# Effect of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio-venous techniques

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**Whole-body protein synthesis, estimated by the irreversible loss rate procedure, and hind-leg protein metabolism determined by arterio-venous techniques were monitored in response to three nutritional conditions (approximately 0·6, 1·2 and 1·8 × energy maintenance (M)) in ten wether lambs (33 kg average live weight). In all lambs and treatments measurements were based on radiolabelled phenylalanine, but the terminal procedures (five at 0·6 × M and five at 1·8 × M) also included infusion of [1-$^{13}$C]leucine; this permitted comparison of amino acids catabolized (leucine) and non-metabolized (phenylalanine) by the hind-limb tissues. Whole-body protein synthesis increased with intake and the relationship with energy expenditure was slightly lower than that reported previously for pigs and cattle. The efficiency of protein retention:protein synthesis did not exceed 0·25 between the two intake extremes. Effects of intake on amino acid oxidation were similar to those observed for cattle. Hind-limb protein synthesis also increased significantly ($P < 0·001$) in response to intake. Estimates of protein gain, from net uptake values, indicated that the tissues made a greater proportional contribution to total protein retention above M and to protein loss below M, emphasizing the role played by muscle tissue in providing mobile protein stores. The rates of protein synthesis calculated depended on the selection of precursor (blood) metabolite, but rates based on leucine always exceeded those based on phenylalanine when precursor from the same pool was selected. The incremental efficiency of protein retained: protein synthesis was apparently unity between 0·6 and 1·2 × M but 0·3 from 1·2 to 1·8 × M. Blood flow through the iliac artery was also proportional to intake. Leucine and oxo-acid catabolism to carbon dioxide increased with intake such that the metabolic fate of the amino acid was distributed in the proportion 2:1 between protein gain and oxidation. The rates of oxidation were only 1–3% the reported capacity of the rate-limiting dehydrogenase enzyme in muscle, but sufficient enzyme activity resides in the hind-limb adipose tissue to account for such catabolism.**

**Food intake: Protein synthesis: Hind-limb protein metabolism: Lamb**

It has long been recognized that mammalian protein metabolism operates in the form of a major substrate cycle, with the rates of the opposing reactions, i.e. protein synthesis and protein breakdown, far exceeding inflow (amino acid supply) and outflow (protein deposition or export). In cattle, for example, dietary amino acid supply and protein gain represent only approximately 0·31 and 0·06 of the rates of protein synthesis and degradation in animals consuming more than maintenance (M) energy (from Lobley *et al.* 1987) and, thus, small changes in the balance of the two processes can produce marked effects on net anabolism and production efficiency. One effective modulator of protein turnover is intake and for a variety of commercial species, including pig (Reeds *et al.* 1980),

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sheep (Oddy & Lindsay, 1986; Pell *et al.* 1986; Teleni *et al.* 1986; Oddy *et al.* 1987) and cattle (Lobley *et al.* 1987), whole-body protein synthesis increases with nutritional improvement.

Alterations in whole-body dynamics need to be interpreted with caution, however, as the protein metabolic activity (i.e. g protein synthesized/d per 100 g tissue protein) of the various tissues varies widely, with gastrointestinal and hepatic turnover being 5–50-fold greater than that for skeletal muscle or skin (e.g. Lobley *et al.* 1980; Davis *et al.* 1981; Schaefer *et al.* 1986; Seve *et al.* 1986; Attaix *et al.* 1988). Thus, skeletal muscle which comprises 0·35–0·55 of body protein probably contributes only 0·12–0·33 of total protein synthesis (Lobley *et al.* 1980; Davis *et al.* 1981; Attaix *et al.* 1988), and significant responses in muscle protein dynamics may be masked, in whole-body terms, by the contributions of the other tissues.

Initial attempts to monitor tissue protein synthesis changes in ruminants in response to various stimuli followed the same approach as adopted for laboratory species, i.e. single, terminal measurements. With large animals the ability to catheterize the main blood vessels which supply tissue beds has led, however, to the introduction of tracer-based methods in which net removal of isotopically-labelled amino acids by organs can be partitioned between synthesis and, where appropriate, oxidation (e.g. Cheng *et al.* 1985, 1987; Oddy & Lindsay, 1986; Pell *et al.* 1986; Teleni *et al.* 1986). The advantages of this technique are several and include the ability to perform repeat measurements in the same animal, coupled with the simultaneous determination of protein synthesis, protein gain and thus, by difference, protein degradation. Furthermore, for appropriate tissues the extent of amino acid catabolism can be followed by measurement of labelled carbon dioxide release.

With ruminants the arterio-venous technique has been applied predominantly in sheep (but see Boisclair *et al.* 1988) with the effect of intake monitored usually in the fed–fasted condition. The results from these studies have not been entirely consistent between the fasted and fed states, with protein synthesis and degradation increasing (Pell *et al.* 1986; Oddy *et al.* 1987) or alternatively degradation decreasing while synthesis is unaltered (Teleni *et al.* 1986). In view of the unique, and changing, conditions which pertain in the fasted state, a more representative view of responses in protein metabolism to intake is obtained from following changes between sub- and supra-M; an approach adopted both in the present study and in cattle (Boisclair *et al.* 1988). In addition, comparisons are also made between amino acids, which are (leucine) or are not (phenylalanine) catabolized by hind-limb tissues, to allow assessment of the combined role of skeletal muscle and adipose tissue in regulation of amino acid oxidation. Comparisons of synthesis rates estimated by the arterio-venous procedure with those determined by the large (flood)-dose and continuous-infusion methods are given in the companion paper (Lobley *et al.* 1992). Preliminary accounts of the bioenergetics and phenylalanine kinetics have been reported elsewhere (Harris *et al.* 1989).

## METHODS

### *Animals and diet*

Ten Suffolk-cross lambs (6–9 months old, 26–35 kg) were trained to metabolism crates and a respiration hood. A pelleted grass diet (10 MJ metabolizable energy (ME)/kg dry matter (DM); 26 g nitrogen/kg DM; DM 0·88 as fed) was supplied in equal portions from twenty-four 1 h automatic feeders.

### *Surgical procedures*

Each animal was prepared, under halothane–nitrous oxide anaesthesia, with two indwelling catheters. A silastic sampling catheter (0·76 mm i.d., 1·65 mm o.d.; Dow Corning Health Care Group, Reading) was inserted via the left femoral artery into the aorta (caudal to the

renal artery; 250 mm from the entry point); in a few animals access to the aorta was via the mesenteric artery. Exterior to the artery the catheter was sheathed with polyvinyl chloride (PVC) tubing (1·57 mm i.d., 2·08 mm o.d., Dural Plastics & Engineering, Dural, NSW, Australia). The second permanent catheter (PVC, 0·8 mm i.d., 1·2 mm o.d.; Dural manufacture) was inserted via the right circumflex iliac artery 10–20 mm into the external iliac artery towards the aorta; this was used for close infusion of *p*-aminohippurate (PAH). A transit-time blood flow probe (either 3 mm or 4 mm dependent on vessel dimensions; Transonic Systems Inc., Ithaca, NY, USA) was placed around the right external iliac artery just distal to the junction with the circumflex iliac artery. A minimum of 3 weeks was allowed for recovery, although most animals were on full appetite within 4–5 d. At least 48 h before each kinetic measurement temporary catheters were inserted, under local anaesthesia, into an external jugular vein and the right external iliac vein. The latter (1·57 mm i.d., 2·08 mm o.d.) was introduced 300 mm via a superficial tarsal vein with the tip approximately adjacent to the transit-time probe position. For the first two infusions the jugular infusion catheter was single-bore PVC (1·0 mm i.d., 1·6 mm o.d.; Portex Ltd, Hythe, Kent) while for the third measurement period a double-bore PVC catheter (0·9 mm i.d., 2·0 mm o.d.; Dural) was inserted to allow both continuous infusion of isotopes and injection of a large (flood) dose of phenylalanine (Lobley *et al.* 1992).

### *Experimental design*

Each sheep was offered three intakes, 300, 600 and 900 g as fed/d for 2 weeks. In alternate sequence five animals were fed ascending intake and five descending intake. Measurements were made over the last 4 d of each period when the animals were harnessed for collection of faeces by bag and urine by suction into 2 M-sulphuric acid, while in a respiration hood for measurement of gaseous exchange by an open-circuit procedure which also allowed quantification of radioactive $CO_2$ excretion (see MacRae *et al.* 1988). On the last day of each experimental period amino acid kinetics were measured; in all cases labelled amino acids were dissolved in sterile saline (9 g sodium chloride/l) containing heparin (100 IU/ml). For the first two periods this consisted of infusion for 7–8 h of [$^3$H-2,6 ring]phenylalanine (55 $\mu$Ci/h, 1·2 $\mu$Ci/ml). For the last period a mixture of [U-$^{14}$C]phenylalanine (0·25 $\mu$Ci/ml, 10 $\mu$Ci/h) and [1-$^{13}$C]leucine (99 atom %, 0·16 mmol/h, 0·004 mmol/ml) was infused for 8 h. Initially a few biopsies were taken, under local anaesthesia, from m. longissimus dorsi at the end of the [$^3$H]phenylalanine infusions; the amounts removed were approximately 1 g and provided information on the specific radioactivity of the tissue homogenate free pool (see Lobley *et al.* 1992). Later this was discontinued to avoid trauma to the animals. During the last 3 h of infusion (plus the period of flood dose; see Lobley *et al.* 1992) exhaust gas from the hood was bubbled through 0·7 M-sodium hydroxide (5 l/min) collected every hour and prepared as barium carbonate as described previously (Lobley *et al.* 1985).

Samples of expired air, arterial and venous blood were collected 24 h before the terminal infusion for determination of background $^{13}$C enrichment in exhaled $CO_2$, blood bicarbonate and the C-1 of protein-bound leucine. As reported for other species (e.g. Read *et al.* 1984) there was a significantly greater background enrichment in arterial compared with venous bicarbonate (values not shown).

*Arterio-venous sampling.* Each infusion was allowed to progress for 4 or 5 h to establish pseudo-plateau conditions for the primary (amino acid) pool. Infusion of PAH (0·2 g/h; 40 ml/h) into the external iliac artery was started at approximately 3 h. Blood samples were then withdrawn on a continuous basis at 30 ml/h from both the aorta and external iliac vein by use of a peristaltic pump. Clotting was prevented by the inclusion of heparin in the infusate and also by passing the blood collection lines through an ice-bath; samples were collected under liquid paraffin in an ice-bath over 20 min periods. Total collection times

were 3 h for the first two infusions with each animal and for 3 h plus the length of the (flood) dose for the terminal infusion (measurements during the flood period are not included in the reported data; see Lobley *et al.* 1992).

Blood samples were then analysed for oxygen and (plasma) $HCO_3$ content (Radiometer ABL3 Blood Gas Analyser; Radiometer Ltd, Crawley, UK) and packed cell volume (PCV) determined by haematocrit. Then, two 0·5 g samples were removed for PAH determination and the remainder subdivided according to whether the first two or the terminal infusions were to be analysed. For [$^3$H]phenylalanine infusions all the blood was deproteinized (see below). For terminal runs, two 0·75 ml samples were injected into evacuated 10 ml red-top vacutainers containing 1 ml frozen lactic acid and anti-foam reagent for blood $H^{13}CO_3$ analysis (see Read *et al.* 1984); 6–7 g (accurately weighed) was then mixed with sufficient 0·5 mM-L-norleucine and 80 $\mu$M-2-oxohexanoate to yield final concentrations of 150 $\mu$M and 15 $\mu$M for the two internal standards respectively. This was then subdivided into two 1 g samples and one 4 g sample for [1-$^{13}$C]leucine, 2-oxo-4-methyl[1-$^{13}$C]pentanoate (MOP) and [$^{14}$C]phenylalanine analysis respectively.

The infusions of [$^{14}$C]phenylalanine and [$^{13}$C]leucine were continued when a large dose of [$^{15}$N]phenylalanine was injected rapidly (0·2 g 99 atoms % [$^{15}$N]phenylalanine plus 3·0 g L-phenylalanine in 110 ml sterile saline). The experiment was terminated either 30 or 60 min after injection of the large dose of phenylalanine when the animal was killed by pentobarbitone overdose and body tissues rapidly excised (see Lobley *et al.* 1992).

### *Analyses*

p-*Aminohippurate and blood flow comparisons.* Gravimetric, rather than volumetric, procedures were found to improve the precision of PAH determination in blood ($< 1$ % for triplicate analyses) by the Bratton–Marshall procedure (Smith *et al.* 1945). Blood flow estimates based on PAH routinely exceeded those from the transit-time probe (for the same time-period) by 2–3-fold. Two additional sheep were examined under terminal anaesthesia and a similar discrepancy noted. Gravimetric blood flow through the iliac artery was then determined, by short periods (30 s) of exsanguination through a catheter. These values agreed, within 1 g/min, with those obtained from probe measurements taken at the same time and were similar to blood flow values obtained from the probe just before exsanguination. Blood flow calculations were, therefore, based on values from the transit-time probes.

[$^3$*H*]- *and* [$^{14}$*C*]*phenylalanine determinations.* To known weights (3–5 g) of blood was added 0·5 mM-L-norleucine (1·0:0·2, w/w) and the mixture lysed with 1 vol. ice-cold water followed by 2 vol. sulphosalicylic acid (120 g/l; SSA). Phenylalanine concentration was determined on 1 ml supernatant fraction using an amino acid analyser (Locarte Co. Ltd, London), with measurement of adjacent arterial and venous samples. The remainder of the supernatant fraction was desalted, the phenylalanine separated by ion-exchange chromatography and the specific radioactivity determined based on earlier procedures (Lobley *et al.* 1980) using Optiphase X (Pharmacia LKB, Milton Keynes) as liquid scintillant.

*Stable isotope measurements.* Blood samples were deproteinized with SSA, derivatized and analysed for free leucine and MOP enrichments by gas–liquid chromatography–mass spectrometry (GCMS: VG 12–250 quadrupole mass spectrometer; VG MassLab, Manchester) as described by Calder & Smith (1988). Values were expressed as atoms % excess (ape) over background samples. MOP concentration was determined from the GCMS peak areas, corrected to the known addition of hexanoate. Blood protein precipitates from the pre-infusions were retained and used to give an indication of background enrichment for body protein (see Lobley *et al.* 1990, 1992).

Blood $H^{13}CO_3$ enrichment determination was based on the procedure described by Read

*et al.* (1984); differences in enrichment of acid-liberated $^{13}CO_2$ between blood, plasma and erythrocytes were found to be not significant (values not shown). In consequence, blood enrichments were measured throughout, for convenience, and these were then assumed to be the same as for plasma. Exhaled $^{13}CO_2$ trapped in alkali and then stored as barium carbonate was liberated and analysed as described by McGaw *et al.* (1988) using a gas-isotope-ratio mass spectrometer (SIRA 12; VG Isogas, Middlewich, Cheshire).

### *Calculations*

*Whole-body irreversible loss rate (ILR; flux).* Basically this followed the accepted convention for radio-tracer procedures based on the specific radioactivity (SRA) of relevant metabolite, i.e.

$$\text{ILR (mmol/h)} = \frac{\text{infusion rate } (\mu\text{Ci/h})}{\text{SRA arterial blood free amino acid } (\mu\text{Ci/mmol})},$$

$$\text{fractional oxidation } (FO_c) = \frac{\text{radioactivity as expired } ^{14}CO_2 \ (\mu\text{Ci/h})}{\text{infusion rate } (\mu\text{Ci/h}) \times s},$$

$$\text{amino acid for protein synthesis } (ILR_{syn}; \text{ mmol/h}) = (1 - FO) \times \text{ILR},$$

where $s$ allows for the sequestration of $CO_2$ label over infusions of this duration in sheep, and is 0·64 for both low and high intakes (H. Rocha and G. E. Lobley, unpublished results).

The same theoretical basis was used to calculate whole-body ILR when [$^{13}$C]leucine was infused except for modifications to accommodate the use of non-tracer quantities of the amino acid. As leucine is generally in excess relative to other amino acids in rumen microbial protein (the major amino source for sheep fed on grass pellets; see MacRae & Reeds, 1980), additional leucine supplied in the infusate was assumed to increase oxidation by an equivalent amount. Therefore,

$$\text{leucine ILR (mmol/h)} = \frac{\text{infusion rate (mmol/h)} \times \text{infusate enrichment (ape)}}{\text{arterial MOP enrichment (ape)}},$$

$$\text{total leucine oxidation (LO; mmol/h)} = \frac{CO_2 \text{ production (mmol/h)} \times CO_2 \text{ enrichment (ape)}}{\text{arterial MOP enrichment (ape)} \times s}$$

$$\text{endogenous leucine oxidation (ELO; mmol/h)} = \text{LO} - \text{infused leucine},$$

$$\text{leucine for protein synthesis } (ILR_{syn}; \text{ mmol/h}) = \text{ILR} - \text{LO}.$$

Conversion of ILR (mmol/h) to whole-body protein synthesis (g protein/d) was by the factor 113·1 or 47·6 for phenylalanine and leucine respectively (see Lobley *et al.* 1980: based on amino acid contents for total body protein in sheep of 3·5 and 6·6 g/16 g N respectively; J. C. MacRae, personal communication).

*Hind-limb amino acid kinetics.* These were based on the models shown in Fig. 1 (Oddy & Lindsay, 1986). For phenylalanine it was assumed that no amino acid catabolism occurs across the limb; this assumption was supported by a preliminary study in which no release of $^{14}CO_2$ occurred across the limb during infusion of [1-$^{14}$C]phenylalanine (values not shown). Similarly no evidence could be found, using mass spectrophotometric analysis, of the presence in iliac vein blood of the oxo-acid (*p*-hydroxy-phenylpyruvate) which would be formed during phenylalanine (tyrosine) oxidation.

Net retention of phenylalanine, presumed to be exclusively for protein gain (i.e. no oxidation, synthesis of phenylalanine-derived metabolites nor expansion or contraction of the intracellular and extracellular free phenylalanine pools occurred), was calculated as:

$$\text{net retention of phenylalanine (mmol/min)} = (P_a - P_v) \times BF,$$

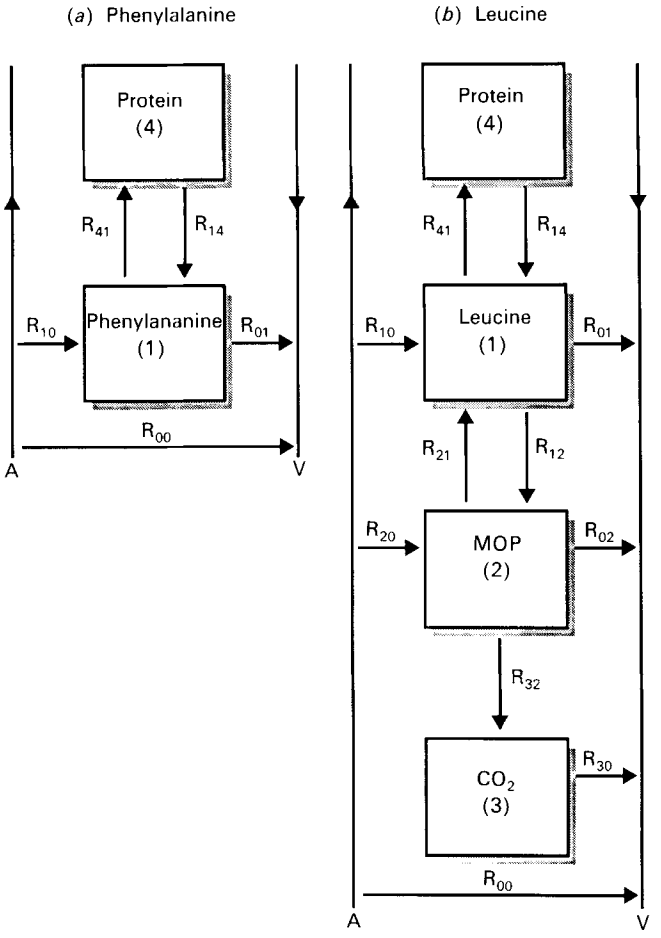


Fig. 1. Kinetic transfers of substrates and isotope tracers through the various body pools, including intracellular free leucine or phenylalanine (1), oxo-acid (2), carbon dioxide (3) and protein (4), of the lamb hind-limb: (*a*) phenylalanine, which is not catabolized by the tissues involved and assumes no changes in the free pool size, (*b*) leucine where interconversions to the oxo-acid, with associated further decarboxylation, can occur. $R_{xy}$, the movement of material to $pool_x$ from $pool_y$. Each pool is represented as a single entity, i.e. further compartmentalization does not occur, and flows of, for example, leucine to carbon dioxide or 4-methyl-2-oxo-pentanoate (MOP) to protein must pass through and equilbrate with the intervening pools (but see Pell *et al.* 1986). Net uptake of amino acid ($\mu$mol/min) for phenylalanine (P) and leucine (L) is represented by, $R_{10}-R_{01}$ (P); and $(R_{10}-R_{01})+(R_{20}-R_{02})-R_{30}$ (L). Kinetic flow of isotope ($\mu$Ci/min) is represented by the same equation; this is converted into movement of amino acid (mmol/min) by division with $S_y$, where $S_y$ represents the isotopic activity ($\mu$Ci/mmol) of the precursor pool(s) (see pp. 394–395). A, arterial; V, venous. For details of procedures, see pp. 390–395.

over each time interval (20 min), where $P$ represents free phenylalanine concentration (mM) for arterial (a) and venous (v) blood and $BF$ is blood flow (kg/min).

Phenylalanine for protein synthesis was estimated from the net removal of isotope as phenylalanine, i.e.:

$$\text{phenylalanine for protein synthesis (mmol/min)} = \frac{(P_a \times S_a - P_v \times S_v)}{S_y} \times BF,$$

where $S$ represents the SRA for free phenylalanine, and y is whichever free pool is selected

to represent best the true precursor pool for protein synthesis. In the present study rates were calculated based on either arterial or venous values (see Table 3).

Because leucine is metabolized by certain tissues of the hind-limb the calculations are more complex. Net leucine retention is the difference between arterial and venous concentrations of leucine corrected for changes in the net output of oxo-acid and oxidation to $CO_2$ of the C-1 atom. Thus,

$$\text{net leucine retention (mmol/min)} = ((L_a - L_v) - (M_a - M_v)) \times BF - \left(\frac{(C_v \times E_{c,v} - C_a \times E_{c,a})}{E_{m,v}} \times BF(1 - \text{PCV})\right),$$

where $L$ and $M$ are the respective concentrations (mM) of free leucine and MOP in blood and $C$ the concentration (mM) of $CO_2$ in plasma. $E$ signifies the enrichment (as ape above appropriate background samples) of the metabolites. $E_{m,v}$ is the enrichment of MOP in iliac vein blood and was taken as most representative of the isotopic activity in the site of the branch-chain 2-oxo-acid-dehydrogenase in the tissues (but see p. 403). Plasma flow (PF) is represented by $BF \times (1 - \text{PCV})$; because the gas analysis measurement gives $CO_2$ concentration in plasma and not total blood, this approach was necessary.

In a similar manner to the phenylalanine observations, leucine for protein synthesis (mmol/min) was calculated as:

$$\frac{((L_a \times E_{l,a} - L_v \times E_{l,v}) - (M_a \times E_{m,a} - M_v \times E_{m,v})) \times BF - (C_v \times E_{c,v} - C_a \times E_{c,a}) \times PF}{S_y},$$

where $S_y$ again represents the single most practical substitute for the true precursor; in line with other workers three alternatives, $E_{l,a}$, $E_{l,v}$ and $E_{m,v}$, were selected. Other models can be considered in which multiple components are introduced (see e.g. Oddy & Lindsay, 1986). In practice such refinements made no qualitative and little quantitative difference compared with the appropriate single value. This coupled with the uncertainty as to which, if any, of the easily sampled pools was the most suitable (see Lobley *et al.* 1992) determined the adoption of this simpler procedure.

Leucine from protein degradation was then calculated by difference, i.e. leucine for protein synthesis − net leucine retention.

Leucine oxidation ($LO_h$) across the hind-limb was that isotope lost as $CO_2$,

$$LO_h \text{ (mmol/min)} = \frac{(C_v \times E_{c,v} - C_a \times E_{c,a})}{E_{m,v}} \times PF.$$

### *Statistical analysis*

For regression analyses, the data were first tested for differences in slopes and intercepts between animals. In no case were these significant and so a common slope and intercept were applied. Comparison of the effects of all three levels of intake (i.e. the phenylalanine data) required analysis by two-way analysis of variance, with animals as blocks, intakes as treatments, with period as a covariate. There was no evidence of a linear time trend within the analyses. The design adopted enabled five animals to be examined at low and high intakes during the terminal periods (Lobley *et al.* 1992) but, in consequence, it proved impossible to distinguish differences between quadratic time effects and the treatment comparison (i.e. medium *v.* high, low average). On analysis it was found that only in two situations was such a comparison significant ($PS_{phe,art}$; $PS_{phe,ven}$; Table 3). Since related biological phenomena were not significant for quadratic time effects and since the time difference between the three intake studies within each animal encompassed only 4 weeks

Table 1. *Whole-body phenylalanine and protein kinetics in sheep offered different daily intakes of grass pellets (300 g (L), 600 g (M), 900 g (H))**

(Values are means for ten lambs measured at each intake)

| Intake... | L | M | H | SED | Residual df† | Statistical significance of intake effects: *P* |
|---|---|---|---|---|---|---|
| ILR (mmol/h) | 1·446 | 1·975 | 2·544 | 0·087 | 17 | < 0·001 |
| $FO_c$‡ | 0·118 | nd | 0·168 | 0·039 | 8 | 0·233 |
| $PhO_c$§ (mmol/h) | 0·160 | 0·282 | 0·430 | 0·033 | 17 | < 0·001 |
| PS‖ (g/d) | 144 | 191 | 239 | 10 | 17 | < 0·001 |
| PD¶ (g/d) | 153 | 183 | 216 | 10 | 17 | < 0·001 |
| $Phe_{art}$ (μM) | 36·9 | 45·8 | 57·2 | 11 | 17 | < 0·001 |

ILR, irreversible loss rate; $FO_c$, corrected fractional oxidation; $PhO_c$, corrected phenylalanine oxidation; PS, protein synthesis; PD protein degradation; $Phe_{art}$, arterial phenylalanine concentration; SED, standard error of difference; nd, not determined.

* For details of procedures, see pp. 390–395.

† For a complete data set residual df is 17, except for $FO_c$, which was based on five animals measured at L and H intakes when residual df is 8.

‡ Calculated from measured fractional oxidation/*s*, where *s* is the fractional sequestration of labelled carbon dioxide set at 0·64 for both L and H intakes (H. Rocha and G. E. Lobley, unpublished results) and assumed similar for M.

§ Calculated from (ILR × $FO_c$); values for M intake are based on value for $FO_c$ of 0·143, i.e. mean of $FO_c$ values at L and H intakes.

‖ Calculated from ILR × (1 − $FO_c$) × 113·1 (see pp. 393–395).

¶ Calculated from PS − (nitrogen retention × 6·25).

and the total experiment spanned many months, it was considered more likely (but not conclusive) that the differences were due to treatment rather than period. For the complete data set, i.e. ten animals each at three intakes, plus appropriate covariate correction, the residual df were 17. For all leucine values and phenylalanine oxidation, data exclusive to period 3, effects of intake between animals were compared by one-way analysis of variance (8 residual df).

## RESULTS

N and energy balance observations have been reported previously (Harris *et al.* 1989) and followed the expected pattern with the lambs in negative N and energy retention (−1·4 g/d and −0·9 MJ/d respectively) when offered 300 g grass pellets per d (L), in slight positive balance at 600 g (M) intake (1·5 N g/d and 0·7 MJ/d) and in positive retention at 900 g/d (H; 4·1 N g/d and 1·8 MJ/d). The apparent incremental efficiencies of utilization of dietary N and energy were 0·41 (SE 0·02) for dietary N and 0·53 (SE 0·04) for ME between 300 and 900 g/d intake.

### *Whole-body amino acid kinetics*

ILR values and derivatives for phenylalanine kinetics are shown in Table 1. ILR, phenylalanine oxidation, protein synthesis, protein degradation and the arterial free phenylalanine concentration all increased significantly with each increment of intake. Corrected fractional oxidation ($FO_c$) increased between the L and H intakes, but not significantly. Based on a body composition of 155 g protein per kg live weight (from N × 6·25; J. C. MacRae, personal communication), the minimum whole-body fractional synthesis rates (FSR; × 100 and based on arterial phenylalanine kinetics) were 2·7, 3·9 and 4·9 %/d at L, M and H intakes respectively. The incremental change in protein gain, i.e. N

retention × 6·25: whole-body protein synthesis, was 0·32 (SE 0·03) between the lower and upper intakes.

The corresponding values for whole animal leucine kinetics are shown in Table 2. Although based on fewer observations, five measurements only on different animals at the L and H intakes, the trends were very similar with significant increases in all variables (including $FO_c$) at the higher ration. The derived total protein synthesis values were greater when based on leucine rather than phenylalanine (for the ten periods on which both determinations were made the difference was 45 g, $P < 0·001$). The relationship between energy expenditure and protein synthesis is shown in Fig. 2.

### *Hind-limb metabolism*

*Blood flow.* This increased significantly in response to each increment of intake (Table 3). During the experiment animals were generally allowed postural freedom; while this had the advantage of producing a more normal situation it introduced complications on the bioenergetics and haemodynamics for the hind-limb (Harris *et al.* 1989). Standing quietly approximately doubled blood flow, compared with the lying condition (values not shown) and, as the fractional extraction of $O_2$ was hardly altered (values not shown), there was a corresponding increase in $O_2$ exchange.

Based on dissections of lambs of similar age and weight, the de-fleeced leg tissues probably drained by the iliac vein at the point of catheter placement comprised 0·078 of body-weight and in gross composition were 0·62 muscle (and fat), 0·22 bone and 0·16 shaved skin (similar to values for Merinos reported by Oddy *et al.* 1984). The tissue masses obtained by dissection do not necessarily relate to their metabolic contributions since FSR of bone and skin greatly exceed that of muscle (Lobley *et al.* 1980; Preedy *et al.* 1983; Attaix *et al.* 1988; Lobley *et al.* 1992). Mean blood flow ranged from 26 to 91 g/kg leg tissue per min, in line with values based on acute measurements with $^3H_2O$ or tritiated ethanol as marker for sheep-limb leg muscle (e.g. 70–136 ml/kg per min, Oddy *et al.* 1981; 57–139 ml/kg per min, Teleni *et al.* 1986; 73 ml plasma flow/kg per min, Brown *et al.* 1990). Blood flow through the separate tissues could not be evaluated in the present study but blood volumes in muscle, bone and skin from anaesthetized sheep are similar (12·3, 13·6 and 16·1 g/kg respectively; Weaver *et al.* 1989) and skin blood flow in thermoneutral conditions is similar per unit wet tissue to muscle (Hales, 1973).

*Phenylalanine kinetics.* Net uptake of phenylalanine across the leg tissues followed whole-body N retention and changed significantly from negative to positive between the low and high intakes (Table 3). The correlation between arterial phenylalanine concentration and net uptake (Fig. 3) was superior to that of supply of phenylalanine (i.e. arterial concentration × blood flow) *v.* uptake ($r$ 0·59; values not shown). While at the lowest intake blood-free phenylalanine concentrations were similar (mean 37 (SE 2) $\mu$M), more variation was observed at the greatest intake (mean 57 (SE 5) $\mu$M).

As the transit-time flow probes measure total blood flow rather than flow per unit tissue, as is provided by $^3H_2O$ methods (Oddy *et al.* 1981), absolute rates of uptake, protein synthesis and degradation are estimated rather than fractional rates. Based on the dissections and protein-bound phenylalanine (leucine) contents (g/kg wet tissue) of 3·4 (5·5) for shaved skin and 3·3 (6·4) for mixed bone and muscle (J. C. MacRae, personal communication) then compared with whole-body retention (calculated from N balance) daily fractional rates of protein gain for the hind-limb tissues were greater above maintenance, while at sub-maintenance the rate of loss from the leg, based on phenylalanine transfers, was larger (see Table 5).

Hind-limb synthesis was related to intake ($P < 0·001$) at all levels, regardless of whether the calculations were based on arterial or venous blood-free phenylalanine SRA (Table 3).

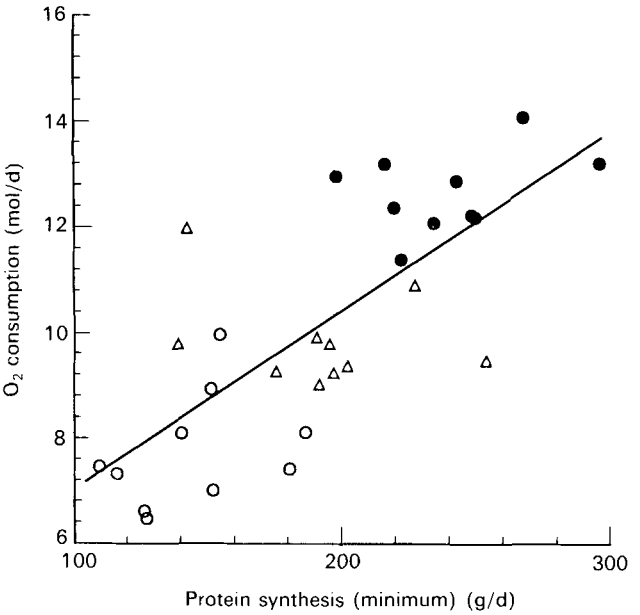


Fig. 2. The relationship between whole-body oxygen consumption and protein synthesis in lambs fed on either 300 g (○), 600 g (△) or 900 g (●) grass pellets daily. Whole-body synthesis was based on the irreversible loss rate of phenylalanine through arterial blood (mmol/h), corrected for oxidation. Adjustment to protein synthesis (g/d) was by the factor 113·14 (based on phenylalanine content of body protein of 3·5 g/16 g protein-nitrogen). The best fit line for the values shown is:

$O_2$ uptake (mol/d) = 0·0349 (+0·0056) × protein synthesis (g/d) + 3·41, residual standard deviation 1·47, $r$ 0·76.

For details of procedures, see pp. 390–395.

Table 2. *Whole-body leucine and protein kinetics in sheep offered either 300 g (L intake) or 900 g (H intake) of grass pellets daily**

(Values are means for five different lambs at each intake)

| Intake... | L | H | SED | Residual df | Statistical significance of intake effects: $P$ |
|---|---|---|---|---|---|
| ILR (mmol/h) | 4·188 | 7·791 | 0·408 | 8 | < 0·001 |
| $FO_c$† | 0·145 | 0·225 | 0·028 | 8 | 0·021 |
| $LO_c$‡ (mmol/h) | 0·590 | 1·710 | 0·200 | 8 | < 0·001 |
| PS§ (g/d) | 164 | 281 | 18 | 8 | < 0·001 |
| PD‖ (g/d) | 172 | 258 | 18 | 8 | < 0·001 |
| $Leu_{art}$ ($\mu$M) | 116 | 169 | 27 | 8 | < 0·004 |
| $MOP_{art}$ ($\mu$M) | 13 | 15 | 3 | 8 | 0·474 |

ILR, irreversible loss rate; $FO_c$, corrected fractional oxidation; $LO_c$, corrected leucine oxidation; PS, protein synthesis; PD, protein degradation; $Leu_{art}$, $MOP_{art}$, arterial leucine and 4-methyl-2-oxo-pentanoate concentrations respectively; SED, standard error of differences.

* For details of procedures, see pp. 390–395.

† Calculated from measured fractional oxidation/$s$, where $s$ is the fractional sequestration of labelled carbon dioxide set at 0·64 for L and H intakes (H. Rocha and G. E. Lobley, unpublished results) and then corrected for exogenous infusion of leucine.

‡ Calculated from (ILR × $FO_c$).

§ Calculated from ILR × (1 − $FO_c$) × 47·6 (see pp. 393–395).

‖ Calculated from PS − (nitrogen retention × 6·25).

Table 3. *Phenylalanine kinetics across the external iliac vein drained tissues of the hind-limb of lambs offered different daily intakes of grass pellets (300 g (L), 600 g (M), 900 g (H))**

(Values are means for ten lambs measured at each intake)

| Phenylalanine kinetics (μmol/h) | Intake | | | SED | Residual df | Statistical significance of intake effects: *P* |
|---|---|---|---|---|---|---|
| | L | M | H | | | |
| Net uptake | −13·9 (−1·6)† | 7·1 (0·8) | 24·3 (2·7) | 5·1 | 17 | < 0·001 |
| $PS_{phe,art}$ | 35·1 (4·0) | 54·9 (6·2) | 104·0 (11·8) | 5·2 | 17 | < 0·001 |
| $PD_{phe,art}$ | 49·2 (5·6) | 48·0 (5·4) | 80·1 (9·1) | 6·7 | 17 | < 0·001 |
| $PS_{phe,ven}$ | 44·1 (5·0) | 63·7 (7·2) | 125·7 (14·2) | 7·4 | 17 | < 0·001 |
| $PD_{phe,ven}$ | 58·6 (6·6) | 57·1 (6·5) | 101·8 (11·5) | 9·0 | 17 | < 0·001 |
| Blood flow (g/min) | 114 | 135 | 164 | 10 | 17 | < 0·001 |

$PS_{phe,art}$, $PS_{phe,ven}$, $PD_{phe,art}$, $PD_{phe,ven}$, protein synthesis and degradation respectively with calculations based on specific radioactivity of free-phenylalanine (phe) in either arterial (art) or venous (ven) blood; SED, standard error of difference.

* For details of procedures, see pp. 390–395.

† Values in parentheses represent equivalent of protein (g/d) based on a phenylalanine content in hind-limb protein of 3·5 g/16 g N.

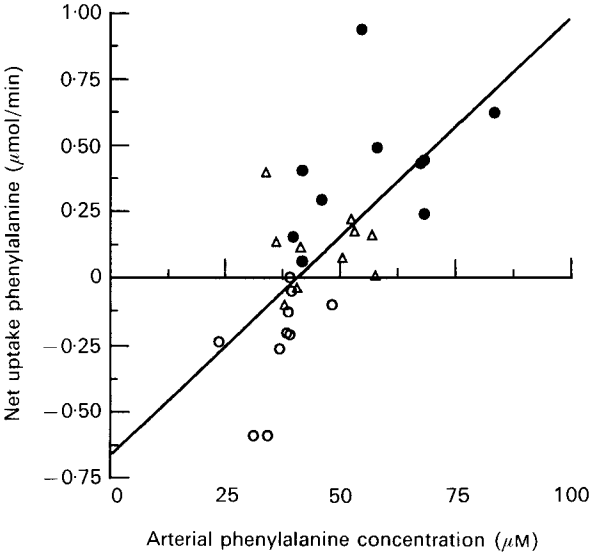


Fig. 3. The relationship between arterial concentration of free phenylalanine and the net uptake of the amino acid across the hind-limb of ten lambs fed at three intakes, 300 (○), 600 (△) or 900 (●) g grass pellets/d. The equation for the best fit line is:

uptake (μmol/min) = 0·0166 (+0·004) × arterial concentration (μM) − 0·68,
residual standard deviation 0·254, *r* 0·66.

Table 4. *Leucine kinetics across the external iliac vein drained tissues of the hind-limb of lambs offered either a low (L; 300 g) or high (H; 900 g) daily intake of grass pellets**

(Values are means for five different lambs at each intake)

| Leucine kinetics (μmol/h) | Intake L | Intake H | SED | Residual df† | Statistical significance of intake effects: *P* |
|---|---|---|---|---|---|
| Net uptake | −15·6 (−0·7)‡ | 77·4 (3·7) | 12·3 | 8 | < 0·001 |
| $LO_h$ | 18·0 (0·9) | 69·6 (3·3) | 12·7 | 8 | 0·004 |
| $PS_{leu,art}$ | 124 (5·9) | 273 (13·0) | 30 | 7 | < 0·001 |
| $PD_{leu,art}$ | 137 (6·5) | 196 (9·3) | 25 | 7 | 0·060 |
| $PS_{leu,ven}$ | 154 (7·3) | 319 (15·2) | 42 | 7 | < 0·001 |
| $PD_{leu,ven}$ | 169 (8·0) | 242 (11·5) | 41 | 7 | 0·067 |
| $PS_{mop,ven}$ | 137 (6·6) | 304 (14·5) | 33 | 7 | < 0·001 |
| $PD_{mop,ven}$ | 150 (7·1) | 226 (10·8) | 35 | 7 | 0·044 |
| MOP uptake | 2·0 | 8·9 | 11 | 7 | 0·301 |

$LO_h$, leucine oxidation; $Ps_{leu,art}$, $PS_{leu,ven}$, $PS_{mop,ven}$, $PD_{leu,art}$, $PD_{leu,ven}$, $PD_{mop,ven}$, protein synthesis and degradation respectively with calculations based on enrichment of either free leucine (leu) or 4-methyl-2-oxo-pentanoate (MOP) in arterial (art) or venous (ven) blood; SED, standard error of difference.

* For details of procedures, see pp. 390–395.

† For a full data set residual df is 8.

‡ Values in parentheses represent equivalent of protein (g/d) based on a leucine content in hind-limb mixed tissue protein of 6·6 g/16 g N.

Calculated daily FSR ( × 100) for the mixed tissue preparation varied between 1·2 and 3·3 % for L and H intakes based on venous SRA (see Table 5). The amount of muscle (plus bone) monitored represents 0·14 of total and skin 0·18 so, if the kinetic values obtained are representative of total carcass plus integument, then together these would comprise approximately 0·19, 0·23 and 0·35 of whole-body protein synthesis, at L, M and H intakes respectively, when arterial phenylalanine SRA is used as reference. Thus, in common with net uptake, the synthetic component of peripheral tissues makes, apparently, a larger contribution as intake is increased.

Protein degradation exhibited rather different kinetics for, although there was a significant increase ($P < 0·001$) between M and H intakes, the values at L and M were similar. Above maintenance both synthesis and degradation increased, the former more so, with the apparent retention of newly synthesized material at approximately 0·3.

*Leucine kinetics.* Again uptake was significantly related to the level of intake (Table 4). Because no within-animal comparisons were made for this amino acid, analysis of relationships between supply and net gain are not possible although, in general, arterial blood-free leucine concentrations were lower when net retention was negative compared with the positive uptake situation (Tables 2 and 4). If net retention (μmol leucine/h) was scaled to equivalent g protein/d the absolute values were different from those estimated from phenylalanine transfers (cf Tables 3 and 4), although the incremental change between the L and H intakes was similar for the two amino acids (Table 5).

Regardless of which precursor was selected, protein synthesis was correlated with intake

Table 5. *Calculated daily fractional rates ( × 100) of protein (amino acid) gain* ($k_g$) *and protein synthesis* ($k_s$)* *for whole-body and hind-limb tissues for sheep fed at various intakes*†‡

(Values are means at each intake for lambs of 33 kg for ten sheep per intake for phenylalanine values, five for leucine values (four for kinetic measurements at 300 g/d intake))

| Intake (g/d)... | | 300 | 600 | 900 |
|---|---|---|---|---|
| Whole body | | | | |
| Phenylalanine | | | | |
| | $k_g$ | −0·17 | 0·18 | 0·50 |
| | $k_s$§ | 2·83 | 3·76 | 4·73 |
| Leucine | | | | |
| | $k_g$ | −0·17 | | 0·52 |
| | $k_s$§ | 3·24 | | 5·18 |
| Hind-limb | | | | |
| Phenylalanine | | | | |
| | $k_g$ | −0·36 | 0·18 | 0·64 |
| | $k_{s,a}$ | 0·95 | 1·48 | 2·81 |
| | $k_{s,v}$ | 1·16 | 1·67 | 3·30 |
| Leucine | | | | |
| | $k_g$ | −0·17 | | 0·86 |
| | $k_{s,a}$ | 1·38 | | 3·02 |
| | $k_{s,v}$ | 1·71 | | 3·53 |
| | $k_{s,m}$ | 1·51 | | 3·36 |

* Values for synthesis calculated based on isotopic activity of free amino acid in either arterial ($k_{s,a}$) or venous ($k_{s,v}$) blood or of oxo-acid in venous blood ($k_{s,m}$).
† For details of procedures, see pp. 390–395.
‡ Estimates of fractional degradation ($k_{d,x}$) can be obtained from ($k_{s,x} - k_g$).
§ Based on arterial blood-free amino acid isotopic activity.

($P < 0·001$; Table 4). Based on venous leucine enrichment daily FSR ( × 100) were, at 1·7 and 3·5 %, slightly greater than those calculated from phenylalanine kinetics (Table 5). The proportional differences between the FSR estimates reflect the relationships between the enrichments of the various amino acid and oxo-acid pools. The enrichment of the venous blood MOP was, in sheep, 0·9 that of arterial leucine. Other studies in sheep (Oddy & Lindsay, 1986; Pell *et al.* 1986; Oddy *et al.* 1987) have found the SRA of MOP: free leucine to range from 0·62 to 0·91; the lower ratios were with plasma and the higher with whole blood. Such differences may reflect slow equilibration between plasma and the erythrocyte. At both L and H intakes venous blood MOP enrichment was greater than that of venous leucine (1·11 (SE 0·03) and 1·05 (SE 0·03) respectively). Protein degradation rates increased between L and H, similar to the situation with phenylalanine (cf. Tables 2 and 4).

The amount of leucine decarboxylated changed with intake, although the increase in oxidation was less than the improvement in retention (Table 4). Together, these two fates represent the net metabolism of leucine so between L and H intakes the proportion of retention:oxidation was 0·64:0·36; the peripheral tissues thus contribute to the inefficient utilization of (branched-chain) amino acids since the net retention of absorbed amino acids from rumen microbial protein (the major source for animals fed on grass pellets) is also, on an incremental basis, 0·53–0·79 (for review see Lobley, 1986). The single isotope procedure adopted in the present study does not distinguish $CO_2$ arising from decarboxylation of either leucine or MOP supply, nor separate whether venous MOP is from leucine metabolism across the hind-limb tissues or derives from arterial oxo-acid input. To ascertain this would require the use of either two tracers, infused on separate occasions (e.g. Schwenk *et al.* 1985), or a bis-tracer (e.g. Cheng *et al.* 1985, 1987). Net

movements of MOP were small, however, compared with both leucine net uptake and oxidation (Table 4). Again, assuming the hind-limb tissues are representative of carcass components, in the whole animal approximately 0·3 (0·4 if sequestration is ignored) of leucine decarboxylation occurs in peripheral tissues.

## DISCUSSION

### *Whole-animal kinetics*

The use of the ILR (or flux) method for estimation of whole-body protein synthesis has proved very popular in studies with the larger species (e.g. Reeds *et al.* 1980; Lobley *et al.* 1987; Krishnamurti & Janssens, 1988; Muramatsu *et al.* 1988) although it must be borne in mind that the values obtained are minimum estimates, as isotopic activity is monitored in the primary (arterial) pool. In any response-analysis situation, such as the present study, it needs to be assumed, therefore, that the relationship between the activities of the precursor pool for protein synthesis and circulating free amino acid tracer is unaltered by treatment; this may not be correct in all situations (see Lobley *et al.* 1992). With this reservation in mind, the kinetic changes in phenylalanine (and leucine) metabolism follow those expected of chronic changes in appetite from studies on pigs (Reeds *et al.* 1980) and cattle (Lobley *et al.* 1987). The increase in protein synthesis with intake accompanied that in energy expenditure and, based on phenylalanine kinetics, equated to 17 kJ heat energy per incremental g protein synthesized. Minimum estimates of protein synthesis from leucine dynamics averaged 1·27 (SE 0·04) those based on phenylalanine, so the associated energy costs reduce to 13 kJ/g increment of protein synthesis. This estimate is lower than values obtained during radioactive leucine infusion in both pigs and cattle (21 and 24 kJ/g protein synthesis respectively); this may be due to the restraint imposed by the respiration hood, which restricted postural movements that otherwise could add considerably to energy expenditure, but would not affect amino acid kinetics to any large extent (Brockway & Lobley, 1983). Such general correlations only link together related cellular mechanisms and indeed the real contribution of protein synthesis to energy expenditure is probably of the order of 12–33% (for example, see Lobley, 1990).

The increase in $FO_c$ of phenylalanine (non-significant) and leucine ($P = 0{\cdot}007$) between the L and H intakes was similar to the pattern observed for cattle between M and $1{\cdot}6 \times M$ (Lobley *et al.* 1987), but the opposite of that in pigs between $2 \times M$ and $3 \times M$. In the pig study urinary N elimination, the corollary of amino acid-C oxidation, was unaltered with the intake change (Reeds *et al.* 1980) and, thus, a smaller fraction of the increased leucine flux was catabolized to maintain this constancy. In the ruminant species the situation is different with increased urinary N elimination as intake is raised (e.g. Lobley *et al.* 1987; Harris *et al.* 1989) and, although a part of this may originate from ammonia absorbed from the rumen, a large contribution, on an incremental basis, must be derived from amino acid catabolism (see Lobley *et al.* 1987). Indeed, based on the phenylalanine oxidation values only 39% of the urinary N at the low intake could be attributed to protein oxidation, whereas at the higher intake the contribution was greater than 80%; leucine presents a similar picture. These protein oxidation values may be somewhat misleading as the supply of either phenylalanine and leucine from rumen microbial protein will not restrict growth (see MacRae & Reeds, 1980; Storm & Ørskov, 1984) and, as they are in excess compared with methionine and lysine, may yield overestimates of protein (mixed amino acid) oxidation.

### *Use of the arterio-venous procedure*

*General considerations.* While the arterio-venous procedure has proved popular, especially applied to limb studies, several reservations must be borne in mind. First, the technique suffers from the same short-comings prevalent in all tracer-based protein metabolism

studies, i.e. the various pools (e.g. vascular, extracellular, intracellular) label to different extents, so the final values obtained depend on which is selected by the researcher as most representative of the true precursor (the appropriate aminoacyl-t-RNA(s)). Thus, while net isotope uptake can be easily determined, as can the associated amino acid removed with the label from the arterial input (expressed as an absolute or fractional extraction), the amount of amino acid which enters the venous blood cannot be quantified. This is because the venous drainage includes amino acid which has not left the circulation (bypass material), which will be of similar isotopic activity to the arterial metabolite, plus that which has entered the cell and mixed, to a greater or lesser extent, with amino acid of low (zero) activity from protein breakdown before exit into the venous blood. Thus, the free amino acid isotopic activity in both arterial and venous blood will exceed that within the cells of the hind-limb tissues and, if the precursor is more closely related to the intracellular condition (see Lobley *et al.* 1992), underestimates of protein synthesis will be obtained.

Use has been made of blood (plasma) oxo-acid isotopic activity to overcome this problem since this is formed intracellularly and may derive from a pool similar to that used for protein synthesis. This approach is also inadequate, however, both for the bypass situation discussed previously and because not all cells produce the oxo-acid and, thus, with mixed tissue preparations such as the hind-limb distortions may arise.

One further concern involves the assumption that only free amino acids are involved. Suggestions have been made that plasma proteins (Elwyn *et al.* 1968; Chalmers *et al.* 1982), peptides (Jois *et al.* 1985) and amino acids non-covalently bound to protein (McCormick & Webb, 1987) may exchange across the hind-quarters. Such transfers, in both directions, would not only interfere with net uptake determinations but, because both proteins and peptides are likely to be of much lower isotopic activity, also provide a further potential dilution of the intracellular pool; this would then confound estimates of constitutive tissue protein degradation based on isotopic dilution of homogenate free amino acids. The net extent of the problem can be assessed partly by comparison of amino acid uptake with expected, or measured, protein gain (see p. 401).

These several reservations, which are important, should not detract from the usefulness of the arterio-venous technique which affords considerable scope to analyse responses across tissues and organs in the larger species and where intra-animal comparisons can minimize variance and identify key areas where more detailed studies should be undertaken.

*Hind-limb kinetics.* The previously reported responses in sheep hind-limb metabolism to the fed–fast cycle have encompassed a wide age-range, from the milk-fed lamb (Oddy *et al.* 1987) to mature adults of both sexes (Pell *et al.* 1986; Teleni *et al.* 1986). Stage of maturity may be an important factor in determining metabolic responses since, while both milk-fed lambs and young adult sheep increased protein synthesis between the fed and fasted condition (Pell *et al.* 1986; Oddy *et al.* 1987), there was no change for mature Merino wethers (Teleni *et al.* 1986). The lambs in the present study were in the ruminant condition but still growing and, thus, can be considered to be intermediate between those of Oddy *et al.* (1987) and Pell *et al.* (1986) and the increase in synthesis with chronic changes in intake is compatible with their earlier observations. The unaltered synthesis in mature sheep has analogies with the situation in single-stomached animals because muscle protein synthesis in mature female rats does not change with overnight fast (Baillie *et al.* 1988), whereas the younger growing animal exhibits prandial responses (e.g. Garlick *et al.* 1983).

In the earlier observations on sheep, protein degradation was greater at zero intake when preferential and extensive mobilization of peripheral tissue protein occurs. More representative information on response analysis may be obtained, therefore, from supra-fasting contrasts, as adopted in the present study. Here, hind-limb (and whole-body) protein breakdown was greatest at the highest intake. This follows the classical description, established for rodent muscle, of increases in both protein synthesis and degradation

occurring in response to food intake (e.g. Millward *et al.* 1976; Jepson *et al.* 1988). Of particular interest is the observation that protein degradation appeared unchanged between the L and M intakes; in consequence the improvement in protein gain is due entirely to increases in protein synthesis, i.e. the apparent efficiency of retention of newly synthesized protein is unity. This effect cannot be attributed to a compensatory mechanism because only half the lambs were measured in the transition from L to M. While some caution must be observed, since the calculation depends on accurate description of the true precursor for synthesis (see Lobley *et al.* 1992), the findings follow the trend in the human forearm (from Cheng *et al.* 1985, 1987) and young adult sheep hind-limb (from Pell *et al.* 1986), where 83–85% of the net anabolism induced by feeding could be attributed to changes in protein synthesis. The corresponding values in old sheep and milk-fed lambs ranged from 0 to 65% (from Teleni *et al.* 1986; Oddy *et al.* 1987), which again raises the question of age-related differences.

In contrast to the high efficiency of retention of synthesized protein between L and M, that between M and H was much lower (0·30 (SE 0·07); based on venous SRA). While the various tissues of the hind-limb may respond differently, this observation has similarities to the rodent muscle where incremental protein gain was between 40 and 70% of protein synthesis increases (Jepson *et al* 1988).

One further factor which requires consideration is substrate supply; in both lactation (Linzell, 1974; Rulquin, 1986) and hind-quarter metabolism (Oddy & Lindsay, 1986) links between blood flow or arterio-venous differences for amino acids with protein synthesis or output have been suggested. Although the correlations between amino acid supply and net gain were not strong in the present study, the response in protein gain per extra blood flow, as intake increased, was 55 mg protein/l compared with 70 mg/l during lactation in goats (Linzell, 1974). Similar constraints on response may, therefore, exist on hind-limb (muscle) and mammary metabolism, but this requires further investigation.

*Leucine oxidation.* The liver is the major site of catabolism for most amino acids but substantial oxidative capacity for the branched-chain amino acids (leucine, isoleucine and valine) resides also in ovine skeletal muscle and adipose tissue (Goodwin *et al.* 1987; Bergen *et al.* 1988), which contain both the transaminase and the branched-chain oxo-acid dehydrogenase. The activity of the dehydrogenase is tightly regulated by the phosphorylation status; the phosphorylated form is inactive and the dephosphorylated is active (Randle *et al.* 1984). Two separate enzymes, a phosphatase and a protein kinase, determine the activity state and these, in turn, are regulated by a range of physiological factors including cellular ATP status, systemic hormone concentrations (especially insulin and the glucocorticoids), products of amino acid catabolism and exercise (see Harper *et al.* 1984; Block *et al.* 1987*a*, *b*; Lobley, 1988). This regulation of activity by phosphorylation affords a very rapid control, while the wide range of effectors offers sensitivity to prevailing physiological conditions.

The absolute amino acid catabolism rates measured across the sheep hind-limb in the present study were 7–27 $\mu$mol/kg per h, which is lower than the values for the milk-fed lamb (63–105 $\mu$mol/kg per h, Oddy *et al.* 1987) but similar to young adult ewes (13–17 $\mu$mol/kg per h, Pell *et al.* 1986), and valine oxidation in mature Merino wethers (11–12 $\mu$mol/kg per h, Teleni *et al.* 1986). Such oxidation rates represent only 1–6% of total dehydrogenase capacity in the L and H condition respectively (values based on tissue enzyme activities reported for mature ewes by Goodwin *et al.* 1987; these are approximately twofold less than those for preruminant lamb muscle, Papet *et al.* 1988). These values are similar to the proportion of active form reported for rat muscle in vivo, but much lower than estimates of the dephosphorylated : phosphorylated enzyme ratio determined in vitro (0·07–0·62 Goodwin *et al.* 1987; Papet *et al.* 1988), although further regulation through the enzyme $K_m$ may operate under conditions in vivo.

Despite the low amount of enzyme capacity utilized, the peripheral tissues still account for a substantial proportion of leucine oxidation; if the activity of the hind-limb is representative of muscle, skin and fat in the whole body then these regulate at least 0·3 of branched-chain amino acid catabolism. This value is similar to those which can be calculated from the other studies with sheep and where the proportion of leucine catabolized by non-hepatic tissues is similar at low and high intakes, different for the situation reported for human muscle (see Harris & Lobley, 1990). The peripheral regulation of branched-chain amino acid catabolism through dehydrogenase activity may be a feature of a putative regulatory mechanism by which leucine acts as both a signal and a modulator of protein metabolism (see Harris & Lobley, 1990). Such a control system would, ideally, be directed at muscle but in the arterio-venous preparation it is not possible to resolve how much of the oxidation can be ascribed to the separate tissues. Skin probably plays a negligible role since the infusion of [$^{14}$C]leucine across a sheep skin arterio-venous preparation did not yield any labelled $CO_2$ (Dellow & Harris, as quoted in Harris & Lobley, 1990). The dehydrogenase activity per g tissue or per mg protein is higher for adipose tissue (Goodwin *et al.* 1987; Bergen *et al.* 1988) than muscle, but based on a fat content of 50 g/kg for ruminant tissue then 75% of the total enzyme resides in muscle. However, the decarboxylation rates observed in vivo would still be easily achieved with only 15% of the potential activity present in adipose tissue. It thus remains to be resolved whether muscle tissue *per se* does contribute significantly to leucine catabolism and whether the low rates of total decarboxylation observed in vitro by Bergen *et al.* (1988) reflect a further regulation, perhaps based on the transaminase enzyme, as has been suggested for the preruminant lamb (Papet *et al.* 1988). The answer to such a question may help address the wider issues as to why extra-hepatic amino acid catabolism has evolved and how integration of visceral and peripheral protein demands is achieved.

The ruminant, as with all mammals, is a complex entity in which the contributions and metabolism of individual organs must be integrated in order to maintain the overall well-being of the organism. Our understanding of intra- and inter-organ interactions is limited and studies on the larger species using trans-tissue preparations affords opportunities to advance our knowledge with a general applicability to all species including man. The ability to produce perturbations at specific sites within the body (e.g. Oddy *et al.* 1987) offers real opportunities for dissociating primary from secondary effects on metabolism.

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