

Branched-chain amino acids in catabolic states

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In states of protein loss, such as starvation, injury and diabetes mellitus, the circulating concentrations of those amino acids with branched chains (BCAA), i.e. leucine, isoleucine and valine, are selectively increased. This finding, together with the known differences in metabolism between the BCAA and other amino acids (Adibi, 1976), and the suggestion that leucine may influence protein synthesis (Buse & Reid, 1975), has led to considerable work on the BCAA in catabolic states in rats and man (see Walser & Williamson, 1981). This account briefly examines whether the special interest in these amino acids is justified and how far recent advances may be applied to clinical states in man.

Metabolism of BCAA

The metabolism of BCAA has been elucidated in the rat rather than in man and species differences probably exist. The BCAA differ from the other amino acids in their initial metabolism in muscle (Fig. 1). This important feature appears to follow from the distribution and activity of the enzymes responsible for their degradation. In the rat the amount and activity of the specific branched-chain amino acid transferase (*EC* 2.6.1.42), which is responsible for the transamination of BCAA to branched-chain keto or oxo acids (BCKA), are far greater in muscle than in the liver, whereas the branched-chain keto or oxo acid dehydrogenase (*EC* 1.2.4.4) is found predominantly in the liver (Shinnick & Harper, 1976). As a result most branched-chain keto acids are produced from BCAA in muscle (and to a lesser extent in other non-hepatic tissues); but since BCKA dehydrogenase is a rate-limiting step in muscle, some of the keto acids are released into the circulation and transported to the liver for further metabolism (Krebs & Lund, 1977).

In muscle, BCAA such as leucine may be used for protein synthesis or completely oxidized after conversion to 2-ketoleucine; some of the ketoleucine is released from muscle for further metabolism in the liver. Likewise, the amino group is transferred either to alanine, which is dealt with by the liver, or to glutamine (see below), which is dealt with by the gut and kidneys. Within the liver, in contrast to muscle, ketoleucine may also act as a precursor for lipids or ketone bodies. Adipose tissue may also be an important site of removal of BCAA and their conversion into lipids.

The practical result of the preferential initial metabolism of BCAA by extrahepatic tissues, especially muscle, is that changes in their concentration reflect events in muscle rather than in the liver. The fate of an administered BCAA will

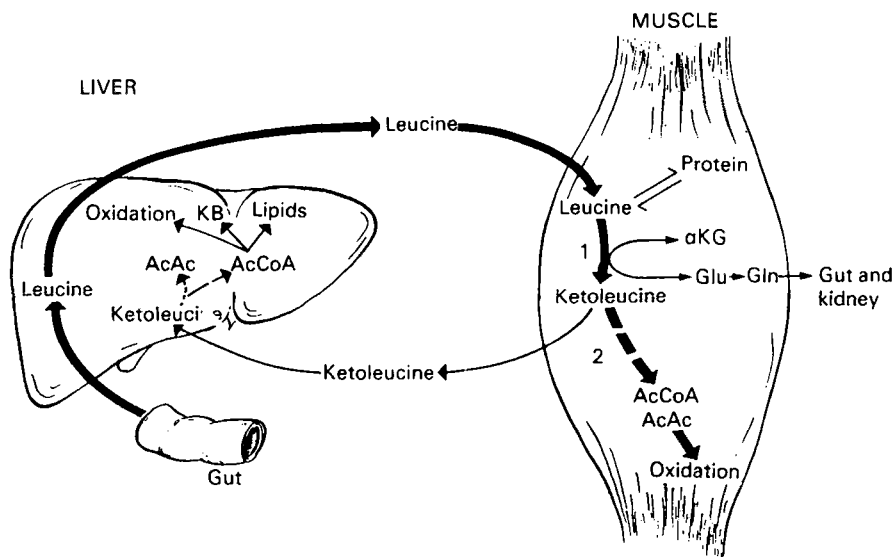


Fig. 1. The metabolic pathway of leucine, as an example of the metabolism of branched-chain amino acids. 1, Branched-chain amino acid transferase (*EC* 2.6.1.42); 2, branched-chain keto acid dehydrogenase (*EC* 1.2.4.4); α KG, ketoglutarate; AcCoA, acetyl CoA; AcAc, acetoacetate; KB, ketone bodies. The thick arrows represent the major pathway of leucine after oral administration as protein.

partly depend on whether it is given on its own or with other amino acids as part of a protein meal. In the latter instance, more leucine will be utilized for protein synthesis.

Catabolic states

The basal BCAA concentration increases to a variable extent in different catabolic states (Table 1). The increase is most marked after a short period of starvation (Gelfand *et al.* 1979) and after severe accidental injury. It affects the circulating concentration of the three individual BCAA to about the same extent. The rise in BCAA occurs within 24 h of starvation in man but is delayed in the rat. The effects of starvation are to some extent reproduced by a period of dietary carbohydrate restriction and may be partly attributed to it.

After severe accidental injury there is a significant rise in BCAA concentration within the first 24 h. Wedge *et al.* (1976) compared the venous blood concentrations of BCAA in sixteen normal adults after road traffic accidents and in four adults after elective skin graft operations. There were no significant changes after skin grafting. The increase in BCAA concentration in the injured subjects was immediate and sustained. It was proportional to the extent of the urinary nitrogen loss and greatest in those severely-injured subjects who remained normoketonaemic. In contrast, the concentration of other amino acids tended to fall. Since the initially normoketonaemic patients had a lower N intake than those with initial hyperketonaemia, the difference between the two groups was not related to

Table 1. Basal branched-chain amino acid (BCAA) concentrations and rates of protein turnover in catabolic states

Physiological or pathological state	Circulating BCAA concentration	Protein degradation rate	Protein synthetic rate	Reference
Nutritional				
Well nourished, adequate protein	~ 350 µmol/l	Normal	Normal	Fujita <i>et al.</i> 1979;
Well nourished, low protein, high energy	Decreased	Low	Low	Motil <i>et al.</i> 1981
Well nourished, adequate protein, high fat	Increased	?	?	Swendsen <i>et al.</i> 1967
Well nourished, normal protein, low energy	Temporary increase	Low	Low	Marliss <i>et al.</i> 1978;
Short-term starvation	Increased	Normal	Low	Winterer <i>et al.</i> 1980
	Decreased	Low	Low	Gelfand <i>et al.</i> 1979;
				Elia <i>et al.</i> 1980;
				Winterer <i>et al.</i> 1980*
				Saunders <i>et al.</i> 1967;
				Smith <i>et al.</i> 1974;
				Golden <i>et al.</i> 1977
Post traumatic				
Orthopaedic surgery	Moderately increased	Normal†	Decreased†	Askanazi <i>et al.</i> 1978
Severe accidental injury	Increased	High	High	Birkhan <i>et al.</i> 1980
Burns	Increased	High	High	Kien <i>et al.</i> 1978;
				Aulick & Wilmore, 1979;
				Wolfe <i>et al.</i> 1983
Other				
Immobility	Normal	Normal	Low	Schonheyder <i>et al.</i> 1954
Muscular dystrophy	Normal	Decreased	Decreased	Bank <i>et al.</i> 1971;
				Haymond <i>et al.</i> 1978;
Insulinoma	Decreased	?	?	Rennie <i>et al.</i> 1982
Diabetes (mild to moderate insulin deficiency)	Increased	Increased	Normal or increased	Berger <i>et al.</i> 1978
				Berger <i>et al.</i> 1978;
				Saunders <i>et al.</i> 1982
Cirrhosis ± encephalopathy	Normal or decreased	Normal or decreased	Normal	Ansley <i>et al.</i> 1978;
				O'Keefe <i>et al.</i> 1981
Fulminant hepatic failure	Normal or decreased	Increased	Increased	O'Keefe <i>et al.</i> 1981

*After protein-modified fast in obese people.

†Depends on nutritional state.

exogenous protein. Although the initial difference in ketonaemia may have a direct effect on BCAA release from muscle (Sherwin *et al.* 1975), this could only have occurred in the first 24 h after injury, since the ketone body concentrations were normal thereafter whilst the BCAA concentrations remained abnormally high.

After elective surgery, such as total hip replacement, the increase in BCAA is less marked. Basal BCAA concentrations are also known to be increased in diabetes mellitus and decreased in patients with insulinomas and in those with cirrhotic liver disease where the circulating insulin concentration is above normal. In prolonged starvation the blood BCAA concentration is also reduced.

In most catabolic conditions the intake of BCAA in protein is variably reduced and the increase in BCAA concentration is therefore not determined by dietary excess, but is due either to a decreased peripheral metabolism of BCAA or to an increased release of BCAA from lean tissue. In order to clarify the cause of these changes and to assess how the body deals with BCAA, studies have been made after oral protein loading and after oral or intravenous leucine administration.

The fate and effects of BCAA

Early studies showed that the perfused liver did not readily metabolize BCAA (McMenamy *et al.* 1962) and that hepatectomy did not cause an increase in blood BCAA concentration or prevent their oxidation, whereas the circulating concentration of other amino acids increased and their oxidation virtually ceased (Miller, 1962; McMenamy *et al.* 1965).

When steak (oral protein) is given to carbohydrate-restricted or to starved normal subjects, there is a rapid and persistent rise in BCAA concentration, which has been likened to the glucose intolerance of the diabetic (Gelfand *et al.* 1979). These authors fed a lean beef meal to six adults on a normal diet containing 8.4 MJ/d (2000 kcal/d), or on a diet consisting of less than 25 g carbohydrate/d but unrestricted in protein and fat, or after 3 d fasting. Both carbohydrate restriction and fasting exaggerated the protein-induced increase in circulating BCAA concentration. Intravenous leucine in carbohydrate-restricted subjects also produced a greater increment in plasma leucine than in normal subjects. These alterations in the handling of BCAA were all corrected by hypo-energetic carbohydrate refeeding. It was postulated that the decreased disposal of ingested BCAA was partly due to the fall in basal and protein-stimulated insulin levels which carbohydrate restriction and starvation produced; however, induced variations in insulin concentration in normal subjects do not appear to significantly alter the peripheral disposal of intravenously-administered leucine (Eriksson *et al.* 1982). It is possible that the increased circulating concentration and oxidation of fatty acids and ketone bodies produced by these dietary changes could also inhibit the disposal of BCAA.

When oral protein was given at various times after elective surgery, an increase in BCAA occurred which was greatest in the early postoperative days when dietary intake was at its minimum (Elia *et al.* 1979). This observation suggested that the uptake of exogenous BCAA by the peripheral tissues (mainly muscle) was delayed

after surgery, and the situation appeared to be similar to that produced by starvation.

Further studies, involving the measurement of the rate of disposal of an intravenous leucine load (3.8 g) in adequately-fed, postoperative patients, produced somewhat different results (Table 2) (Elia *et al.* 1980); whilst in normal healthy subjects starvation for 72 h prolonged the half-life of infused leucine (in agreement with the previous observations of Sherwin, 1978), total hip replacement did not and, after accidental injury, infused leucine was disposed of more rapidly than normal. The extent to which such observations explain the changes in endogenous leucine concentration is not clear since it is likely that an exogenous load of leucine produces its own unphysiological effects; nevertheless, the fact that exogenous leucine is dealt with differently in the starved than in the post-traumatic subject does suggest that the cause of the increase in basal leucine in the two conditions is probably different.

Administration of leucine produces a number of metabolic effects. Hypoglycaemia normally occurs, partly due to an increase in insulin secretion, and there is an increase in the blood ketone body and glutamine concentrations. In addition, Sherwin (1978) showed that after prolonged fasting there was an improvement in N balance together with an unchanged excretion of 3-methyl-histidine which suggested a stimulation of (muscle) protein synthesis (see below).

The study of Elia *et al.* (1980) dealt with a number of other clinical conditions and showed the following: the blood concentration of BCAA was increased in diabetes, unchanged in muscular dystrophy and decreased in cirrhosis; the clearance rate of infused leucine was reduced in diabetes and muscular dystrophy and increased in cirrhosis; infusion with 'Intralipid®', which increased circulating free fatty acid and ketone body concentrations (as in starvation, carbohydrate restriction and diabetes) had no effect on the removal of a leucine load; the effects of infused leucine on blood glucose and ketone body concentrations varied with the

Table 2. *The rate of removal of intravenous leucine in man (modified from Elia et al. 1980)*

Subjects	Basal concentration of leucine ($\mu\text{mol/l}$)	Half-life of infused leucine (min)
Starvation		
Before	140	27.8
Day 4	264	55.5
Refed	134	31.8
Surgery		
Before	107	28.3
Day 4	143	26.3
Day 8	129	29.0
Severe accidental injury		
Day 4	168	22.6
Insulin deficient		
diabetes mellitus	162	42.4
Normal	120	28.1

groups studied, so that leucine infusion did not produce the normal reduction in blood sugar in subjects who had been starved for 3 d, and the increment in ketone body concentration produced by leucine was several-fold greater in diabetic than in normal subjects.

To investigate further the effects of BCAA, measurements have been made of amino acid exchange across the human forearm after giving oral steak or intravenous leucine. There are two recent complementary studies on this. Aoki *et al.* (1981) measured bilateral forearm flux after oral leucine and Elia & Livesey (1981, 1983) measured changes in plasma concentrations after oral steak or intravenous leucine.

In the first study (Aoki *et al.* 1981), a bolus (14.7 g) of oral leucine was given to six normal, post-absorptive individuals. The arterio-venous (A-V) differences in metabolic flux were measured in both forearms and the dominant arm was also lightly exercised for 15 min. After oral leucine, large uptakes of leucine were detected across both forearm muscle beds. Glutamine outflow from the dominant forearm increased three times after ingestion of leucine and eight times after exercise, and smaller increments occurred for alanine. Similar but less marked changes were seen in the opposite forearm. It was concluded that the main way in

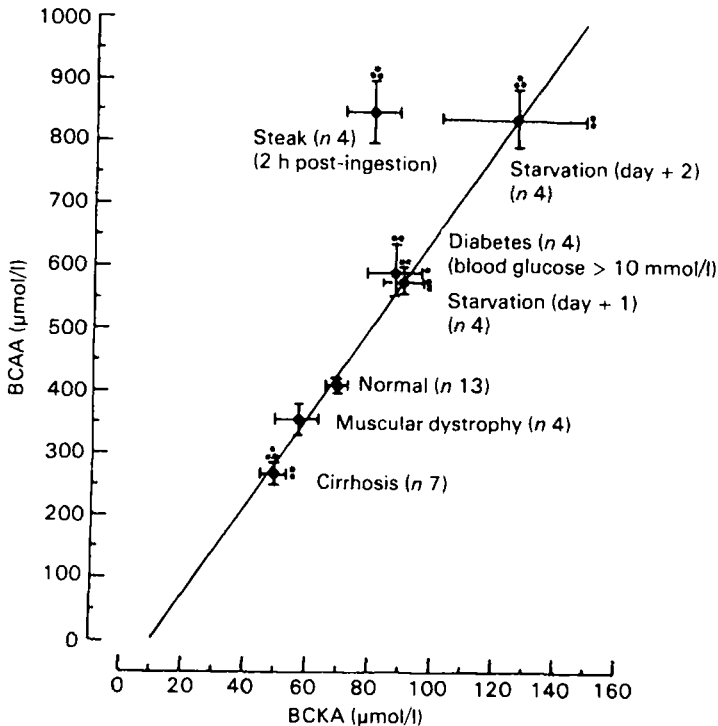


Fig. 2. Effects of various clinical conditions on the plasma concentrations of branched-chain amino acids (BCAA) and branched-chain keto acids (BCKA). Values ($\mu\text{mol/l}$) are mean concentrations with SEM; vertical bars refer to the BCAA and horizontal bars to the BCKA. The coefficient of correlation, r , = 0.989. Values significantly different from normal: • P <0.05, •• P <0.01, ••• P <0.001 (after Elia & Livesey, 1983).

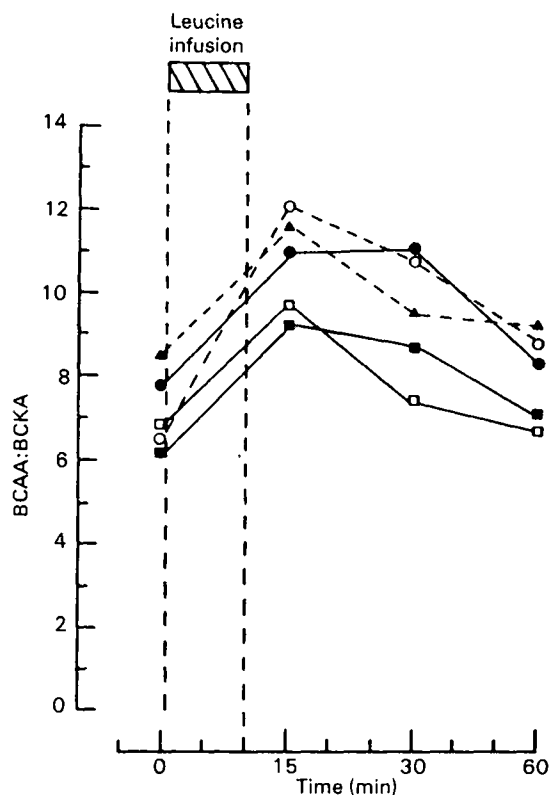


Fig. 3. Effects of leucine infusion on the ratio of plasma concentrations of branched-chain amino acids (BCAA): branched-chain 2-keto acids (BCKA) in various clinical conditions ($n = 4$ for each condition): ●, normal; ○, diabetic; ■, muscular dystrophy; □, cirrhosis; ▲, starvation (4th day) (after Elia & Livesey, 1983).

which excess N is released from muscle after leucine ingestion and light exercise is by the formation of glutamine. An unexpected observation was that the ingestion of leucine in these normal subjects resulted in a large net N loss from both forearms, possibly due to a stimulation of proteolysis in muscle, despite observed increases in circulating insulin. It is possible that leucine displaces free amino acids from the intracellular pool.

These investigators did not measure the BCKA concentrations, a deficiency made up for by the work of Elia & Livesey (1983) who studied the effects of ingested steak and of intravenous leucine (3.8 g) on human forelimb metabolism of four fasted men. They also compared the basal concentrations of BCAA and BCKA in patients with the clinical conditions which they had previously studied with intravenous leucine loads. They found that the ratio between basal BCAA and BCKA was constant in a number of clinical conditions (Fig. 2) and that it increased after leucine or protein administration (Fig. 3). In the fasted normal subjects the mean basal A-V difference in concentrations of BCKA was small ($-3.6 \mu\text{mol/l}$) and this increased very little after leucine or steak administration,

despite large increases in BCAA uptake. This observation suggested that in man BCKA was preferentially oxidized rather than released from peripheral tissues. This contrasts with the situation in rat tissue which shows a large negative A-V difference of BCKA across the hind-limb in the basal state and a dramatic increase in the release of BCKA after a leucine infusion which achieves a similar positive A-V difference for BCAA as in man (Elia & Livesey, 1981). These differences may partly relate to the distribution of BCKA dehydrogenase since in man, 60% of the total body BCKA dehydrogenase is found in muscle compared with only 30% in the rat (Khatra *et al.* 1977). The distribution of BCAA transaminases is similar in rat and man (Shinnick & Harper, 1976; Goto *et al.* 1977). When the subjects were

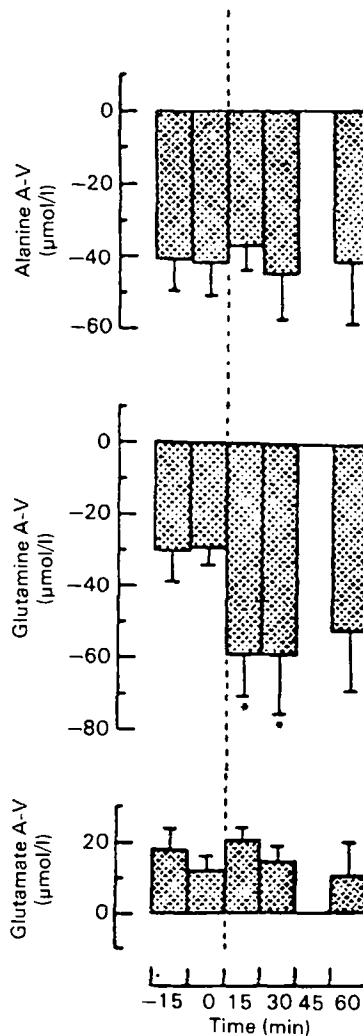


Fig. 4. Effect of leucine (3.8 g) infusion on the release of alanine and glutamine and uptake of glutamate by forearm muscle (pre-infusion values appear on the left of the broken horizontal line, post-infusion values on the right). A-V, arterio-deep venous concentration difference. Values are means \pm 1 SEM for four subjects. Values significantly different from the mean of two pre-infusion basal measurements: * $P < 0.05$.

infused with leucine alone, the negative A-V difference for glutamine promptly increased but that for alanine did not (Fig. 4).

After ingestion of steak, the large uptake of BCAA by human forearm tissues did not alter the negative A-V difference for glutamine but decreased the release of other amino acids including alanine (and of pyruvate which is in a near equilibrium state with alanine). By 2 h after steak ingestion BCAA accounted for 50% of the positive A-V difference and glutamine up to 75% of the negative A-V difference (Fig. 5). These authors (Elia & Livesey, 1983) concluded that glutamine, not

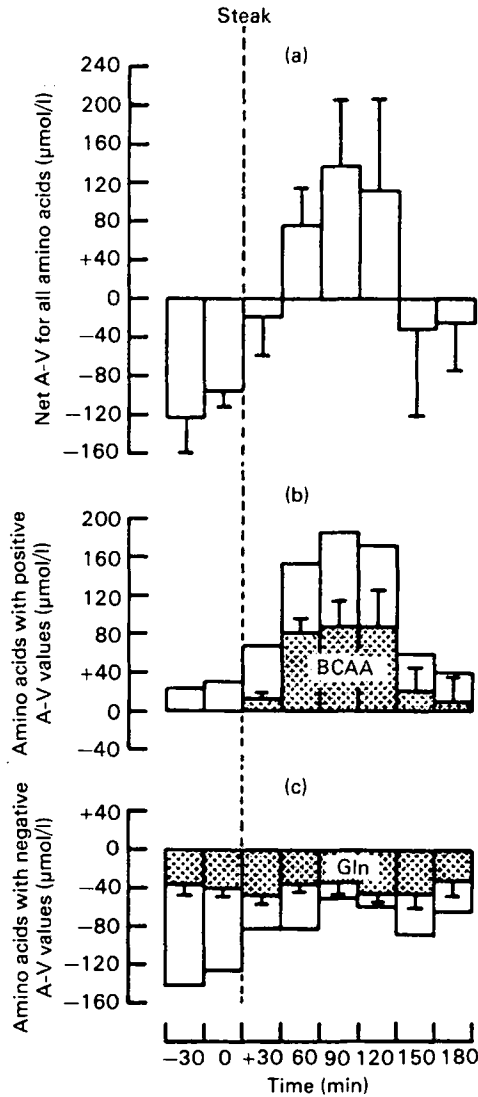


Fig. 5. Effect of steak ingestion (250 g raw weight) on amino acid exchange across the forearm. (a) Net arterio-venous (A-V) difference of all amino acids. (b) Sum of A-V values for those amino acids with positive A-V values. (c) Sum of A-V values for those with negative A-V values. Contributions made by branched-chain amino acids (BCAA) and glutamine (Gln) are indicated by the shaded areas ($n = 4$) (after Elia & Livesey, 1983).

alanine, is the major amino-group carrier leaving the forearm after leucine administration (Fig. 4) (in agreement with Aoki *et al.* 1981) and after a protein meal (Fig. 5).

Because of the strong correlation between BCAA and BCKA in fasted, rested subjects with a variety of conditions, it seems likely that the concentration of these metabolites is closely linked by equilibrium reactions catalyzed by the widely distributed BCAA transaminases. Since the ratio of BCAA:BCKA increases similarly after leucine infusion in conditions associated with widely varying rates of leucine removal (Fig. 3), it also seems unlikely that the observed changes in the rate of disposal are due to defects in cellular uptake or transamination of BCAA; possibly they reflect changes in the permeability of the mitochondrial membrane to BCKA or alterations in the phosphorylation of the BCKA dehydrogenase complex (Randle *et al.* 1981). It is of interest that the distribution of BCAA and BCKA between plasma and red cells differs between rat and man (Elia & Livesey, 1983).

BCAA and protein turnover

It has become widely accepted that BCAA, and leucine in particular, can directly stimulate protein synthesis. This conclusion has been derived largely from work on the perfused or isolated tissues of the rat, and there are few relevant direct measurements in man. Whilst most evidence continues to suggest that the BCAA have an effect on protein synthesis (and N balance) not shared by other amino acids, this conclusion is not unanimous.

In rats. In rats it can be shown that protein synthesis of the incubated diaphragm, perfused hemicorpus (Li & Jefferson, 1978) and perfused heart (Chua *et al.* 1980) responds rapidly to leucine. However, McNurlan *et al.* (1982) were recently unable to demonstrate any stimulation of protein synthesis by leucine alone *in vivo* in fed or starved rats or in those deprived of dietary protein. This lack of effect was shown both in fed and catabolic rats and in groups with variable initial concentrations of BCAA and insulin. McNurlan *et al.* (1982) point out that since the reported positive effects of leucine have been observed in tissues in catabolic states (either *in vitro*, where conditions are generally catabolic, or *in vivo* on stressed or starved animals or man), they particularly chose to study animals during starvation or protein depletion, conditions both characterized by loss of body N and low rates of tissue protein synthesis. The circulating BCAA concentrations in these two groups of animals were different and there was no correlation between them and the rates of protein synthesis in any tissues. This confirms previous findings (Millward *et al.* 1976) and is also suggested by Table 1. It is also clear from the analysis of McNurlan *et al.* (1982) that the choice of method used to estimate the rate of protein synthesis, both in the whole animal and in the tissues, is important; these authors found that a large dose of [³H]phenylalanine, rather than a continuous infusion of [¹⁴C]tyrosine, gave results for tissues which were easier to interpret.

Such criticisms raise considerable doubt about the ability of leucine to increase protein synthesis in catabolic states. They also emphasize the importance of the

nutritional status of the subject (rat or man) being investigated. It has been pointed out elsewhere that the apparent reduction in protein synthesis following elective surgery may be as much related to the temporary reduction in nutritional intake as to the stress of the operation (Clague, 1981).

The effects of giving various percentages of BCAA in isonitrogenous amino acid regimens to a fractured, septic rat model have been studied by Blackburn *et al.* (1979, 1981) and several others (e.g. Freund *et al.* 1982). Amino acid mixtures are (as would be expected) more effective than glucose alone, and where there is a difference the most effective N sparing appears to be obtained by solutions containing 50% BCAA rather than 15 or 100%.

In man. Sherwin (1978) showed that intravenous leucine reduced N loss in the starved human, but Aoki *et al.* (1981) found that it induced N loss from human forearm muscles. In a preliminary study in seven patients undergoing major abdominal surgery, Blackburn *et al.* (1981) found (as in the rat) that mixtures containing 50% BCAA appeared to produce the most marked N sparing. Freund *et al.* (1981) looked at thirty-five adults undergoing operations of moderate severity, and could find no significant difference in the effects on N balance of amino acid mixtures containing from 22 to 100% BCAA. In a different approach we have given 3-d infusions of leucine to patients after total hip replacement who continued on a constant intake of energy and N; we found that in comparison with similar patients on the same diet not given leucine, there was no significant improvement in postoperative N balance (Fig. 6). Since leucine may produce its N-sparing effect best in the most catabolic states, these patients who were fed throughout may not be ideal subjects to demonstrate this effect. A more practical problem is that the variation in N loss from one postoperative patient to another is normally so great that it would require large numbers of patients to demonstrate a significant reduction in N balance. However, Cerra *et al.* (1982) have shown a significant decrease in N loss on the 3rd postoperative day, but not on day 0 or day 6, in a group of fifteen patients given amino acid solutions containing 50% BCAA compared with fifteen given 15.5% BCAA. In contrast Schmitz *et al.* (1982), who studied thirty patients in an intensive care unit for 5 d, found a significantly worse N balance in those given an amino acid solution containing 45% rather than 10% BCAA. Similarly Gouin *et al.* (1982), in a preliminary study of eighteen patients undergoing major abdominal surgery, found that the cumulative N loss was greater in those receiving a solution containing 36% BCAA in comparison with 21% BCAA.

Conclusions

The BCAA provide much biochemical interest because of their special metabolic pathways and their apparently unusual biochemical functions and effects. Of necessity many studies on their physiology and tissue distribution have been done in animals, and there seem to be real differences between the handling of BCAA by the rat and man. In man, most investigators have been concerned with the increase in the circulating concentration of BCAA in states of catabolism and the possible

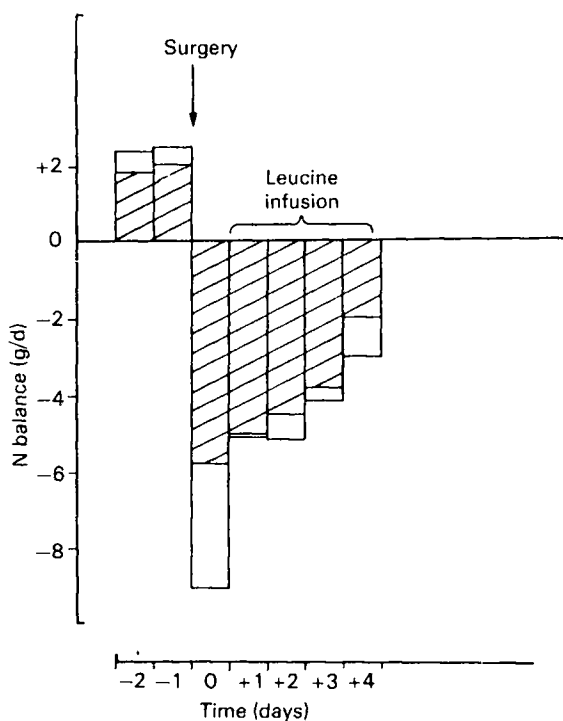


Fig. 6. The effect of continuous intravenous leucine (0.15 g/kg body-weight per d) on nitrogen loss in patients maintained on a constant N intake after total hip replacement. The shaded area represents the mean N loss in a group of similar subjects after hip replacement not given intravenous leucine. For clarity the values for SEM are omitted. There was no significant difference between the values in the two groups.

effects of BCAA, especially leucine, on protein synthesis. Present results suggest that the BCAA concentration is increased for different reasons after starvation and accidental injury. Since there appears to be no delay in the peripheral disposal of infused leucine after injury and since both intracellular and extracellular concentrations of leucine are increased at this time, it seems illogical to give further BCAA to try to augment muscle protein synthesis. Table 1 emphasizes that in a variety of conditions there is no relationship between circulating BCAA concentration and rates of protein synthesis or breakdown. The rate of protein synthesis is important in maintaining muscle mass in a variety of clinical states (Smith & Williamson, 1983) but in severe injury, where the extent of N loss is considerable, increased proteolysis may be equally important. In such states, possible inhibition of the newly described mediators of proteolysis in fever and injury (Beisel, 1983) may turn out to be a much more important approach therapeutically than administration of BCAA in post-traumatic muscle wasting.

There remain two reasons which encourage the administration of amino acid solutions enriched with BCAA in catabolic states. The first stems from the idea that the amino acid composition of solutions infused by parenteral nutrition should

be similar to that in blood leaving the liver, which has a greater proportion of BCAA than is present in ingested protein; and the second is that the apparent effects of BCAA on protein synthesis *in vitro* might occur *in vivo*. There are several possible explanations for the conflicting results obtained in injured man. One is the individual biochemical response to trauma, which depends on the age, sex, and nutritional state of the patient, the severity of the injury and the coexistence of immobility and infection (Elia, 1982). Another is the different ways in which the supplemental BCAA has been given, either in addition to the previous N intake (in which case it is difficult to know whether to apportion an improved N balance to the increased N intake or to the added BCAA (Sherwin, 1978)) or as part of an isonitrogenous regimen, which must imply a decrease in the intake of other amino acids. In the latter instance the decrease in non-BCAA has not always been uniform and some have been increased (Freund *et al.* 1978, 1979), even to a considerable extent (see Hartig *et al.* (1982) on experimental injury in pigs). The infusion of BCAA as the sole source of N is nutritionally unsound since other amino acids are necessary if protein synthesis is to occur; whether lower concentrations of BCAA can produce a useful improvement in N balance in catabolic states remains in doubt and will be difficult to demonstrate, especially in states of rapidly changing N intake.

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