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# Effect of feed intake on ovine hindlimb protein metabolism based on thirteen amino acids and arterio–venous techniques

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It has been suggested that protein synthesis in peripheral tissues: (1) responds in a curvilinear manner to increasing feed intake over a wide range of feeding levels; and (2) has a greater sensitivity to intake than protein breakdown. The aim of the present experiment was to test these hypotheses across the ovine hindlimb. Six growing sheep (6–8 months, 30–35 kg), with catheters in the aorta (two), posterior vena cava and jugular vein, received each of four intakes of dried grass pellets (0·5, 1·0, 1·5 and 2·5 × maintenance energy; M) for a minimum of 7 d. A U-$^{13}$C-labelled algal hydrolysate was infused intravenously for 10 h and from 3–9 h *para*-aminohippuric acid was infused to measure plasma flow. Arterial and venous plasma were obtained over the last 4 h and the concentrations and enrichments of thirteen $^{13}$C-labelled amino acids (AA) were determined by GC–MS. As intake increased, a positive linear response was found for plasma flow, arterial concentrations of the aromatic and branched-chain AA, total flow of all AA into the hindquarters and net mass balance across the hindquarters (except glycine and alanine). Based on two separate statistical analyses, the data for protein synthesis showed a significant linear effect with intake (except for phenylalanine, glycine and alanine). No significant curvilinear effect was found, which tends not to support hypothesis 1. Nonetheless, protein synthesis was not significantly different between 0·5, 1·0 and 1·5 × M and thus the 2·5 × M intake level was largely responsible for the linear relationship found. There was no significant response in protein breakdown to intake, which supports hypothesis 2.

**Amino acids: Protein metabolism: Feed intake: Hindlimb: Arterio–venous: Sheep**

Enhanced nutrition of growing animals results in improved protein gain. This gain is associated with changes in both whole-body and peripheral tissue protein synthesis and degradation in non-ruminant, pre-ruminant and ruminant animals (e.g. Reeds *et al.* 1980; Lobley *et al.* 1987; Oddy *et al.* 1987; Dawson *et al.* 1991; Thomson *et al.* 1997).

It has been proposed (Lobley, 1993*a*, 1998), based on a combination of data from various sources, that ruminant animal peripheral tissue protein synthesis and breakdown both respond in a curvilinear manner to increasing feed intake between fasting and supra-maintenance levels. When linking differential actions of anabolic hormones to feed intake, it was also proposed that the response differs between synthesis and degradation, with synthesis more sensitive to changes in intake.

Although a number of studies have investigated the effect of nutrition on hindquarter (or muscle) protein synthesis in growing ruminant animals, most have involved limited levels of feed intake per study, over a restricted range (e.g. Lobley *et al.* 1987, 1992; Dawson *et al.* 1991; Harris *et al.* 1992; Boisclair *et al.* 1993; Thomson *et al.* 1997; Liu *et al.* 1998). Thus, it is difficult to assess the overall response pattern of protein synthesis to feed intake. Data on protein degradation are even more limited due to the lack of suitable methods for measurement of degradation, and only the arterio–venous (A–V) technique used in certain studies (e.g. Harris *et al.* 1992; Boisclair *et al.* 1993) allows simultaneous estimation of protein gain, synthesis and breakdown.

The aim of the current experiment was to investigate whether the protein kinetic basis of the hypotheses

**Abbreviations:** AA, amino acid; A–V, arterio–venous; M, maintenance energy; PF, plasma flow.

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mentioned earlier were correct, within the context of hindlimb protein metabolism of growing lambs across levels of feed intake from 0·5 to 2·5 × maintenance energy (M), using A–V techniques. The infusion of a multiple U-$^{13}$C-labelled amino acid (AA) mixture, derived from hydrolysis of algal protein allowed the kinetics of thirteen different AA to be measured and provided a comparison for the single or dual tracers used in other studies. Part of these findings, corresponding to lysine kinetics, have been presented elsewhere (Savary *et al.* 2001).

## Materials and methods

### *Animals and treatments*

Suffolk-cross wether lambs (*n* 6, aged 6–8 months, mean live weight 32·5 (SD 2·0)kg) were surgically prepared with hindlimb A–V catheters according to the methods described by Savary *et al.* (2001). In short, permanent indwelling polyvinyl or silicone rubber catheters were inserted into the aorta (two, 3 cm apart; caudal for infusion of plasma flow (PF) marker *para*-aminohippurate and cranial for arterial blood sampling) and posterior vena cava (venous blood sampling). As required, temporary polyvinyl catheters were inserted into the jugular vein (for labelled algal hydrolysate infusion), posterior vena cava via tarsal vein (if permanent vena cava catheter lost patency) and auricular artery (if one of the aortal catheters lost patency), or occasionally into the heart via jugular vein (if the auricular artery also failed).

The sheep were fed a pelleted dried grass diet (10 MJ estimated metabolisable energy/kg DM; 26 g N/kg DM) from automatic feeders, supplied daily in twenty-four equal portions, one per hour, whilst housed in metabolism cages. Originally in a Latin square design, but with two sequences repeated, the animals were offered each of four levels of feed intake corresponding to 0·5, 1·0, 1·5 and 2·5 × M (based on daily M requirements of 400 kJ/kg body weight$^{0.75}$). Each level of intake was fed for 2 weeks prior to measurement, except for 0·5 × M, which was only fed for 1 week following a prior week at 1·0 × M. During the week leading up to measurement, animals were housed in metabolism cages, otherwise they were individually housed in floor pens with straw bedding and fed twice per day. The minimum of 14 d between measurements was considered sufficient to allow animal recovery and adjustment to the new intake level. The experiment was conducted between November and February and animals were kept under standard lighting conditions of 10 h light–14 h dark, with free access to water and a multi-mineral salt block.

### *Infusion and blood sampling*

Measurements of AA kinetics were made on the last day of each 2-week feeding period and involved infusion of a U-$^{13}$C-labelled algal hydrolysate, prepared as described by Savary *et al.* (2001), based on Lobley *et al.* (1996). Samples of plasma were obtained on the day prior to infusion to provide background (natural abundance) samples for gas GC–MS analyses. The same amount of algal hydrolysate was continuously infused (20 g infusate/h; 22–198 μmol various AAh) into the jugular vein over a 10 h period, regardless of level of feed intake. From 3 to 9 h following the start of the algal hydrolysate infusion, a solution of 0·15 M-sodium *para*-amino hippuric acid (pH 7·4, 20 g/h) containing sodium heparin (400 IU/g) was infused into the more caudal aortal catheter to allow the determination of PF. Integrated blood samples were withdrawn continuously (10 g/h) by peristaltic pump from both the other aortal catheter (or auricular artery or heart) and the vena cava over 30 min intervals during the last 4 h of *para*-aminohippuric acid infusion. Muscle and skin tissue biopsy samples were obtained from the hindlimb (*m. vastus lateralis*) as described by Savary *et al.* (2001) for analysis of AA transport rates; these will be presented in a companion paper.

### *Laboratory analyses*

Blood (5 g) was centrifuged for 10 min at 1000 ***g***, to obtain 3 g plasma. PF (kg/min) was determined from 1 g plasma according to the gravimetric method described by Lobley *et al.* (1995). To another, 1 g plasma was added 0·3 g 2·5 × diluted solution of the algal infusate for measurement of AA concentration by the isotope dilution technique using the *t*-butyldimethylsilyl derivatives (Calder *et al.* 1999). The final volume of plasma was stored in a microcentrifuge tube for later preparation of freeze-dried eluates (Savary *et al.* 2001). Enrichments of AA (as molar percent excess) were determined from freeze-dried eluates as *n*-butylheptafluorobutyryl derivatives, with the D and L proportions of lysine resolved on a chiral column (Lobley *et al.* 1996). Results for glutamate, aspartate, asparagine, glutamine, arginine, cysteine and tryptophan are not presented due to technical reasons. Losses of most of these AA occur during acid hydrolysis of the algal powder, and in addition to this problem, the method used to create derivatives for GC–MS analyses is associated with oxidation and losses of some of these amino acids (see Lobley *et al.* 1996).

### *Calculations*

The hindlimb AA A–V kinetic calculations are based upon those described for phenylalanine by Harris *et al.* (1992). For simplicity, it was assumed that for all AA no catabolism occurred across the hindquarters, even though this is known to be untrue for the non-essential AA and branched chain AA measured (Pell *et al.* 1986).

Flow of AA into (A flow) or from (V flow; results not shown) the hindquarters was calculated as:

$$\text{Flow of AA } (\mu\text{mol/min}) = C_{(a \text{ or } v)} \times PF, \quad (1)$$

where for each sample, C represents free AA concentrations (μmol) for arterial (a) and venous (v) plasma).

Net retention of all AA, presumed to be exclusively for protein gain (i.e. no oxidation, nor synthesis of metabolites, nor expansion or contraction of the intracellular or extracellular free AA pool occurs), was calculated as:

$$\text{Net retention of AA } (\mu\text{mol/min}) = (Ca - Cv) \times PF. \quad (2)$$

Amino acids for protein synthesis were estimated from

the net removal of isotope as the respective AA, using V as the precursor pool:

$$\text{AA for protein synthesis } (\mu\text{mol/min}) = \text{PF} \times (\text{Ca} \times \text{Ea} - \text{Cv} \times \text{Ev})/\text{Ev}, \quad (3)$$

where for each sample, E represents the enrichment (molar percent excess) of the AA in arterial (a) and venous (v) plasma. To convert this value into daily protein synthesis (g protein/d) equation 3 was multiplied by a conversion factor (CF) given by:

$$\text{CF} = 60 \times 24 \times (\text{MW}_{\text{AA}}/\text{PC}_{\text{AA}})/10^4,$$

where $\text{MW}_{\text{AA}}$ represents the molecular weight of the AA and $\text{PC}_{\text{AA}}$ represents the protein content of the AA (g) per 100 g ovine mixed protein deposited during growth, from MacRae *et al.* (1993).

Protein breakdown (g protein/d) was calculated as the difference between net retention of AA (μmol/min; equation 2) and AA synthesis (μmol/min; equation 3); this was then multipied by CF to derive values as g protein/d.

*Statistics*

All statistical analyses were carried out with Genstat 5.4.1 (Lawes Educational Trust, Rothamsted, Herts., UK). Results having $P$ values $>0{\cdot}05$ were considered non-significant, but $P$ values $<0{\cdot}10$ are presented.

Data for the individual AA were analysed by ANOVA, where animals were treated as blocks and intake as treatment. In all cases there was no significant effect of period and this was excluded in order to increase the residual d.f. Data were also analysed for both linear and quadratic effects.

To investigate any interaction between AA and intake, a repeated–measures analysis was conducted on the protein synthesis estimates obtained from each of the individual essential AA, plus tyrosine, from Table 1. The random effects consisted of AA nested within period within animals. AA, intake and their interaction were treated as fixed effects. The dependency between the repeated measures was modelled by means of both uniform and unstructured covariance matrices (using the REML and VSTRUCTURE directives in Genstat Lawes Educational Trust). The former assumes that the AA have equal variances and that

**Table 1.** Effect of feed intake (× maintenance energy, M) on essential amino acid arterial concentration (μM), arterial inflow (μmol/min), net retention (μmol/min), protein synthesis and breakdown (both g protein/d) across the ovine hindquarters*

| | Feed intake (× M) | | | | *P* values | | |
|---|---|---|---|---|---|---|---|
| Amino acid | 0·5 | 1·0 | 1·5 | 2·5 | Intake | Linear | SED |
| Histidine† | | | | | | | |
| Arterial concentration | 52·7 | 54·4 | 51·4 | 51·9 | NS | NS | 4·74 |
| Arterial flow | 28·1 | 28·7 | 31·7 | 42·0 | 0·044 | 0·008 | 4·89 |
| Net retention | −0·8 | 0·3 | 0·6 | 2·4 | <0·001 | <0·001 | 0·42 |
| Synthesis | 40·9 | 40·3 | 44·8 | 67·7 | 0·032 | 0·007 | 9·30 |
| Breakdown | 49·4 | 36·8 | 38·2 | 42·3 | NS | NS | 9·76 |
| Lysine‡ | | | | | | | |
| Arterial concentration | 78·9 | 89·1 | 91·0 | 91·8 | NS | NS | 18·18 |
| Arterial flow | 36·8 | 47·9 | 56·1 | 76·4 | 0·008 | <0·001 | 9·86 |
| Net retention | −0·8 | 1·7 | 3·2 | 7·2 | <0·001 | <0·001 | 0·92 |
| Synthesis | 33·1 | 36·4 | 45·6 | 65·3 | <0·001 | <0·001 | 6·40 |
| Breakdown | 35·7 | 30·6 | 35·0 | 41·7 | NS | NS | 6·43 |
| Methionine† | | | | | | | |
| Arterial concentration | 13·6 | 15·3 | 17·4 | 21·0 | NS | 0·027 | 3·16 |
| Arterial flow | 8·5 | 8·4 | 10·6 | 19·3 | 0·002 | <0·001 | 2·49 |
| Net retention | −0·4 | −0·1 | 0·4 | 1·9 | <0·001 | <0·001 | 0·40 |
| Synthesis§ | 30·2 | 22·6 | 36·4 | 79·2 | 0·002 | <0·001 | 12·54 |
| Breakdown§ | 35·8 | 23·8 | 32·0 | 55·5 | 0·053 | 0·032 | 10·54 |
| Phenylalanine‖ | | | | | | | |
| Arterial concentration | 38·4 | 42·3 | 48·8 | 64·2 | 0·009 | 0·001 | 6·47 |
| Arterial flow | 22·8 | 25·3 | 31·4 | 60·2 | 0·002 | <0·001 | 7·80 |
| Net retention | −1·1 | 0·0 | 0·0 | 1·92 | <0·001 | <0·001 | 0·33 |
| Synthesis | 49·4 | 44·2 | 50·6 | 71·4 | NS | NS | 12·05 |
| Breakdown | 57·8 | 44·3 | 50·9 | 56·6 | NS | NS | 12·41 |
| Threonine† | | | | | | | |
| Arterial concentration | 68·4 | 100·6 | 108·6 | 124·1 | NS | NS | 23·02 |
| Arterial flow | 43·1 | 51·8 | 61·3 | 102·0 | 0·002 | <0·001 | 13·10 |
| Net retention | −1·9 | 0·6 | 0·9 | 3·8 | 0·001 | <0·001 | 1·11 |
| Synthesis | 47·2 | 44·4 | 53·5 | 85·6 | 0·007 | 0·001 | 10·86 |
| Breakdown | 55·3 | 41·4 | 49·6 | 69·8 | NS | NS | 13·24 |

* For details of diets and procedures, see p. 578 and Savary *et al.* (2001).
† d.f. 14.
‡ d.f. 15.
§ Quadratic effect for synthesis $P$=0·059, degradation $P$=0·077.
‖ d.f. 12.

correlations between each pair of AA observations are equal, whereas the latter allows for different variances and correlations. It was found that the unstructured covariance matrix gave a significantly better fit ($P<0{\cdot}001$, using the deviance test) so that this was used throughout.

The efficiency of net retention was investigated by linear regression for each essential AA, with net retention as response variable and protein synthesis as explanatory variable. Sheep effects and interactions between sheep and protein synthesis were found not to be significant and were excluded from the regression analysis.

## Results

All sheep completed the experiment within the original design, and consumed all feed. Mean body weight changes during each 2-week period of feeding were (g/d): 0·5 × M −321 (SE 60·5), 1·0 × M −24 (SE 39·8), 1·5 × M 48 (SE 57·3), 2·5 × M 381 (SE 79·6).

Hindquarter PF increased linearly with intake ($P<0{\cdot}05$ intake, $P<0{\cdot}01$ linear), corresponding to 515, 534, 607 and 836 ml/min (SED 103·5) for 0·5, 1·0, 1·5 and 2·5 × M respectively. A positive linear effect of feed intake on AA concentration in the arterial plasma was found for both the aromatic AA (phenylalanine and tyrosine, $P<0{\cdot}001$; Tables 1 and 3) and the branched chain AA ($P<0{\cdot}05$; Table 2). The flow of AA into the hindquarters increased in a positive linear manner with intake for all AA ($P<0{\cdot}05$; Tables 1–3), except for glycine. For all the essential AA, the effect of intake on net mass balance across the hindlimbs was significant and linear ($P<0{\cdot}001$; Tables 1 and 2). The incremental nature of AA retention with level of intake was obvious for all essential AA, except phenylalanine. Linear relationships were also found for the non-essential AA ($P<0{\cdot}01$; Table 3), except for glycine and alanine which were unaffected by intake.

Feed intake did not significantly alter protein breakdown for any AA, although a tendency was observed for methionine ($P=0{\cdot}053$). In contrast, for all the essential AA (except phenylalanine) and the non-essential AA (except glycine and alanine) there were significant effects on protein synthesis, with a strong linear component ($P<0{\cdot}01$). This apparent linearity of protein synthesis may result from the constraints applied by the conventional ANOVA where the treatment extremes dominate the slope and then the extent of deviations of the intermediate points from linearity is assessed. This statistical indication of linearity contrasts with the visual impression of curvilinearity gained from the mean values (Tables 1–3; see Fig. 1(a), methionine, and 1(b), serine). Only for methionine, however, was there a statistical tendency towards a quadratic effect (synthesis $P=0{\cdot}059$, degradation $P=0{\cdot}077$). Despite this, for all AA there were no significant differences between the 0·5, 1·0 and 1·5 × M intakes for arterial AA concentration, flow into the hindquarter, net retention of AA or protein synthesis. Indeed, the major response was that values at 2·5 × M differed significantly from those at both 0·5 and 1·0 × M ($P<0{\cdot}001$) and, in most cases, the data at 1·5 × M were also lower than for the highest intake ($P<0{\cdot}05$). This emphasises the dominant effect of the 2·5 × M intake on the linear-relationships derived.

Conversion of the original protein synthesis data (μmol AA/min) to absolute protein synthesis (g protein/d) provides a comparative base across all the AA. This calculation assumes that the ratios of AA present in deposited protein

**Table 2.** Effect of feed intake (× maintenance energy, M) on branched chain amino acid arterial concentration (μM), arterial inflow (μmol/min), net retention (μmol/min), protein synthesis and breakdown (both g protein/d) across the ovine hindquarters*

(Mean values for six sheep)

| | Feed intake (× M) | | | | *P* values | | |
|---|---|---|---|---|---|---|---|
| Amino acid | 0·5 | 1·0 | 1·5 | 2·5 | Intake | Linear | SED |
| Isoleucine† | | | | | | | |
| Arterial concentration | 60·2 | 76·0 | 90·2 | 116·8 | 0·003 | <0·001 | 12·57 |
| Arterial flow | 40·5 | 40·8 | 54·0 | 105·0 | <0·001 | <0·001 | 11·68 |
| Net retention | −0·5 | 2·3 | 3·3 | 8·3 | <0·001 | <0·001 | 1·14 |
| Synthesis | 57·2 | 46·7 | 67·8 | 116·9 | 0·006 | 0·001 | 17·43 |
| Breakdown | 60·4 | 33·2 | 48·2 | 68·2 | NS | NS | 16·03 |
| Leucine† | | | | | | | |
| Arterial concentration | 106·4 | 113·6 | 133·8 | 174·3 | 0·032 | 0·004 | 21·80 |
| Arterial flow | 50·9 | 59·9 | 81·1 | 146·9 | <0·001 | <0·001 | 16·68 |
| Net retention | −0·6 | 3·4 | 5·3 | 12·0 | <0·001 | <0·001 | 1·52 |
| Synthesis | 48·7 | 56·3 | 67·2 | 110·8 | 0·004 | <0·001 | 14·85 |
| Breakdown | 50·7 | 45·8 | 50·8 | 73·7 | NS | NS | 12·54 |
| Valine‡ | | | | | | | |
| Arterial concentration | 164·9 | 189·0 | 240·2 | 321·1 | 0·002 | <0·001 | 33·51 |
| Arterial flow | 56·4 | 101·9 | 151·3 | 243·9 | 0·002 | <0·001 | 38·13 |
| Net retention | −0·9 | 2·2 | 3·8 | 9·0 | 0·001 | <0·001 | 1·90 |
| Synthesis | 58·6 | 45·6 | 67·8 | 136·8 | 0·006 | 0·001 | 22·03 |
| Breakdown | 60·8 | 36·2 | 51·9 | 92·4 | NS | 0·084 | 22·46 |

* For details of diets and procedures, see p. 578 and Savary *et al.* (2001).
† d.f. 14.
‡ d.f. 13.

**Table 3.** Effect of feed intake (× maintenance energy, M) on non-essential amino acid arterial concentration (μM), arterial inflow (μmol/min), net retention (μmol/min), protein synthesis and breakdown (both g protein/d) across the ovine hindquarters*

(Mean values for six sheep)

| | Feed intake (× M) | | | | *P* values | | |
|---|---|---|---|---|---|---|---|
| Amino acid | 0·5 | 1·0 | 1·5 | 2·5 | Intake | Linear | SED |
| Alanine† | | | | | | | |
| Arterial concentration | 105·2 | 118·7 | 129·0 | 118·3 | NS | NS | 16·91 |
| Arterial flow | 52·4 | 62·0 | 75·3 | 93·2 | 0·010 | 0·001 | 10·84 |
| Net retention | −6·2 | −5·0 | −6·0 | −7·6 | NS | NS | 2·77 |
| Synthesis | 39·4 | 41·8 | 55·5 | 57·7 | NS | NS | 12·19 |
| Breakdown | 51·0 | 51·1 | 66·7 | 70·3 | NS | 0·080 | 12·04 |
| Glycine‡ | | | | | | | |
| Arterial concentration | 429·2 | 414·4 | 362·9 | 370·0 | NS | NS | 53·93 |
| Arterial flow | 211·1 | 216·4 | 216·9 | 300·1 | NS | NS | 38·81 |
| Net retention | −3·6 | −2·6 | −3·7 | −4·8 | NS | NS | 2·36 |
| Synthesis | 33·2 | 28·1 | 32·7 | 44·1 | NS | NS | 9·73 |
| Breakdown | 37·7 | 31·6 | 37·4 | 50·1 | NS | NS | 8·59 |
| Proline‡ | | | | | | | |
| Arterial concentration | 56·6 | 65·0 | 68·6 | 72·5 | NS | NS | 10·69 |
| Arterial flow | 27·5 | 33·9 | 41·3 | 60·4 | 0·003 | <0·001 | 7·26 |
| Net retention | −0·5 | 0·8 | 1·7 | 3·6 | <0·001 | <0·001 | 0·58 |
| Synthesis | 15·5 | 17·7 | 22·8 | 29·7 | 0·026 | 0·003 | 4·33 |
| Breakdown | 16·8 | 15·4 | 18·1 | 19·6 | NS | NS | 4·25 |
| Serine§ | | | | | | | |
| Arterial concentration | 30·3 | 40·8 | 44·0 | 52·7 | NS | 0·027 | 8·90 |
| Arterial flow | 19·5 | 22·1 | 25·7 | 44·1 | 0·001 | <0·001 | 5·26 |
| Net retention | −0·4 | 2·1 | 2·2 | 6·1 | 0·002 | <0·001 | 1·27 |
| Synthesis | 55·7 | 47·9 | 63·5 | 100·5 | 0·020 | 0·005 | 15·45 |
| Breakdown | 56·9 | 38·7 | 54·0 | 74·3 | NS | NS | 16·40 |
| Tyrosine‡ | | | | | | | |
| Arterial concentration | 34·5 | 46·9 | 48·6 | 71·2 | 0·001 | <0·001 | 7·00 |
| Arterial flow | 17·3 | 24·3 | 30·3 | 60·7 | <0·001 | <0·001 | 5·92 |
| Net retention | −0·8 | −0·1 | −0·3 | 0·6 | 0·037 | 0·008 | 0·43 |
| Synthesis | 43·1 | 47·2 | 55·0 | 81·8 | 0·036 | 0·006 | 12·49 |
| Breakdown | 51·5 | 48·6 | 58·4 | 75·9 | NS | 0·050 | 13·03 |

* For details of diets and procedures, see p. 578 and Savary *et al.* (2001).
† d.f. 15.
‡ d.f. 13.
§ d.f. 14.

are the same as that in the amounts synthesised and that no conversion of the AA to other metabolites occurs. The former assumption is not strictly correct, but can be used as a first approximation. The second assumption is probably valid for most AA in Table 1 (plus tyrosine), but not for the branched-chain AA which are catabolised by hindquarter tissues (Pell *et al.* 1986; Oddy *et al.* 1987; Harris *et al.* 1992). In practise, comparison of the absolute protein synthesis values (g protein/d) obtained from the repeated-measures analysis on the essential AA (except for the branched-chain AA) resulted in no significant differences, except that the threonine- and tyrosine-derived values were greater than for the other essential AA ($P<0·01$). The repeated-measures analysis was also used to test for linear and quadratic effects of intake on protein synthesis of the combined essential AA (except for the branched-chain AA). As for the individual AA analyses, this combined analysis also gave a strong linear response ($P<0·001$), with quadratic effects non-significant. Furthermore, protein synthesis values for 0·5, 1·0 and 1·5 × M were not significantly different from each other, whereas values for 2·5 × M were significantly greater than for 0·5, 1·0 ($P<0·01$) and 1·5 × M ($P<0·05$).

For each of the essential AA, the efficiency of protein retained *v.* protein synthesised was obtained from the slopes of linear regression of net retention on protein synthesis. The efficiency ranged from 6 % for phenylalanine, to (%): threonine 14, tyrosine 18, valine 22, methionine 31, isoleucine 33, leucine 37, histidine 44, lysine 51.

## Discussion

This experiment set out to challenge two hypotheses (Lobley, 1998): (1) that protein synthesis in peripheral tissues responds in a curvilinear manner to increasing feed intake; and (2) that protein synthesis is more sensitive to changes in feed intake than protein breakdown. These were investigated using A–V approaches across the ovine hindquarters but two major constraints were involved.

First, the surgical preparation encompasses the muscle, skin, bone and fat of the total hindquarters. Therefore, any observed responses to intake will be the combined result of metabolism in all these peripheral tissues. The muscle, skin

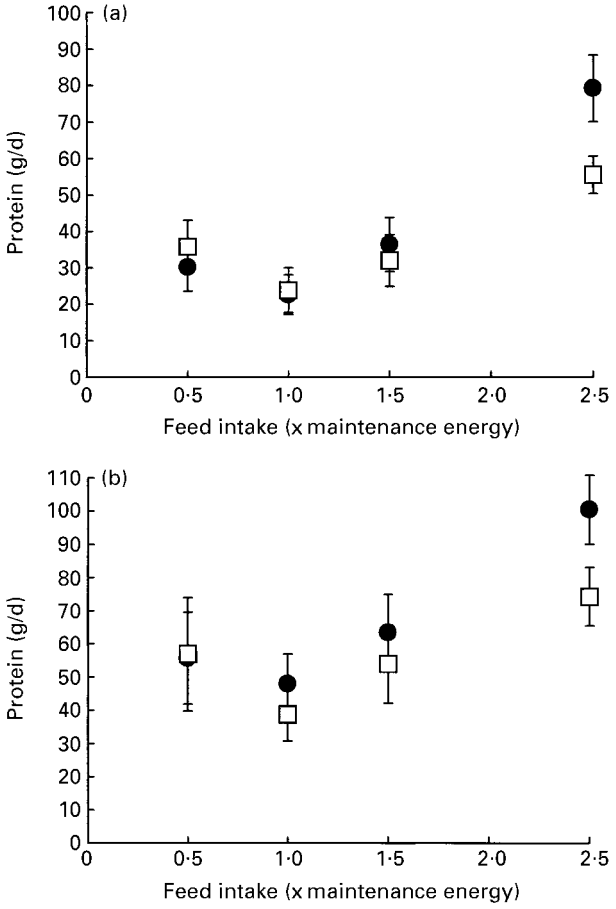


**Fig. 1.** Effect of feed intake on mean protein synthesis (●) and degradation (□) in the hindquarters of sheep, calculated from (a) methionine and (b) serine arterio–venous transfers. For details of diets and procedures, see p. 578 and Savary *et al.* (2001). Values are means for six sheep, with their standard errors represented by vertical bars.

and bone drained by the catheterised vasculature vary both in mass and metabolic activity (e.g. Lobley *et al.* 1992). Nonetheless, increased intake has been shown in a number of species to increase protein synthesis in muscle, skin and bone (e.g. Seve *et al.* 1986; Lobley *et al.* 1992, 1993*b*; Ponter *et al.* 1994), although differences with skin have been reported (Zhang *et al.* 1998). Therefore, these peripheral tissues exhibit similar general responses, even if subtle differences may exist.

The second issue relates to the absolute values obtained by the A–V kinetic approach and these depend on the choice of precursor pool(s). A number of alternatives are possible. In theory, the most acceptable choices would involve measurement of enrichments from either nascent polypeptide chains (Ilan & Singer, 1975) or the aminoacyl-tRNA pools (Airhart *et al.* 1974). The latter has been used in a number of studies (e.g. Airhart *et al.* 1974; Khairallah & Mortimore, 1976; Schneible & Young, 1984; Ljungqvist *et al.* 1997; Davis *et al.* 1999), but suffers from concerns that charging times of the aminoacyl-tRNA encompass only a few seconds and may alter during removal of the tissue sample. In the current study, it was considered important that a wide range of AA were studied to ensure there was no bias due to specific metabolic reactions and to extend this into analysis of all the aminoacyl-tRNA was not feasible. Another option would involve use of homogenate ('intracellular') free AA enrichments (Ljungqvist *et al.* 1997), but not all tissues (notably bone) could be sampled within the longitudinal design. Uncertainties also existed as to how representative the small biopsies of skin and muscle would be of the total tissue masses. In addition, even use of these tissue enrichments would not necessarily be appropriate, because previous ovine studies have shown that while the precursor pool for muscle may be intracellular, that for skin is closer to arterial samples (Lobley *et al.* 1992). For these various reasons it was decided to use the enrichment of the plasma free AA as the 'precursor'. This is a commonly adopted approach with A–V techniques in both animals and human subjects (e.g. Pell *et al.* 1986; Boisclair *et al.* 1993; Fryburg *et al.* 1995). Often the arterial sample is used, but the venous sample has the advantage of being closer to the intracellular enrichment (indeed if all the plasma AA exchanged with the tissue then the intracellular and venous enrichments would be identical). Although use of the venous (or arterial) free AA will not necessarily yield a correct answer in absolute terms, the interpretations will be valid if the enrichment ration of the selected:true precursor is unaltered by the dietary treatments. This assumption appears to hold for the ovine skin and muscle between 0·6 and 1·8 × M intakes (Lobley *et al.* 1992), but not for the more extreme situations when pigs and dogs are infused with insulin and AA (Watt *et al.* 1992; Caso *et al.* 2000).

Hypothesis 1 was developed from ovine data from many sources (Lobley, 1998) and, as such, is susceptible to differences in breed, age, technique employed (continuous infusion, large dose, A–V etc.) and the labelled AA selected as the tracer. The current study permitted analysis within a discrete age and breed of sheep, across a range of intakes (although not at the extremes of fasting and *ad libitum* intake used in the hypothesis development) and encompassed kinetics for most AA.

Across the selected intakes, the net balances of AA progressed from negative to positive in an incremental manner, in line with the changes in body weight. The two exceptions, alanine and glycine, are well known to perform major functions in peripheral tissues in addition to involvement in protein turnover. For example, maintenance of glucose homeostasis involves transport of 3C units as alanine from skeletal muscle for gluconeogenesis (Wolff & Bergman, 1972). Glycine on the other hand, is required for glutathione synthesis and retention within ovine peripheral tissues (Lee *et al.* 1993).

The current study showed significant effects of feed intake on hindlimb protein synthesis for the majority of AA. The various statistical methods used indicated this relationship to be linear, but this could possibly still be misleading. Comparisons between the four levels of feed intake revealed that the 0·5, 1·0 and 1·5 × M values were not significantly different from each another. In contrast, all 0·5 and 1·0 × M and most of the 1·5 × M values were less than values for 2·5 × M (see protein synthesis in Fig. 1). This analysis would suggest a relatively reduced responsiveness

of peripheral tissue protein synthesis to changes in intake at low levels. This has a mechanistic basis in that the changes in protein turnover that occur at lower intakes appear to involve insulin. In a number of studies of ruminant animals (e.g. Oddy *et al.* 1987; Wester *et al.* 2000) insulin has been shown to increase net protein (AA) balance across peripheral tissues, but mainly through changes in protein degradation, rather than synthesis (Oddy *et al.* 1987; Tesseraud *et al.* 1993; Grizard *et al.* 1999; Wester *et al.* 2000). At higher intakes, the growth hormone–insulin-like growth factor-I axis plays a more dominant role and, in most studies, this involved stimulation of protein synthesis (e.g. Pell & Bates, 1987; Crompton & Lomax, 1989; Boisclair *et al.* 1994). The original hypothesis (Lobley, 1998) was derived from analysis that included data from fasted animals and these values markedly influenced the apparent curvilinear nature of the response. Fasting was not included in the present study as it was considered to be aphysiological, rarely occurring in practical animal production systems.

Hypothesis 2, the less sensitive response of protein degradation to intake compared with protein synthesis across a wide range of feeding levels, was supported by the present study, as no significant changes in protein breakdown were found. Studies by Harris *et al.* (1992; 0·6–1·8 × M, leucine results only), Crompton & Lomax (1993; 0·26–3·4 × M) and Thomson *et al.* (1997; 0·6–1·0 × M) with sheep and Boisclair *et al.* (1993; 0·6–2·2 × M) with cattle, have found that the increased protein gain associated with higher intake occurred due to increases in protein synthesis, with no alteration in degradation. In the study by Harris *et al.* (1992), however, based on phenylalanine kinetics and conducted with sheep of similar genotype, age and live weight and fed the same diet as the current study, hindleg protein breakdown significantly increased from 1·2–1·8 × M, with no difference between 0·6 and 1·2 × M. In contrast, the same study showed no significant difference in protein breakdown between 0·6 and 1·8 × M when leucine was used as the tracer (Harris *et al.* 1992). This agrees with the results for leucine from the present study and indicates that kinetic observations may depend on the AA selected as precursor and the various assumptions inherent in the approach. Interestingly, Oddy *et al.* (1987) observed that changing milk-fed lambs from fasting to maximum intake led to an increase in protein synthesis, based on leucine kinetics, but protein degradation in the hindlimb decreased. This may be a feature of including fasting as a treatment, when protein catabolism is markedly accelerated to provide C from AA as gluconeogenic precursors.

The phenylalanine results of Harris *et al.* (1992) where protein synthesis increased across all intake levels, yet protein breakdown increased only between the two higher levels of intake supports hypothesis 2 of the present study. However, contradictory results exist for phenylalanine in that while the present study failed to find any effect of intake on protein breakdown, protein synthesis also showed no significant change, despite the greater number of intake levels used. This is in direct contrast with the study of Harris *et al.* (1992). These apparently opposing observations may be a feature of the higher variance (compared with data from other AA) associated with phenylalanine measurements in the current study. The other aromatic AA, tyrosine, that acts as an essential AA in muscle or across the hindlimb (Chang & Goldberg, 1978; Harris *et al.* 1992; Boisclair *et al.* 1993) did achieve significance for protein synthesis. Of all the essential AA, the current phenylalanine data are unique in not showing significant effects for synthesis.

AA tracers used in hindlimb (or muscle) AA metabolism studies in ruminant animals have been mostly limited to branched-chain AA (leucine and/or valine), phenylalanine (e.g. Teleni *et al.* 1986; Oddy *et al.* 1987; Harris *et al.* 1992; Lobley *et al.* 1992; Rocha *et al.* 1993; Thomson *et al.* 1997) or tyrosine (e.g. Dawson *et al.* 1991; Crompton & Lomax, 1993). The branched-chain AA are oxidized across the ruminant animals' hindquarters (e.g. Pell *et al.* 1986; Teleni *et al.* 1986; Harris *et al.* 1992) and may also effect the sensitivity of protein turnover within muscle to insulin (Garlick & Grant, 1988; Wester *et al.* 2000). In general, the branched-chain AA in the current study gave higher apparent values for protein synthesis than the other essential AA, but this is probably due to inclusion of the oxidation within the anabolic component. The trends with intake for the branched-chain AA may thus reflect responses in protein turnover (Pell *et al.* 1986; Harris *et al.* 1992), but yield overestimated absolute values.

For the non-essential AA, there is the capacity for synthesis *de novo*, plus oxidation and inter-conversions within the hindlimb and these additional processes might be expected to result in higher apparent net retention and protein synthesis–breakdown values than for the essential AA. In practice, however, similar values were obtained for protein turnover from both essential AA and non-essential AA kinetics. For example, arterial AA inflow, net AA retention, protein synthesis and protein breakdown of proline and serine responded to feed intake similar to the essential AA. The hindquarters have a high requirement for synthesis of collagen within the skin and this may dominate proline metabolism and requirements (Pell & Bates, 1987). The synthesis *de novo* and inter-conversion of glycine and serine, as reported in both the rat (Fern & Garlick, 1974) and across the ovine placenta (Cetin *et al.* 1992), occurs to only a minor extent in non-pregnant sheep, either in terms of whole-body plasma fluxes (GE Lobley, unpublished results) or within the skin (Liu *et al.* 2000). In the absence of metabolic transformations in peripheral tissues, serine may only have two fates, synthesis and oxidation, and thus similar values to the essential AA would be obtained.

The current data show that, across the hindlimbs, most of the AA gave similar kinetic data, with the major effect being changes in synthesis. In consequence, the 'efficiency' of gain (relative to incremental synthesis) remained high. The relative insensitivity of peripheral tissue protein breakdown to intake may have implications for strategies based on increasing proteolysis *post-mortem* as a mechanism for production of more tender meat (see Lobley *et al.* 2000).

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## References

Airhart J, Vidrich A & Khairallah EA (1974) Compartmentation of free amino acids for protein synthesis in rat liver. *Biochemical Journal* **140**, 539–545.

Boisclair YR, Bauman DE, Bell AW, Dunshea FR & Harkins M (1994) Nutrient utilisation and protein turnover in the hindlimb of cattle treated with bovine somatotropin. *Journal of Nutrition* **124**, 664–673.

Boisclair YR, Bell AW, Dunshea FR, Harkins M & Bauman DE (1993) Evaluation of the arteriovenous difference technique to simultaneously estimate protein-synthesis and degradation in the hind-limb of fed and chronically underfed steers. *Journal of Nutrition* **123**, 1076–1088.

Calder AG, Garden KE, Anderson SE & Lobley GE (1999) Quantitation of blood and plasma amino acids using isotope dilution mass spectrometry impact gas chromatography/mass spectrometry with U-$^{13}$C amino acids as internal standards. *Rapid Communications in Mass Spectrometry* **13**, 2080–2083.

Caso G, Ford C, Nair KS, Garlick PJ & McNurlan MA (2000) Insulin with euaminoacidemia stimulates muscle protein synthesis in dogs. *FASEB Journal* **14**, A768.

Cetin I, Fennessey PV, Sparks JW, Meschia G & Battaglia FC (1992) Fetal serine fluxes across fetal liver, hind-limb, and placenta in late gestation. *American Journal of Physiology* **263**, E786–E793.

Chang TW & Goldberg AL (1978) The origin of alanine produced in skeletal muscle. *Journal of Biological Chemistry* **253**, 3677–3684.

Crompton LA & Lomax MA (1989) The effect of growth hormone on hind-limb muscle protein metabolism in growing lambs. *Proceedings of the Nutrition Society* **48**, 96A.

Crompton LA & Lomax MA (1993) Hind-limb protein-turnover and muscle protein-synthesis in lambs – a comparison of techniques. *British Journal of Nutrition* **69**, 345–358.

Davis TA, Fiorotto ML, Nguyen HV & Burrin DG (1999) Aminoacyl-tRNA and tissue free amino acid pools are equilibrated after a flooding dose of phenylalanine. *American Journal of Physiology* **277**, E103–E109.

Dawson JM, Buttery PJ, Lammiman MJ, Soar JB & Essex CP (1991) Nutritional and endocrinological manipulation of lean deposition in forage-fed steers. *British Journal of Nutrition* **66**, 171–185.

Fern EB & Garlick PJ (1974) The specific radioactivity of the tissue free amino acid pool as a basis for measuring the rate of protein synthesis in the rat *in vivo*. *Biochemical Journal* **142**, 413–419.

Fryburg DA, Jahn LA, Hill SA, Oliveras DM & Barrett EJ (1995) Insulin and insulin-like growth factor-1 enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. *Journal of Clinical Investigation* **96**, 1722–1729.

Garlick PJ & Grant I (1988) Amino acid infusion increases the sensitivity of muscle protein synthesis *in vivo* to insulin. Effect of branched chain amino acids. *Biochemical Journal* **254**, 579–584.

Grizard J, Dardevet D, Balage M, Larbaud D, Sinaud S, Savary I, Grzelkowska K, Rochon C, Tauveron I & Obled C (1999) Insulin action on skeletal muscle protein metabolism during catabolic states. *Reproduction, Nutrition and Development* **39**, 61–74.

Harris PM, Skene PA, Buchan V, Milne E, Calder AG, Anderson SE, Connell A & Lobley GE (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.

Ilan J & Singer M (1975) Sampling of the leucine pool from the growing peptide chain: difference in leucine specific activity of peptidyl-transfer RNA from free and membrane-bound polysomes. *Journal of Molecular Biology* **91**, 39–51.

Khairallah EA & Mortimore GE (1976) Assessment of protein turnover in perfused rat liver. Evidence for amino acid compartmentation from differential labeling of free and tRNA-bound valine. *Journal of Biological Chemistry* **251**, 1375–1384.

Lee J, Harris PM, Sinclair BR & Treloar BP (1993) Whole body metabolism of cysteine and glutathione and their utilization in the skin of Romney sheep: consequences for wool growth. *Journal of Agricultural Science, Cambridge* **121**, 111–124.

Liu SM, Mata G, Figliomeni S, Powell BC, Nesci A & Masters DG (2000) Transsulfuration, protein synthesis rate and follicle mRNA in the skin of young Merino lambs in response to infusions of methionine and serine. *British Journal of Nutrition* **83**, 401–409.

Liu SM, Mata G, Odonoghue H & Masters DG (1998) The influence of live weight, live-weight change and diet on protein synthesis in the skin and skeletal muscle in young Merino sheep. *British Journal of Nutrition* **79**, 267–274.

Ljungqvist OH, Persson M, Ford GC & Nair KS (1997) Functional heterogeneity of leucine pools in human skeletal muscle. *American Journal of Physiology* **36**, E564–E570.

Lobley GE (1993*a*) Protein metabolism and turnover. In *Quantitative Aspects of Ruminant Digestion and Metabolism*, pp. 313–339 [JM Forbes and JM France, editors] Oxon, UK: CAB International.

Lobley GE (1993*b*) Species comparisons of tissue protein metabolism: effects of age and hormonal action. *Journal of Nutrition* **123**, 337–343.

Lobley GE (1998) Nutritional and hormonal control of muscle and peripheral tissue metabolism in farm species. *Livestock Production Science* **56**, 91–114.

Lobley GE, Connell A & Buchan V (1987) Effect of food intake on protein and energy metabolism in finishing beef steers. *British Journal of Nutrition* **57**, 457–465.

Lobley GE, Connell A, Lomax MA, Brown DS, Milne E, Calder AG & Farningham DAH (1995) Hepatic detoxification of ammonia in the ovine liver- possible consequences for amino-acid catabolism. *British Journal of Nutrition* **73**, 667–685.

Lobley GE, Connell A, Revell DK, Bequette BJ, Brown DS & Calder AG (1996) Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through use of a multiple U-$^{13}$C-labelled amino acid mixture. *British Journal of Nutrition* **75**, 217–235.

Lobley GE, Harris PM, Skene PA, Brown D, Milne E, Calder AG, Anderson SE, Garlick PJ, Nevison I & Connell A (1992) Responses in tissue protein synthesis to sub- and supra-maintenance intake in growing young sheep: comparison of large-dose and continuous-infusion techniques. *British Journal of Nutrition* **68**, 373–388.

Lobley GE, Sinclair KD, Grant CM, Miller L, Mantle D, Calder AG, Warkup CC & Maltin CA (2000) The effects of breed and level of nutrition on whole-body and muscle protein metabolism in pure-bred Aberdeen Angus and Charolais beef steers. *British Journal of Nutrition* **84**, 275–284.

MacRae JC, Walker A, Brown D & Lobley GE (1993) Accretion of total protein and individual amino-acids by organs and tissues of

growing lambs and the ability of nitrogen-balance techniques to quantitate protein retention. *Animal Production* **57**, 237–245.
Oddy VH, Lindsay DB, Barker PJ & Northrop AJ (1987) Effect of insulin on hind-limb and whole-body leucine and protein metabolism in fed and fasted lambs. *British Journal of Nutrition* **58**, 437–452.
Pell JM & Bates PC (1987) Collagen and non-collagen protein turnover in skeletal muscle of growth hormone-treated lambs. *Journal of Endocrinology* **115**, R1–R4.
Pell JM, Caldarone EM & Bergman EN (1986) Leucine and α-ketoisocaproate metabolism and interconversions in fed and fasted sheep. *Metabolism* **35**, 1005–1016.
Ponter AA, Cortamira NO, Seve B, Salter DN & Morgan LM (1994) The effects of energy source and tryptophan on the rate of protein synthesis and on hormones of the entero–insular axis in the piglet. *British Journal of Nutrition* **71**, 661–674.
Reeds PJ, Cadenhead A, Fuller MF, Lobley GE & McDonald JD (1980) Protein turnover in growing pigs. Effects of age and food intake. *British Journal of Nutrition* **43**, 3–455.
Rocha HJG, Nash JE, Connell A & Lobley GE (1993) Protein-synthesis in ovine muscle and skin – sequential measurements with 3 different amino-acids based on the large-dose procedure. *Comparative Biochemistry and Physiology B – Biochemistry & Molecular Biology* **105**, 301–307.
Savary IC, Hoskin SO, Dennison N & Lobley GE (2001) Lysine metabolism across the hindquarters of sheep; effect of intake on transfers from plasma and the red blood cells. *British Journal of Nutrition* **85**, 565–573.
Schneible PA & Young RB (1984) Leucine pools in normal and dystrophic chicken skeletal muscle cells in culture. *Journal of Biological Chemistry* **259**, 1436–1440.
Seve B, Reeds PJ, Fuller MF, Cadenhead A & Hay SM (1986) Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early weaning. Possible connection to the energy metabolism. *Reproduction, Nutrition and Development* **26**, 849–961.
Teleni E, Annison EF & Lindsay DB (1986) Metabolism of valine and the exchange of amino-acids across the hind-limb muscles of fed and starved sheep. *Australian Journal of Biological Sciences* **39**, 379–393.
Tesseraud S, Grizard J, Debras E, Papet I, Bonnet Y, Bayle G & Champredon C (1993) Leucine metabolism in lactating and dry goats: effect of insulin and substrate availability. *American Journal of Physiology* **265**, E402–E413.
Thomson BC, Hosking BJ, Sainz RD & Oddy VH (1997) The effect of nutritional status on protein degradation and components of the calpain system in skeletal muscle of weaned wether lambs. *Journal of Agricultural Science, Cambridge* **129**, 471–477.
Watt PW, Corbett ME & Rennie MJ (1992) Stimulation of protein synthesis in pig skeletal muscle by infusion of amino acids during constant insulin availability. *American Journal of Physiology* **263**, E453–E460.
Wester TJ, Lobley GE, Birnie LM & Lomax MA (2000) Insulin stimulates phenylalanine uptake across the hind-limb in fed lambs. *Journal of Nutrition* **130**, 608–611.
Wolff JE & Bergman EN (1972) Gluconeogenesis from plasma amino acids in the fed sheep. *American Journal of Physiology* **223**, 455–460.
Zhang X, Chinkes DL, Doyle D Jr & Wolfe RR (1998) Metabolism of skin and muscle protein metabolism is regulated differently in response to nutrition. *American Journal of Physiology* **274**, E484–E492.