

## Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on plasma amino- and keto-acid concentrations and branched-chain keto-acid dehydrogenase activity

Stefan Langer\*, Peter W. D. Scislawski, David S. Brown, Peter Dewey and Malcolm F. Fuller†  
Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

(Received 2 June 1999 – Accepted 22 June 1999)

The present experiment was designed to elucidate the mechanism of the methionine-sparing effect of excess branched-chain amino acids (BCAA) reported in the previous paper (Langer & Fuller, 2000). Twelve growing gilts (30–35 kg) were prepared with arterial catheters. After recovery, they received for 7 d a semipurified diet with a balanced amino acid pattern. On the 7th day blood samples were taken before (16 h postabsorptive) and after the morning meal (4 h postprandial). The animals were then divided into three groups and received for a further 7 d a methionine-limiting diet (80% of requirement) (1) without any amino acid excess; (2) with excess leucine (50% over requirement); or (3) with excesses of all three BCAA (leucine, isoleucine, valine, each 50% over the requirement). On the 7th day blood samples were taken as in the first period, after which the animals were killed and liver and muscle samples taken. Plasma amino acid and branched-chain keto acid (BCKA) concentrations in the blood and branched-chain keto-acid dehydrogenase (BCKDH; EC 1.2.4.4) activity in liver and muscle homogenates were determined. Compared with those on the balanced diet, pigs fed on methionine-limiting diets had significantly lower ( $P < 0.05$ ) plasma methionine concentrations in the postprandial but not in the post-absorptive state. There was no effect of either leucine or a mixture of all three BCAA fed in excess on plasma methionine concentrations. Excess dietary leucine reduced ( $P < 0.05$ ) the plasma concentrations of isoleucine and valine in both the postprandial and postabsorptive states. Plasma concentrations of the BCKA reflected the changes in the corresponding amino acids. Basal BCKDH activity in the liver and total BCKDH activity in the *biceps femoris* muscle were significantly ( $P < 0.05$ ) increased by excesses of leucine or all BCAA.

### Branched-chain amino acids: Methionine: Amino acid utilization

Interactions between leucine, isoleucine and valine in growing pigs have been reported by numerous authors (Oestemer *et al.* 1973; Henry *et al.* 1976; Taylor *et al.* 1984) who showed that even moderate dietary excesses of leucine (i.e.  $>3$  g/kg) reduced isoleucine and valine concentrations in plasma. Much larger quantities of leucine were required to reduce growth performance (Taylor *et al.* 1984). Changes in plasma and tissue branched-chain amino acid (BCAA) concentrations of rats (Rogers *et al.* 1962; Tannous *et al.* 1966), poultry (D'Mello & Lewis, 1970; Smith & Austic, 1978), kittens (Hargrove *et al.* 1988), lambs (Papet *et al.* 1988) and human subjects (Synderman *et al.* 1959; Nair *et al.* 1992) receiving leucine in excess were similar to the changes reported in pigs (Oestemer *et al.* 1973; Henry *et al.*

1976; Taylor *et al.* 1984) indicating a common regulatory mechanism in these species. All three BCAA share a common enzyme complex, the branched-chain keto-acid dehydrogenase (EC 1.2.4.4; BCKDH) complex, in their degradative pathways. Activation of BCKDH by dietary leucine excess increased the oxidation of isoleucine and valine in rats and chicks (Calvert *et al.* 1982; Block & Harper, 1984) and this has been proposed as the reason for the altered BCAA concentrations (Harper *et al.* 1984; Block, 1989).

Methionine is catabolized not only via the trans-sulfuration pathway but also by a transamination pathway (Case & Benevenga, 1976; Benevenga, 1984), by which methionine is transaminated to  $\alpha$ -keto- $\gamma$ -methylbutyric

**Abbreviations:** BCAA, branched-chain amino acids; BCKA, branched-chain keto acids; BCKDH, branched-chain keto-acid dehydrogenase; KIC,  $\alpha$ -ketoisocaproic acid; KIV,  $\alpha$ -ketoisovaleric acid; KMTB,  $\alpha$ -keto- $\gamma$ -methylbutyric acid; KMV,  $\alpha$ -keto- $\beta$ -methyl-n-valeric acid.

\* **Present address:** Agribrands Europe Espana, Aribau 197-199, escalera 1, Planta 3, puertas 1 y 2, 08021 Barcelona, Spain.

† **Corresponding author:** Dr Malcolm Fuller, fax +44 (0)1224 716687, email aw@rri.sari.ac.uk

acid (KMTB) followed by an irreversible decarboxylation to 3-methylthiopropionic acid and CO<sub>2</sub> (Mitchell & Benevenga, 1978). The decarboxylation reaction is also catalysed by BCKDH which raises the possibility that the interactions amongst the BCAA may extend to the metabolism of methionine.

Another connection between the BCAA and methionine is their transport via the L-system (Oxender & Christensen, 1963; Baumrucker, 1985; Guidotti & Gazzola, 1992) which may involve direct competition for uptake into tissues, especially the brain (Eriksson *et al.* 1981; Wahren *et al.* 1981). Changes in brain amino acid concentrations appear to regulate food intake (Peng *et al.* 1973; Harper & Peters, 1983; Tackman *et al.* 1990).

Furthermore, leucine has been proposed as a specific regulator of protein metabolism and effects on protein synthesis and degradation *in vitro* have been observed in a number of experiments (Buse & Reid, 1975; Li & Jefferson, 1978; Goldberg & Tischler, 1981; Mitch & Clark, 1984). This could affect amino acid utilization and consequently animal performance as well as amino- and keto-acid concentrations in plasma and tissues.

However, the response of growing pigs to leucine or BCAA appears to depend on the limiting amino acid. Langer & Fuller (2000) reported a reduced protein utilization (measured as N retention) in growing pigs fed on isoleucine-limiting diets after increasing the leucine concentration from 7.2 g/kg to 10.8 g/kg. In contrast, protein utilization was improved rather than reduced by additions of leucine or a mixture of BCAA to a methionine-limiting diet. In order to explore the mechanism underlying these observations the following experiment was carried out.

## Materials and methods

### Animals

Twelve growing gilts (Cotswold crossbred) with an average initial weight of 30–35 kg were used in the experiment. About 2 weeks before the experiment all animals were prepared with two blood sampling catheters (PVC – NT2; 0.99 mm internal and 1.60 mm external diameter, inserted length 275 mm; Portex Ltd, Hyde, Kent, UK) which were inserted into the lumbar aorta, via the medial saphenous arteries of the hindlegs, as described by Fuller *et al.* (1977), except that Fluothane® (Pitman-Moore, Crewe, Cheshire, UK) anaesthesia was used. Animals were housed in open pens for about 1 week after surgery; after that they were kept in individual metabolism cages in a temperature-controlled room (22–24°) where they were allowed a second week of recovery, and where they remained for the duration of the experiment. All management and experimental procedures in this study were carried out in strict accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 by staff licensed under this Act to carry out such procedures.

### Diets and experimental design

The isoenergetic and isonitrogenous diets based on casein and synthetic amino acids were identical to the diets

described in the previous paper (Langer & Fuller, 2000): there was one diet with an 'ideally' balanced amino acid pattern according to Wang & Fuller (1989) (control; no limiting amino acid, no excess) and three methionine-deficient diets (80 % of requirement). The methionine-deficient diets contained either no excess, excess leucine (50 % over requirement) or excesses of all three BCAA (each 50 % over requirement). Leucine, isoleucine, valine and methionine were added or removed isonitrogenously at the expense of aspartic acid and monosodium glutamate. Before the start of the experiment the animals were adapted to a semipurified low-protein (100 g crude protein/kg) diet for 7 d. During the experiment feed was given twice daily (at 07.00 and 15.00 hours) at a rate of 80 g/kg body weight<sup>0.75</sup>. The animals consumed all the feed given, usually within 15 min. The planned daily N intake on all diets was 1.25 g/kg body weight<sup>0.75</sup>. Water was available *ad libitum* from nipple drinkers and also added to the diets during feeding to reduce spillage.

The experiment consisted of two 7 d periods. In the first period the animals received a diet with the 'ideally' balanced amino acid pattern. On day 7 blood samples were taken before (16 h postabsorptive, before morning feeding) and after the morning feeding (4 h postprandial). In the second period the twelve pigs were randomly divided into three groups and received either a methionine-limiting diet without any amino acid excess, or the same diet with excess leucine or with excesses of all three BCAA. Blood samples were taken as in the first period. After the postprandial blood samples had been taken a lethal dose of Euthesate® (pentobarbitone sodium Ph Eur 200 mg/ml; Willows Francis Veterinary, Crawley, Sussex, UK) was given and samples of liver, biceps femoris and adductor muscle were taken immediately.

### Sample collection, storage and chemical analysis

**Diets.** Diets were analysed for crude protein (N × 6.25) by the macro Kjeldahl procedure (Davidson *et al.* 1970) and for amino acid concentrations (except methionine, cyst(e)ine and tryptophan) by ion-exchange chromatography. Methionine and cyst(e)ine concentrations were determined after oxidation with performic acid as described by Bech-Andersen *et al.* (1990) and tryptophan was measured according to Naumann & Bassler (1993).

**Plasma.** After collection in prechilled heparinized tubes the blood samples (10 ml) were kept on ice for not more than 2 h before separating the plasma by centrifugation (2800 g, 15 min, 4°). The plasma was divided into portions of 0.8 ml, to which 0.1 ml 200 μM-norleucine standard with 1.0 mM-dithiothreitol and 0.1 ml α-ketocaproic acid standard were added. Samples and standards were mixed using a vortex mixer and stored in Eppendorf tubes at –70° until required for analysis. For the determination of amino and keto acids plasma was deproteinized with 0.2 ml ice-cold 5-sulfo-salicylic acid (1.9 M), vortex mixed and centrifuged at 15 000 g for 15 min at 4°. An ion-exchange column (Dowex 50W (X8), H<sup>+</sup> form) was used to separate amino and keto acids. Keto acids were eluted with 2 ml distilled water added slowly to the column. The column was then washed twice with 2 ml distilled water before eluting the amino acids

twice with 2 ml 4 M-NH<sub>4</sub>OH and 1 ml water. The eluate was freeze-dried twice to remove the NH<sub>3</sub> and redissolved in citrate buffer pH 2.2 before amino acid analysis by ion-exchange chromatography using a Pharmacia Alpha-Plus analyser with a Li-form high performance 202 × 4.6 mm column (Pharmacia, Cambridge, Cambs., UK). Keto acids were measured as their dimethylsilylated quinoxalinol derivatives by GC with *o*-phenylenediamine and *N*-(tert-butyl-dimethylsilyl)-*N*-methyltrifluoroacetamide as derivatives using a Hewlett-Packard model 5890A analyser with flame-ionization detector and a Chrompack SIL-5 column (25 m × 0.25 mm) (Packard Instruments, Downers Grove, IL, USA).

**Tissue samples.** Liver and muscle samples were removed as quickly as possible, frozen with Wollenberg tongs cooled in liquid N<sub>2</sub>, immersed in liquid N<sub>2</sub> for 2–3 h and then stored at –70° until further analysis. BCKDH activity in sample homogenates was measured as <sup>14</sup>CO<sub>2</sub> released from α-keto[carboxyl-<sup>14</sup>C]isovaleric acid ([<sup>14</sup>C]KIV) or L-[carboxyl-<sup>14</sup>C]valine (L-[<sup>14</sup>C]valine). L-[<sup>14</sup>C]valine was purchased from American Radiolabeled Chemicals Ltd (Tocris-Cookson, Bristol, UK). [<sup>14</sup>C]KIV was produced from the radiolabelled amino acid according to the method described by Rüdiger *et al.* (1972). The yield was approximately 84% keto acid with a purity of about 97.8% (checked by TLC). The activation state of the enzyme was measured as the ratio basal activity:total activity. The basal activity was determined in the presence of phosphatase and kinase inhibitors, 100 mM-KF1 and 10 mM-dichloroacetic acid respectively. Total activity was estimated after preincubation of the enzyme with Mg<sup>2+</sup>. The homogenization buffer (pH 7.4 at 4°) contained: 2 ml/l Triton X-100, 70 mM-sucrose, 200 mM-D-mannitol, 10 mM-HEPES and 0.4 mM-ethylene glycol bis(β-aminoethyl ether)*N,N'*-tetraacetic acid (EGTA). The radioactive cofactor solution (pH 7.4 at 37°) contained: 4 mM-NAD<sup>+</sup>, 0.8 mM-CoA-lithium salt, 0.8 mM-thiamine pyrophosphate, 4 mM-dithiothreitol, 10 mM-HEPES, 4 mM-EGTA, 10 mM-sodium carbonate, KIV and [<sup>14</sup>C]KIV. Frozen liver samples were quickly weighed and a 75 g/l homogenate was prepared using a Polytron homogenizer (15 s at setting 3; Kinematica AG, Lucerne, Switzerland). Muscle samples were crushed and weighed before preparing a 160 g/l homogenate with a Polytron homogenizer (30 s at setting 3). Homogenates were centrifuged for 5 min at 600 g for liver and 800 g for muscle. To remove larger particles the supernatant fraction was filtered through gauze. BCKDH assays were optimized for each tissue. Until incubation the homogenates were stored and processed on ice. Total enzyme activity was measured after preincubation at 37° with 5 mM-MgSO<sub>4</sub> for 20 min (liver) or 75 min (muscle). The incubations were carried out in sealed 25 ml Pyrex vials with a hanging centre well containing 0.3 ml phenylethylamine-methanol (1:1, v/v) to trap the released <sup>14</sup>CO<sub>2</sub>. The reaction was started by adding 0.6 ml homogenate to 0.6 ml cofactor solution. For liver BCKDH activity about 60 000 disintegrations/min (dpm) and 1 mM-KIV and for muscle BCKDH activity about 50 000 dpm and 2 mM-KIV were used per incubation. For basal and total activity liver was incubated for 4 min and muscle for 10 min. The reaction was stopped with 0.3 ml 1 M-HClO<sub>4</sub>. Tissue homogenates treated

with 1 M-HClO<sub>4</sub> before the incubation served as blanks for background. CO<sub>2</sub> was trapped for 2 h. Centre wells were placed in scintillation vials with 5 ml Ultima Gold® scintillation fluid (Hewlett-Packard) and the radioactivity was counted using a Hewlett-Packard Tri-carb 1900CA liquid scintillation analyser.

For the indirect determination of basal BCKDH activity in adductor muscle samples the method used for muscle samples was modified. The cofactor solution contained instead of [<sup>14</sup>C]KIV, L-[<sup>14</sup>C]valine (150 000 dpm/incubation), 0.2 mM-unlabelled KIV, 1 mM-L-valine and 100 μM-pyridoxal 5' phosphate. L-[<sup>14</sup>C]valine was more resistant to chemical decarboxylation than [<sup>14</sup>C]KIV which led to a reduced background and improved the sensitivity. The method is based on the assumption that in muscle, transamination is not a limiting factor in BCAA catabolism. However, the method only gives information about the valine oxidative flux and the percentage of enzyme that is active and does not allow conclusions to be drawn about absolute activities. Nevertheless, knowing the total activity measured with [<sup>14</sup>C]KIV and the percentage of active enzyme it is possible to predict the basal activity indirectly.

Protein in tissue samples was assayed by the biuret method using the Total Protein Reagent (Sigma Chemical Company Ltd, Poole, Dorset, UK).

#### Statistical analysis

Data were analysed using GENSTAT 5.2 (Rothamsted Experimental Station, Harpenden, Herts., UK). Plasma amino- and keto-acid concentrations were analysed by ANOVA using a split-plot analysis for a 3 × 2 factorial design. The three-level factor defining treatment group determined main plots and the two level factor (control, treatment) the sub-plot factor. Each treatment mean was compared against its own control and treatment differences were assessed by (t<sub>1</sub> – c<sub>1</sub>) – (t<sub>2</sub> – c<sub>2</sub>), i.e. by difference between changes. From the analysis of previous experiments of similar design (e.g. Wang & Fuller, 1989) the effect of time (i.e. period) was considered negligible. BCKDH activities in liver and muscle samples, as well as flux rates in adductor muscle, were analysed by ANOVA using a simple block design.

## Results

### Postprandial plasma amino acid concentrations

Reducing dietary methionine by 20% caused a 55% reduction in plasma methionine concentration (Table 1). Plasma concentrations of both threonine and valine increased significantly in animals given the methionine-deficient diet, compared with those given the balanced control diet.

The greatest changes in plasma amino acid concentrations were caused by excesses of leucine or BCAA in the methionine-deficient diets. A 50% excess of leucine increased plasma leucine concentration by about 60%; at the same time isoleucine and valine concentrations both dropped by more than 35% when compared with the

**Table 1.** Postprandial and postabsorptive plasma amino acid concentrations in pigs fed on a control diet with a balanced amino acid pattern and on a methionine-deficient diet with no excess amino acids, excess leucine or an excess of the branched-chain amino acids (BCAA) for 7 d\* (Values are means for four pigs)

|                              | Amino acid concentration ( $\mu\text{mol/l}$ ) |                   |                  |                  |                  |                  |          |            |
|------------------------------|--|-------------------|------------------|------------------|------------------|------------------|----------|------------|
|                              | No excess                                      |                   | Leucine excess   |                  | BCAA excess      |                  | SED      |            |
|                              | Control  | Treatment         | Control          | Treatment        | Control          | Treatment        | Control† | Treatment‡ |
| <b>Postprandial (4 h)</b>    |  |                   |                  |                  |                  |                  |          |            |
| Valine                       | 278 <sup>a</sup>                               | 355 <sup>b</sup>  | 289 <sup>a</sup> | 227 <sup>c</sup> | 286 <sup>a</sup> | 530 <sup>d</sup> | 31.0     | 43.8       |
| Cyst(e)ine                   | 84   | 91                | 88               | 79               | 85               | 77               | 9.6      | 13.5       |
| Methionine                   | 41 <sup>a</sup>                                | 18 <sup>b</sup>   | 45 <sup>a</sup>  | 13 <sup>b</sup>  | 42 <sup>a</sup>  | 23 <sup>b</sup>  | 5.9      | 8.3        |
| Isoleucine                   | 94 <sup>a</sup>                                | 120 <sup>a</sup>  | 82 <sup>ab</sup> | 73 <sup>b</sup>  | 97 <sup>a</sup>  | 222 <sup>c</sup> | 13.3     | 18.8       |
| Leucine                      | 213 <sup>a</sup>                               | 216 <sup>a</sup>  | 188 <sup>a</sup> | 345 <sup>b</sup> | 207 <sup>a</sup> | 369 <sup>b</sup> | 27.3     | 38.6       |
| Threonine                    | 182 <sup>a</sup>                               | 260 <sup>b</sup>  | 175 <sup>a</sup> | 241 <sup>b</sup> | 176 <sup>a</sup> | 257 <sup>b</sup> | 21.9     | 31.0       |
| <b>Postabsorptive (16 h)</b> |  |                   |                  |                  |                  |                  |          |            |
| Valine                       | 249 <sup>a</sup>                               | 307 <sup>b</sup>  | 230 <sup>a</sup> | 161 <sup>c</sup> | 244 <sup>a</sup> | 368 <sup>d</sup> | 14.0     | 19.8       |
| Cyst(e)ine                   | 86   | 76                | 78               | 75               | 93               | 69               | 20.4     | 28.8       |
| Methionine                   | 19   | 19                | 16               | 19               | 20               | 22               | 4.4      | 6.2        |
| Isoleucine                   | 79 <sup>a</sup>                                | 104 <sup>b</sup>  | 62 <sup>a</sup>  | 67 <sup>a</sup>  | 74 <sup>a</sup>  | 128 <sup>b</sup> | 9.9      | 14.0       |
| Leucine                      | 154 <sup>a</sup>                               | 168 <sup>ab</sup> | 141 <sup>a</sup> | 180 <sup>b</sup> | 151 <sup>a</sup> | 185 <sup>b</sup> | 7.3      | 10.3       |
| Threonine                    | 99 <sup>a</sup>                                | 165 <sup>b</sup>  | 85 <sup>a</sup>  | 149 <sup>b</sup> | 88 <sup>a</sup>  | 147 <sup>b</sup> | 17.7     | 25.1       |

<sup>a,b,c,d</sup> Mean values within a row not sharing a common superscript letter indicate significant ( $P < 0.05$ ) differences between a treatment and its own control, or between two treatments.

\* For details of diets and procedures, see pp. 50–51.

† For comparison of group mean against its own control.

‡ For comparison of treatment means against each other.

methionine-deficient diet without excess. Plasma concentrations of valine and isoleucine were significantly higher on the BCAA-supplemented diet. Neither methionine nor cyst(e)ine concentrations in the plasma of pigs given the methionine-deficient diets were altered by excesses of leucine or of all BCAA.

#### Postabsorptive plasma amino acid concentrations

In general plasma amino acid concentrations were lower in the postabsorptive than in the postprandial state (Table 1).

Threonine, isoleucine and valine concentrations were significantly increased by methionine deficiency compared with the control diet. Although plasma leucine concentrations were similar in all three methionine-deficient treatment groups, isoleucine and valine concentrations were significantly reduced by leucine excess. Whereas plasma isoleucine was reduced by approximately 35%, as it was in the postabsorptive state, valine decreased rather more, by nearly 50%. Addition of BCAA significantly increased the plasma concentration of valine but the increase in isoleucine was not significant.

**Table 2.** Postprandial and postabsorptive plasma keto acid concentrations in pigs fed on a control diet with a balanced amino acid pattern and on a methionine-deficient diet with no excess amino acids, excess leucine, or an excess of the branched-chain amino acids (BCAA) for 7 d\* (Values are means for four pigs)

|                              | Keto acid concentration ( $\mu\text{mol/l}$ ) |                  |                  |                  |                 |                  |          |            |
|------------------------------|---|------------------|------------------|------------------|-----------------|------------------|----------|------------|
|                              | No excess                                     |                  | Leucine excess   |                  | BCAA excess     |                  | SED      |            |
|                              | Control                                       | Treatment        | Control          | Treatment        | Control         | Treatment        | Control† | Treatment‡ |
| <b>Postprandial (4 h)</b>    |   |                  |                  |                  |                 |                  |          |            |
| KIV                          | 21 <sup>a</sup>                               | 19 <sup>a</sup>  | 16 <sup>a</sup>  | 6 <sup>b</sup>   | 23 <sup>a</sup> | 19 <sup>a</sup>  | 3.2      | 4.5        |
| KMV                          | 55 <sup>a</sup>                               | 49 <sup>a</sup>  | 40 <sup>ab</sup> | 28 <sup>b</sup>  | 58 <sup>a</sup> | 56 <sup>a</sup>  | 6.6      | 9.3        |
| KIC                          | 80 <sup>a</sup>                               | 77 <sup>ab</sup> | 67 <sup>a</sup>  | 100 <sup>b</sup> | 80 <sup>a</sup> | 90 <sup>ab</sup> | 11.9     | 16.8       |
| <b>Postabsorptive (16 h)</b> |   |                  |                  |                  |                 |                  |          |            |
| KIV                          | 11 <sup>a</sup>                               | 18 <sup>bc</sup> | 14 <sup>a</sup>  | 9 <sup>ab</sup>  | 10 <sup>a</sup> | 20 <sup>c</sup>  | 3.5      | 4.9        |
| KMV                          | 29 <sup>a</sup>                               | 46 <sup>ab</sup> | 32 <sup>a</sup>  | 31 <sup>ab</sup> | 28 <sup>a</sup> | 54 <sup>b</sup>  | 8.9      | 12.5       |
| KIC                          | 53 <sup>a</sup>                               | 56 <sup>ab</sup> | 49 <sup>a</sup>  | 62 <sup>b</sup>  | 57 <sup>a</sup> | 56 <sup>ab</sup> | 5.8      | 8.2        |

KIV,  $\alpha$ -ketoisovaleric acid; KMV,  $\alpha$ -keto- $\beta$ -methyl-n-valeric acid; KIC,  $\alpha$ -ketoisocaproic acid.

<sup>a,b,c</sup> Mean values within a row not sharing a common superscript letter indicate significant ( $P < 0.05$ ) differences between a treatment and its own control, or between two treatments.

\* For details of diets and procedures, see pp. 50–51.

† For comparison of group mean against its own control.

‡ For comparison of treatment means against each other.

Postabsorptive methionine and cyst(e)ine concentrations were similar for all treatments, regardless of the deficiency of methionine or the excess of leucine or the BCAA.

#### Branched-chain keto acid concentrations

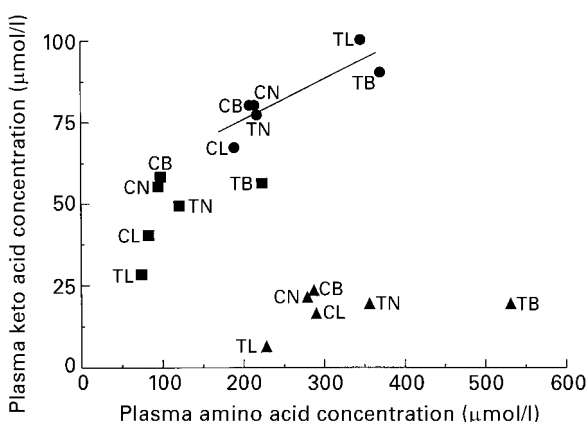
Changes in plasma keto acid concentrations caused by diet and time after feeding are shown in Table 2. The concentrations of  $\alpha$ -keto- $\beta$ -methyl-*n*-valeric acid (KMV) and KIV, the keto acids of isoleucine and valine, were altered in the same direction as the corresponding amino acid concentrations. Postprandial plasma concentrations of KIV and KMV were significantly ( $P < 0.05$ ) reduced by dietary leucine excess whereas in the postabsorptive state the effect was not significant. Additions of isoleucine and valine together with leucine (BCAA excess treatment) reversed the effects of leucine on KIV and KMV. It was not possible to determine the concentration of KMTB, the keto acid of methionine as this keto acid appears to be unstable in the conditions used for analysis of the BCAA.

#### The relationship between branched-chain amino acid and keto acid concentrations

In the postprandial state (Fig. 1) there was not a strong relationship between the plasma concentrations of valine or isoleucine and their corresponding keto acid concentrations. However, plasma  $\alpha$ -ketoisocaproic acid (KIC) increased with plasma leucine concentration and both were increased with dietary leucine excess. The relationship between the plasma concentrations of KIC ( $y$ ) and leucine ( $x$ ) can be described by the equation:

$$y = 50.2 + 0.125x; r^2 0.760.$$

In the postabsorptive state, there was a linear relationship between isoleucine and KMV as well as between valine



**Fig. 1.** Relationships between 4 h postprandial plasma concentrations of branched-chain amino acids and branched-chain keto acids in pigs fed on diets containing different proportions of amino acids. C, control treatment (balanced amino acid pattern); T, treatment; N, methionine-deficient diet with no excess amino acids; L, methionine-deficient diet with excess leucine; B, methionine-deficient diet with excess branched-chain amino acids. The relationships shown are: (■), isoleucine:  $\alpha$ -keto- $\beta$ -methyl-*n*-valeric acid; (●), leucine:  $\alpha$ -ketoisocaproic acid ( $y = 0.125x + 50.2$ ,  $r^2 0.760$ ); (▲), valine:  $\alpha$ -ketoisovaleric acid.

and KIV (Fig. 2). The relationship between KMV ( $y$ ) and isoleucine ( $x$ ) can be described by the equation:

$$y = 2.76 + 0.396x; r^2 0.845,$$

and that between KIV and valine by the equation:

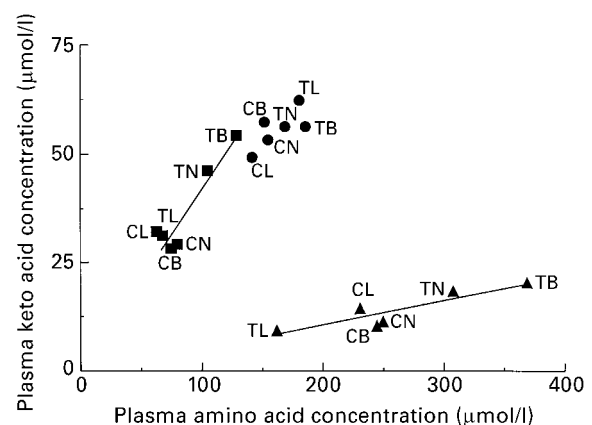
$$y = -1.20 + 0.057x; r^2 0.758.$$

#### Changes in branched-chain keto-acid dehydrogenase activity in liver

**Measurements with [ $^{14}$ C] $\alpha$ -ketoisovaleric acid.** Enzyme activities are expressed per g tissue and per mg protein (Table 3). Pig liver contained approximately 170 mg extractable protein per g tissue. Whether expressed per g tissue or per mg protein, basal BCKDH activity in the liver was significantly increased ( $P < 0.05$ ) by leucine or BCAA excess. The percentage enzyme active was significantly increased ( $P < 0.05$ ) by leucine excess and even more by BCAA excesses.

#### Changes in branched-chain keto-acid dehydrogenase activity in muscle

**Measurements with [ $^{14}$ C] $\alpha$ -ketoisovaleric acid.** Using the radioactive keto acid as substrate the basal activities were below the detection limit of the assay. The total BCKDH activities of adductor and biceps femoris muscles, measured with [ $^{14}$ C]KIV, are shown in Table 4. Pig muscle contained approximately 60 mg extractable protein per g tissue. Both muscles had similar total activities but the activity in adductor muscle was not affected by the dietary treatments. Total BCKDH activity in biceps femoris muscle was significantly ( $P < 0.05$ ) increased by excess leucine and excess BCAA.



**Fig. 2.** Relationships between 16 h postabsorptive plasma concentrations of branched-chain amino acids and branched-chain keto acids in pigs fed on diets containing different proportions of amino acids. C, control treatment (balanced amino acid pattern); T, treatment; N, methionine-deficient diet with no excess amino acids; L, methionine-deficient diet with excess leucine; B, methionine-deficient diet with excess branched-chain amino acids. The relationships shown are: (■), isoleucine:  $\alpha$ -keto- $\beta$ -methyl-*n*-valeric acid ( $y = 0.396x + 2.76$ ,  $r^2 0.876$ ); (●), leucine:  $\alpha$ -ketoisocaproic acid; (▲), valine:  $\alpha$ -ketoisovaleric acid ( $y = 0.057x - 1.20$ ,  $r^2 0.806$ ).

**Table 3.** Hepatic branched-chain keto-acid dehydrogenase activities measured with  $\alpha$ -keto[carboxyl- $^{14}\text{C}$ ]isovaleric acid in pigs fed on methionine-deficient diets with either no excess, excess leucine or excess branched-chain amino acids (BCAA)\*  
(Values are means for four pigs)

|                                    | Branched-chain keto-acid dehydrogenase activity |                   |                   | Pooled SED |
|------------------------------------|---|-------------------|-------------------|------------|
|                                    | No excess                                       | Leucine excess    | BCAA excess       |            |
| Activity (nmol/min per g tissue)   |   |                   |                   |            |
| Basal                              | 18 <sup>a</sup>                                 | 38 <sup>b</sup>   | 54 <sup>b</sup>   | 8.1        |
| Total                              | 224   | 250               | 272               | 26.0       |
| Active (%)†                        | 7.8 <sup>a</sup>                                | 15.2 <sup>b</sup> | 19.7 <sup>b</sup> | 2.32       |
| Activity (nmol/min per mg protein) |   |                   |                   |            |
| Basal                              | 0.11 <sup>a</sup>                               | 0.22 <sup>b</sup> | 0.34 <sup>c</sup> | 0.052      |
| Total                              | 1.32  | 1.44              | 1.71              | 0.205      |
| Active (%)                         | 7.8 <sup>a</sup>                                | 15.2 <sup>b</sup> | 19.7 <sup>b</sup> | 2.32       |

<sup>a,b,c</sup> Mean values within a row not sharing a common superscript letter were significantly different:  $P < 0.05$ .

\* For details of diets and procedures, see pp. 50–51.

† Active (%) is the basal activity/total activity  $\times 100$ .

*Measurements with L-[ $^{14}\text{C}$ ]valine.* Basal BCKDH activity in adductor muscle was not significantly increased by excesses of leucine or BCAA, whether expressed per g tissue or per mg protein.

When L-[ $^{14}\text{C}$ ]valine was used as substrate, there was no difference in total enzyme activity amongst the treatments (Table 5). The percentage of active enzyme rose significantly from about 10% on the treatment without excess to 14% with leucine excess. Using the ratio basal:total activity determined with L-[ $^{14}\text{C}$ ]valine the indirect calculation of absolute basal activity was possible using the results for total activity obtained with [ $^{14}\text{C}$ ]KIV. Assuming that adductor muscle BCKDH was about 10% active the basal activity would be approximately 0.50 nmol KIV/min per g tissue.

### Discussion

This work was undertaken to elucidate the mechanisms by which dietary branched-chain amino acids affect the

**Table 4.** Total branched-chain keto-acid dehydrogenase activities in adductor and biceps femoris muscles of pigs fed on methionine-deficient diets with either no excess, excess leucine or excess branched-chain amino acids (BCAA)\*  
(Values are means for four pigs)

|                                    | Branched-chain keto-acid dehydrogenase activity |                    |                    | Pooled SED |
|------------------------------------|---|--------------------|--------------------|------------|
|                                    | No excess                                       | Leucine excess     | BCAA excess        |            |
| Activity (nmol/min per g tissue)   |   |                    |                    |            |
| Adductor                           | 4.8   | 5.3                | 3.9                | 0.74       |
| Biceps femoris                     | 4.2 <sup>a</sup>                                | 6.5 <sup>b</sup>   | 7.0 <sup>b</sup>   | 1.07       |
| Activity (nmol/min per mg protein) |   |                    |                    |            |
| Adductor                           | 0.079 <sup>a,b</sup>                            | 0.091 <sup>a</sup> | 0.064 <sup>b</sup> | 0.0119     |
| Biceps femoris                     | 0.072 <sup>a</sup>                              | 0.114 <sup>b</sup> | 0.115 <sup>b</sup> | 0.0147     |

<sup>a,b</sup> Mean values within a row not sharing a common superscript letter were significantly different:  $P < 0.05$ .

\* For details of diets and procedures, see pp. 50–51.

**Table 5.** Branched-chain keto-acid dehydrogenase activities measured with L-[ $^{14}\text{C}$ ] valine in the adductor muscle of pigs fed on methionine-deficient diets with either no excess, excess leucine or excess branched-chain amino acids (BCAA)\*  
(Values are means for four pigs)

|                                   | Branched-chain keto-acid dehydrogenase activity |                   |                  | Pooled SED |
|-----------------------------------|---|-------------------|------------------|------------|
|                                   | No excess                                       | Leucine excess    | BCAA excess      |            |
| Activity (nmol/min per g tissue)  |   |                   |                  |            |
| Basal                             | 0.041   | 0.060             | 0.054            | 0.0089     |
| Total                             | 0.55  | 0.42              | 0.57             | 0.196      |
| Active (%)†                       | 9.9 <sup>a</sup>                                | 14.3 <sup>b</sup> | 9.6 <sup>a</sup> | 2.13       |
| Activity (nmol/min per g protein) |   |                   |                  |            |
| Basal                             | 0.0008  | 0.0011            | 0.0010           | 0.00018    |
| Total                             | 0.0104  | 0.0078            | 0.0101           | 0.00385    |
| Active (%)                        | 9.5 <sup>a</sup>                                | 14.0 <sup>b</sup> | 9.6 <sup>a</sup> | 2.01       |

<sup>a,b</sup> Mean values within a row not sharing a common superscript letter were significantly different:  $P < 0.05$ .

\* For details of diets and procedures, see pp. 50–51.

† Active (%) is the basal activity/total activity  $\times 100$ .

utilization of a methionine-deficient diet, as reported in the previous paper (Langer & Fuller, 2000). To try to understand how the alterations in amino acid utilization were brought about we examined changes in plasma concentrations of amino acids and keto acids and changes in the activity of BCKDH in muscle and liver.

### Plasma amino acid concentrations

The results demonstrate that BCAA interactions occur in the growing pig even at moderate levels of excess, and even when methionine, rather than one of the BCAA, is the limiting amino acid in the diet. The consumption of a moderate excess of leucine resulted in significant ( $P < 0.05$ ) depressions in the plasma concentrations of the other two BCAA as previously reported in pigs (Oestemer *et al.* 1973; Henry *et al.* 1976; Taylor *et al.* 1984), other animal species (D'Mello & Lewis, 1970; Block, 1989) and human subjects (Abumrad *et al.* 1984). These observed changes could be caused by various metabolic reactions: (1) effects on intestinal absorption or transport between tissues and blood; (2) effects on protein accretion due to (a) changes in protein synthesis, (b) changes in protein degradation; (3) effects on transamination to branched-chain keto acids (BCKA) and further decarboxylation.

The observed depressions of isoleucine and valine concentrations in plasma are probably not due to the inhibition by leucine of isoleucine or valine absorption or transport because leucine excess appears to have negligible effects on isoleucine and valine absorption (Rogers *et al.* 1962; Smith & Austic, 1978; Calvert *et al.* 1982; Block & Harper, 1984) and because intravenous infusion of leucine or KIC into human forearm resulted in a similar decrease in isoleucine and valine (Abumrad *et al.* 1984). Inhibition of isoleucine and valine uptake into tissues by leucine excess should increase their plasma concentrations rather than reduce them.

Increased accretion of protein due to leucine excess as observed by Langer & Fuller (2000) using exactly the same diets cannot be completely excluded as part of the reason for the changes in plasma isoleucine and valine concentrations. However, enhanced protein accretion should also affect the plasma concentrations of other amino acids, which was not observed. A selective accretion of protein with higher isoleucine and valine concentrations seems very unlikely and the changes in their plasma concentrations seem to be the result of a selective mechanism other than incorporation into protein (Harper *et al.* 1984).

Several authors (e.g. Harper *et al.* 1984; Block, 1989) have suggested that an excess of leucine or KIC can increase both the transamination of isoleucine and valine to KMV and KIV and their further decarboxylation and this could account for the changes observed here. Various experiments in other animal species showed that leucine excess supplied via the feed or by intravenous infusion can activate the catabolic pathway of BCAA causing a reduction in the plasma concentrations of isoleucine and valine (Calvert *et al.* 1982; Block & Harper, 1984).

#### *Branched-chain keto acid concentrations*

It seems that this is the first report of plasma keto acid concentrations and the effect of leucine or its metabolites on the keto acids of isoleucine and valine in pigs. Similar experiments investigating the effects of leucine or BCAA on their metabolism in other animals have usually been carried out using greater excesses. In rats, addition of large amounts of leucine (i.e. 15 g/kg diet) to a low-protein diet reduced the plasma concentrations of KMV and KIV but increased KIC significantly (Block & Harper, 1991). Similarly, the addition of KIC to a low-protein diet increased the plasma concentration of KIC and reduced the concentrations of KMV and KIV in rats (Crowell *et al.* 1990). This suggests that leucine and KIC cause the same response.

The present results show that similar changes in KMV and KIV occur in the pig even at moderate levels of excess. These could be explained by an activation of BCKDH due to leucine or KIC excess and an increase in the decarboxylation of KMV and KIV.

#### *The relationship between branched-chain amino and keto acids*

There was in general a close relationship between the plasma concentration of a BCAA and its corresponding keto acid. Similar observations were made by Matthews *et al.* (1982) in man. This relationship is presumably due to the presence of a near equilibrium transaminase in most

tissues (Krebs & Lund, 1977). Transamination of BCAA to BCKA occurs very rapidly (Matthews *et al.* 1982) and therefore it is not surprising that plasma BCAA and BCKA concentrations were closely related. However, these relationships were closer in the postabsorptive than in the postprandial state. This suggests that the absorption and the transport of BCAA might play an important role. Transamination of the BCAA appears to occur mainly in skeletal muscle (Shinnick & Harper, 1977); therefore it is necessary to transport BCAA to the muscle before the BCKA can be released (Harper & Zapalowski, 1981). This might explain the weaker relationship during the postprandial state when the absorption is much higher than in the postabsorptive state. Furthermore, in both postprandial and postabsorptive states, the ratios BCAA : BCKA were always higher when all three BCAA were supplied together which indicates that all three BCAA or BCKA might compete for transamination or transport. Although there is no *in vivo* evidence, this is consistent with the different  $K_m$  values for the transaminases and the fact that all three BCAA are transported by the L-system.

#### *Branched-chain keto-acid dehydrogenase activity in liver and muscle*

In the pig, total liver BCKDH activity was much higher than total skeletal muscle activity. Expressed per g tissue, liver had about forty times higher total activity than muscle. Expressed per mg protein, the total activity in liver was still eighteen times higher than in muscle. This may be related to the higher concentration of mitochondria in liver.

Excesses of both leucine and BCAA enhanced the activity of BCKDH in liver. This effect could be explained by the larger amounts of KIC produced. It has been shown that enhanced levels of BCKA, especially KIC, inhibit the BCKDH kinase (EC 2.7.1.115), which results in a less phosphorylated BCKDH complex and therefore a higher BCKDH activity (Paxton & Harris, 1984). This response of BCKDH to KIC would explain why leucine excess reduced the concentrations of isoleucine and valine and their keto acids in plasma: the increased BCKDH activity would result in greater oxidation of isoleucine and valine.

#### *Branched-chain amino acid metabolism in the whole animal*

Comparing the values for liver and muscle basal BCKDH capacity (Table 6), the 40 kg pig fed on a methionine-deficient diet without excess amino acids was able to decarboxylate about 25.9 mmol KIV/d in liver and about 11.5 mmol in muscle. In the rat, the contribution of other

**Table 6.** Basal activities of branched-chain keto-acid dehydrogenase (BCKDH) in a 40 kg pig

| Organ  | Organ weight (g) | Organ : body (%) | BCKDH activity (nmol/min per g) | Organ BCKDH capacity |          | Liver : muscle ratio |
|--------|------------------|------------------|---------------------------------|----------------------|----------|----------------------|
|        |                  |                  |                                 | (nmol/min)           | (nmol/d) |                      |
| Liver  | 1000             | 2.5              | 18.0                            | 18 000               | 25.9     | 2.3 : 1              |
| Muscle | 16 000           | 40.0             | 0.5*                            | 8000                 | 11.5     |                      |

\* Adductor muscle.

**Table 7.** Branched-chain amino acid intake, excretion in ileal digesta (i.e. passing into caecum), body concentrations, retention and oxidation in a 40 kg pig

| Amino acids | Intake (g/d) | Passing into caecum* (g/d) | Concentration in body protein† (g/kg) | Retention (g/d) | Oxidation‡ |          |
|-------------|--------------|----------------------------|---------------------------------------|-----------------|------------|----------|
|             |              |                            |                                       |                 | (g/d)      | (mmol/d) |
| Leucine     | 9.3          | 0.98                       | 74                                    | 6.3             | 2.0        | 15.2     |
| Valine      | 6.4          | 0.92                       | 47                                    | 4.0             | 1.5        | 12.8     |
| Isoleucine  | 5.1          | 0.74                       | 35                                    | 3.0             | 1.4        | 10.7     |

\* Wang &amp; Fuller (1989).

† Kyriazakis &amp; Emmans (1993).

‡ Amino acid oxidation is calculated as the difference between the amino acid digested (intake – that passing into the caecum) and that retained (calculated as N retention  $\times$  6.25  $\times$  amino acid concentration in body protein).

organs was relatively low compared with liver and muscle, except kidney with 6.5% of the total decarboxylation capacity (Shinnick & Harper, 1977). Liver and muscle seem to be the major tissues degrading BCAA. The diet supplied about 70.9 mmol (9.3 g) leucine, 54.7 mmol (6.4 g) valine and 38.9 mmol (5.1 g) isoleucine per day. Knowing the daily BCAA intake, the N retention (taken from Langer & Fuller, 2000), the whole-body BCAA concentrations (Kyriazakis & Emmans, 1993) and the daily flow of BCAA in ileal digesta passing into the caecum (Wang & Fuller, 1989) it is possible to calculate how much BCAA (via BCKA) had to be degraded (Table 7) by animals given the methionine-deficient diet. These are simplified and approximate calculations in which it is assumed that the enzyme was completely extracted, that its activity was measured under nearly optimal conditions and that all three BCKA are equally good substrates for BCKDH. Nevertheless, the degradation of further excess of leucine or BCAA would require the activation of BCKDH or an increase in the amount of BCKDH. Both were observed in the present experiment. Total activation of the BCKDH after feeding BCAA in excess would allow for a daily degradation of up to 392 mmol KIV in liver and 161 mmol in muscle.

#### *Possible effects of branched-chain keto-acid dehydrogenase activity on methionine metabolism*

In the previous paper (Langer & Fuller, 2000) we reported that the addition of excess leucine or BCAA to a methionine-deficient diet enhanced N retention. This suggests that these amino acids reduced the oxidative loss of methionine, making more available for body protein accretion. We were unable to measure directly either the concentrations of KMTB or its oxidation so our discussion of this issue must be incomplete. The low  $K_m$  and non-inducibility of S-adenosyl methionine synthase (*EC* 2.5.1.6) probably limits the degradation of methionine by the trans-sulfuration pathway, whereas the  $K_m$  for transamination of methionine is high relative to plasma and tissue concentrations of this amino acid (Harper, 1983; Livesey, 1984); therefore regulation of the flux through the transamination pathway by supply of other substrates (i.e. BCAA and BCKA) might regulate methionine catabolism (Livesey, 1984). Besides activating a degradative enzyme for methionine, excessive amounts of leucine, isoleucine or valine, or of their keto acids, could affect methionine transamination as well as decarboxylation by supplying competitive substrates.

Depression of methionine transamination and of methionine oxidation by addition of BCAA during incubation of rat skeletal muscle homogenates has been reported by Wu & Thompson (1989). However, BCAA had no effect on the transamination of methionine in rat liver (Livesey & Lund, 1980). Because BCKA, and particularly KIC, activate BCKDH this would be expected to increase rather than reduce the oxidation of KMTB. However, whereas the  $K_m$  values for the BCKA are in the range 10–20  $\mu$ M, that for KMTB is at least three times higher, 67  $\mu$ M (Jones & Yeaman, 1986). Thus the more important effect of additional BCAA might be the provision of alternative substrate. Dixon & Benevenga (1980) reported that addition of 1 mM-KIC depressed the decarboxylation of KMTB in rat liver mitochondria by 78%. Studies with rat hepatocytes (Livesey, 1981) showed inhibition of the decarboxylation of KMTB by KIV, KMV and KIC.

It seems likely that the dominant effect of excess leucine or BCAA on methionine was to reduce the oxidation of KMTB by competitive inhibition, leading to increased availability of methionine for body protein synthesis.

#### Acknowledgements

This work was supported by the Scottish Office Agriculture, Environment and Fisheries Department and the financial support (S.L.) of Eurolysine SA and Degussa AG is gratefully acknowledged. We are grateful to Degussa AG for the estimation of methionine, cystine and tryptophan, to Dr M. Franklin and the staff of BioSS for their help with the statistical analyses and Mrs Ann White for her help with the preparation of the manuscript.

#### References

- Abumrad NN, Abumrad NA, Sandler MP & Lacy WW (1984) The metabolic effects and fate of branched-chain amino and keto acids across human skeletal muscle. In *Advances in Hepatic Encephalopathy and Urea Cycle Diseases*, pp. 505–518 [G Kleinberger, P Ferenci, P Riederer and H Thaler, editors]. Basel: Karger.
- Baumrucker CR (1985) Amino acid transport systems in bovine mammary tissue. *Journal of Dairy Science* **68**, 2436–2451.
- Bech-Andersen S, Mason VC & Dhanoa MS (1990) Hydrolysate preparation for amino acid determinations in feed constituents. 9. Modifications to oxidation and hydrolysis conditions for streamlined procedures. *Journal of Animal Physiology and Animal Nutrition* **63**, 188–197.
- Benevenga NJ (1984) Evidence for alternative pathways of



- methionine catabolism. In *Advances in Nutritional Research*, vol. 6, pp. 1–18 [HH Draper, editor]. New York, NY: Plenum Press.
- Block KP (1989) Interactions among leucine, isoleucine, and valine with special reference to the branched-chain amino acid antagonism. In *Absorption and Utilization of Amino Acids*, vol. 1, pp. 229–244 [M Friedman, editor]. Boca Raton, FL: CRC Press.
- Block KP & Harper AE (1984) Valine metabolism in vitro: effects of high dietary levels of leucine and isoleucine. *Metabolism* **33**, 559–566.
- Block KP & Harper AE (1991) High levels of dietary amino and branched-chain  $\alpha$ -keto acids alter plasma and brain amino acid concentrations in rats. *Journal of Nutrition* **121**, 663–671.
- Buse MG & Reid SS (1975) Leucine. A possible regulator of protein turnover in muscle. *Journal of Clinical Investigation* **56**, 1250–1261.
- Calvert CC, Klasing KC & Austic RE (1982) Involvement of food intake and amino acid catabolism in the branched-chain amino acids antagonism in chicks. *Journal of Nutrition* **112**, 627–635.
- Case GL & Benevenga NJ (1976) Evidence for S-adenosyl-methionine independent catabolism of methionine in the rat. *Journal of Nutrition* **106**, 1721–1736.
- Crowell PL, Block KP, Repa JJ, Torres N, Nawabi MD, Buse MG & Harper AE (1990) High branched-chain  $\alpha$ -keto acid intake, branched-chain  $\alpha$ -keto acid dehydrogenase activity, and plasma and brain amino acid and plasma keto acid concentrations in rats. *American Journal of Clinical Nutrition* **52**, 313–319.
- D’Mello JPF & Lewis D (1970) Amino acid interactions in chick nutrition. 2. Interrelationships between leucine, isoleucine and valine. *British Poultry Science* **11**, 313–323.
- Davidson J, Mathieson J & Boyne AW (1970) The use of automation in determining nitrogen by the Kjeldahl method, with final calculation by computer. *Analyst* **95**, 181–193.
- Dixon JL & Benevenga NJ (1980) The decarboxylation of  $\alpha$ -keto-gamma-methylbutyrate in rat liver mitochondria. *Biochemical and Biophysical Research Communications* **97**, 939–946.
- Eriksson S, Hagenfeldt L & Wahren J (1981) A comparison of the effects of intravenous infusion of individual branched-chain amino acids on blood amino acid levels in man. *Clinical Science* **60**, 95–100.
- Fuller MF, Weekes TEC, Cadenhead A & Bruce JB (1977) The protein-sparing effect of carbohydrate. 2. The role of insulin. *British Journal of Nutrition* **38**, 489–496.
- Goldberg AL & Tischler ME (1981) Regulatory effects of leucine on carbohydrate and protein metabolism. In *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*, pp. 205–216 [M Walser and JR Williamson, editors]. New York, NY: Elsevier/North-Holland.
- Guidotti GG & Gazzola GC (1992) Amino acid transporters: systematic approach and principles of control. In *Mammalian Amino Acid Transport*, pp. 3–29 [MS Kilberg and D Häusinger, editors]. New York, NY: Plenum Press.
- Hargrove DM, Rogers QR, Calvert CC & Morris JG (1988) Effects of dietary excesses of branched-chain amino acids on growth, food intake and plasma amino acid concentrations of kittens. *Journal of Nutrition* **118**, 311–320.
- Harper AE (1983) Some recent developments in the study of amino acid metabolism. *Proceedings of the Nutrition Society* **42**, 437–449.
- Harper AE, Miller RH & Block KP (1984) Branched-chain amino acid metabolism. *Annual Review of Nutrition* **4**, 409–454.
- Harper AE & Peters JC (1983) Amino acid signals and food intake and preference: relation to body protein metabolism. In *Nutritional Adequacy, Nutrient Availability and Needs, Nestlé Nutrition Research Symposium/Experientia Supplementum*, vol. 44, pp. 107–134 [J Mauron, editor]. Basel: Birkhäuser Verlag.
- Harper AE & Zapalowski C (1981) Metabolism of branched chain amino acids. In *Nitrogen Metabolism in Man*, pp. 97–115 [JC Waterlow and JML Stephen, editors]. London and New Jersey: Applied Science Publishers.
- Henry Y, Duée PH & Rérat A (1976) Isoleucine requirement of the growing pig and leucine–isoleucine interrelationship. *Journal of Animal Science* **42**, 357–364.
- Jones SMA & Yeaman SJ (1986) Oxidative decarboxylation of 4-methylthio-2-oxobutyrate by branched-chain 2-oxo acid dehydrogenase complex. *Biochemical Journal* **237**, 621–623.
- Krebs H & Lund P (1977) Aspects of the regulation of the metabolism of branched-chain amino acids. *Advances in Enzyme Regulation* **15**, 375–394.
- Kyriazakis I & Emmans GC (1993) Whole body amino acid composition of the growing pig. *Journal of the Science of Food and Agriculture* **62**, 29–33.
- Langer S & Fuller MF (2000) Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. *British Journal of Nutrition* **83**, 43–48.
- Li JB & Jefferson LS (1978) Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochimica et Biophysica Acta* **544**, 351–359.
- Livesey G (1981) Metabolism of ‘essential’ 2-oxo acids by liver and a role for branched-chain oxo acid dehydrogenase in the catabolism of methionine. In *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*, pp. 143–148 [M Walser and JR Williamson, editors]. New York, NY: Elsevier/North-Holland.
- Livesey G (1984) Methionine degradation: ‘anabolic and catabolic’. *Trends in Biochemical Science* **5**, 27–29.
- Livesey G & Lund P (1980) Methionine metabolism via the transamination pathway in rat liver. *Biochemical Society Transactions* **8**, 540–541.
- Matthews DE, Schwarz HP, Yang RD, Motil KJ, Young VR & Bier DM (1982) Relationship of plasma leucine and  $\alpha$ -ketoisocaproate during a L-[1-<sup>13</sup>C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. *Metabolism* **31**, 1105–1112.
- Mitch WE & Clark AS (1984) Specificity of effects of leucine and its metabolites on protein degradation in skeletal muscle. *Biochemical Journal* **222**, 579–586.
- Mitchell AD & Benevenga NJ (1978) The role of transamination in methionine oxidation in the rat. *Journal of Nutrition* **108**, 67–78.
- Nair KS, Schwartz RG & Welle S (1992) Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *American Journal of Physiology* **263**, E928–E934.
- Naumann C & Bassler R (1993) *Methodenbuch (Band III) Die chemische Untersuchung von Futtermitteln*. Darmstadt: VDLUFA-Verlag.
- Oestemer GA, Hanson LE & Meade RJ (1973) Leucine–isoleucine interrelationship in the young pig. *Journal of Animal Science* **36**, 674–678.
- Oxender DL & Christensen HN (1963) Distinct mediating systems for the transport of neutral amino acids by the Ehrlich cell. *Journal of Biological Chemistry* **238**, 3686–3699.
- Papet D, Breuille F, Glomot F & Arnal M (1988) Nutritional and metabolic effects of dietary leucine excess in preruminant lambs. *Journal of Nutrition* **118**, 450–455.
- Paxton R & Harris RA (1984) Regulation of branched-chain  $\alpha$ -ketoacid dehydrogenase kinase. *Archives of Biochemistry and Biophysics* **231**, 48–57.
- Peng Y, Gubin J, Harper AE, Vavich MG & Kemmerer AR (1973) Food intake regulation: amino acid toxicity and changes in rat brain and plasma amino acids. *Journal of Nutrition* **103**, 608–617.
- Rogers QR, Spolter PD & Harper AE (1962) Effect of leucine–isoleucine antagonism on plasma amino acid

- pattern of rats. *Archives of Biochemistry and Biophysics* **97**, 497–504.
- Rüdiger HW, Langenbeck U & Goedde HW (1972) A simplified method for the preparation of <sup>14</sup>C-labelled branched-chain  $\alpha$ -oxo acids. *Biochemical Journal* **126**, 445–446.
- Shinnick FL & Harper AE (1977) Effects of branched-chain amino acid antagonism in the rat on tissue amino acid and keto acid concentrations. *Journal of Nutrition* **107**, 887–895.
- Smith TK & Austic RE (1978) The branched-chain amino acid antagonism in chicks. *Journal of Nutrition* **108**, 1180–1191.
- Synderman SE, Cusworth DC, Roitman E & Holt LE (1959) Amino acid interrelationship: the effect of variations in leucine intake. *Proceedings of the Society for Experimental Biology and Medicine* **18**, 546 Abstr.
- Tackman JM, Tews JK & Harper AE (1990) Dietary proportions of amino acids in the rat: effect on food intake, plasma and brain amino acids and brain serotonin. *Journal of Nutrition* **120**, 521–533.
- Tannous RI, Rogers QR & Harper AE (1966) Effect of leucine–isoleucine antagonism on the amino acid pattern of plasma and tissues of the rat. *Archives of Biochemistry and Biophysics* **113**, 356–361.
- Taylor AJ, Cole DJA & Lewis D (1984) Amino acid requirements of growing pigs. 5. The interactions between isoleucine and leucine. *Animal Production* **38**, 257–261.
- Wahren J, Eriksson S & Hagenfeldt L (1981) Influence of branched-chain amino acids and ketoacids on arterial concentrations and brain exchange of amino acids in man. In *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*, pp. 471–480 [M Walser and JR Williamson, editors]. New York, NY: Elsevier/North-Holland.
- Wang TC & Fuller MF (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *British Journal of Nutrition* **62**, 77–89.
- Wu G & Thompson JR (1989) Is methionine transaminated in skeletal muscle? *Biochemical Journal* **257**, 281–284.