Responses in tissue protein synthesis to sub- and supra-maintenance intake in young growing sheep: comparison of large-dose and continuous-infusion techniques

BY G. E. LOBLEY, PATRICIA M. HARRIS*, PAT A. SKENE, D. BROWN, E. MILNE, A. G. CALDER, SUSAN E. ANDERSON, P. J. GARLICK, I. NEVISON AND ALEXMARY CONNELL

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB

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In ten lambs (average live weight 33 kg), five offered 300 g/d (approximately 0·6 × maintenance; L) and five 900 g/d (1·8 × maintenance; H), tissue protein synthesis was measured by three procedures simultaneously. The techniques involved continuous infusion of [U-^{14}C]phenylalanine and [1-^{13}C]leucine over 7–8 h followed by a terminal large dose of [^{15}N]phenylalanine during the last 30 or 60 min. Rates of protein synthesis were then calculated based on the free amino acid or oxo-acid isotopic activity in either arterial, iliac venous blood or tissue homogenate for the continuous-infusion studies, or on plasma or tissue homogenate for the large-dose procedure. For muscle (> 99 %), and to a lesser extent skin (85–93 %), effective flood conditions were achieved with the [^{15}N]phenylalanine but were either not established or maintained for liver and tissues of the gastrointestinal tract (< 50 %). The large dose of phenylalanine also caused changes in the concentration and isotopic activity of blood leucine and 4-methyl-2-oxo-pentanoate. Based on the assumption that the large-dose procedure yields the closest value for the true rate of protein synthesis (L 1·97 %/d, H 2·85 %/d) then, for muscle, only values based on the homogenate as precursor gave comparable results for both leucine (L 1·83 %/d, H 3·01 %/d) and phenylalanine (L 1·67 %/d, H 2·71 %/d) continuous infusion. The values based on the arterial or venous amino or oxo-acid were significantly less, more so at the lower intake. In contrast, for skin, a tissue dominated by export protein synthesis, values from the large-dose procedure (L 6·37 %/d, H 10·98 %/d) were similar to those derived with arterial or venous metabolites as precursor (L 5·23 and 6·93 %/d, H 9·98 and 11·71 %/d for leucine), but much less than those based on homogenate data. Based on the large-dose technique, protein synthesis increased with intake in muscle ($P < 0·001$), skin ($P = 0·009$) and liver (26·7 *v.* 30·5 %/d; $P = 0·029$). The contributions of muscle and skin to total protein synthesis were approximately equal. The incremental efficiency of conversion for muscle of synthesized protein into deposition appeared to be similar to values reported for rodents.

Tissue protein synthesis: Protein intake: Lamb

The dynamic nature of protein metabolism has been much investigated over the past two decades, with particular emphasis on understanding responses in tissues to a variety of nutritional and physiological stimuli. In both laboratory and commercial species most attention has focused on measurement of protein synthesis, for which a range of tracer-based techniques are available (see Lobley, 1988). Data for the larger species are more limited than for rodents, due to both accessibility and cost. Furthermore, much of the information on protein metabolism in individual tissues of farm animals is based on the continuous infusion of tracer amino acid developed by Waterlow and his colleagues (e.g. Garlick *et al.* 1973). The problem with this technique is that the various free amino acid pools of the body (e.g. vascular, interstitial, intracellular) become labelled to different

* Present address: Biotechnology Division, DSIR, Private Bag, Palmerston North, New Zealand.

extents. Thus, considerable uncertainty exists as to which, if any, best represents the true precursor (the aminoacyl-tRNA) isotopic activity. The doubt caused is aggravated if the relationship between the various pools changes as a result of the treatment under investigation: this has, thus, restricted the usefulness of the many interesting studies reported on farm animals (e.g. sheep, Buttery *et al.* 1975, Davis *et al.* 1981; pigs, Garlick *et al.* 1976, Simon *et al.* 1978, Bergen *et al.* 1990; cattle, Lobley *et al.* 1980, Eisemann *et al.* 1989).

This problem has been overcome, to a large extent, by the introduction of the large (flood)-dose procedure (Henshaw *et al.* 1971; McNurlan *et al.* 1979; Garlick *et al.* 1980) in which a substantial quantity of unlabelled amino acid is introduced with the tracer and which raises the concentration in the body free amino acid pools with equalization of their isotopic activity. The method has become very much that of choice in rodent studies but, partly on ground of cost, has only been applied in a few instances with commercial species, the majority of which have involved preweaning animals (e.g. lambs, Attaix *et al.* 1986, 1987, 1988; piglets, Seve *et al.* 1986). Only two studies have been reported for ruminant lambs (Pell & Bates, 1987; Lobley *et al.* 1990*b*); both of these dealt with hormonal rather than nutritional responses and neither provided a comparison with the older methodology.

In the current investigation the rates of protein synthesis in various tissues, particularly muscle and skin, determined by the methods of continuous infusion, involving both leucine and phenylalanine as tracer, and large dose are compared in ruminant lambs fed either below or above energy and nitrogen equilibrium. These are further compared with hind-limb rates of protein synthesis estimated, at the same time, by arterio-venous isotopic exchange (Harris *et al.* 1992).

METHODS

Preliminary studies

Three wether lambs (40–45 kg; 12–15 months) were studied for the assessment of appropriate large-dose conditions with phenylalanine. The animals were offered grass pellets to approximately 1·5 × maintenance supplied from twenty-four 1 h feeders. Temporary catheters (polyvinylchloride, 1·0 mm i.d., 1·6 mm o.d.; Portex, Hythe, Kent) were inserted, under local anaesthesia, into both external jugular veins and through one of these was injected L-phenylalanine, containing either 50 or 500 μCi [2,6-ring ^{3}H]-phenylalanine, dissolved in 120 ml sterile saline (37°; 9 g sodium chloride/l). Blood samples (5 ml) were withdrawn at 5 or 10 min intervals over the next 1 h. One sheep received 0·5 g phenylalanine and the other two either 1·5 or 3 g amino acid and muscle biopsies (0·5 g) were taken from m. longissimus dorsi, under local anaesthesia, on five occasions between 5 and 60 min.

Main study

Animals and dietary treatments were as described earlier (Harris *et al.* 1992). The ten animals (five fed 300 g/d and five fed 900 g/d grass pellets) were infused for 8 h with both [1-^{13}C]leucine and [U-^{14}C]phenylalanine while arterio-venous measurements were made across the right hind-limb (Harris *et al.* 1992). The continuous infusions were maintained while a large dose of labelled phenylalanine (0·2 g 99 atoms % [^{15}N]phenylalanine plus 3·0 g L-phenylalanine, in 110 ml sterile saline) was injected into the jugular vein. Blood samples (5 ml), for plasma preparation, were taken from the jugular vein catheter at frequent intervals (every 5 min up to 30 min and every 10 min thereafter). The experiment was terminated either 30 (the first three sheep) or 60 min after the large-dose injection when the animal was killed by pentobarbitone overdose and tissues rapidly excised, washed in cold saline, diced and frozen in liquid N_2 before storage at −20°. Tissue removal was rapid: hind-limb shaved skin and total mixed muscle from the right hind-leg thigh was placed in

liquid N_2 within 60 s, followed by m. longissimus dorsi, liver, rumen wall, small intestine and finally mixed muscle from the left hind-leg thigh (approximately 5 min later). Experiments were conducted over a 12-month period; seasonal effects on wool growth and, thus, skin metabolism cannot be excluded but animals were alternated between diet levels to avoid bias.

Protein separation and measurements of isotopic activity

Tissues and pre-infusion blood samples were homogenized in 100 g sulphosalicylic acid/l and prepared as dried protein extracts essentially as described previously (Nicholas *et al.* 1977), except that the ethanol stage was excluded. Because of the insolubility of collagen and the bias that this would introduce the treatment with sodium hydroxide was excluded for skin samples.

Approximately 800 mg dried protein were hydrolysed in 200 ml constant-boiling hydrochloric acid, with phenol present, under reflux for 18 h. The hydrolysate was filtered, dried under reduced pressure and dissolved in 5 ml 0·1 M-HCl. Then [1-^{13}C]leucine (7–10 μmol) was separated from the hydrolysate equivalent of 20 mg protein, desalted and the enrichment determined as described by Lobley *et al.* (1990*b*).

For separation of [^{14}C]phenylalanine and [^{15}N]phenylalanine (60–80 μmol) the equivalent of 330 mg hydrolysed protein was separated on a 12 × 10000 mm ion-exchange column (resin size 10 μm; The Locarte Co., London) using 0·2 M-citrate buffer pH 3·70 at 50° with a flow rate of 50 ml/h, and fractions collected at 3 min intervals at the appropriate times. The chromatography took 18 h and resolution of phenylalanine from tyrosine was confirmed by spectrophotometric analysis. Care was taken to ensure that the complete peak was collected, as fractionation of the ^{14}C-labelled (e.g. Nicholas *et al.* 1977) and ^{15}N-labelled amino acids (results not shown) occurs during separation. The pooled fraction (60–80 ml) was then desalted through 9 ml AG-50 (H^+ form, 100–200 mesh, 8% cross-linked; Bio-Rad Laboratories Ltd, Watford) and, after thorough washing with organic N-free water (Elgastat, Elga Ltd, High Wycombe, Bucks.), the bound phenylalanine was eluted with 30 ml 0·1 M-NaOH. The sample was then dried under reduced pressure at 40°, washed with purified water, redried and taken up in 10 g 0·3 M-HCl. A portion (3 g) of this was retained for determination of radioactivity (10 ml Optiphase X; Pharmacia LKB, Milton Keynes) and a further 0·5 g diluted 40-fold (by weight) with 0·1 M-HCl for phenylalanine content quantification (Locarte Amino Acid Analyzer, Locarte Co., London), using L-norleucine as standard. The remainder (approximately 6·5 ml) was freeze-dried and, if necessary, stored at −20°. This procedure was adopted to ensure that volatile N sources from, for example, buffers were removed before analysis for ^{15}N enrichment.

The [^{15}N]phenylalanine was digested to $(^{15}NH_4)_2SO_4$ by a micro-Kjeldahl technique (1·5 g potassium chloride, 1·5 ml concentrated sulphuric acid, 50 mg mercuric oxide in 1 ml 4 M-H_2SO_4), with heating continued for 30 min after the solution clarified. Ammonia was liberated in a Hoskins still, with the addition of 1 ml sodium thiosulphate (80 g/l) and 10 ml 10 M-NaOH, and collected into boric acid solution (20 g/l) containing bromocresol green and methyl red as mixed indicator, essentially as described by Bremner & Mulvaney (1982). Steam was generated from water free of organic N. The distillate was dried under reduced pressure at 40°, dissolved in approximately 5 ml purified water and freeze-dried. Powder, equivalent to 0·5 mg N, was then treated with alkaline hypobromite by the Sprinson & Rittenberg procedure as detailed by Hauck (1982), and enrichment of the liberated N measured in a gas-isotope-ratio mass spectrometer (SIRA 12; VG Isogas, Middlewich, Cheshire).

The enrichment of blood and tissue free [1-^{13}C]leucine, blood [1-^{13}C]4-methyl-2-oxo-pentanoate (MOP) and specific radioactivity (SRA) of blood free [U-^{14}C]phenylalanine

were determined as previously described (Lobley *et al.* 1990*b*; Harris *et al.* 1992). Both plasma and tissue free [^{15}N]phenylalanine were extracted and converted to the tertiary butyldimethylsilyl derivative before gas–liquid chromatography–mass spectrometry (Calder & Smith, 1988).

Assessment of 'background' protein-bound amino acid enrichments

To provide a 'background' enrichment sample for protein-bound leucine 'zero-time' blood proteins were used (Lobley *et al.* 1990*b*), since the animals had been maintained on the same diet for a considerable period before measurement. Although the muscle protein leucine C-1 enrichment slightly exceeded that of blood protein (Lobley *et al.* 1990*b*), this would cause an underestimate in the fractional synthesis rates of no more than 3–6%. For 'background' [^{15}N]phenylalanine the ^{15}N enrichment in total protein from mixed muscle was used. In preliminary tests it was shown that the natural enrichment of [^{15}N]phenylalanine in muscle pre-injection was the same as the enrichment of total N from muscle protein at the end of the experiment, i.e. the increase in phenylalanine enrichment (range 0·0024–0·0067 atoms % excess (ape)) was very greatly diluted by all the other non-labelled amino-N. These values were also not significantly different from the 'background' ^{15}N enrichment of protein-bound phenylalanine from plasma and blood protein taken pre-injection.

Calculations and statistics

Continuous-infusion studies. Calculation of tissue fractional synthesis rates (FSR; g protein synthesis/d per g tissue protein) followed the general principles described by Garlick *et al.* (1973) in the version adopted by Nicholas *et al.* (1977), i.e.

$$\frac{S_B}{S_x} = \frac{\phi}{\phi - k_s} \times \frac{1 - e^{-kst}}{1 - e^{\phi t}} - \frac{k_s}{\phi - k_s},$$

where S_x and S_B are the specific radioactivities or enrichment of the precursor and protein-bound amino acid respectively, ϕ is a rate constant describing the change in S_x (ascribed, on empirical grounds, a value of 40/d, see e.g. Nicholas *et al.* 1977; Schaefer *et al.* 1986), t is time of incorporation (d) and k_s is the fractional rate of protein synthesis.

A variety of choices is available for S_x, arterial amino- or oxo-acid (in the case of leucine), iliac venous blood amino- or oxo-acid or homogenate amino acid (a mixture of intracellular and extracellular). All these have been used comparatively in the present study, where the subscripts $_a$, $_v$, $_t$ and $_m$ refer to arterial, venous and tissue homogenate free amino acid and venous MOP respectively for either leucine, $_l$, or phenylalanine, $_p$, as appropriate. For blood metabolites, the plateau isotopic activity was calculated as the mean over the last 180–240 min of infusion: in the case of leucine kinetics this included the period of the large-dose procedure, whilst for [^{14}C]phenylalanine it was assumed that the flood dose swamped all free phenylalanine pools, their SRA declined to zero and radioactive incorporation ceased at the time of injection. In consequence, no values for free [^{14}C]phenylalanine SRA tissue homogenate were obtained from terminal samples and instead a value for muscle was used based on isotopic activities measured in biopsies taken on a few occasions in earlier studies on the same sheep (see p. 378–379). Because the administration of the 3·2 g phenylalanine caused changes in both concentration and enrichment of blood-free leucine, it is possible that changes may have occurred in the tissue-free amino acid: therefore, the enrichment of homogenate leucine over the total infusion period was calculated in two ways. The first (to yield $k_{s,lt}$) used the terminal value coupled with a ϕ of 40/d: the second ($k_{s,lt'}$) adjusted the enrichment under the arterial leucine curve by the homogenate:arterial blood ratio for free leucine isotopic activity in terminal samples.

Large-dose procedure. Calculations follow those described by Garlick *et al.* (1980):

$$k_s = \frac{E_1 - E_0}{S_y} \times \frac{1}{t},$$

where E represents the protein-bound phenylalanine enrichment either as 'background' natural enrichment, E_0, or at the end of the large-dose period, E_1. S_y is the area under the enrichment curve, either for the jugular plasma samples (to yield FSR P′) or where the plasma curve value has been adjusted for the ratio of appropriate tissue homogenate:plasma-free [^{15}N]phenylalanine enrichment in terminal samples (to give FSR values M′, S′ and L′ for muscles, skin and liver respectively).

Statistical procedures. For intake effects, muscle data within each calculation procedure were analysed as a split-plot type analysis with intake as a main effect, muscle as a sub-plot effect and with animals treated as blocks. Each of the treatment effects, i.e. muscle, calculation and muscle × calculation was tested against its interaction with replicates as the appropriate error term. The data comparisons for the types of calculation (i.e. the various methods of estimating protein synthesis in muscle) were interpreted with the aid of analysis of variance. Where appropriate, estimates of missing values are included in means presented in the tables. Analyses of skin data were, for between-calculation procedures, by two-way analysis of variance and by one-way analysis of variance for between intakes. Two-way analysis of variance was also used to determine effects of calculation (i.e. method and precursor selection) within animal for liver but, for comparisons of intake differences, the variances were not always similar and the data were, therefore, analysed by unpaired t test, not assuming common variance.

RESULTS

Kinetics of 'flooding' with phenylalanine

The preliminary trials established that injection of either 0·5 or 1·5 g phenylalanine did not achieve adequate 'flood' conditions for either the plasma- or muscle-free amino acid. With 1·5 g, for example, plasma [^{3}H]phenylalanine SRA declined by 30% between 5 and 30 min, while the muscle SRA did not become equal to that of plasma until after 20 min, even though maximal concentrations were established within 5 min (Fig. 1(*a*)). The 3 g dose proved suitable, however, with a rapid rise (2–5 min; Fig. 1(*b*)) to plateau for free [^{3}H]phenylalanine in muscle homogenate; and after 15 min the amino acid enrichment remained similar between plasma and muscle for at least 60 min. The 3 g dose was approximately 15-fold the free phenylalanine content of the body (values not shown).

Effect of large dose on phenylalanine and leucine kinetics

The free [^{14}C]phenylalanine SRA in blood and muscle declined to very low values immediately after the large-dose adminstration (values not shown) but the $^{14}CO_2$ release decreased only between 0 and 20% compared with the pre-injection values and there were no consistent differences between animals at the two intakes.

Between 30 and 60 min after administration of phenylalanine the concentration of leucine in pooled blood samples was 0·83 (SE 0·04) of the pre-injection value at H intake and 0·92 (SE 0·03) at L intake, with declines in MOP concentration to 0·65 (SE 0·03) and 0·66 (SE 0·10) respectively (Fig. 2). Similar changes occurred for the other branched-chain amino acids, valine and isoleucine (declines of 10–20%, values not shown), while plasma tyrosine increased, presumably through conversion of the excess phenylalanine. After injection of phenylalanine the enrichment of leucine and MOP increased (for leucine to 1·17 (SE 0·03),

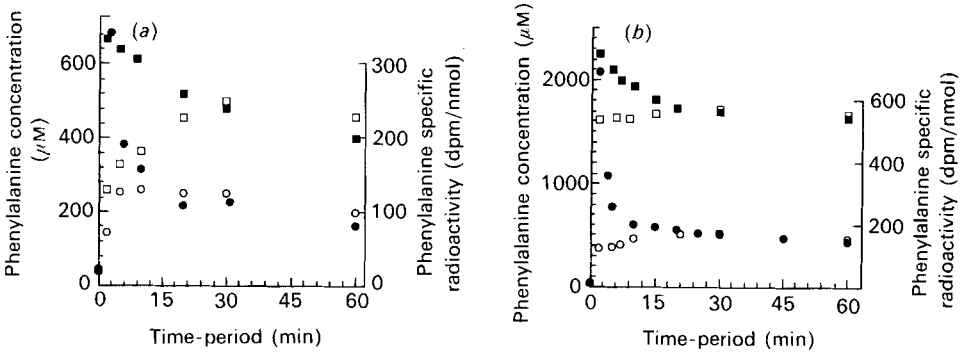

Fig. 1. Changes in concentration and specific radioactivity of plasma (●, ■) and muscle (○, □) free phenylalanine following intravenous injection of either (*a*) 1·5 g or (*b*) 3·0 g [^{3}H]phenylalanine respectively in 40 kg wether lambs; values are the means of two sheep. For details of procedures, see pp. 374–376. dpm, disintegrations/min.

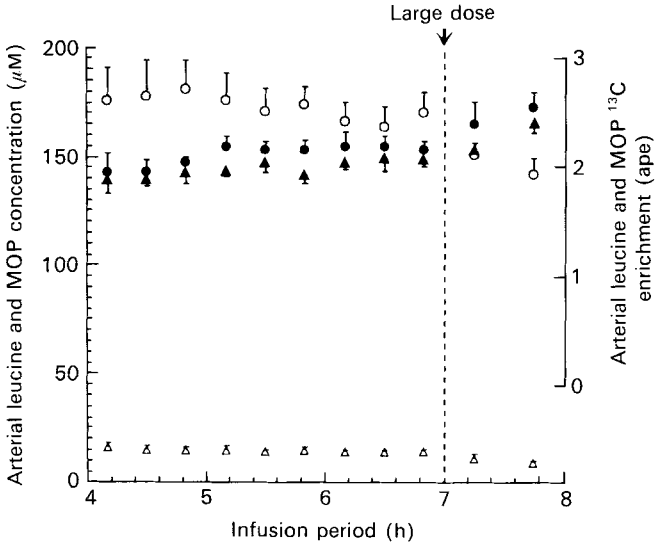

Fig. 2 Effect of intravenous injection ('large dose') of 3 g [^{15}N]phenylalanine on concentration (μM) and enrichment (atoms % excess; ape) of free leucine (○, ●) and 4-methyl-2-oxo-pentanoate (MOP; △, ▲) in arterial blood during continuous intravenous infusion of [1-^{13}C]leucine in 33 kg sheep fed on 900 g dried-grass pellets/d supplied in twenty-four 1 h portions. Values are means with their standard errors represented by vertical bars for five sheep and each point represents a pooled sample over either a 20 min ('pre-flood') or 30 min ('post-flood') period.

1·16 (SE 0·03) and for MOP to 1·16 (SE 0·05), 1·10 (SE 0·05) at H and L intakes respectively). There were no discernible effects on whole-body leucine oxidation as assessed by total production of CO_2 and exhaled $^{13}CO_2$ enrichment.

Tissue homogenate:blood-free amino acid isotopic activity

It was decided not to take biopsy samples during the tracer-infusion for fear of causing trauma. During earlier measurements made on these animals (Harris *et al.* 1992), biopsies were taken from m. longissimus dorsi towards the end of infusions of [^{3}H]phenylalanine and the homogenate free phenylalanine SRA determined. Five samples were taken (two at

Table 1. *Fractional rates of protein synthesis* (× *100*) *in either mixed muscle from the right* (*RHS*) *or left* (*LHS*) *leg or m. longissimus dorsi* (*LD*), *determined by use of a large-dose of* [^{15}N]*phenylalanine, in sheep fed on either a high* (*900 g*) *or low* (*300 g*) *daily intake of dried-grass pellets**

(Mean values for five separate sheep measured at each intake)

Intake...	High		Low	
Calculation...	P′	M′	P′	M′
Muscle				
LHS	2·89	2·87	1·91	1·95
LD	2·69	2·71	2·09	2·18
RHS	2·99	2·98	1·73	1·77
Statistical significance of effects of				
Muscle‡	NS		NS	
Calculation‡	NS		NS	
Intake§	P′ 0·024, M′ 0·042			

NS, not significant.

* For details of procedures, see pp. 374–376.

† Calculations based either on area under the plasma-free [^{15}N]phenylalanine enrichment curve (P′) or on that curve adjusted for the terminal muscle:plasma [^{15}N]phenylalanine enrichment ratio (M′). For details, see p. 377.

‡ The appropriate standard error of difference (SED) values were, for high intake, between muscles 0·140 and between calculations 0·067; for low intake the corresponding SED were 0·160 and 0·056. Residual df between muscles were 8 and between calculation 4.

§ The appropriate SED values were, for 8 residual df, between P′ calculations 0·340 and between M′ calculations 0·367.

H, two at M and one at L intakes) and with such a small number it was not possible to detect difference due to intake. The mean value for homogenate:blood free [^{14}C]-phenylalanine was 0·52 (SE 0·04) and this was used for subsequent estimations of muscle protein synthesis ($k_{s,\,pt}$), for all animals.

For the purposes of calculation it was assumed that the tissue homogenate:blood enrichment ratio for free leucine represented that during the full plateau period and was not altered by the large dose of phenylalanine. This ratio did not significantly vary between the three muscles within each intake, but for all pooled muscles the ratios were lower for the L intake (0·61 (SE 0·03) *v.* 0·73 (SE 0·03), $P < 0·05$). For the remaining tissues there were no significant differences with intake, although the tendency was for a lower ratio at the L intake: the mean values (pooled for both intakes) for skin, rumen wall, small intestine and liver were 0·21, 0·08, 0·10 and 0·29 respectively.

Estimates of muscle protein synthesis

Large-dose procedure. The method of calculation, i.e. whether based on the plasma free [^{15}N]phenylalanine curve (P′) or the final enrichment ratio for muscle homogenate:plasma amino acid (M′), did not significantly influence the values obtained (Table 1), i.e. homogenate:plasma enrichment ratio at slaughter for all muscles was 0·99 (SE 0·05; *n* 30). In subsequent comparisons, therefore, the M′ value was used. The FSR values (× 100) obtained for the two mixed muscle preparations from the left and right hind legs and m. longissimus dorsi were similar within each animal (Table 1) although variation between animals was considerable, e.g. for right leg mixed muscle at H intake values ranged from 2·33 to 3·75, and at L intake from 1·69 to 2·54.

Table 2. *Fractional rates of protein synthesis (× 100) in either mixed muscle from the right (RHS) or left (LHS) leg or m. longissimus dorsi (LD) based on continuous infusion of [1-^{13}C]leucine, in sheep fed on either high (900 g) or low (300 g) daily intakes of dried-grass pellets**

(Mean values for five separate sheep at each intake)

Intake	Muscle	Fractional synthesis rates (× 100)†					Statistical significance of effects of‡	
		$k_{s,ta}$	$k_{s,tv}$	$k_{s,m}$	$k_{s,tt}$	$k_{s,tt'}$	Muscle	Calculation
High								
	LHS	2·27	2·61	2·51	2·69	3·13		
	LD	2·15	2·47	2·39	2·69	3·01	0·500	< 0·001
	RHS	2·11	2·44	2·34	2·54	2·89		
Low								
	LHS	1·17	1·48	1·32	1·73	1·96		
	LD	1·08	1·37	1·22	1·50	1·79	0·103	< 0·001
	RHS	1·09	1·39	1·23	1·52	1·76		
Intake SED		0·14	0·17	0·23	0·30	0·37		
Statistical significance of effects§: *P*		< 0·001	< 0·001	< 0·001	0·007	0·013		

SED, standard error of difference.

* For details of procedures, see pp. 374–376.

† Values are calculated based on the areas under the enrichment curves for either free leucine in arterial blood ($k_{s,ta}$), iliac venous blood ($k_{s,tv}$) or iliac venous 4-methyl-2-oxo-pentanoate ($k_{s,m}$) or based on tissue-free leucine enrichment at end of infusion unadjusted ($k_{s,tt}$) or adjusted to ratio of blood:tissue amino acid ($k_{s,tt'}$; for details, see p. 376).

‡ The appropriate SED at high intakes were 0·145 (muscle) and 0·171 (calculation); the corresponding values at low intake were 0·064 (muscle) and 0·130 (calculation). Residual df between muscles were 8 and between calculation 16.

§ For residual df of 8.

For mixed muscles from both legs k_s declined significantly with intake, while there was only a trend for m. longissimus dorsi (Table 1).

Continuous-tracer infusions. The selection of 'precursor' had significant effects on the calculated k_s from leucine kinetics: the lowest values obtained were based on arterial enrichments and the greatest on tissue enrichments (Table 2). Only $k_{s,tt}$ (H intake) and $k_{s,tt'}$ (both intakes) were not significantly different from the M′ values derived from the large-dose procedure (Fig. 3).

Within each method of calculation, FSR was significantly reduced at the lower intake (Table 2).

FSR values based on [^{14}C]phenylalanine kinetics were significantly lower than those from [^{13}C]leucine when calculated by the same method (compare Tables 2 and 3; Fig. 3). When the estimated value for homogenate SRA (set at 0·52 of arterial SRA) was adopted the calculated $k_{s,pt}$ values were similar to $k_{s,tt}$ and $k_{s,tt'}$ (Table 2) and, at high intake, were not significantly different from M′ derived from the large-dose procedure. At the lower intake, the values for $k_{s,pt}$ were lower than M′ but were still much closer than estimates based on blood metabolite SRA.

Again, regardless of calculation procedure adopted FSR was significantly less at the lower intake (Table 3).

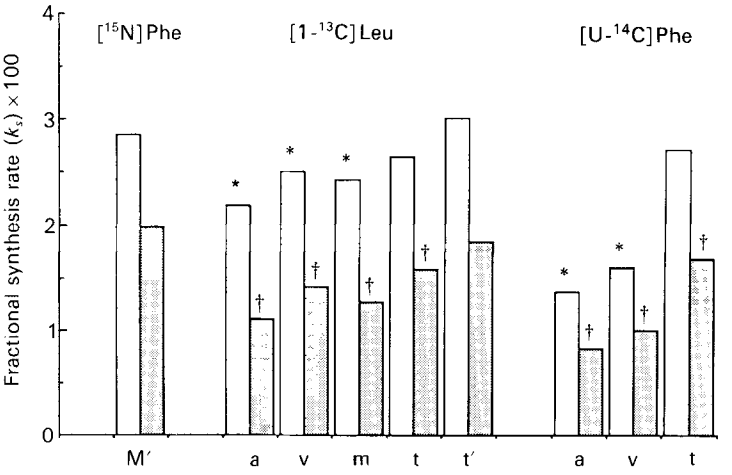

Fig. 3. Comparison of estimates of fractional rates of muscle protein synthesis calculated from the various body pools sampled during continuous infusion of [1-^{13}C]leucine (Leu) and [U-^{14}C]phenylalanine (Phe) with values obtained from terminal large-dose administration of [^{15}N]phenylalanine (Phe) in sheep offered either a high (900 g; □) or low (300 g; ▨) daily intake of dried-grass pellets; where *a*, *v*, *t*, *t′* and *m* represent values based on the precursor pools of arterial, iliac venous, tissue, or tissue proportionally adjusted to arterial values, amino acids or iliac venous 4-methyl-2-oxo-pentanoate enrichment respectively. Combined analyses of mixed muscle from the right and left legs and m. longissimus dorsi, with effects compared against the large-dose procedure (M′): high intake, * $P < 0{\cdot}05$ or less, standard error of difference (SED 0·20), residual df 32; low intake. † $P < 0{\cdot}05$ or less (SED 0·09, residual df 32).

Other tissues

Although the 'flood' condition had been well achieved in muscle this was not the case for the remaining tissues, those with higher rates of protein synthesis generally being the least satisfactory.

In skin the homogenate:plasma-free [^{15}N]phenylalanine enrichments at 60 min were 0·87 (SE 0·02, *n* 9) and this was reflected in the calculations based on plasma and homogenate as precursors (P′ and S′; Table 4). The k_s values were approximately 3-fold those in skeletal muscle, but likewise showed similar and significant responses to intake (Table 4). The effect of intake was also significant for k_s calculated from [^{13}C]leucine infusion, except for those based on homogenate enrichments (Table 4). These latter rates were greatly in excess of either value (P′ or S′) determined from the flood-dose procedure (Table 4). When compared with S′ values, estimates based on venous blood leucine enrichment were not significantly different, while arterial values were only different at the high intake (Table 4; based on exclusion of $k_{s,lt'}$ data for SE of difference (SED) values). In contrast to the situation with leucine, values based on arterial or venous blood-free phenylalanine were significantly lower than S′ at both intakes (Table 4), although within each calculation procedure synthesis rates differed significantly with intake.

Flooding was less effective in liver where homogenate:plasma-free [^{15}N]phenylalanine enrichments varied from 0·50 to 0·84. These will probably overestimate the extent to which true 'flooding' occurred, over the total period, as no effort was made to exsanguinate the liver portions taken and, as a contamination of 10–15% with blood is likely, intracellular values will, therefore, be lower. By comparison with the L′ values from the large-dose procedure, the arterial calculations gave severe underestimates for infusion of both leucine and phenylalanine (Table 4). Only the value calculated from homogenate leucine was not significantly different from L′, although caution should be exercised in interpretation as,

Table 3. *Fractional rates of protein synthesis (× 100) in either mixed muscle from the right (RHS) or left (LHS) leg or m. longissimus dorsi (LD), based on continuous infusion of [U-^{14}C]phenylalanine, in sheep fed on either high (900 g) or low (300 g) daily intakes of dried-grass pellets**

(Mean values for five separate animals at each intake)

Intake	Muscle	Fractional synthesis rates (× 100)†			Statistical significance of effects‡	
		$k_{s,pa}$	$k_{s,pv}$	$k_{s,pt'}$	Muscle	Calculation
High						
	LHS	1·40	1·63	2·80		
	LD	1·32	1·55	2·64	0·623	< 0·001
	RHS	1·35	1·58	2·70		
Low						
	LHS	0·89	1·09	1·78		
	LD	0·78	0·94	1·57	0·539	< 0·001
	RHS	0·80	0·96	1·65		
Intake SED		0·20	0·22	0·41		
Statistical significance of effects §: *P*		0·029	0·030	0·034		

SED, standard error of difference.

* For details of procedures, see pp. 374–376.

† The calculations were based on the area under the specific radioactivity (SRA) curves obtained, following continuous infusion of [U-^{14}C]phenylalanine, for the free amino acid in either arterial blood ($k_{s,pa}$), iliac venous blood ($k_{s,pv}$) or a mean estimate for tissue-free phenylalanine based on an SRA 0·52 that in arterial blood ($k_{s,pt'}$; for details, see p. 378).

‡ The appropriate SED were, for high intake, 0·110 (muscle) and 0·120 (calculation); corresponding values for low-intake analyses were 0·141 (muscle) and 0·118 (calculation). Residual df for between muscles were 8 and between calculation 8.

§ For residual df of 8.

again, blood contamination will affect the values obtained for both free and protein-bound amino acid.

In all comparisons, k_s was greater at the higher intake, although because of the reduced df associated with unequal variances the differences were only significant for L′ (Table 4).

The problems with incomplete flood conditions were worse with rumen wall and small intestine samples. Homogenate:plasma terminal ratios averaged 0·58 (SE 0·052) for rumen wall and 0·253 (SE 0·028) for small intestine; the corresponding values for the ratio with [^{13}C]leucine infusion were even lower at 0·08 (SE 0·04) and 0·10 (SE 0·04) respectively. In consequence, no calculations are presented for these tissues.

DISCUSSION

The aim of the present study was twofold. First, to compare procedure for measurement of protein synthesis with the same animals and time-scale. Second, to investigate the effects of intake on protein synthesis within some major tissues.

Comparison of techniques of protein synthesis

The large-dose procedure was chosen as base reference; its theoretical advantages have been generally accepted for rodent studies and recently it has been extended to studies on man (Garlick *et al.* 1989). Despite its wide acceptance, there are several potential problems

Table 4. *Comparison of estimates of fractional rates of skin and liver protein synthesis* (× *100*) *after continuous infusion of both [1-*^{13}*C]leucine and [U-*^{14}*C]phenylalanine with values obtained from terminal large-dose administration of [*^{15}*N]phenylalanine in sheep fed either a high (900 g) or low (300 g) daily intake of dried-grass pellets**

(Mean values for five separate sheep at each intake)

	Fractional rates of protein synthesis (× 100)†							
Intake	P′	S′ (L′)	$k_{s,ta}$	$k_{s,tv}$	$k_{s,lt'}$	$k_{s,pa}$	$k_{s,pv}$	Statistical significance of calculation‡§
				Skin				
High	9·48	10·98	9·98	11·71	44·68	6·56	7·62	< 0·001
Low	5·65	6·37	5·23	6·93	31·73	3·28	3·92	< 0·001
Intake‖ SED	1·11	1·14	1·23	1·38	7·81	1·14	1·22	
Statistical significance of effects: *P*	0·009	0·004	0·005	0·009	0·147	0·021	0·016	
				Liver				
High	30·5	49·4	18·0		56·8	7·1		< 0·001
Low	26·3	32·4	14·5		36·5	4·7		< 0·001
Intake df	3	3	7		1	4		
Statistical significance of effects¶: *P*	0·10	0·05	0·09		0·61	0·17		

SED, standard error of difference.

* For details of procedures, see pp. 374–376.

† Estimates of protein synthesis rates (k_s) based on isotopic activity in either arterial, $_a$, or iliac venous, $_v$, blood, tissue homogenate, $_{t'}$, for either free leucine, $_l$, or phenylalanine, $_p$, following simultaneous continuous infusion of [^{13}C]leucine and [^{14}C]phenylalanine. These are compared with values obtained during the last 30–60 min of infusion by use of a large dose of [^{15}N]phenylalanine and based either on the enrichment curve for plasma-free amino acid (P′) or the curve corrected for the final tissue homogenate:plasma enrichments (S′ and L′ for skin and liver respectively).

‡ For skin the appropriate SED were 3·27 (high intake) and 2·06 (low intake), for 23 residual df (1 missing value). If all values for $k_{s,lt'}$ are excluded the SED were 0·39 and 0·78, at high and low intakes respectively, for 20 residual df, calculation effects were still significant ($P < 0·001$).

§ For liver the appropriate SED were 6·95, for high intake, with 10 residual df (six missing values; this includes three estimates of $k_{s,lt'}$ which exceeded 100, i.e. homogenate-free leucine enrichment was very low and where label recycling could cause faulty estimates) and 2·02, for low intake, with 13 residual df (three missing values). If all values for $k_{s,lt'}$ are excluded the SED value at high intake is 3·35 (9 residual df, three missing values) and at low intake 1·34 (12 residual df).

‖ For intake effects the residual df were 8 except for $k_{s,lt'}$ (6 df).

¶ The variances between intake effects were different and, therefore, the data were analysed by unpaired *t* test, not assuming common variance.

which may arise. One involves the need to maintain metabolic 'steady-state' if measurements of short duration are to be extrapolated to a longer interval (usually expressed as 'per d'); in ruminants the large-dose time-periods have ranged from 25 to 180 min (Attaix *et al.* 1986, 1988; Pell & Bates, 1987; Lobley *et al.* 1990*b*). Another is that the amino acid used may alter protein metabolism either directly (Schaefer *et al.* 1986; see also Lobley *et al.* 1990*b*) or through changes in normal endocrine or substrate status (Lobley *et al.* 1990*a*, *b*). In companion studies 3 g phenylalanine was shown to provoke a transient increase in circulating insulin concentration (Lobley *et al.* 1990*a*) and it is probable that similar changes occurred in the current investigation. Furthermore, a large

dose of phenylalanine depresses the plasma concentration of the branched-chain amino acids (Lobley *et al.* 1990*a*).

Despite these observations, the net effects on protein metabolism, over the time-periods concerned, may be minimal. Serial measurements of protein synthesis by the large-dose procedure have yielded linear rates between 5 and 180 min (Laurent, 1982; Attaix *et al.* 1986; Pell & Bates, 1987). Furthermore, proline is less likely to induce insulin secretion than phenylalanine or leucine (e.g. Rocha *et al.* 1972) yet FSR measured by flooding with that amino acid in sheep were greater (3·9 %/d Pell & Bates, 1987) than those obtained with either the phenylalanine or leucine (2·6 %/d Lobley *et al.* 1990*b*; 2·9 %/d present study). Even when allowance is made for the age and intake differences between the various studies there appears to be no stimulation of synthesis with amino acids which act as insulin secretagogues. Whereas these arguments do not rigorously preclude erroneous data from the large-dose technique, in the absence of conclusive evidence to the contrary it will be assumed that the values obtained are representative of the correct rates in skeletal muscle from fed sheep.

With the previously stated provisos in mind then, for skeletal muscle, the best estimates of protein synthesis with continuous tracer infusion of either leucine or phenylalanine are those based on tissue homogenate isotopic activity under the physiological and nutritional conditions employed in the present study. On a proportional basis, the error introduced by using the blood enrichment as precursor is greater at L than H intake e.g. based on arterial blood the value of M′ is 0·76 at H intake *v.* 0·56 at L intake. This is compatible with a greater recycling of intracellular amino acids at the lower intake, possibly a result of proportionally reduced supply of amino acids by the circulation (based on either concentration or concentration × blood flow; Harris *et al.* 1992). Although venous amino- and oxo-acid isotopic activity gave closer values than arterial metabolites, none was particularly satisfactory. Despite the popularity of using arterial or oxo-acid values in measurement of irreversible loss rate (flux), or arterio-venous difference by continuous infusion, blood metabolite variables do not represent the actual state across drained tissues, because only a fraction (unknown) is of tissue origin, the remainder being bypass material (for discussion, see Harris *et al.* 1992). Furthermore, in the samples used here venous blood drains a mixture of muscle, bone, skin and fat and, thus, will reflect their relative contributions based on metabolic activity rather than mass (see Harris *et al.* 1992), and so for individual tissues the blood precursors or products are unlikely to provide representative values. A further discrepancy between blood- and homogenate free amino acid isotopic activities may involve peptide contribution as precursors of protein synthesis (see Harris *et al.* 1992).

Because the other tissues exhibited less thorough 'flood' conditions, clarification of their most suitable 'precursor' pool is difficult. For skin, the extensive dilution of the tissue tracer pool eliminated intracellular leucine from consideration, but while an intermediate value between arterial and venous amino acid seemed most appropriate for leucine, both yielded low results for phenylalanine. This emphasizes both the differences that can occur between amino acids and the need for caution in making extrapolation from one situation to another. Several reasons can be advanced for such differences, including those of greater involvement of intracellular phenylalanine (from proteolysis of skin proteins or peptides) or extracellular phenylalanyl-peptides, as discussed for muscle. Certainly, differences in precursor pools have been observed in rabbit skin where, following multiple tracer infusions, the SRA of free amino acids in skin homogenate were compared with that bound in procollagen. The skin procollagen pool is small with a short half-life (20–30 min; Robins, 1979) and, thus, reaches plateau isotopic activity only slightly less quickly than the appropriate precursor pool. Compared with skin homogenate free amino acid SRA

procollagen-bound tyrosine and proline were lower than leucine, indicating that a less-labelled pool would be needed to obtain similar values (Robins, 1979). An alternative explanation might involve the synthesis and processing of the secreted proteins. First, the amino acid composition of synthesized protein may differ from that present in analysed shaved skin. Wool proteins (the 'hard keratins'), for example, both have greater quantities of leucine (6·5 *v.* 5·2 g/16 g N) and phenylalanine (4·6 *v.* 2·8 g/16 g N) than skin proteins (J. C. MacRae, personal communication) including the 'soft keratins' and other non-keratin intermediate filament proteins. Thus, synthesis of keratin would distort k_s values based on skin protein extracts. Second, the processing peptides of prepro- and pro-protein of collagen, profilaggrin and filaggrin, and the type I and type II keratin groupings have different compositions from the secreted protein (Dale *et al.* 1989) and, therefore, alter apparent synthesis rates. Although production of secreted proteins, involving polypeptide processing, is an important feature of skin metabolism (collagen, keratin), these will remain within the skin matrix and, therefore, differences between the various kinetic procedures will not be as acute as for, say, liver.

The situation with flooding in the liver was less satisfactory. Probably, the 'flood' condition was established initially but this was steadily lost due to high intracellular hepatic protein turnover coupled with lower systemic phenylalanine concentrations, as occurred with [^{13}C]leucine in human liver (Ballmer *et al.* 1990). Moreover, since the longer continuous-infusion study will measure predominantly constitutive liver protein, as export protein (for plasma) can be synthesized, processed and secreted within about 30 min, lower rates of protein synthesis would be expected compared with the large-dose procedure.

Intake effects

Regardless of which precursor was selected, muscle protein synthesis responded to intake. This finding matches observations in rodents subjected to either acute (e.g. Garlick *et al.* 1983; Bates & Millward, 1983) or chronic (El Haj *et al.* 1986) alterations in food quantity or quality.

In most studies on ruminants hitherto the effect of intake has focused on the fed *v.* fasted state and, except for the study of Patureau-Mirand *et al.* (1985), has involved use of arterio-venous preparations to follow hind-limb tissue metabolism. The implications of most of these reports, and the metabolic peculiarities associated with the fasted condition, have been discussed previously (see Harris *et al.* 1992). One difficulty in making comparisons from different studies is that various factors, including age and intake, can effect muscle protein synthesis. However, in a range of studies with sheep under different physiological conditions, intake rather than age would appear to be the more important variable. Thus, when muscle FSR (%/d; calculated from homogenate precursors, as suggested by the present study) and metabolizable energy intake (kJ/kg body-weight$^{0.75}$) respectively are compared there appears to be reasonable correlation (1·6, 210 present study; 2·1, 710 Schaefer *et al.* 1986; 2·6, 693 Lobley *et al.* 1990*b*; 2·6, 620 present study; 3·0, 675 Bryant & Smith, 1982; 4·2, 823 Davis *et al.* 1981; 6·5, 1268 Bohorov *et al.* 1987), despite the fact that the sheep studied varied in age from 0·7 to 3·0 years. In rats it has been shown that the decline in k_s with age approximates to an exponential with 0·85–0·90 of the curve determined before the animal reaches 0·4 of mature body size (Lewis *et al.* 1984; see also Lobley, 1988): hence, in the growth of fattening lambs to adulthood chronological changes are minimized.

Between L and H intakes the change in muscle growth was 0·68 %/d calculated from amino acid uptake data across the hind-limb tissues and 1·00 %/d from total N retention (Harris *et al.* 1992). The incremental change in muscle protein synthesis determined here for the same right leg was 1·22 %/d, yielding efficiencies for retained:synthesized muscle

protein of 0·56–0·82, which are comparable with efficiencies observed with rodents (e.g. Garlick *et al.* 1973; Bates & Millward, 1983) and in cattle treated with exogenous growth hormone (from Eisemann *et al.* 1989). The maximum efficiency for retention:synthesis between the same intakes on whole-body terms was only 0·25 (from Harris *et al.* 1992), illustrating the differences that exist between slow-turning over tissues, such as the musculature, and the metabolically more active viscera, even when only incremental changes are considered.

Skin has a higher synthesis rate than muscle but responds to intake in a similar manner. The higher rate of protein synthesis in skin compared with muscle leads to the two tissues making approximately equal contributions to total body synthesis of 31 and 24 g/d at L intake and 45 and 40 g/d at H intake for muscle and skin respectively (assuming protein contents for 33 kg lambs of 1·56 and 0·42 kg for muscle and skin respectively and that the samples of tissue analysed are representative of the total). These similar proportions are in agreement with estimates in cattle and sheep (Lobley *et al.* 1980; Davis *et al.* 1981) but differ from those reported for pre-ruminant lamb by Attaix *et al.* (1988). Indeed, there is a noticeable difference between developmental changes in protein metabolism for the two tissues. In neonatal lambs muscle and skin have similar FSR (22–24%/d; Attaix *et al.* 1988) but by the time lambs attain 33 kg (present study) the rates in muscle have declined by 6–7-fold, while that for skin has only halved: this occurs despite the fact that muscle has become the dominant protein mass in the animal during this time-scale.

Liver protein synthesis was also sensitive to intake. This again has analogies with rodents in which hepatic synthesis is responsive to both acute and chronic alterations in food intake (Merry *et al.* 1987). In studies with rats subjected to chronic food restriction the changes in FSR of liver protein were influenced by the tissue mass and the age of the animals (Merry *et al.* 1987). In the present investigation liver protein mass was not determined but was probably less at the L intake so that total synthesis was decreased even more than the fractional changes, although the involvement of export protein production further complicates matters.

Measurements of hind-limb k_s values for these animals by arterio-venous procedures yielded values of 1·7 and 3·5%/d at L and M intakes (Harris *et al.* 1992). Assuming that bone:muscle k_s was 3·8:1 (from Lobley *et al.* 1980) then summated total tissue FSR by the large-dose procedure was 3·8 and 5·8%/d respectively. Similarly, while the trends in fractional degradation rates at the L and H intakes (1·9 and 2·7%/d respectively by arterio-venous determination and 4·0 and 4·9%/d estimated from large-dose procedures) were similar, the proportional changes were not. Overall, therefore, while the qualitative nature of hind-limb protein dynamics estimated from arterio-venous measurements appears to be valid, under these nutritional and physiological conditions, the quantitative estimates are unlikely to be accurate.

REFERENCES

Attaix, D., Aurousseau, E., Bayle, G., Manghebati, A. & Arnal, M. (1987). Protein synthesis and degradation in growing lambs. In *5th International Symposium on Protein Metabolism and Nutrition. European Association of Animal Production Publication* no. 35, pp. 24–27. Rostock: Wilhelm-Reck University

Attaix, D., Aurousseau, E., Manghebati, A. & Arnal, M. (1988). Contribution of liver, skin and skeletal muscle to whole-body protein synthesis in the young lamb. *British Journal of Nutrition* **60**, 77–84.

Attaix, D., Manghebati, A., Grizard, J. & Arnal, M. (1986). Assessment of *in vivo* protein synthesis in lamb tissues with [^{3}H] valine flooding doses. *Biochimica et Biophysica Acta* **882**, 389–397.

Ballmer, P. E., McNurlan, M. A., Milne, E., Heys, S. D., Buchan, V., Calder, A. G. & Garlick, P. J. (1990). Measurement of albumen synthesis in man: a new approach employing stable isotopes. *American Journal of Physiology* **259**, E797–803.

Bates, P. C. & Millward, D. J. (1983). Myofibrillar protein turnover. Synthesis rates of myofibrillar and sarcoplasmic protein fractions in different muscles and the changes observed during postnatal development and in response to feeding and starvation. *Biochemical Journal* **214**, 587–592.

Bergen, W. G., Johnson, S. E., Skjaerlund, D. M., Babiker, A. S., Ames, N. K., Merket, R. A. & Anderson, D. B. (1990). Muscle protein metabolism in finishing pigs fed ractoparine. *Journal of Animal Science* **67**, 2255–2262.

Bohorov, O., Buttery, P. J., Curreia, J. H. R. D. & Soar, J. B. (1987). The effect of the β-2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. *British Journal of Nutrition* **57**, 99–107.

Bremner, J. M. & Mulvaney, C. S. (1982). Nitrogen-total. In *Methods of Soil Analysis*, pp. 612–616 [A. L. Page, R. H. Miller and D. R. Keeney, editors]. Madison, WI: American Society of Agronomy Inc.

Bryant, D. T. W. & Smith, R. W. (1982). The effect of lactation on protein synthesis in ovine skeletal muscle. *Journal of Agricultural Science, Cambridge* **99**, 319–323.

Buttery, P. J., Beckerton, A., Mitchell, R. M., Davies, K. & Annison, E. F. (1975). The turnover rate of muscle and liver protein in sheep. *Proceedings of the Nutrition Society* **34**, 91A.

Calder, A. G. & Smith, A. (1988). Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. Use of the tertiary butyldimethylsilyl derivatives. *Rapid Communications in Mass Spectrometry* **2**, 14–16.

Dale, B. A., Resing, K. A., Haydock, P. V., Fleckman, P., Fisher, C. & Holbrook, K. (1989). Intermediate filament associated protein of epidermis. In *The Biology of Wool and Hair*, pp. 97–116 [G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marschall, editors]. London: Chapman & Hall.

Davis, S. R., Barry, T. N. & Hughson, G. A. (1981). Protein synthesis in tissues of growing lambs. *British Journal of Nutrition* **46**, 409–419.

Eisemann, J. H., Hammond, A. C. & Rumsey, T. S. (1989). Tissue protein synthesis and nucleic acid concentrations in steers treated with samatotropin. *British Journal of Nutrition* **62**, 657–671.

El Haj, A. J., Lewis, S. E. M., Goldspink, D. J. & Holehan, A. M. (1986). The effect of chronic and acute dietary restriction on the growth and protein turnover of fast and slow types of rat skeletal muscle. *Comparative Biochemistry and Physiology* **85A**, 281–287.

Garlick, P. J., Burk, T. L. & James, W. P. T. (1973). The diurnal response of muscle and liver protein synthesis *in vivo* in meal-fed rats. *Biochemical Journal* **136**, 935–945.

Garlick, P. J., Burk T. L. & Swick, R. W. (1976). Protein synthesis and RNA in tissues of the pig. *American Journal of Physiology* **230**, 1108–1112.

Garlick, P. J., Fern, M. & Preedy, V. R. (1983). The effect of insulin infusion and food intake on muscle protein synthesis in post absorptive rats. *Biochemical Journal* **210**, 669–676.

Garlick, P. J., NcNurlan, M. A. & Preedy, V. R. (1980). A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. *Biochemical Journal* **192**, 719–723.

Garlick, P. J., Wernerman, J., McNurlan, M. A., Essen, P., Lobley, G. E., Milne, E., Calder, A. G. & Vinnars, E. (1989). Measurement of the rate of protein synthesis in muscle of postabsorptive, young men by injection of a 'flooding dose' of [1-^{13}C]leucine. *Clinical Science* **77**, 329–336.

Harris, P. M., Skene, P. A., Buchan, V., Milne, E., Calder, A. G., Anderson, S. E., Connell, A. & Lobley, G. E. (1992). Effect of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio-venous techniques. *British Journal of Nutrition* **68**, 389–407.

Hauck, R. D. (1982). Nitrogen-isotope-ratio analysis. In *Methods of Soil Analysis*, pp. 759–765 [A. L. Page, R. H. Miller and D. R. Keeney, editors]. Madison, WI: American Society of Agronomy Inc.

Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971). Control of protein synthesis in mammalian tissues through changes in ribosome activity. *Journal of Biological Chemistry* **246**, 436–446.

Laurent, G. J. (1982). Rates of collagen synthesis in lung, skin and muscle obtained *in vivo* by a simplified method using [^{3}H] proline. *Biochemical Journal* **206**, 535–544.

Lewis, S. E. M., Kelly, F. J. & Goldspink, D. F. (1984). Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. *Biochemical Journal* **217**, 517–526.

Lobley, G. E. (1988). Protein turnover and energy metabolism in animals: interactions in leanness and obesity. In *Leanness in Domestic Birds*, pp. 331–361 [B. Leclerq and C. C. Whitehead, editors]. London: Butterworths.

Lobley, G. E., Connell, A., Buchan, V., Milne, E., Calder, A. G. & Skene, P. A. (1990*a*). Use of a large-dose, stable isotope procedure to measure muscle protein synthesis in sheep. *Proceedings of the Nutrition Society* **49**, 134A.

Lobley, G. E., Connell, A., Milne, E., Buchan, V., Calder, A. G., Anderson, S. E. & Vint, H. (1990*b*). Muscle protein synthesis in response to testosterone administration in wether lambs. *British Journal of Nutrition* **64**, 691–704.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). Whole body and tissue protein synthesis in cattle. *British Journal of Nutrition* **43**, 491–502.

McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979). The effect of starvation on the rate of protein synthesis in rat liver and small intestine. *Biochemical Journal* **178**, 373–379.

Merry, B. J., Holchan, A. M., Lewis, S. E. M. & Goldspink, D. F. (1987). The effects of ageing and chronic dietary restriction on *in vivo* hepatic protein synthesis in the rat. *Mechanisms of Ageing and Development* **39**, 189–199.

Nicholas, G. A., Lobley, G. E. & Harris, C. I. (1977). Use of the constant infusion technique for measuring rates of protein synthesis in the New Zealand White rabbit. *British Journal of Nutrition* **38**, 1–17.

Patureau-Mirand, P., Bernard, O., Prugnaud, J. & Arnat, M. (1985). Métabolisme protéique de l'agneau nouveau-né. II. Influence de l'alimentation sur les flux et au taux de renouvellement des protéines (Protein metabolism in the new-born lamb. II. Influence of intake on protein flux and turnover rate). *Reproduction, Nutrition, Développement* **25**, 1007–1112.

Pell, J. M. & Bates, P. C. (1987). Collagen and non-collagen protein turnover in skeletal muscle of growth hormone-treated lambs. *Journal of Endocrinology* **115**, R1–R4.

Robins, S. P. (1979). Metabolism of rabbit skin collagen. Differences in the apparent turnover rates of type-I- and type-III-collagen precursors determined by constant intravenous infusion of labelled amino acids. *Biochemical Journal* **181**, 75–82.

Rocha, D. M., Faloona, G. R. & Unger, R. H. (1972). Glucagon-stimulating activity of 20 amino acids in dogs. *Journal of Clinical Investigation* **51**, 2346–2351.

Schaefer, A. L., Davis, S. R. & Hughson, G. A. (1986). Estimation of tissue protein synthesis in sheep during sustained elevation of plasma leucine concentration by intravenous infusion. *British Journal of Nutrition* **56**, 281–288.

Seve, B., Reeds, P. J., Fuller, M. F., Cadenhead, A. & Hay, S. M. (1986). Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early weaning. Possible connection to energy metabolism. *Reproduction, Nutrition, Développement* **26**, 849–861.

Simon, O., Münchmeyer, R., Bergner, H., Zebrowska, T. & Buraczewska, L. (1978). Estimation of rate of protein synthesis by constant infusion of labelled amino acids in pigs. *British Journal of Nutrition* **40**, 243–252.

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