Comparison of the Effects of Hepatic-Aid and a Casein Modular Diet on Encephalopathy, Plasma Amino Acids, and Nitrogen Balance in Cirrhotic Patients

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Hepatic-Aid is purported to ameliorate encephalopathy and promote positive nitrogen balance in protein-intolerant, cirrhotic patients by correcting their imbalanced amino acid profile. This study evaluated Hepatic-Aid by comparing a 50-g Casein diet with an identical diet with 20-g Casein/30-g Hepatic-Aid per day in a cross-over study. Four patients with biopsy-proven stable cirrhosis, encephalopathy, and undernutrition were studied. Each study period included three days of equilibration and eight days of metabolic balance, with the following measured at baseline and on balance days 5 and 8: routine biochemistry, fasting ammonia, psychometric tests, EEG, and plasma amino acid profiles. There was no significant change in clinical status, routine biochemistry, fasting ammonia, psychometrics or EEG between the two study periods. Mean (±SD) nitrogen balance on the Casein diet at 1.5 ± 1.5 g/day was not significantly different from that on the Hepatic-Aid diet at 1.5 ± 1.2 g/day. Plasma amino acid profiles showed a significant fall ($p < 0.05$) in fasting and intraprandial tyrosine (tyr) and phenylalanine (phe) on Hepatic-Aid, but only intraprandial leucine (leu), isoleucine (ile), and valine (val) were significantly increased ($p < 0.05$) on Hepatic-Aid. The ratio leu + ile + val to tyr + phe was significantly increased ($p < 0.05$) on Hepatic-Aid. It is concluded that Hepatic-Aid, as given in this study, maintains N balance similar to Casein, alters the amino acid profile towards normal, but does not ameliorate encephalopathy.

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HEPATIC ENCEPHALOPATHY often limits the amount of protein that can be given to the patient with cirrhosis. Although the biochemical mechanism of encephalopathy remains unknown, gastrointestinal bleeding, infection, electrolyte abnormalities, and protein intolerance are recognized precipitating factors.[1] The abnormalities of protein metabolism shown to correlate with encephalopathy are elevation of plasma ammonia,[2] elevated mercaptans,[3] elevated tyramine and octopamine,[4,5] a disturbed plasma amino acid profile,[6] and reduced capacity to synthesize urea.[7] The abnormal plasma amino acid profile seen in these patients consists of increases in the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and in methionine and decreases in the branched chain amino acids (valine, leucine, and isoleucine). The normal molar ratio of the three branched chain amino acids to phenylalanine plus tyrosine (BCAA/AAA), is 3.0 to 3.5. In patients with encephalopathy, this ratio is reduced to approximately 1. It has been postulated that as a result of this imbalance, cerebral uptake of aromatic amino acids increases since they successfully compete against the depleted branched chain amino acids at the blood brain barrier.[8] This excess, in turn, causes overproduction of normally minor metabolites of phenylalanine and tyrosine degradation, such as octopamine and tyramine and displacement of the neurotransmitters by these false neurotransmitters that results in hepatic encephalopathy.

This hypothesis predicts that correction of the abnormal amino acid profile, with restoration of BCAA/AAA to normal, will ameliorate encephalopathy. To correct the abnormal aminogram, intravenous (F080) and enteral amino acid formulas (Hepatic-Aid) have been developed. These products contain reduced levels of aromatic and increased levels of branched chain amino acids and are the mirror image in composition

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TABLE 1. *Prestudy Evaluation of the Four Patients with Cirrhosis and Encephalopathy*

Patient Normal	Sex	Age	LBM 100%	Bilirubin <1 mg/dL	Albumin <3.5 g/dL	Protime 12.3 sec	FNH_3 <35 μg/dL	Enceph. 0	EEG 0	HPI 0.66	GEC 450–550 mg/min	Galactose Plasma Clearance 1300 ml/min
1	F	54	55%	1.30	3.0	14.3	97–142	2+	IIB	0.50	169	1667
2	M	65	101%	1.27	3.8	12.6	83–209	4+	IIB	0.00	344	739
3	F	57	80%	7.49	3.0	16.1	77–128	2+	IIB	0.04	164	—
4	F	62	73%	1.14	4.1	13.8	42–134	4+	nl	0.27	202	604

LBM = Lean body mass.
FNH_3 = Fasting plasma ammonia.
HPI = Hepatic perfusion index.
GEC = Galactose elimination capacity.
Baseline assessment was on 40 g regular protein diet.

of the typical encephalopathic aminogram. The requirements are that such a formula 1) will correct the abnormal amino acid profile, 2) will improve the patient's encephalopathy, and 3) will be an adequate protein source for patients. This study seeks to answer these questions by comparing Hepatic Aid with a Casein modular diet in a cross-over study.

Materials and Method

Subjects

Four patients, one man, aged 65, and three women, aged 54–62 years, were studied on the Clinical Research Facility for one month. They met the following criteria for inclusion: stable biopsy proven cirrhosis, and encephalopathy precipitated by increasing dietary protein above 40 g per day. Excluded were patients with active liver disease, as judged on routine biochemistry or liver biopsy, recent gastrointestinal bleeding, hepatorenal syndrome, encephalopathy due to infection, bleeding, constipation, or dehydration, or altered mental status due to causes other than protein-induced hepatic encephalopathy. The study protocol was approved by the Emory University Hospital Human Investigations Committee.

Prestudy Evaluation

A comprehensive hepatic data base was completed on all patients in the three to five days before the protocol began. At this time all received a 40-g protein solid food diet. Clinical, biochemical, hemodynamic,[9,10] hepatic function,[11] and neurologic data are summarized in Table 1. Nutritional status was assessed from the 24-hour urine creatinine output, and by anthropometric measurements.

Study Protocol

Diets: 1) Casein Modular diet. This diet furnished 2000 Kcal, with 50-g high quality protein given as Casein (Mead Johnson, Evansville, IN), 260 g carbohydrate and 80 g fat. The complete composition is given in Table 2.

2) Hepatic-Aid diet. This diet was identical to diet 1, except that 30 g of the protein source was given as Hepatic-Aid (American McGaw, Irvine, CA). The inclusion of some carbohydrate and fat in the Hepatic-Aid package mix, but not in the Casein protein source, necessitated careful equilibration of all components of the two diets to assure an identical intake of all nonprotein components. The two diets are given in Table 2, and the amino acid composition is given in Table 3. Each diet was given in blenderized form in a volume of approximately 2000 ml/day and was consumed between 8 am and 10 pm each day. Three of the patients drank this in six divided feedings, while one was fed through a small-bore feeding tube. The only additional intake

TABLE 2. *Composition of Hepatic-Aid and Casec Modular Diets*

	G	Kcal	Protein g/day	Carbohydrate g/day	Fat g/day
Casein Diet					
Casec	60	203	50.9	—	—
Polycose*	595	1029	—	257.3	—
Corn Oil	80	720	—	—	80
Flavor Package†					
Vitamin & Mineral Mix‡					
Total		1932	50.9	257.3	80
Hepatic-Aid Diet					
Hepatic aid	250	1118	29.0	195.4	24.6
Casec	25	85	21.3	—	—
Polycose*	145	250	—	62.7	—
Corn Oil	55	495	—	—	55
Flavor Package†					
Vitamin & Mineral Mix‡					
Total		1948	50.3	258.1	79.6

* Ross Laboratories.
† Norwich Eaton.
‡ Twice the RDA of the following vitamins and minerals were added to the diet: pantothenic acid, copper, zinc, manganese, biotin, iodine, magnesium, potassium, phosphorous, and calcium. The diet contained 4 g NaCl. Two tablets of Dayalets with Fe were taken daily by each patient.

TABLE 3. *Composition of Casein and Hepatic-Aid Diets* Compared with Estimated Daily Requirements for 70 Kg Man*

Amino Acids	Casein (g)	Hepatic-Aid (g)	Rose's MDR g/day
Essential			
Isoleucine	2.56	4.41	0.84
Leucine	4.90	6.11	1.12
Lysine	4.20	4.93	0.84
Phenylalanine	2.65	1.43	1.12
Methionine	1.55	1.00	.70
Threonine	2.20	2.57	0.56
Tryptophan	0.53	0.45	0.21
Valine	3.45	4.55	0.98
Nonessential			
Alanine	1.55	3.50	
Arginine	1.90	3.02	
Aspartic Acid	3.65	1.46	
Glutamic Acid	12.30	4.92	
Glycine	0.98	3.78	
Histidine	1.55	1.53	
Proline	5.80	5.34	
Serine	3.10	3.13	
Cystine	0.11	0.04	
Tyrosine	2.70	1.08	

* Casein and Hepatic-Aid diets are based on 50 g protein.

in the study periods was distilled water, tea or coffee, and six pieces of hard candy per day.

Study Periods

Each diet was administered for 11 days, three days equilibration and eight days metabolic balance. Patients were randomly allocated to commence with diet 1 or 2, and on completion crossed over to the other diet.

The 8-day metabolic balance periods comprised of serial 24-hour urine collections and two 4-day pooled stool collections. These were analyzed for total nitrogen, sodium, chloride, calcium, magnesium, potassium, and phosphorus. Total creatinine was measured for each 24-hour urine collection to assess completeness of the collection. In addition, prepared blenderized diets 1 and 2 were both analyzed for the same elements.

The other indices measured on days 5 and 8 of each balance period were: routine biochemistry, fasting venous plasma ammonia,[12] fasting amino acid profiles,[13] EEG,[14] and eight psychometric tests. Two of the patients also had amino acid profiles drawn on days 5 and 8 of each balance period at 5 pm, while the diet was being given; these were designated intraprandial. The eight psychometric tests were number connection A and B,[15] cancelling Z's[16] Williams' test of delayed recall,[17] simple reaction time to light (SRTL), simple reaction time to sound (SRTS), choice reaction time to light (CRTL), and choice reaction time to sound (CRTS).[18]

Data Analysis

Data from all measured parameters on the two diets were compared by a repeated measures analysis of variance.[19] These comparative analyses were based on the mean of the two measurements of each variable for each patient in the two study periods.

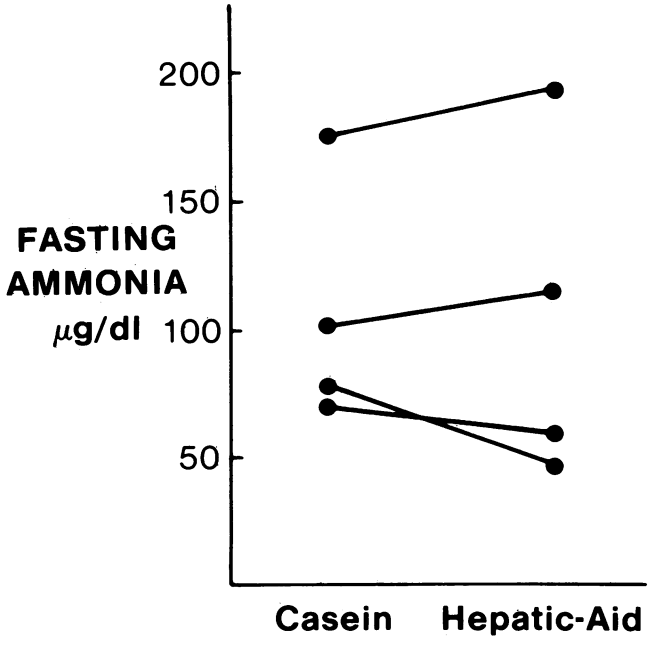

FIG. 1. Fasting ammonia in individual patients shows no significant difference between the two diets.

Results

Clinical

Baseline evaluation documented subclinical encephalopathy in all patients: they all showed hyperammonemia, three had abnormal EEGs, and all had at least two abnormal tests on psychometric evaluation. There were no demonstrable clinical changes in the patients in either study period. The routine biochemistry (BUN, creatinine, sodium, chloride, potassium, bilirubin, SGOT, alkaline phosphatase, total protein, albumin, calcium, phosphate, uric acid, glucose) was not significantly different in the two study periods.

Ammonia: The mean fasting plasma ammonia on the Hepatic-Aid diet at 103 ± 66 μg/dl was not significantly different from that on the Casein diet at 106 ± 48 μg/dl. The individual fasting venous plasma ammonia for the two study periods are summarized in Figure 1.

EEGs and psychometric data: All four patients showed some abnormality on EEG on the Casein diet with deterioration in one during the study period; the abnormality improved in one, persisted in two, and deteriorated in one patient on the Hepatic-Aid diet. A mean

of six psychometric tests were abnormal on the Casein diet, which was not significantly different from the mean of five abnormal tests on the Hepatic-Aid diet.

Metabolic balance: The mean (±SD) nitrogen balance for the four patients was 1.5 ± 1.5 g N/day on the Casein diet, which was not significantly different from 1.5 ± 1.2 g N/day on the Hepatic-Aid diet. The changes in nitrogen balance on the two diets are illustrated in Figure 2.

Plasma amino acids: All four patients showed elevated tyrosine and phenylalanine and depressed valine, leucine and isoleucine on the Casein diet, which is the typical abnormal profile of cirrhosis. The mean (±SD) values for the 21 amino acids measured in the four encephalopathic patients on the two diets are given in Table 4. The significant fall in tyrosine ($p < 0.05$) and phenylalanine ($p < 0.01$) on the Hepatic-Aid diet is the prime factor in the significant ($p < 0.05$) rise in the ratio of BCAA/AAA seen in these patients (Fig. 3). However, as measured on the fasting aminogram, this ratio did not return to normal. Glutamic acid was significantly reduced on Hepatic-Aid ($p < 0.05$); serine and arginine ($p < 0.05$) were significantly higher; glutamine, glycine and ornithine ($p < 0.1$), tended to be higher on the Hepatic-Aid diet.

The reduction in aromatic and elevation in branched chain amino acids were seen much more dramatically in the two patients studied intraprandially (Table 5). The conversion of their BCAA/AAA from 1.31 to 5.62 and 0.75 to 2.94 illustrates the capability of Hepatic-Aid to correct the disturbed amino acid profile.

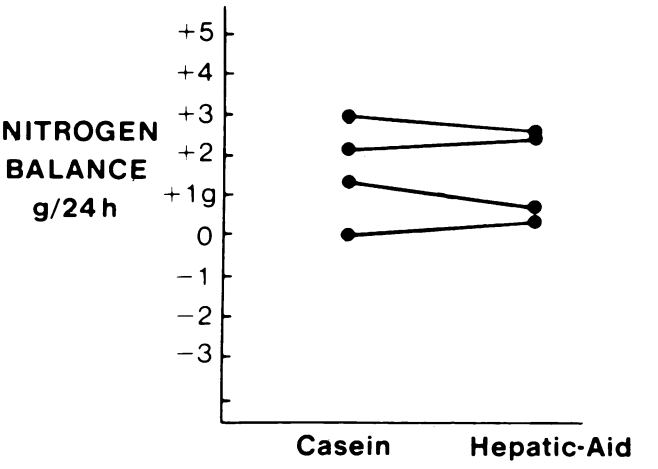

FIG. 2. Nitrogen balance in individual patients shows no significant difference on the two diets.

Discussion

Plasma Aminograms

Fasting levels of plasma amino acids were analyzed during both diet periods. The finding that the ratio BCAA/AAA of fasting aminograms was significantly improved illustrates that Hepatic-Aid can significantly improve this ratio without being given on a continuous basis. However, it has been shown that postprandial aminograms may be more informative in dietary studies,[20] so in an attempt to quantitate the maximal effect of the dietary regime, intraprandial aminograms were

TABLE 4. *Plasma Amino Acids on Casein and Hepatic-Aid*

	Normal (Range) μmol/L	Mean Value on Casein (±SD)	Mean Value on Hepatic-Aid (±SD)	Significant difference Casein vs Hepatic-Aid p
Amino Acid				
Taurine	33–66	61.23 (±7.03)	72.71 (±24.60)	
Threonine	119–181	137.50 (±46.90)	200.77 (±107.60)	
Serine	95–147	82.74 (±28.05)	107.72 (± 41.00)	$p < 0.05$
Glutamine	585–733	639.28 (±226.41)	738.80 (±177.82)	$p < 0.1$
Proline	144–262	329.47 (±115.18)	315.65 (±100.97)	
Glutamic acid	20–34	54.05 (±20.02)	38.61 (±15.16)	$p < 0.05$
Citrulline	26–46	58.09 (±16.38)	63.10 (±20.49)	
Glycine	190–346	266.97 (±75.39)	335.57 (±80.77)	$p < 0.1$
Alanine	308–484	298.28 (±122.83)	319.82 (±86.68)	
α-Aminobutyric acid	16–32	10.22 (±4.87)	11.66 (±2.46)	
Valine	196–264	135.03 (±43.94)*	159.93 (±32.94)	
Cystine	46–66	75.96 (±42.51)	91.62 (±36.50)	
Methionine	23–35	49.97 (±24.37)	42.01 (±8.80)	
Isoleucine	59–89	49.88 (±21.13)*	51.08 (±15.93)	
Leucine	116–166	76.78 (±20.14)*	80.13 (±12.57)	
Tyrosine	38–94	157.94 (±60.56)†	92.64 (±9.03)	$p < 0.05$
Phenylalanine	51–69	79.38 (±15.41)†	65.86 (±15.66)	$p < 0.01$
Ornithine	41–77	54.52 (±10.27)	72.39 (±18.63)	$p < 0.1$
Lysine	157–223	146.02 (±25.03)	173.97 (±42.81)	
Histidine	74–98	72.58 (±24.57)	78.38 (±14.34)	
Arginine	57–107	95.37 (±35.76)	116.19 (±44.82)	$p < 0.05$

* Significantly lower than normal ($p < 0.01$).

† Significantly higher than normal ($p < 0.05$).

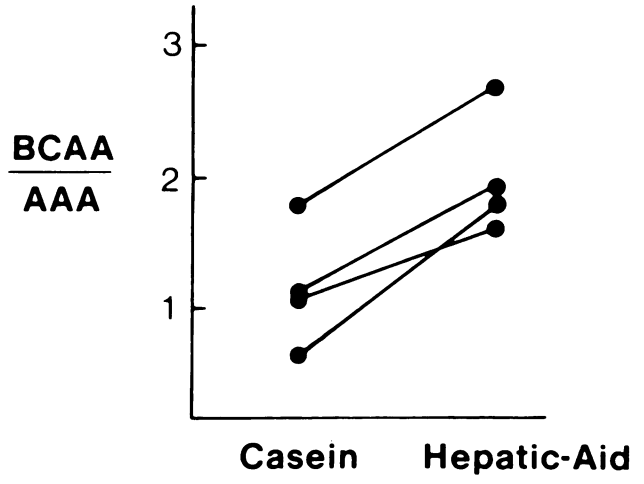

FIG. 3. The ratio BCAA/AAA in each patient rises giving an overall significant ($p < 0.05$) rise on the Hepatic-Aid diet for patients with encephalopathy.

studied in the last two patients. The greater effect on BCAA/AAA observed on these aminograms demonstrates that the intake of Hepatic-Aid used in this study was sufficient to achieve the biochemical aims of the product, namely restoration of this ratio to normal. The question, however, is raised as to how much greater an effect on the fasting BCAA/AAA ratio can be achieved by increasing Hepatic-Aid intake. The observed "normalization" of the BCAA/AAA is attained largely through significant reduction in tyrosine and phenylalanine rather than increases in valine, leucine, and isoleucine. It may be that continuing to increase the Hepatic-Aid component alone may be associated with a serious reduction in tyrosine as observed in the studies on F080.[21] The correct balance of regular protein required to avoid this potential problem, and also to optimize correction of BCAA/AAA, will probably vary from patient to patient depending on the intrinsic impairment on their hepatic metabolic pathways.[22]

The changes from the baseline induced by Hepatic-Aid extend to other amino acids beyond the intended changes in aromatic and branched chain amino acids (Table 4). Additional benefit might accrue from the concomitant reduction towards normal of methionine and glutamic acid, but the significant elevation of glutamine, glycine, ornithine, and arginine on the Hepatic-Aid diet may be detrimental. This study addressed these further alterations only as they affect the fasting amino acid profile, at one intake of Hepatic-Aid, and over an 11-day study period. The effects on intraprandial or postprandial aminograms, at higher intakes, for longer periods of time may prove to be of clinical significance.

TABLE 5. *Intraprandial Aromatic and Branched Chain Amino Acids on Two Patients Receiving Casein and Hepatic-Aid Diets*

	Normal μmol/L	Casein		Hepatic-Aid	
Patient I.D.		2	1	2	1
Tyrosine	38–94	85	256	57	95
Phenylalanine	51–69	64	79	44	30
Valine	196–264	104	134	270	221
Leucine	116–166	45	68	156	78
Isoleucine	59–89	46	48	142	69

Nitrogen Balance

The biologic value of proteins can be estimated by calculating their amino acid scores, using for comparison a reference protein.[23] This score depends on the amino acid content and the ratio of one amino acid to another: the higher the score, the greater the nutritional value. For these purposes, Casein was used as the reference protein. The limiting amino acids in Hepatic-Aid are phenylalanine and tyrosine: its protein score is 56% of the reference protein. However, in the population studied, Hepatic-Aid proved to be as good a protein source as the Casein reference protein as shown by virtually identical nitrogen balances on the two diets. This finding was unexpected, as the low protein score of the Hepatic-Aid diet would predict lower, or even negative, nitrogen balance on that diet. The authors have previously demonstrated the reduced ability of these patients to degrade tyrosine[24] and methionine[25] and to synthesize urea.[7] It is postulated that the decreased content of tyrosine and methionine in the combined Casein/Hepatic-Aid diet is sufficient for the cirrhotic patient with an impaired ability to metabolize these amino acids, although they are below the minimal daily requirement for normal subjects.

Encephalopathy

The ability to improve the BCAA/AAA in this study was not accompanied by improvement in encephalopathy. The four patients who had measurable impairment in neuropsychological function on EEG and by psychometric testing showed no significant improvement on the Hepatic-Aid regime. This finding is in conflict with previous reports of improvement in encephalopathy with Hepatic-Aid.[26]

The patients in this study had documented encephalopathy, which could be precipitated by protein excess. Acute encephalopathy, often precipitated by bleeding, constipation, dehydration, or infection, is much harder to evaluate because of the unpredictable changes associated with the precipitating factor. The latter patients were deliberately excluded. Encephalopathy is difficult to quantitate, and at present has no universal definition.

Clinical assessment is notoriously inaccurate,[2] but addition of quantitative psychometric measurements and EEG analysis offers the best methods available for measuring encephalopathy at this time. Previously reported studies[26] have not used these methods, and the authors seriously question the validity of clinical assessment. In this study a stable patient population was utilized, with demonstrable encephalopathy, changing only one variable—the protein source—between the two study periods, and measured encephalopathy with the most sensitive methods available. Utilizing such methods the authors were unable to demonstrate improvement in encephalopathy, despite attaining the anticipated improvement in metabolic indices.

The data presented in this paper lead the authors to conclude that Hepatic-Aid, used as a supplement to regular protein can maintain nitrogen balance and correct the BCAA/AAA abnormality in some cirrhotics. However, in patients with subclinical encephalopathy, there was no evidence that it improved their neurologic state.

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