Maternal undernutrition during mid-pregnancy in sheep. Placental size and its relationship to calcium transfer during late pregnancy

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The aim of the present experiment was to determine the relationship between placental and fetal weight after placental growth had been retarded by maternal undernutrition. Placental weight and fetal weight were measured in single-lamb-bearing ewes which were well-fed throughout pregnancy, or severely undernourished between the 30th and 96th day of pregnancy. Placental transfer of calcium and whole-body metabolism of both glucose and Ca were measured during late pregnancy. The change in fleece-adjusted live weight between the 30th and 96th day of pregnancy was 99 (SE 9·8) and −146 (SE 9·6) g/d for the well-fed and undernourished ewes respectively. The condition score of well-fed ewes did not significantly change between the 96th (2·9 (SE 0·08)) and 140th (3·0 (SE 0·13)) day of pregnancy, while it increased from 1·6 (SE 0·15) to 2·3 (SE 0·11) for the previously undernourished group. Undernutrition caused an increase ($P < 0·01$) in placental weight measured on the 96th (21 %) and 140th (30 %) day of pregnancy. In contrast fetal growth was not significantly affected by maternal undernutrition. While the voluntary dry matter intakes (g/d) of previously undernourished ewes after the 97th day of pregnancy were higher than for their well-fed counterparts, there was no significant difference between whole-body glucose or Ca metabolism, or the placental transfer of Ca measured during late pregnancy. This experiment confirms earlier reports of an increase in placental weight as a result of maternal undernutrition during mid-pregnancy; but the factors causing and the functional significance of this response have not been identified. Contrary to earlier proposals, placental weight *per se* did not limit fetal growth during late pregnancy. It is hypothesized that a combination of factors originating from maternal, placental and fetal sources act together to regulate growth of the fetus.

Undernutrition: Placenta: Calcium: Pregnancy: Sheep

When pregnant ewes have been subjected to a period of undernutrition before the 100th day of pregnancy, growth of the placenta has been reported to be retarded (Everitt, 1964; Morris, 1973; Mellor, 1983) or stimulated (Faichney, 1981; Owens *et al.* 1986*b*). The level of body reserves in the ewe, as well as the timing and severity of the period of undernutrition, may explain some differences in the response of the placenta. However, the significance of placental weight arises from possible effects on the rate of fetal growth and, therefore, the birth weight attained. Mellor (1983, 1987) proposed that, in the well-fed ewe placental weight is the major determinant of growth rate of the fetus. This can only be true first, if placental weight is an appropriate index of the maximum functional ability of the placenta (i.e. its functional element), and second if the placenta is functioning at its maximum capacity as parturition approaches. In contrast to this hypothesis a preliminary experiment reported by Everitt (1965) demonstrated that a smaller placenta retarded by maternal undernutrition was not consistently associated with lower fetal weight at birth. Therefore, a clear relationship between placental weight and fetal growth has not yet been

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established. Attempts to clarify this relationship have been made by manipulation of placental weight using heat-stress (Bell *et al.* 1987*b*) or a premating carunculectomy operation (Robinson *et al.* 1979; Owens *et al.* 1986*a*).

The objective of the experiment was to evaluate the consequence of different placental weight, resulting from maternal undernutrition before the 97th day of gestation, on birth weight of the lamb and on the transport of calcium across the placenta during late pregnancy. Ca was chosen as an index of placental transport because it is a nutrient essential for fetal growth and required at maximum levels during late pregnancy (Grace *et al.* 1986). It is transported across the placenta by means of a membrane-bound carrier mechanism (Comar, 1956; Braithwaite *et al.* 1972) as are other metabolically important substrates such as glucose (Widdas, 1952; Stacy *et al.* 1978) and some amino acids (Lemons *et al.* 1976). Ca transport across the placenta is readily determined because it is transferred across the placenta by a one-way process and deposited in the fetal body (Symonds *et al.* 1966; Braithwaite *et al.* 1972) without being mobilized or retained by the placenta. The transfer of Ca across the ovine placenta has been reviewed recently (Lester, 1986). If fetal growth is determined by placental weight then a change in placental weight will be associated with a proportional change in the net movement of nutrients across the placenta.

The present experiment confirmed earlier reports (e.g. Faichney & White, 1987) that placental weight can be increased by maternal undernutrition during mid-pregnancy. However the larger placenta did not confer higher rates of fetal growth during late pregnancy. The birth weight of the lamb and the transport of Ca were not improved. Some of these results have been reported in a preliminary communication (McCrabb *et al.* 1987).

MATERIALS AND METHODS

Animals and management

Two groups of twelve 5-year-old Corriedale/Comeback ewes with oestrous cycles synchronized with medroxyprogesterone acetate (Repromap; Upjohn, Rydalmere, N.S.W. 2116) were treated 3 weeks apart, to permit sequential handling for experimental measurements. Two harnessed Merino rams, both weighing 74·8 kg, were introduced to the flock and the days of mating were recorded. All ewes were run as one flock until the 30th day of pregnancy when the nutritional treatments began.

Ewes in each mating group were allocated to one of two treatment groups. The well-fed (control, C) group was fed as one flock in the field with lucerne (*Medicago sativa*) hay (11·0 MJ metabolizable energy (ME)/kg dry matter (DM) and 13·8 g nitrogen/kg DM) *ad lib.* plus lupin (*Lupinus angustifolius*) grain (13·6 MJ ME/kg DM and 44·6 g N/kg DM), during the period between the 30th and 96th day of pregnancy. The level of feed was calculated (Ministry of Agriculture, Fisheries and Food, 1984) to support a gain of approximately 100 g/d in live weight between the 30th and 96th day of pregnancy. The under-fed (restricted, R) group was fed to produce a loss of 8 kg live weight between the 30th and 96th day of pregnancy. Group R ewes were housed in individual pens from the 30th day of pregnancy, while group C ewes remained in the field until the 65th day of pregnancy and then housed until the time of slaughter.

At the 96th day of pregnancy, six ewes were selected for slaughter from each treatment group, according to live weight and condition score. At the time of slaughter one of the six group R ewes was found to be non-pregnant, and was excluded from the experiment. The remaining ewes in each group were given the same lucerne hay *ad lib.* until the 140th day of pregnancy, when they were also slaughtered. *Ad lib.* feeding during the final 44 d of the experiment was achieved by supplying 20 % more DM than the previous days' consumption.

All animals were treated for internal parasites with levamisole hydrochloride (Nilverm LV; Coopers, North Ryde, NSW 2113) and for external parasites with cyhalothrin (Grenade; ICI Aust. Operations Pty Ltd, Melbourne, Victoria 3000), before both the time of joining and housing. Animals were fed daily at 09.00 hours and fresh water was available at all times. The animals were weighed before feeding and condition scored (Russel *et al.* 1969) at least once each week during the study.

Ewe slaughter and uterine dissection

Ewes slaughtered on the 96th day of pregnancy were fasted for 24 h before their slaughter. Ewes slaughtered on the 140th day of pregnancy were not fasted before slaughter, to avoid interference with the metabolism of the ewe during the study of Ca transfer.

At the time of slaughter, the ewes were stunned with a captive bolt pistol before exsanguination. The uterus was removed through a 120 mm mid-line incision and tied off as close as possible to the cervix with cotton thread. After weighing the gravid uterus an incision was made along the greater curvature of the pregnant horn and the fetus exposed. The umbilical cord was tied off with cotton thread and severed. The fetus was immediately removed and killed with a lethal dose of pentobarbitone (Euthatal® 350; May and Baker, West Footscray, Victoria 3012), dried with paper towelling and weighed. The curved crown–rump length and thoracic girth were measured in triplicate with a length of cotton thread. The fetal membranes were dissected from the cotyledons using curved scissors. All cotyledons, including the fetal cotyledon and maternal caruncle, were then dissected from the uterine wall, counted and the total weight recorded.

Whole-body entry rate of glucose

The entry rate of glucose was determined on the 126th day of pregnancy. A PVC catheter was implanted in the jugular vein of each ewe at least 48 h before the beginning of the study. PVC catheter tubing (1·0 mm i.d., 1·5 mm o.d.; Dural Plastics and Engineering, Dural, NSW 2158) was implanted into the left jugular vein via a 50 mm × 14 gauge hypodermic needle (Portland Surgical Products, Portland, Victoria 3305) and fixed in place with surgical suture (size 1/2·0 metric). A three-way tap (TOP Surgical Manufacturing Co. Ltd, Tokyo, Japan) was attached to the catheter tubing and fixed to the wither of the ewe for ease of sampling. Patency of the catheter was maintained by flushing daily with 1 ml sterile (autoclaved) saline (9 g sodium chloride/l) containing 33 IU sodium heparin (Weddel Pharmaceuticals, Thornleigh, NSW 2120).

The feeding strategy adopted on the day of study of glucose entry involved feeding one-eighth of the total daily ration at two-hourly intervals beginning at 06.00 hours, and ending at 20.00 hours (see Steel & Leng, 1968). At 09.00 hours 5 ml sterile saline containing 50 μCi (1·85 mBq) [6-^{3}H]glucose (Amersham International Plc, Amersham, Bucks, UK) and glucose carrier (1 g glucose/l) was injected into the non-catheterized jugular vein. Twelve blood samples were taken at 20 min intervals for the first 4 h, and an additional five blood samples at less frequent intervals until 24 h after injection of the dose. At each time of sampling 10 ml whole blood was withdrawn, placed in a tube containing 125 IU lithium heparin (Disposable Products Pty Ltd, Technology Park, South Australia 5098) and placed on ice. The blood was then centrifuged at 750 ***g*** for 20 min and the plasma stored at −20 ° for later analysis.

Plasma was deproteinized with barium hydroxide and zinc sulphate (Somogyi, 1945) and assayed for glucose (Marks, 1959). The activity of [^{3}H]glucose was determined by liquid scintillation counting (LS 3801; Beckman Instruments Inc., Irvine, CA 92713, USA) using

a freeze-dried sample of deproteinized solution (Brockman & Halvorson, 1981) reconstituted with 1 ml water. ACS II scintillant (7 ml; Amersham Corporation, Arlington Heights, IL 60005, USA) was added before counting.

Placental transfer of Ca

The procedure of Durand *et al.* (1983) was used to measure the rate of transfer of Ca from the dam to the fetus. A PVC catheter was implanted into the jugular vein of each ewe on the 131st day of pregnancy, using the same procedure as for the determination of the entry rate of glucose. At the 133rd day of pregnancy 500 μCi (18·5 MBq) ^{45}Ca as $CaCl_2$ (Australian Atomic Energy Commission, Lucas Heights, NSW 2234) in 5 ml sterile (autoclaved) saline containing a carrier (100 μg Ca as $CaCl_2$/ml) was injected into the jugular vein of the dam at 09.00 hours. Following the injection of the dose, samples of whole blood (8 ml) were taken; nineteen during the first 24 h and a further thirteen during the following 6 d until slaughter. The blood was immediately placed on ice in tubes containing 125 IU lithium heparin, centrifuged at 750 ***g*** for 20 min and the plasma stored at -20 ° for later analysis. Immediately following withdrawal of the final blood sample on the 140th day of pregnancy, each ewe was slaughtered as described previously. The fetus was frozen, and minced through a 3 mm sieve, allowing a representative sample to be taken for ashing. Fetal tissue was ashed at 600 ° for 16 h in a muffle furnace. The concentration of Ca in both maternal plasma and fetal ash was determined by atomic absorption spectroscopy (Willis, 1960, 1961). Plasma samples were deproteinized with trichloroacetic acid (200 g/l), freeze-dried and reconstituted with 1 ml 2 M-hydrochloric acid. To suppress any interference by protein or phosphorus during Ca determination, strontium ($SrCl_2.6H_2O$) was added to plasma (Willis, 1960) and fetal ash (Willis, 1961). Fetal ash dissolved in 2 M-HCl (about 75 μl) was made up to a volume of 1 ml with distilled water. The activity of ^{45}Ca in both plasma and fetal ash was determined by liquid scintillation counting (LS 5801; Beckman Instruments Inc., Irvine, CA 92713, USA), in 7 ml of ACS II scintillant (Amersham Corporation, Arlington Heights, IL 60005, USA), against a set of quenched standards.

Calculations

The decay curve for the specific activity of both glucose and Ca was described by calculating the line of best fit in the form:

$$SR_t = \sum_{i=1}^{n} A_i e^{-m_i t} \quad (1)$$

where SR_t is the specific radioactivity of glucose (disintegrations/min (dpm) per mg glucose) or Ca (dpm/μg Ca) at time t; A is the zero time intercept of each exponential component (dpm/mg glucose or dpm/μg Ca); m is the rate-constant of each component (/min); n is the number of components; i is the component identification and t is the time (min). Parameters were fitted by the least-squares method using the SAAM/CONSAAM computer program (Berman & Weiss, 1978). Pool size, total entry rate and irreversible loss of glucose were calculated as described by White *et al.* (1969). The rate of Ca deposition was calculated using the method of Twardock *et al.* (1973). The mean specific activity of plasma Ca during the study of Ca transfer was calculated by dividing the area under equation 1 by the time duration of the study, that is (dpm × min)/min.

Ca transfer from the dam to the fetus was calculated as follows:

$$\frac{\text{percentage } ^{45}\text{Ca dose in fetus at slaughter}}{\text{mean specific activity of } ^{45}\text{Ca in maternal plasma}} \quad (2)$$

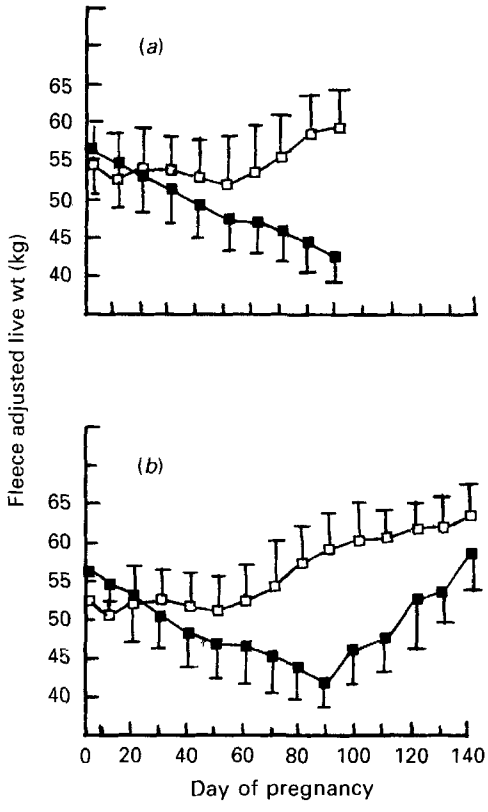

Fig. 1. Fleece-adjusted live weight of control (□) and restricted (■) ewes slaughtered at (*a*) 96 and (*b*) 140 d of pregnancy. For details of dietary regimes and procedures, see pp. 158–159. Values are means with their standard errors represented by vertical bars.

Statistical analysis

Both *t* test and two-way (treatment and day) analysis of variance (Nie, 1983) were used to test statistical differences between the treatment means. A least squares regression approach was incorporated into the analysis of variance to eliminate bias caused by unequal observations per cell (Nie, 1983).

RESULTS

Live weight and condition score

Fig. 1 shows the changes in fleece-adjusted live weight (FALW) of group C and R ewes slaughtered at the 96th and 140th day of pregnancy. The changes in FALW between the 30th and 96th day of pregnancy for group C and R ewes were 99 (SE 9·8) and −146 (SE 9·6) g/d respectively. The FALW for group C ewes was greater ($P < 0·01$) at the 96th day of pregnancy, but not significantly different at the 140th day of pregnancy. The increase in FALW between the 96th and 140th day of pregnancy was greater ($P < 0·05$) for group R than group C. The condition scores of control and restricted ewes during the various stages of gestation are reported in Table 1.

Table 1. *Condition score of control and restricted ewes† during four stages of pregnancy*

(Mean values with their standard errors for no. of observations shown in parentheses)

Day of pregnancy	Control			Restricted		
	Mean	SE	*r*	Mean	SE	*n*
Joining	2·3	0·09	(12)	2·5 NS	0·12	(11)
30	2·5	0·16	(12)	2·4 NS	0·10	(11)
96	2·9	0·08	(12)	1·6***	0·15	(11)
140	3·0	0·13	(6)	2·3***	0·11	(6)

NS, not significant.

Mean values were significantly different from control value (*t* test, pooled estimate of variance used): ***$P < 0·001$.

† For details of dietary regimen and procedures, see pp. 158–159.

Table 2. *Voluntary dry matter intake (g/d) of chaffed lucerne* (Medicago sativa) *hay by control and restricted ewes‡ during late pregnancy*

(Mean values with their standard errors)

Day of pregnancy	Control (*n* 6)		Restricted (*n* 6)	
	Mean	SE	Mean	SE
116–120	1022	66·6	1722***	123·4
121–125	1276	78·6	1773**	134·8
126–130	1261	69·3	1693*	167·5
131–135	1379	80·0	1671†	132·6

Mean values were significantly different from control values (*t* test, pooled estimate of variance used): *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$, †$P < 0·10$.

‡ For details of dietary regimen and procedures, see pp. 158–159.

DM intake

The DM intake of group R ewes adjusted to provide the loss of weight described previously gradually declined from 800 to 210 g DM/ewe per d between the 30th and 96th day of pregnancy respectively. Voluntary DM intake during late pregnancy for group C was significantly lower than that for group R ewes (Table 2).

Placental and fetal components

There was no significant effect of undernutrition on fetal weight or dimensions when measured at either the 96th or 140th day of pregnancy (Table 3). No significant difference was observed at the 96th day of pregnancy between the group C (*n* 6) and R (*n* 5) fetuses in the weight (g) of the brain (19 (SE 0·9), 19 (SE 0·8)), liver (41 (SE 3·8), 39 (SE 2·7)), heart (8 (SE 0·9), 7 (SE 0·9)), lung (33 (SE 1·6), 30 (SE 2·2)), right kidney (5 (SE 0·3), 5 (SE 0·4)) or left kidney (5 (SE 0·3), 5 (SE 0·4)) respectively, or when they were expressed as a proportion of fetal weight. The weight of the fetus, its crown–rump length and thoracic girth increased ($P < 0·01$) between the 96th and 140th day of pregnancy. The weight of the placenta of group R ewes was greater ($P < 0·01$) than for group C ewes at both the 96th and 140th day of pregnancy (Table 3). There was no significant difference in the number of cotyledons for groups C and R ewes at either stage of gestation.

Table 3. *Contents of the gravid uterus of control and restricted ewes* at the 96th and 140th day of pregnancy*

(Mean values for no. of ewes in parentheses)

	Fetus† (g)		Crown–rump length (mm)		Thoracic girth (mm)		Placenta (g)		Cotyledon no.	
Day of pregnancy...	96	140	96	140	96	140	96	140	96	140
Control	644 (6)	4552 (6)	303 (6)	549 (6)	170 (6)	339 (6)	496 (6)	430 (6)	83 (6)	87 (6)
Restricted	686 (5)	4561 (6)	296 (5)	554 (6)	170 (5)	339 (6)	600 (5)	560 (6)	88 (5)	82 (6)
Statistical significance‡										
Day of pregnancy	$P < 0.01$		$P < 0.01$		$P < 0.01$		NS		NS	
Control *v.* restricted	NS		NS		NS		$P < 0.01$		NS	
Interaction	NS		NS		NS		NS		NS	
SE	0·030		6·0		3·9		29·9		5·2	

NS, not significant.

* For details of dietary regimen and procedures, see pp. 158–159.

† Statistical analysis done on log transformed data, SE from log analysis.

‡ Two-way (treatments and days) analysis of variance.

Glucose metabolism

There was no significant difference between group C (n 4) and R (n 5) ewes in the concentration of glucose in maternal plasma (mmol/l; 3·8 (SE 0·21), 3·4 (SE 0·07)), pool size (mmol; 42 (SE 3·8), 36 (SE 3·6)), entry rate (mmol/min; 0·72 (SE 0·059), 0·84 (SE 0·075)) or irreversible loss (mmol/min; 0·63 (SE 0·048), 0·72 (SE 0·078)) of glucose on the 126th day of pregnancy respectively. When both entry rate and irreversible loss of glucose were expressed as proportions of ewe live weight, they were lower ($P < 0.05$) for group C (mmol/min per kg; 0·011 (SE 0·0004), 0·010 (SE 0·0003)) than group R (mmol/min per kg; 0·017 (SE 0·0014), 0·014 (SE 0·0015)) ewes respectively. There was no significant difference in pool size when expressed as a proportion of ewe live weight.

Metabolism and transfer of Ca across the placenta

There was no significant difference between group C (n 6) and R (n 6) ewes in the concentration of Ca in maternal plasma (μmol/ml; 2·6 (SE 0·06), 2·5 (SE 0·08)), pool size (mmol; 47 (SE 1·7), 47 (SE 4·9)), entry rate (mmol/h; 5·5 (SE 0·30), 5·5 (SE 0·52)) or irreversible loss (mmol/h; 3·6 (SE 0·24), 3·7 (SE 0·31)) of Ca measured during the period between the 133rd and 140th day of pregnancy respectively. There was no significant difference in the percentage of the ^{45}Ca dose deposited in the fetus, or the rate of deposition of total (stable) Ca in the fetus between the 133rd and 140th day of pregnancy in group C and group R ewes (Table 4). The percentge of the ^{45}Ca dose deposited ($P < 0.05$), and the rate of deposition of Ca ($P < 0.05$) when expressed as a proportion of the weight of the placenta was higher for group C than group R ewes. The rate of deposition of Ca expressed as a proportion of the weight of the fetus was not significantly different between group C and R ewes.

DISCUSSION

Fetal growth

The period of severe maternal undernutrition between the 30th and 96th day of pregnancy did not affect growth of the fetus, measured in terms of either its body-weight or the size

Table 4. *Variables of placental transfer of calcium in control and restricted ewes* measured between the 133rd and 140th day of pregnancy*

(Mean values with their standard errors)

	Control (*n* 6)		Restricted (*n* 6)		Statistical significance of difference†
	Mean	SE	Mean	SE	
Percentage of dose deposited in fetus:					
%	39·4	1·34	37·5	2·49	NS
%/g placenta × 100	9·3	0·68	6·9	0·67	$P < 0·05$
%/g fetus × 1000	8·8	0·52	8·4	0·69	NS
Rate of Ca deposition:					
mmol/d	35·0	2·5	35·0	2·5	NS
mmol/d per kg placenta	80	5·2	62	3·0	$P < 0·05$
mmol/d per kg fetus	7·5	0·35	7·5	0·30	NS
Total Ca in fetus:					
mol	0·97	0·034	0·98	0·063	NS
mol/kg placenta	2·3	0·01	1·8	0·01	$P < 0·05$
mol/kg fetus	0·22	0·009	0·22	0·007	NS

NS, not significant.

* For details of dietary regimen and procedures, see pp. 158 and 160.

† *t* test, pooled estimate of variance used.

of the individual organ and tissue components. This is in contrast to a number of studies on the effect of maternal undernutrition which have reported either an increase (Everitt, 1965; Russel *et al.* 1981; Holst *et al.* 1986; Faichney & White, 1987) or a decrease (Everitt, 1965; Morris, 1973; Curll *et al.* 1975; Rattray & Trigg, 1979; Russel *et al.* 1981; Mellor, 1983; Nordby *et al.* 1986) in birth weight of the lamb. The reasons for these various responses have not been established, but it seems likely that in the present experiment, the mobilization of the maternal body tissue and a shift in the factors regulating the partition of nutrients by the dam, were able to buffer growth of the fetus from any effect of severe maternal feed restriction. It has previously been hypothesized that these different responses in fetal growth and birth weight of the lamb are directly related to placental weight (Davis *et al.* 1981; Mellor, 1983; Holst *et al.* 1986; Kelly, 1986; Faichney & White, 1987), but a clear relationship has not been demonstrated. Both Davis *et al.* (1981), Kelly (1986) and Kelly & Ralph (1988) have proposed that feeding the ewe in specific ways can alter placental weight and affect fetal growth. The relationship between placental weight and fetal weight at the 140th day of pregnancy in the present experiment was poor. Clearly variability in fetal weight was not determined by placental weight alone.

Intake and metabolism

Voluntary DM intakes during late pregnancy were higher for the previously under-fed (group R) ewes than for their well-fed (control) counterparts, and confirm similar observations made in mature wethers of the same genetic background (Djajanagara, 1986). During the periods between the 116th and 120th, and the 131st and 135th day of pregnancy the intake of group R ewes was 68% ($P < 0·001$) and 21% (not significant) greater respectively than group C ewes. The calculated intakes of ME, N and Ca of group C (+17%, +19%, +300%) and group R (+25%, +44%, +600%) ewes were well above the currently recommended allowances (Agricultural Research Council, 1980), and indicate that intake was not limiting the supply of nutrients during late pregnancy. Despite the

difference in intake, and the higher rate of gain in live weight and condition score of group R compared with group C ewes, the entry rates of both glucose and Ca by the dam during late pregnancy were similar between treatment groups. In addition the whole-body entry rate of glucose was higher (20%) than in other reports (Leng, 1970). Therefore, the maternal pool of nutrients available for transfer to the fetus was assumed to be not limiting the supply of nutrients for fetal growth. The potential supply of glucose, which is one of the major metabolic substrates required by the fetus (Battaglia & Meschia, 1981; Mellor, 1983), was similar for all ewes and was not affected by the level of feeding during early and mid-pregnancy. In addition, the concentration of glucose in maternal plasma, which is the driving force for glucose transport (Jodarski *et al.* 1985; Rankin *et al.* 1986), was similar for both groups of ewes. It is supposed that the rate at which glucose was partitioned to the fetus during late pregnancy was similar for both groups, as reflected in the fetuses of similar weight when measured at the 140th day of pregnancy.

Placental growth

Placental growth was more sensitive than fetal growth to maternal undernutrition during mid-pregnancy, and confirms the observations made in an earlier study (McCrabb *et al.* 1986). The nutritional restriction resulted in a 104 and 130 g larger ($P < 0{\cdot}01$) placental weight in group R than group C when measured at the 96th and 140th day of pregnancy respectively. These differences are intermediate between 148 g (Faichney & White, 1987) and 25 g (Owens *et al.* 1986*b*), both measured at the 135th day of pregnancy. The decline in weight of the placenta during late pregnancy of control (well-fed) ewes followed the normal pattern of growth (Bell, 1984). In addition, the commonly observed non-significant decline in weight of the placenta during late pregnancy of group R ewes was lower than for group C ewes in both the present (40 *v.* 68 g) and an earlier (10 *v.* 44 g) experiment (McCrabb *et al.* 1986).

The observations of Faichney & White (1987) demonstrate that ewes which had a larger placenta as a result of maternal feed restriction, also exhibited higher rates of fetal growth. In contrast, the present experiment has demonstrated that the smaller placenta of group C ewes has, at least, the same capacity as the larger placenta of group R ewes for the transport of nutrients for growth of the fetus.

Placental transport

The transport of Ca across the placenta was chosen as a suitable indicator of fetal growth because it is actively transported across the placental barrier as are other metabolically important substrates such as glucose and some amino acids (Lemons *et al.* 1976). Second, the major sink of Ca in the growing fetus is its skeleton. Since the size of the fetal skeleton is related to fetal weight (Gabbedy, 1974), the placental transfer of Ca should be an index of fetal growth. It is possible that the compartmental kinetics of ^{45}Ca irreversible loss in the pregnant ewe may provide a sufficiently sensitive estimate of fetal growth during late pregnancy to be used in future studies of fetal growth. Validation of this hypothesis is required to determine its potential uses.

The rate of Ca transfer observed in the present study (7·5 mmol/d per kg fetus) was similar to previous measurements made by Field & Suttle (1967), 8·2 mmol/d per kg; Braithwaite *et al.* (1970), 9·0 mmol/d per kg; and Twardock *et al.* (1973), 7·4 mmol/d per kg. Placental weight *per se* was not an accurate indicator of the rate of transport of nutrients across the placenta during late pregnancy, as the rate of movement of Ca across the placenta was 35 mmol/d for both previously restricted (group R) and control (well-fed, group C) ewes. Alternatively, when the rate of transfer of Ca was expressed relative to placental weight, it was higher for control (80 mmol/d per kg placenta) than restricted (62

mmol/d per kg placenta) ewes. This is consistent with the proposal that the mechanisms involved in the transfer of Ca across the placenta are not limiting nutrient transfer during normal growth. The factors regulating the rate of growth of the fetus during late pregnancy appear to originate from sources other than the placenta alone. Ca may not have limited fetal growth, although its transport was probably controlled by fetal growth processes, in turn limited by another nutrient. This hypothesis is supported by recent evidence which has shown that a hormone (parathyroid hormone-related protein), produced in the parathyroid gland of the ovine fetus, is responsible for stimulating the transport of Ca across the placenta and thus maintaining fetal hypercalcaemia (Loveridge *et al.* 1988; Rodda *et al.* 1988). Since the rate of deposition of Ca across the placenta was the same for both control and previously restricted groups, it is proposed that the fetus itself may be regulating Ca transport.

It is, therefore, hypothesized that placental weight *per se* does not limit fetal growth but rather that a combination of maternal, fetal and placental factors all interact to regulate fetal growth. The similar rates of transfer of ^{45}Ca across placentas of different weights support this proposal. Twardock *et al.* (1973) demonstrated that the lower placental weight associated with twin fetuses was not associated with an alteration in the rate of transport of Ca across the placenta. Since placental weight associated with individual twin fetuses is smaller than their single-bearing counterparts (Stegeman, 1974), it can be inferred that the smaller placental weight for twin fetuses does not limit the transport of Ca across the placenta.

Clearly the physiological settings which were regulating the partition of nutrients were vastly different between the two treatment groups. During late pregnancy leaner ewes had less body reserves to mobilize, consumed a greater amount of feed and increased in live weight and condition score more than did the previously well-fed control ewes. Despite these differences, growth of the fetus remained unaffected, suggesting that either (1) the factors regulating growth of the fetus were independent of the physiological status of the ewe, or (2) that the control of the partition of nutrients in the ewe was altered, despite severe maternal undernutrition, to maintain similar rates of fetal growth (see Bauman & Currie, 1980; Bell *et al.* 1987*a*). The main assumptions contained in the hypothesis proposed by Mellor (1983) are not supported by the present findings. It is concluded that placental weight *per se* does not limit fetal growth in ewes with adequate current nutrition during late pregnancy.

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