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Partitioning of Nutrients in Merino Ewes. II* Glucose Utilization by Skeletal Muscle, the Pregnant Uterus and the Lactating Mammary Gland in Relation to Whole Body Glucose Utilization

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Abstract

The net uptake and oxidation of glucose by leg muscle, pregnant uterus, and lactating mammary gland, together with the rate of irreversible loss and oxidation of glucose in the whole body of Merino ewes are reported. The ewes were fed on either chaffed oaten hay (OH), chaffed lucerne hay (L), or a mixture of chaffed oaten and lucerne hays (OHL). Measurements were made during five different physiological states: dry (non-pregnant), at 94 and 125 days of pregnancy, and at 20 and 50 days after lambing.

Whole body glucose irreversible loss was related significantly to intake of metabolizable energy and fleece-free maternal body weight and this relation was the same in dry, pregnant and lactating ewes.

The proportion of glucose oxidized in the whole body was unaffected by diet, but was lower in pregnant than in dry or lactating ewes. Some 6% of whole body carbon dioxide (CO_2) production was derived from oxidation of glucose, and in ewes eating the OH diet this proportion was lower than for ewes fed on other diets. The proportion of CO_2 derived from glucose was lower in pregnant ewes than in dry and lactating ewes.

Leg (muscle) glucose uptake was lower in ewes fed on the OH diet than in ewes given the other diets. This arose partly because of decreased blood flow to the leg in ewes fed OH. Muscle glucose uptake, corrected for lactate output, accounted for 20, 44 and 34% of glucose irreversible loss in ewes fed OH, OHL and L respectively. There was no significant effect of physiological state on glucose uptake by leg muscle. The maximum contribution glucose uptake, corrected for output of lactate, could make to leg muscle oxygen consumption was 31% and there were no differences due to diet or physiological state.

Uterine glucose uptake was 10·5 mg min^{-1} kg^{-1}, and was unaffected by diet and stage of pregnancy. Glucose uptake was maintained, despite a decline in blood flow per kilogram of uterus from 399 to 237 ml min^{-1} kg^{-1}, between 94 and 125 days of pregnancy by an increase in arteriovenous difference of glucose over the same period from 2·8 to 4·4 mg 100 ml^{-1}. Total uptake of glucose by the uterus increased from 26 to 47 mg min^{-1} between 94 and 125 days of pregnancy. The proportion of glucose irreversible loss accounted for by uterine uptake increased from 46 to 65% between 94 and 125 days, and was greater for ewes fed OH (84%) than L (46%) at 125 days of pregnancy.

A maximum of 71% of milk lactose could have been derived directly from glucose; 17% of glucose taken up by the mammary gland was oxidized, contributing to 20% of mammary CO_2 output. Mammary glucose uptake was lower in ewes fed OH than in ewes fed the other diets.

The results indicate that during pregnancy there was a decline in the amount of glucose available for maternal tissues, with the exception of muscle which was unaffected. In lactating ewes it appeared that there was no decline in the amount of glucose available to maternal tissues.

Introduction

The priorities of different tissues for glucose utilization during pregnancy and lactation have a bearing on the productive capacity of the dam. Virtually all the glucose used by ruminants is derived from gluconeogenesis and glucose is a major substrate of the

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pregnant uterus (Setchell *et al.* 1972), and lactating mammary gland (Linzell 1974). As much as 80% of whole body glucose utilization may occur in the pregnant uterus of ewes (Setchell *et al.* 1972), or in the mammary gland of high-producing dairy cows (Bickerstaffe *et al.* 1974) and goats (Linzell 1974). Lindsay (1971) suggested that redirection of glucose utilization from peripheral tissues to meet the demands of high-producing organs may occur. Since then, few attempts have been made to determine the extent to which glucose utilization is altered during pregnancy and lactation, or indeed in which tissues this may occur.

We recently reported the simultaneous application of isotope dilution, arteriovenous (A–V) difference and blood flow measurements to determine the partitioning of whole body energy utilization between skeletal muscle, the pregnant uterus and lactating mammary gland in ewes fed on a range of diets (Oddy *et al.* 1984). In this paper we present data on the partitioning of whole body glucose utilization between these tissues during pregnancy and lactation. Preliminary results of this work have been published (Gooden *et al.* 1981; Oddy *et al.* 1981*b*).

Materials and Methods

Animals, Diets, Design and Experimental Procedure

Merino ewes (medium Peppin type), 5 years old, and initially with 3 months of wool growth were used. The management, diets, surgical procedures and experimental treatments have been described previously (Oddy *et al.* 1984). Catheters were inserted into the jugular vein, deep femoral artery via the saphenous artery, the deep femoral vein via the recurrent tarsal vein, and either a utero-ovarian vein during pregnancy, or the medial subcutaneous mammary vein in lactation.

The experiment was designed as a 3 × 5 factorial, with three diets and five physiological states. A group of 36 ewes, mated to Merino rams on known dates and selected by ultrasonic imagery at 35–40 days after mating to have a single lamb, and 18 unmated ewes were selected. They were offered either chopped oaten hay (OH), chopped lucerne hay (L), or a 50/50 (w/w) mixture of the two (OHL). Experiments were conducted on the ewes when dry (non-pregnant), 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). The metabolizable energy (ME), the fleece-free body weight (FFBW), mammary gland weight at 50 days after lambing, estimated mammary gland weight at 20 days after lambing and milk production of the ewes were published previously (see Oddy *et al.* 1984).

The schedule of experiments on each animal was as follows: whole body glucose kinetics, tissue glucose A–V difference, tissue blood flow, O_2 consumption and CO_2 production, and the concentration of other metabolites and hormones were measured on day 1. On day 3, tissue blood flow, and acetate A–V difference, O_2 consumption and CO_2 production were determined. Carbon dioxide entry rate was measured on day 4 (Oddy *et al.* 1984).

Each measurement period began with an infusion of [U-^{14}C]glucose for at least 8 h at 2·2 kBq min^{-1} (74–150 mBq mmol^{-1} glucose) into the jugular vein in order to reach a relatively constant specific radioactivity (SRA) of blood glucose and CO_2. Blood was then withdrawn continuously over 1 h (six 10-ml samples) at 1 ml min^{-1} from the deep femoral artery, deep femoral vein (leg) and either a utero-ovarian or medial subcutaneous mammary vein during blood flow measurements (see Oddy *et al.* 1984). Samples of blood for the determination of O_2 and CO_2 concentration were collected anaerobically from both artery and veins before and after the blood flow measurements.

Mean values for the SRA of blood glucose and CO_2 were obtained from the six samples taken during the blood flow measurement. Blood from the same six samples was assayed for lactate.

Chemical Analyses

Blood glucose concentration was determined on an aliquot of a neutral supernatant (Somogyi 1945), by a semi-automated glucose oxidase–peroxidase procedure using *o*-toluidine as the electron acceptor (Cramp 1967). Penta-acetate derivatives of blood glucose were prepared as described by Jones (1965) and counted in a Philips model PW4540 liquid scintillation analyser. Lactate was measured by the procedure of Gutmann and Wahlefeld (1974), on deproteinized whole blood (0·5 ml) precipitated within 1 h of collection with ice-cold 0·5 M perchloric acid (0·5 ml). The supernatant was stored at −20°C until analysis.

The methods for analysis of blood O_2 and CO_2 concentration, and the specific activity of CO_2 and milk lactose and the assumptions used in estimation of uterine and fetal weight, and mammary gland weight in early lactation were outlined previously (Oddy *et al.* 1984).

Calculations of Glucose Metabolism in Whole Body and Tissues

Whole body glucose irreversible loss (A, mg C min^{-1}) was derived from the equation:

$$A = (I_{gl}/S_{gl}) \times 1000 ,$$

where I_{gl} was the rate of infusion of [U-^{14}C]glucose (Bq min^{-1}) and S_{gl} was the SRA of glucose [Bq (g C)$^{-1}$] in arterial blood (Leng 1970).

The percentage of CO_2 derived from glucose (B,%) was calculated as

$$B = (S_{CO_2} \times 100)/S_{gl} ,$$

where S_{CO_2} was the SRA of $^{14}CO_2$ [Bq (g C)$^{-1}$] in arterial blood at plateau during the [U-^{14}C]glucose infusion.

The rate of oxidation of glucose (C, mg C min^{-1}) was calculated from

$$C = (B/100) \times D \times 1000 ,$$

where D was the entry rate of CO_2, (mg C min^{-1}) calculated as

$$D = I_{CO_2}/S_{CO_2} ,$$

where I_{CO_2} was the rate of infusion of $NaH^{14}CO_3$ (Bq min^{-1}) and S_{CO_2} is the corresponding plateau $^{14}CO_2$ SRA [Bq (g C)$^{-1}$].

The percentage of glucose oxidized (E,%) was calculated from the equation

$$E = (C/A) \times 100 .$$

Since arterial and venous glucose SRA were not significantly different, glucose utilization by tissues was determined using the following relationships. Tissue glucose uptake [F, mg min^{-1} (kg tissue)$^{-1}$] was calculated from

$$F = [f \times (A_{gl} - V_{gl})]/100 ,$$

where f was tissue blood flow (ml min^{-1} kg^{-1}) and A_{gl} and V_{gl} were the concentrations of glucose in arterial and venous blood respectively (mg 100 ml^{-1}).

Carbon dioxide output (G, mg C min^{-1} kg^{-1} tissue) was calculated as

$$G = [f \times (V_{CO_2} - A_{CO_2})] \times 0{\cdot}012 ,$$

where V_{CO_2} and A_{CO_2} were the venous and arterial concentrations (mM) in blood respectively.

The percentage of glucose uptake oxidized to CO_2 in tissues (H, %) was calculated using the following equations:

$$J = [12 (A_{CO_2} \times S_{A,CO_2}) - (V_{CO_2} \times S_{V,CO_2})] \times f ,$$

where J was the amount of $^{14}CO_2$ produced by the tissue (Bq l^{-1}), and S_{A,CO_2} and S_{V,CO_2} were the plateau SRA's of $^{14}CO_2$ in arterial and venous blood [Bq (g C)$^{-1}$] respectively, and

$$K = [(A_{gl} - V_{gl})/0{\cdot}25] \times S_{gl} \times f ,$$

where K was the amount of [^{14}C]glucose taken up by the tissue (Bq min^{-1} kg^{-1}). Therefore, the percentage of glucose taken up by the tissue and oxidized (H) was

$$H = (J/K) \times 100 .$$

Glucose oxidation rate in tissue (L, mg C min^{-1} kg^{-1}) was then derived from the equation:

$$L = (H \times F)/100 ,$$

and the percentage of CO_2 derived from glucose (M,%) was

$$M = (40 \times L)/G .$$

After calculation in terms of glucose carbon, parameters were converted to either milligrams or moles of glucose. Tissue uptake of lactate was calculated as for glucose.

Statistical Analyses

Unequal numbers of ewes completed the experimental schedule in each group, largely because of the difficulty of maintaining catheter patency for the necessarily long period. In the OHL group in early pregnancy no measurements could be made because of failure of catheters. This precluded statistical analysis by conventional analysis of variance. Consequently, the experiment was analysed using a Generalized Linear Model with the terms diet, physiological state and their interaction fitted in turn using Genstat MK 4.03 (Rothamsted Experiment Station, Lawes, U.K.). Relationships between parameters, within diets and physiological states were determined using least squares regression techniques.

Results

Whole Body Glucose Metabolism

The arterial concentration of glucose was significantly lower ($P < 0{\cdot}05$) in ewes fed on OH than in those given OHL or L, mainly because of a reduction in arterial glucose concentration in pregnancy in the OH ewes (Table 1).

Table 1. Arterial glucose concentration (mg 100 ml^{-1}), rate of irreversible loss of glucose (GIL, mg min^{-1}), percentage of carbon dioxide derived from glucose, and percentage of glucose oxidized in relation to diet and physiological state

Diets: OH, chaffed oaten hay; OHL, mixture of equal parts by weight of chaffed oaten hay and lucerne chaff; L, lucerne chaff. Physiological states: dry, non-pregnant; EP, 94 days after mating; LP, 125 days after mating; EL, 20 days after lambing; LL, 50 days after lambing. The weights of the ewes, and their ME intake are shown in the preceding paper (Oddy *et al.* 1984). Values are means ± s.e.m., with numbers of sheep in parentheses

Diet		Physiological state				
		Dry	EP	LP	EL	LL
OH	Arterial glucose	40·1±2·5(2)	34·8±1·5(4)	31·4±1·1(4)	44·0±1·1(4)	39·4±1·4(4)
	GIL	41·9±16·2	43·3±5·3	43·5±2·2	66·6±6·8	47·1±4·9
	CO_2 from glucose	7·0±1·1	5·5±0·4	2·7±0·5	5·3±0·4	5·5±0·4
	Glucose to CO_2	51·3±3·0	48·3±1·9	25·3±1·2	54·2±4·9	49·2±2·3
OHL	Arterial glucose	40·9±1·3(6)	—	38·8±2·7(3)	42·7±0·7(6)	46·2±1·3(6)
	GIL	70·1±3·1	—	61·4±7·2	95·7±9·4	81·8±2·9
	CO_2 from glucose	6·8±0·4	—	5·6±0·8	5·9±0·3	6·6±0·6
	Glucose to CO_2	40·4±2·1	—	52·5±5·9	46·9±3·3	50·9±6·2
L	Arterial glucose	44·5±2·2(3)	42·5±2·0(5)	42·4±1·9(5)	41·9±2·1(5)	42·7±1·3(5)
	GIL	84·3±1·7	74·4±4·1	85·4±4·6	103·1±7·0	105·0±5·2
	CO_2 from glucose	9·5±0·4	3·4±0·3	4·9±0·4	8·1±0·5	7·6±1·1
	Glucose to CO_2	52·2±3·5	28·7±6·1	30·2±3·7	65·5±4·5	48·1±4·5

The rate of irreversible loss of glucose (GIL) differed significantly between diets ($P < 0{\cdot}001$), and physiological states ($P < 0{\cdot}001$). However, these effects could be due to differences in ME intake and ewe body weight. GIL (mg min^{-1}) was correlated with ME intake (MJ day^{-1}) and fleece-free maternal body weight according to the following equation:

$$\text{GIL} = -17{\cdot}8\ (\pm 11{\cdot}89) + 4{\cdot}192(\pm 0{\cdot}428)\text{ME} + 2{\cdot}956(\pm 0{\cdot}715)\text{FFBW}^{0{\cdot}75}$$

($r = 0{\cdot}87$, $P < 0{\cdot}001$; r.s.d. = 11·67, d.f. = 58; s.e. are in parentheses).

This relationship between GIL, ME and $\text{FFBW}^{0{\cdot}75}$ was similar in dry, pregnant, or lactating sheep.

The mean proportion of glucose oxidized in the whole body was 46%, with no difference due to diet. During pregnancy the proportion of glucose oxidised was significantly lower ($P < 0{\cdot}05$) than for dry and lactating ewes.

Approximately 6% of the whole body carbon dioxide was derived from glucose. The value for ewes fed OH was significantly lower ($P < 0{\cdot}05$) than for ewes fed OHL or L. The oxidation of glucose was significantly lower ($P < 0{\cdot}01$) for pregnant than for dry and lactating ewes.

The rate of GIL was unrelated to arterial glucose concentration in dry and lactating ewes. But there was a strong relationship between GIL (Y, mg min^{-1}) and concentration of glucose (X, mg %) in pregnant ewes

$$Y = -43{\cdot}4(\pm 17{\cdot}9) + 2{\cdot}78(\pm 0{\cdot}46)X$$
$$(r = 0{\cdot}80,\ P < 0{\cdot}001;\ \text{r.s.d.} = 11{\cdot}81,\ \text{d.f.} = 19)\ .$$

Metabolism of Glucose in Leg Muscle

The uptake and oxidation of glucose per kilogram of hind limb muscle (blood sampled from the preparation arises predominantly from muscle, Domanski *et al.* 1974; Oddy *et al.* 1981*a*; Teleni 1984) and the measurements from which they were derived are shown in Table 2.

Table 2. Glucose A–V difference (mg 100 ml^{-1}), extraction of glucose (%) by, blood flow (ml min^{-1} kg^{-1}) to, and glucose uptake (mg min^{-1} kg^{-1}) by the hind limb of ewes in relation to diet and physiological state

For details of abbreviations, see Table 1

Diet		Physiological state				
		Dry	EP	LP	EL	LL
OH	Glucose A–V	0·94 ± 0·44(2)	1·93 ± 0·39(3)	1·36 ± 0·45(3)	2·27 ± 0·34(2)	2·17 ± 0·36(4)
	Extraction[A]	2·40 ± 1·30	5·60 ± 0·90	4·50 ± 1·60	5·10 ± 0·90	5·50 ± 0·80
	Blood flow	76 ± 1	56 ± 8	77 ± 13	76 ± 6	95 ± 12
	Glucose uptake	0·71 ± 0·32	1·07 ± 0·25	0·99 ± 0·34	2·15 ± 0·15	1·94 ± 0·19
OHL	Glucose A–V	1·86 ± 0·21(5)	—	3·30 ± 1·30(2)	2·36 ± 0·59(5)	3·13 ± 0·41(5)
	Extraction	4·40 ± 0·40	—	9·00 ± 3·30	5·40 ± 1·30	6·70 ± 0·90
	Blood flow	118 ± 10	—	107 ± 27	104 ± 22	90 ± 10
	Glucose uptake	2·21 ± 0·28	—	3·19 ± 0·48	1·83 ± 0·45	2·86 ± 0·59
L	Glucose A–V	1·70 ± 0·16(3)	3·23 ± 0·54(4)	3·16 ± 1·12(5)	2·18 ± 0·24(4)	2·25 ± 0·55(4)
	Extraction	3·90 ± 0·50	7·50 ± 0·90	7·30 ± 2·20	5·10 ± 0·60	5·50 ± 1·50
	Blood flow	106 ± 11	100 ± 20	99 ± 13	101 ± 27	100 ± 15
	Glucose uptake	1·76 ± 0·08	2·96 ± 0·27	2·79 ± 0·71	2·28 ± 0·76	2·05 ± 0·28

[A] Extraction of glucose = $[(A_{gl} - V_{gl})/A_{gl}] \times 100$.

The A–V difference of glucose was not significantly ($P > 0{\cdot}05$) related to diet or physiological state, although there was a tendency for glucose A–V difference to be lowest in ewes fed OH. The extraction of glucose [$100 \times (A_{gl} - V_{gl})/A_{gl}$], was significantly greater ($P < 0{\cdot}05$) for pregnant than for dry or lactating ewes. This was a reflection of the increased A–V difference in ewes fed on L, and the maintenance of the A–V difference in the face of declining arterial concentration in the ewes fed OH.

Glucose uptake was significantly lower ($P < 0{\cdot}01$) in ewes fed on OH because of the significant decrease ($P < 0{\cdot}05$) in blood flow, and the generally lower glucose A–V difference. There was no significant effect ($P > 0{\cdot}05$) of physiological state on glucose uptake nor blood flow to leg muscle. Approximately 25% of hind limb muscle glucose uptake could have returned as lactate in ewes fed OH, compared with about 10% in ewes fed the other diets. There was no significant effect of physiological state in lactate output by muscle.

The proportions of glucose oxidized and of CO_2 produced from glucose did not differ between diets and physiological state (mean ± s.e.m., 26 ± 6·5% and 7 ± 1·5% respectively). The maximum contribution that glucose could have made to O_2

Table 3. Arterial concentration (mg 100 ml^{-1}), and A–V difference (mg 100 ml^{-1}) of lactate, across the hind limb, uterus and mammary gland of ewes in relation to diet and physiological state
For details of abbreviations, see Table 1

Diet		Physiological state				
		Dry	EP	LP	EL	LL
OH	Arterial lactate	6·04 ± 1·95	4·08 ± 0·15	5·32 ± 0·21	6·12 ± 0·52	4·54 ± 0·42
	Leg A–V	−0·16 ± 0·54	−1·03 ± 0·46	−0·40 ± 0·04	−0·60 ± 0·22	0·11 ± 0·19
	Uterine A–V	—	−0·58 ± 0·32	−0·34 ± 0·12	—	—
	Mammary A–V	—	—	—	0·46 ± 0·16	−0·15 ± 0·20
OHL	Arterial lactate	4·97 ± 0·81	—	7·05 ± 0·81	5·31 ± 0·41	5·23 ± 0·20
	Leg A–V	−0·10 ± 0·19	—	−0·68 ± 0·47	−0·26 ± 0·14	0·23 ± 0·19
	Uterine A–V	—	—	−0·27 ± 0·20	—	—
	Mammary A–V	—	—	—	0·87 ± 0·22	0·62 ± 0·18
L	Arterial lactate	5·19 ± 0·46	6·73 ± 0·53	6·38 ± 0·29	5·97 ± 0·32	5·96 ± 0·37
	Leg A–V	−0·68 ± 0·18	0·40 ± 0·42	−0·56 ± 0·09	−0·29 ± 0·08	0·17 ± 0·15
	Uterine A–V	—	−0·53 ± 0·33	−0·19 ± 0·08	—	—
	Mammary A–V	—	—	—	0·77 ± 0·22	0·87 ± 0·30

Table 4. Glucose A–V difference (mg min^{-1}) across, extraction of glucose (%) by, blood flow (ml min^{-1} kg^{-1}) to, glucose uptake (mg min^{-1} kg^{-1}) by, the percentage of carbon dioxide arising from glucose, and the percentage of glucose oxidized by the pregnant uterus in ewes eating three diets at two stages of pregnancy
For details of abbreviations, see Table 1

Diet		Physiological state	
		EP	LP
OH	Glucose A–V	2·68 ± 0·55(3)	3·37 ± 0·53(4)
	Extraction[A]	7·80 ± 1·90	12·30 ± 2·20
	Blood flow	389 ± 155	217 ± 25
	Glucose uptake	8·90 ± 1·80	8·20 ± 1·40
	CO_2 from glucose	16·60 ± 2·50	6·20 ± 2·80
	Glucose oxidized	31·50 ± 7·40	12·10 ± 5·80
OHL	Glucose A–V	—	5·11 ± 0·33(3)
	Extraction	—	13·20 ± 0·90
	Blood flow	—	274 ± 32
	Glucose uptake	—	14·20 ± 2·50
	CO_2 from glucose	—	7·00 ± 3·60
	Glucose oxidized	—	8·50 ± 4·30
L	Glucose A–V	2·84 ± 0·57(5)	4·05 ± 0·93(5)
	Extraction	6·60 ± 1·10	9·80 ± 2·60
	Blood flow	409 ± 43	220 ± 20
	Glucose uptake	12·50 ± 3·80	8·90 ± 1·90
	CO_2 from glucose	23·80 ± 3·90	12·70 ± 5·10
	Glucose oxidized	33·00 ± 9·40	20·50 ± 7·00

[A] Extraction of glucose = $[(A_{gl} - V_{gl})/A_{gl}] \times 100$.

consumption [glucose A–V (mM) × 6]/[O_2 A–V (mM) × 100], the glucose oxygen quotient, was 36%. Since some glucose was converted to lactate in the leg, glucose uptake corrected for lactate output (Table 3) could account for 31 ± 4·0% of O_2

uptake. Mean ± s.e.m. values were 21 ± 6·5, 40 ± 8·9, and 35 ± 8·5% for ewes fed OH, OHL and L respectively, but there was no significant difference ($P > 0·05$) due to diet or physiological state.

Uterine Glucose Metabolism

Table 4 shows the uptake and oxidation of glucose per kilogram of uterine tissue during pregnancy. Glucose uptake was not affected by diet or stage of pregnancy and averaged 10·54 ± 2·95 mg min^{-1} kg^{-1} uterine tissue. Uptake was maintained as pregnancy advanced from 94 to 125 days after mating because glucose A–V difference increased significantly ($P < 0·05$), despite a significant decline ($P < 0·05$) in blood flow (per unit weight of tissue). There were no effects of diet on glucose extraction or blood flow.

Table 5. Glucose A–V difference (ml min^{-1} kg^{-1}) to, glucose uptake (mg min^{-1} kg^{-1}) by, the percentage of carbon dioxide arising from glucose, the percentage of glucose oxidized by the lactating mammary gland, and the percentage lactose in milk of ewes eating three diets at two stages of lactation

For details of abbreviations, see Table 1

Diet		Physiological state	
		EL	LL
OH	Glucose A–V	6·9 ± 2·9(3)	4·3 ± 0·8(4)
	Extraction[A]	17 ± 8	11 ± 3
	Blood flow	231 ± 127	180 ± 51
	Glucose uptake	19·4 ± 15·3	8·8 ± 3·6
	CO_2 from glucose	18·2 ± 1·7	10·1 ± 4·3
	Glucose oxidized	17·7 ± 3·3	12·8 ± 4·9
	Lactose in milk	4·1	4·9
OHL	Glucose A–V	9·5 ± 1·2(6)	10·2 ± 1·2(6)
	Extraction	22 ± 3	21 ± 3
	Blood flow	260 ± 25	233 ± 27
	Glucose uptake	23·7 ± 5·3	23·9 ± 3·4
	CO_2 from glucose	25·9 ± 4·0	25·6 ± 3·5
	Glucose oxidized	20·2 ± 1·6	19·8 ± 3·3
	Lactose in milk	4·9 ± 0·2	4·8 ± 0·2
L	Glucose A–V	10·7 ± 0·8(5)	9·3 ± 2·0(3)
	Extraction	25 ± 2	22 ± 4
	Blood flow	273 ± 32	265 ± 46
	Glucose uptake	29·2 ± 4·2	26·0 ± 9·6
	CO_2 from glucose	20·5 ± 2·4	16·1 ± 3·9
	Glucose oxidized	16·4 ± 2·5	14·8 ± 2·5
	Lactose in milk	4·6 ± 0·2	4·0 ± 1·3

[A] Extraction of glucose = $[(A_{gl} - V_{gl})/A_{gl}] \times 100$.

The proportion of CO_2 derived from glucose declined significantly ($P < 0·05$), from 21% at 94 days to 9·5% at 125 days, and during this period the percentage of glucose oxidized declined from 32 to 14% ($P < 0·05$). These changes were similar across diets, but contrasted with the uterine glucose oxygen quotient, [glucose A–V (mM) × 6]/[O_2 A–V (mM)], which was 0·53 and 0·56 (OH), and 0·79 and 1·04 (L) at 94 and 125 days of pregnancy respectively. Lactate output by the pregnant uterus declined significantly ($P < 0·05$) from 2·2 ± 0·58 mg min^{-1} kg^{-1} at 94 days to 0·6 ± 0·15 mg min^{-1} kg^{-1} at 125 days of pregnancy.

Mammary Glucose Metabolism

Glucose uptake per kilogram of mammary tissue was not significantly affected ($P > 0{\cdot}05$) by diet nor stage of lactation, although the ewes on the OH diet had consistently lower A–V difference, extraction, and blood flow, particularly in late lactation (Table 5). However, substantially less glucose was taken up by the udder of ewes eating OH, ($3{\cdot}2$ mg glucose min^{-1}), than in ewes fed OHL and L diets ($14{\cdot}2$ and $17{\cdot}2$ mg glucose min^{-1} respectively).

Of the CO_2 output 20% was derived from glucose, irrespective of diet or stage of lactation. Glucose contributed significantly less ($P < 0{\cdot}05$) to CO_2 output in ewes fed OH than in ewes fed other diets. The percentage of glucose uptake oxidized (17%) was similar between diets and stages of lactation. Milk yield and lactose production were correlated poorly with mammary glucose uptake when the latter was expressed as mg min^{-1} kg^{-1}, but were significantly correlated when expressed as mg min^{-1}. Mammary glucose uptake (X, mg min^{-1}), corrected for oxidation, was related to milk lactose output (Y, mg min^{-1}) as follows

$$Y = 5{\cdot}86(\pm 2{\cdot}03) + 0{\cdot}862(\pm 0{\cdot}158)X$$
$$(r = 0{\cdot}73,\ P < 0{\cdot}001,\ \text{r.s.d.} = 6{\cdot}3\ \text{d.f.} = 24)\ .$$

Mean ± s.e.m. values for all diets and stages of lactation for glucose uptake (corrected for oxidation) and lactose output were $11 \pm 1{\cdot}5$ and $15 \pm 1{\cdot}7$ mg min^{-1} respectively. These results show that a maximum of 71% of lactose was derived from glucose. There was a substantial uptake of lactate by the mammary gland ($1{\cdot}0 \pm 0{\cdot}22$ mg min^{-1}) which, if incorporated into lactose, would account for a further 6·5% of lactose output. Values for SRA of glucose in mammary arterial and venous blood were $30{\cdot}1 \pm 2{\cdot}2$ and $30{\cdot}4 \pm 1{\cdot}8$ kBq (g C)$^{-1}$ respectively. This indicates that, if mammary gluconeogenesis had occurred, it was of minor importance.

Mammary glucose uptake in early and late lactation, respectively, was 8 and 4% of GIL in ewes fed OH, 16 and 14% in ewes fed OHL and 20 and 13% in ewes fed L.

Discussion

Whole Body

Judson and Leng (1968) and Lindsay (1971) first suggested that feed intake rather than physiological state was the major factor influencing GIL. The results shown in this paper are in agreement with this hypothesis and extend the range of ME intake and physiological states beyond those previously investigated. Although Steel and Leng (1973) reported an increase in GIL of underfed pregnant ewes bearing a single lamb, and Wilson *et al.* (1983) observed a 22% increase in GIL of ewes with one lamb eating a fixed ration and a 40% increase with twinbearing ewes at 120 days of pregnancy, these effects are small compared with those elicited by increased food intake. In lactation, although there is some evidence that GIL may be increased by the stimulus of suckling twins rather than single lambs (Wilson *et al.* 1983), the influence of feed intake overwhelms such effects.

The proportion of glucose oxidized was reduced from 48% in non-pregnant to 36% in pregnant ewes, which is consistent with the observations of Ford (1972), but contrasts with those of Wilson *et al.* (1983) who found glucose oxidation unchanged in pregnancy. We believe that the reduction observed here was due to the relatively large demands for glucose by the pregnant uterus. This in turn resulted in a decrease in glucose available

for utilization by maternal tissues (see below). These changes occurred when the proportion of uterine glucose that was oxidized was less than the proportion of glucose oxidized in the whole body, particularly during late pregnancy.

The amounts of milk produced, and the proportion of GIL taken up by the mammary gland, by the ewes in the present study were comparatively small. Consequently there was no reduction in the contribution of glucose oxidation to CO_2 production in the whole body during lactation. Although others (Bergman and Hogue 1967; Wilson *et al.* 1983) have found whole body glucose oxidation was reduced in lactation when the quantities of milk produced by the ewes were greater than those reported here, this is likely to be due only to the low proportion of mammary glucose that is oxidized (in this study, 17%). For, unlike pregnancy where there was a decline in the proportion of GIL available for maternal non-muscle utilization, there was no decline in the proportion of GIL available to these tissues during lactation (see Fig. 1). These results are consistent with the finding that non-mammary glucose metabolism was similar in lactating and non-lactating cows (Bruckenthal *et al.* 1980).

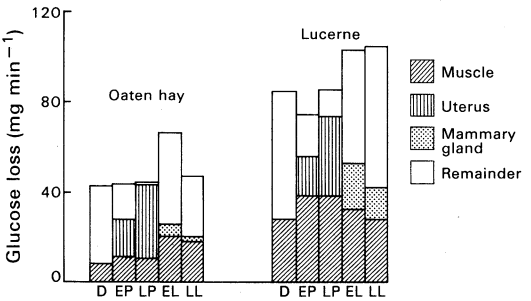

Fig. 1. The contribution of skeletal muscle, pregnant uterus and lactating mammary gland to glucose irreversible loss (GIL, mg min^{-1}) in ewes fed either on chaffed oaten hay (OH) or chaffed lucerne hay (L). GIL was measured in dry, early (EP) and late pregnancy (LP), and in early (EL) and late lactation (LL).

Leg

Uptake of glucose per kg of muscle was similar across all physiological states, but was less in ewes eating OH (where ME intake was generally less than requirement) than in ewes on the other diets (which were eaten in quantities close to requirement). It was notable that the effect of diet was not mediated primarily through A–V difference of glucose, but through change in blood flow to muscle. Furthermore, the diet effect appears to be mediated through the effect of feed intake on weight, for muscle blood flow was influenced more by body weight than by current feed intake (Oddy *et al.* 1984). Blood flow has previously been shown to be the major component affecting uptake of glucose by exercising leg muscle in sheep (Bird *et al.* 1981) and man (Wahren *et al.* 1971).

Oxidation of glucose by muscle appears to be unaffected by diet or physiological state, although the comparatively low proportion of glucose taken up that was oxidized (29%) could mask differences if they occurred. There were substantial differences in the proportions of O_2 consumption which could be accounted for by assuming complete oxidation of glucose uptake–lactate output (31%), and the proportion of glucose oxidized to CO_2 by direct measurement of appearance of $^{14}CO_2$ from [U-^{14}C]glucose (7% of CO_2 output and 29% of glucose oxidized). Such differences could arise because either insufficient time elapsed to permit equilibrium of [U-^{14}C]glucose with muscle glycogen (Lindsay 1981) or $^{14}CO_2$ derived from oxidation of [U-^{14}C]glucose was not in

equilibrium with tissue CO_2. Studies of perfused rat hind limb led Ruderman *et al.* (1971) to suggest that glycogen synthesis was a major fate of glucose in muscle, and speculate that oxidation of recently transported glucose thus represents a small part of glucose uptake. In such studies (Ruderman *et al.* 1971) infused [^{14}C]glucose for 15–30 min, and found that just 1% of glucose uptake was oxidized, compared with the present study where 29% of muscle glucose uptake was oxidized after infusion of [^{14}C]glucose for at least 8 h. If glucose metabolism in rat and sheep skeletal muscle were similar, these results suggest that the period over which the present study was conducted was also too short to permit equilibrium of glucose carbon with glycogen. This explanation seems more likely than a failure to achieve equilibrium between $^{14}CO_2$ and CO_2 within muscle. For these reasons our estimate of 7% of CO_2 derived from glucose using isotopic exchange should be considered a minimal estimate, and the value obtained by considering the proportion of oxygen consumption potentially used in complete oxidation of glucose uptake (corrected for lactate output, 31%) the maximal, but more likely, estimate of the glucose contribution to the oxidative metabolism of skeletal muscle.

We were unable to demonstrate significant effects of diet or physiological state on lactate output from muscle, although there was a tendency for lactate output to increase in late pregnancy and early lactation; Pethick and Lindsay (1982) also found that lactate output from the leg muscle in ewes tended to be higher in early lactation than in late lactation. We found that these changes were accompanied by increased concentrations of FFA in pregnancy, decreased insulin in early lactation and elevated concentrations of β-hydroxybutyrate in both late pregnancy and early lactation (J. M. Gooden and V. H. Oddy, unpublished results). These changes are similar to those which Ruderman *et al.* (1979) have shown are associated with increased output of lactate in the perfused hindquarter.

To determine the extent to which muscle contributed to whole body glucose utilization, leg muscle glucose uptake (mg min^{-1} kg^{-1}) was extrapolated to total skeletal muscle uptake on the basis that leg muscle is representative of all skeletal muscle and skeletal muscle constitutes 24% of maternal fleece-free liveweight (Butterfield *et al.* 1983). Although it cannot be safely assumed that muscle accounts for a fixed fraction of maternal fleece-free weight, or that the metabolic activities of hind limb muscle are identical to all skeletal muscles in sheep eating different diets and in different physiological states, these assumptions are plausible. On this basis, muscle glucose utilization was least in ewes fed OH (12 mg min^{-1}), compared with 29 and 31 mg min^{-1} in OHL and L ewes respectively. After correction for lactate output, muscle glucose uptake could account for 18% of GIL in the OH ewes, and 40 and 31%, respectively, in OHL and L ewes. There was no difference in whole body muscle glucose uptake with physiological states, indicating that the reduction in maternal glucose utilization seen during pregnancy [i.e. whole body GIL minus uterine glucose uptake (Fig. 1; see also Lindsay 1971)], does not occur in muscle.

Pregnant Uterus

Uterine glucose uptake was maintained across a wide range of maternal ME intakes [during pregnancy ME intake of the ewes ranged from 0·3 to 1·1 times maternal heat production (Oddy *et al.* 1984)], and over a significant part of the gestational period (94–125 days)—see also Morriss *et al.* (1974), Christenson and Prior (1978). Glucose uptake was maintained, in spite of a decline in uterine blood flow per kg of uterus between 95 and 125 days of pregnancy, by an increased extraction of glucose. Such constancy

of glucose uptake by the pregnant uterus of fed ewes is consistent with the view that transplacental glucose uptake is limited by rate of membrane transport, rather than by blood flow (Simmons *et al.* 1974, 1979). However, it contrasts with recent reports that indicate a reduction in uterine glucose uptake in fasted ewes (Hay *et al.* 1983*b*), and during exercise (Bell *et al.* 1982).

The importance of a constant uterine uptake of glucose can be seen in the way in which uterine glucose utilization contributes to whole body glucose utilization. Our data permit calculation of total uterine glucose uptake based on predicted uterine weight at 94 and 125 days of pregnancy of 2·47 and 4·46 kg [predicted using the equations of Geisler and Jones (1979), as described by Oddy *et al.* (1984)]. Mellor (1983) has shown that individual uterine weight can be predicted with only limited accuracy from birth weight, especially if maternal nutrition is variable. Nonetheless, on the basis of the above uterine weights, mean uterine glucose uptake increased from 26 mg min^{-1} to 47 mg min^{-1} between 94 and 125 days of pregnancy. The proportion of GIL which could therefore be attributed to uterine uptake increased with advancing pregnancy and declining feed (ME) intake. In ewes being fed OH (eating approximately half ME requirements) uterine glucose uptake could account for 51 and 84% of GIL at 94 and 125 days of pregnancy, respectively, compared to 41 and 46% at similar stages of pregnancy in ewes consuming L (intake equivalent to requirement). These results contrast with Hay *et al.* (1983*b*) who found the proportion of whole body glucose utilization attributable to the pregnant uterus was similar in both fed and fasted ewes. It is difficult to make valid comparisons between studies where intake has been restricted for a considerable period prior to the experiment, as here, or imposed rapidly (Hay *et al.* 1983*b*), for the adaption to treatment may well have occurred by different routes, and to different degrees. In the case of chronic treatment the ewe and uterine contents would be expected to reach a compromise over the partition of nutrients conducive with survival of both. In acute (starvation) experiments the immediate priority is to maternal survival, and the crisis is greater; accordingly, the relationship between nutrient use by uterine and maternal tissues would be expected to favour the mother, as indeed has been observed by Hay *et al.* (1983*b*).

Values for the glucose/oxygen quotient (range 50% on the OH diet to 100% on the L diet) lay within the range normally encountered in fetal sheep (see Battaglia and Meschia 1978). As with leg muscle there was a discrepancy between oxidation measured using isotopes and the glucose/oxygen quotient. The possible reasons for such a difference have already been discussed in the case of muscle. However, unlike skeletal muscle, uterine tissue was growing during the course of the experiment, and it would be reasonable to assume some glucose carbon would be retained. We calculate that the amount of glucose carbon retained, i.e. not oxidized or returned as lactate, was 4·7 and 14·6 g C day^{-1} at days 94 and 125 respectively. These values, when compared with total uterine C deposition of 4 and 9·6 g C day^{-1} at days 94 and 125 respectively [calculated from the data of Langlands and Sutherland (1965)], clearly indicate that glucose carbon retention calculated using isotopic estimates of oxidation are excessive. However, because there is no information on the proportion of uterine carbon deposited which was derived from glucose, it is difficult to say by how much oxidation was underestimated using isotopic measurements. Comparison with the results of Hay *et al.* (1983*a*), which showed that 61% of fetal glucose uptake was oxidized over 3 h of infusion, indicates that the degree of underestimation of uterine glucose oxidation was substantial in the present study.

There was a reduction in the amount of glucose available to tissues other than uterus and, as shown in Fig. 1, this occurred predominantly in tissues other than muscle. Since arterial glucose concentration did not decline, except in severely undernourished ewes, a mechanism which spared glucose utilization in these tissues but did not rely solely on glucose concentration as an indicator of glucose availability must have been in action. Insulin and ovine placental lactogen (oPL) are likely candidates for such a role. Insulin concentration is reduced during pregnancy in sheep (Hove and Blom 1976, Vernon *et al.* 1981), while oPL concentration increases during pregnancy, and is responsive to feed intake (Oddy and Jenkin 1981). Furthermore, removal of oPL by specific antibody in pregnant sheep leads to increased insulin concentration (M. J. Waters, V. H. Oddy, C. E. McCloghry, unpublished results), suggesting that increased oPL concentration may be partly responsible for the reduction in insulin concentration in the pregnant ewe. A reduction in insulin concentration may be in part responsible for reduced glucose utilization in maternal non-muscle tissues, and at the same time allow an increase in material fat mobilization (see Vernon *et al.* 1981). However, there is still no direct evidence of a link between the changes seen in these two hormones during pregnancy and glucose metabolism.

Mammary Gland

Mammary glucose uptake accounted for 4–20% of GIL. This was substantially less than 41% reported for ewes (Pethick and Lindsay 1982), 66% for cows (Bickerstaffe *et al.* 1974) and goats (Linzell 1974). However, the quantity of milk produced by our ewes in this study (in which the lambs were removed at birth and the ewes hand-milked) was substantially less than that reported by Pethick and Lindsay (1982) and that obtained from the same ewes in previous studies in which lambs were left with the ewes (range 200–2500 ml day^{-1}; V. H. Oddy, unpublished results).

Values for the extraction of glucose, (13–25%, mean 20%), proportion of glucose oxidized (17%), and the contribution made to total CO_2 production by the mammary gland (20%) are similar to values reported for the goat (Annison and Linzell 1964), cow (Bickerstaffe *et al.* 1974), and ewe (Davis and Bickerstaffe 1978; Pethick and Lindsay 1982). Glucose uptake by the mammary gland was well correlated with milk and milk lactose production (see Linzell 1974). In the present study there was sufficient glucose uptake, after allowing for oxidation, to account for 71% of lactose production. This was substantially less than indicated for goats and cows by Linzell (1974) and for ewes (Davis and Bickerstaffe 1978), but close to that of 75% (Chaiyabutr *et al.* 1980) and 85% (Annison 1971) obtained following incorporation of labelled glucose into milk lactose in the fed goat.

Because we were unable to account for all of the output of lactose by glucose uptake, this raises the possibility of gluconeogenesis within the mammary gland. In this regard, Mepham and Linzell (1974) have indicated that glutamate C is incorporated into lactose. This prompted Linzell (1974) to suggest that glycerol, lactate, and some amino acids could be incorporated into lactose, and indeed all the gluconeogenic enzymes with the exception of glucose-6-phosphatase have been found in mammary tissue. In spite of this, Scott *et al.* (1976), although able to demonstrate glycerol incorporation into lactose, could find no evidence for incorporation of lactate, alanine or glutamate C into lactose. The evidence for mammary gluconeogenesis is clearly contradictory and further study is warranted.

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