Glucose metabolism in shorn and unshorn pregnant sheep

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1. Whole-body, hind-limb and uterine tissue metabolism of glucose was studied using a combination of isotopic and arterio-venous difference techniques in shorn and unshorn pregnant sheep over the final 4 weeks of pregnancy. This was combined with the measurement of the concentrations of oxygen and carbon dioxide in arterial blood and plasma concentrations of lactate, acetate, non-esterified fatty acids, 3-hydroxybutyrate, glycerol, growth hormone (GH), insulin, glucagon, cortisol, thyroxine and 3, 5, 3'-triiodothyronine (T_3).

2. Glucose entry rate was 28 % higher in shorn ewes compared with unshorn controls, even though there was no difference in the arterial plasma concentration of glucose. This effect may have been caused by a decrease in the molar rate, insulin: glucagon (I:G), which was 40 % lower in shorn ewes as a result of a significant decrease in the plasma concentration of insulin. There was no difference in the plasma concentration of cortisol or GH.

3. Blood flow across the hind-limb or uterine tissues was not significantly different between shorn and unshorn groups, neither were the net glucose uptake, glucose oxidation rate or contribution of glucose to O_2 consumption across these tissues.

4. Insulin-tolerance tests performed on a separate group of shorn and unshorn ewes showed an increased sensitivity to the hypoglycaemic effects of insulin in the shorn group.

5. There was no significant difference between shorn and unshorn animals in the contribution of glucose to CO_2 output or in the proportion of glucose entry rate oxidized. CO_2 entry rate was 18 % higher in shorn ewes compared with unshorn controls which resulted in a 26 % higher estimated value for heat production. There was a 47 % increase in glucose oxidation rate in shorn ewes but there was no significant difference in the proportion of total heat production which was derived from glucose. The arterial concentrations of O_2 and CO_2 were significantly higher in shorn ewes, which may be an indication of the higher metabolic rate in these animals. This effect may be mediated via a significant rise in plasma T_3 concentration in the shorn group.

6. It is concluded that as a result of long-term cold exposure there is a significant increase in whole-body glucose entry and oxidation rates in the shorn pregnant ewe. The increase in insulin sensitivity at the same time as a decrease in plasma insulin concentration may represent a mechanism to ensure continued glucose supply to insulin-sensitive tissues while the concomitant decrease in plasma I:G stimulates hepatic gluconeogenesis.

Winter shearing of pregnant ewes during the final 10 weeks of gestation has been shown to both increase lamb birth weight (LBW) and maternal fat oxidation (Symonds *et al.* 1986*a*). Thompson *et al.* (1982) have proposed the hypothesis that in the shorn animal, there is an increase in glucose supply to the fetus as a result of adaptations in maternal glucose metabolism in response to cold stress. Studies involving the effect of short-term cold exposure of non-pregnant sheep have demonstrated a significant increase in hepatic glucose production (Thompson *et al.* 1978*a*, *b*). A rise in maternal glucose production might be expected to stimulate fetal growth since Wilson *et al.* (1981) have demonstrated a linear correlation between the maternal glucose entry rate measured over the final 7 weeks of pregnancy and LBW.

We have shown that the concentration of glucose in plasma is increased in the shorn pregnant ewe during the final 3 weeks of pregnancy (Symonds *et al.* 1985, 1986*a*). This change in glucose homeostasis may be the result of an increase in maternal glucose

production or a decrease in glucose utilization due to the increase in maternal fat oxidation, or both. The former appears more likely since long-term cold exposure of non-pregnant sheep results in a higher oxidative requirement for glucose (McKay *et al.* 1974).

The aim of the present experiment was to investigate the extent to which glucose metabolism is altered in shorn pregnant ewes housed in the cold environment of a British winter. This involved the measurement of whole-body glucose entry and oxidation rates in shorn and unshorn pregnant ewes over the final 4 weeks of pregnancy, whilst simultaneously measuring glucose metabolism across the hind-limb and uterine tissues. The effect of shearing on the recycling of glucose-carbon via the Cori and alanine cycles was measured by comparing the glucose entry rates obtained with $[U-^{14}C]$- and $[6-^3H]$glucose (Wilson *et al.* 1981; Baird *et al.* 1983). Measurements of circulating hormone levels were made in order to assess the role of growth hormone (GH), insulin, glucagon, cortisol, thyroxine (T_4) and 3, 5, 3'-triiodothyronine (T_3) in the hormonal regulation of energy metabolism following long-term cold exposure. A preliminary report of this work has already been communicated (Symonds *et al.* 1986*b*).

MATERIALS AND METHODS
Animals and diets

Twelve Bluefaced Leicester × Swaledale ewes, diagnosed as being pregnant by X-ray (Institute of Grassland and Animal Production, Hurley, Maidenhead, Berks SL6 5LR), were paired with respect to body-weight (range 62–87 kg). Ewes were housed individually at ambient temperature and 8 weeks before lambing one ewe from each pair was shorn. All ewes were fed on a diet identical to that described by Symonds *et al.* (1986*a*). The diet was given in two equal portions at 08.30 and 17.00 hours and the refusals weighed at 08.30 hours. The estimated metabolizable energy (ME; calculated from the measured intake of the diet) and nitrogen intakes over the experimental period were 9.84 MJ/d and 20 g/d. The mean minimum and maximum daily temperatures recorded at 09.00 hours were 3·7 (SD 3·3) and 6·6 (SD 3·4)° respectively.

Surgery

Ewes were fasted for 24 h, and water removed 12 h before surgery. The operations were performed on ewes carrying fetuses aged between 103 and 125 d, estimated from the known mating date. Anaesthesia was induced with intravenous administration of 1 g thiopentone and maintained with halothane in oxygen via a closed-circuit anaesthetic machine. The uterine vein was then catheterized as described by Hatfield *et al.* (1984). The carotid artery was exposed and a single lumen silastic catheter (inside diameter (i.d.) 0·76 mm, outside diameter (o.d.) 1·65 mm; Dow Corning Corporation, Medical Products, Midland, Michigan 48640, USA) was inserted towards the animal's head for approximately 70 mm. The catheter was secured around a cuff using 4/0 mersilk thread (Mersutures; Ethicon Ltd). All catheters were filled with fresh saline (9 g sodium chloride/l) containing heparin (500 units/ml) and Crystapen (0·15 g/ml; Glaxo Laboratories Ltd, Greenford, Middx) and were then capped. A 4 ml intramuscular injection of procaine penicillin G with dihydrostreptomycin (Streptopen; Glaxo Laboratories Ltd) was also administered to the ewe daily for 3 d post-surgery. The duration of surgery rarely exceeded 2 h.

Assessment of the recovery of animals from operative stress

All ewes were allowed at least a 7 d period from which to recover from surgery before any measurements of glucose kinetics were made, by which time food intake had returned to pre-operative levels. The only exception to this criteria was a shorn animal on which an

additional operation was performed in order to recover its uterine vein catheter, and results from this animal have therefore been discarded.

All catheters were maintained patent by a daily administration of sterile saline, and refilled with heparinized saline (carotid artery 500 units/ml; uterine vein 200 units/ml) and Crystapen (0·6 g/l). The catheterization of a vessel was only deemed successful if blood samples could be obtained with ease at 6 d and more following the operation. Using this criterion the success rate was as follows: carotid artery 1·0 (shorn and unshorn), uterine vein 0·6 (shorn) and 0·67 (unshorn).

Experimental design

On the day before the measurement of glucose kinetics a jugular vein was catheterized to allow continuous infusion of the isotope. The femoral vein, via recurrent tarsal vein, was also catheterized under local anaesthesia with a polyvinyl catheter (i.d. 1·0 mm, o.d. 2·0 mm) and filled with heparinized saline (200 units/ml). The tip of the catheter was positioned approximately 30–50 mm anterior to the head of the femur. Measurements of glucose kinetics were made between 28 and 10 d before lambing, followed 4 d later by the measurement of carbon dioxide entry rate.

Either the day before or day after, measurements of glucose kinetics were made. Blood and plasma flow-rates across the hind-limb and uterine tissues were made with tritiated water (TOH) (Amersham International plc, Amersham, Bucks) (Oddy *et al*, 1981; Brown *et al*. 1982).

A further five pairs of shorn and unshorn ewes also had insulin-tolerance tests carried out on day 128 (SEM 3) of pregnancy. Human insulin (Wellcome Reagents Ltd, Beckenham, Kent; 0·08 units/kg in 2 ml sterile saline) (Hart *et al*. 1984) was injected via a jugular vein catheter at approximately 14.00 hours. Arterial blood samples (2 ml) were taken immediately before insulin administration and at 5, 10, 20, 30 and 40 min after insulin injection and placed in heparinized tubes on ice and the plasma collected and stored at $-20°$.

Experimental procedures

Measurements of glucose entry and oxidation rates were made simultaneously on each pair of shorn and unshorn ewes. At approximately 09.00 hours a priming dose of radioactive glucose (Amersham International plc; 2·8 μCi [U-^{14}C]glucose + 3·3 μCi [6-^{3}H]glucose + 1·2 mg carrier glucose) in sterile saline was infused into a jugular vein over a 5 min period, followed by a constant infusion of 0·28 μCi [U-^{14}C]glucose/min + 0·33 μCi [6-^{3}H]glucose/min and 0·012 % carrier glucose, which was maintained for 5 h using a peristaltic pump (Gilson Minipulse 2; Anachem House, 20 Charles Street, Luton, Beds LU2 0EB). This procedure is similar to that described by Chaiybutar *et al*. (1982) and produced a plateau specific radioactivity (SRA) of plasma glucose throughout the final 1·5 h of infusion. During the final 1·5 h four sets of 20 ml blood samples were taken simultaneously from the carotid artery, femoral and uterine veins into heparinized syringes and then immediately placed in ice-cold test tubes. The plasma was then collected and stored at $-20°$. Blood samples were taken every 0·5 h from these vessels and the sampling times were approximately 12.30, 13.00, 13.30 and 14.00 hours.

At 3 or 4 d after the infusion of radiolabelled glucose the whole-body CO_2 entry rate was measured. At approximately 09.00 hours a priming dose of radioactive sodium bicarbonate (Amersham International plc; 2·3 μCi NaH^{14}CO$_3$ plus 5 mg carrier NaHCO$_3$) in sterile saline was infused into a jugular vein for a 5 min period followed by a constant infusion of 0·23 μCi NaH^{14}CO$_3$/min, which was maintained for 5 h using a peristaltic pump (Gilson Minipulse 2). During the final 1·5 h, 5-ml blood samples were taken from the carotid artery

into heparinized syringes. Blood samples were taken every 0·5 h and the sampling times were approximately 12.30, 13.00, 13.30 and 14.00 hours. This procedure produced a constant SRA of blood CO_2 throughout the final 1.5 h of infusion.

Laboratory procedures

The SRA of glucose was determined in plasma which was deproteinized according to the method of Somogyi (1945), by the passage of 3 ml deproteinized extracts through successive ion exchange resins (Katz *et al.* 1974). The columns were Dowex 1 (acetate form; 200–400 mesh) and Dowex 50 (H^+ form; 200–400 mesh), with about 2 ml of the resin being placed in disposable 2 ml syringes which were arranged in tandem. The eluate and washings (total volume 10 ml) were collected in scintillation vials and frozen, and tritiated water removed by freeze drying. The [U-^{14}C]- and [6-^{3}H]glucose was then measured in 5 ml liquid scintillant (Cocktail T; BDH Chemicals) using a Packard Liquid Scintillation counter (Tri-Carb 460 CD). Recovery of [U-^{14}C]- and [6-^{3}H]glucose was 96·4 (SEM 1·0) and 70·0 (SEM 1·6) % (*n* 9) respectively, which was used to correct measured values. The intra-assay coefficient of variation (CV) was 3·5% (*n* %). A possible reason for the low recovery of [6-^{3}H]glucose is that its ionization properties are altered during its passage down the ion exchange columns, thereby resulting in some ^{3}H being retained within the column (C. Ford, personal communication).

The SRA of CO_2 in arterial and venous blood samples was measured using the gravimetric method of Hinks *et al.* (1966). The recovery of $NaH^{14}CO_3$ was 96·5 (SEM 0·3) % (*n* 6) and the intra-assay CV was 3·0% (*n* 5). The $NaH^{14}CO_3$ infusion solution radioactivity was measured by the addition of 0·1 ml infusate to 1 ml phenylethylamine in Cocktail T (MacRae & Wilson, 1978).

The concentrations of glucose, non-esterified fatty acids (NEFA), 3-hydroxybutyrate, GH and insulin in plasma were measured by the methods described by Symonds *et al.* (1986*a*). The concentration of lactate was determined in neutralized perchloric acid plasma extracts using the enzyme lactate dehydrogenase (*EC* 1.1.2.27; Gutmann & Wahlefield, 1974), acetate by the method of Snowswell *et al.* (1978) and glycerol by the enzymic method of Wieland (1974).

The plasma concentration of glucagon was determined from blood samples to which 250 Kallikrein-inactivating units aprotinin (Sigma Chemical Co., Poole, Dorset) per ml blood were added immediately after sampling, in order to minimize the proteolytic breakdown of glucagon (e.g. Bassett, 1972). The assay was a 'double-antibody' radioimmunoassay using porcine glucagon standards (NIBSC 1st International Standard for glucagon (69/194); NIBSC, Holly Hill, Hampstead, London NW3 6RB), a rabbit antiserum specific for porcine pancreatic glucagon (D. Altamar & A. Lewis, personal communication; Guildhay Antisera), ^{125}I-labelled glucagon (New England Nuclear) and donkey antiserum to rabbit gamma-globulin (Guildhay Antisera). The intra- and interassay CV were 6·8% (*n* 8) and 10·5% (*n* 4) respectively. The molar ratio of the plasma concentrations, insulin: glucagon (I:G) were calculated using the values of 5734 (Humbel *et al.* 1972) and 3485 (Bromer, 1972) for the molecular weights of insulin and glucagon respectively. The assay for cortisol was identical to that described by Symonds *et al.* (1986*a*) with the addition that blanks were run for each sample in order to correct for the ^{3}H present in the samples. The plasma concentrations of T_4 and T_3 were measured using Quantaphase T_4 and Quantimune T_3 RIA kits (Bio-Rad Laboratories Ltd, Caxton Way, Watford). The interassay CV for T_4 was 6·0% (*n* 4) and for T_3 10·5% (*n* 4).

Blood for the determination of O_2 and CO_2 concentrations was withdrawn anaerobically into 1 ml heparinized syringes and kept on ice until analysis. The pO_2, pCO_2 and pH were determined within 30 min of sampling using a IL213 blood gas analyser (Instrumentation Laboratories UK Ltd, Altrincham, Cheshire). Packed cell volume (PCV) was measured

after centrifugation for 5 min in a microhaematocrit centrifuge (Hawksley & Sons Ltd, Lancing, Sussex). Haemoglobin was measured using a co-oximeter (Model 182; Instrumentation Laboratories UK Ltd). The concentrations of O_2 and CO_2 in blood were then calculated using the equations described by Oddy *et al.* (1984).

Calculations

Whole animal.

$$ER = I/\text{glucose SRA},$$

where *ER* is the glucose entry rate (mmol/min), *I* is the infusion rate of [U-^{14}C]- or [6-^{3}H]glucose (disintegrations/min (dpm) per min) and SRA is the specific activity of [U-^{14}C] or [6-^{3}H] (dpm/mmol) in arterial plasma at equilibrium.

$$\text{Recycling of glucose-C (\%)} = (ER_H - ER_C) \times 100/ER_H,$$

where ER_H is the glucose entry rate obtained using [6-^{3}H]glucose and ER_C is that obtained using [U-^{14}C]glucose and gives the percentage of the C of newly formed glucose that comes from metabolically recycled glucose-C (Katz *et al.* 1974).

The proportion of blood CO_2 derived from glucose (transfer quotient; *TQ*) = plateau arterial CO_2 SRA/plateau arterial glucose SRA, during [U-^{14}C]glucose infusion where SRA is expressed as ^{14}C dpm per mAtom C at equilibrium.

$$ER_{CO_2} = I/CO_2 \text{ SRA},$$

where ER_{CO_2} is the CO_2 entry rate (mmol/min), *I* is the infusion rate of $NaH^{14}CO_3$ (dpm/min) and SRA is the specific activity of $^{14}CO_2$ (dpm/mmol) in arterial blood at equilibrium.

$$\text{Proportion of glucose entry rate (} ER \text{) oxidized} = (TQ \times ER_{CO_2})/ER_H$$

where both ER_{CO_2} and ER_H are in mAtom C.

$$\text{Glucose oxidation rate (mmol/min)} = \text{proportion of glucose entry rate oxidized} \times ER_H.$$

Total heat production (*H*) (MJ/d) was estimated from the measured ER_{CO_2} with the conversion of CO_2 from mmol/d to l/d which was made on the basis that 1 mol occupies 22·4 litres at standard temperature and pressure, using the equation:

$$H = (0\cdot0257 \times ER_{CO_2}) - 0\cdot81,$$

where ER_{CO_2} is expressed in l/d. This equation was derived from the linear relation observed between *H* and CO_2 production measured using indirect open-circuit calorimetry by Symonds (1986).

H due to glucose oxidation (MJ/d) was calculated by multiplying the glucose oxidation rate (mol/d) by 2·82 (the energy content of 1 mol glucose).

Tissues.

$$\text{Net fractional extraction of glucose} = (A - V)/A,$$

where *A* and *V* are the concentrations of glucose (mM) in arterial and venous plasma respectively.

Total plasma flow (*PF*) and blood flow rates (*BF*) (l/min per kg tissue) through the hind-limb and uterine tissues were calculated using the Fick equation as described by Oddy *et al.* (1981) and Brown *et al.* (1982).

$$\text{Net utilization of glucose (mmol/min per kg tissue)} = (A - V) \times PF.$$
$$\text{Estimated contribution of glucose to tissue oxygen consumption}$$
$$= (\text{net utilization of glucose} + ((A - V) \text{ lactate} \times PF)/2) \times 6)/\text{net utilization of } O_2.$$

The oxidation of 1 mol glucose produces 6 mol O_2.

The measured contribution of glucose uptake simultaneously accounted for by production of CO_2 $(OX_F) = (V-A)^{14}CO_2 \times BF/(^{14}C_A - {}^{14}C_V) \times PF$, where $(V-A)^{14}CO_2$ is the average $^{14}CO_2$ released from the tissue at equilibrium (dpm/ml blood) and $(^{14}C_A - {}^{14}C_V)$ is the average $[^{14}C]$glucose taken up by the tissue at equilibrium (dpm/ml plasma).

$$\text{Gross utilization of glucose} = (^{14}C_A - {}^{14}C_V)/^{14}C_A \times A \times PF,$$

where $^{14}C_A$ and $^{14}C_V$ are the amounts of radioactivity (dpm/ml plasma) in arterial and venous plasma respectively.

$$\text{The measured rate of glucose oxidation (Ox) (mmol/min per kg tissue)}$$
$$= \text{gross utilization} \times OX_F.$$

$$\text{The measured contribution of leg } O_2 \text{ uptake spent in immediate oxidation of glucose}$$
$$= (6 \times Ox)/\text{net utilization of } O_2.$$

Statistical analysis

Comparison of values obtained between shorn and unshorn groups. Statistical analysis of the whole-body entry rates and concentration of metabolites and hormones was performed using a Student's paired t test (shorn $v.$ unshorn), as were significant differences between shorn and unshorn groups at each sampling point following insulin injection. Regression analysis was also performed between the period after insulin injection and plasma glucose injection for shorn and unshorn animals.

Analysis of change in values of measurements with time during sampling. Regression analyses were carried out for each group of sampling values obtained in shorn and unshorn animals, to calculate the mean percentage change with time over the 1·5 h sampling period.

RESULTS
Fetal weight and feed intake

All ewes produced live fetuses which were delivered by Caesarian section on day 138 (SEM 4) of pregnancy. In the unshorn group there was one single and one triplet pregnancy and the remainder bore twin lambs. In the shorn group all animals bore twins except for one ewe that bore triplets. There was no significant difference in fetal weight (shorn 3·74 (SEM 0·39) kg (n 11); unshorn 3·63 (SEM 0·28) kg (n 12)) or of the weight of the uterus plus fluids and placenta (shorn 3·49 (SEM 0·67) kg (n 5); unshorn 3·58 (SEM 0·52) kg (n 6)).

There was no difference in ewe live weight between the two groups (shorn 75 (SEM 3) kg (n 5); unshorn 76 (SEM 4) kg (n 6)). The fresh weight intake of ammonia-treated straw was 35% higher (shorn 0·91) (SEM 0·09) kg (n 5), unshorn 0·68 (SEM 0·07) kg (n 6)) over the experimental period in the shorn ewes.

Plasma metabolites and blood gases

Table 1 shows that there were no significant differences between shorn and unshorn ewes in the arterial concentration of glucose, lactate, acetate, NEFA, 3-hydroxybuytyrate or glycerol. Shorn animals did exhibit significantly higher arterial blood CO_2 and O_2 concentrations and this latter change was associated with a significant increase in the blood haemoglobin concentration and PCV.

Whole-body entry rate and oxidation

The regression analysis results given in Table 2 demonstrate that the mean changes in the magnitude of the various criteria over the sampling period was always less than 10% in

Table 1. *Packed cell volume (PCV), arterial blood concentrations of haemoglobin (Hb), oxygen and carbon dioxide, and plasma concentrations of glucose, lactate, acetate, non-esterified fatty acids (NEFA), 3-hydroxybutyrate and glycerol in shorn (n 5) and unshorn (n 5) pregnant ewes*

| | Shorn | Unshorn | |
	Mean	Mean	SED
PCV	0·25	0·23	0·009
Hb (g/l)	89*	81	3
O_2 (mM)	5·10*	4·70	0·11
CO_2 (mM)	26·25*	23·88	0·76
Glucose (mM)	2·79	2·57	0·15
Lactate (mM)	0·46	0·44	0·03
Acetate (mM)	1·07	1·52	0·37
NEFA (mM)	0·66	0·48	0·10
3-Hydroxybutyrate (mM)	0·60	0·83	0·32
Glycerol (mM)	0·11	0·12	0·01

SED, standard error of difference.
Significant differences between means (shorn v. unshorn) assessed by a paired *t* test: * $P < 0.05$.

Table 2. *Regression analysis of changes in the values of arterial glucose and carbon dioxide specific radioactivities (SRA) with time during sampling in shorn (S) (n 5) and unshorn (US) (n 6) pregnant ewes*

Infusate	Measurement		Mean	SEM	Slope of regression line (change/0·5 h)	Change during sampling period (%)*
[U-14C]glucose	Glucose SRA	S	978	3	0·52	0·4
	(dpm/mmol)	US	1255	13	0·37	−0·8
	CO_2 SRA	S	26	1	−0·45	3·9
	(dpm/mmol)	US	29	0·5	0·13	0·5
	Glucose (mM)	S	2·71	0·01	−0·07	−0·2
		US	2·62	0·02	0·60	0·6
	CO_2 (mM)	S	26·39	0·10	−0·80	−0·2
		US	24·13	0·17	−0·23	−0·7
[6-3H]glucose	Glucose SRA	S	988	5	−0·60	1·1
	(dpm/mmol)	US	1151	11	0·64	3·3

dpm, disintegrations/min.
* No statistically significant changes with sampling time.

both shorn and unshorn groups of ewes, and there were no consistent trends with time. It was therefore possible to apply steady-state equations to the sampling means for each criterion (Baird *et al.* 1983). From Table 3 it can be seen that there was a significantly higher glucose entry rate as measured using either [U-14C]- or [6-3H]glucose in shorn ewes. There was no significant difference between shorn and unshorn animals in either the calculated value for glucose recycling or in the fractional contribution of glucose to CO_2 output or in the proportion of glucose entry rate oxidized. However, glucose oxidation rate was 47% greater in the shorn group.

Table 3. *Whole-body glucose metabolism in shorn (n 5) and unshorn (n 5) pregnant ewes*

| | Shorn | Unshorn | |
	Mean	Mean	SED
Entry rate (mmol/min) using:			
[U-^{14}C]glucose	0·677*	0·561	0·040
[6-^{3}H]glucose	0·746*	0·628	0·030
Recycling of glucose-C (%)	9·0	10·3	3·6
Contribution to carbon dioxide output (%)	17·0	15·0	0·6
Proportion of glucose entry rate oxidized	0·76	0·62	0·04
Oxidation rate (mmol/min)	0·560*	0·381	0·043

SED, standard error of difference.
Significant differences between means (shorn *v.* unshorn) assessed by a paired *t* test: * $P < 0.05$.

Table 4. *Carbon dioxide entry rate, whole-body heat production and whole-body heat production due to glucose oxidation in shorn (n 5) and unshorn (n 5) pregnant ewes*

| | Shorn | Unshorn | |
	Mean	Mean	SED
CO_2 entry rate (mmol/min)	18·12*	15·35	0·71
Estimated whole-body heat production† (MJ/d)	15·02*	11·93	1·09
Whole-body heat production from glucose oxidation† (MJ/d)	2·25*	1·44	0·21
Proportion of total heat production from glucose oxidation	0·15	0·12	0·01

SED, standard error of difference.
Significant differences between means (shorn *v.* unshorn) assessed by a paired *t* test: * $P < 0.05$.
† For details of calculations, see p. 253.

A significant increase in the whole-body CO_2 entry rate was observed in shorn animals (Table 4) which resulted in a 26% increase in the estimated heat production. The contribution of glucose oxidation towards whole-body heat production was 56% greater in the shorn group but when this value was expressed as a proportion of total heat production there was little difference between shorn and unshorn animals.

Tissue utilization and oxidation

No significant difference between shorn and unshorn animals was apparent with respect to the hind-limb tissue plasma or blood flows, nor in the net utilization and oxidation rates of glucose by these tissues (Table 5). There was also no apparent effect on either the estimated or measured contribution of glucose to tissue O_2 consumption or CO_2 production. However, the estimated contribution of glucose to tissue oxidative metabolsim gave values approximately 80% greater than actually measured values using ^{14}C-labelled glucose.

There was also no significant difference between shorn and unshorn groups in the plasma or blood flow across the uterine tissues nor was there any effect on the net extraction or utilization of glucose across the uterine tissues (Table 6). No effect on either the estimated or measured contribution of glucose to CO_2 consumption was observed. In addition there

Table 5. *Metabolism of glucose by the hind-limb tissues of shorn (n 4) and unshorn (n 6) pregnant ewes*

(Mean values with their standard errors)

	Shorn		Unshorn	
	Mean	SEM	Mean	SEM
Blood flow (l/min per kg tissue)	0·166	0·012	0·150	0·005
Plasma flow (l/min per kg tissue)	0·133	0·010	0·123	0·005
Arterio-venous difference (mM)	0·19	0·05	0·14	0·05
Net fractional extraction	0·06	0·01	0·05	0·01
Net utilization (mmol/min per kg × 10^{-1})	0·25	0·05	0·17	0·02
Oxidation rate (nmol/min per kg × 10^{-1})	0·10	0·05	0·08	0·02
Measured contribution to tissue O_2 consumption	0·14	0·07	0·17	0·04
Net production of lactate (mmol/min per kg × 10^{-1})	0·14	0·03	0·08	0·04
Estimated contribution to tissue O_2 consumption*	0·27	0·09	0·26	0·04

* Estimated contribution of glucose oxidation to tissue O_2 consumption is corrected for tissue lactate production and assumes complete oxidation of glucose.

Table 6. *Metabolism of glucose by the uterine tissues of shorn (n 3) and unshorn (n 4) pregnant ewes*

(Mean values with their standard errors)

	Shorn		Unshorn	
	Mean	SEM	Mean	SEM
Blood flow (l/min per kg tissue)	0·286	0·015	0·288	0·026
Plasma flow (l/min per kg tissue)	0·234	0·010	0·233	0·022
Arterio-venous difference (mM)	0·41	0·04	0·35	0·06
Net fractional extraction	0·14	0·02	0·13	0·01
Net utilization (mmol/min per kg × 10^{-1})	0·96	0·09	0·79	0·09
Oxidation rate (mmol/min per kg × 10^{-1})	0·25	0·04	0·30	0·03
Measured contribution to tissue O_2 consumption	0·39	0·08	0·37	0·03
Net production of lactate (mmol/min per kg × 10^{-1})	0·01	0·02	0·05	0·02
Estimated contribution to tissue O_2 consumption*	1·48	0·19	1·19	0·14

* Estimated contribution of glucose oxidation to tissue O_2 consumption is corrected for tissue lactate production and assumes complete oxidation of glucose.

was a marked difference in the measured and estimated contribution of glucose to tissue oxidative metabolism, with the latter method giving values three to four times larger than actually measured values using ^{14}C-labelled glucose.

Plasma hormone concentration

Table 7 shows that there were no significant differences in the arterial concentrations of GH, glucagon, cortisol or T_4, but T_3 concentration was higher in the shorn group. The plasma concentration of insulin was also significantly ($P < 0.05$) lower in the shorn group and as a result I:G was 40 % lower in shorn animals.

Table 7. *Mean arterial plasma concentrations of growth hormone (GH), insulin, glucagon, cortisol, thyroxine (T_4) and 3, 5, 3'-triiodothyronine (T_3) and molar insulin:glucagon (I:G) ratio in shorn (n 5) and unshorn (n 5) pregnant ewes*

	Shorn	Unshorn	
	Mean	Mean	SED
GH (ng/ml)	12·82	10·71	4·60
Insulin (ng/ml)	0·30*	0·40	0·02
Glucagon (ng/ml)	0·11	0·09	0·02
I:G	2·1	3·5	0·6
Cortisol (ng/ml)	6·0	4·8	0·6
T_4 (nM)	63	53	9
T_3 (nM)	1·40†	0·62	0·30

SED, standard error of difference.
Significant differences between means (shorn v. unshorn) assessed by a paired t test: † $P < 0.06$ * $P < 0.05$.
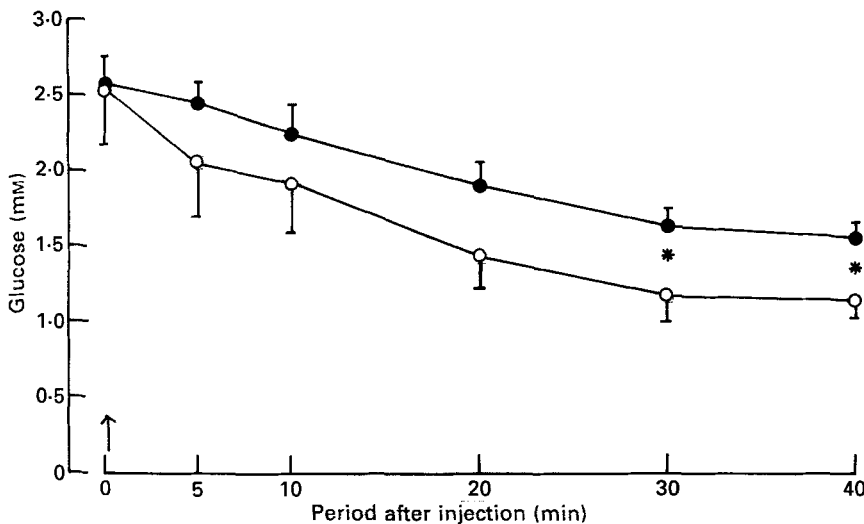
Fig. 1. Effect of an intravenous insulin injection (↑, 0·08 units/kg body-weight) on plasma glucose concentrations in unshorn (●) (*n* 5) and shorn (○) (*n* 5) pregnant sheep. Values are means with their standard errors represented by vertical bars. * $P < 0.05$.

Insulin tolerance test

Fig. 1 illustrates that intravenous injection of insulin resulted in a decrease in the arterial plasma concentration of glucose in both shorn and unshorn ewes over the 40 min sampling period. The mean plasma glucose concentration was significantly lower in the shorn group compared with unshorn controls when measured 30 and 40 min after insulin injection. Furthermore the slope of the change in plasma glucose concentration with time was 25% greater in the shorn animals (shorn −0·035; unshorn −0·028 (SED 0·003) mmol/min (*n* 5), $P < 0.1$).

DISCUSSION
Whole-body glucose metabolism

The present study has demonstrated that the glucose entry rate in shorn pregnant ewes is 28 % higher than in unshorn controls. This effect was observed even though there was no significant difference in the arterial concentration of glucose between the two groups, as measured between 4 and 5·5 h after feeding. The values for glucose entry rate in the present experiment (shorn 3·04, unshorn 2·39 g C/d per kg live weight$^{0.75}$) are in the same range (i.e. 2·16–2·45 g C/d per kg live weight$^{0.75}$) as those reported by other workers using unshorn pregnant sheep, fed on a similar ME and at the same stage of pregnancy (e.g. Hodgson *et al.* 1980, 1981; Oddy *et al.* 1985).

Studies by McKay *et al.* (1974) in fed shorn non-pregnant ewes have shown that glucose entry rate is increased from 2·07 g C/d per kg live weight$^{0.75}$ when measured at 18°, to 5·07 g C/d per kg live weight$^{0.75}$ after being maintained at an environmental temperature of $-2°$ for 6 weeks. The environmental temperature at which the measurements of McKay *et al.* (1974) were made was 6° lower than in the current study. These different results suggest that the extent to which glucose entry rate is stimulated in the shorn pregnant ewes may depend on the environmental temperature and the length of time an animal is maintained at this temperature. Alternatively, the response of pregnant ewes to chronic cold exposure could be reduced because glucose production may have already been increased as a result of pregnancy (Steel & Leng, 1973).

The higher glucose entry rate in the shorn ewe is likely to be the result of an increase in exogenous or endogenous gluconeogenic precursors, or both. There was no difference in the intake of barley concentrate between shorn and unshorn groups but shorn animals did eat 35 % more ammonia-treated straw. This is unlikely to result in a substantially increased absorption of nutrients, because Symonds *et al.* (1986*a*) reported that there was a significant decrease in the apparent digestibility of gross energy and N of the diet in shorn ewes. However, the possibility that the primary precursor responsible for the increased glucose entry rate may be of dietary origin cannot be ruled out. Studies on the effect of short-term cold exposure of non-pregnant sheep (Thompson *et al.* 1978*b*) have shown that an increase in the portal supply and hepatic uptake of propionate could partly account for the increase in glucose production, even though feed intake was similar in thermoneutral and cold environments.

Endogenous gluconeogenic precursors may be more important in stimulating glucose entry rate in the shorn pregnant ewe. However, the observation that glucose re-cycling was unchanged suggests that glucose resynthesis due to the operation of the Cori and alanine cycles was not increased. It is possible that glucose synthesis from glycerol was stimulated because it has been shown that glycerol entry rate was 41 % higher in shorn pregnant sheep (Symonds, 1986), even though there was no difference in the plasma concentration of glycerol between shorn and unshorn groups. However, if all the extra glycerol released into the circulation in the shorn ewe were converted to glycerol it would only account for an 8 % increase in glucose synthesis. It has been suggested that catabolism of liver proteins may be stimulated during cold exposure and gluconeogenic amino acids converted to glucose *in situ* (Thompson *et al.* 1978*a*). However, results from Symonds *et al.* (1986*a*) did not show any significant difference in urinary N excretion between shorn and unshorn groups, which indicates that amino acids are unlikely to have been responsible for increased glucose synthesis. The present study therefore does not provide information as to which are the gluconeogenic precursors responsible for the increase in glucose synthesis in the shorn ewe. This effect may be the result of a combination of a slightly larger supply of both exogenous and endogenous gluconeogenic precursors.

The increased glucose entry rate in shorn ewes may have been due to a change in the plasma I:G since there was a decrease in the plasma concentration of insulin in the shorn animals while that of glucagon was unchanged. A decrease in I:G was observed in cold-compared with warm-acclimated non-pregnant sheep (Sasaki *et al.* 1982). It seems unlikely that the adreno-cortical system played any direct role in stimulating glucose production since this and the previous studies (Symonds *et al.* 1986*a*) have not shown any difference in the circulating concentration of cortisol between shorn and unshorn ewes.

Hind-limb and uterine tissue metabolism

There was no difference in either the net or gross uptake of glucose across the hind-limb tissues between shorn or unshorn animals despite a significantly lower plasma concentration of insulin in the shorn group. This suggests that there may be a change in the sensitivity of these tissues to the stimulatory effect of insulin on glucose uptake by the hind-limb (Hay *et al.* 1984*a*) in the shorn ewe as a result of chronic cold exposure. This proposal is supported by the results of the insulin-tolerance test in which a greater decrease in plasma glucose concentration was measured in the shorn ewes. These results are consistent with the suggestion of Weekes *et al.* (1983) that insulin secretion may be reduced as a result of chronic cold exposure but its effects on glucose uptake by skeletal muscle remain unaffected.

The measured contribution of glucose to oxidative metabolism across the hind-limb tissues using [^{14}C]glucose was nearly half the value estimated assuming complete oxidation of glucose uptake, even allowing for re-cycling of glucose via lactate production. One explanation for this may be that the period over which the present study was conducted was too short to permit equilibrium of glucose-C with the muscle CO_2 pool. Another possibility is that ^{14}C may enter pools with a long half-life, such as muscle protein and glycogen, and result in an underestimate of the contribution of glucose to oxidative metabolism (e.g. Oddy *et al.* 1985). It is therefore possible that in both groups of animals glucose was being spared from oxidation and may have been stored as glycogen or used in non-essential amino acid metabolism, or both.

There was no difference in the uterine blood flow between the small number of shorn and unshorn animals in which measurements were made. This suggests that any effect of being shorn on nutrient supply to the uterine tissues is not the result of an increased blood flow to the uterus. There was also no difference in the net uptake of glucose across the uterine tissues between shorn and unshorn animals. This result may be due to the absence of any difference in plasma glucose concentration between shorn and unshorn groups, because it has been shown that in the unshorn pregnant ewe maternal glucose concentration is the primary factor influencing glucose uptake by the uterine tissues (Hay *et al.* 1983, 1984*b*). However, the possibility that the partition of glucose between the utero-placenta and fetus may be signficantly different between shorn and unshorn animals cannot be ruled out.

The measured values of glucose oxidation across the uterus were only 20–30% of the estimated values assuming complete oxidation of glucose uptake, after correcting for the re-cycling of glucose via lactate production. This is not unexpected because several studies have shown that the potential contribution of glucose to O_2 consumption by the uterus is greater than 1 (Chandler & Bell, 1981; Faichney *et al.* 1981; Bell *et al.* 1982; Oddy *et al.* 1985) which indicates that glucose is being used for synthetic purposes.

The predicted LBW, based on the linear relation between glucose entry rate and LBW reported by Wilson *et al.* (1981) was 4·62 kg (shorn) and 3·61 kg (unshorn). Therefore the increase in glucose entry rate reported in the present study can more than account for the 0·61 kg difference in LBW between shorn and unshorn ewes reported by Symonds *et al.* (1986*a*). However, in the present study the increased glucose entry rate was not associated

Table 8. *Comparison of indirect and direct measurements of heat production from carbohydrate and acetate oxidation in shorn and unshorn pregnant sheep*

	Heat production (MJ/kg live weight$^{0.75}$ per d)	
	Shorn	Unshorn
Carbohydrate and acetate (Symonds *et al.* 1986*a*)	(a) 0·205	0·229
Glucose (present study)	(b) 0·087	0·056
Acetate (a − b)	0·118	0·173
Acetate*		
Annison *et al.* (1967)	0·028	
Pethick *et al.* (1981)	0·132	

* Estimates of possible contribution of heat production due to acetate oxidation, based on measurements made in non-pregnant sheep and assuming that 92 % of the entry rate is oxidized (value obtained for late pregnant sheep by Wilson (1984)).

with an increase in fetal weight. This was probably due to the stress of surgery or to the error in measuring fetal weight at a wide range of gestational ages in a small number of animals. It is also conceivable that a rise in maternal plasma glucose concentration is necessary to stimulate fetal growth since the study of Symonds *et al.* (1986*a*) demonstrated an increase in plasma glucose concentration in shorn ewes while in the present study this effect was not recorded. Thompson *et al.* (1982) proposed that cold stress would stimulate both maternal and fetal plasma glucose concentrations and enhance fetal growth due to higher fetal insulin concentrations.

Oxidative metabolism

The increase in glucose entry rate in the shorn group was used entirely to meet the higher whole-body oxidative requirement for glucose. However, since the whole-body CO_2 entry rate also increased in the shorn ewes there was no change in the proportion of total heat production due to glucose oxidation. Symonds *et al.* (1986*a*) reported that there was no difference in the rate of carbohydrate oxidation between shorn and unshorn animals. However, carbohydrate oxidation was measured using values for non-protein respiratory quotient which include the oxidation of acetate. Therefore a comparison of the results from the present study and those of Symonds *et al.* (1986*a*; Table 8) suggests that acetate oxidation may be decreased in the shorn ewe. The derived values for acetate oxidation are in the same range as previous reports (Table 7).

The shorn animals exhibited an 18 % increase in whole-body CO_2 entry rate, which resulted in a 26 % rise in estimated total heat production, indicating that the response to chronic cold exposure in these sheep was similar to that measured by Symonds *et al.* (1986*a*). There was also a significantly higher concentration of O_2 and CO_2 in the arterial blood of the shorn group which may be an indication of the greater energy requirements of these animals. The higher concentration of O_2 in arterial blood of shorn animals was associated with an increased haemoglobin concentration which is similar to results from adult rams during acute cold exposure (Thompson *et al.* 1978*a*).

An increase in the plasma concentration of T_3 could represent the endocrine mechanism responsible for this change in heat production. T_3 is thought to be the metabolically active thyroid hormone and its concentration may be stimulated by either an increase in its rate of secretion from the thyroid gland, or by a higher rate of conversion of T_4 to T_3 by

peripheral tissues (see Danforth, 1983). The absence of any difference in the circulating level of T_4 between shorn and unshorn groups may be the result of an increased rate of conversion of T_4 to T_3.

It is concluded that the whole-body glucose entry rate is stimulated in the winter-shorn pregnant ewe and this extra glucose is oxidized by tissues other than those of the hind-limb and pregnant uterus. This increase in glucose oxidation may be due to a rise in the metabolic rate of tissues with an obligatory requirement for glucose in winter-shorn ewes. The increase in insulin sensitivity at the same time as a decrease in circulating insulin concentration may be interpreted as a mechanism to maintain glucose supply to insulin-sensitive tissues while the concomitant decrease in plasma I:G stimulates hepatic glucose production in order to meet this increased obligatory requirement for glucose.

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