Tissue protein synthesis in lactating and dry goats

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Intravenous infusion of L-[^{3}H]phenylalanine (Phe) was carried out for 8 h in dry, non-pregnant and lactating dairy goats. Nitrogen balance was positive in the dry group and negative in the lactating group. Whole-body Phe flux was 50% greater in lactating goats ($P < 0.01$). Fractional synthesis rates (K_s) of tissue proteins were estimated from plasma- (K_{sp}) and tissue- (K_{sh}) specific radioactivities of Phe. In lactating goats, K_{sp} for mammary gland, duodenum and diaphragm was increased ($P < 0.05$). K_{sp} also tended to increase in liver, kidney and rumen ($P < 0.08$) of lactating goats, but was not different in uterus, spleen, caecum or heart. Values of K_{sh} were higher than K_{sp}; however, these measures agreed qualitatively. When absolute rates of protein synthesis were calculated, an increased contribution of mammary and visceral organs was seen in lactating goats. K_s and absolute rates of protein synthesis of hind-limb skin were less in lactating goats ($P < 0.05$). A decreased proportion of skeletal muscle ($P < 0.01$) and decreased K_s resulted in lower absolute synthesis of hind-limb muscle protein in lactating animals ($P < 0.05$). Decreased rates of muscle and skin protein synthesis would appear to participate in alterations of protein metabolism, permitting lactation to occur at the expense of body reserves.

Lactation: Phenylalanine flux: Protein synthesis: Goat

In high-producing dairy ruminants, early lactation is recognized as a period of negative nitrogen balance (Brun-Bellut *et al.* 1984). In order to maintain milk output, a utilization of maternal sources of protein and energy is necessary to supplement the dietary supply (Brun-Bellut *et al.* 1984; Giger, 1987; Barnes & Brown, 1990). Dairy goats have attracted some attention for studies of this phenomenon, because of their high milk output per unit body-weight and small body size (Wilkinson & Stark, 1987).

Net body protein loss may result from an increase in protein degradation, a decrease in body synthesis, or both. However, the changes in protein metabolism which take place to permit high rates of milk synthesis in the face of negative N balance over-all remain to be fully characterized. Few studies of whole-body or tissue protein metabolism have been performed on ruminants early in lactation (Bryant & Smith 1982; Vincent & Lindsay, 1984; Oddy *et al.* 1988; Riis, 1988). Some attention has been paid to skeletal muscle (Smith *et al.* 1981; Bryant & Smith, 1982; Vincent & Lindsay, 1984; Millican *et al.* 1987) because it is viewed as a primary site of protein mobilization. At the same time, the nature of any alterations of muscle protein content and metabolism occurring during lactation is not a topic of general agreement amongst these reports.

There is little information concerning how the over-all flux of amino acids available for protein synthesis is divided amongst tissues and organs in large animals. Lobley *et al.* (1980) characterized the percentage contribution of muscle, carcass, gastrointestinal tract, liver and skin to total protein synthesis in cattle. In lactation the distribution of amino acids for protein synthesis is clearly altered, but the quantitative significance of changes in the

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Table 1. *Composition of the diets (g/kg dry matter (DM))*

	DM (g/kg diet)	Crude protein (nitrogen × 6·25)	Organic matter	Gross energy (MJ)
Dried beet-pulp pellets	901	83	912	16·8
Dried lucerne (*Medicago sativa*) pellets	931	170	880	18·4
Meadow hay	850	81	916	18·3
Soya-bean meal	878	509	923	19·4

Dry animals were offered a diet consisting of (g/kg) 750 beet-pulp pellets, 125 hay and 125 lucerne. Lactating goats were offered 540 beet-pulp pellets, 250 lucerne, 150 hay and 60 soya-bean meal.

different tissues is not known. These changes may relate to the increased need for protein synthesis in the mammary gland, possibly at the expense of that occurring in some other tissues.

In the present study, we describe measurements of whole-body phenylalanine (Phe) flux and protein synthesis in lactating and dry dairy goats. In order to identify the respective contributions of each tissue, protein synthesis in diverse organs including the mammary gland, uterus, digestive tract and visceral organs was determined. In order to identify changes occurring in the skeletal tissues, a quantitative analysis of hind-limb composition and protein synthesis in muscle, skin and bone marrow was also conducted.

MATERIALS AND METHODS

Animals and management

One Alpine and two Saanen goats were allocated to each treatment. All were adult with two or three previous lactations. The lactating goats were studied during the third week post partum. The dry goats were non-pregnant and non-lactating. Animals were kept in individual pens and 10 d before infusion they were transferred to metabolism crates. During the 7 d before infusion, daily N balance measurements were carried out. Faeces and urine were collected daily. Ambient temperature was 21° and natural light conditions prevailed. The lactating goats were milked twice daily by machine at 06.00 and 18.00 hours.

Feeding

All goats were fed on meadow hay, sugar-beet-pulp pellets and dehydrated lucerne (*Medicago sativa*) pellets (Table 1). The quantity and composition of the diets fed were calculated for each individual animal to meet maintenance requirements of the dry animals, and the maintenance and milk production needs of the lactating animals, according to the levels suggested by the National Research Council (1981) and Morand-Fehr *et al.* (1987). Maintenance requirements were 270 kJ net energy and 2·5 g digestible crude protein (N × 6·25)/kg body-weight$^{0.75}$. An additional 2950 kJ net energy and 45 g digestible crude protein were provided per kg milk produced. The greatly elevated protein requirement of animals in early lactation necessitated the addition of a protein supplement (soya-bean-meal) to the diet of the lactating goats.

During the day of the infusion and the 7-d N balance period which preceded it, the diet was divided into four equal portions and fed at 06.00, 12.00, 18.00 and 24.00 hours. Feed not consumed during any period was collected and recorded. Animals were given continuous access to water and a mineralized salt block. For measurements of N balance, the dry matter and N contents of feed, urine, milk and faeces were determined daily for each animal.

Infusion procedure

Each animal was bilaterally implanted with jugular venous catheters on the day before infusion. Infusions and venous blood samplings were made via the left and right jugular catheters respectively. The length of the infusion catheter within the vein (300 mm) was longer than that of the sampling catheter (100 mm) to avoid direct sampling of the infusate. L-[2,3,4,5,6-^{3}H]Phe was obtained from Amersham (Paris); the specific radioactivity of the Phe was 3·92 TBq/mmol and the radiochemical purity 98 %. The infusate prepared was calculated to deliver 1·48 MBq (40 μCi)/kg body-weight. A further 1 μmol non-radioactive Phe/ml was added as carrier. The infusate was made up in 50 ml sterile saline (9 g sodium chloride/l) and was infused at a constant rate (6 ml/h) for 8 h using an infusion pump. Infusions were commenced at 09.00 hours and feed distributions were continued throughout the infusion period.

Blood and tissue sampling

On the day before infusion, hind-limbs were shaved of hair to facilitate sampling of the skin and subsequent analyses. Venous blood was sampled at 0, 5, 10, 15, 20, 25, 30, 40 and 50 min, and 1, 2, 3, 4, 5, 6, 7 and 8 h of infusion. Samples were drawn into heparinized tubes and centrifuged to separate the plasma. Immediately before the start of the infusion and just before its end, lactating goats were milked dry by machine and then by hand. The milk collected at the end of the infusion was mixed, and two portions (250 ml) were immediately frozen.

The sampling protocol was designed to obtain tissues rapidly, in order to minimize post-mortem alteration of the intracellular specific radioactivity of Phe by tissue metabolism. At the end of the infusion goats were anaesthetized with pentobarbitone (60 g/l). Anaesthetized animals were transferred rapidly to a slaughter table after interruption of the infusion and immediately killed by exsanguination. The time elapsed between the start of exsanguination and tissue freezing in liquid N_2 was recorded for each sample. Within 100 s after the beginning of exsanguination, the left m. longissimus dorsi was sampled, the left hind-limb was disarticulated at the hip joint and frozen in liquid N_2, and the organs of the abdominal and thoracic cavities were removed and plunged into ice-cold saline. Samples (30–200 g) of the large intestine (caecum), small intestine (duodenum), rumen and diaphragm, and the whole uterus, spleen, liver, one lung, one kidney, half the mammary gland and the heart were frozen in liquid N_2 within a further 3 min. Frozen tissues weighing more than 50 g were cooled in liquid N_2 and broken in pieces with a hammer for sub-sampling. After sampling was completed, one kidney and the remainder of the rumen, small intestine and mammary gland were carefully dissected and stored frozen for determination of tissue Phe mass.

Samples of bone marrow, skin and mixed skeletal muscle were taken from the left hind-limb. The frozen limb was first cut into 20 mm thick slices along its length. Muscle tissue (about 300 g) from one slice taken at the mid-thigh level and one slice taken from the mid-calf level were pooled, for each animal, for determination of tissue rates of protein synthesis. Skin samples were taken from the same slices. Bone marrow was removed from all of the bone slices from each leg and pooled. In addition to the rapidly sampled left leg, the right m. longissimus dorsi was carefully dissected and stored frozen for protein and Phe determination. The right hind-limb was dissected free, weighed, and then muscle tissue, skin and bone separated by dissection and stored frozen.

Analytical procedure

Plasma was deproteinized by the addition of perchloric acid (700 g/l; PCA) to a final concentration of 20 g/l. After separation of the protein by centrifugation (2000 ***g***; 20 min), the supernatant fractions were brought to pH 6·3 by the addition of saturated tripotassium citrate. Precipitated potassium perchlorate was removed by centrifugation. Weighed tissue samples were cooled to liquid N_2 temperatures, pulverized, then homogenized in 5 vol. ice-cold PCA (20 g/l) in a Waring blender. The homogenates were then centrifuged (3000 ***g***; 20 min). The same operation was repeated three times on each protein precipitate in order to eliminate free [^{3}H]Phe from the protein fraction. The first acid supernatant fraction obtained from each tissue fraction was neutralized by the addition of saturated tripotassium citrate. Precipitated potassium perchlorate was removed by centrifugation. The neutralized supernatant fraction containing free amino acids was stored at $-30°$ for determination of Phe specific radioactivity (see below). Protein precipitates from tissues were de-fatted by homogenization with chloroform:methanol (2:1, v/v), and finally with ethanol, before drying at 80°. Tissue proteins were hydrolysed by refluxing in 6 M-hydrochloric acid at 110° for 24 h. The HCl was removed by rotary evaporation and the hydrolysate made up to a fixed volume in sodium citrate buffer (0·5 M; pH 6·3).

The specific radioactivities of Phe in plasma, in the tissue intracellular pool and in tissue proteins were determined after conversion of Phe to β-phenethylamine by the action of L-tyrosine decarboxylase (*EC* 4.1.1.85) (Garlick *et al.* 1980). Concentrations of Phe in plasma and tissue hydrolysates, and β-phenethylamine were determined by high-performance liquid chromatography (Jones & Gilligan, 1983). Skin, bone marrow and muscle from the right hind-limb were pooled and thoroughly mixed before sub-sampling for determinations of N, Phe, crude lipid, water and ash. The values presented for these analyses represent the averages of two to five replicates per animal. N was determined by the Kjeldahl method and protein contents are expressed as N × 6·25. Results are expressed as group means and were analysed by one-way analysis of variance. Pooled standard errors of means are also presented.

Calculations

The flux of plasma Phe was calculated from the formula: $F = I/Sp_{max}$, where I is the rate of infusion of isotope (disintegrations/min (dpm)/h), F is the flux (mmol/h) and Sp_{max} is the plateau specific radioactivity of Phe in plasma (dpm/mmol). The protein fractional synthetic rate (K_s) in the tissues was calculated as described by Schaefer *et al.* (1986), using the specific radioactivity of free Phe in plasma (K_{sp}) and in tissue proteins (K_{sh}). The specific radioactivity of plasma Phe at plateau, and the rate-constant (λp) were determined by non-linear regression analysis using the formula: $y = A - B_e^{-kt}$, where y is the plasma specific radioactivity of Phe at any time (t), A is the plateau specific radioactivity, B is the y intercept and k is the rate constant. The absolute rate of tissue protein synthesis was calculated as K_{sp} multiplied by tissue Phe mass. Milk protein synthesis was taken as Phe secreted in milk protein (net protein synthesis).

RESULTS

Animal measurements

Animal measurements are shown in Table 2. Since the animals used varied in absolute body-weight, an eviscerated carcass weight is also presented. The mean body and carcass weights of dry animals used in the present study were similar to those of the lactating group. The average milk production of the lactating group was 2·44 kg/d; the milk contained 31 g crude protein/kg. All lactating goats produced as much or more milk during the infusion as during the prior 3 days. Lactating animals showed increased N intake and digestibility

Table 2. *Animal measurements and nitrogen balance in dry and lactating goats**

(Mean values for three animals)

	Dry	Lactating	SEM†	Statistical significance of difference: $P <$
Body wt (kg)	51.3	53.3	2·99	0·66
Carcass wt‡ (kg)	25·8	27·6	2·12	0·59
Dry matter intake (g/d)	671	1347	174	0·05
N intake (g/d)	12·1	28·9	2·36	0·01
Faecal N (g/d)	4·7	8·5	1·2	0·09
N digestibility	0·61	0·71	0·03	0·05
Urine N (g/d)	5·4	12·6	1·2	0·02
Milk output				
kg/d	—	2·44	0·32	—
g N/d	—	12·2	2·4	—
mmol Phe/d	—	3·43	0·35	—
N balance (g N/d)	1·9	−4·4	1·7	0·06
Calculated energy balance§ (MJ/d)	1·14	−4·38	1·10	0·04

Phe, phenylalanine.

* For details of procedures, see pp. 452–454.

† Pooled SEM.

‡ The carcass weight is equal to the body-weight at death minus the mammary gland and the contents of the abdominal and thoracic cavities.

§ Energy balance was calculated as the difference between energy intake (determined from feed intake and digestibility of organic matter) and calculated energy requirements (270 kJ net energy/kg body-weight$^{0·75}$ plus 2950 kJ/kg milk production).

of N. At the same time, increased urinary and faecal N, and the secretion of milk N resulted in a net negative N balance for the lactating goats. The degree of negative N balance varied proportionately with milk production, so that goats producing 8·1, 12·0 and 16·4 g N/d in milk, had N balances of −0·1, −4·5 and −8·5 g N/d respectively. Dry goats were in slightly positive N balance throughout the period of study. Based on calculated values (Table 2), lactating goats were in negative energy balance, while dry goats were in slightly positive energy balance.

Phe flux and organ protein synthesis

Plasma Phe concentration was lower in lactating animals (17·7 μmol/l) than in dry animals (21·9 μmol/l; $P < 0·02$), and did not vary throughout the period of infusion. The specific radioactivity of Phe in plasma rose rapidly during the infusion period (Fig. 1). Values for plateau specific radioactivity and the rate-constant (λp) were calculated from non-linear regression analysis of data for each animal. In addition, the values for each animal were expressed as a percentage of their respective plateau specific radioactivities and pooled by treatment before regression analysis. Values of r^2 for the regressions (regression sum of squares/total sum of squares) were greater than or equal to 0·98. The average λp for the dry animals was 127/d, reflecting a rapid rise to plateau specific activity. The rise in specific radioactivity was even faster in lactating animals; in one case the earliest sample (5 min) was at 91% of the plateau value. Therefore the λp from the pooled values for the lactating animals (157/d) and dry animals (127/d) was used in subsequent calculations. Whole-body Phe flux was 52% greater in lactating (4·63 mmol/h) than in dry goats (3·04 mmol/h; $P < 0·01$).

K_s values calculated from protein-bound specific radioactivities and either (a) plasma specific radioactivities of Phe (K_{sp}), or (b) intracellular specific radioactivities of Phe (K_{sh})

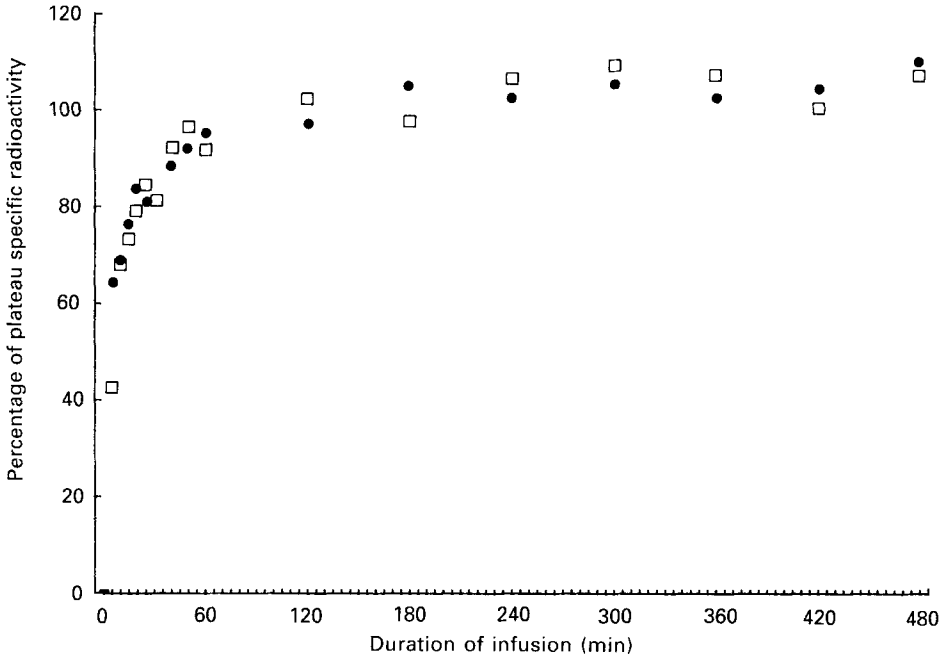

Fig. 1. The rise of plasma specific radioactivity of phenylalanine (expressed as percentage of the plateau value obtained during infusion) in lactating (●) and dry (□) goats. Points represent treatment means for three lactating and three dry animals. For details of procedures, see pp. 453–454.

were determined for diverse tissues and organs (Table 3). Values of K_{sh} were higher than those of K_{sp} in all cases; however, the two sets of calculations agreed well when expressed as lactating:dry. K_s values varied in tissues and were lowest in diaphragm muscle and heart, and highest in duodenum and lactating mammary gland.

The results in Table 3 show a fourfold increase in the fractional rate of mammary protein synthesis ($P < 0{\cdot}002$) in lactating animals. In addition, in lactating animals the K_s of diverse organs and tissues of the abdominal and thoracic cavities, including liver, lung, kidney, caecum, duodenum, rumen, heart and diaphragm, tended to rise. These effects were significant ($P < 0{\cdot}05$) for the K_{sp} of lung, small intestine and diaphragm, and for the K_{sh} of spleen and kidney.

Tissue Phe mass values are presented in Table 4. The lactating mammary gland contained more than fourfold more Phe than that of dry animals. The involuting uterus of the lactating group also tended to have a greater Phe mass. Organs of the digestive tract also tended to show increased Phe mass in lactating animals; this was particularly marked in the rumen ($P < 0{\cdot}05$) and small intestine. The different tissues varied in their Phe contents (mg/g wet weight): 3·48 mammary gland, 7·81 uterus, 9·00 liver, 5·89 rumen, 5·50 small intestine, 8·28 skeletal muscle, 7·35 heart, 5·04 kidney and 8·47 spleen. This variable was not different in dry and lactating animals.

The absolute synthesis rates of tissue proteins are presented in Table 5. These values were calculated as K_{sp} multiplied by tissue Phe mass. The value for milk was taken as the net Phe secretion in milk protein. The synthesis of protein in the uterus plus mammary gland was 0·145 g Phe/d in dry animals, or about 1·2% of the daily plasma Phe flux. Lactating animals secreted 3·36 g Phe/d as milk protein (net protein synthesis). Lactating animals incorporated 5·84 g Phe/d into proteins of the milk, mammary gland and uterus, or about

Table 3. *Fractional synthesis rates* (K_s) *of tissue proteins calculated using the specific radioactivities of free phenylalanine* (*Phe*) *in plasma* (K_{sp}) *or tissue* (K_{sh}), *in dry and lactating goats**

(Mean values for three animals)

	K_{sp} (%/d)				K_{sh} (%/d)			
Tissue	Dry	Lactating	SEM	Statistical significance of difference: $P <$	Dry	Lactating	SEM	Statistical significance of difference: $P <$
Uterus	6·81	6·18	0·71	0·5	14·6	12·3	1·50	0·4
Mammary gland	8·70	41·6	3·01	0·00	28·5	107	3·96	0·01
Spleen	7·95	8·79	0·85	0·5	38·3	23·7	2·39	0·01
Liver	10·7	12·6	0·58	0·07	26·5	30·6	1·77	0·18
Lung	6·95	9·28	0·57	0·04	18·0	20·0	1·56	0·42
Kidney	16·4	20·4	1·06	0·05	39·8	49·5	2·42	0·04
Caecum	15·4	18·6	3·14	0·5	35·1	42·1	7·48	0·55
Duodenum	21·0	27·5	1·25	0·02	107	131	11·7	0·22
Rumen	16·4	23·3	2·05	0·07	27·7	39·6	3·89	0·09
Heart	3·93	5·20	0·76	0·3	6·35	8·21	0·99	0·25
Diaphragm	1·79	2·29	0·16	0·01	2·87	3·13	0·13	0·22

* For details of procedures, see pp. 453–454.

Table 4. *Tissue phenylalanine mass* (*g*) *in dry and lactating goats**

(Mean values for three animals)

Tissue	Dry	Lactating	SEM	Lactating:dry	Statistical significance of difference: $P <$
(A) Reproductive system					
Uterus	0·384	0·949	0·056	2·47	0·06
Mammary gland	1·363	5·880	0·208	4·31	0·01
(B) Other organs					
Spleen	0·802	0·915	0·061	1·14	0·40
Liver	7·635	9·522	0·596	1·25	0·19
Lung	3·731	9·912	0·290	1·05	0·77
Kidneys	0·796	0·898	0·038	1·13	0·26
Duodenum	2·217	3·089	0·162	1·39	0·05
Rumen	5·256	10·78	0·760	2·05	0·02
Heart	1·693	1·921	0·139	1·13	0·46
Organs					
Sub-total	26·15	33·62	0·396	1·29	0·01

* For details, see pp. 453–454.

31·7% of the daily Phe flux. The absolute synthesis of protein in the abdominal and thoracic organs of the lactating animals also tended to rise; this was particularly marked in the rumen, small intestine and kidney ($P < 0·05$). When the synthesis of the visceral organs was summed (Table 5), it was seen that protein synthesis in this compartment was increased twofold in lactating animals. The total protein synthesis (based on K_{sp}) accounted for in the specific organs and tissues studied here may be compared with the daily plasma Phe flux, since these measures are based on the same precursor pool. In the dry animals, 9·264 g Phe/d, or 77% of the daily Phe flux was not accounted for in the present analysis.

Table 5. *Absolute synthesis rates of tissue protein synthesis (g phenylalanine (Phe)/d)* in dry and lactating goats†*

(Mean values for three animals)

Tissue	Dry	Lactating	SEM	Lactating:dry	Statistical significance of difference: $P <$
(A) Reproductive system					
Uterus	0·026	0·060	0·007	2·31	0·06
Milk	0	3·360	0·566	NA	NA
Mammary gland	0·119	2·420	0·089	20·3	0·01
Sub-total	0·145	5·840	0·344	40·3	0·01
(B) Other organs					
Spleen	0·064	0·079	0·004	1·23	0·15
Liver	0·827	1·206	0·085	1·46	0·09
Lung	0·263	0·361	0·027	1·37	0·14
Kidneys	0·106	0·182	0·009	1·72	0·04
Duodenum	0·465	0·855	0·057	1·84	0·02
Rumen	0·839	2·514	0·182	3·00	0·01
Heart	0·067	0·097	0·009	1·45	0·16
Sub-total	2·656	5·305	0·279	2·00	0·01
(C) Total	2·801	11·145	0·578	3·98	0·01
Plasma Phe flux	12·1	18·4	1·63	1·52	0·01
Plasma Phe flux not accounted for	9·264	7·215	0·360	0·78	0·04

NA, not applicable.

* Absolute synthesis rates were calculated as fractional synthesis rate derived from specific radioactivity of free Phe in plasma multiplied by tissue Phe mass (for details, see p. 454).

† For details of procedures, see pp. 453–454.

By contrast, in lactating animals, only 7·215 g Phe/d or 40% of the daily Phe flux was not accounted for.

Hind-limb composition and protein synthesis

Hind-limb variables are shown in Table 6. The hind-limbs weighed slightly (−10%), but not significantly less in the lactating animals. When these values were expressed per unit carcass weight, the hind-limbs of the lactating group were 15% lighter ($P < 0·05$). The wet weights of muscle, skin and bone from the right hind-limbs were recorded. When these values were expressed as a percentage of the wet weight of the whole leg, it was seen that muscle comprised significantly less (−10%), and bone comprised significantly more (+32%) of the leg weight in the lactating group. This latter result was not due to an increase in the absolute amount of bone present, but rather to a decrease in total muscle mass. Leg muscle also comprised a lower proportion of carcass weight ($P < 0·01$) in the lactating group. The proportion of skin in the leg (about 120 g/kg wet weight) was not different between groups.

The tissues of the hind-limb were further analysed for N and Phe contents (Table 7). Because we lacked suitable materials and expertise for determination of N and Phe in hard bone, these were not included. Where total hind-limb N and Phe are mentioned hereafter, it should be noted that bone is not included. Bone marrow separated from bone segments appeared to be mostly fat, and for this tissue only chloroform–methanol extraction was carried out first. The de-fatted bone marrow fraction in both dry and lactating animals contained a very small percentage (< 0·3) of the N and Phe present in muscle, skin and

Table 6. *Hind-limb tissue variables in dry and lactating goats**

(Mean values for three animals)

	Dry	Lactating	SEM	Lactating:dry	Statistical significance of difference: $P <$
Left plus right hind-limbs wt:					
kg	5·41	4·85	0·30	0·90	0·41
g/kg CW	21·3	18·0	0·86	0·85	0·04
Right hind-limb components (kg wet wt)					
Limb	2·66	2·38	0·16	0·89	0·46
Muscle	1·74	1·41	0·13	0·81	0·24
Skin	0·32	0·29	0·02	0·91	0·53
Bone	0·59	0·70	0·04	1·19	0·20
Marrow	0·05	0·06	0·01	0·83	0·52
Right hind-limb composition (g/kg)					
Muscle	65·5	58·9	1·66	0·90	0·02
Skin	12·0	12·1	0·28	1·01	0·87
Bone	22·4	29·5	1·68	1·32	0·01
Leg muscle (two limbs)					
g/kg CW	13·5	10·2	0·80	0·76	0·01
Lipid (g/kg)	7·7	7·3	0·74	0·95	0·72
Total lipid (g)	262	208	10·6	0·79	0·06
Water (g/kg)	70·9	73·3	0·80	1·03	0·10
Ash (g/kg)	1·00	0·97	0·02	0·97	0·37

CW, carcass weight.

* One whole hind-limb was disarticulated at the hip joint, and muscle, skin and bone marrow dissected. For details of procedures, see pp. 453–454.

Table 7. *Hind-limb nitrogen and phenylalanine (Phe) composition in dry and lactating goats**

(Mean values for three animals. Tissue samples were thoroughly mixed to obtain homogeneous and representative sub-samples; analyses were done in triplicate for tissue N and in duplicate for tissue Phe)

	Dry	Lactating	SEM	Lactating:dry	Statistical significance of difference: $P <$
N (g/kg limb) in:					
Skin	5·44	5·47	0·19	1·01	0·95
Bone marrow	0·026	0·032	0·01	1·23	0·52
Muscle	21·2	17·7	0·24	0·83	0·01
Phe (g/kg limb) in:					
Skin	0·715	0·642	0·03	0·90	0·26
Bone marrow	0·007	0·009	0·00	1·29	0·52
Muscle	5·248	4·392	0·06	0·84	0·01
Total	5·970	5·043	0·08	0·84	0·01

* One whole hind-limb was disarticulated at the hip joint, and muscle, skin and bone marrow dissected. For details of procedures, see pp. 453–454.

Table 8. *Hind-limb protein synthesis in dry and lactating goats**

(Mean values for three animals)

	Dry	Lactating	SEM	Lactating:dry	Statistical significance of difference: $P <$
K_{sh} (%/d)					
Skin	11·1	6·43	0·57	0·58	0·02
Bone marrow	37·4	16·8	2·09	0·48	0·14
Muscle	4·24	3·34	0·18	0·79	0·43
K_{sp} (%/d)					
Skin	2·56	1·64	0·21	0·64	0·09
Bone marrow	5·12	4·03	0·30	0·79	0·14
Muscle	2·14	1·93	0·09	0·95	0·46
Absolute synthesis rate (mg Phe/kg limb per d)					
Calculated from K_{sh}					
Skin	79·4	41·1	4·74	0·52	0·02
Bone marrow	2·25	1·60	0·44	0·71	0·50
Muscle	223	147	8·80	0·66	0·01
Total	305	190	10·9	0·62	0·01
Calculated from K_{sp}					
Skin	18·3	10·5	1·15	0·57	0·04
Bone marrow	0·36	0·36	0·10	1·00	0·86
Muscle	105	84·8	5·21	0·80	0·01
Total	124	95·6	5·84	0·77	0·01

K_{sh}, fractional rate of protein synthesis calculated from tissue free phenylalanine (Phe) specific radioactivity; K_{sp}, fractional rate of protein synthesis calculated from plasma free Phe specific radioactivity.

* For details of procedures, see pp. 453–454.

bone marrow combined. Skeletal muscle contained 76% of hind-limb N and 88% of hind-limb Phe. Skin contained 20% of hind-limb N but only 12% of hind-limb Phe, due to its relatively low Phe content. The crude fat, water and ash contents of mixed hind-limb muscle from animals on the two treatments did not differ (Table 6).

Fractional and absolute rates of tissue protein synthesis calculated from the specific radioactivities of tissue free Phe (K_{sh}) and plasma Phe (K_{sp}) are shown in Table 8. Skeletal muscle had the lowest K_s, , followed by skin and bone marrow. The intracellular specific radioactivities of Phe in skin and bone marrow were the lowest observed in any tissue in the study (15–30% of plasma specific radioactivity), resulting in considerably higher K_{sh} than K_{sp}) in these tissues. K_s values tended to be lower in all three tissues of lactating goats; this was significant only in the case of K_{sh} in skin. Absolute rates of protein synthesis were calculated using both K_{sh} and K_{sp} (Table 8). When K_{sh} was used, the absolute synthesis rate of skin protein was 48% lower ($P < 0·05$), of muscle protein 34% lower ($P < 0·05$), and of bone marrow 29% lower (not significant) in the lactating group. When K_{sp} was used, similar reductions of protein synthesis were seen in skin (−42%) and muscle (−20%) of lactating animals, while bone marrow protein synthesis was not affected. When the sum of the absolute synthesis rates for the three tissues was computed, it was evident that bone-marrow protein synthesis comprised a small percentage (< 1) of the total. Muscle and skin comprised the bulk of protein synthesis: however, the relative proportions made up by these tissues varied, depending on whether K_{sh} or K_{sp} was used in the calculations. When K_{sh} was used, muscle protein synthesis comprised 73 and 77% of hind-limb protein synthesis in dry and lactating goats respectively. Skin made up 26 and 22% of hind-limb

protein synthesis in dry and lactating animals respectively. Thus, the proportions of protein synthesis taking place in the three tissues were not different between the two treatments. When K_{sp} was used in the same calculations, muscle protein synthesis comprised 85–89% of hind-limb protein synthesis, while skin made up 11–15%.

Values obtained for the longissimus dorsi muscle agreed qualitatively with those obtained for leg muscle (values not shown). The longissimus dorsi muscle tended to weigh less (−31%; not significant) in the lactating animals, and this muscle comprised a significantly smaller proportion of the carcass weight. The Phe mass of the longissimus dorsi was reduced in the lactating animals; this effect was also significant when expressed per unit of carcass weight. Fractional rates of protein synthesis (K_{sp} and K_{sh}) were not significantly different in longissimus dorsi muscles of dry and lactating animals, and the absolute synthesis rate tended to be lower in the lactating group, because of the reduction in tissue Phe mass.

DISCUSSION

Methodological considerations

Rates of protein synthesis may be measured by the administration of isotopically labelled amino acids (Garlick *et al.* 1973, 1980; Schaefer *et al.* 1986). For large animals, the continuous infusion of a tracer dose of amino acid remains a method of choice for the study of protein synthesis although, at least for the most rapidly turning over tissues, there is some uncertainty concerning the use of plasma or extracellular amino acids as the precursor pool (Schaefer *et al.* 1986). The flooding-dose method developed in the rat (Garlick *et al.* 1980) minimizes the differences between plasma and tissue free amino acid specific radioactivities, but is complicated by a short labelling period and is expensive in large animals. Despite these limitations, there is a need for measures of protein synthesis in different tissues of large animals in different physiological and pathological states.

During infusion, plateau specific radioactivity of Phe was achieved very rapidly compared with other tracer amino acids reported in the literature. For example, in studies with adult sheep infused with leucine (Schaefer *et al.* 1986), 2–3 h of infusion were required to reach plateau. The amount of Phe contributed by the infusate was small (6 μmol/h) compared with the plasma Phe flux > 3000 μmol/h) and, thus, was not likely to have affected the rate of rise of plasma specific radioactivity. The use of Phe as a tracer is convenient because plateau is attained rapidly, and the method for chemical estimation of Phe specific radioactivity is simple. The relatively low plasma concentration of Phe and the higher value of the time-rate constant (λp) in lactating animals compared with dry animals imply that the free pool of extracellular Phe is small and may also have a high turnover rate. The continuous feeding protocol (6 h intervals) was adequate to generate a constant metabolic milieu for the study, since plasma specific radioactivity remained quite constant.

The calculation of rates of protein synthesis presents a number of conceptual problems which have been discussed by others (Garlick *et al.* 1973; Lobley *et al.* 1980; Schaefer *et al.* 1986). It should be noted that the use of plasma Phe as precursor gives a lower estimate of protein synthesis (K_{sp}), while the use of intracellular Phe gives an upper estimate (K_{sh}). Both the K_{sp} and K_{sh} values obtained are presented here. The values for K_{sp} and K_{sh} were generally in agreement when expressed as lactating:dry, although there were some differences. We used values of K_{sp} and tissue Phe mass in the calculation of absolute rates of protein synthesis. Since the Phe content of tissue proteins varies, we suggest that tissue protein mass is more correctly expressed in Phe units, since this was the tracer used. The choice of K_{sp} in this case permits comparison of tissue synthesis rates with plasma Phe flux, since both are based on the plasma Phe pool.

The data presented here are measures of rates of tissue Phe incorporation into protein,

and do not necessarily distinguish the synthesis of proteins which are secreted from cells and the synthesis of resident cellular protein. This problem may be considered in a tissue-specific fashion. For the liver and bone marrow, which export protein into the blood, K_s may be underestimated, as proteins leave the tissue during the infusion period. The lactating mammary gland synthesizes a substantial amount of protein which is ultimately secreted. We attempted physically to separate milk from endogenous mammary protein by milking the goats just before the start of the infusion and at its end. There may be a high turnover of milk protein before secretion (Oddy *et al.* 1988); this component cannot be quantified with the methods used here. Thus our estimate of milk protein synthesis (net Phe secretion) represents a minimum value for this variable. Since new hair protein is added from the base of the follicle, hair is included in the over-all skin K_s. It is conceivable that skin and hair protein synthesis might be differentially affected during periods of nutritional stress such as lactation; however, this distinction cannot be made with current methodology. Samples of fresh hind-limb hide from dry and lactating goats were 171–174 g hair/kg ($n = 6$). The inclusion of hair in a skin sample results in a lower apparent rate of protein synthesis than in shaved skin as used here. The act of shaving may also have had some impact on skin protein synthesis.

The role of changes in protein synthesis can be placed in context of whole-body protein metabolism if rates of protein degradation are also known. The most widely used method is to calculate degradation as the difference between protein synthesis and net protein gain (or loss). The latter may be estimated from sequential slaughter and was not within the scope of the present study.

Comparison of lactating and dry animals

Dry, non-pregnant goats were compared with lactating goats in the third week post partum. Daily Phe flux was 52% greater in the lactating group in the present study. Animals in these two states differ in feed intake and milk production, and also differ in that one group has just completed a cycle of gestation. The differences seen in the protein metabolism of the two groups reflect adaptation to all of the previously mentioned variables. In the lactating animals, increased feed intake was associated with greater total protein synthesis in the digestive system. This resulted in part from increased tissue Phe mass, as also documented by Fell *et al.* (1972) in lactating ewes, and from increased fractional rates of tissue protein synthesis. Feed intake and dietary protein levels are known to be important influences on whole-body amino acid flux and tissue protein synthesis (Waterlow, 1984). However, since Bryant & Smith (1982) demonstrated a 50 % increase in plasma tyrosine flux in lactating ewes fed the same amount as dry ewes, it is clear that protein intake is not the sole variable resulting in increased plasma Phe flux. It is also known that changes in tissue protein mass occur during the latter part of gestation (Robinson *et al.* 1978), and it would also be useful to clarify the effects of gestation *v.* lactation on protein metabolism. Although the scope of the present study was limited in terms of numbers of treatments and numbers of animals, it was possible to identify marked differences in the synthesis of protein in different tissues.

Tissue K_s values observed here were similar to those obtained in other ruminants such as sheep (Schaefer *et al.* 1986) and cattle (Lobley *et al.* 1980), but were generally lower than those reported for the rat (Garlick *et al.* 1980). This would be expected, since protein turnover is known to be related to metabolic mass (Waterlow, 1984). The highest rates of protein synthesis were seen in the duodenum, as shown for the rat by Garlick *et al.* (1980). Lactating mammary gland also showed high rates of protein synthesis, which agrees with the results of Millican *et al.* (1987) in the mouse. Although lactating animals were milked immediately before tissue sampling, the tissue may have contained some milk protein. In

spite of this, it seems clear that milk-protein synthesis was associated with high rates of endogenous mammary protein synthesis.

The absolute synthesis rates of different organs may be compared with the daily Phe flux, in order to estimate their respective contributions. These comparisons may be used to draw a qualitative picture rather than a quantitative one, due to the many methodological considerations discussed previously. It should be noted that the lower estimates of organ contributions to total Phe flux are presented in Table 5. In spite of this limitation it seems clear that Phe utilization for the synthesis of proteins in the mammary gland and other organs comprised a larger proportion of the daily whole-body Phe flux in the lactating goats. A proportion of the total Phe flux in dry and lactating animals was not accounted for, and would probably comprise Phe used for synthesis of proteins in the remaining tissues (principally skeletal muscle, skin and bone; see p. 464), and the oxidative degradation of Phe. The observation that the flux not accounted for in the lactating animals was significantly less than in the dry group implies that lactating animals reduce protein synthesis in carcass tissues or reduce Phe degradation, or both. The oxidative catabolism of amino acids in dry and lactating animals has not been extensively examined and requires further study.

Hind-limb composition and protein synthesis

High levels of milk production maintained in the face of negative N balance over-all imply that N metabolism is altered in some way in the whole body. This could be achieved by net protein degradation in non-mammary tissues. Skeletal muscle has received some attention in this regard, since it is considered a primary site of protein mobilization, but the published reports do not agree. Muscle protein synthesis has been reported to increase (Vincent & Lindsay, 1984), decrease (Bryant & Smith, 1982) or not change (Millican *et al.* 1987) in lactating animals, and muscle protein mass or content has been reported to fall or stay the same (Smith *et al.* 1981; Bryant & Smith, 1982). There may be differences between muscles in different anatomical locations (Bryant & Smith, 1982). Furthermore, the species and the approaches used by the different investigators vary considerably.

Compositional analysis reported here shows decreased hind-limb protein in lactating animals resulting in large part from a reduced proportion of skeletal muscle. Muscle protein, but not that of skin, appeared to have undergone a net mobilization. It is not known whether this occurred during the onset of lactation or during the period of gestation which preceded it (Robinson *et al.* 1978). Bone-marrow protein comprised an insignificant proportion of hind-limb protein. This result is in accord with those of Russell *et al.* (1968), who showed that bone in adult ewes contains considerable fat deposits. The fat, water and ash contents of mixed hind-limb muscle were not different between treatments.

The rates of synthesis of muscle protein are similar to those obtained by others with adult ruminants (Bryant & Smith, 1982; Schaefer *et al.* 1986; Early *et al.* 1988). Our values for skin protein synthesis are higher than those reported for hide of cattle by Lobley *et al.* (1980). This may be due to our choice of skin shaved of hair, rather than 'hide' in the Lobley *et al.* (1980) study which had the hair on. Bone-marrow protein synthesis has not previously been reported in large animals. The relatively high rate of bone-marrow protein synthesis agrees with work by Garlick and coworkers (summarized by Waterlow, 1984) in the rat. This may be attributable to the biosynthetic activity of haematopoietic stem cells in the bone marrow. Since bone marrow in these mature goats contained a high proportion of fat and very little protein, this high synthesis rate had little impact on leg metabolism as a whole.

The wet weight of the hind-limb of adult dairy goats was approximately (g/kg) 600 muscle, 120 skin, 230 hard bone and 20 bone marrow. The relative muscle:skin ratios in

hind-limb protein synthesis based on the analyses done here falls within the range of 75:25–89:11. These proportions were not different in dry and lactating animals. We are not aware of other reports in the literature of similar analyses of the hind-limb protein synthesis of lactating animals. These results may have important implications for the interpretation of measures of hind-limb metabolism derived from arterio-venous differences of substrates and from the perfused hemicorpus preparation. In the present study the wet weight of the hind-limb was not different between treatments; at the same time there were highly significant differences in the proportional composition of the limb. It is often assumed that muscle is the principal (if not the only) constituent of hind-limb protein metabolism (e.g. Oddy & Lindsay, 1986; Barrett *et al.* 1987; Early *et al.* 1988). Protein synthesis in the skin may be as much as 25% of the total and must be taken into consideration. It would seem prudent to study hind-limb metabolism in a tissue-specific fashion, in order to interpret correctly over-all measures of hind-limb metabolism. The hind-limb composition of the laboratory rat, and the protein synthetic activity of muscle, skin and bone marrow may be different than seen here for large animals, and requires clarification. Finally, the protein synthesis rate of hard bone would be valuable additional information.

Our results suggest an adaptive mechanism sparing protein peripherally in order to make substrates available for milk synthesis. In the case of skin this may reflect a reduction in hair growth as in other instances of nutritional stress or reduced skin protein turnover, or both. Skeletal muscle would appear to undergo net protein mobilization, which may be due to increased rates of protein degradation, since K_s values were not significantly less in lactating than in dry goats. Studies of muscle proteolysis are needed to clarify this possibility.

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