Plasma amino-acid patterns in liver disease

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SUMMARY Plasma amino-acid concentrations were measured in 167 patients with liver disease of varying aetiology and severity, all free of encephalopathy, and the results compared with those in 57 control subjects matched for age and sex. In the four groups of patients with chronic liver disease (26 patients with chronic active hepatitis, 23 with primary biliary cirrhosis, 11 with cryptogenic cirrhosis, and 48 with alcoholic hepatitis±cirrhosis) plasma concentrations of methionine were significantly increased, while concentrations of the three branched chain amino-acids were significantly reduced. In the first three groups of patients plasma concentrations of aspartate, serine, and one or both of the aromatic amino-acids tyrosine and phenylalanine were also significantly increased, while in the patients with alcoholic hepatitis±cirrhosis plasma concentrations of glycine, alanine, and phenylalanine were significantly reduced. In the three groups of patients with minimal, potentially reversible liver disease (31 patients with alcoholic fatty liver, 10 with viral hepatitis, and 18 with biliary disease) plasma concentrations of proline and the three branched chain amino-acids were significantly reduced. Patients with alcoholic fatty liver also showed significantly reduced plasma phenylalanine values. Most changes in plasma amino-acid concentrations in patients with chronic liver disease may be explained on the basis of impaired hepatic function, portal-systemic shunting of blood, and hyperinsulinaemia and hyperglucagonaemia. The changes in patients with minimal liver disease are less easily explained.

The effects of liver disease on amino-acid metabolism have received much attention. Several early workers found abnormally high concentrations of methionine, cysteine, and the aromatic amino-acids in patients with cirrhosis,¹⁻⁸ and a generalised or selective amino-aciduria has been reported.^{2,3,9-14} Conversely, in other early studies no consistent changes in plasma or urinary amino-acids could be found in patients with cirrhosis.¹⁵⁻¹⁷

In recent years, with improving techniques for measurement, specific and reproducible plasma amino-acid patterns have been shown in patients and experimental animals with chronic liver failure,¹⁸⁻²⁵ and in patients with fulminant hepatic failure.^{25,26} Almost without exception these studies have concentrated on patients with appreciable hepatocellular dysfunction evidenced by the presence of either acute or chronic hepatic encephalopathy. Little information is available on the plasma amino-acid patterns in patients with chronic though well-compensated liver disease, or in patients with minimal liver damage.^{27,28} Addition-

ally, although certain animal work suggests that changes in plasma amino-acids after liver damage may be specifically linked to the nature of the damaging agent, few attempts have been made to relate this work to liver disease in man.^{13,20,29-32}

We have therefore studied the plasma amino-acid concentrations in a large number of patients with liver disease of varying aetiology and severity, but without hepatic encephalopathy, to look for patterns of change related to the nature of the damage present.

As age and sex both influence plasma amino-acid concentrations^{33,34} careful matching of the control group was necessary. Sex differences are more pronounced in the fasting state than post-prandially;³⁴ thus in all cases blood samples were taken two hours after a standard meal.

Methods

PATIENTS

The study group comprised 167 patients in hospital. The aetiology and severity of their liver disease was assessed clinically, with biochemical and serological tests, liver histology and, where necessary, by oral,

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intravenous, or retrograde cholangiography. None of the patients had clinical or electroencephalographic evidence of portal systemic encephalopathy. None of the alcoholic patients had taken alcohol for at least three days.²⁹

The control group of 57 individuals, of whom 21 were laboratory personnel and the remainder hospital inpatients without hepatic or renal disease, was carefully matched by age and sex to the patient groups.

Subjects in both control and patient groups were weighed, their mean daily protein intake was estimated, and note made of any medications taken.

All plasma samples were collected at 10.00, two hours after a standard breakfast.³⁴ The plasma was separated and deproteinised in a final concentration of 3% sulphosalicylic acid. Norleucine was added to the supernatant after deproteinisation to a final concentration of 25mM to act as an internal standard. All samples were stored at -20°C until analysed using a Technicon TSM amino-acid analyser.³⁴ In most patients two or more samples taken under the same standardised conditions were examined.

Although the values for individual amino-acids in the control group were normally distributed, those obtained in the various patient groups were not; thus non-parametric statistical analyses were used. Amino-acid values in control and patient groups were compared using Kruskal-Wallis analysis of variance. Values of amino-acids showing significant intergroup differences were then compared in each disease group with control values using the Mann-Whitney U test.

Because of the difficulty in obtaining reliable results from analysis for tryptophan, glutamic acid,

and glutamine,³⁴ these amino-acids have been omitted from this study.

Results

DETAILS OF PATIENTS (Table 1)

The patient group of 167 comprised 26 patients with chronic active hepatitis with progression to cirrhosis, 23 with primary biliary cirrhosis with stage 3 or 4 change on liver biopsy, 11 with cryptogenic cirrhosis, 48 with alcohol-related hepatitis with or without cirrhosis, 31 with alcohol-related fatty change, 10 with viral hepatitis (non-B), and 18 with cholelithiasis and cholangitis with only minimal necrosis and inflammatory infiltration in the portal and periportal areas of the liver.

The mean age and age range of all the patient groups were similar to those of the control group, with the exception of the patients with viral hepatitis who tended to be younger.

The mean weight for most of the patient groups was similar to the control mean. The patients with primary biliary cirrhosis had a lower mean weight and the patients with alcohol-related fatty change a higher mean weight than the controls. This probably reflects the predominance of women in the primary biliary cirrhosis group and of men in this alcoholic group.

The mean daily protein intake per kg body weight in the disease groups did not differ significantly from the control mean.

Most patients received vitamin supplements, and the patients with chronic active hepatitis received prednisolone in doses of from 2.5 to 20 mg daily. No women in the patient or control groups were taking an oral contraceptive.³⁴

Table 1 Details of control subjects and patient groups

Group	No.	Sex ratio M:F	Age (mean range) (yr)	Weight (mean range) (kg)	Daily protein intake (mean) (g/kg)	Medications
Control subjects	57	31:26	47.1 (17-94)	67.5 (35-112)	0.99	—
Chronic active hepatitis	26	6:20	41.3 (17-81)	64.8 (40-89)	0.97	Prednisolone 2.5-20 mg/day
Primary biliary cirrhosis	23	3:20	56.3 (37-73)	56.2 (45-78)	0.95	Vitamins A, D, K
Cryptogenic cirrhosis	11	9:2	49.6 (22-65)	66.6 (49-90)	0.72	—
Alcoholic hepatitis±cirrhosis	48	27:21	52.4 (27-73)	66.3 (40-108)	0.84	Vitamins B, C
Alcoholic fatty liver	31	29:2	47.0 (29-61)	71.4 (40-101)	0.99	Vitamins B, C
Viral hepatitis	10	6:4	36.1 (19-61)	66.6 (55-76)	0.86	Vitamins B, C
Biliary disease	18	9:9	50.7 (22-75)	67.5 (50-86)	0.89	Vitamin K

PLASMA AMINO-ACID CONCENTRATIONS (Table 2)

No significant differences in plasma amino-acid concentrations were observed in repeat samples obtained from any given patient. The overall reproducibility of results was consistent within $\pm 5\%$.

Values of seven amino-acids – asparagine, alpha-aminobutyric acid, cysteine, ornithine, lysine, histidine, and citrulline – showed no significant intergroup differences. Comparisons of the values of the remaining 13 amino-acids in each disease group with control values showed several significant increases and decreases ($p < 0.05$) as indicated in Table 2.

CHRONIC ACTIVE HEPATITIS (Fig. 1)

Plasma concentrations of aspartate, threonine, serine, methionine, and the aromatic amino-acid tyrosine were significantly raised, while concentrations of proline and of the three branched chain amino-acids valine, isoleucine, and leucine were significantly reduced.

PRIMARY BILIARY CIRRHOSIS

Plasma concentrations of aspartate, threonine, serine, arginine, methionine, and tyrosine were significantly raised, while concentrations of the

three branched amino-acids were significantly reduced.

CRYPTOGENIC CIRRHOSIS

Plasma concentrations of aspartate, serine, methionine, and the aromatic amino-acids tyrosine and phenylalanine were significantly raised, while the concentrations of the three branched chain amino-acids were significantly reduced.

ALCOHOLIC HEPATITIS $\pm$ CIRRHOSIS

(Fig. 2)

The plasma concentration of methionine was significantly raised while concentrations of glycine, alanine, phenylalanine, and the three branched chain amino-acids were significantly reduced.

Thus in the four groups of patients with chronic liver disease a common pattern of change in plasma amino-acid was seen in which the concentration of methionine was increased and the concentrations of the three branched chain amino-acids were reduced. Concentrations of aspartate, serine, and one or both of the aromatic amino-acids tyrosine and phenylalanine were raised in the groups with chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis, but not in the patients with alcoholic hepatitis $\pm$ cirrhosis. Changes in the

Fig. 1 Plasma amino-acid concentration in patients with chronic active hepatitis (CAH) that show significant differences from control values ($p < 0.05$). Blocks represent median values.

Table 2 Plasma amino-acid concentrations

Plasma amino-acid concentrations								
Median + range (μmol/l)								
	Control subjects (n=57)	Chronic active hepatitis (n=26)	Primary biliary cirrhosis (n=23)	Cryptogenic cirrhosis (n=11)	Alcoholic hepatitis ± cirrhosis (n=48)	Alcoholic fatty liver (n=31)	Viral hepatitis (n=10)	Biliary disease (n=18)
Aspartate	14.8 (5.0-92.9)	30.0* (8.9-108.1)	22.3* (14.1-126.2)	23.2* (7.9-71.5)	16.3 (4.0-89.9)	18.3 (3.5-115.7)	30.4* (13.6-83.1)	18.5 (8.0-61.9)
Threonine	126.4 (39.6-265.2)	150.6* (83.5-301.8)	157.1* (96.4-311.3)	164.2 (70.9-208.9)	116.0 (34.5-357.4)	129.3 (51.8-271.3)	150.8 (61.3-271.5)	123.2 (62.7-245.7)
Serine	118.9 (43.3-243.1)	149.3* (92.4-269.3)	154.0* (99.5-312.1)	171.9* (63.8-254.6)	114.2 (44.9-211.0)	122.0 (37.9-227.0)	125.2 (66.2-184.5)	114.3 (70.9-222.3)
Asparagine	50.1 (17.9-242.7)	71.1 (34.5-197.2)	69.0 (29.0-284.5)	62.2 (37.1-170.9)	70.5 (25.6-200.0)	80.9 (19.9-216.0)	56.5 (37.7-166.7)	54.5 (36.2-191.8)
Proline	215.4 (130.2-449.7)	178.7* (116.5-341.2)	216.3 (90.5-316.2)	248.2 (124.4-466.0)	203.9 (99.4-439.4)	186.7* (71.7-464.3)	177.3* (115.7-294.6)	177.6* (97.0-306.9)
Glycine	256.8 (97.3-471.2)	237.7 (156.5-396.7)	282.5 (199.3-456.8)	265.1 (178.6-374.8)	193.6* (86.2-740.0)	248.7 (115.4-356.2)	217.7 (137.6-365.6)	219.6 (105.4-377.8)
Alanine	353.3 (169.3-875.5)	325.7 (222.9-626.6)	352.6 (171.1-519.5)	380.9 (245.0-614.0)	293.1* (85.1-605.4)	325.1 (210.0-490.4)	288.3 (190.0-557.1)	300.3 (174.1-687.5)
Alpha-aminobutyric acid	28.7 (8.6-66.4)	32.0 (10.5-50.2)	23.2 (10.9-43.0)	26.3 (15.3-83.6)	23.4 (7.4-59.2)	25.0 (9.6-99.8)	33.2 (20.8-64.2)	28.1 (17.6-55.5)
Valine	237.6 (145.2-430.1)	160.6* (72.8-286.2)	159.8* (85.9-238.8)	177.3* (74.4-275.9)	122.1* (46.2-283.2)	207.5* (85.6-672.3)	180.5* (99.6-239.3)	174.3* (96.4-267.2)
Cysteine	73.0 (17.0-224.0)	83.0 (40.0-189.7)	84.7 (41.4-202.0)	81.9 (48.5-122.7)	91.1 (21.1-245.5)	72.5 (31.0-206.3)	78.1 (58.1-98.4)	78.3 (51.5-129.9)
Methionine	33.6 (13.8-77.4)	58.4* (24.1-223.6)	48.2* (24.5-149.4)	81.4* (35.5-340.0)	43.0* (21.0-191.3)	34.8 (16.2-114.4)	55.3* (24.9-127.2)	38.7 (17.1-89.4)
Isoleucine	88.1 (48.1-166.3)	62.1* (32.1-153.9)	62.6* (32.3-166.8)	62.7* (32.1-109.0)	53.8* (24.6-115.7)	77.3* (52.4-230.7)	64.4* (32.4-85.8)	63.3* (23.8-97.1)
Leucine	165.8 (85.8-296.4)	108.0* (46.9-209.3)	111.7* (47.7-176.9)	113.6* (46.7-208.5)	69.1* (32.2-207.5)	138.8* (55.3-615.9)	116.8* (65.1-145.5)	110.2* (56.0-178.1)
Tyrosine	76.4 (33.6-155.2)	93.5* (53.7-245.7)	112.7* (78.8-214.4)	149.6* (69.3-224.8)	85.4 (23.8-211.4)	67.3 (38.5-227.4)	75.1 (50.3-206.0)	77.1 (46.4-151.5)
Phenylalanine	72.2 (44.6-168.4)	73.4* (50.4-139.2)	74.2 (57.0-153.5)	101.1* (70.9-163.5)	63.5* (29.5-191.2)	55.3* (29.6-173.3)	126.7 (102.8-167.7)	70.4 (48.4-119.6)
Ornithine	117.8 (43.5-209.9)	101.0 (48.0-139.7)	142.7 (91.0-346.7)	120.7 (63.6-324.1)	100.1 (42.9-218.4)	124.2 (60.4-245.7)	126.7 (102.8-167.7)	131.5 (72.5-257.0)
Lysine	234.7 (109.0-453.2)	216.3 (134.5-490.2)	218.9 (125.3-366.3)	119.0 (59.2-399.8)	186.0 (67.1-602.6)	208.8 (77.8-436.1)	223.9 (108.5-325.8)	223.6 (104.6-388.5)
Histidine	100.4 (40.4-229.5)	138.5 (57.2-267.6)	111.1 (54.7-306.9)	105.3 (67.8-301.7)	111.5 (27.5-452.9)	99.3 (62.3-283.1)	105.3 (61.5-237.3)	106.8 (61.9-237.6)
Arginine	101.7 (33.7-291.8)	114.7 (43.2-286.3)	116.0* (54.2-404.3)	136.7 (55.3-309.9)	82.4 (22.3-201.3)	80.4* (23.4-224.7)	116.6 (51.8-182.2)	92.7 (55.9-211.6)
Citrulline	28.2 (12.8-63.6)	30.7 (19.1-54.1)	39.2 (33.6-53.8)	41.6 (22.6-74.2)	32.7 (22.4-68.6)	31.5 (20.4-50.4)	26.4 (18.6-30.6)	26.4 (17.4-83.3)

Amino-acid concentrations significantly different from control *p<0.05.

Individual amino-acid concentrations mg/100 ml = (µmol/l) × mol. wt. amino acid

1000

Fig 2 Plasma amino-acid concentrations in patients with alcoholic hepatitis ± cirrhosis that show significant differences from control values ($p < 0.05$). Blocks represent median values.

concentrations of certain other amino-acids occurred in each group in addition to the common changes observed.

ALCOHOLIC FATTY LIVER

Plasma concentrations of proline, phenylalanine, arginine, and the three branched chain amino-acids were significantly reduced.

ACUTE VIRAL HEPATITIS

Plasma concentrations of aspartate and methionine were significantly raised, while concentrations of proline and of the three branched chain amino-acids were significantly reduced.

BILIARY DISEASE (Fig. 3)

Plasma concentrations of proline and of the three branched chain amino-acids were significantly reduced.

Thus even in patients with minimal, potentially reversible liver disease significant changes were seen in plasma amino-acid profile – notably, reduced

concentrations of proline and of the three branched chain amino-acids.

Discussion

This study has shown that significant changes occur in plasma amino-acid concentrations in patients with chronic well-compensated liver disease and also in patients with minimal liver damage. Certain changes were common to all groups of patients while others appeared to relate to the severity of the liver disease, its activity, or even to its aetiology.

Specific and reproducible plasma amino-acid patterns have been shown in patients and experimental animals with chronic liver failure.^{18–25} The typical changes are increased concentrations of one or both of the aromatic amino-acids tyrosine and phenylalanine together with methionine and decreased concentrations of the three branched chain amino-acids – valine, isoleucine, and leucine. This pattern was confirmed in the patients with chronic liver disease in the present study with

Fig. 3 Plasma amino-acid concentrations in patients with biliary disease that show significant differences from control values ($p < 0.05$). Blocks represent median values.

the exception that patients with alcoholic hepatitis±cirrhosis showed reduced plasma phenylalanine concentrations. The patients with chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis also showed increased concentrations of aspartate and serine, while, in contrast, the patients with alcoholic hepatitis±cirrhosis showed reduced concentrations of glycine and alanine. Plasma proline concentrations were significantly reduced in the group with chronic active hepatitis. Previous studies have not differentiated patients on the aetiology of their liver disease so that comparisons cannot be made.

In the three groups of patients with minimal potentially reversible liver disease the consistent abnormality was of a reduction in the concentrations of the three branched chain amino-acids and of proline. The patients with alcoholic fatty liver, in common with the patients with alcoholic hepatitis±cirrhosis, showed significantly reduced plasma phenylalanine values. No previous studies are available for comparison.

Explanations are available for several of these findings. In all patient groups the plasma concentrations of the three branched chain amino-acids were significantly reduced. In normal man the liver is of major importance in amino-acid transamination. Its ability to use the three branched chain amino-acids, however, is limited,³⁵ and these

amino-acids are primarily catabolised via the skeletal muscle.^{18 36 37} The capacity of the extra-hepatic tissues to metabolise these amino-acids may assume compensatory proportions if liver gluconeogenesis decreases, with the result that plasma concentrations will fall. Insulin acts directly on the liver inhibiting glucogenesis and peripherally it suppresses muscle output of branched chain amino-acids.^{38 39} Hyperinsulinaemia may occur in chronic liver disease⁴⁰ as a result of portal-systemic shunting and decreased hepatic catabolism.⁴¹⁻⁴⁵ The low levels of branched chain amino-acids may be related to the raised circulating insulin levels. Fasting insulin and amino-acids values do not correlate,⁴⁶ however, and branched chain amino-acid concentrations have been shown to fall in rats after hepatectomy when peripheral and portal insulin levels are low.^{36 47 48} Thus the low concentrations of circulating branched chain amino-acids in these patients cannot be attributed solely to hyperinsulinism. It has recently been suggested that spontaneous portal-systemic shunting itself is closely related to the reduction in plasma branched chain amino-acids independently of the presence of liver disease;⁴⁶ the mechanism is not clear. Plasma glucagon values tend to be raised in cirrhotic patients^{44 49 50} and could influence plasma branch chain amino-acid concentrations. Similarly, plasma noradrenaline concentrations may be raised in

cirrhotic patients⁵¹ and infusion of noradrenaline in healthy subjects results in decreased plasma amino-acid concentrations.⁵² None of these explanations serve, however, to explain the reduced circulating branch chain amino-acid concentrations in the patients with minimal liver disease. Hyperinsulinaemia has been reported in patients with viral hepatitis²⁸ but would at best provide only a partial explanation for these findings.

The aromatic amino-acids and methionine are catabolised mainly in the liver;³⁶ their raised values in patients with chronic liver disease probably result from impaired hepatic metabolism and portal systemic shunting of blood.³⁰ The presence of a catabolic state with hyperglucagonaemia and increased gluconeogenesis could also contribute to the abnormal amino-acid pattern.⁵⁰

High plasma aspartate concentrations were found in patients with chronic active hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis but not in patients with alcoholic hepatitis±cirrhosis. Increased plasma aspartate concentrations have previously been reported in cirrhotic patients.²³ Recently, a significant reduction in whole blood aspartate concentration was reported in a group of well-compensated cirrhotic patients, suggesting partial intracellular depletion of this amino-acid.⁵³ Aspartate serves as a nitrogen donor in the urea cycle; if intracellular values were low, impaired ammonia detoxification could result. Aspartate may also serve as an excitatory neurotransmitter in the brain,⁵⁴ so that low intracellular levels could result in impaired cerebral function. The majority of patients in this recent study⁵² were alcoholics with cirrhosis; in this group of patients in the present study we found normal plasma aspartate levels. Information is not available on the whole blood aspartate values in cirrhotic patients in whom plasma aspartate concentrations are high.

Plasma proline concentrations were significantly reduced in patients with chronic active hepatitis and in all three groups of patients with minimal liver damage. Proline is used in collagen synthesis, and low levels might indicate increased collagen production. The normal proline levels in the other groups of patients are unexplained.

A percentage of patients with liver disease are malnourished, and protein-calorie malnutrition is associated with reduced plasma concentrations of all amino-acids except glycine and alanine.⁵⁵ The patients in the present study, however, were of normal body weight and took a normal diet, suggesting that malnutrition is an unlikely explanation for the changes observed.

In animal studies acute administration of ethanol *in vivo* or *in vitro* inhibits the active intestinal

transport of L-phenyl-alanine, L-leucine, L-glycine, L-alanine, L-methionine, and L-valine.⁵⁶⁻⁵⁸ Although none of the alcoholic patients in the present study had taken alcohol within three days of the study, it is still possible that the low phenylalanine concentrations seen in these patients, in contrast with the findings in the other groups of patients, might be explained as an effect of alcohol.

The patients with chronic active hepatitis were receiving prednisolone, which is known to influence protein and purine metabolism.⁵⁹ Peripherally, prednisolone mobilises protein and amino-acids from skeletal muscle, thus increasing their circulating concentrations. Steroids will probably have contributed to some of the plasma amino-acid changes seen in this group.

Significant changes occur in plasma amino-acid concentrations in patients with well-compensated chronic liver disease and also in patients with minimal liver damage. Most of the changes occurring in patients with chronic liver disease can be explained on the basis of impaired hepatic function, portal-systemic shunting of blood, and hyperinsulinaemia and hyperglucagonaemia. The changes occurring with patients with minimal liver damage, however, are less readily explained; nevertheless their presence indicates that amino-acid metabolism can be significantly disturbed by liver injury that is judged by all other criteria to be minor.

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