

Partitioning of Nutrients in Merino Ewes

I. Contribution of Skeletal Muscle, the Pregnant Uterus and the Lactating Mammary Gland to Total Energy Expenditure

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Abstract

The contribution of leg muscle, pregnant uterine tissue and lactating mammary gland to overall energy utilization was determined in Merino ewes. Ewes were offered one of three diets based on chaffed oat hay (7.9 MJ metabolizable energy per kilogram dry matter); chaffed lucerne hay (8.6 MJ/kg); or a 50:50 (w/w) mixture of chaffed oat hay and lucerne hays (8.2 MJ/kg). Measurements were made during five different physiological states: dry (non-pregnant), at 94 and 125 days after mating, and at 20 and 50 days after lambing.

Tissue energy use was calculated from oxygen uptake and carbon dioxide output obtained from measurement of blood flow and arteriovenous difference. Whole-body energy use was calculated from carbon dioxide energy rate. Energy use by leg muscle was 144 ± 8 (mean \pm s.e.) $\text{kJ kg}^{-1} \text{day}^{-1}$, and unrelated to metabolizable energy intake, but leg energy use increased with ewe body weight. On the basis that leg muscle was representative of all muscle, total muscle energy use accounted for $26 \pm 4\%$ of whole-body energy expenditure in dry ewes. Uterine energy use per unit weight was respectively 348 ± 53 and $254 \pm 23 \text{ kJ kg}^{-1} \text{day}^{-1}$ at 94 and 125 days after mating.

Milk production was highly correlated with weight of secretory tissue, and with blood flow to the mammary gland. The ratio of blood flow to milk produced was 473:1 in ewes producing from 200 to 1000 ml of milk per day. The mammary gland used energy to produce milk with an efficiency of 0.90 ± 0.01 , a value close to the theoretical estimate of 0.89. On the basis that metabolic rate does not increase during lactation, the efficiency of use of metabolizable energy for milk production was 0.51 ± 0.05 .

Examination of energy use by different tissues indicated that energy use by muscle was related to weight, but energy use by remaining tissues (whole body less muscle, uterus and mammary gland) was related to metabolizable energy intake. The results reveal an increase in energy use by the remaining tissue in lactating ewes ($8500 \pm 569 \text{ kJ/day}$) compared with dry ($5634 \pm 216 \text{ kJ/day}$) and pregnant ewes ($5815 \pm 393 \text{ kJ/day}$).

Introduction

There have been substantial advances in the procedures for determining the nutrient requirements of tissues, largely through the simultaneous measurements of arteriovenous (A-V) concentration differences and blood flow. These techniques have proved invaluable for studying metabolism in specific tissues, as exemplified by the studies of the mammary gland by Linzell (1974), and similar work on the liver and gut (Bergman and Wolff 1971), a defined muscle mass in the leg (Domanski *et al.* 1974), the pregnant uterus (Setchell *et al.* 1972) and the fetus and placenta (Battaglia and Meschia 1978).

In such studies, the energy expenditure of tissues may be calculated from oxygen uptake and carbon dioxide output, and total energy expenditure may be measured from the entry rate of carbon dioxide determined using isotope-dilution procedures (Corbett *et al.* 1971).

This isotope-dilution procedure complements the classical A-V difference and the isotope-dilution procedures developed for measurements of nutrient turnover and oxidation in specific tissues (see Linzell and Annison 1975). These combined procedures make it possible to examine the manner in which energy expenditure in different tissues contributes to overall energy expenditure. Comparison of whole-body and tissue energy expenditure derived by these means with the results of conventional calorimetric studies is useful in two ways. Firstly, to demonstrate the validity of the A-V difference methods, in particular the site of venous sampling, and measurement of blood flow. Secondly, to provide insights into partition of energy use between different tissues which cannot be obtained by conventional calorimetry.

In this paper we report studies designed to investigate the manner in which the major energy-yielding metabolites used by hind limb skeletal muscle, the pregnant uterus, and the lactating mammary gland contribute to whole-body metabolism in adult Merino ewes. Data are presented here on the influence of nutritional and physiological state on patterns of nutrient utilization by these tissues in relation to total energy expenditure. Whenever possible, simultaneous measurements were made of metabolism in muscle and either pregnant uterus or mammary gland (see Gooden *et al.* 1980).

Materials and Methods

Animals, Diets and Management

Merino ewes (medium Peppin type), 5 years old, initially with 3 months wool growth, were used for the studies. The oestrous cycles of 55 ewes were synchronized with progesterone-impregnated vaginal sponges (Repromap, Upjohn Pty Ltd), and ewes were mated to Merino rams on known dates. At approximately 50 days after mating the number of lambs carried by each ewe was determined using real-time ultrasonic scanning (Fowler and Wilkins 1982). Thirty-six ewes, each bearing a single lamb, were selected for subsequent studies. A further 18 ewes which were not mated served as a control group.

From 2 weeks after mating three groups of 18 ewes were fed either chaffed oaten hay—7.9 MJ metabolizable energy (ME) and 11 g nitrogen (N) per kilogram dry matter (DM); chaffed lucerne hay—8.6 MJ ME and 33 g N per kilogram DM; or a 50:50 (w/w) mixture of chaffed oaten hay and chaffed lucerne hay—8.2 MJ ME and 21 g N per kilogram DM. The ME content of the feed was derived from *in vivo* organic matter digestibility in dry matter (DOMD%)—viz $ME = 0.15 \text{ DOMD}\%$ (Anon. 1975). The ewes were offered, respectively, 810, 1260 and 1620 g DM per day of the oaten hay, the 50:50 and the lucerne diets when non-pregnant and throughout pregnancy. In lactation, the oaten hay diet was supplemented with 180 g DM of oats per day (10.8 MJ ME and 20 g N per kilogram DM) and the amounts of the mixed diet and lucerne offered were increased to 2250 g DM per day. For at least 10 days before and during the experimental periods feed was offered continuously from automatic feeders. ME intakes during the subsequent measurement periods are shown in Table 1. Water was freely available. The ewes were weighed at intervals of 4 weeks, except during lactation when they were weighed weekly.

After lambing the ewes and lambs were weighed, and the lambs removed permanently from the ewes. The ewes were hand-milked daily at 0800 and 1600 h. Milk production was recorded daily and samples bulked for subsequent analyses. At the end of the experiment each ewe was shorn, killed and the mammary glands and individual muscles of the hind limb were dissected and weighed. Mammary skin, teats, visible fat and secretory tissue were weighed individually.

The sheep used in this experiment had been routinely handled for intensive experimental procedures during the previous 2 years, and were well accustomed to handling. Every effort was made to ensure that the animals were stressed as little as possible during the experiment. Air temperature in the sheep-shed remained within the range of minimum 5°C, maximum 25°C throughout the experiment.

Surgical Procedures

One week before measurements were made, polyethylene or polyvinyl chloride catheters (1.0 mm i.d., 1.5 mm o.d., Dural Plastics, N.S.W.) were introduced under general anaesthesia into the jugular vein and femoral artery via the saphenous artery and, in pregnant ewes, catheters were placed into both utero-ovarian veins as described by Brown *et al.* (1982). Two days before measurements were

made, similar catheters were placed in the deep femoral vein via the recurrent tarsal vein (Oddy *et al.* 1981) and in the case of the lactating ewes, the medial subcutaneous mammary vein (Gooden *et al.* 1980) by a percutaneous Seldinger technique using local anaesthesia. The catheters were filled with sterile heparinized saline (250 i.u./ml) containing streptomycin (2 g/l) and catheter patency was checked twice weekly.

Experimental Design and Statistical Analysis

The experiment was designed as a 3×5 factorial with three diets and five physiological states. The ewes, which were fed chaffed oat hay (OH), chaffed lucerne (L), or a 50:50 mixture of the two (OHL), were studied when non-pregnant (dry), in early pregnancy (94 days after mating, EP), in late pregnancy (125 days after mating, LP), in early lactation (about 20 days after lambing, EL) and in late lactation (about 50 days after lambing, LL).

Measurements of tissue blood flow, oxygen consumption and carbon dioxide production were made on two occasions over a 4-day period at the end of which whole-body entry rate of carbon dioxide was measured. During each period of measurement the animals stood quietly.

Numbers of ewes completing the experimental schedule in each group varied, largely because of difficulty in maintaining catheter patency for the necessarily long period. It was necessary, therefore, to use some ewes on which measurements had been made during pregnancy for the lactation studies. The number of sheep on which measurements were successfully completed are shown in Table 1.

The unbalanced structure of the results precluded application of conventional analysis of variance methods. Accordingly, statistical analysis using a generalized linear model with the terms diet, physiological state, and their interaction, was fitted using the regression techniques contained within GENSTAT MK 4.03 (Rothamsted Experimental Station, United Kingdom). Other relationships between observations were explored using similar techniques.

Measurement of Blood Flow

Blood flow rates in hind limb tissues, the pregnant uterus, and lactating mammary gland were measured with an indicator-diffusion technique using tritiated water (TOH) as a marker (Oddy *et al.* 1981; Brown *et al.* 1982). Blood samples were withdrawn continuously over six consecutive intervals of 10 min to obtain the required integral of A-V concentration difference and of TOH concentration at equilibrium.

Total blood flow per kilogram of perfused tissue was measured by this technique. In the case of hind limb, perfused tissue consisted predominantly of muscle but included skin, bone and fat. The proportions by weight were muscle 0.61 ± 0.01 (s.e.), bone 0.22 ± 0.005 , skin 0.12 ± 0.004 and fat 0.05 ± 0.004 . Perfused tissues of the uterus included fetus, placenta, uterine tissue and fluids.

To confirm the suitability of TOH as a marker for measurement of total udder blood flow, a comparison between total blood flow measured using TOH and by a microsphere technique was made. The microsphere technique of Hales (1974) was used to measure capillary blood flow to the mammary gland. Microspheres (New England Nuclear Co., Boston, Mass.) were 15 ± 3.0 (mean \pm s.d.) μm in diameter and were labelled with either ^{46}Sc or ^{113}Sn .

A dose of microspheres (approximately 15 million) was injected into the left ventricle of the free-standing conscious ewe, while blood was being withdrawn from the abdominal aorta at approximately 25 ml/min (to provide the reference organ for determination of cardiac output) and from the medial subcutaneous mammary vein catheter at approximately 15 ml/min during the time of injection of microspheres and for 45 s after, to determine total flow passing through A-V anastomoses in the mammary gland (Archie *et al.* 1973).

The estimates of total mammary blood flow for seven ewes measured with TOH was 268 ± 75 ml $\text{min}^{-1} \text{kg}^{-1}$, and 371 ± 40 ml $\text{min}^{-1} \text{kg}^{-1}$ when measured using microspheres within the same hour. The values obtained by the two methods were not significantly different ($P > 0.05$). In calculation of blood flow using the TOH method a correction factor was used to account for the difference in water content of blood and lactating mammary gland. Udder water content was 0.700 ± 0.011 g/g, the water content of blood was 0.834 g/g and a factor of 1.04 was used to account for dilution of blood with heparin. These were combined to form a correction factor of 0.81 and blood flow was calculated as described by Oddy *et al.* (1981). Blood flow to individual mammary tissues obtained using microspheres showed that $91.9 \pm 1.1\%$ of mammary blood flow was to secretory tissue. The proportions of total blood flow passing through teats, skin and adipose tissue were 0.9 ± 0.1 , 2.9 ± 0.4 and $4.3 \pm 1.1\%$ respectively. A-V anastomoses in the udder accounted for $3.0 \pm 0.9\%$ of total blood flow (B. W. Brown, V. H. Oddy, J. M. Gooden and G. M. Hough, unpublished data).

Measurement of Blood Gases

Blood for determination of oxygen and carbon dioxide concentrations was withdrawn anaerobically into heparinized syringes immediately before and after blood-flow measurements, and kept on ice until analysis. The pO_2 , pCO_2 and pH were determined within 30 min of sampling with a Radiometer PHM 72 blood gas analyser. Haemoglobin was measured using a colorimetric procedure (Boehringer, West Germany, Kit No. 142729).

Oxygen concentration ($mm = 0.446 [O_2]$, vol.%) was calculated from measured pO_2 , pH and haemoglobin concentration, H (g/100ml), using the equation:

$$[O_2] \text{ (vol.\%)} = \{1.34 H [O_2] \text{ (sat.\%)}\} / 100,$$

where $[O_2]$ (sat.%) at 37°C and pH 7.40 equals $(pO_2/34 \cdot 1)^{2.68} / [1 + (pO_2/34 \cdot 1)^{2.68}]$ after a correction of pO_2 for the Bohr effect (Anon. 1971) thus:

$$\log pO_2 = -0.48 \text{ per } 0.1 \text{ pH unit different from pH } 7.40.$$

Carbon dioxide concentration (mm) was calculated from pCO_2 and pH measurements using the Henderson-Hasselbach equation:

$$[CO_2] = \text{antilog} (\text{pH} - 6.12) \times 0.0314 pCO_2 + 0.0314 pCO_2.$$

Tissue energy use ($\text{kJ kg}^{-1} \text{ day}^{-1}$) was calculated from tissue oxygen uptake and carbon dioxide output ($A-V$ difference \times blood flow, $\mu\text{mol min}^{-1} \text{ kg}^{-1}$) by a modification of the equation of Brouwer (1965) in which the term for nitrogen was ignored and conversion of oxygen and carbon dioxide from litres per day to moles $\text{min}^{-1} \text{ kg}^{-1}$ was made on the basis that 1 mole occupies 22.4 litres at standard temperature and pressure. The relationship used was as follows:

$$\text{Tissue energy use} = 0.552 \text{ oxygen uptake} + 0.162 \text{ carbon dioxide output.}$$

Measurement of Carbon Dioxide Entry Rate

Whole-body entry rate of carbon dioxide was determined by constant infusion of $\text{NaH}^{14}\text{CO}_3$ (1.8 kBq/min) into the jugular vein for at least 12 h. Arterial blood samples for determinations of $^{14}\text{CO}_2$ were taken at hourly intervals for 4 h, from 8 h after commencement of the infusion. Specific radioactivity of arterial blood $^{14}\text{CO}_2$ was determined by the gravimetric method of Leng and Leonard (1965), but modified to permit solubilization of BaCO_3 using Tris-EDTA solution as described by Hinks *et al.* (1966). Entry rate of carbon dioxide (mg C/min) was calculated as the ratio of the infusion rate (Bq/min) to the specific radioactivity of arterial carbon dioxide (Bq/mg C).

Whole-body energy use E (kJ/day) was calculated from the carbon dioxide entry rate R (mg C/min) using the equation derived by Corbett *et al.* (1971), viz:

$$E = 20.564R + 3018.07.$$

Milk Analyses

The energy content of milk was calculated from measured fat and solids-not-fat contents as described by Tyrell and Reid (1965). The predicted milk energy content was not significantly different from that obtained by bomb calorimetry. Predicted and measured milk energy contents for six samples were 4.5 ± 0.4 and 4.3 ± 0.3 MJ/kg respectively.

Milk fat content was determined by the Babcock method (Davis and McDonald 1953), content of total solids by the method of Davis (1959), and content of solids-not-fat was measured by difference.

Prediction of Uterine Weight

The total weights of uterine tissue and the fetus, on the days when measurements were made, were predicted from the equations of Geisler and Jones (1979), after correction for measured birth weights, which in this study was 3.9 kg (mean of the 12 lambs). Uterine weight was calculated using liveweight at mating of 45 kg. The predicted uterine tissue weights were respectively 2470 and 4460 g and predicted fetal weights 640 and 2180 g at 94 and 125 days after mating.

Table 2. Blood flow to, O₂ arteriovenous (A-V) difference across, and calculated energy utilization by the hind limb (predominantly muscle tissue) and estimated total muscle energy utilization in the body of ewes eating three different diets

Values are means \pm s.e. Number of sheep given in parentheses

Parameter	Physiological state ^a				
	Dry	EP	LP	EL	LL
Blood flow (ml min ⁻¹ kg ⁻¹)	70.0 (2)	Diet: chaffed oatens hay	79.4 \pm 9.5 (3)	84.7 \pm 0.5 (2)	83.4 \pm 7.4 (4)
O ₂ (A-V) (mm)	1.99	49.4 \pm 5.2 (3)	2.96 \pm 0.52	2.46 \pm 0.14	2.41 \pm 0.34
Leg energy use (kJ kg ⁻¹ day ⁻¹)	101.6	2.84 \pm 0.19	145.5 \pm 38.0	140.7 \pm 19.0	142.8 \pm 5.3
Estimated whole body muscle energy use (kJ/day) ^b	1150	111.5 \pm 14.6	1155	1310	1380
Blood flow (ml min ⁻¹ kg ⁻¹)	116.0 \pm 11.5 (5)	Diet: chaffed oatens hay—lucerne chaff	103.9 \pm 1.62 (2)	109.6 \pm 15.7 (5)	102.2 \pm 14.0 (5)
O ₂ (A-V) (mm)	2.31 \pm 0.12	—	1.74 \pm 0.23	1.53 \pm 0.31	2.37 \pm 0.49
Leg energy use (kJ kg ⁻¹ day ⁻¹)	177.5 \pm 17.5	—	131.9 \pm 16.7	107.3 \pm 27.4	130.0 \pm 13.9
Estimated whole body muscle energy use (kJ/day)	2570	—	1710	1370	1650
Blood flow (ml min ⁻¹ kg ⁻¹)	131.8 \pm 32.2 (3)	Diet: lucerne chaff	93.0 \pm 8.6 (5)	114.9 \pm 23.6 (4)	85.1 \pm 7.3 (4)
O ₂ (A-V) (mm)	2.42 \pm 0.42	101.7 \pm 18.2 (4)	2.89 \pm 0.32	1.74 \pm 0.32	2.92 \pm 0.26
Leg energy use (kJ kg ⁻¹ day ⁻¹)	153.9 \pm 47.6	2.54 \pm 0.29	184.6 \pm 16.2	127.8 \pm 14.1	192.5 \pm 14.2
Estimated whole body muscle energy use (kJ/day)	2420	162.0 \pm 29.8	2670	1800	2690

^aSee footnote to Table 1.

^bCalculated on the basis that 26% of fleece-free maternal body weight is muscle, and assuming that energy use per kilogram of hind limb muscle of quietly standing sheep is representative of energy use by all skeletal muscle.

Results

Whole-body Energy Metabolism

The mean liveweight, fleece weight and fleece-free maternal body weights of the ewes, their estimated ME intake, measured carbon dioxide entry rates, and heat production calculated from those rates are shown in Table 1.

Heat production (Y_1 , kJ/day) was significantly related to ME intake (X_1 , kJ/day) over all diets and physiological states. The relationship was:

$$Y_1 = 6050 (\pm 469) + 400 (\pm 47) X_1,$$

$r = 0.75$, $P < 0.001$, r.s.d. = 1400, d.f. = 57. After correction for ME intake, heat production was significantly greater ($P < 0.005$) during lactation than while dry or pregnant. The largest increase occurred in early lactation.

Muscle Energy Use

The blood flow to, oxygen uptake by, and calculated energy utilization ($\text{kJ kg}^{-1} \text{day}^{-1}$) by the leg (predominantly muscle) are shown in Table 2. Energy utilization values were extrapolated to whole-body use by muscle by assuming that leg muscle was representative of muscle in general, and that dissectable muscle was 26% of maternal, fleece-free body weight in Merino ewes (J. Thompson and R. Butterfield, personal communication).

Energy use by leg muscle was significantly affected by diet. Ewes fed OH used less energy in their leg muscles than ewes on the other diets ($P < 0.01$). There was no relationship between muscle energy use and ME intake. The relationship between daily muscle energy use (Y_2 , kJ/kg) and body weight (X_2 , kg) for all animals was:

$$Y_2 = 61 (\pm 36) + 1.6 (\pm 0.6) X_2,$$

$r = 0.31$, $P < 0.05$, r.s.d. = 43, d.f. = 48.

Partitioning of the variance between blood flow, oxygen uptake and carbon dioxide production, which were the values used to calculate muscle energy use, revealed that each contributed similarly to the variation in energy use. Leg muscle blood flow (Y_3 , $\text{ml min}^{-1} \text{kg}^{-1}$), however, was significantly less in ewes eating OH than in those fed the other diets ($P < 0.05$), and was related to fleece-free body weight (X_3 , kg):

$$Y_3 = 40 (\pm 24) + 1.2 (\pm 0.5) X_3,$$

$r = 0.38$, $P < 0.05$, r.s.d. = 30, d.f. = 51.

Uterine Energy Use

Table 3 shows the blood flow and oxygen A-V difference, energy utilization per kilogram of pregnant uterus at 94 and 125 days after mating and lamb birth weights. Blood flow (per unit weight of tissue) to the pregnant uterus was greater at 94 days than at 125 days mating ($P < 0.05$), and although there was a trend for oxygen A-V difference to be less at 94 days than at 125 days the difference was not significant. Oxygen consumption per kilogram of uterus was 0.444 ± 0.065 and $0.334 \pm 0.035 \text{ mmol kg}^{-1} \text{min}^{-1}$ at 94 and 125 days after mating respectively. This was reflected in energy use per kilogram pregnant uterus, which was 348 ± 53 and $254 \pm 23 \text{ kJ kg}^{-1} \text{day}^{-1}$ at 94 and 125 days after mating, respectively.

Mammary Gland Energy Use

The weight of mammary tissue, milk yield, mammary gland energy use and milk energy output, blood flow and oxygen uptake by the udders of ewes fed each diet during early and late lactation are shown in Table 4. There was a significant decrease ($P < 0.05$) in milk energy output, and mammary gland energy use in late lactation. Those ewes eating the OH ration produced significantly less milk than those eating the other diets ($P < 0.001$).

Although milk production (Y_4 , g/day) was significantly but comparatively weakly related to blood flow expressed as $\text{ml min}^{-1} \text{kg}^{-1}$ ($P < 0.05$, $r = 0.37$), it was well correlated with blood flow expressed as ml/min (X_4):

$$Y_4 = 6.8 (\pm 6.71) + 3.39 (\pm 0.45) X_4$$

$r = 0.83$, $P < 0.005$, r.s.d. = 39, d.f. = 24. The mean ratios of blood flow to milk yield were 787.5 ± 81.1 , 479.8 ± 56.7 and 464.8 ± 84.8 for ewes fed OH, OHL and L diets respectively. The relationship between blood flow (ml/min) and milk production was largely due to a strong relationship between milk production (Y_5 , g/day) and udder weight (X_5 , g):

$$Y_5 = -238 (\pm 110) + 1.37 (+0.24) X_5$$

$r = 0.84$, $P < 0.005$, r.s.d. = 133, d.f. = 12. Weight of secretory tissue was highly correlated with milk yield, and accounted for the greatest part of the variation in the relationship between milk production and udder weight, for there was no relationship between the weight of mammary skin or connective tissue and milk production. The relationship between milk production (Y_6 , g/day) and weight of secretory tissue (X_6 , g) was:

$$Y_6 = 0.21 (\pm 0.80) + 2.31 (\pm 0.31) X_6$$

$r = 0.90$, $P < 0.005$, r.s.d. = 106, d.f. = 12.

Energy use by the mammary gland (Y_7 , kJ/day) was positively related to milk energy output (X_7 , kJ/day):

$$Y_7 = 55 (\pm 33) + 0.084 (\pm 0.013) X_7$$

$r = 0.79$, $P < 0.005$, r.s.d. = 82, d.f. = 23. The slope of the regression implies that the efficiency of milk energy production by the mammary gland was about 0.92; which compares well with the mean value of 0.90 ± 0.01 calculated from the expression:

$$\text{Efficiency} = \frac{\text{Milk energy output}}{\text{Milk energy output} + \text{mammary gland energy use}}$$

If it is assumed that the maintenance energy requirements of the lactating ewe are similar to those of the dry and pregnant ewe, the efficiency of use of ME above maintenance for milk production was 0.51 ± 0.05 .

Partition of Whole-body Energy Use

The results obtained were combined to partition whole-body energy use into that by muscle, the pregnant uterus, lactating mammary gland, and by difference, remaining tissues. Milk was assumed to be produced from exogenous (gut-derived) and endogenous (stored-tissue-derived) precursors with an energetic efficiency of 75% (Kronfield 1976). The direct energy cost of milk production (25% of milk energy output) was then subtracted from whole-body energy use after correction for measured mammary gland energy use.

The mean energy use by remaining tissues was 5634 ± 216 kJ/day whilst dry, and 5815 ± 393 kJ/day during pregnancy, with no difference due to diet or physiological state. During lactation, energy use by remaining tissues was greater than in dry or pregnant ewes (8500 ± 569 kJ/day, $P < 0.01$). There were no differences due to diet, but more energy was used in early (9660 ± 184 kJ/day) than in late lactation (7342 ± 491 kJ/day, $P < 0.05$).

Energy use by remaining tissues (Y_8 , kJ/day) was related to ME intake (X_8 , MJ/day):

$$Y_8 = 4102 (\pm 605) + 301 (\pm 61) X_8$$

$r = 0.58$, $P < 0.01$, r.s.d. = 1662, d.f. = 45, but not to their weight.

Discussion

Before considering the implications of the present results it must be recognized that the methods used have important limitations. In particular, the measurement of carbon dioxide entry rate is subject to a systematic error of about 20% due to participation of $^{14}\text{CO}_2$ in fixation reactions in various tissues not likely to return $^{14}\text{CO}_2$ to the measured pool during the course of the experiment. This source of error was acknowledged and

Table 3. Blood flow to, O_2 arteriovenous (A-V) difference across, and energy utilization by the pregnant uterus at 94 and 125 days of pregnancy of ewes eating three different diets

Values given are means \pm s.e. Lamb birth weights were 3.6 ± 0.3 kg (mean for four lambs), 3.7 ± 0.3 kg (three lambs) and 4.2 ± 0.3 kg (five lambs) for sheep eating the chaffed oat hay, chaffed oat hay-lucerne chaff and lucerne chaff diet respectively.

No. of sheep	Stage of pregnancy (days)	Blood flow ($\text{ml min}^{-1} \text{kg}^{-1}$)	O_2 (A-V) (mm)	Energy use ($\text{kJ kg}^{-1} \text{day}^{-1}$)
Diet: chaffed oat hay				
3	94	300 ± 73	1.23 ± 0.20	386.1 ± 107.4
4	125	218 ± 29	1.82 ± 0.26	268.4 ± 52.2
Diet: chaffed oat hay-lucerne chaff				
3	125	294 ± 73	1.20 ± 0.06	285.5 ± 41.5
Diet: lucerne chaff				
5	94	433 ± 43	1.16 ± 0.20	324.5 ± 64.1
5	125	236 ± 15	1.29 ± 0.22	222.9 ± 29.8

Table 4. Mammary gland weight, blood flow, O_2 arteriovenous (A-V) difference, energy utilization by the mammary gland, milk production and output of energy in milk 20 and 50 days after lambing in ewes eating three different diets

No. of sheep	Days after lambing	Mammary gland wt (g)	Blood flow ($\text{ml min}^{-1} \text{kg}^{-1}$)	O_2 (A-V) (mm)	Energy use by gland (kJ/day^A)	Milk production (g/day)	Energy in milk (kJ/day)
Diet: chaffed oat hay							
2	20	273 ^A	203.3	2.29	122	136	651
3	50	231 ± 24	151.5 ± 35.9	2.28 ± 0.20	68 ± 9	63 ± 16	285 ± 79
Diet: chaffed oat hay-lucerne chaff							
5	20	637 ^A	293.5 ± 53.5	2.64 ± 0.16	368 ± 58	635 ± 92	2874 ± 499
6	50	489 ± 39	254.8 ± 50.8	1.96 ± 0.34	170 ± 21	419 ± 53	1854 ± 228
Diet: lucerne chaff							
5	20	690 ^A	293.3 ± 58.3	2.23 ± 0.14	290 ± 49	708 ± 101	3086 ± 409
3	50	527 ± 46	288.0 ± 72.9	2.78 ± 0.17	288 ± 62	499 ± 125	2180 ± 535

^AMammary gland weight 20 days after lambing was estimated on the basis of yield at 50 days after lambing using the equation shown in the text.

subsequently accounted for by Corbett *et al.* (1971) when they determined the relationship between carbon dioxide entry rate and heat production. There are short-term variations in this rate which accompany feeding activity, postural change and handling and these real variations no doubt contribute to a decrease in the precision of the estimate of heat production from this parameter, compared with direct measurement in a calorimeter, where integration over a long period is used to reduce such variation.

Similarly, the measurement of tissue energy used by calculation from measured consumption of oxygen and production of carbon dioxide is subject to variations in measurement of blood flow, and in A-V difference. The measurement of pO_2 and pCO_2 and subsequent conversion to concentration is inevitably imprecise. Such factors as haemoglobin type, and differences in body temperature could lead to errors in estimating concentrations of both oxygen and carbon dioxide. Although Oddy *et al.* (1981) and Brown *et al.* (1982) have shown that the methods used here to measure blood flow to the hind limb muscle and the pregnant uterus are sound, they and others have pointed out that flow to these tissues varies with time. Changes in oxygen uptake and carbon dioxide output from tissues can complement variation in blood flow to maintain an approximately constant energy metabolism as in the pregnant uterus (Huckabee *et al.* 1972), although our results suggest that no such compensation occurs in muscle.

Leg Muscle

ME intake *per se* was found to have no effect on energy use by leg muscle. This is in agreement with the results of Bird *et al.* (1981) who found no difference in oxygen consumption by the resting leg at two levels of feed intake. Leg energy use increased as ewe weight increased. There are no reported measurements of leg energy use in sheep standing quietly which permit comparison with our findings, although those of Bird *et al.* (1981) with sheep of comparable weight compare closely with the values obtained here. The few reported measurements of energy cost in sheep standing relative to that of sheep lying down, made by Hall and Brody (1933), do not reveal any relationship between weight and energy used by standing.

Although there is no direct evidence that leg muscle energy use is representative of energy use by all muscle, there is considerable indirect evidence at least in sheep standing quietly. In this connection A. W. Bell, J. R. S. Hales, R. King and A. Fawcett (personal communication) found that Merino ewes standing quietly in a thermoneutral environment had similar blood flows to the muscles of the hind leg, foreleg and torso. Furthermore, they found that under these conditions $22 \pm 5\%$ of cardiac output passed through skeletal muscle. This is not very different to the value of $26 \pm 4\%$ for total heat production accounted for by muscle energy use in dry sheep in our studies. On this basis the assumption that leg muscle energy use can be extended to energy use by all muscle seems plausible, but in our studies measurements were made on stationary animals. A different relationship would almost certainly apply during periods of exercise.

Pregnant Uterus

Daily energy utilization by the pregnant uterus (mean of 94 and 125 days after mating, 300 kJ/kg) was not substantially different from 340 kJ/kg calculated from data obtained by calorimetric studies (Graham 1964). Although total energy use (oxygen uptake) increased as uterine weight increased, energy use per kilogram of uterus declined between 94 and 125 days of pregnancy. Meschia *et al.* (1966, 1980) found that the proportion of oxygen utilized by uterine placental tissue accounted for 75% of total uterine plus fetal oxygen uptake at 100 days and 30% at 140 days after mating. Meschia *et al.* (1980) found oxygen uptake per kilogram of fetus was 0.293 mmol per minute and uteroplacental oxygen consumption 1.054 mmol per minute between 130 and 145 days after mating. Using the above relationship it is possible to calculate that uterine oxygen consumption at 94 and 125 days of pregnancy could have been 0.517 and 0.396 mmol $\text{min}^{-1} \text{kg}^{-1}$, respectively, values which compare with the values observed in this study of 0.444 ± 0.065 and 0.334 ± 0.035 mmol $\text{min}^{-1} \text{kg}^{-1}$ at 94 and 125 days, respectively.

This agreement may be fortuitous for the assumptions on which they are based (*viz* that oxygen consumption per kilogram of fetus and oxygen consumption per kilogram of uteroplacental tissue are fixed) may not hold at different gestational ages and/or maternal

nutrient intakes. Recently, Bell *et al.* (1982) demonstrated that both plane of nutrition and exercise affected the partition of oxygen use within the pregnant uterus. Although no quantitative conclusions can yet be drawn it seems plausible to suggest that during periods of high nutritional demand by other tissues, total uterine oxygen consumption may decline, with little change in fetal oxygen use.

In the past it has been assumed that the energy requirements of pregnancy are due largely to fetal requirements. The energy requirements of uterine tissues have been assumed to be low. This, in part, explains the difference in energetic efficiency of fetal gain (variously reported from 10 to 20% with preferred values of 13–16%—Robinson 1978, Geisler and Jones 1979, Anon. 1980) from the gross energetic efficiency of gain of 30% in new-born lambs growing at rates comparable to that found *in utero*.

Lactation

It is well recognized that in other ruminant species, such as the goat and cow, milk production is highly correlated with secretory tissue weight, udder weight and udder blood flow (Linzell 1974). The present data show that this also applies to sheep. The ratios of blood flow to milk yield observed here of 473 : 1 in ewes producing more than 200 ml of milk per day and 788 : 1 in those producing very small amounts of milk (OH group) is almost identical with those observed by others in cows, goats (see Linzell 1974) and, more recently, sheep (Pethick and Lindsay 1982). They differ substantially, however, from that observed in sheep of 870 : 1 (range 530 : 1–1050 : 1) by Davis and Bickerstaffe (1978), who used the A–V difference of plasma methionine as a marker to estimate udder blood flow.

The precision of the A–V difference technique is directly related to the accuracy of blood-flow measurement. Measurement of udder blood flow using TOH was considered by Setchell and Linzell (1974) to underestimate total udder blood flow due to loss of marker through the skin, but our results show that less than 4% of total udder blood flow passes to the skin. This means that errors due to possible loss of marker by that route (if they occurred) were negligible. Furthermore, the lack of concurrence between estimates obtained within the same hour using microspheres and TOH can neither verify nor repudiate the accuracy of the TOH method as it has been demonstrated by Linzell (1974) that mammary blood flow fluctuates by $\pm 20\%$ on a minute to minute basis. For that reason, estimates based on measurements which take approximately 1 h (the TOH method), although subject to variation within that time, are more likely to provide a realistic estimate of average blood flow than a method based on trapping of microspheres during an interval of 30 s. This assertion is supported by the fact that our results for mammary blood flow in the sheep conform well with those for goats and cows obtained using a variety of different methods (Linzell 1974).

During lactation, estimates of energy demand and feed requirements rely heavily on estimates of the efficiency of energy use for milk production above that required for maintenance. It is instructive, therefore, to compare the observed lactational efficiency with theoretical estimates of efficiency of energy use by the mammary gland.

Calculated theoretical efficiencies for the mammary gland were derived from estimates of Baldwin (1968), Milligan (1971) and Kronfeld (1976). In so doing, we have assumed that 80% of milk lactose was derived from glucose with an efficiency of 96%, and the remaining 20% of lactose was synthesized *in situ* from C₃ precursors with an efficiency of 78%. Approximately 90% of milk protein is synthesized in the mammary gland with an efficiency of 82–87% (Baldwin 1968; Kronfeld 1976). Further, we have assumed that 10% of milk proteins are of exogenous origin and that their efficiency of incorporation in milk (about 98%) reflects the low energy cost of transport. About 60% of milk fat in the cow is from triglyceride, transported into and reassembled in milk with an efficiency of 98% and the remainder is synthesized *de novo* largely from acetate and 3-hydroxybutyrate with an efficiency of 71% (Baldwin 1968). For ewes milk, which in this study contained about 5% lactose, 5% protein and 6% fat, the theoretical efficiency is

therefore 89%. This is close to the observed efficiencies of milk synthesis by the mammary gland of between 90 and 92% and is also close to 92% which can be calculated from the data of Pethick and Lindsay (1982) obtained using similar A-V difference techniques.

Calculations of the efficiency of use of ME for milk production are fraught with errors of assumption. For instance, on the basis that maintenance energy requirements of lactating ewes are the same as for dry ewes, $51 \pm 5\%$ of ME above maintenance was secreted as milk energy. This is less than that usually considered to be the efficiency of conversion of ME to milk energy in sheep ($63 \pm 3\%$, Robinson 1978), but is close to the value of 50% obtained by Graham (1964), Peart (1968), and Peart *et al.* (1972). Theoretical considerations suggest a maximum efficiency of conversion of feed energy to milk of 75%, on which basis we calculate an increased heat production during lactation.

Whole Body

The results of the present study suggest that, of whole-body energy use, muscle is the component which is related to weight, and the remainder to feed intake. Webster (1980) showed that approximately half of the heat increment of feeding was due to digestion in, and metabolism by, gut tissues and indicated that the source of remaining heat increment accompanying feeding was metabolism by body tissues. Our results indicate that muscle is unlikely to be the tissue contributing to a major part of heat increment of feeding, but demonstrate a substantial increase in resting metabolic rate during lactation. It therefore seems reasonable to suggest that this was due to changes in rates and sites of metabolism, largely in the gut and liver, which accompany rapid increases in feed intake particularly in early lactation. These changes also occur in other physiological states where feed intake increases rapidly (Graham 1982).

The present data illustrate that the techniques used in combined studies of tissue and whole-body metabolism provide results not greatly different to those obtained by other methods. Furthermore, the results indicate that the preparations described are appropriate for studies on the utilization of discrete nutrients (e.g. glucose, acetate and amino acids).

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