Hypercysteinemia and delayed sulfur excretion in cirrhotics after oral cysteine loads[1–3]

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ABSTRACT Biosynthesis of cysteine from methionine via the hepatic transsulfuration pathway is impaired in some cirrhotic patients, who therefore might require cysteine in the diet. However, because further metabolism of cysteine also occurs primarily in the liver, the metabolic clearance of this amino acid could be impaired in cirrhosis. We administered oral loads of L-cysteine to cirrhotic patients and healthy volunteers. Plasma cyst(e)ine (free and protein-bound cysteine, and ½ cystine) and urinary sulfur-containing constituents were measured at various times postload. Cirrhotic subjects exhibited a greater maximal plasma cyst(e)ine concentration and plasma elimination half-life ($t_{1/2}$) and a delayed excretion of metabolic end products after an oral L-cysteine load. The postload increase in total plasma cyst(e)ine was accounted for primarily by an increase in the disulfide form (cystine). These studies show that cirrhotics have an impaired ability to clear cyst(e)ine from the plasma. *Am J Clin Nutr* 1989;50:1401–6.

KEY WORDS Hypercysteinemia, sulfur, cirrhosis

Introduction

Early studies by Rose et al (1) established that omission of any of eight amino acids from the diet of healthy adult males resulted in a reversible negative nitrogen balance. Subsequent studies showed that humans are unable to synthesize these amino acids de novo. With the advent of chemically defined enteral and parenteral nutritional formulations and the development of better criteria for defining individual protein nutriture, other amino acids originally classified as nonessential have been found to be indispensible in certain clinical conditions and are referred to as conditionally essential (2) or acquired indispensable amino acids (3).

Methionine utilization, and hence cysteine biosynthesis, are impaired in cirrhotics (4). A subset of these patients became hypocysteinemic when maintained on cysteine-free total parenteral nutrition (TPN) formulations and showed signs of deteriorating nutritional status despite the provision of excess calories and repletion of protein N (5). Simultaneous declines have been noted in the concentrations of α-aminobutyric acid, taurine, and glutathione in plasma, three compounds derived from cysteine (6). Improvement in several nutritional variables and a positive N balance can be attained within 1 wk of cysteine (cystine) supplementation (5). Accordingly, a source of cysteine has been suggested as a necessary component of the cirrhotic diet (2–5).

Because most currently available elemental nutritional formulas either lack or contain only minor amounts of cysteine (as cystine), formula revision and/or cysteine supplementation when providing these mixtures to cirrhotic patients may be indicated. However, cysteine is degraded primarily by hepatic enzymes; therefore, disorders in cysteine processing by the cirrhotic liver could cause hypercysteinemia. Elevated plasma cysteine was noted in some cirrhotic patients consuming mixed foods (2) and, depending on the relative amounts of the various forms of cysteine in the plasma, could contribute to additional medical complications including widespread alterations in the function of plasma proteins with critical thiol groups. The thiol and disulfide status of cysteine is altered in cirrhotic patients in favor of the disulfide forms [cystine, cysteine-mixed disulfides, and protein-bound cysteine (6)]. Problems also could arise from a secondary build-up of free and protein-bound homocysteine, a transsulfuration intermediate proximal to cysteine. This metabolite has been implicated in causing damage to proteins and to the vascular endothelium and other tissues (7).

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Received March 15, 1988.
Accepted for publication December 14, 1988.

TABLE 1
Clinical data of the four patients with biopsy-proven micronodular cirrhosis

	1	2	3	4
Age (y)	50	41	61	55
Sex	F	M	M	M
Time since initial diagnosis (y)	3	4	6	8
History of encephalopathy	Yes	Yes	Yes	Yes
History of bleeding esophageal varices	No	No	Yes	Yes
Body weight (% ideal body wt)	98	93	110	106
Serum albumin (mmol/L)*	0.51	0.54	0.63	0.59
Serum bilirubin (μmol/L)*	27	31	20	15
Blood NH_3 (μmol/L)*	27	21	21	34

* Fasting venous blood.

The purpose of this study was to examine the response of cirrhotic patients to oral loads of L-cysteine.

Experimental design and implementation

Subjects

The study population consisted of four cirrhotic patients and five healthy control subjects aged 42–61 y. The cirrhotic group comprised one female and three male adults with biopsy-proven, micronodular (alcoholic) cirrhosis who, by physical and laboratory assessment, did not show any evidence of hepatitis, encephalopathy, bleeding varices, ascites, hepatorenal disease, or diabetes mellitus. They had abstained from alcohol for ≥ 3 mo according to history from patient and family. All had a history of hepatic encephalopathy and were therefore being treated with a protein-restricted diet. Their clinical features are summarized in **Table 1.** The control group consisted of one adult female and four adult male volunteers aged 24–36 y with no history of hepatobiliary disease. Results of all conventional liver tests (serum bilirubin, alkaline phosphatase, glutamate oxaloacetate aminotransferase, lactate dehydrogenase, and prothrombin time) were within the normal range for all control subjects. The investigation was conducted with approval of the Emory University Human Investigations Committee and with the informed consent of all subjects.

Experimental procedures

All participants were equilibrated for 5 d on a diet containing 40 g protein and 2 g Na^+ with calories adequate for weight maintenance. The caloric intake required for weight maintenance was calculated by the Harris and Benedict equation, which adjusts for height, weight, and age (8).

Six consecutive 6-h urine samples were collected beginning at 0800 on the 4th day for analysis of basal excretion of S-containing constituents. At 0800 on the 6th day after commencing the 40-g–protein diet, an oral bolus of L-cysteine (40.6 mg/kg ideal body weight) was given in 150 mL of carbohydrate-free vehicle. The concentration of the cysteine load was the same as that of the methionine load (50 mg/kg ideal body weight) previously utilized at the Emory Clinical Research Center (4). Breakfast, lunch, and dinner were delivered at 0.5, 4, and 9 h postload, respectively.

Subjects were instructed to void 0.5 h before the oral load; urine collections began immediately after cysteine administration. Six consecutive 6-h urine samples were collected postload. Blood samples were drawn immediately before administration of the load and at various times postload (up to 8 h) for analysis of plasma amino acid concentrations.

During the course of the study and particularly after the load tests, cirrhotic subjects were carefully observed for signs of clinical encephalopathy. No significant changes in orientation or motor skills were noted in any of the cirrhotic patients.

Analytical procedures

Determination of plasma amino acid concentrations. Two 10-mL blood samples were drawn into heparinized tubes. One blood sample was immediately treated with iodoacetic acid (0.1 mL of a 500 mg/mL solution) to prevent rapid autooxidation of cysteine by forming the S-carboxymethyl derivative. Both samples were centrifuged for 10 min to pellet the cells. Samples (1.5 mL) of the plasma were filtered and frozen at −80 °C until analysis. Plasma samples were maintained at −80 °C for ≤ 6 wk before analysis. Under these conditions the plasma amino acid concentrations are stable for 8 mo (5).

Plasma aminograms were measured with an amino acid analyzer (Model 121, Beckman Instruments, Palo Alto, CA) (5). Plasma cysteine concentrations (as the S-carboxymethyl derivative) were measured by the method of Brigham et al (9). Protein-bound cysteine was determined by the spectrophotometric method of Malloy et al (10) and Gaitonde (11).

Analysis of urinary sulfur-containing compounds and creatinine. Two 10-mL samples of each 6-h urine sample were frozen at −80 °C for later analysis of amino acids and taurine (5); the remainder were stored for later analysis of S and creatinine. Inorganic S (H_2S and SO_4^{2-}) was measured by the method of Folin (12). Urinary creatinine values were used to correct for inaccurate urine collections. Creatinine was measured on an automated Technicon analyzer in a manner similar to that described by Chasson et al (13).

Pharmacokinetic evaluation and statistical analyses

The rate constant of elimination (K_e) and volume of distribution (V_d) of cysteine were determined from the slope and the *y* intercept, respectively, of the least-squares regression line on a semilogarithmic plot of plasma amino acid concentration vs time. The metabolic clearance rate was calculated by multiplying K_e by V_d. The elimination half-life ($t_{1/2}$) was determined from $t_{1/2} = 0.693/K_e$.

The two-group *t* test or Mann-Whitney U test was used to evaluate group differences in basal plasma amino acid concentrations and urinary S excretion. Postload plasma profiles were compared by using a repeated measures analysis of variance (14).

Results

Plasma amino acid concentrations

The preload, fasting (basal) plasma amino acid concentrations are listed in **Table 2** and differences between the control subjects and cirrhotics are illustrated in **Figure 1.** Cirrhotics exhibited lower concentrations of the branched-chain amino acids ($p < 0.05$ for isoleucine and leucine; $p < 0.10$ for valine) and taurine in plasma ($p < 0.01$) but higher concentrations of plasma methionine ($p < 0.05$), phenylalanine ($p < 0.01$), serine ($p < 0.05$), tyrosine ($p < 0.005$), and cyst(e)ine (free cysteine + cys-

TABLE 2
Basal plasma amino acid concentrations in control and cirrhotic subjects*

Amino acid	Plasma amino acid concentration	
	Control	Cirrhotic
	μmol/L	
Alanine	433.6 ± 25.5	367.3 ± 51.9
Glutamine	606.1 ± 54.5	561.0 ± 29.3
Glycine	272.7 ± 23.8	295.9 ± 20.3
Isoleucine	77.8 ± 4.9	46.8 ± 4.8†
Leucine	128.8 ± 15.3	94.2 ± 13.7†
Methionine	47.7 ± 0.35	63.2 ± 2.8†
Phenylalanine	53.0 ± 6.6	89.4 ± 12.8‡
Serine	73.0 ± 8.4	94.3 ± 4.3†
Taurine	95.1 ± 17.1	46.1 ± 9.2‡
Threonine	126.0 ± 3.2	135.2 ± 12.7
Tyrosine	56.8 ± 0.55	129.7 ± 6.0§
TPC‖	214.8 ± 13.5	288.9 ± 27.1¶

* $\bar{x}$ ± SEM.
† Significantly different from control values, $p < 0.05$.
‡ Significantly different from control values, $p < 0.01$.
§ Significantly different from control values, $p < 0.005$.
‖ TPC, total plasma cysteine (cysteine + cystine + protein-bound cysteine).
¶ Significantly different from control values, $p < 0.025$.

tine + cysteine mixed disulfides + protein-bound cysteine, $p < 0.025$). The increase in plasma cyst(e)ine concomitant with a decrease in plasma taurine suggests that

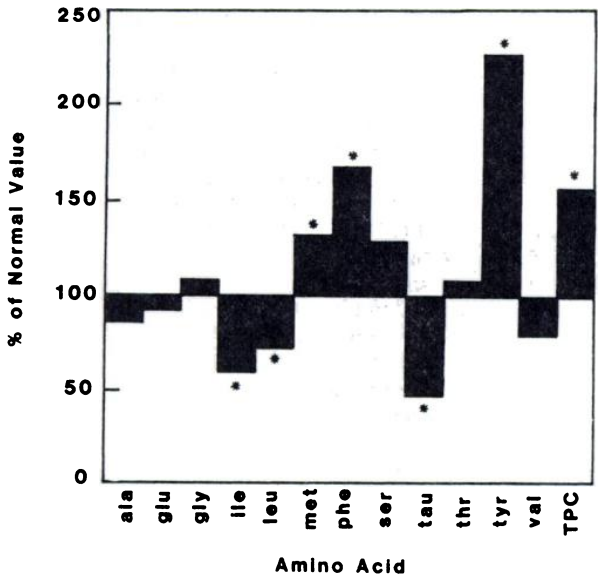

FIG 1. Percent deviation of cirrhotic basal plasma amino acids from control values. The mean plasma values (μmol/L) in control subjects were set at 100% and the percent deviations of the cirrhotic mean values were calculated for each amino acid. TPC is total plasma cysteine and includes free cysteine, cysteine as cystine, and protein-bound cysteine. Statistically significant values are marked with an asterisk (*see* text).

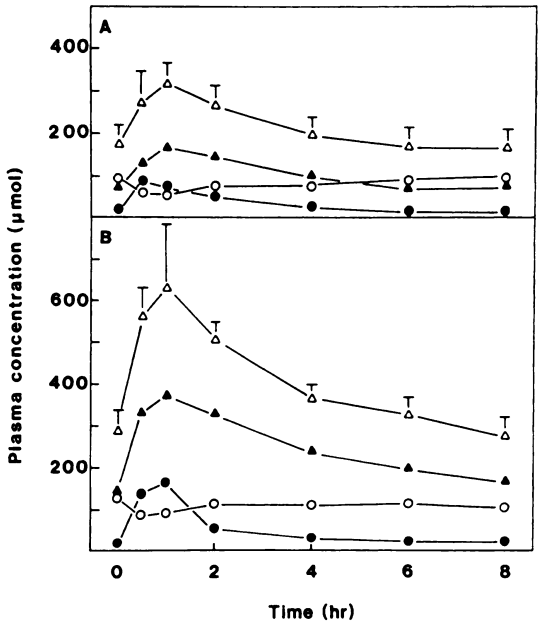

FIG 2. Plasma cyst(e)ine after an oral L-cysteine load. Free cysteine (●), cystine (▲), and protein-bound cysteine (○) were determined as described in the text at various times after the oral L-cysteine load. The mean concentrations for control subjects and cirrhotics are shown in panels A and B, respectively. Total plasma cyst(e)ine (△) mean values ± SEM are also shown for both groups. Total plasma cyst(e)ine and cystine values of cirrhotics are significantly different from control values ($p < 0.001$).

cysteine utilization was impaired in cirrhotic patients relative to control subjects.

Plasma cyst(e)ine increased within 30 min after oral L-cysteine administration and was maximal at 1 h in both control subjects and cirrhotics (**Fig 2**); however, cirrhotics exhibited a twofold greater increase in cyst(e)ine than the control subjects. After 1 h, first-order elimination of cyst(e)ine occurred; elimination was significantly slower in the cirrhotic group as shown by both the plasma half-life and the metabolic clearance rate (**Table 3**).

The elevation in plasma cyst(e)ine was primarily due to an increase in cystine (Fig 2). Plasma cystine was max-

TABLE 3
Pharmacokinetic variables of plasma cyst(e)ine elimination after an oral bolus of L-cysteine*

	Maximal plasma concentration	t1/2	Metabolic clearance rate
	μmol/L	*h*	*L/h*
Control	336.0 ± 37.1	3.48 ± 0.34	1.41 ± 0.15
Cirrhotic†	664.5 ± 64.1	5.18 ± 0.98	0.94 ± 0.16

* $\bar{x}$ ± SEM.
† Significantly different from controls for all values, $p < .01$.

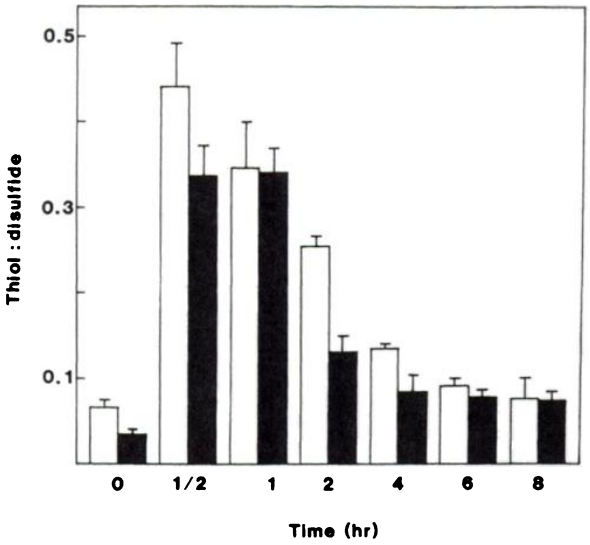

FIG 3. Plasma cysteine thiol:disulfide status after an oral L-cysteine load. The mean plasma cysteine thiol-disulfide ratio was calculated by dividing free cysteine by the cysteine present as cystine and protein-bound cysteine at various times postload. Values represent $\bar{x} \pm$ SEM. Control values, open bars; cirrhotic values, shaded bars. Cirrhotic values are significantly different from control values ($p < 0.005$).

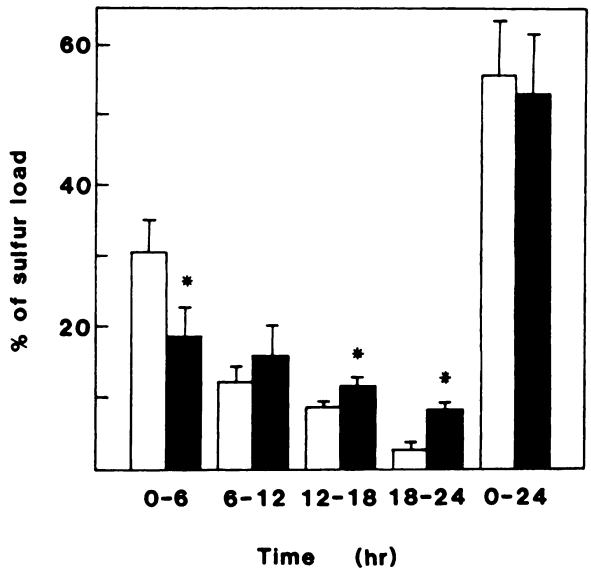

FIG 4. Percent of sulfur load excreted in the urine after an oral L-cysteine load. H_2S, SO_4^{2-} and taurine were determined as described in the text. The mean increase (±SEM) in total S excretion after an oral L-cysteine load over the basal urinary S excretion during the corresponding time period is divided by the total S load. Control values, open bars; cirrhotic values, shaded bars. Cirrhotic values are significantly different from control values ($p < 0.05$) where marked with asterisk.

imal at 1 h and remained elevated for ~4 h in control subjects and ~8 h in cirrhotics. Free cysteine also increased rapidly and returned to preload values by 4 h in both groups. Because cysteine increased over preload values to a greater extent than did cystine, the ratio of thiol to disulfide (moles cysteine per moles cysteine as cystine and protein-bound cysteine) was increased in both groups after the L-cysteine load (**Fig 3**). At every time point the ratio of thiol to disulfide was greater in control subjects than in cirrhotics.

Urinary sulfur-containing metabolites. On the fourth and fifth days of dietary equilibration, urinary excretion of total S averaged 0.50 ± 0.03 g/d ($\bar{x} \pm$ SEM) for control subjects and 0.39 ± 0.04 g/d for cirrhotics (**Table 4**). SO_4^{2-} accounted for ~85% of the urinary S in both groups; the remainder was comprised mostly of taurine. [These percentages are similar to those reported previously (15–17) and reveal that only insignificant amounts (< 1%; data not shown) of the S-containing amino acids methionine, cystine, cysteine, homocysteine, and cystathionine were excreted in the urine of either group.]

TABLE 4
Basal and post-load urinary sulfur excretion

	Control			Cirrhotic		
	Total S	Inorganic S	Taurine	Total S	Inorganic S	Taurine
		g/d			*g/d*	
Basal	0.50	0.43	0.07	0.38	0.32	0.06
Postload	2.11*	1.84*	0.25*	1.42*	1.37*	0.07

* Significantly different from basal values, $p < 0.01$.

Thirty-one percent of the S from the cysteine load was excreted in the urine of control subjects within the first 6 h postload (**Fig 4**). This percentage decreased with each consecutive 6-h urine sample. Approximately 56% was excreted within 24 h of cysteine administration. A similar amount was excreted by the cirrhotic group within this 24-h period (54%) but the distribution of S in the four 6-h urine samples was different. Corresponding to the slower rate of cyst(e)ine clearance from the plasma, less S was excreted in the urine within the first 6 h. After 24 h, S excretion was not significantly different from the basal values in either group although only half of the S load had been accounted for in the urine.

Krijgsheld et al (18) reported a similar inability to account for all S after an oral cysteine load. The discrepancy between cysteine intake and output is probably not due to a concomitant increase in fecal S because this is a very minor route of S excretion (19). The cysteine may have been retained in the body in the form of proteins and/or glutathione (α-glutamyl-cysteinyl-glycine) (20, 21).

Discussion

A recent strategy in the treatment of hospitalized cirrhotic patients has been the use of tailor-made nutri-

tional formulations, which affect nutritional repletion while minimizing the consequences of protein and specific amino acid intolerances (22–25). The purpose of this study was to examine the response of cirrhotic patients to oral loads of L-cysteine, and thus determine the feasibility of supplementing the nutritional intake of these patients with this amino acid.

We found a significant impairment in the ability of the cirrhotic patients to process orally administered L-cysteine, as reflected in a higher plasma elimination t½ and a lower metabolic clearance rate. The increase in total plasma cyst(e)ine after the L-cysteine load was primarily due to an increase in plasma cystine in both groups. Impaired plasma cysteine elimination and the accompanying alteration in the cysteine oxidation state could result in *1*) high circulating concentrations of cysteine in cirrhotic patients receiving cysteine-containing meals; *2*) decreased production of the amino acid taurine, which may be critical for the function of nervous and cardiac tissue (26); and *3*) alterations in the oxidation state of plasma protein sulfhydryl groups and, hence, protein conformation and activity (27). Consistent with this possibility is the known toxicity of excess dietary cysteine in the mammal, manifested by neurologic and hepatic dysfunctions and lesions (28).

The pathophysiological basis for the prolonged postload elevation of plasma cysteine in cirrhotics is not known. Decreased hepatic uptake of cyst(e)ine immediately after absorption is a plausible explanation. Reduced uptake could result from diminished portal perfusion and/or a curtailed ability of the liver to remove cyst(e)ine from the perfusate. Impaired cyst(e)ine uptake from the plasma potentially could be secondary to decreased plasma glutathione. Beatty et al (29, 30) showed that by liberation of cysteine from cystine, glutathione potentiates hepatic cysteine uptake. By a thiol-disulfide interchange reaction with glutathione, one mole of cystine is converted to one mole of cysteine-glutathione–mixed disulfide and one mole of free cysteine. Although we did not have the resources to measure plasma glutathione in this study, Chawla et al (6, 31) reported subnormal plasma glutathione concentrations in cirrhotic patients maintained either on mixed foods, enteral mixtures, or parenteral formulas. Decreased plasma glutathione in cirrhosis suggests decreased hepatic concentrations because plasma glutathione concentrations have been shown to parallel hepatic concentrations in experimental animals (32).

The significantly lower plasma taurine in the cirrhotics than in the control subjects could reflect in part a partial block in conversion of cysteine to taurine by hepatic decarboxylation and oxidation. Taurine, however, is concentrated in platelets and precise measurements of plasma taurine require preparation of platelet-free plasma, which was not done in our study. Consequently, the question of taurine status in cirrhotics requires further evaluation before conclusions will be possible.

The plasma essential amino acid concentrations tend to increase with increasing protein intake (33, 34). In the present study the subjects were equilibrated to a low-protein (40 g) diet, which is often used in cirrhotic patients with a history of hepatic encephalopathy. Adaptation to higher concentrations of protein intake involves increases in the activities of numerous enzymes responsible for the catabolism of the dietary amino acids (35, 36). Accordingly, the present demonstration of impaired cysteine tolerance in cirrhotics on a low-protein diet needs to be extended to higher concentrations of protein intake.

Provision of a diet that supports repletion of the cirrhotic patient is a fundamental component of the medical treatment plan. This is often difficult to achieve because of the severe protein and amino acid intolerances that accompany liver disease. Impaired cysteine biosynthesis from methionine may necessitate the provision of a source of cysteine to some cirrhotic patients. Yet, as these studies indicate, such a supplement could lead to hypercyst(e)inemia, with possible toxic consequences unless the dosage of cysteine is carefully adjusted.

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