

Diurnal variations in plasma neutral amino acid concentrations among patients with cirrhosis: effect of dietary protein¹⁻³

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ABSTRACT The effect of varying dietary protein content on the daily rhythms in plasma neutral amino acid concentrations was studied in patients with chronic cirrhosis. For two consecutive 5-day periods, subjects consumed diets containing either 0 or 75 g of protein per day. Blood samples were drawn at 4-hr intervals on the 4th and 5th days of each dietary period. For most of the neutral amino acids, the changes in plasma concentration associated with time of day or with variations in dietary protein content were similar to those observed previously in normal subjects. Ingestion of the protein-free diet caused significant reductions in the daytime levels of all amino acids studied, except for tryptophan, the concentration of which did not change during the 24-hr period. Ingestion of the 75-g protein diet caused plasma levels of most of the amino acids to increase slightly during the day; these increments were not statistically significant for tryptophan, tyrosine, leucine, and methionine. The absolute plasma concentrations of most of the neutral amino acids were also in the normal range; exceptions included methionine, tyrosine, and phenylalanine, whose plasma levels were significantly elevated above normal values. The plasma ratios of tryptophan, tyrosine, and phenylalanine concentrations to the sum of the concentrations of other large neutral amino acids increased during the day when the protein-free diet was ingested; this effect was moderated by the addition of protein to the food. The plasma ratios for the branched-chain amino acids were depressed below normal; those for tyrosine, phenylalanine, and methionine were significantly increased. The plasma tryptophan ratio was within the normal range. These findings provide a basis for anticipating that the uptake from blood into brain of several of the large neutral amino acids may be modified in patients with chronic cirrhosis. *Am. J. Clin. Nutr.* 32: 1923-1933, 1979.

The plasma concentrations of the large neutral amino acids are important to normal brain function, inasmuch as they affect the rates of uptake into brain of amino acids used in the production of neurotransmitters (1, 2). Certain of these amino acids, tryptophan and tyrosine, are the precursors of the brain neurotransmitters serotonin, dopamine, and norepinephrine. Variations in the ratios of tryptophan or tyrosine to the sum of the plasma concentrations of the other, competing neutral amino acids (phenylalanine, leucine, isoleucine, valine, and possibly methionine) cause parallel changes in their rates of uptake into rat brain. This in turn modifies the rates at which brain neurons synthesize the monoamine neurotransmitters (1-4).

In humans, it is not possible to study directly the relationship between neutral amino

acid concentrations in plasma and brain. However, it can be determined whether the plasma concentrations of these amino acids in humans and rats respond similarly to such treatments as the ingestion of particular diets. If they do, then it may be possible to infer that neutral amino acid levels in brain also change similarly. In a recent report (5), we

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²Supported in part by grants from the John A. Hartford Foundation, the National Aeronautics and Space Administration, the Sugar Association, and the National Institutes of Health. The Clinical Research Center is supported by a grant from the National Institutes of Health.

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showed that, in normal humans and rats, the effects of diets containing various amounts of protein on plasma neutral amino acid levels (and on corresponding plasma concentration ratios) were similar. The plasma concentrations were found to vary with the time of day and with the protein content of the diet, falling during the daytime when a protein-free diet was ingested, but rising after consumption of a high-protein diet. In contrast, plasma glycine and alanine levels fell with addition of protein to the diet. The plasma ratios for the aromatic amino acids tended to fall as dietary protein increased, a relationship similar to that previously observed in rats (1, 6).

Cirrhosis is associated with exaggerated postprandial increases in plasma insulin (7, 8), as well as with other metabolic alterations that might be expected to modify amino acid metabolism (e.g., Reference 9). Any resulting changes in plasma neutral amino acid levels might be expected to influence the availability of these compounds to the brain, and thereby influence neurotransmitter synthesis (10). Such changes might contribute to the neurologic signs and symptoms associated with chronic liver disease.

The present data demonstrate that plasma tyrosine, phenylalanine, methionine, and proline concentrations are significantly elevated in patients with cirrhosis; plasma tryptophan levels are within the normal range. The plasma ratios of tyrosine, phenylalanine, and methionine to competing neutral amino acids were also found to be significantly elevated, while those of the branched-chain amino acids were depressed and that of tryptophan was within the normal range.

Materials and methods

Subjects

Four male long-standing alcoholics with well-documented histories and physical signs of chronic cirrhosis were studied (Table 1). Each had previously been hos-

pitalized elsewhere, and all were admitted to the Massachusetts Institute of Technology's Clinical Research Center for the purpose of this study only. Each patient provided informed consent.

The known duration of cirrhosis was between 3 and 6 years. One patient was under therapy elsewhere for ascites. The others, at the time of the study, were in a chronic but stable condition. Three had been treated surgically, two to relieve portal hypertension (one by a mesocaval shunt and one by a splenorenal shunt) and one for recurrent bleeding from gastritis (partial gastrectomy).

The control subjects were a group of seven, healthy, male undergraduate students at Massachusetts Institute of Technology (MIT), ranging in age from 18 to 24 years and weighing between 62 and 83 kg (5).

Experimental procedure

The subjects were inpatients at the MIT Clinical Research Center for the duration of the study, and all meals were prepared at the Center. After consuming a "house diet" for 5 days, the subjects began a protocol of ingesting three special diets, one during each of three consecutive 5-day periods. The diet provided during the first period was essentially protein-free; those provided during the second and third periods contained 75 and 150 g of protein per day, respectively. Blood samples were drawn on the 4th and 5th days of each period, beginning at 7 AM on day 4, and at eleven 4-hr intervals thereafter. The blood was collected into heparinized tubes and immediately centrifuged; plasma was frozen until assayed. Because the first two patients studied became drowsy on the 150-g protein diet, this portion of the study was discontinued. At the end of the study, all subjects consumed the house diet for 5 days before discharge.

Diets

All meals were prepared in the kitchen of the Clinical Research Center under the supervision of the research dietitian. Each patient's diet was designed to suit his individual caloric needs; daily caloric intakes ranged between 2200 to 3000 kcal. Subjects consumed one-third of their daily caloric intake at each of three identical meals, served at 8 AM, noon, and 5 PM. A member of the staff was present at each meal to ensure that the subjects consumed all of the food provided.

The diet contained protein as dried egg or egg white, and fat as corn oil, Crisco, or butter. Carbohydrate sources included ginger ale, applesauce, Junket danish dessert, oatmeal, and sucrose and dextromaltose in Kool-Aid and specially formulated cookies. Total protein, carbohydrate, and fat contents of each meal were calculated from standard food composition tables. As pro-

TABLE 1
Base-line clinical data on subjects with cirrhosis

Patient	Age	Nutritional status	Ascites	Edema	Hematocrit	Serum bilirubin	Serum albumin	SGOT
	<i>yr</i>				<i>%</i>	<i>mg/100 ml</i>	<i>g/100 ml</i>	<i>Karmen units</i>
1	69	Wasted	Massive	Slight	30-36	0.8-3.2	1.6-2.1	30-43
2	40	Fair	None	None	37-44	0.4-1.3	3.2-3.9	31-41
3	48	Poor	None	None	44-49	0.6-1.6	4.5-4.9	29-36
4	53	Good	None	None	46-52	0.2-0.6	4.1-4.6	15-34

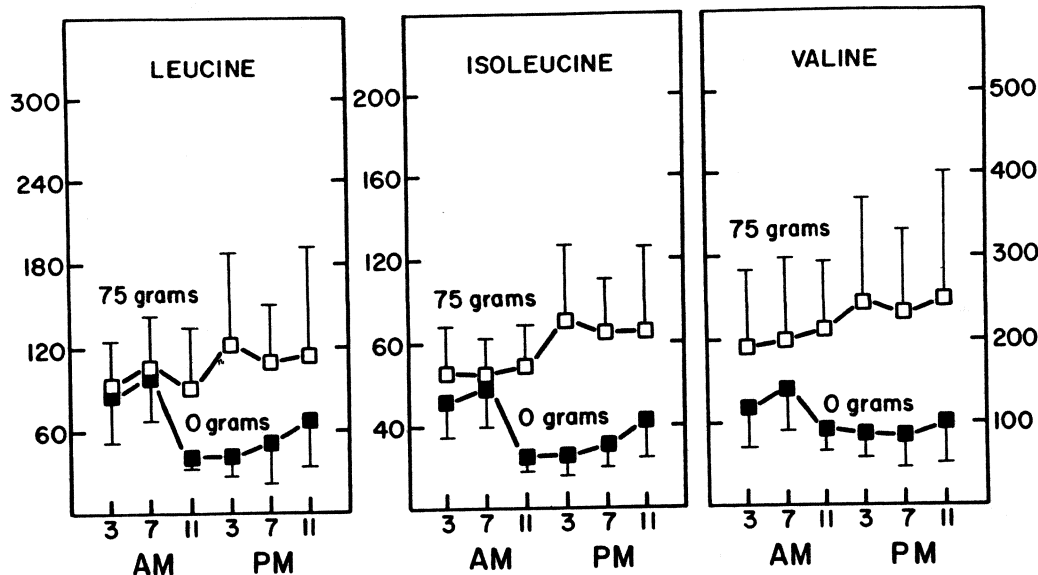


FIG. 1. Diurnal variations in plasma branched-chain amino acid levels in patients with cirrhosis consuming 0 or 75 g of protein per day. Plasma amino acid levels are expressed in nanomoles per milliliter; vertical bars represent SD. In this and all figures, closed squares = protein-free diet, and open squares = 75 g of protein per day diet; moreover, data are from 2 days of sampling, and were combined, since ANOVA revealed no significant differences between days 4 and 5 (see "Materials and Methods"). Identical meals were served at 8 AM, 12 noon, and 5 PM.

tein was added to the diet, carbohydrate was removed; the fat content was thus consistently maintained at about 35% of total daily caloric intake.

Analytical methods

Plasma samples were prepared and analyzed for amino acids as described previously (5). Plasma tryptophan concentrations were determined fluorometrically, by a modification of the method of Denckla and Dewey (11), Lehmann (12), and Bloxam and Warren (13).

Data were analyzed by four-way analysis of variance; the factors were diet (0 or 75 g of protein per day), day (1st or 2nd day of blood sampling), time of day (3 AM, 7 AM, 11 AM, 3 PM, 7 PM, 11 PM), and subject (normal versus cirrhotic). The least significant difference was calculated for comparison among selected means ($P < 0.05$; ref (14)). Data in the text and Figures are presented as the means \pm SD. Analysis of variance revealed no significant differences in the 24-hr patterns of variation for any of the amino acids or ratios between days 4 and 5. Hence, the data from the 2 days were combined, and are so presented in the figures.

Results

Large neutral amino acids

Branched-chain amino acids (Fig. 1). Plasma isoleucine and valine concentrations underwent significant ($P < 0.05$) diurnal variations at both levels of protein intake. They fell during the daytime when the protein-free diet was ingested, and increased slightly with the 75-g protein diet. The daily rhythm in plasma leucine was similar to those of isoleucine and valine when the protein-free diet was ingested; however, no significant daily

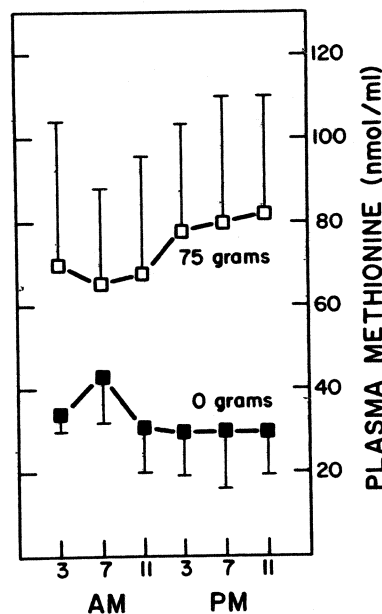


FIG. 2. Diurnal variations in plasma methionine levels in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD. Combined mean levels in cirrhotics (53 ± 22 nmole/ml) are greater than those in normal subjects consuming the same diets (30 ± 10 nmole/ml) (5). Symbols are those in Figure 1.

variation was observed on the 75-g protein diet. Plasma branched-chain amino acid levels increased significantly as protein was added to the diet (0 versus 75 g of protein per day; $P < 0.05$). These levels were statistically indistinguishable from the range observed in healthy subjects (5).

Methionine (Fig. 2). Plasma methionine

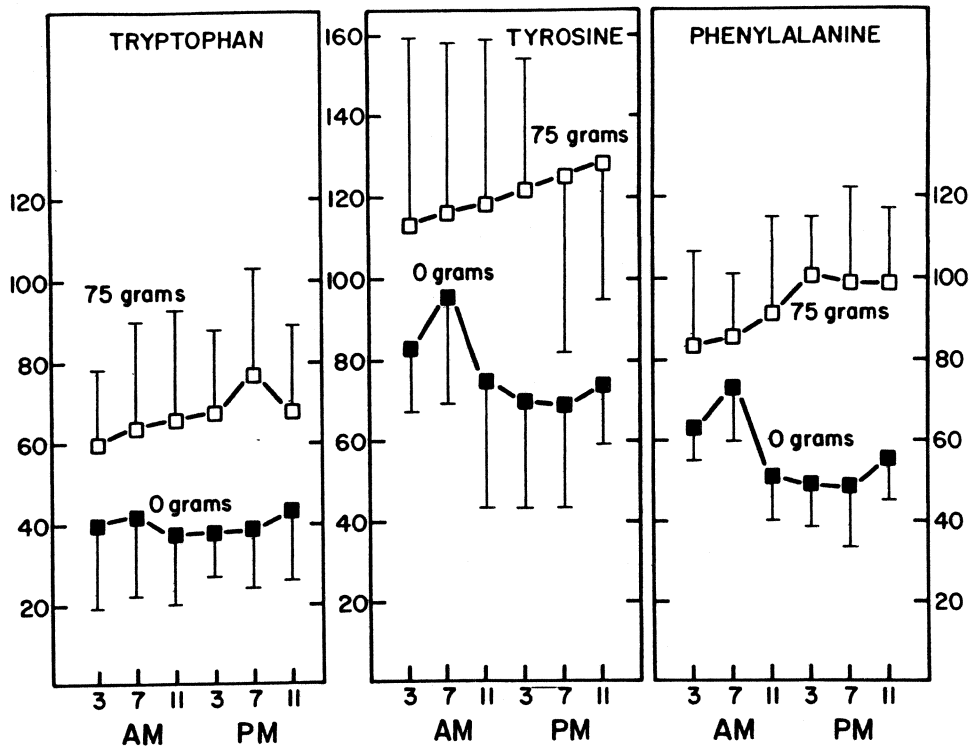


FIG. 3. Diurnal variations in plasma aromatic amino acid levels in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD; ordinate units are nanomoles per milliliter. Symbols are those in Figure 1. Combined mean plasma tyrosine (99 ± 24 nmole/ml) and phenylalanine (75 ± 21 nmole/ml) levels were significantly greater than those in healthy subjects ingesting the same diets (5) (46 ± 11 and 48 ± 10 nmole/ml for tyrosine and phenylalanine, respectively).

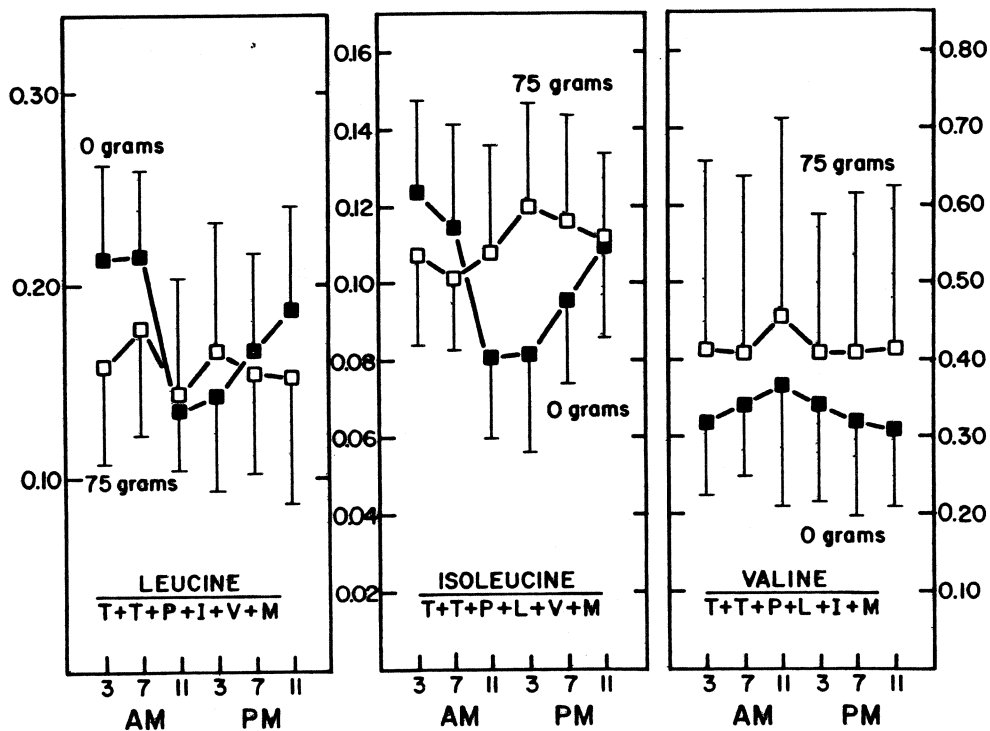


FIG. 4. Diurnal variations in plasma branched-chain amino acid ratios in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD. Symbols are those in Figure 1; letter abbreviations: T + T = tryptophan + tyrosine; P = phenylalanine; L = leucine; I = isoleucine; V = valine; M = methionine.

levels were substantially higher when the 75-g protein diet was ingested than when the protein-free diet was consumed ($P < 0.05$). A small but significant ($P < 0.05$) daily rhythm was evident when the protein-free diet was ingested, but no rhythm occurred with consumption of the 75-g protein diet. By analysis of variance, plasma methionine levels were significantly higher in patients with cirrhosis than in the normal subjects ((5) ; 53 ± 22 versus 30 ± 10 nmole/ml; $P < 0.05$).

Aromatic amino acids (Fig. 3). The plasma concentrations of phenylalanine and tyrosine, but not of tryptophan, underwent significant daily variations when subjects consumed the protein-free diet ($P < 0.05$); daytime levels fell below nocturnal values. Plasma phenylalanine levels increased significantly ($P < 0.05$) during the day when the 75-g protein diet was consumed; a similar trend was seen for both tryptophan and tyrosine, but these effects did not attain statistical significance. The plasma concentration of all three aromatic amino acids bore a direct relationship to dietary protein content (0 versus 75 g of protein per day, $P < 0.05$). Mean plasma phenylalanine and tyrosine concentrations were also significantly higher in patients with cirrhosis than in the normal subjects: 75 ± 21 versus 48 ± 10 nmole/ml for phenylalanine, 99 ± 24 versus 46 ± 11 nmole/ml for tyrosine; $P < 0.05$ (5); plasma tryptophan levels were within the normal range (5).

Neutral amino acid ratios (Figs. 4 to 6). Significant ($P < 0.05$) daily rhythms for each of the branched-chain amino acid ratios occurred when the protein-free diet was consumed, with daytime levels clearly falling below nocturnal values for the leucine and isoleucine ratios (Fig. 4). Although the daily variations in these ratios were also statistically significant ($P < 0.05$) when the subjects consumed 75 g of protein per day, no consistent pattern of variation was noted among the ratios. No significant effect of dietary protein content (0 versus 75 g/day) was noted for the isoleucine or valine ratios; a statistically significant effect was found for the leucine ratio, although the nature of the interaction is not obvious in Figure 4. Each of the branched-chain amino acid ratios was significantly lower in patients with cirrhosis than in the normal subjects ($P < 0.05$); normal values are reported in the companion article (5).

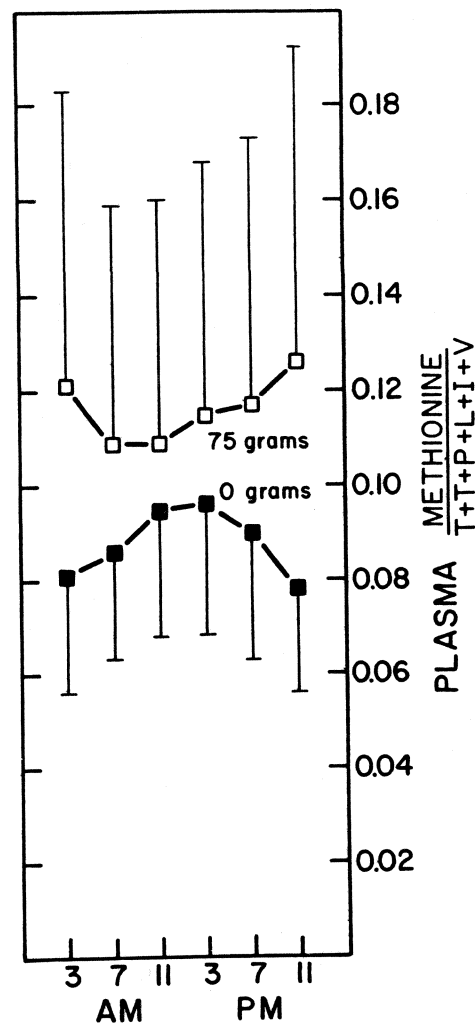


FIG. 5. Diurnal variations in plasma methionine ratios in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD. Symbols are those in Figure 1; letter abbreviations are defined in Figure 4.

A significant diurnal variation in the methionine ratio (Fig. 5) was observed at both levels of dietary protein ($P < 0.05$). Dietary protein content did not significantly influence this ratio. The plasma methionine ratio was significantly greater in cirrhotic patients than in normal subjects ($P < 0.05$; (5)).

Significant increases in each of the aromatic amino acid ratios were noted in the daytime when the protein-free diet was ingested ($P < 0.05$; Fig. 6); no daily rhythms occurred when the 75 g of protein per day diet was consumed. A significant effect of dietary protein content (0 versus 75 g/day) on the plasma tyrosine ratio was observed; however, neither the tryptophan nor the phenylalanine ratio was significantly influenced by dietary protein content. The overall ratios for tyrosine and phenylalanine, but not

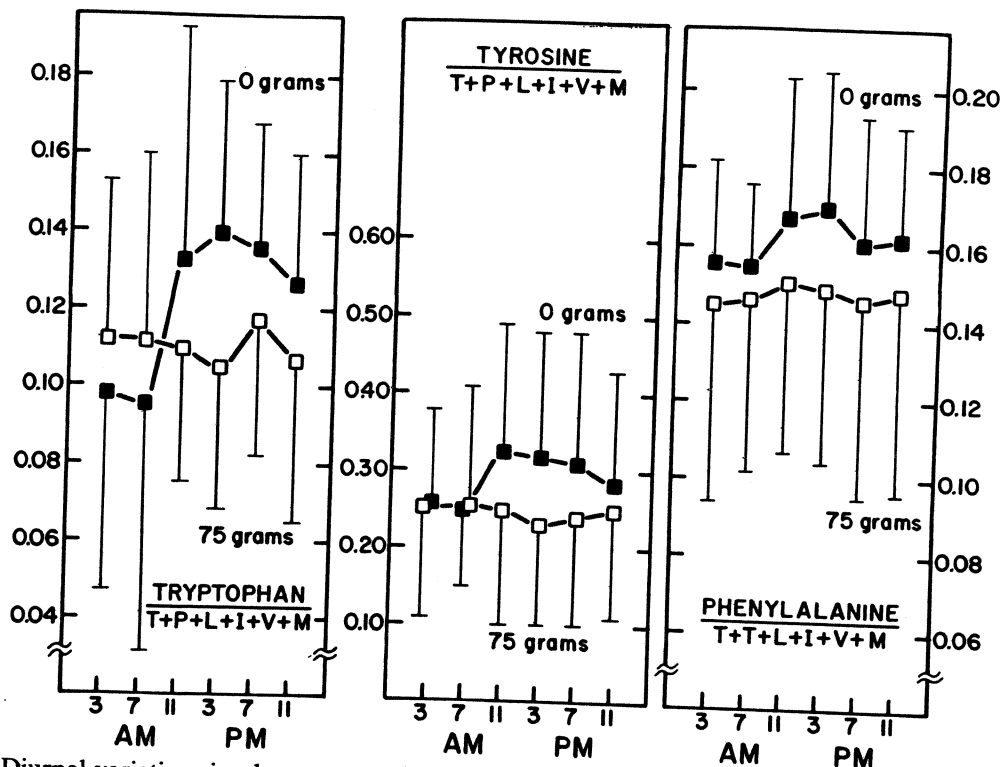


FIG. 6. Diurnal variations in plasma aromatic amino acid ratios in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD. Symbols are those in Figure 1; letter abbreviations: P, L, I, V, and M, as defined in Figure 4; in the left panel T = tyrosine, in the middle panel T = tryptophan, and in the right panel T + T = tryptophan + tyrosine.

for tryptophan, were significantly greater in patients with cirrhosis than in the normal subjects ($P < 0.05$); these latter data are reported in our companion article (5).

Glycine and alanine

Plasma glycine and alanine levels (Fig. 7) fell during the daytime ($P < 0.05$) when the protein-free diet was ingested, and significant diurnal rhythms were discernable ($P < 0.05$). A significant increase in plasma alanine but not in glycine occurred during the daytime when the 75-g protein diet was consumed. A significant effect of dietary protein content on plasma amino acid concentration was present for glycine ($P < 0.05$), but not for alanine; glycine levels appeared to be inversely related to dietary protein content (0 versus 75 g/day). Plasma glycine and alanine levels in subjects with cirrhosis were not statistically different from those noted in normal subjects (see Reference 5), although alanine levels tended to be lower in cirrhotic patients ($P = 0.08$).

Threonine, serine, and proline

Significant 24-hr rhythms ($P < 0.05$) occurred in plasma threonine, serine, and proline levels (Fig. 8) when the protein-free diet

was consumed, with values falling during daylight hours. When the 75 g of protein per day diet was consumed no rhythm was detected in plasma threonine, and statistical significance ($P = 0.05$) was just barely attained for serine and proline. The plasma levels of these two amino acids rose during the day at the 75-g level of dietary protein. Ingestion of 75 g of protein per day caused overall plasma threonine and serine, but not proline, levels to be increased significantly over those observed when the protein-free diet was consumed ($P < 0.05$). Plasma proline concentrations were higher in patients with cirrhosis than in the normal subjects (191 ± 20 versus 153 ± 17 nmole/ml; $P < 0.05$); plasma threonine and serine concentrations were within the normal range (see Reference 5).

Discussion

These data show that the daily rhythms in plasma neutral amino acid levels observed in cirrhotic patients—and the effects of dietary protein content on these rhythms—are, in general, similar to those noted in the normal subjects (see Reference 5). Thus, for all of the amino acids examined except tryptophan (i.e., leucine, isoleucine, valine, methionine,

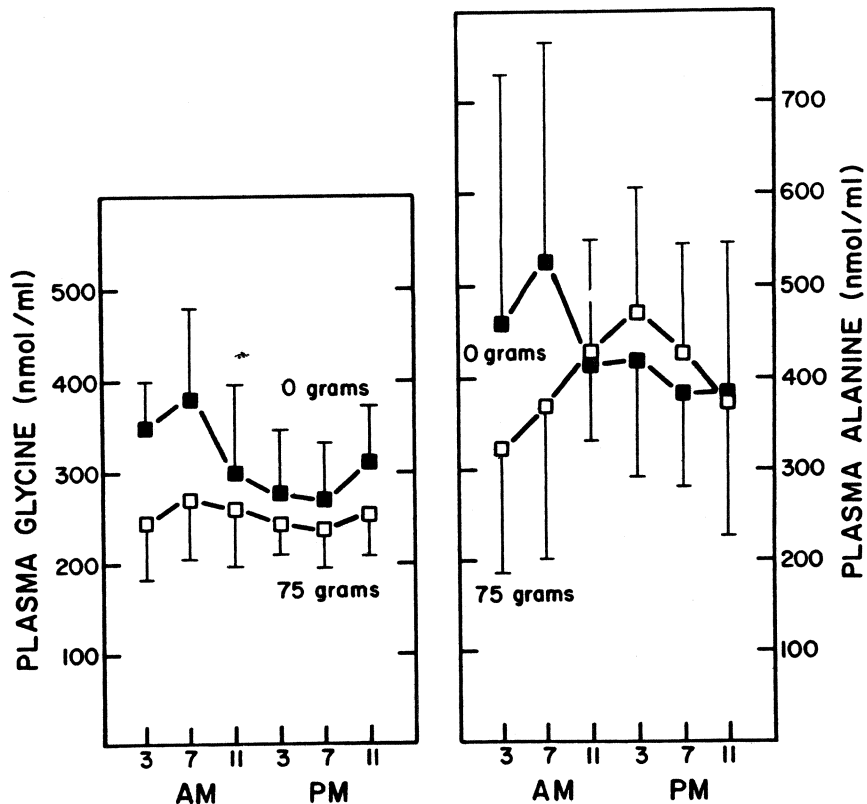


FIG. 7. Diurnal variations in the plasma levels of glycine and alanine in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD. Symbols are those of Figure 1.

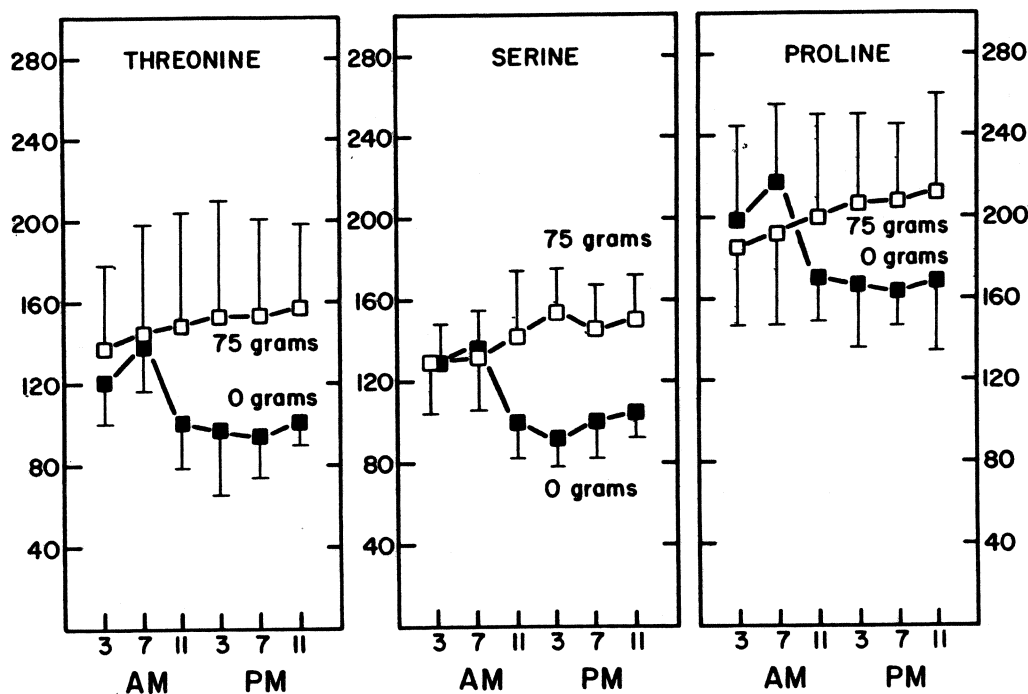


FIG. 8. Diurnal variations in the plasma levels of threonine, serine, and proline in patients with cirrhosis consuming 0 or 75 g of protein per day. Vertical bars represent SD; ordinate units are nanomoles per milliliter. Symbols are those in Figure 1. Combined mean levels of proline in cirrhotics (191 ± 20 nmole/ml) are greater than those in normal subjects consuming the same diets (153 ± 17 nmole/ml) (5).

tyrosine, phenylalanine, glycine, alanine, threonine, serine and proline), plasma concentrations fell during the day when subjects consumed the protein-free diet. When the 75-

g protein diet was ingested, the plasma concentrations of most of these amino acids increased diurnally, as was observed in the normal subjects (5); exceptions were leucine,

methionine, tryptophan, tyrosine, and threonine. Moreover, the absolute concentrations of most of the neutral amino acids averaged over the 24-hr period were not different in patients with cirrhosis from those observed in the normal subjects (5); major exceptions were methionine, phenylalanine, tyrosine, and proline, whose plasma levels in cirrhotics were elevated (by 73, 56, 115, and 25%, respectively) over those in the normal subjects (5). Our group of patients with cirrhosis had daytime plasma insulin concentrations that were about 5-fold higher than those of normal volunteers (8); hence, it is somewhat surprising that no significant abnormalities were detected in the plasma levels of the branched-chain amino acids, since these tend to be most sensitive to insulin's actions (15).

The plasma ratios of tryptophan, tyrosine, and phenylalanine to their competitors showed no major changes with the addition of protein to the diet, except that the daytime increases were more moderate than those observed when the protein-free diet was ingested; this also was true for leucine and isoleucine. Overall, the plasma ratios for leucine, isoleucine, and valine in the cirrhotic patients were significantly below normal values (5); those for methionine, phenylalanine, and tyrosine were significantly elevated. The plasma tryptophan ratio was within the range noted in the normal subjects (5)

Plasma neutral amino acid levels in cirrhosis

Our finding that plasma tyrosine and phenylalanine levels in cirrhotics were elevated (Fig. 3) over values observed in the normal subjects is consistent with the results of other clinical (7, 16–20) and animal (21–23) experiments. Methionine levels in blood have also been reported to be elevated in liver disease (16, 17, 19, 20, 22), but this is not a universal finding (18, 21, 23). This discrepancy might reflect differences in the nutritional states of the subjects examined in different studies, or in the times at which blood was sampled. The slight elevations in plasma proline levels seen in our cirrhotic patients (Fig. 8) are consistent with other reports (17, 21).

A reduction in plasma alanine levels has been noted previously in some (19–21) but not all (16, 17) studies of liver disease. In one of the latter studies (16), fasting blood samples were examined; if the subjects had been

ingesting moderate to low amounts of protein, then their plasma alanine levels might be expected to fall somewhere between the 3 AM and 7 AM values (essentially fasting) for cirrhotic subjects in the present study (Fig. 7); these also did not differ markedly from the values observed in the normal subjects (5).

Of considerable interest are the changes that did *not* occur in cirrhotic subjects. First, a consistent finding in other studies (18–20, 23) has been significant reductions in the plasma levels of leucine, isoleucine, and valine, probably secondary to hyperinsulinemia. In our study, no such reductions occurred (Fig. 1); rather, the plasma concentrations of these amino acids were within the normal range (5). It seems unlikely that this result reflects a less advanced stage of the chronic cirrhosis in our subjects, inasmuch as they were unable to tolerate a 150-g protein diet, their circulating insulin levels attained mean daytime values 5-fold greater than normal (8), and their plasma tyrosine, phenylalanine, and methionine levels were markedly elevated, as has been noted elsewhere in people with cirrhosis (9, 16, 17, 19, 20). Instead, the lack of reductions in plasma branched-chain amino acid levels could reflect the development of insulin resistance. This phenomenon is not uncommon among subjects with cirrhosis who have had high circulating insulin levels for long periods (24), and may contribute to the development of diabetes in such patients (25, 26). It could also reflect the apparent lack of acute liver disease in our subjects.

Second, plasma tryptophan concentrations in cirrhotic subjects in our study were not greater than normal (5). This finding is compatible with the results of other studies (19–21, 23); however, it is somewhat at odds with reports that tryptophan clearance from blood is diminished in patients with cirrhosis (27, 28).

Plasma neutral amino acid ratios in subjects with cirrhosis

The uptake by brain of circulating large neutral amino acids occurs via a competitive transport mechanism localized within brain capillaries (29, 30). Tryptophan uptake into rat brain can thus be made to increase, for example, by elevating plasma tryptophan

concentrations (31) or by reducing the plasma levels of one or more of the other large neutral amino acids (1). In fact, the postprandial changes in the brain level of any large neutral amino acid can be predicted from a corresponding ratio of its plasma concentration to the sum of the concentrations of its competitors (1, 4, 32); for example, brain tryptophan varies in proportion to the ratio of plasma tryptophan to the sum of its major competitors (primarily tyrosine, phenylalanine, leucine, isoleucine, valine, and methionine). By inference, alterations in these blood amino acid ratios in humans should also indicate change in neutral amino acid uptake into the human brain.

The plasma tryptophan ratio was not significantly different from normal in our patients (Fig. 6; (5)). This result stands in contrast to published data showing an increase in cerebrospinal fluid tryptophan levels of humans (23) and dogs (33), and in brain tryptophan concentrations of rats (23, 34-36), suffering from hepatic insufficiency. Our findings suggest either that 1) brain tryptophan concentrations were not elevated in our patients, and thus increased brain tryptophan levels are not necessarily characteristic of chronic hepatic cirrhosis, or that 2) brain tryptophan levels were increased, but the plasma ratio was not an accurate predictor of this increase. Other phenomena besides changes in the plasma tryptophan ratio that might affect brain tryptophan levels include alterations in the activity of the tryptophan transport carrier (independent of competition; (36)), a decrease in tryptophan efflux from brain, an increase in cerebral protein breakdown or a reduction in protein synthesis, or as suggested by some investigators, an increase in the pool of tryptophan that is not bound to circulating albumin in blood (23, 35). This last possibility does not seem compelling, however, inasmuch as serum free tryptophan levels do not always correlate with brain tryptophan concentrations in patients with cirrhosis (37) or in a variety of experimental situations (38, 39). Our findings are not incompatible with the possibility that the plasma tryptophan ratio, and brain tryptophan levels, might be elevated in acute liver disease.

The plasma tyrosine and phenylalanine ratios in our patients were substantially higher

than normal (5), suggesting that abnormally large amounts of these amino acids might be entering the brain (Fig. 6). Consistent with this prediction are the findings of others that brain tyrosine levels are significantly elevated in animals with portocaval shunts (37, 40), and that tyrosine levels in cerebrospinal fluid are elevated in humans with hepatic encephalopathy (41). An increase in brain tyrosine concentration might stimulate the formation of catecholamines, as is known to occur in experimental animals (2, 3). However, very large increases in brain phenylalanine could also conceivably suppress catecholamine formation, by inhibiting the enzyme tyrosine hydroxylase (42).

The plasma ratios of leucine, isoleucine, and valine observed in our patients with cirrhosis were significantly below normal, suggesting that the uptakes of these amino acids into brain might also be depressed. At present, however, the functional consequences of variations in branched-chain amino acid levels in brain are unknown.

Fischer et al. (19, 33) have related the changes in brain aromatic amino acid levels of cirrhotic animals to a plasma ratio of (leucine + isoleucine + valine) to (tyrosine + phenylalanine). They noted that this ratio is low in cirrhosis, and suggested that such reductions might characteristically be associated with the development of hepatic coma. In our patients, even though the plasma levels of the branched-chain amino acids were *not* reduced, nonetheless, the plasma ratio of branched-chain amino acids to (tyrosine + phenylalanine) was below normal. The decline (to about 2), however, was not to the level that has previously been associated with significant encephalopathy (1 or less (33)); our subjects showed no signs of encephalopathy while consuming 0 or 75 g of protein.

Dietary protein intake and hepatic coma

Only a few studies have explored the relationships among dietary protein content and plasma amino acid patterns, brain neurotransmitter levels, and hepatic coma (43, 44). It is generally accepted that patients with cirrhosis cannot tolerate high levels of protein intake; however, the mechanisms relating dietary protein to hepatic encephalopathy are not fully understood. We anticipated that this intolerance might result from an abnormal

response of plasma tryptophan, and of the tryptophan/competitor ratio, to the ingestion of large amounts of protein (10). However, the present results do not constitute a real test of this hypothesis, inasmuch as our subjects were not in impending coma, and neither plasma tryptophan levels nor the ratio of tryptophan to its competitors was significantly different in them from those previously noted (5) in normal subjects. Moreover, no abnormal effect of dietary protein was noted on these parameters. The cirrhotic patients did have greatly elevated plasma concentrations of tyrosine and phenylalanine, and increased ratios for these amino acids, perhaps indicating enhanced uptakes into brain. However, as with tryptophan, no obvious pathological variation in these ratios was evident as a function of dietary protein content. (The plasma ratios, not the plasma levels, of tyrosine and phenylalanine should predict their uptake into brain.) Hence, although our patients may, on the average, have had increased brain uptake rates (based on elevated plasma ratios) for tyrosine, phenylalanine, and also methionine, the failure of increases in dietary protein content to produce abnormalities in these ratios suggests that the mechanism by which dietary protein intake induces hepatic encephalopathy may not critically involve changes in the brain uptake of these large neutral amino acids. Resolution of this question requires that plasma ratios be measured in comatose or precomatose patients.

The authors thank Mr. Sun Shin and Dr. Douglas V. Faller for expert technical assistance.

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