Effect of branched chain amino acid infusions on body protein metabolism in cirrhosis of liver

P D WRIGHT, J D HOLDSWORTH, P DIONIGI, M B CLAGUE, AND O F W JAMES

From the Departments of Surgery and Medicine, University of Newcastle on Tyne, Newcastle on Tyne

SUMMARY Thirty seven patients with established cirrhosis of the liver were subjected to measurement of body protein metabolism using L-(1-^{14}C) labelled leucine as a tracer. The effects of disease severity and those of solutions containing 0%, 16%, 35%, 53%, and 100% branched chain amino acids were evaluated. Significant increases in protein synthesis were noted with solutions containing 35%, 53%, and 100% branched chain amino acids, but in patients receiving 100% branched chain amino acids without additional essential amino acid supplement the increase in synthesis was matched by a significant increase in protein breakdown. Protein balance was thus improved only in patients receiving 35% and 53% branched chain amino acids. It was concluded that the high increase in protein breakdown in patients receiving 100% branched chain amino acids was undesirable, and such a solution should not be recommended for clinical use.

The most characteristic of the changes in plasma amino acids that occur in cirrhosis of the liver are a lowering of the three branched chain amino acids (BCAA) leucine, isoleucine, and valine and an increase in methionine and the aromatic amino acids phenylalanine and tyrosine.[1–6] Infusion of amino acid solutions enriched with BCAA[7–12] and BCAA alone[8 13 14] have been used to modify this amino acid profile with some apparent beneficial effects on portal systemic encephalopathy; little evidence, however, has been documented concerning the effects of these infusions on other metabolic variables, notably protein metabolism.

Body protein metabolism can be measured using various techniques in which the kinetics of a labelled amino acid are analysed.[15] A recent report,[16] using U(^{14}C) tyrosine, suggested that the administration of BCAAs diminished protein breakdown and synthesis in cirrhosis of the liver. The use of tyrosine, a non-essential amino acid that has a small body pool and is metabolised by the liver, makes interpretation of these results difficult. Leucine is a more appropriate choice for studying patients with cirrhosis of the liver as this amino acid has a larger pool size, is an essential amino acid, and is metabolised by skeletal muscle. Recent refinements in technique using a double isotope infusion of sodium (^{14}C) bicarbonate followed by L-(1-^{14}C)

leucine[17] have shown that a single metabolic study can be completed in four hours with all measurements carried out on plasma samples, obviating the need to collect urine or expired air.[18] The aims of this study were twofold: firstly, to evaluate body protein metabolism and plasma amino acids in patients with cirrhosis of the liver with reference to disease severity; and secondly, to assess the effects of intravenous infusions of different amino acid solutions of varying BCAA content on protein metabolism. As the metabolism of BCAAs may be influenced by insulin[19 20] plasma insulin was also measured during these studies.

Patients and methods

Thirty seven patients with histologically confirmed stable cirrhosis were studied. The severity of the disease was assessed on the basis of abnormalities in the serum bilirubin and albumin prothrombin time, the quantity of ascites and the level of encephalopathy in accordance with the Pugh modification of Child's classfication.[21] Patients were excluded if there was evidence of current infection or variceal bleeding, or if there had been a recent noticeable change in ascites. Patients were also excluded if they had had surgery or blood transfusion within the preceding four weeks, if they had diabetes mellitus, or had received steroids.

Five different intravenous fluid regimens were

Correspondence to: Mr P D Wright, Freeman Hospital, Freeman Road, High Heaton, Newcastle upon Tyne NE7 7DN, England.

Table 1 *Details of patients within nutritional groups and of those additional patients who failed to complete full study protocol but who provided sufficient data to be used for assessing disease severity*

	Sex M:F	Age (mean(SD))	Child's grading system		
Group (n=)			A	B	C
100% BCAA (7)	5 2	58 (7)	1	2	4
53% BCAA (6)	3 3	60 (5)	1	4	1
35% BCAA (6)	1 5	63 (12)	3	2	1
16% BCAA (6)	3 3	59 (10)	3	3	0
Dextrose (7)	3 4	58 (12)	2	2	3
Additional 5	4 1	58 (8)	2	1	2
Totals 37	19 18	59 (9)	12	14	11

used and patients were randomly allocated into groups on this basis (32 patients). Table 1 gives details of the patients and groups. Those patients who failed to complete the full study schedule, but who provided additional data for the assessment of disease severity, were also included (n=5). The nutritional groups were of comparable ages, but there was some variability in the sex allocation and child's grades within each group.

STUDY SCHEDULE
Each of the 32 patients in the five study groups underwent two protein metabolic studies (two patients failed to complete the second study due to difficulties with blood sampling, but provided sufficient blood to complete the plasma amino acid data). The first study was used to assess disease severity (Child's classification) in all subjects and each first study was also used as a control against which metabolic changes brought about by the intravenous fluid regimens could be assessed. An oral liquid feed was given during the first study, whereas during the second study patients received the intravenous solution to which they had been allocated. After an overnight fast the oral feed was started, given as aliquots each half hour and continued throughout the first metabolic study, which began one and a half hours after the start of the oral feeding. When the first study had been completed nutrition was changed to the appropriate intravenous formulation and continued for a further 24 hours, the second protein metabolic study being carried out during the final four hours.

NUTRITION
Clinifeed 400 was selected for the control studies as this is a liquid oral feed and thus easy to administer. It was given at the rate of 0.8 ml/kg^{-1}/hour^{-1},

equivalent to 3.84 mg nitrogen and 0.64 cal/kg^{-1}/hour^{-1}.

Four amino acid solutions of varying BCAA content were used: a) 100% BCAA (4% BCAA, Travenol Laboratories Ltd); b) 53% BCAA (Synthamin 17+4% BCAA); c) 35% BCAA (Hepatamine); and d) 16% BCAA (Synthamin 14). All of these amino acid solutions were given with 10% dextrose, and therefore 10% dextrose alone was also evaluated. Patients with liver disease may be intolerant of intravenous amino acids [4], and to avoid this the infusion rate of the amino acid solutions was adjusted so that the nitrogen input was 2.11 mg/kg^{-1}/hour^{-1}. The carbohydrate content of each solution was adjusted so that the calorific input was 0.64 cal/kg^{-1}/hour^{-1}.

Details of the calorific content and volumetric infusion rates of the five intravenous infusions.

MEASUREMENTS OF BODY PROTEIN METABOLISM
Primed constant rate infusions of sodium $(1-^{14}C)$ bicarbonate and L-$(1-^{14}C)$ leucine were given, as described by Clague *et al.*[17] The sodium (^{14}C) bicarbonate was used to calibrate the body bicarbonate pool so that the entry rate of $^{14}CO_2$ into the pool from the oxidation of L-$(1-^{14}C)$ leucine could be determined during the second isotope infusion. After taking the blood for background radioactivity

Table 2 *Volumetric composition (%), infusion rates (ml/kg^{-1}/hour^{-1}), and calorific infusion rates (cal/kg^{-1}/hour^{-1}) of components of each solution**

Solution	BCCA (%)	Volume %	Volumetric infusion rate	Calorific infusion rate
4% BCAA		24·97		0·068†
+	100		1·906	+
10% dextrose		75·03		0·572
Synthamin 17		4·78		
+				0·053†
4% BCAA	53	9·57	1·714	+
+				
10% dextrose		85·65		0·587
Hepatamine		10·56		0·051†
+	35		1·647	+
10% dextrose		89·44		0·589
Synthamin 14		9·05		0·045†
+	16		1·636	+
10% dextrose		90·95		0·595
10% dextrose		100·00	1·600	0·64

†Allowances made for calories that may be derived from amino acids (3·57 calg^{-1})
*Final rate of nitrogen was 2.11 mg and that of calories 0·64 kg^{-1}/hour^{-1}.

the sodium (^{14}C) bicarbonate was infused for one and a half hours, with blood sampling during the final half hour to determine the plateau of $^{14}CO_2$ in plasma. The isotope infusion was then changed to the L1–(1–^{14}C) leucine, which continued for a further two and a half hours, blood being taken three times during the last hour to determine plateau activity of $^{14}CO_2$ and ^{14}C–leucine in plasma. Separation of ^{14}C-activity from blood was carried out on the day of collection and counted in a Packard Tricarb liquid scintillation counter.

Permission was granted by the isotope advisory panel and local ethical committee for two studies (50 μCi total radiation) in subjects 45 years or older who had given informed consent.

CALCULATION OF BODY PROTEIN METABOLISM
The body is composed of a number of intracellular and extracellular pools of amino acids. The number and size of these pools is not considered to be important when administering a primed constant rate infusion of a labelled amino acid to measure protein metabolism, provided sufficient time elapses to permit the isotope to equilibrate within each pool. Based on this assumption, the following mathematical relation can be applied to relate the various components of leucine metabolism:

$$q = b + i = s + c \quad (gm/kg^{-1}/d^{-1})$$

where,

q=leucine turnover,
b=leucine entering the amino acid pool from protein breakdown,
i=intake (oral or intravenous) of leucine,
s=leucine incorporated into newly synthesised protein,
c=leucine catabolism or oxidation.

Having calculated the rates of leucine released from (b) and incorporated into (s) protein, these values were converted into those for whole body

protein, of which leucine represents 8%. Protein balance was calculated by subtracting breakdown from synthesis (s–b).

DETERMINATION OF PLASMA INSULIN
This was measured on each of the blood samples B and C, using a single antibody radioimmunoassay.[22]

STATISTICAL ANALYSIS
Results were presented as the mean+1SD. Paired *t* tests were used within nutritional groups and for comparison between group analysis of variance (Anovar) and linear regression analysis. The Child's data were subjected to analysis of variance for regression on grouped data (Regova).[23]

Results

EFFECT OF DISEASE SEVERITY
Comparison was made between results obtained in patients with Child's grade A disease *v* patients with grades B and C disease.

Leucine and protein metabolism (Table 3)
There were no significant differences in leucine turnover or catabolism, nor in body protein synthesis, breakdown, or balance between the three Child's groups.

Plasma insulin (Table 3)
No significant differences were detected in the concentration of insulin between the three grades of disease, although the concentration of insulin was noted to be higher in the patients with the least severe disease (grade A) than in the patients with more severe disease (grades B and C).

EFFECT OF THE INTRAVENOUS INFUSIONS
Leucine and protein metabolism (Table 4)
When the comparison was made within each study group between the control study and the infusion study (study 1 *v* 2) infusion of dextrose alone

Table 3 *Results for body leucine and protein metabolism (mean (SD)g/kg^{-1}/days^{-1}) and plasma insulin (mean (SD)μU/ml^{-1})*

Metabolic measurement	Child's grading system			Regova p=
	A (n=12)	*B (n=14)*	*C (n=11)*	
Leucine turnover	0·25 (0·08)	0·27 (0·08)	0·27 (0·06)	NS
Leucine catabolism	0·04 (0·02)	0·04 (0·01)	0·06 (0·02)	NS
Protein synthesis	2·52 (0·81)	2·83 (0·89)	2·63 (0·56)	NS
Protein breakdown	2·28 (0·97)	2·62 (1·01)	2·58 (0·70)	NS
Protein balance	0·24 (0·30)	0·21 (0·15)	0·05 (0·25)	NS
Insulin	20·2 (9·9)	14·6 (12·0)	11·6 (9·1)	NS

Table 4 *Leucine and protein metabolism (mean(SD)g/kg⁻¹/day¹) and plasma insulin (mean (SD) μU/ml¹) for five nutritional groups during first and second metabolic studies*

Metabolic variable	Study	BCAA 100% (n=6)		BCAA 53% (n=6)		BCAA 35% (n=6)		BCAA 16% (n=6)		Dextrose (n=6)		Anovar
Leucine turnover	1	0·24	(0·06)	0·31	(0·07)	0·23	(0·06)	0·27	(0·10)	0·29	(0·07)	NS
	2	0·47	(0·06)***	0·32	(0·09)	0·26	(0·02)	0·26	(0·09)	0·24	(0·06)*	
	2–1	0·23	(0·04)	0·01	(0·06)	0·03	(0·06)	−0·01	(0·03)	−0·05	(0·03)	p<0·001
Leucine catabolism	1	0·05	(0·02)	0·05	(0·01)	0·04	(0·01)	0·04	(0·02)	0·06	(0·03)	NS
	2	0·14	(0·04)**	0·05	(0·02)	0·04	(0·01)	0·03	(0·02)**	0·04	(0·02)*	
	2–1	0·09	(0·04)	0·00	(0·02)	0·00	(0·01)	−0·01	(0·01)	−0·02	(0·02)	p<0·001
Protein synthesis	1	2·47	(0·64)	3·25	(0·77)	2·34	(0·68)	2·82	(1·04)	2·76	(0·59)	NS
	2	4·06	(0·61)***	3·28	(0·85)	2·71	(0·19)	2·93	(0·91)	2·49	(0·63)	
	2–1	1·59	(0·24)	0·03	(0·63)	0·37	(0·77)	0·11	(0·35)	−0·27	(0·36)	p<0·001
Protein breakdown	1	2·28	(0·78)	3·03	(0·91)	2·11	(0·71)	2·53	(1·19)	2·81	(0·90)	NS
	2	3·85	(0·74)***	3·10	(1·07)	2·67	(0·27)	3·06	(1·11)**	2·95	(0·80)	
	2–1	1·57	(0·47)	0·07	(0·77)	0·56	(0·78)	0·53	(0·32)	0·14	(0·35)	p<0·001
Protein balance	1	0·19	(0·20)	0·21	(0·23)	0·23	(0·04)	0·28	(0·16)	−0·05	(0·41)	NS
	2	0·21	(0·51)	0·18	(0·27)	0·04	(0·15)*	−0·13	(0·21)***	−0·46	(0·24)**	
	2–1	0·02	(0·47)	−0·03	(0·20)	−0·19	(0·15)	−0·41	(0·12)	−0·41	(0·25)	p<0·05
Insulin	1	25·2	(18·6)	18·9	(6·0)	47·6	(27·2)	10·4	(9·0)	25·0	(20·0)	NS
	2	33·2	(17·6)	43·1	(25·6)	58·3	(22·0)	26·9	(15·0)	34·0	(25·4)	
	2–1	8·0	(7·4)	24·2	(25·6)	10·7	(13·8)	16·5	(16·9)	9·0	(13·9)	NS

Significantly different 1 *v* 2 p=*<0·05; **<0·01; ***<0·001.

resulted in a significant fall in both leucine turnover and catabolism, although no significant changes were seen in body protein synthesis and breakdown. The net effect of these small changes in synthesis and breakdown led to negative protein balance (p<0·01). By contrast, infusion of the 100% BCAA solution led to high increases in leucine turnover and catabolism and body protein synthesis and breakdown, but as the changes in synthesis and breakdown were of a similar order there was no net change in protein balance. When the effects of infusion of amino acid solutions with increasing amounts of BCAA (16%, 35% and 53%) were examined, it was seen that the metabolic variables did not change to the same extent as with dextrose alone or 100% BCAA. The 16% BCAA solution led to a significant increase in body protein breakdown and consequent negative protein balance (p<0·001); 35% BCAA induced a significant fall in protein balance (p<0·05); and 53% BCAA did not lead to significant changes in any of the calculated components.

Comparison between the groups showed no significant differences between the control studies (study 1), but highly significant differences were detected in all the metabolic variables on the basis of changes from the first to the second studies (study 2–1). The significance of these differences for leucine turnover and catabolism and body protein synthesis and breakdown were attributed to the high increases that occurred in those patients given the 100%

BCAA. The significance of the differences in the changes in protein balance was due to the negative balance observed with infusion of the 16% BCAA and dextrose alone.

Linear regression analysis of the leucine and protein metabolic measurements against BCAA content of the infused solution showed a close correlation with leucine catabolism (r=0·90) and turnover (r=0·88), a detectable correlation with protein synthesis (r=0·76), but only a poor correlation with protein breakdown and balance (r=0·59 and 0·52, respectively).

Plasma Insulin (Table 4)
A wide range in the mean concentrations of insulin was observed between the five groups. The concentrations of insulin were increased by the intravenous infusions in all groups (study 2). No significance, however, was attached to these findings, probably because of the wide variation in insulin concentration within the groups.

Discussion

This study suggests that on the basis of Child's classification body protein metabolism does not change with increasing severity of cirrhosis. This is probably because skeletal muscle forms the bulk of body protein and as the method used to measure protein metabolism cannot distinguish between metabolism in different tissues changes in protein

synthesis and breakdown due to liver function will be masked, liver protein being only a relatively small component of whole body protein. As no differences were detected in protein metabolism between the three grades of disease severity it is unlikely that the uneven distribution of patients with different grades of disease between the five nutritional groups will have adversely influenced the results obtained from these groups. This contention is further supported by the absence of differences in the control studies between the nutritional groups.

It has been suggested that infusion of large quantities of leucine at the same time as measuring protein metabolism with L–$(1-^{14}C)$ leucine may invalidate the technique.[24] As the quantity of leucine used in protein breakdown and synthesis must remain fixed in relation to the total amino acids utilised, however, the method is valid in these circumstances. The same cannot be said for leucine turnover and catabolism, and therefore these two components were not converted into whole body protein equivalents. Infusion of large amounts of leucine will increase turnover by enlarging the leucine content of the body amino acid pool and will increase catabolism, because any leucine present in excess of the amount required for protein synthesis will be oxidised, the body being unable to store excess amino acids. This was supported by our results in that both leucine turnover and catabolism correlated with the increasing amounts of leucine in the infused solutions. As the correlation of protein synthesis and breakdown with the BCAA content of the infusions was much poorer, the high increases observed in those patients receiving only BCAA (100% BCAA) may thus be a feature particular to giving only these three amino acids and not simply due to increasing the BCAA content of the infusion.

Patients with cirrhosis differ metabolically from subjects in whom L–$(1-^{14}C)$ leucine has previously been used to measure body protein metabolism,[17 25 26] particularly because of the presence or absence of ascites. The difference in the quantity of ascites between the patients with cirrhosis might be regarded as a source of error in the measurement of protein metabolism in this disease, as the ascites represents a variable extracellular amino acid pool. With a constant rate of infusion, however, this possible variation in pool size is not regarded as important, provided sufficient time is allowed for equilibration of the labelled amino acid within all amino acid pools.

Another aspect of metabolism that has to be considered when measuring protein metabolism with L–$(1-^{14}C)$ leucine is the effect of insulin on leucine metabolism. Leucine is a potent stimulant to the secretion of insulin,[19] and in turn, insulin lowers the plasma concentration of BCAA, including leucine.[20] This relation could represent another potential source of error in the measurement of protein metabolism as the turnover and catabolism of leucine may be influenced by fluctuations in the concentration of plasma insulin, especially when cirrhosis is associated with increased peripheral insulin concentration.[27] Furthermore, the results could have been influenced in a similar manner during the intravenous infusions as a consequence of the differing BCAA content of the solutions. The results of plasma insulin concentration do not seem to support these arguments. Insulin concentrations were observed in the patients with grades B and C severity and although all five infusions induced a rise in insulin, this was similar for each infusion.

The findings of this study do not agree with those of O'Keefe *et al.*[16] In their study, however, a different labelled amino acid, U(^{14}C) tyrosine, was used to determine protein metabolism. Furthermore, the rates of the metabolic components were given in g/d^{-1}, no allowance being made for variations in patient weight, and BCAA were given orally, thus making comparison with the current study difficult.

Infusion of both dextrose alone and the 16% BCAA solution resulted in negative protein balance, although the underlying mechanism with each infusion, due to changes in protein synthesis and breakdown, was different. The small fall in synthesis and increase in breakdown with dextrose alone can be interpreted as body protein metabolism responding to an absence of dietary amino acids. With infusion of 16% BCAA the negative protein balance was due to breakdown, increasing to a greater extent than synthesis. As the infusions were given for 24 hours and the amounts infused were less than is normally recommended for total intravenous nutrition,[28] the quantity of amino acids in the 16% BCAA formulation may have been sufficient to preserve protein synthesis, but, nevertheless, the suboptimal amount of amino acids would have been insufficient to prevent a rise in breakdown after 24 hours. It has previously been reported that protein metabolism changes in response to nutrition by an increase in protein synthesis, without a corresponding increase in breakdown.[29] These responses, however, were documented over a few hours and not 24 hours as in this study.

Better protein balance was observed in the two groups receiving the branched chain enriched amino acid solutions (35% and 53% BCAA) and the group receiving BCAA alone (100% BCAA). This phenomenon is again related to different patterns of protein synthesis and breakdown within the groups; possibly the most interesting feature being the high

increase in both measurements observed with the 100% BCAA infusion. The increase in protein synthesis with this infusion was not entirely unexpected: experimental work has shown that leucine can increase protein synthesis,[30][31] that BCAA infusions can increase total liver nitrogen[32]; and that branched chain keto acids stimulate albumin synthesis.[33] The rise in protein breakdown associated with this infusion was of particular interest as this has not been reported previously. These findings may be explained if the above mentioned in vitro effects of leucine on skeletal muscle[30][31] also occur in vivo. By infusing large quantities of leucine we may have invoked this response. To have fuelled the high level of muscle protein synthesis with amino acids, when muscle protein breakdown was diminished and only three of the essential amino acids were infused, the observed total net increase in protein breakdown would only have been due to a secondary increase in other tissues. If we analyse our results on this basis the possible benefits, in terms of protein synthesis and balance conferred by infusion of BCAA alone (100% BCAA), may thus be counteracted by the increase in protein breakdown.

Although the intravenous solutions administered during this study were given to subjects with stable cirrhosis, in clinical practice it is not these patients who would receive such infusions. The clinical role of tailored branched chain amino acid preparations would be in the management of acutely ill cirrhotic patients, such as those with bleeding from oesophageal varices or postoperative patients. As such patients are limited in number and because they are metabolically unstable, it was considered necessary to evaluate the infusions in patients with stable cirrhosis. The findings suggest that if amino acid solutions were used in the management of unstable cirrhosis then to obtain optimal protein metabolic function the composition should conform to that of either the 53% or the 35% BCAA formulations, these being the two solutions which led to satisfactory protein balance without affecting underlying protein synthesis and breakdown to the extent observed with 100% BCAA. Furthermore, both these solutions contained all the essential amino acids, and they were considered to have the most desirable effect on plasma amino acid concentrations. More work is now required to assess the effects of these infusions in patients with unstable cirrhosis.

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