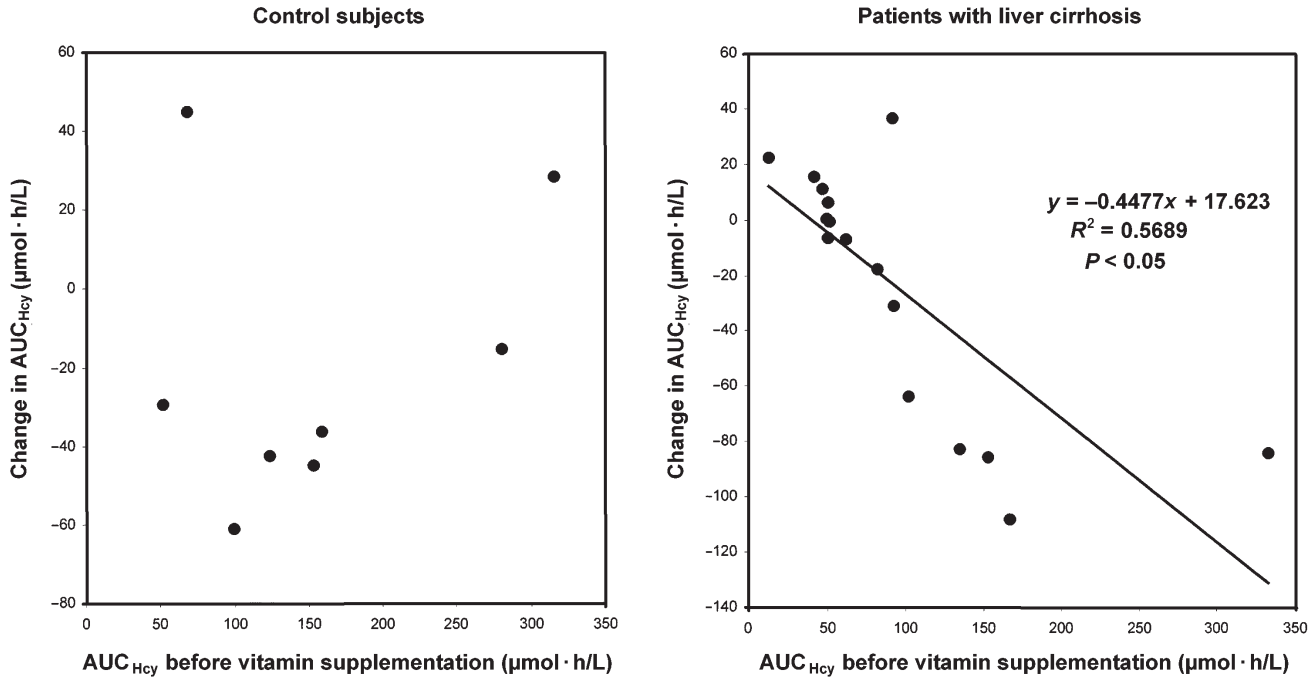

FIGURE 3. Change in the area under the time-dependent plasma concentration curve of total homocysteine (AUC_{Hcy}) after supplementation with folic acid and vitamin B-6 plotted against the AUC_{Hcy} before vitamin supplementation in 8 healthy control subjects and in 16 patients with liver cirrhosis.

disease. Hyperhomocysteinemia is in part explained by impaired renal function. Serum creatinine showed a positive correlation with tHcy in patients with cirrhosis and after OLT. Renal function is also a determinant of plasma tHcy in patients with normal liver function (27, 28). However, in cirrhosis, mean tHcy remained elevated after the exclusion of patients with elevated serum creatinine concentrations (Table 2).

Physiologic determinants of tHcy are nutritive cofactors of its metabolism. Folic acid and vitamin B-12 showed an inverse correlation with tHcy plasma concentrations in all patient groups and in healthy control subjects (Table 3). In cirrhosis, however, this association was confined to folic acid in Child A patients. By contrast, plasma concentrations of vitamin B-12 were elevated and increased with the severity of liver disease (Table 2). A cellular leakage of vitamin B-12 with a subsequent intracellular vitamin B-12 deficiency has been proposed for liver cirrhosis (17). This might lead to the so-called folate trapping mechanism in which intracellular vitamin B-12 deficiency leads to an accumulation of methyl tetrahydrofolate with a reduction in synthesis of tissue folate polyglutamates and a concomitant increase in plasma folate (29). This would argue in favor of intracellular vitamin B-12 as well as folate deficiency in cirrhotic patients. These findings suggest that, compared with their effects in healthy control subjects, nutritional cofactors have a minor influence on Hcy metabolism in cirrhosis.

Plasma tHcy concentrations were positively correlated with fibrinogen concentrations in hepatitis and liver cirrhosis and with C-reactive protein and leukocyte counts in cirrhosis. These indexes are part of the inflammatory response. It is tempting to speculate that chronic tissue damage resulting from ischemia, autoimmune processes, viral infection, or alcohol will induce cell repair and proliferation concomitantly, accelerating specific methylation reactions, generating *S*-adenosylhomocysteine, and releasing tHcy (30). This idea may explain the elevated tHcy concentrations seen after myocardial infarction (31) and stroke (32) and in hyperproliferative disorders (33), malignancy (34), or inflammatory diseases (35, 36). In addition, tHcy concentrations showed a close correlation with variables of the interleukin 6–dependent acute phase response in studies screening for cardiovascular disease risk factors (37, 38).

The elevated tHcy concentrations seen in patients with hepatitis and liver cirrhosis might be explained in part by tissue damage occurring directly through increasing tHcy leakage or indirectly by initiated cell repair. However, in our study, the portohepatovenous concentration gradients obtained after the TIPS were variable (protocol 2). We observed a negative portohepatovenous tHcy gradient in 75% of our patients. These data may suggest an increased leakage of tHcy from the liver in cirrhotic patients. The value of these data are limited, however, because of disturbances in hepatic hemodynamics due to both liver disease and the TIPS. It is possible that the tHcy and methionine gradients are also affected by dilution of hepatic venous blood with portal blood bypassing the liver. However, dilution would decrease hepatovenous substrate concentrations and thus reduce tHcy and methionine gradients. This would have further increased rather than decreased the prevalence of negative tHcy gradients.

Postprandial homocysteine metabolism

An impairment of post-methionine-load tHcy metabolism was recently reported in patients with cirrhosis (3). Contrary to these results, we did not find a significant difference in time-dependent changes in plasma concentrations of tHcy (AUC_{Hcy}) between patients and control subjects (Table 4). However, our subgroup of cirrhotic patients for investigation of post-methionine-load tHcy metabolism had significantly higher plasma PLP concentrations than did the whole group of 168 cirrhotic patients (128 compared

with 53 pmol/mL; $P < 0.001$). Because vitamin B-6 concentrations were not reported in the above-mentioned study (3), differences in this vitamin might explain the discrepant results. Vitamin B-6 is a cofactor for enzymes of transsulfuration. Low concentrations of plasma vitamin B-6 are common in patients with liver disease (39, 40). Low concentrations are also found in 35% of cirrhotic patients, and particularly low concentrations were seen in patients after OLT (a prevalence of vitamin B-6 deficiency of 61%; Table 2). An increased extracellular degradation of PLP by an increased activity of alkaline phosphatase might contribute to vitamin B-6 deficiency in chronic liver disease (41).

Accordingly, inverse correlations were observed between plasma PLP and alkaline phosphatase in patients with hepatitis, liver cirrhosis, and after OLT. As to this mechanism, 90% of PLP in plasma is bound to proteins; thus, in cirrhosis, reduced hepatic albumin synthesis might accelerate degradation of free PLP by alkaline phosphatase. A positive correlation between albumin and vitamin B-6 concentrations was found in OLT patients ($r = 0.394$, $P < 0.01$). There is also direct and indirect evidence for hepatic tissue vitamin B-6 deficiency in cirrhosis (8, 39, 42, 43).

The AUC_{Hcy} did not differ significantly between patients and control subjects. This is likely explained by an impaired degradation of methionine by a diminished activity of MAT in cirrhosis (44). Impaired MAT activity would lead to basal and postload elevations in methionine concentrations as well as a methionine $t_{1/2}$ associated with a lower k_e (Table 4). These data suggest that formation of tHcy from methionine was reduced in cirrhosis (Figure 1). The inverse association between the $t_{1/2}$ for methionine and the maximum rise in tHcy after the methionine load (Figure 2) suggests that patients with impaired methionine degradation are "protected" from tHcy elevation after a methionine load.

Effect of vitamin supplementation

There was no significant effect of 10 d of supplementation with 5 mg folic acid and 20 mg vitamin B-6 on basal tHcy concentrations (Table 4). However, the intervention decreased basal plasma tHcy concentrations in all but 2 patients. The lack of significance is possibly due to the small number of patients. By contrast, postload tHcy metabolism was significantly improved in both patients and control subjects (Table 4). Cirrhotic patients with high pretreatment AUC_{Hcy} values showed the greatest reduction in AUC_{Hcy} in response to vitamin supplementation (Figure 3). We propose that a higher AUC_{Hcy} probably suggests no impairment of MAT activity and thus methionine degradation to tHcy. However, impaired MAT activity would lead not only to reduced tHcy formation but also to impaired tHcy degradation because it results in a deficiency of *S*-adenosylmethionine. *S*-Adenosylmethionine activates the transsulfuration pathway and therefore directs tHcy metabolism toward the irreversible conversion to cysteine. We conclude from our data that patients with normal concentrations of basal methionine would benefit from supplementation with folic acid and vitamin B-6 alone. It is tempting to speculate that patients with basal hypermethioninemia and impaired tHcy degradation may benefit from a combination of vitamin supplements with *S*-adenosylmethionine.

To summarize, hyperhomocysteinemia together with intracellular vitamin deficiency are highly prevalent in patients with liver disease and after OLT. Although the influence of physiologic determinants of Hcy metabolism disappears with deteriorating liver function, vitamin supplementation improves postprandial Hcy metabolism.

Author contributions were as follows: study design, MJM and AB-W; data collection, AB-W, MR, GO, EL, MP, HH, and WF; data analysis, AB-W, MR, and NC; and writing of the manuscript, AB-W and MJM. The authors had no conflicts of interest.

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