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# Metabolism of Valine and the Exchange of Amino Acids across the Hind-limb Muscles of Fed and Starved Sheep

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*Abstract*

A combination of the isotope-dilution and arterio-venous (AV) difference techniques was used to study simultaneously the metabolism of valine in the whole body and in the hind-limb muscles of fed and starved (40 h) sheep. The net exchange of gluconeogenic amino acids across hind-limb muscles was also studied.

Valine entry rate was unaffected by nutritional status. There was significant extraction of valine by hind-limb muscles in both fed and starved sheep. The percentage of valine uptake decarboxylated was higher ($P < 0{\cdot}05$) in fed sheep but the amount of valine decarboxylated was not significantly different. The proportion of valine uptake that was transaminated was about 30 times higher in starved sheep. About 54% of valine taken up by hind-limb muscle of starved sheep was metabolized. The corresponding value for fed sheep was 21%. The contribution of $CO_2$ from valine decarboxylation to total hind-limb muscle $CO_2$ output was about 0·2%.

The output of alanine in both fed and starved sheep was low but the output of glutamine was relatively high and roughly equivalent to the amounts of aspartate, glutamate and branched-chain amino acids that were catabolized. This study has confirmed that valine is catabolized in sheep skeletal muscle, and shown that glutamine is a major carrier of amino nitrogen out of muscle.

## Introduction

The measurement of amino acid arterio-venous (AV) concentration differences across muscle tissue has provided indirect evidence that the movement of amino acids into and out of muscle reflects not only the net synthesis and degradation of muscle protein, but also *de novo* synthesis of some amino acids in muscle. Alanine (London *et al.* 1965; Pozefsky *et al.* 1969) and glutamine (Marliss *et al.* 1971) have been shown to be released by human forearm muscles into the circulation in greater proportion than could be accounted for by muscle proteolysis alone. Mallette *et al.* (1969) and Felig *et al.* (1970) postulated that a glucose–alanine cycle exists in which alanine is synthesized in muscle by transamination of glucose-derived pyruvate: alanine released from muscle is taken up by the liver where its carbon (C) skeleton is used for glucose synthesis. This implies that the glucose is recycled, with no net gain of glucose C, and that the amino group ($-NH_2$) for alanine synthesis is derived from the degradation of other amino acids.

Glutamine, the other proposed $-NH_2$ carrier from muscles (see Ruderman 1975), derives its $-NH_2$ and C skeleton from other amino acids, whereas alanine is able to contribute net C for glucose synthesis in the liver or kidney only if its C skeleton is derived from non-glucose precursors. Although the sources of C of alanine

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synthesized in the muscle have been described, the relative importance of glucose and amino acids as sources of alanine-C is largely unresolved. Recent evidence, reviewed by Palmer *et al.* (1985), indicates that amino acid C is not a major source of alanine in the rat, but there are no corresponding data for ruminants.

It is well established in non-ruminant animals that the branched-chain amino acids (BCAA) are catabolized mainly in muscles (see Adibi 1976). There is also evidence that aspartate and glutamate are catabolized to a limited extent in muscle (see Lindsay and Buttery 1980). It is therefore likely that these amino acids are the major contributors of $-NH_2$ for *de novo* synthesis of alanine and glutamine. In addition, except for leucine, they could also provide carbon via pyruvate for alanine synthesis, or supply glutamate for glutamine synthesis.

In ruminants, there is evidence that the 'glucose–alanine cycle' is of little quantitative importance in muscle metabolism (Ballard *et al.* 1976; Heitmann and Bergman 1980). Alanine efflux from muscles of fed and fasted sheep (Ballard *et al.* 1976; Lindsay *et al.* 1977) and of steers exposed to cold (Bell *et al.* 1975) is approximately four times less than that reported for the human forearm and leg muscles (Pozefsky *et al.* 1969; Felig *et al.* 1970; Felig and Wahren 1971).

While there have been studies of leucine metabolism in ruminants (e.g. Egan and Macrae 1979; Nissen and Ostaszewski 1985) there is little information on valine, which is the only fully glucogenic branched-chain amino acid. The present study was designed to measure directly the extent of catabolism of valine in fed and starved sheep hind-limb muscle *in vivo* and simultaneously to characterize the pattern of circulating amino acids and examine amino acid uptakes by muscle. Preliminary accounts of some of this work have been presented earlier (Teleni *et al.* 1983*a*, 1983*b*).

## Materials and Methods

### *Experimental Animals*

Nine Merino wethers (4 years old; mean liveweight 57 kg) were fed a pelleted ration (9·5 MJ metabolizable energy/kg dry matter; 12·0% crude protein) containing rolled barley, lucerne chaff and oaten chaff in the ratio 40 : 20 : 20 to calculated maintenance requirements (MAFF 1975) from 8 weeks prior to the start of experiments. At least 4 weeks before measurements were made the animals were fed from continuous belt feeders to produce an approximately constant rate of fermentation in the rumen. Measurements in starved sheep were made on five of the wethers which were deprived of feed for approximately 40 h. Water was available at all times.

### *Experimental Procedure*

#### *Hind-limb preparation*

Sheep were surgically prepared for measurements of AV differences and blood flow across a defined muscle mass in the hind limb by the method of Domanski *et al.* (1974), with modifications described by Teleni and Annison (1986). The procedure requires the insertion of indwelling catheters into the jugular vein, femoral artery and lateral saphenous vein. The sheep were all within the size range (body length 104–112 cm, height at withers 70–76 cm) which made it appropriate to place the tip of the venous blood sampling catheter 26 cm from the junction of the cranial and caudal branches of the lateral saphenous vein (Teleni and Annison 1986), in the deep femoral vein.

L-[1-$^{14}$C]valine (Radiochemical Centre, Amersham) was diluted in sterile saline (0·9% w/w), and L-valine added as carrier to give a solution containing 13 kBq, 31 mg valine per litre. This solution was infused into the jugular vein at constant rate (1 ml/min) for 4 h using a peristaltic pump (Minipuls 2, Gilson, France). Samples of arterial and deep femoral venous blood (AV pairs) were taken simultaneously at 30-min intervals during the first 3 h of infusion, and during the fourth hour arterial and venous blood were withdrawn continuously (1 ml/min) and collected as integrated samples at intervals of 10 min.

Pairs of blood samples were taken hourly for 5 h after the end of the infusion. Specific radioactivities of valine, 2-ketoisovalerate and carbon dioxide were determined in the blood samples, which were also assayed for amino acids, glucose and lactate.

*Measurement of blood flow*

Muscle blood flow was determined by the diffusion-equilibrium technique of Pappenheimer and Setchell (1972) as modified by Oddy *et al.* (1981). Tritiated water (TOH) was infused during the third hour of the valine infusion, using a Y connection on the valine infusion line. The integrated blood samples taken over the third hour of infusion were assayed for TOH.

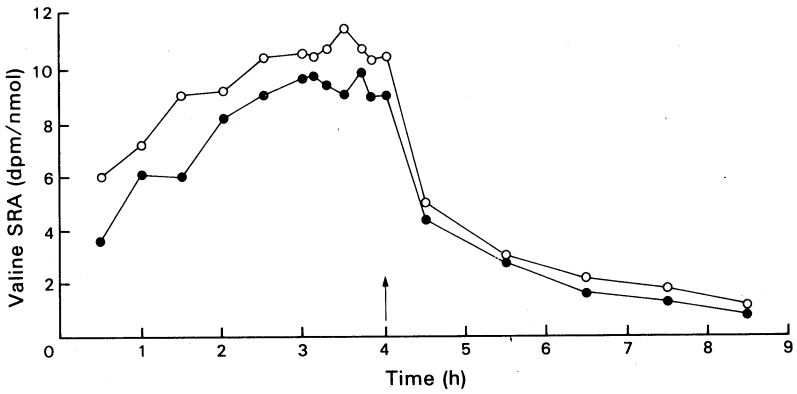


**Fig. 1.** SRA–time course curve in arterial (○) and lateral saphenous venous (●) blood in a starved sheep infused with L-[1-$^{14}$C]valine for 4 h. Arrow indicates when infusion was stopped.

*Infusion of L-[1-$^{14}$C]valine*

Constant specific radioactivity (SRA) of circulating [1-$^{14}$C]valine was achieved after 3 h of infusion (Fig. 1), and blood samples for AV difference and blood flow measurements were taken between the third and fourth hour of infusion. The consistently lower [1-$^{14}$C]valine SRA in deep femoral vein blood in starved sheep relative to arterial blood SRA demonstrated the continuous release of valine from muscle tissue.

*Blood Analysis*

*Amino acids*

An internal standard (0·2 ml of 2·5 mM norleucine) was added to whole blood (2 ml), which was frozen and thawed to disrupt red cells. Sulfosalicylic acid (0·5 ml; concentration 500 g per litre of water) was added, with shaking, to deproteinize the sample, which was then maintained at 4°C for about 1 h to ensure complete auto-oxidation of reduced glutathione (Heitmann and Bergman 1980). After centrifugation at 4°C, the supernatant was placed in a polyethylene vial, the pH adjusted to 2·0–2·5 with 1 M lithium hydroxide, and passed through a filter (0·22 μm, Millipore, U.S.A.). About 20 μl of this filtrate was loaded into an auto-injector for analysis by ion-exchange chromatography on a Dionex D-300 amino acid analyser (Dionex, U.S.A.).

The ion-exchange column (4 mm internal diameter, length 150 mm) packed with DC-5A resin (Dionex) was calibrated using a standard mixture of amino acids (Pierce No. 20077, Pierce, U.S.A.) supplemented with freshly prepared glutamine and asparagine to give final concentrations of amino acids and amides of 50 nmol/ml. The initial elution temperature of 41°C was maintained until the isoleucine peak emerged, when the temperature was raised to 67°C for the remainder of the run. The eluent flow rate was 18 ml/h, and that of the detection reagent (*o*-phthalaldehyde) 9 ml/h.

*Glucose*

Blood glucose concentration was determined colorimetrically by the automated glucose oxidase method of Cramp (1967).

*Lactate*

L-Lactate concentration in deproteinized whole blood (using perchloric acid as a protein precipitant) was determined by the enzyme–spectrophotometric method described by Gutmann and Wahlefeld (1974).

*Blood gases*

Blood $O_2$ and $CO_2$ concentrations were analysed (within 15–20 min of anaerobic collection of blood samples) by a blood gas analyser (ABL1 Acid-base Laboratory, Radiometer, Copenhagen) as described by Oddy *et al.* (1984). Blood $CO_2$ SRA was determined by the gravimetric method described by Leng and Leonard (1965). The $BaCO_3$ formed, however, was solubilized using Tris–EDTA buffer described by Hinks *et al.* (1966).

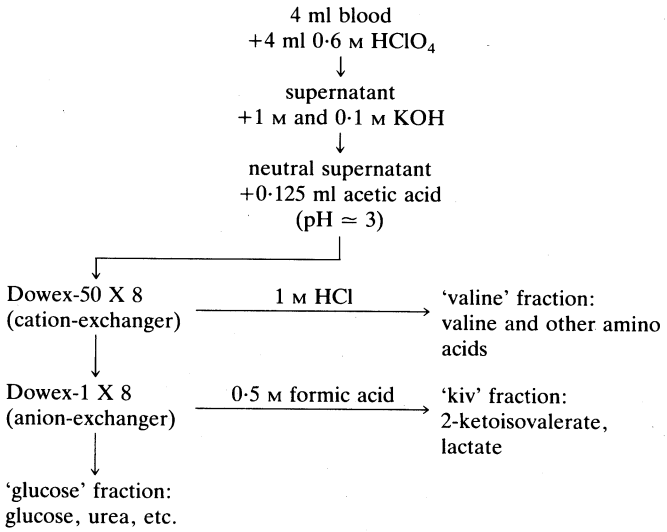


**Fig. 2.** Elution of L-[1-$^{14}$C]valine and L-[1-$^{14}$C]2-ketoisovalerate from the Dowex cation and anion exchanger respectively.

*Specific Radioactivity of Valine and 2-Ketoisovalerate*

The method described by Gulli and Searle (1973) and Faichney *et al.* (1981) was used to measure the SRA of valine, 2-ketoisovalerate and lactate, with modifications. The method, outlined in Fig. 2, involves the retention and subsequent elution of amino acids on a cation-exchange column (Dowex-50X8 column; resin 200–400 mesh, Bio-Rad Laboratories, U.S.A. int. diam. 10 mm, length 40 mm), and the similar use of an anion-exchange column differing only in the characteristics of the resin (Dowex-1X8) to isolate 2-ketoisovalerate.

The recoveries (mean ± s.e.m.) of L-[1-$^{14}$C]valine added to seven aliquots (4 ml) of sheep blood at two levels of radioactivity (2000 and 9200 dpm) were 100 ± 0·74 and 99·9 ± 0·63 respectively. The possible presence in the 2-ketoisovalerate fraction of labelled lactate was checked by the method of Gulli and Searle (1973), based on the isolation of the dimedone–acetaldehyde derivative.

*Calculations for Hind-limb Muscles*

Specific radioactivity (SRA) is expressed as dpm/μmol and muscle blood flow rate (MBF) as litre $min^{-1}$ $kg^{-1}$ muscle. Arteriovenous (AV) difference is the difference in concentration or radioactivity per unit volume of blood between arterial (A) and deep femoral venous (V) sites. For valine (val), 2-ketoisovalerate (kiv) and $CO_2$, the quantity of each is expressed as μmol/l (μM) or as amount of radioactivity ($^{14}$C) in dpm/ml. Muscle mass is assumed to be 0·25 times liveweight (LW) (Pethick *et al.* 1981; Butterfield *et al.* 1983). Thus:

Valine decarboxylation (% uptake)

$$= \frac{^{14}CO_2\,(AV) \times 100}{[1\text{-}^{14}C]\text{val (AV)}}. \quad (1)$$

Valine transaminated but not further oxidized (% uptake)

$$= \frac{[1\text{-}^{14}C]\text{kiv (AV)}}{[1\text{-}^{14}C]\text{val (AV)}} \times 100. \quad (2)$$

2-Ketoisovalerate formed from valine but not oxidized (val : kiv) ($\mu$mol/ml)

$$= \frac{[1\text{-}^{14}C]\text{kiv (AV)}}{[1\text{-}^{14}C]\text{val(V)SRA}}. \quad (3)$$

$CO_2$ produced from valine (val : $CO_2$) ($\mu$mol/ml)

$$= \frac{^{14}CO_2\,(AV)}{[1\text{-}^{14}C]\text{val(V)SRA}}. \quad (4)$$

Contribution of valine $CO_2$ to muscle $CO_2$ output (%)

$$= \frac{\text{val} : CO_2 \times 100}{CO_2\,(AV)}. \quad (5)$$

Total $^{14}CO_2$ produced in muscle (TM $^{14}CO_2$) ($\mu$mol min$^{-1}$ kg$^{-1}$ muscle)

$$= \text{val} : CO_2 \times \text{MBF}. \quad (6)$$

Whole body muscle $^{14}CO_2$ (WBM $^{14}CO_2$) ($\mu$mol/min)

$$= \text{TM } ^{14}CO_2 \times 0{\cdot}25 \times \text{LW}. \quad (7)$$

Valine extraction (val ext) ($\mu$M)

$$= \text{val (A)} - \text{val (V)}, \quad (8)$$

i.e. AV difference of valine concentration.

Valine fractional extraction (%)

$$= \frac{[1\text{-}^{14}C]\text{val (AV)}}{[1\text{-}^{14}C]\text{val (A)}} \times 100. \quad (9)$$

True extraction of valine ($\mu$M)

$$= \text{val (A)} \times [\text{valine fractional extraction}/100]. \quad (10)$$

Valine formed by proteolysis (PD rate) ($\mu$mol min$^{-1}$ kg$^{-1}$ muscle)

$$= \left\{\text{val (A)} \times \left[\frac{\text{SRA val(A)}}{\text{SRA val(V)}} - 1\right]\right\} \times \text{MBF}. \quad (11)$$

Valine used for protein synthesis (PS rate) ($\mu$mol min$^{-1}$ kg$^{-1}$ muscle)

= valine extraction − valine catabolized (through transamination, including oxidation) + valine formed by proteolysis

$$= [(\text{val ext}) - (\text{val} : \text{kiv} + \text{val} : CO_2)] \times \text{MBF} + \text{PD}. \quad (12)$$

Muscle protein turnover (MPS, percentage of muscle protein mass per day)

$$= \frac{\text{PS rate} \times 1440 \times 100}{83{\cdot}2 \times 1000}, \quad (13)$$

assuming that the valine content of ovine muscle is 83·2 mmoles/kg muscle (Teleni and Annison 1986).

Total muscle protein synthesized (TMPS) (g/day)

$$= \text{MPS} \times 0{\cdot}18 \times 0{\cdot}25\text{LW}/100, \quad (14)$$

where 0·18 is the assumed fraction of protein in ovine muscle, from data of Williams (1978) for the preruminant calf.

*Calculations for Whole Body*

Output of $^{14}CO_2$ (dpm/min)

$$= 0{\cdot}235 \times \text{LW} \times \text{arterial } CO_2 \text{ SRA} \quad (15)$$

[arterial $CO_2$ SRA expressed as dpm/mmol; 0·235 is an empirical factor obtained by D. B. Lindsay (unpublished data) for the mean respiratory output of mature sheep (mmole min$^{-1}$ kg$^{-1}$ LW)].

% valine released as $CO_2$ at plateau (VI)

$$= \frac{^{14}CO_2 \text{ output} \times 100}{\text{valine infusion rate (dpm/min)}}. \quad (16)$$

$CO_2$ from valine at plateau ($\mu$mol/min)
= valine entry rate (see 19 below) × VI/100. (17)

Valine decarboxylated in whole body by muscle (%)
$$= \frac{\text{whole body muscle } CO_2 \text{ (see 7 above)}}{CO_2 \text{ from valine at plateau (see 17 above)}} . \quad (18)$$

Valine entry rate (mmol/h)
= (I × 1000)/arterial valine SRA (19)
(I is infusion rate of valine (dpm/h) or valine SRA (dpm/$\mu$mol).

Rate of whole-body protein synthesis (g/day)
= (entry rate $-CO_2$ derived from valine (17 above)/valine content of body). (20)
[assumed to be 33·3 mmol/100 g protein (Williams 1978)].

*Statistical Methods*

Significance of difference in estimates of values in fed and starved sheep have been assessed using *t*-tests.

## Results

### *Glucose and Lactate Metabolism in Hind-limb Muscles*

Essential constancy of glucose and lactate metabolism was achieved in fed and starved sheep during the third hour of the [1-$^{14}$C]valine infusion, when the coefficient of variation in both arterial and venous concentrations was less than 5%. Data on the arterial concentrations and AV differences of glucose and lactate across hind-limb muscles in fed and starved sheep are shown in Table 1. Glucose uptake

**Table 1. Arterial blood concentrations (mean, mM) for glucose, lactate, $O_2$ and $CO_2$ and the corresponding hind-limb muscle AV differences in fed and starved sheep**

Each value is the mean of at least three determinations during the third hour of infusion of [1-$^{14}$C]valine. Negative values indicate net output

| | Nutritional state | | S.E. of difference | $P^A$ |
|---|---|---|---|---|
| | Fed ($n=4$) | Starved ($n=5$) | | |
| | **Arterial concentration** | | | |
| Glucose | 3·43 | 3·1 | 0·10 | <0·05 |
| Lactate | 0·58 | 0·64 | 0·10 | n.s. |
| $O_2$ | 6·11 | 4·92 | 0·62 | <0·1 |
| $CO_2$ | 24·15 | 22·46 | 1·11 | n.s. |
| | **AV differences** | | | |
| Glucose | 0·07 | 0·18 | 0·05 | <0·1 |
| Lactate | −0·05 | −0·08 | 0·02 | <0·1 |
| $O_2$ | 1·93 | 1·67 | 0·33 | n.s. |
| $CO_2$ | −1·93 | −1·45 | 0·36 | n.s. |

[A] In this and other tables, n.s. = not significant ($P > 0·1$).

(A − V) and lactate output (V − A) both increased during starvation. Except for glucose, which had a lower ($P < 0·05$) arterial concentration in starved sheep, there were no significant differences in glucose, lactate and blood gas exchange. About 36 and 22% of glucose uptake by the muscles of fed and starved sheep respectively appeared as lactate.

Starvation had no significant effect on respiratory quotient.

**Table 2. Arterial blood concentrations (mean, μM) and arterio-venous differences (mean, μM) for acid and neutral amino acids across the hind-limb muscles of fed and starved (40 h) sheep**

Negative values indicate net output

| Amino acid | Nutritional state Fed ($n=4$) | Starved ($n=5$) | S.E. of difference | $P$ | Nutritional state Fed ($n=4$) | Starved ($n=5$) | S.E. of difference | $P$ |
|---|---|---|---|---|---|---|---|---|
| | **Arterial blood** | | | | **AV differences** | | | |
| Taurine | 132·6 | 95·5 | 14·9 | <0·05 | −4·5 | 10·4 | 8·3 | n.s. |
| Aspartate | 44·3 | 40·3 | 11·0 | n.s. | 0·9 | −10·9 | 5·6 | n.s. |
| Threonine | 155·0 | 172·0 | 32·0 | n.s. | 7·6 | −4·5 | 5·4 | n.s. |
| Serine | 112·1 | 94·5 | 7·8 | n.s. | 4·6 | −3·3 | 2·5 | <0·02 |
| Asparagine | 61·9 | 51·0 | 10·6 | n.s. | 6·6 | −0·6 | 3·6 | n.s. |
| Glutamate | 190·0 | 156·0 | 39·7 | n.s. | 12·3 | −1·1 | 9·9 | n.s. |
| Glutamine | 334·0 | 425·0 | 48·7 | n.s. | −22·1 | −50·4 | 8·6 | <0·02 |
| Glycine | 497·0 | 897·0 | 89·4 | <0·001 | −22·0 | −10·0 | 33·1 | n.s. |
| Alanine | 126·0 | 149·0 | 20·7 | n.s. | −8·0 | −12·4 | 5·5 | n.s. |
| Citrulline | 143·0 | 173·0 | 23·2 | n.s. | 0·6 | 2·9 | 3·1 | n.s. |
| Valine | 177·0 | 202·0 | 27·6 | n.s. | 11·3 | −4·1 | 4·1 | <0·01 |
| Isoleucine | 77·6 | 77·6 | 13·0 | n.s. | 5·4 | −4·1 | 3·0 | <0·05 |
| Leucine | 111·0 | 124·0 | 20·8 | n.s. | 10·6 | −0·6 | 3·6 | <0·05 |
| Tyrosine | 54·5 | 52·3 | 6·0 | n.s. | 0·8 | −3·6 | 2·4 | n.s. |
| Phenylalanine | 56·6 | 46·7 | 5·5 | n.s. | 1·8 | −8·2 | 4·0 | <0·05 |

**Table 3. Hind-limb muscle blood flow (ml $min^{-1}$ $kg^{-1}$ muscle) and valine arterial blood concentration (A, μM) and hind-limb AV blood concentration differences (AV, μM) in fed and starved sheep**

Each blood valine concentration and difference is the mean of at least four and three determinations, respectively, on samples taken during the third hour of infusions of [1-$^{14}$C]valine. Negative AV difference values indicate net output

| Sheep No. | Body wt (kg) Fed | Starved | Muscle blood flow | Valine A | AV |
|---|---|---|---|---|---|
| | | | **Fed sheep** | | |
| 1 | 53·7 | – | 78·5 | 174·5 | 5·5 |
| 2 | 51·5 | – | 76·5 | 144·9 | 13·1 |
| 3 | 61·0 | – | 84·1 | 160·9 | 4·0 |
| 4 | 59·9 | – | 56·7 | 229·1 | 22·7 |
| Mean | 56·5 | – | 74·0 | 177·0 | 11·3 |
| | | | **Starved sheep** | | |
| 5 | 59·0 | 56·0 | 63·8 | 189·0 | −2·7 |
| 6 | 57·1 | 54·2 | 80·0 | 193·7 | −9·8 |
| 7 | 55·8 | 55·2 | 64·0 | 164·0 | −4·7 |
| 8 | 59·3 | 56·5 | 139·0 | 184·1 | −1·5 |
| 9 | 52·9 | 50·2 | 71·9 | 278·8 | −4·6 |
| Mean | 56·8 | 54·4 | 83·7 | 202·0 | −4·7 |
| S.E. of difference | | 2·4 | 16·8 | 27·7 | 4·1 |
| $P$ | | n.s.[A] | n.s. | n.s. | <0·01 |

[A] Mean body weight of starved group was significantly less than their mean fed body weight (weight loss on starving 2·4 ± 0·45 kg).

*Blood Amino Acid Concentrations*

Arterial blood concentrations and AV differences of amino acids across hind-limb muscles in fed and starved sheep are shown in Table 2. Although circulating levels of amino acids tended to increase in response to starvation, a significant increase occurred only with glycine ($P < 0{\cdot}001$), and the concentration of taurine decreased significantly ($P < 0{\cdot}05$). The release from muscle of all amino acids appeared to increase in starvation, but significant ($P < 0{\cdot}05$) changes were seen only for serine, glutamine, the BCAA and phenylalanine. Glutamine, glycine, alanine and taurine were the only amino acids consistently released from muscle in fed sheep, the remaining amino acids showing a net uptake.

*Valine Metabolism in Hind-limb muscle*

Data on AV differences across hind-limb of muscle valine and muscle blood flows in fed and starved sheep are shown in Table 3. Nutritional status had no significant effects on muscle blood flow, or on arterial concentration of valine, but whereas valine was consistently taken up by muscle in fed sheep, starvation resulted in the net release of significant amounts of valine (Table 3).

Corresponding data on the uptake and release of radioactive valine and on labelled 2-ketoisovalerate and $CO_2$ production are shown in Table 4. Valine is extracted from blood by muscle in both fed and starved sheep, the uptakes tending to be higher in starvation.

The release of $^{14}CO_2$ from hind-limb muscles, a measure of decarboxylation, was not significantly different in starved sheep, but the most notable effect of starvation was the increase in transamination revealed by the higher output of [1-$^{14}$C]2-ketoisovalerate (Table 4). The pattern of [1-$^{14}$C]2-ketoisovalerate release is shown in Fig. 3.

Data collated in Tables 3 and 4 were used to calculate valine uptake, decarboxylation and transamination in hind-limb muscles and whole body muscle mass, and to estimate valine entry rate, and whole body protein synthesis (Table 5).

Valine uptake by hind-limb muscles was not significantly changed in starvation, but the extent of valine transamination increased 30-fold. In contrast, decarboxylation of valine remained unchanged.

Quantitative data on the metabolism of valine in the hind-limb muscles of fed and starved (40 h) sheep are summarized in Fig. 4.

*Valine Metabolism in Whole Body*

In the whole body, valine entry rates and rates of overall protein synthesis were not significantly influenced by starvation, but $CO_2$ production was reduced. About 40% of total valine decarboxylation occurred in muscles.

Assay of the 'glucose' fraction eluted from the Dowex anion-exchange column (Fig. 2) showed that in fed sheep there was no transfer of radioactivity from [1-$^{14}$C]valine into glucose, or any other unchanged material. In starvation, however, the 'glucose' fraction showed a low level of radioactivity (SRA 0·5% of that of valine).

*Amino Acid Release from Hind-limb Muscles in Starved Sheep*

Quantitative data on AV differences of amino acids across hind-limb muscles in starved sheep (Table 2) and the known amino acid to tyrosine ratio in sheep

**Table 4. Infusion rates of L-[1-$^{14}$C]valine and mean values of blood arterial concentration (A) and arterio-venous concentration difference (AV) of [1-$^{14}$C)valine ($^{14}$C val), [1-$^{14}$C]2-ketoisovalerate ($^{14}$C kiv) and $^{14}CO_2$, and specific radioactivity (SRA) of arterial valine and $CO_2$ and their corresponding venous SRA expressed as percentage of arterial SRA (V/A) in fed and starved (40 h) sheep**

Blood samples taken during fourth hour of infusion

| Sheep No. | Infusion rate (dpm/min) | A (dpm/ml) $^{14}$C val | A (dpm/ml) $^{14}$C kiv | A (dpm/ml) $^{14}CO_2$ | AV (dpm/ml) $^{14}$C val | AV (dpm/ml) $^{14}$C kiv | AV (dpm/ml) $^{14}CO_2$ | Valine A (dpm/ $\mu$mol) | SRA V/A (%) | $CO_2$ A (dpm/ $\mu$mol) | SRA V/A (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **Fed sheep** | | | | | | |
| 1 | 787347 | 1762 | 13 | 129 | 166 | −8 | −31 | 10097 | 94 | 5645 | 117 |
| 2 | 789238 | 1807 | 48 | 144 | 174 | 0 | −24 | 12743 | 99 | 6195 | 107 |
| 3 | 695377 | 1223 | 0 | 81 | 95 | 0 | −24 | 7601 | 95 | 3447 | 124 |
| 4 | 781167 | 2191 | 65 | 169 | 181 | −1 | −36 | 9564 | 102 | 6261 | 109 |
| Mean | 763282 | 1746 | 32 | 131 | 154 | −2 | −29 | 10001 | 98 | 5387 | 114 |
| | | | | | **Starved sheep** | | | | | | |
| 5 | 789351 | 1905 | 773 | 89 | 318 | −72 | −10 | 10079 | 82 | 4218 | 105 |
| 6 | 787118 | 1889 | 1154 | 114 | 164 | −63 | −14 | 9750 | 87 | 5266 | 103 |
| 7 | 797440 | 1863 | 891 | 113 | 231 | −101 | −14 | 11363 | 85 | 5011 | 106 |
| 8 | 716495 | 1511 | 1006 | 52 | 91 | −58 | −16 | 8208 | 95 | 2342 | 124 |
| 9 | 790580 | 2149 | 781 | 106 | 186 | −89 | −29 | 7809 | 89 | 4274 | 121 |
| Mean | 776197 | 1863 | 921 | 95 | 198 | −77 | −17 | 9442 | 88 | 4222 | 112 |
| S.E. of difference of means | 26224 | 210 | 83 | 21 | 47 | 9 | 4 | 1140 | 3 | 822 | 6 |
| *P* | n.s. | n.s. | <0·01 | <0·05 | n.s. | <0·01 | <0·05 | n.s. | <0·025 | n.s. | n.s. |

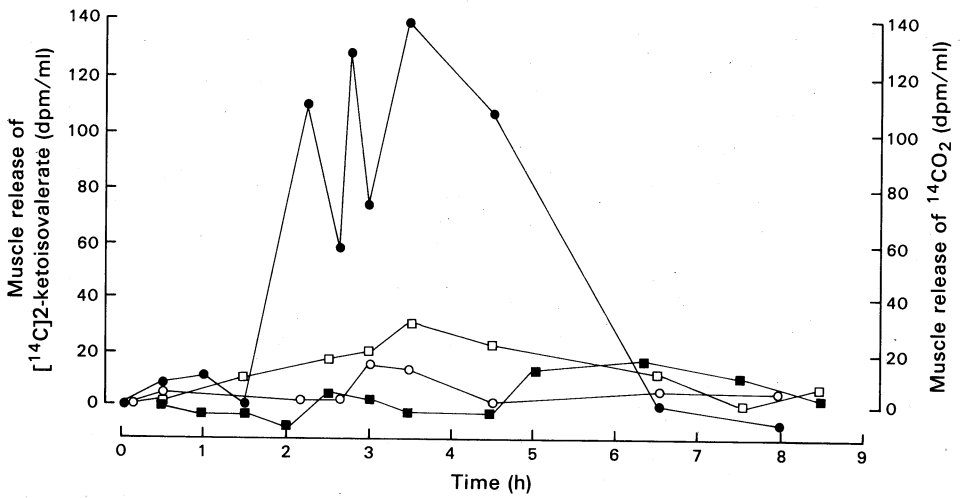


**Fig. 3.** Release from the hind-limb muscles of $^{14}CO_2$ (□) and [$^{14}$C]2-ketoisovalerate (■) in a fed sheep and $^{14}CO_2$ (○) and [$^{14}$C]2-ketoisovalerate (●) in a starved sheep.

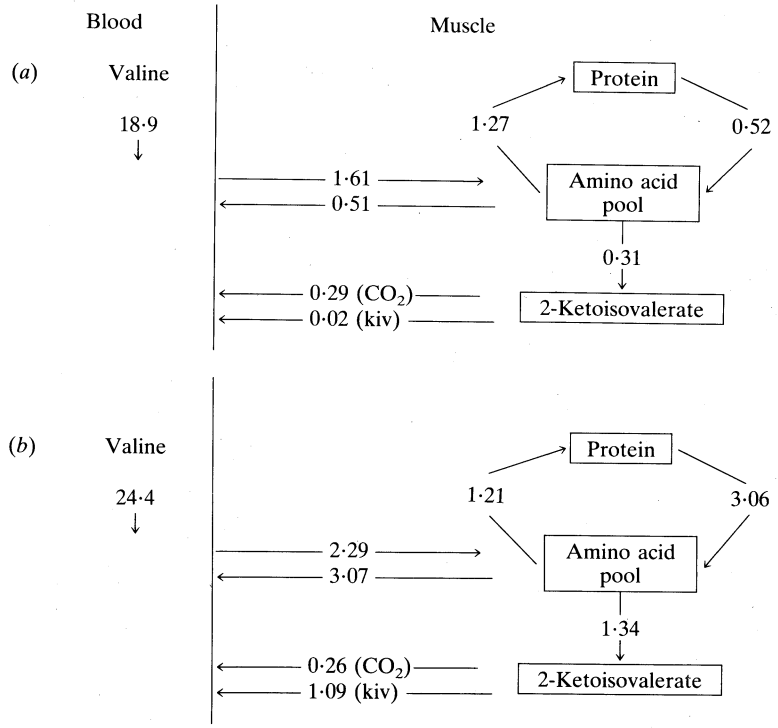


**Fig. 4.** Valine metabolism in the hind-limb muscle of fed (*a*) and starved (*b*) sheep. All values are mmol $kg^{-1}$ $day^{-1}$. Although transamination is reversible, only the net movement could be measured, and is shown as a single flux.

muscle (Teleni and Annison 1986) made it possible to compare the actual release of amino acids with the maximum possible value, assuming that tyrosine is not

**Table 5. Valine metabolism in the hind-limb and whole body and protein synthesis in fed and starved sheep**

| | Nutritional state | | S.E. of difference | *P* |
|---|---|---|---|---|
| | Fed ($n=4$) | Starved ($n=5$) | | |
| **Whole body** | | | | |
| Valine entry rate (mmol/day) | 117 | 137 | 12·0 | n.s. |
| Protein synthesis (g/day) | 307 | 339 | 32 | n.s. |
| $CO_2$ produced from valine (mmol/day) | 10·67 | 9·36 | 1·35 | n.s. |
| **Hind-limb muscle** | | | | |
| Valine extracted (%) | 8·8 | 10·5 | 2·1 | n.s. |
| True uptake of valine (μM) | 15·5 | 20·8 | 4·0 | n.s. |
| Total valine catabolized (% of uptake) | 20·5 | 53·5 | 10·7 | <0·05 |
| Valine converted to 2-ketoisovalerate only (% of uptake) | 1·4 | 43·2 | 7·6 | <0·01 |
| $CO_2$ from valine (% of total) | 0·17 | 0·15 | 0·06 | n.s. |
| Daily rate of protein synthesis (%) | 1·53 | 1·45 | 0·64 | n.s. |
| **Total muscle[A] of body** | | | | |
| Protein synthesis (g/day) | 39 | 35 | 16·0 | n.s. |
| Muscle/whole body (protein synthesis, %) | 12·9 | 10·8 | 5·0 | n.s. |
| $CO_2$ from valine (mmol/day) | 4·32 | 3·02 | 1·01 | n.s. |
| Muscle/whole body (valine, decarboxylated, %) | 43 | 42 | 18 | n.s. |

[A] Muscle mass is assumed to be 25% of liveweight and protein 18% of muscle.

**Table 6. Amino acid to tyrosine ratio (Aa : Tyr) in sheep skeletal muscle (Teleni and Annison 1986) and the maximum possible and actual amino acid outputs from starved sheep hind-limb muscles**

| Amino acid | Aa : Tyr | Possible output (μM) | Actual output (μM) | Possible – actual (μM) |
|---|---|---|---|---|
| Serine | 2·15 | 7·9 | 3·3 | 4·6 |
| Aspartate | 4·06 | 14·9 | 10·9 | 4·0 |
| Glutamate | 6·16 | 22·5 | 1·1 | 21·4 |
| Glycine | 3·31 | 12·1 | 10·0 | 2·1 |
| Alanine | 3·64 | 13·3 | 12·4 | 0·9 |
| Valine | 2·27 | 8·3 | 4·1 | 4·2 |
| Isoleucine | 1·79 | 6·6 | 4·1 | 2·5 |
| Leucine | 3·48 | 12·7 | 0·6 | 12·1 |

catabolized by muscle (Table 6). The results showed that alanine production was negligible, but that a relatively large amount of glutamine was released under the conditions of the experiment.

## Discussion

An intrinsic weakness of the experimental procedure was that neither valine nor 2-ketoisovalerate was isolated, identified and checked for purity. The procedure assumed that there was no significant transfer of radioactivity from [1-$^{14}$C]valine into other amino acids, and that the 2-ketoisovalerate fraction was essentially free of labelled lactate. The latter assumption was largely correct, since efforts to detect labelled lactate in the ketoisovalerate fraction were unsuccessful. The small fixation of $^{14}CO_2$ into glucose in starved sheep, however, raised the possibility of contamination of the labelled valine fraction with other labelled amino acids. The possible transfer of this label into aspartate via $^{14}CO_2$ from oxaloacetate would have led to an overestimate of valine radioactivity. Significant labelled aspartate production is unlikely, however, in view of the channelling of oxaloacetate into gluconeogenesis, particularly in starvation (Ballard *et al.* 1969).

The limited decarboxylation of valine in hind-limb muscles, which was not significantly influenced by feed deprivation (40 h), presumably reflected the low activity of branched-chain ketoacid (BCKA) dehydrogenase in skeletal muscle. Shinnick and Harper (1977) found that in rats, BCKA dehydrogenase was mainly concentrated in the liver. In contrast, branched-chain aminotransferase is higher in rat skeletal muscle than in liver (Krebs 1975; Shinnick and Harper 1977). These observations led to the suggestion that skeletal muscle and liver work in concert to metabolize BCAA, by transaminating them in muscle and oxidizing the resultant keto-acids in liver (Wohlhueter and Harper 1970; Shinnick and Harper 1976). The results obtained here *in vivo* are consistent with these views.

Spydevold (1979) showed that octanoate, and to a lesser extent palmitate, stimulated the oxidation of 2-ketoisovalerate in perfused rat hindquarter although Tessari *et al.* (1986) reported that elevated plasma FFA levels reduced leucine oxidation. The absence of any effect of fasting (and thus elevated FFA) in the present study support neither of these findings.

If it is assumed that tyrosine is not catabolized by hind-limb muscles, the amino acid to tyrosine ratio in muscle may be used to calculate the maximum possible release of each amino acid (Table 6). By subtracting the measured release from this value, an estimate of amino acid catabolism is obtained. Published data (see Lindsay and Buttery 1980) indicates that the amino acids which are catabolized to an appreciable extent in ruminant muscles include aspartate, glutamate and BCAA. In the present study the sum of the difference between maximum possible and actual release of these amino acids (44·2 $\mu$mol) is similar to the output of glutamine (50·4 $\mu$mol), suggesting that glutamine is the major carrier of $-NH_2$ from muscle. The difference between possible and actual output of alanine was only 0·9 $\mu$mol, indicating that in this study alanine had no significant role even as a carrier of $-NH_2$.

The contribution of muscle to whole-body protein synthesis in this study was small relative to values reported earlier for sheep (Buttery *et al.* 1975) and cattle (Lobley *et al.* 1980). The sheep used in this study were mature Merino wethers, however, which would be expected to have relatively low rates of muscle protein synthesis (see Millward *et al.* 1975). The main reason for the low value relative to those reported by Buttery *et al.* (1975) were the assumptions in the present study that muscle containing 18% protein accounted for 25% of body weight, whereas the corresponding values used by Buttery *et al.* (1975) in their calculations were 20% and 47% respectively.

Comparison of values for fractional rate of protein synthesis (FRS) in this and other studies (Lobley *et al.* 1980; Bryant and Smith 1982) reveals similar values for animals of the same age, and indeed FRS values for the semitendinosus and gastrocnemius muscles reported by Bryant and Smith (1982) are similar to the values obtained in the present studies. Differences in FRS between types of skeletal muscle imply that extrapolation from a single muscle type to whole-body skeletal muscle should be undertaken with caution.

The calculation of protein synthesis from whole-body valine flux in the present study has made allowance for valine degradation. By using the value of 0·24 mmol $min^{-1}$ $kg^{-1}$ for total $CO_2$ production (D. B. Lindsay, unpublished data) it can be shown that oxidative decarboxylation accounted for 10 and 7% of valine flux in fed and starved sheep respectively. Corresponding calculated values for protein synthesis are 319 and 384 g/day respectively. Correction for net transamination, a second degradative pathway, although of negligible significance in fed sheep, would reduce still further the calculated rates of protein synthesis in starved animals. Lower rates of protein synthesis would then increase the calculated percentage contribution of muscle protein synthesis (Table 5).

Fasting did not have any significant effect on protein synthesis in muscle, but did result in a highly significant increase in the rate of proteolysis (Fig. 4).

The uncertainty surrounding the true precursor pool for protein synthesis in muscle remains unresolved. Whether the best estimate of the rate of protein synthesis would be from the use of SRA of the free amino acids in tissue (Fern and Garlick 1974) or from a 'pool that equilibrated with extracellular amino acids' (Hider *et al.* 1971) is still not clear. Nevertheless, the use of either is likely to result in an increased estimate of both amino acid entry rate and whole-body protein synthesis. The current study used the arterial valine SRA as an estimate of the valine SRA in the precursor pool. The error involved in this assumption is not known, but the arterial SRA is less 'tissue-biased' than muscle venous SRA. In a recent study in muscle of neonatal lambs (Oddy and Lindsay 1986) it was shown that the estimate of rate of protein synthesis from AV studies was not significantly different from that obtained more conventionally by slaughter of animals and comparison of the SRA of free and protein-bound BCAA (leucine).

The errors involved in the calculations of whole-body protein synthesis would appear to act in opposite directions. However, as has been pointed out by Bryant and Smith (1982), it is unlikely that they cancel out exactly and caution in the interpretation of results should be exercised. Furthermore, in fed sheep net splanchnic valine utilization would be expected to be high, and would not contribute to the measured valine entry rate, whereas, in fasted sheep, little net valine utilization would be expected by the splanchnic bed. This would result in systematic underestimation of protein synthesis rate in fed compared with fasted sheep by the procedure used in this study.

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