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Determination of leucine metabolism and protein turnover in sheep, using gas–liquid chromatography–mass spectrometry

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1. Whole-body protein synthetic rates in non-pregnant ewes were determined by the continuous infusion of L-[^{15}N]- and [1-^{13}C]leucine and measuring the plasma enrichment of leucine, α-ketoisocaproate (α-KIC) and expired carbon dioxide by gas–liquid chromatography–mass spectrometry.

2. The mean whole-body protein synthesis estimated from plasma leucine flux corrected for oxidation was 5·38 (SE 0·54) g/kg per d.

3. Under the conditions of the present study leucine oxidation was 0·323 (SE 0·067) mmol/kg per d and accounted for 10·71 (SE 2·26) % of plasma [^{13}C]leucine flux. Deamination of leucine was 0·55 (SE 0·035) mmol/kg per d and accounted for approximately 17% of plasma [^{15}N]leucine flux.

4. The rate of α-KIC reamination to leucine, calculated by subtracting ^{13}C flux from ^{15}N flux, was 0·228 (SE 0·101) mmol/kg per d.

5. The rate of whole-body protein degradation was 4·49 (SE 0·54) g/kg per d and there was a net protein gain of 0·89 (SE 0·21) g/kg per d.

Technical advances in gas–liquid chromatography–mass spectrometry (GCMS) have improved the sensitivity and reproducibility of analysis and have led to the extensive use of stable isotopes in measuring protein turnover in man under different physiological and pathological conditions (Matthews & Bier, 1983; Wolfe *et al.* 1982, 1983, 1984; Tessari *et al.* 1985). In agricultural animals in which the efficiency of protein synthesis is of great economic importance, only a few reports are available wherein the GCMS technique has been employed for the measurement of the enrichment of stable isotopes of amino acids (Krishnamurti & Schaefer, 1984).

Recently, using [^{15}N]leucine infusions, Krishnamurti & Schaefer (1987) determined protein synthesis and degradation in pregnant ewes. However, no consideration was given to the oxidation of leucine which would have influenced the estimates of rate of protein synthesis. The objectives of the present study were to determine [^{15}N]- and [^{13}C]leucine flux using GCMS and to estimate the rate of whole-body protein synthesis and degradation in sheep after accounting for leucine oxidation by measuring the enrichment of α-ketoisocaproate (α-KIC) in plasma and carbon dioxide in expired air.

EXPERIMENTAL

Animals and their management

Five Dorset × Suffolk ewes, aged between 1 and 2 years and weighing an average of 46 kg each, were used in the study. The ewes were housed indoors at 10–15° in raised metabolic pens designed for the collection of faeces and urine separately. They were fed twice daily on a ration of lucerne (*Medicago sativa*) cubes and barley. Before the isotopic-infusion study a digestibility trial was conducted by measuring the feed consumed and faeces and urine excreted for seven consecutive days. Based on the actual amount of feed consumed it was estimated that the mean daily intake of each ewe was 22·87 (SE 2·77) g nitrogen and 20·72 (SE 2·61) MJ gross energy.

Isotope administration

The day before the experiment polyethylene catheters (PE 90) were introduced into both jugular veins of each sheep and were kept patent by flushing with sterile heparinized saline (9 g sodium chloride/l). The infusate consisted of approximately 75 μmol L-[^{15}N]leucine (99 atom % excess; MSD Isotopes, Pointe Claire, Quebec)/ml saline which was infused through one of the jugular catheters at a rate of approximately 2 ml/h for 8 h using a Sage constant infusion pump (Model 352, Cambridge, Mass.). The ewes had free access to feed and water during the infusions. After 2 d the experiment was repeated with an infusion of 75 μmol L-[1-^{13}C]leucine (99 atom % excess; MSD Isotopes)/ml saline (ewe nos. 248, 287 and 203) at 2 ml/h for 8 h.

Experimental protocol

Blood samples (5 ml) were collected from the non-infusion catheter into chilled, heparinized test-tubes before and at 1·5, 3, 4·5, 6, 7, 7·5, and 8 h after the commencement of the infusion. Portions of whole blood were taken for packed cell volume determination and for blood gas determination using a Radiometer pH meter (PHM 72) fitted with specific electrodes for CO_2 and oxygen measurement. The remaining blood was then centrifuged at 1000 rev/min for 10 min to separate the plasma which was removed with a Pasteur pipette, placed in a clean tube, sealed and stored at $-20°$ until analysis.

During L-[1-^{13}C]leucine infusion samples of expired air were collected using a mask fabricated to fit snugly around the nose of the animal. The presence of one-way valves in the mask permitted air intake freely and collection of expired air from sheep quantitatively. The breath was collected for 2–3 min at 0·5 h intervals between 7 and 8 h of infusion into 30 litre Douglas bags. Using a gas-tight syringe, 50 ml expired air were withdrawn from the bag and transferred to an evacuated rubber-stoppered serum bottle for subsequent GCMS analysis. The remainder of the expired air in the Douglas bag was run through a capnograph (Godart Type KK, Bilthoven, Holland) at a constant flow-rate to determine the percentage CO_2 in the expired air and to estimate the volume of air expired during the collection interval. Values were corrected to standard temperature and pressure.

Ion-exchange chromatography

In order to separate the branched-chain keto acid (BCKA) and branched-chain amino acid (BCAA) fractions the frozen plasma was thawed and subjected to ion-exchange chromatography following the method used by Nissen *et al.* (1982). Briefly, 1 ml plasma was deproteinized with 200 μl 1 M-hydrochloric acid and added to a prepared ion-exchange column containing 2 ml aqueous cation-exchange resin (AG5OW-X8, 400 mesh, hydrogen form; Bio-Rad Labs, Richmond, CA). The column was rinsed with four 1 ml portions of 0·1 M-HCl and the eluate plus washings containing the BCKA were collected in a screw-capped test-tube. The column was washed with four 1 ml portions of 4 M-ammonium hydroxide to elute the adsorbed amino acids from the resin. This eluate which contained the BCAA was collected in a different test-tube. The BCAA fraction was frozen and lyophilized directly whereas the BCKA fraction was first neutralized by the addition of 300 μl 1 M-sodium hydroxide, frozen and lyophilized.

Derivatization of leucine

The trimethylsilyl (TMS) derivative of leucine for the enrichment analysis of [^{15}N]leucine was prepared as described by Krishnamurti & Schaefer (1987). The n-acetyl, n-propyl (NAP) derivative of leucine for the enrichment analysis of [1-^{13}C]leucine was prepared according to the derivatization procedure of Adams (1974). To the dried amino acid eluate

were added 50 μl dimethoxypropane and 1 ml 3·5 M-propanolic hydrobromic acid. The tubes were flushed with N_2, capped and heated at 110° in a sand-bath for 20 min. After cooling briefly, the tubes were uncapped and dried at 110° using a stream of N_2. Azeotrope reagent (n-propanol–benzene, 70:30, v/v; 1 ml) was added to eliminate the acid vapours and the samples were redried. Acylation reagent (acetone–triethylamine–acetic anhydride 5:2:1, by vol; 1 ml) was added and the tubes were flushed with N_2, capped and heated at 60° for 2–3 min. After heating, they were uncapped and dried with a slow stream of N_2 not exceeding a flow-rate of 50 ml/min at a temperature of 60°. The residue was dissolved in 2 ml ethyl acetate and 1 ml saturated NaCl solution was added. This was shaken to extract the derivatives into the top ethyl acetate layer which was removed with a Pasteur pipette and then dried at 45° under a slow stream of N_2 and finally redissolved in 100 μl ethyl acetate. Standards of varying proportions of [^{15}N]- and [^{14}N]leucine and [1-^{13}C]- and [1-^{12}C] leucine were prepared and derivatized in the same manner as the TMS and NAP-leucine derivatives respectively. Leucine concentration in plasma and feed was determined after derivatization with *o*-phthaldialdehyde by high-performance liquid chromatography (HPLC) as described by Krishnamurti *et al.* (1984).

Derivatization of α-KIC

The silylquinoxalinol (SQ) derivative of α-KIC was prepared according to the procedure of Cree *et al.* (1979). To the dried BCKA fraction were added 1·7 ml of a hot solution containing 1·5 ml water, 0·17 ml concentrated HCl and 42 mg *o*-phenylenediamine dihyrochloride (OPDA). The tube was capped and heated in a boiling water-bath for 30 min.

After cooling briefly, 2·5 ml methylene chloride were added and the tube shaken vigorously to extract the derivatives. The bottom layer containing the methylene chloride and the derivatives was removed with a Pasteur pipette and transferred to a clean tube. The extraction was repeated adding the second extract to the first. The pooled quinoxalinol extracts were dried at room temperature under N_2. The dried quinoxalinols were then silylated by adding 10 μl pyridine and 50 μl N,O-bis–trimethylsilyl–trifluoroacetamide (BSTFA) and heating in a 70° water-bath for 30 min. Standards of varying proportions of α-[^{13}C]KIC (99 atom % excess; MSD Isotopes) and α-[^{12}C]KIC were prepared and derivatized in the same manner. Plasma α-KIC concentration was determined fluorometrically by HPLC as its quinoxalinol derivative according to the procedure described by Hayashi *et al.* (1982) with modifications to simplify sample clean up.

Preparation of CO_2 standards

Standard solutions containing varying proportions of $NaH^{13}CO_3$ (99 atom % excess; MSD Isotopes) and $NaH^{12}CO_3$ were prepared in water. Portions (200 μl) were injected through the rubber septa of red-stoppered vacutainer tubes. Then 200 μl perchloric acid (300 ml/l) were injected into each tube to liberate the CO_2 from the bicarbonate standards. The remainder of the vacuum was filled with N_2 from a gas cylinder to facilitate withdrawal of air from the tube for GCMS analysis.

GCMS analysis

Analysis of the derivatives of leucine, α-KIC and CO_2 for isotopic enrichment was performed on a Hewlett Packard 5985B quadrupole GCMS.

The NAP–leucine ester was separated by gas–liquid chromatography on a Carbowax 20M (0·32 mm × 30 m) column using a thermal gradient of 30–200° at 25°/min. Single ion monitoring and chemical ionization with methane as a carrier and reactant gas were used with source pressure set at $0{\cdot}8 \times 10^{-4}$ Torr. Source temperature was 200°, the injection-port

temperature 280° and interface temperature was 285°. The mass peak areas were monitored at m/z 216 and 217 corresponding to ^{12}C and ^{13}C fragments. The observed peak areas of the labelled species ([1-^{13}C]leucine) were corrected for the contributions of natural abundance by mass spectral analysis of the ion clusters at 217 and 216 m/z according to Campbell (1974). The enrichment of [^{15}N]leucine was determined as described previously (Krishnamurti & Schaefer, 1987) from the peak areas of ion fragments at m/z 158 and 159 following electron impact ionization.

The SQ derivative of α-KIC was separated using an SE-54 gas–liquid chromatographic column (0·32 mm × 30 m; Supelco). The thermal gradient was programmed from 60 to 285° at 15°/min. Helium was used as a carrier gas at a flow-rate of 30 ml/min under EI conditions with electron impact energy at 70 eV. Ions at m/z 232 and 233 were monitored with 20 ms dwell time. The observed peak areas were corrected for natural abundance.

The expired air samples were chromatographed on a Poropak Q column (0·39 mm × 1·8 m). The temperature was isothermal at 50° and helium was the carrier gas at a flow-rate of 25 ml/min. Peaks were measured in the GCMS directly at m/z 44 and 45 corresponding to $^{12}CO_2$ and $^{13}CO_2$ respectively.

Calculations

The isotopic enrichment of the metabolites in the plasma was estimated from standard calibration curves constructed according to the procedure outlined by Tserng & Kalhan (1983). The isotopic enrichment, $x/x+y$ (%), was plotted against the isotope peak abundance, $a/a+b$ (%), where x and y are the amounts and a and b are the peak areas of labelled and unlabelled standards respectively. For this purpose the peak areas of ion fragments at m/z 217 and 216 for [^{13}C]- and [^{12}C]leucine, m/z 233 and 232 for α-[^{13}C]- and α-[^{12}C]KIC, and m/z 45 and 44 for $^{13}CO_2$ and $^{12}CO_2$ were used respectively.

Plasma leucine flux, leucine oxidation and protein synthesis and degradation were calculated from the mass spectrometric values using the following equations:

$$\text{Leucine flux (mmol/kg per d)} = \left[\frac{100}{Ep} - 1\right] \times RI, \quad (1)$$

where Ep is isotopic enrichment of [^{15}N]- or [^{13}C]leucine in plasma at plateau (mol % excess) and RI is the rate of infusion of [^{15}N]- or [^{13}C]leucine (mmol/kg per d).

$$\text{Leucine oxidation} = \frac{(IE_{CO_2}/0{\cdot}81) \times V_{CO_2}}{IE_{\alpha\text{-KIC}}}, \quad (2)$$

where IE_{CO_2} is isotopic enrichment of expired CO_2 at plateau, $IE_{\alpha\text{-KIC}}$ is isotopic enrichment of plasma α-KIC at plateau, and V_{CO_2} is CO_2 production rate. The division of IE_{CO_2} by 0·81 accounts for the retention of bicarbonate in the body.

$$\text{Protein synthesis (g/kg per d)} = \frac{([^{13}\text{C]leucine flux} - \text{leucine oxidation})}{\text{Leucine concentration in carcass protein}} \times 0{\cdot}131. \quad (3)$$

The value of 6·6% was used for leucine concentration in sheep carcass protein (Reeds & Harris, 1981). Multiplication by 0·131 converts the units to g/kg per d. The calculation of whole-body protein degradation in ruminants by using the mass balance equation (eqn (4)) (Waterlow *et al.* 1978) will result in inaccuracies due to the extensive degradation of dietary N in the rumen by microbial action.

$$\text{Leucine flux} = \text{synthesis} + \text{excretion} = \text{degradation} + \text{intake}. \quad (4)$$

To overcome this problem leucine absorption from the small intestine was estimated (Davis *et al.* 1981) using the relation between the flow of duodenal non-amino-N and dietary N intake (MacRae & Ulyatt, 1974):

$$\text{Flow of duodenal non-amino-N} = 0{\cdot}56\ \text{N} + 2{\cdot}7.$$

Leucine absorption was calculated assuming 75 g leucine/kg crude protein in duodenal digesta (non-ammonia-N × 6·25) and an apparent absorption efficiency of 73% (Davis *et al.* 1981) replaced intake in eqn (4):

$$\text{Protein degradation} = \frac{[^{13}\text{C}]\text{leucine flux} - \text{leucine absorption}}{\text{leucine concentration in carcass protein}}. \quad (5)$$

The difference between [^{15}N]- or [^{13}C]leucine flux gives the rate of α-KIC reamination to leucine:

$$[^{15}\text{N}]\text{leucine flux} - [^{13}\text{C}]\text{leucine flux} = \alpha\text{-KIC reamination to leucine.} \quad (6)$$

$$\text{Leucine deamination to } \alpha\text{-KIC} = \underset{\text{(eqn (2))}}{\text{Leucine oxidation}} + \underset{\text{(eqn (6))}}{\alpha\text{-KIC reamination.}} \quad (7)$$

Statistics

Values are given as means with their standard errors. Student's *t* test was employed to test significant differences between means.

RESULTS

The mean blood pH, O_2 pressure, CO_2 pressure and packed cell volume values of the ewes were 7·52 (SE 0·019), 21·10 (SE 1·39) mmHg, 33·56 (SE 5·38) mmHg and 0·334 (SE 0·0174) respectively. The daily excretion of N in the faeces was 13·45 (SE 2·41) g and in the urine 14·77 (SE 1·31) g with an apparent N balance of −5·32 (SE 0·92) g/d per head. The mean leucine intake was 1·68 (SE 0·16) mmol/kg per d. Plasma leucine and α-KIC concentrations were 0·465 (SE 0·073) and 0·024 (SE 0·004) μmol/ml respectively and did not change significantly due to the isotopic infusion.

The partial mass spectra of selected ion monitoring of NAP-leucine (CI), SQ-α-KIC (EI) and CO_2 standards are shown in Fig. 1. The molecular ions (+1) for NAP-[1-^{13}C]leucine, SQ-α-[^{13}C]KIC and $^{13}CO_2$ occurred at m/z 217, 233 and 45 respectively. There was a high correlation ($r^2 > 0{\cdot}999$) between the isotopic enrichment ($x/x+y$, %) and the isotopic peak abundance ($a/a+b$, %) at the range of concentrations used (Fig. 2).

The means of the last three plasma enrichment values at 7, 7·5 and 8 h after the commencement of the infusion were used as the plateau isotopic enrichment of the metabolities in the plasma (*Ep*) and in expired CO_2. The plateau enrichments of [^{13}C]leucine, α-[^{13}C]KIC and $^{13}CO_2$ are given in Table 1. The mean plasma [^{15}N]leucine flux (3·258 (SE 0·286) mmol/kg per d) was higher (although not significant, $P < 0{\cdot}1$) than the [1-^{13}C]leucine flux (3·029 (SE 0·279) mmol/kg per d) (Table 2).

Leucine oxidation was 0·323 (SE 0·067) mmol/kg per d and accounted for 10·71 (SE 2·26) % of plasma [^{13}C]leucine flux. The rate of deamination of leucine (eqn (7)) was 0·55 (SE 0·035) mmol/kg per d amounting to 17·0 % of [^{15}N]leucine flux. The rate of reamination of α-KIC to leucine (eqn (6)) was 0·228 (SE 0·101) mmol/kg per d. The percentage of α-KIC reaminated to leucine was 40·42 % while the remainder (59·58 %) underwent oxidation.

The rate of protein synthesis under the conditions of the present study was 5·38 (SE 0·54) g/kg per d and the rate of protein degradation was 4·49 (SE 0·54) g/kg per d indicating that

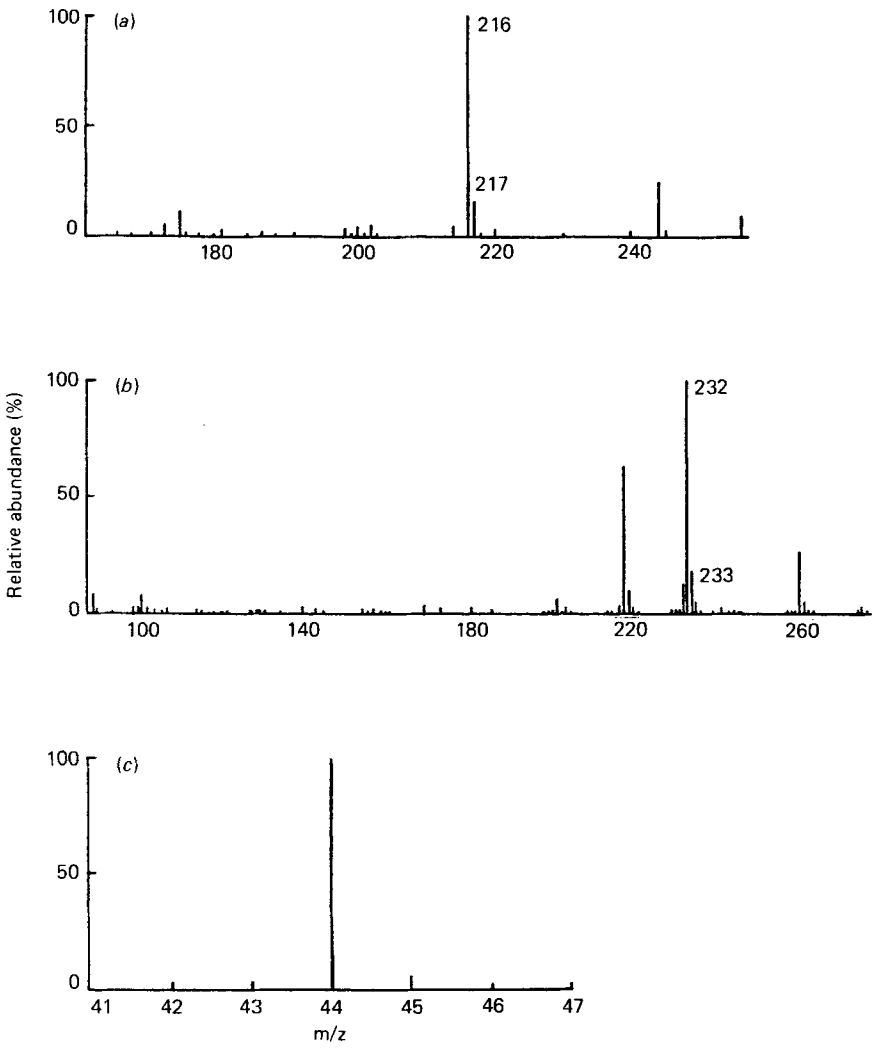

Fig. 1. Partial mass spectra of stable isotopes. (*a*) n-Acetyl, n-propyl leucine derivative (NAP), m/z 216 and 217. (*b*) Silylquinoxalinol derivative of α-KIC, m/z 232 and 233. (*c*) Carbon dioxide, m/z 44 and 45.

there was a net protein gain of 0·89 (SE 0·21) g/kg per d (Table 3). According to digestibility balance studies there was a net apparent loss of 0·83 (SE 0·13) g protein/kg per d from the ewes.

DISCUSSION

Although TMS-[^{15}N]leucine has been shown to yield good peaks at m/z 159 by electron impact ionization in GCMS, this derivative has been found unsuitable when [1-^{13}C]leucine was used for infusion. Similar difficulties have been reported by Matthews *et al.* (1979). This problem was overcome by the formation of the NAP-leucine derivative followed by chemical ionization using methane which produced satisfactory peaks at m/z 217 and 216 for [^{13}C]- and [^{12}C]leucine respectively.

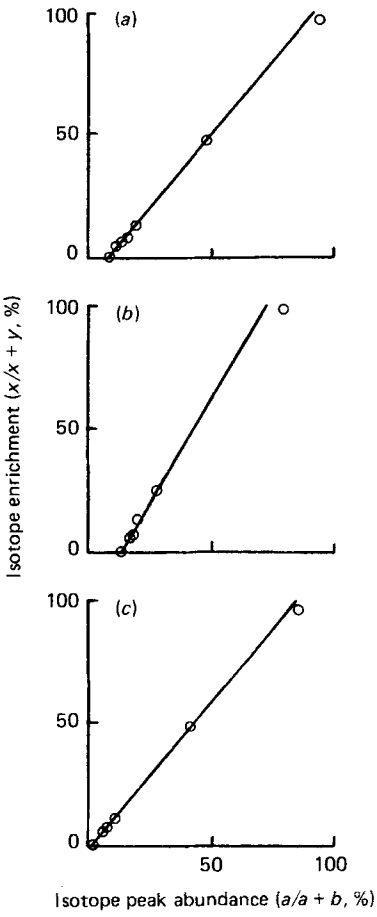

Fig. 2. Linear regression of isotope enrichment ($x/x+y$, %) *v.* isotope peak abundance ($a/a+b$, %) for (*a*) n-acetyl, n-propyl leucine (r^2 0·999); (*b*) silylquinoxalinol α-ketoisocaproate (α-KIC) (r^2 0·999) and (*c*) carbon dioxide (r^2 0·999). *x* and *y* are the amounts and *a* and *b* are the peak areas of labelled and unlabelled standards respectively. Peak areas of ion fragments of [^{13}C]- and [^{12}C]leucine were measured at m/z 217 and 216 respectively; α-[^{13}C]- and α-[^{12}C]KIC at m/z 233 and 232 respectively; and $^{13}CO_2$ and $^{12}CO_2$ at m/z 45 and 44 respectively.

Table 1. *Plateau enrichment of metabolites in plasma and expired carbon dioxide of sheep infused with [^{15}N]leucine or [1-^{13}C]leucine*

Ewe no.	Body-wt (kg)	Enrichment at plateau (mol % excess)			
		[^{15}N]leucine	[^{13}C]leucine	α-[^{13}C]KIC	$^{13}CO_2$
248	49·3	2·084	2·130	1·270	0·0005
287	61·3	2·073	2·007	1·315	0·0011
203	47·2	2·077	2·170	1·447	0·0011
296	37·2	1·218	—	—	—
291	37·2	1·565	—	—	—
Mean	46·4	1·803	2·126	1·344	0·0009
SE	8·9	0·354	0·038	0·075	0·0003

α-KIC, α-ketoisocaproate.

Table 2. *Plasma leucine flux and oxidation in the ewes infused with [^{15}N]leucine or [1-^{13}C]leucine*

Ewe no.	Leucine infusion rate (mmol/h)		Plasma leucine flux (mmol/kg per d) (eqn (1))*		Leucine oxidation (mmol/kg per d) (eqn (2))*
	[^{15}N]leucine	[^{13}C]leucine	[^{15}N]leucine	[^{13}C]leucine	
248	0·148	0·136	3·448	3·081	0·233
287	0·153	0·138	2·854	2·665	0·340
203	0·143	0·144	3·472	3·343	0·394
Mean	—	—	3·258	3·029	0·323
SE	—	—	0·286	0·279	0·067

* For details, see p. 158.

Table 3. *Rate of whole-body protein synthesis and degradation calculated from plasma leucine flux and oxidation*

Ewe no.	Protein synthesis (eqn (3))* (g/kg per d)	Protein degradation (eqn (5))* (g/kg per d)	Protein balance (g/kg per d)
248	5·66	4·47	1·19
287	4·62	3·83	0·79
203	5·86	5·16	0·70
Mean	5·38	4·49	0·89
SE	0·54	0·54	0·21

* For details, see pp. 158–159.

Table 4. *Comparison of whole-body protein synthetic rates in sheep estimated by the isotope-dilution technique*

Animal	Approximate body-wt (kg)	Amino acid infused	Whole-body protein synthesis (g/kg per d)	Source
Growing lambs	17–26	[^{3}H]leucine	11·41–12·42	Davis *et al.* (1981)*
Non-pregnant ewes	65	[^{3}H]tyrosine	7·2	Bryant & Smith (1982*a*)*
Lactating ewes	53–62	[^{3}H]tyrosine	9·60–10·20	Bryant & Smith (1982*a*)*
Wethers	67	[^{3}H]tyrosine	5·29 (hay), 3·82 (straw)	Bryant & Smith (1982*b*)*
Pregnant ewes	60	[^{15}N]leucine	2·87	Krishnamurti & Schaefer (1987)*
Ewes and wethers	42–67	[1-^{14}C]leucine	6·7	Brockway & Lobley (1982)
Non-pregnant ewes	46	[^{15}N]leucine, [1-^{13}C]leucine	5·38	Present study

*Oxidative loss of amino acid excluded from calculation.

The isotopic enrichment of $^{13}CO_2$ in human breath has been determined by isotope ratio mass spectrometry preceded by elaborate sample preparation to minimize variations in ^{13}C abundance which could be a major source of error (Schoeller *et al.* 1984), especially at low ^{13}C enrichments as observed in the present study (Table 1). However the simplicity of the direct GCMS analysis of the expired air used in the present study makes it suitable and convenient for routine measurement of $^{13}CO_2$ enrichment, particularly in agricultural animals in which the quantitative collection of CO_2 itself without stress poses additional problems.

The rate of protein synthesis in non-pregnant ewes (5·38 (SE 0·054) g/kg per d) was higher than the values (2·87 g/kg per d) reported earlier for pregnant ewes (Krishnamurti & Schaefer, 1987). Since the ewes were gaining body-weight during the trial the negative N balance may be ascribed to possible errors in the collection or analysis of excreta, or both. A comparison of the rates of protein synthesis in sheep by infusion of radioactively labelled isotopes or stable isotopes of amino acids reported in the literature is given in Table 4. All the protein synthetic rates reported are subject to the general criticism that only the enrichment or specific activity of plasma amino acids has been used to calculate protein synthesis rather than that of the corresponding tRNA, the immediate precursor of proteins. Also, the assumption that the metabolic fate of the tracer amino acid is representative of the metabolism of all the other non-limiting amino acids may be open to question.

As expected, plasma leucine flux was higher following the infusion of [^{15}N]leucine than [1-^{13}C]leucine due to recycling of N via the reversible leucine transamination step. However, the ratio, ^{15}N:^{13}C leucine flux (1·08) was lower than the value of 1·32 reported in man by Tsalikian *et al.* (1984). The advantage of using [1-^{13}C]leucine in preference to [^{15}N]leucine as an optimal non-recycling tracer in protein turnover studies has been reported (Matthews *et al.* 1981). In the case of ruminants the estimation of oxidative loss by enrichment analysis of urinary urea when [^{15}N]leucine is used as a tracer is rendered more difficult by the large pool size and extensive recycling of urea.

Of interest is the observation in the present study that deamination of leucine accounts for approximately 17·0% of [^{15}N]leucine flux. Recently Nissen & Ostaszewski (1985) have reported that 18·5% of leucine is converted to α-KIC in wethers under post-absorptive conditions. Their isotopic infusion lasted only for 3 h which may not be adequate to achieve a steady state. Using in vitro techniques Wijayasinghe *et al.* (1984) observed that in the external intercostal muscle fibres obtained from wethers, the irreversible decarboxylation of leucine at physiological concentrations was 35% of its metabolism under fed and 58% under starvation conditions. Although the previously described studies are not directly comparable, leucine deamination values (15·1–18·5%) are lower than those (62·2–82·1%) reported for simple-stomach animals (Nissen & Ostaszewski, 1985; Matthews *et al.* 1981). The percentage of [^{13}C]leucine flux oxidized (10·71%) is close to the value of 11·9% reported in man under post-absorptive conditions by Matthews *et al.* (1981). The percentage of α-KIC reaminated to leucine (40·42%) is very close to the value reported by Nissen & Ostaszewski (1985) in sheep and confirms their conclusion that the metabolism of leucine in ruminants is similar to that in non-ruminants with the exception of the leucine–α-KIC transamination step. The use of [1-^{13}C]leucine as a tracer thus appears to be suitable for protein turnover studies in ruminants. The accuracy of the measurements may be further enhanced by improving the $^{13}CO_2$ enrichment analysis (Garlick *et al.* 1987) and by actually determining portal absorption of leucine simultaneously using techniques similar to those reported by Wolff *et al.* (1972) and Hammond *et al.* (1987).

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