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Effects of Maternal Nutritional Status on Fetal and Placental Growth and on Fetal Urea Synthesis in Sheep

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Abstract

Fetal and placental growth, and fetal and maternal urea synthesis in late gestation, were studied in 2-year-old Corriedale ewes on a maintenance ration (M) except when subjected to moderate dietary restriction from day 50 to day 100 (RM), day 100 to day 135 (MR) or day 50 to day 135 (RR). In comparison with fetuses of ewes maintained throughout the experiment (MM), RR fetuses were smaller and RM fetuses were larger whereas MR fetuses were unaffected; all restrictions were associated with increased placental size. Fetal urea synthesis at day 133 in the well-nourished ewes (MM) was 21.5 mg N h^{-1} kg^{-1} increasing to, respectively, 25.7, 27.3 and 38.8 mg N h^{-1} kg^{-1} in groups MR, RM and RR; these values were 1.6, 3.9, 2.2 and 3.8 times the maternal rates of synthesis. On the basis of the observed urea synthesis rates, amino acid oxidation could have accounted for up to, respectively, 32, 38, 40 and 57% of fetal oxygen consumption in groups MM, MR, RM and RR. Amino acids, in addition to their role in tissue accretion, may be key energy substrates for the fetus.

Introduction

The influence of maternal nutrition on placental and fetal growth in sheep has been reviewed recently (Robinson 1977; Robinson and McDonald 1979; Black 1983). Generally, placental weight does not appear to increase after about day 100 and severe undernutrition can reduce the number of cotyledons and also the total weight of cotyledonary tissue. Fetal growth, more than 80% of which occurs during the last trimester of gestation, can be more or less affected by maternal undernutrition, depending on its degree and duration. Fetal metabolism is supported by glucose and amino acids taken up from the maternal circulation and lactic acid taken up (Faichney *et al.* 1981) and produced in the placenta from glucose (Sparks *et al.* 1983). Although placental urea clearance increases in acute starvation (Simmons *et al.* 1974), little is known of the metabolic changes that occur in the fetus when moderate levels of undernutrition occur over extended periods during gestation. This paper reports the results obtained in an experiment in which fetal and placental growth and fetal and maternal urea metabolism were studied following moderate dietary restrictions during the second, third or second plus third trimesters of gestation in sheep. Preliminary reports of some of the data have been published (Faichney and White 1980; Faichney 1981).

Materials and Methods

Animals

Subgroups of 2-year-old, nulliparous, Corriedale ewes were randomly selected each week from a flock of 60 ewes. After oestrus synchronization, they were mated individually to Corriedale rams and allocated to a treatment group (see below). Ewes were penned with harnessed, vasectomized rams for 36 days after mating and those not marked were transferred to individual pens. They were X-rayed at 78 days, at which stage ewes with multiple fetuses, together with any non-pregnant at this stage, were discarded.

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At 114 days, self-retaining rumen catheters were fitted surgically (Faichney and Colebrook 1979) and the ewes were transferred to metabolism cages in a room which was maintained between 20 and 23°C and continuously illuminated. On day 124, indwelling catheters were established in the maternal and fetal circulations. First, a polyvinylchloride catheter (1.5 mm o.d., 1.0 mm i.d., Dural Plastics and Engineering, Dural, N.S.W.) was inserted into a jugular vein by percutaneous puncture. General anaesthesia was then induced with pentobarbitone sodium and maintained using halothane with positive ventilation. Throughout surgery, a dextrose solution (5% in 0.9% NaCl; Travenol Laboratories Ltd, Sydney) was administered via the jugular catheter. After exposure of the uterus through a midline incision, the hind limbs of the fetus were withdrawn through a small incision in the uterus and catheters (1.5 mm o.d., 1.0 mm i.d.) were advanced in the dorsal aorta via a femoral artery and into a femoral vein via a recurrent tarsal vein. Prior to closure of the incisions, 1 ml of oxytetracycline solution (50 mg/ml; Terramycin, Pfizer Agricare Pty Ltd) was instilled into the amniotic space and the fetus was given an intramuscular injection of cloxacillin (250 mg in 1 ml 0.9% NaCl; Orbenin, Beecham Research Laboratories). The catheters were exteriorized through the right flank of the ewe and a catheter (2.0 mm o.d., 1.0 mm i.d.) was then placed in a carotid artery. The catheters were filled with sterile 0.9% NaCl containing 500 i.u. heparin/ml; the solution used for the fetal catheters also contained neomycin sulfate (4 mg/ml; Neobiotic, Upjohn Pty. Ltd.). The catheters were maintained by daily flushing and refilling with these solutions. Following surgery, the ewe was given penicillin and streptomycin (Depomycin, Ethnor Pty Ltd) intramuscularly and the fetus was given Neobiotic (8 mg) and benzylpenicillin (150 mg) intravenously each day for 3 days.

Diet and design

The diet was a pelleted mixture of lucerne hay and oat grain (3:2); the hay and grain were passed through a hammermill (screen 6 mm) before mixing and pelleting. Its average composition (g/kg dry matter) was: organic matter, 936; total N, 24.4; cell wall organic matter, 387; acid-detergent lignin, 59; α-linked glucose polymers (starch), 220; crude fat, 36. The ewes were offered the diet at 900 g/day each whilst in group pens from 2 weeks before mating until 36 days of gestation and then in individual pens until day 50. This level of feeding was intended to ensure that, if given throughout gestation, the ewes' bodyweights (liveweight less gravid uterus) would not fall below their liveweights at mating and was known to be sufficient to allow twin fetuses of similar Corriedale ewes to grow throughout gestation at a rate similar to that of single fetuses (Faichney and White, unpublished data).

The treatments to which the ewes were allocated at mating consisted of one of two levels of feeding (maintenance, M = 900 g/day; moderate restriction, R = 500 g/day) during mid-gestation, i.e. from day 50 to day 100, and during late gestation, i.e. from day 100 to slaughter at day 135. The first group, MM, continued to receive the maintenance level of feeding throughout gestation, group MR was restricted from day 100 to day 135, group RM from day 50 to day 100 and group RR from day 50 to day 135. The daily ration was given as a single daily meal until day 114, after which it was given continuously by means of a conveyor belt in order to minimize fluctuations in nutrient supply and utilization.

Experimental Procedures

Parameters relating to digestion were studied using the markers ^{51}Cr-EDTA (Downes and McDonald 1964) and ^{103}Ru-labelled tris-(1, 10-phenanthroline)-ruthenium (II) chloride (^{103}Ru-phen) (Tan *et al.* 1971) as described by Faichney (1975*a*). The marker solution was made up to contain 37 kBq ^{51}Cr, 7.4 kBq ^{103}Ru and 0.3 mg Cr-EDTA per ml. Following a priming dose (30 ml), a continuous infusion of the marker solution into the rumen was begun on day 131 and maintained at 47 ml/day until slaughter at day 135. Daily urine output during the infusion was collected into sufficient HCl to maintain pH <2.

Urea labelled with ^{14}C was infused continuously for 9 h into the maternal jugular vein or the fetal femoral vein on day 132 and into the fetal femoral vein or the maternal jugular vein on day 134. The infusate contained 25.9 kBq [^{14}C]urea per ml sterile 0.9% NaCl, to which was added 0.8 mg urea/ml as carrier, and was infused at *c.* 10 ml/h. Blood samples were taken from the maternal carotid artery and the fetal dorsal aorta at intervals throughout the infusions. Each 2 ml sample of blood was lysed by mixing with 2 ml water in a tube held in an ice slurry; 4 ml 10% w/v trichloracetic acid was added and, after mixing, the tubes were allowed to stand in the slurry for 15 min before centrifugation. The supernatants were stored at -10°C.

On day 133, blood samples were taken into heparinized syringes on three occasions at intervals of 2 h. The plasma was separated by centrifugation and stored at -10°C. On day 135 the ewes were killed by the intravenous injection of concentrated pentobarbitone sodium, after which the abdomen was opened. The gastro-intestinal (GI) tract was removed and the contents of the rumen, abomasum and caecum-proximal

colon were weighed and sampled as described by Faichney and Barry (1986). The gravid uterus was then removed, weighed and separated into its components. The weights of the fetus, the fluids, the cotyledons (placenta), the cord plus membranes, and the uterus were recorded; the weight loss on dissection averaged 110 g (*c.* 2%).

Analytical

Urea in plasma and blood supernatant was determined by a modification (Technicon Clinical Method No. 01) of the method of Marsh *et al.* (1965). Blood supernatant was assayed for ^{14}C by liquid scintillation counting. One ml of sample solution was added to 10 ml scintillation fluid (3 g *p*-terphenyl + 0.1 g 1,4-bis, 2-(phenyloxazolyl) benzene in 1 litre toluene plus 500 ml Teric X10) and was assayed in a model 3375 Tri-Carb spectrometer (Packard Instrument Co., Illinois). Counting efficiency was determined by the addition of an internal standard. Other methods of analysis were those used by Faichney and White (1988*b*).

Calculations

The concentrations of ^{51}Cr-EDTA in digesta and fluid were corrected for absorption from the GI tract on the basis that absorption is proportional to sectional mean retention time (Faichney 1975*b*). The mean retention times of ^{51}Cr-EDTA (solutes) and ^{103}Ru-phen in the rumen, abomasum and caecum-proximal colon were calculated by the continuous infusion-total sampling procedure (Faichney 1975*a*). Values reported for the caecum-proximal colon are the mean of those for ^{51}Cr-EDTA and ^{103}Ru-phen because digesta constituents do not behave independently distal to the pylorus (Faichney 1975*b*; Faichney and Boston 1983). Faecal output was estimated using ^{103}Ru-phen as the marker and digesta flow was calculated by the double-marker technique (Faichney 1975*a*, 1980) using ^{51}Cr-EDTA and indigestible acid-detergent lignin as the markers (Faichney 1982). The rumen mean retention time of particles was calculated as the ratio rumen pool: faecal output of acid detergent lignin (Faichney 1980).

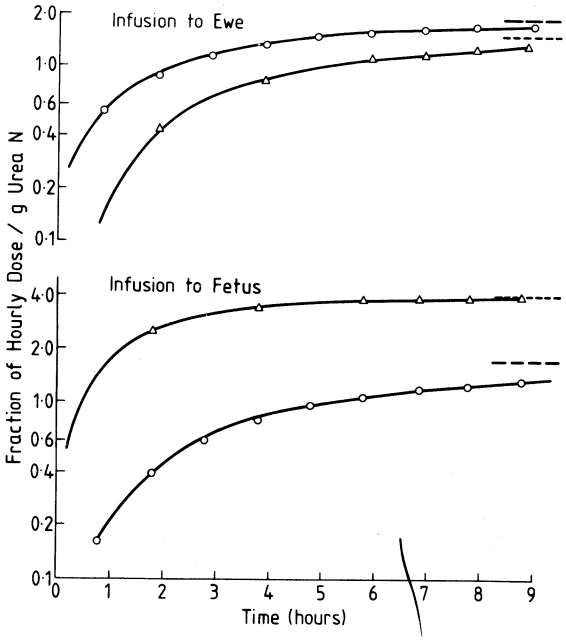

Fig. 1. Specific radioactivity of blood urea in a ewe (○) and her fetus (△) during continuous infusions of [^{14}C] urea. Calculated plateau values are shown for the ewe (— —) and her fetus (- - -).

The plateau value for specific radioactivity (SR) of urea, which was expressed as a proportion of the infusion rate, was determined from the relationship between SR and time (t) using the equation

$$SR = A - B \exp(-Ct),$$

where A is the plateau SR and B and C are constants. The curves obtained for one ewe are shown in Fig. 1. Urea N flows between the maternal and fetal pools were calculated from the plateau SR values assuming no significant metabolic change between days 132 and 134. Irreversible loss from an infused pool was calculated by dividing the infusion rate by that pool's plateau SR; transfer quotients, i.e. the fraction of the urea in the non-infused pool that originated in the infused pool, were calculated by dividing the plateau SR of the non-infused pool by that of the infused pool. Initially, the unrestricted, two-pool model was used (Faichney and White 1980) and it was found that there was no significant irreversible loss from the fetal pool other than via the maternal pool. It was therefore assumed that differences in maternal plateau SR values between the maternal and fetal infusions were due to experimental error. The restricted, two-pool model used to calculate urea flows is shown in Fig. 2.

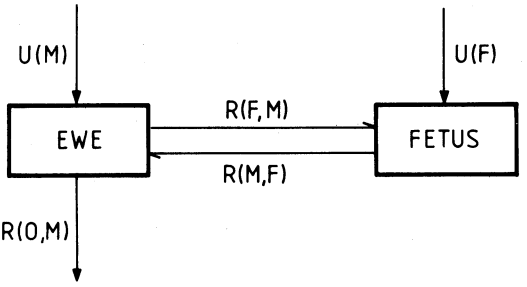

Fig. 2. Model used to describe the synthesis (U) of urea in a ewe (M) and her fetus (F), its flow (R) between the ewe and fetus and its irreversible loss (O).

Seventeen ewes were subjected to the surgical and measurement procedures: 5 in group MM, 4 in group MR, 5 in group RM and 3 in group RR. No urea kinetic data were available for one ewe in group MR because a fault developed in the infusion pump during [^{14}C]urea infusion. Two ewes in group MM did not eat their full ration after surgery, and one ewe in group RM refused some feed during the measurement period and was subsequently found to be hypoglycaemic; as these were no longer on the maintenance level of feeding, their urea kinetic data have not been included in the group means. One ewe in group RR did not eat all the feed offered during the measurement period; its intake and digestion data were discarded.

The treatments were compared by one-way analysis of variance and the pooled standard deviation of the means, i.e. root error mean square, is reported.

Results

Digestion

The treatments did not affect the digestibility of organic matter (Table 1). The passage of non-ammonia N to the intestines indicated a net gain due to recycling of N to the rumen; the differences between treatments reflected the differences in intake.

Retention of N was reduced ($P < 0.01$) when feed intake was restricted, particularly in the chronically restricted ewes which also showed a doubling of urine acid excretion as ammonium ($P < 0.05$). There were no significant differences in the proportion of the organic matter digestion occurring in the rumen nor in the proportion represented by crude protein digestion in the intestines.

The digesta load and metabolite concentrations in the rumen at slaughter on day 135 were related to feed intake (Table 2); a similar tendency was apparent in the digesta content of the caecum-proximal colon. The mean retention times of digesta constituents in the rumen were not affected but that of the particle-associated marker, ^{103}Ru-phen, in the abomasum was higher ($P < 0.05$) in the restricted ewes (groups MR and RR). Digesta mean retention times in the caecum-proximal colon were also higher ($P < 0.05$) in these ewes.

Weight Changes

The body weight of group MM ewes at day 135 was almost the same as their liveweight at mating (Table 3). Group RM ewes lost only 1.8 kg whereas group MR lost 2.9 and group

RR lost 7.2 kg. Fetal growth was not affected by dietary restriction during the last third of gestation (MR cf. MM) but chronic restriction (RR) reduced fetal growth by 10%. By contrast, restriction during mid-gestation followed by a return to maintenance feeding (RM) resulted in an increase of about 12% ($P < 0.10$) in fetal growth to day 135. Placental size increased by 44% ($P < 0.05$) in RM ewes and by 30% ($P < 0.10$) in group MR and 23% in group RR. The amount of fetal fluids tended to be higher in group RM. As a result of these changes, the gravid uterus in group RM ewes was heavier ($P < 0.05$) than in the other groups.

Table 1. Effect of nutritional regime on organic matter (OM) and N digestion and on N retention by ewes at day 135 of gestation

MM = maintenance throughout; MR = restriction days 50–100; RM = restriction days 100–135; RR = restriction days 50–135. Number of ewes in parentheses. $*P < 0.05$, $**P < 0.01$. ^{a,b,c}Means within rows with the same superscript do not differ at $P < 0.05$. RDOM = organic matter digested in the rumen; DOMI = digestible organic matter; DCP_i = crude protein (non-ammonia N × 6.25) digested in the intestines

	MM (3)	MR (4)	RM (4)	RR (2)	Pooled s.d.
OM intake (g day^{-1})	753	417	751	418	
OM digestibility	0.694	0.711	0.717	0.704	0.0234
N intake (g day^{-1})	19.3	11.0	19.8	10.8	
Non-ammonia N:					
Entering intestines (g day^{-1})	22.2^a	14.2^b	21.3^a	12.1^b	3.40*
Fraction digested in intestines	0.803	0.826	0.790	0.796	0.0343
Urine N (g day^{-1})	7.9^a	4.8^b	7.2^a	6.5^a	0.94**
N retention (g day^{-1})	7.2^a	3.8^b	8.3^a	1.8^c	0.83**
Urine ammonium (meq. day^{-1})	4.5^a	5.1^a	5.0^a	10.7^b	2.24*
RDOM/DOMI (g kg^{-1})	653	574	602	578	71.2
DCP_i/DOMI (g kg^{-1})	215	248	197	207	54.1

Urea N Transactions

The values for the urea N fluxes calculated from the maternal and fetal irreversible losses and transfer quotients using the restricted model (Fig. 2) are presented in Table 4. Dietary restriction in late pregnancy (MR) reduced maternal synthesis and irreversible loss by, respectively, 52% ($P < 0.05$) and 45% ($P < 0.05$); by contrast, chronic restriction (RM) reduced them by only 35% ($P < 0.05$) and 25% ($P < 0.10$). Fetal urea synthesis was affected ($P < 0.10$) by the variations in maternal nutrition; it increased by 15% when ewes were restricted in late gestation but by 60% when chronically restricted. Fetal urea synthesis was 40% greater in fetuses of ewes that had been restricted in mid-gestation (RM) than in those of ewes maintained throughout gestation (MM). When expressed in terms of body weight, maternal urea synthesis was reduced by 69% by feed restriction in late gestation (Table 5) but, with chronic restriction, it was reduced by only 25%, being 55% greater than in the ewes restricted in late gestation. Fetal urea synthesis per kg of fetal weight increased by 20% when ewes were restricted in late gestation but by 80% when chronically restricted; in fetuses of ewes restricted in mid-gestation it increased by 27%. Fetal synthesis was less than twice the rate of maternal synthesis when ewes were maintained throughout gestation and about twice the maternal rate when dietary restriction was imposed in mid-gestation. However, fetal synthesis increased to about four times the maternal rate during dietary restriction (MR and RR). It was calculated that the maximum contribution that could be made by amino acid oxidation to fetal oxygen consumption was 32% when ewes were maintained throughout gestation, increasing to about 40% when restrictions were imposed in mid or late gestation, but was considerably higher at nearly 60% when chronic dietary restriction, sufficient to affect fetal growth, was imposed.

The amounts of urea degraded in the maternal GI tract, urinary urea excretion and the renal clearance of urea showed similar patterns of response to that seen with maternal irreversible loss. Thus restriction in late gestation resulted in substantial decreases but the effect was less in chronically restricted ewes (Table 5). As a result, the proportion of the urea synthesized that was degraded in the GI tract was similar for all groups.

Table 2. Effect of nutritional regime on water intake and on rumen metabolite concentrations, rumen digesta load and mean retention times in the rumen, abomasum and caecum-proximal colon of ewes at day 135 of gestation

MM = maintenance throughout; MR = restriction days 100–135; RM = restriction days 50–100; RR = restriction days 50–135. Number of ewes in parentheses. †$P < 0.10$, *$P < 0.05$, **$P < 0.01$. ^{ab}Means within rows with the same superscript do not differ at $P < 0.05$. $^{\alpha\beta}$Means within rows with the same superscript do not differ at $P < 0.01$

	MM (3)	MR (4)	RM (4)	RR (2)	Pooled s.d.
Water intake[A] (l day^{-1})	3.54	6.20	4.20	4.84	1.640
Rumen volatile fatty acids:					
Total (mM)	117^{α}	96^{β}	113^{α}	84^{β}	9.8**
Acetic (molar %)	67.3	69.9	67.4	69.9	2.49
Propionic (molar %)	16.7	16.6	15.8	12.6	3.15
Butyric (molar %)	12.7	9.7	12.7	13.3	2.28
Rumen ammonia N (mg l^{-1})	286	245	304	264	49.8
Digesta load:					
Rumen (kg)	2.34^{α}	1.56^{β}	2.48^{α}	1.59^{β}	0.196**
Abomasum (g)	264	212	241	370	88.7
Caecum-proximal colon (g)	598^{ab}	503^{a}	674^{b}	556^{ab}	85.6†
Mean retention time (h)					
Rumen: Solutes	6.30	7.23	6.57	7.80	1.522
^{103}Ru-phen	10.5	11.8	11.2	11.7	2.73
Particles	34.7	34.5	32.4	33.6	7.35
Abomasum: Solutes	0.63^{a}	0.80^{a}	0.69^{a}	1.63^{b}	0.333*
^{103}Ru-phen	1.68^{a}	2.82^{ab}	1.76^{a}	4.53^{b}	0.899*
Caecum-proximal colon	7.57^{a}	14.0^{b}	9.32^{a}	13.3^{ab}	2.178*

[A]Water intake is the mean for the 7 days prior to slaughter.

Table 3. Effect of nutritional regime on ewe body weight (liveweight less gravid uterus) at slaughter (day 135 of gestation) and the weight of the gravid uterus and its components

MM = maintenance throughout; MR = restriction days 100–135; RM = restriction days 50–135; RR = restriction days 50–135. Number of ewes in parentheses. *$P < 0.05$. ^{CD}Means within rows with the same superscript do not differ at $P < 0.10$. ^{ab}Means within rows with the same superscript do not differ at $P < 0.05$

	MM (5)	MR (4)	RM (5)	RR (3)	Pooled s.d.
Liveweight (kg, day 0)	43.7	45.1	44.8	44.8	4.54
Bodyweight (kg, day 135)	43.5^{a}	42.2^{a}	43.0^{a}	37.6^{b}	2.38*
Gravid uterus (kg)	5.21^{a}	5.54^{ab}	6.29^{b}	4.98^{a}	0.620*
Fetus (kg)	3.36^{ab}	3.31^{ab}	3.78^{a}	3.03^{b}	0.332
Placenta (g)	337^{a}	437^{ab}	485^{b}	413^{ab}	77.2*
Cord + membranes (g)	246	272	301	252	64.8
Fluids (g)	607^{CD}	838^{CD}	971^{C}	588^{D}	242.0
Uterus (g)	545	582	648	571	82.1

Plasma Metabolites

Plasma glucose concentrations were reduced by 27% ($P < 0.01$) in ewes restricted in late gestation and by 45% ($P < 0.01$) in chronically restricted ewes (Table 6). Fetal plasma glucose concentrations reflected these changes; there was a close relationship between fetal and maternal plasma glucose concentrations which is shown in Fig. 3. Maternal plasma D-3-OH-butyrate concentrations increased as those of glucose fell (Fig. 4); D-3-OH-buytrate concentrations exceeded 100 mg/l^{-1} only for MR and RR ewes and the two MM ewes that refused some feed. Fetal urea synthesis was inversely related to fetal plasma glucose concentrations; the relationship is shown in Fig. 5.

Table 4. Effect of nutritional regime on urea N exchange between ewes and their fetuses on day 133 of gestation (see Fig. 2)

MM = maintenance throughout; MR = restriction days 100–135; RM = restriction days 50–100; RR = restriction days 50–135. Number of ewes in parentheses. †P < 0.10, * P < 0.05, **P < 0.01. ^{DEF}Means within rows with the same superscript do not differ at $P < 0.10$. ^{ab}Means within rows with the same superscript do not differ at $P < 0.05$

	MM (3)	MR (3)	RM (4)	RR (3)	Pooled s.d.
Urea flux (mg N h^{-1}):					
Maternal synthesis (U(M))	588^{a}	282^{b}	544^{a}	380^{b}	70.1**
Fetal synthesis (U(F))	72^{D}	83^{DE}	101^{EF}	115^{F}	19.0†
To fetus from ewe (R(F,M))	396^{a}	157^{b}	362^{a}	360^{ab}	103.4†
To ewe from fetus (R(M,F))	468^{D}	240^{E}	463^{D}	475^{D}	114.4†
Maternal irreversible loss (R(O,M))	660^{a}	365^{b}	645^{a}	495^{a}	81.3**
Fetal irreversible loss	289^{a}	168^{b}	295^{a}	273^{a}	53.3*
Transfer quotients (fraction):					
To fetus from ewe	0.84^{a}	0.65^{b}	0.78^{a}	0.76^{a}	0.041**
To ewe from fetus	0.43^{a}	0.46^{ab}	0.46^{ab}	0.55^{b}	0.051†
Fetal synthesis					
(Fraction of maternal irreversible loss)	0.11^{a}	0.23^{b}	0.16^{a}	0.23^{b}	0.029**
(Fraction of fetal irreversible loss)	0.25^{a}	0.49^{b}	0.34^{ac}	0.42^{bc}	0.048**

Table 5. Effect of nutritional regime on maternal and fetal urea N synthesis and on maternal urea N recycling and excretion in sheep on day 133 of gestation

MM = maintenance throughout; MR = restriction days 100–135; RM = restriction days 50–100; RR = restriction days 50–135. Number of ewes in parentheses. †$P < 0.10$, *$P < 0.05$, **$P < 0.01$. ^{CD}Means within rows with the same superscript do not differ at $P < 0.10$. ^{abc}Means within rows with the same superscript do not differ at $P < 0.05$. Amino acid oxidation was calculated assuming that fetal oxygen consumption was 420 ml h^{-1} kg^{-1} (Battaglia and Meschia 1978) and that 6.183 ml O_2/mg N was required for complete oxidation of protein (16% N, 53% C)

	MM (3)	MR (3)	RM (4)	RR (3)	Pooled s.d.
Maternal synthesis (mg N h^{-1} kg^{-1})	13.6^{a}	6.6^{b}	12.6^{a}	10.2^{a}	1.99**
Fetal synthesis (mg N h^{-1} kg^{-1})	21.5^{a}	25.7^{ab}	27.3^{ab}	38.8^{b}	4.72†
Fetal : maternal ratio	1.6^{a}	3.9^{b}	2.2^{a}	3.8^{b}	0.71**
Fetal amino acid oxidation					
(Fraction of oxygen consumption)	0.32^{a}	0.38^{ab}	0.40^{ab}	0.57^{b}	0.113†
Maternal metabolism:					
Degraded in gut (mg N h^{-1})	415^{ab}	247^{c}	427^{a}	300^{bc}	60.4*
(Fraction of irreversible loss)	0.633	0.675	0.656	0.602	0.0631
Urine excretion (mg N h^{-1})	245^{a}	118^{b}	224^{a}	195^{ab}	43.8*
Renal clearance (ml min^{-1})	34.6^{C}	25.1^{D}	34.4^{C}	27.9^{CD}	4.82†

The concentrations of α-amino N (i.e. total amino acids) were much higher in fetal than maternal plasma and were not affected by maternal dietary restriction (Table 6). Maternal concentrations were maintained in all except the chronically restricted ewes in which amino acid concentrations fell by 30% ($P < 0.01$).

Table 6. Effect of nutritional regime on plasma glucose, α-amino N and urea N in pregnant ewes on day 133 of gestation

MM = maintenance throughout; MR = restriction days 100–135; RM = restriction days 50–100; RR = restriction days 50–135. Number of ewes in parentheses $*P < 0.05$, $**P < 0.01$. ^{ab}Means within rows with same letter do not differ at $P < 0.05$. $^{\alpha\beta\gamma}$Means within rows with same letter do not differ at $P < 0.01$.

		MM (3)	MR (3)	RM (4)	RR (3)	Pooled s.d.
Plasma glucose (mg l^{-1})	ewe	654^{α}	478^{β}	634^{α}	360^{γ}	55.7**
	fetus	192^{a}	138^{ab}	168^{ab}	110^{b}	32.9*
Plasma α-amino N (mg l^{-1})	ewe	36^{α}	33^{α}	34^{α}	25^{β}	2.0**
	fetus	66	65	65	67	6.3
Plasma urea N (mg l^{-1})	ewe	118	79	108	125	32.2
	fetus	143	99	130	147	35.1

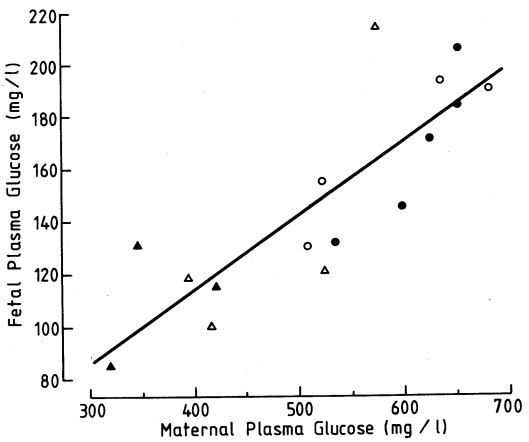

Fig. 3. Relationship between fetal and maternal plasma glucose concentrations.
○ Group MM
△ Group MR
● Group RM
▲ group RR
The line of the fitted equation $y = 1.09 + 0.282x$, $r^2 = 0.706$ is shown.

Urea N concentrations were always higher in fetal than in maternal plasma; the close relationship between them is shown in Fig. 6 and was described by the equation

$$y = 15.1 + 1.07x, \qquad r^2 = 0.972,$$

where y is the fetal and x the maternal concentration (mg N l^{-1}). Maternal concentrations were reduced by 33% in ewes restricted in late gestation (MR) but, in chronically restricted ewes (RR), urea concentrations were 6% higher than in ewes maintained throughout gestation (Table 6) and nearly 60% greater than in the ewes restricted in late gestation. Changes in fetal concentrations were of similar magnitude as would be expected from the close relationship between fetal and maternal concentrations (Fig. 6).

Discussion

Digestion

There were no differences between the treatments in the digestibility of the diets in this experiment so that differences in nutrient supply reflected the differences in feed intake. In view of the effect of feed intake on digesta mean retention times and digestibility (Faichney 1986; Faichney and Gherardi 1986), an effect of intake may have been expected here. However, gestation substantially reduces rumen mean retention times (Faichney and White 1988*a*), leading to an increased supply of protein to the tissues of the pregnant ewe (Faichney and White 1988*b*). The rumen mean retention times recorded for groups MM and RM were similar to those reported by Faichney and White (1988*a*) and it may well be that the effect of gestation masked any effect due to feed intake.

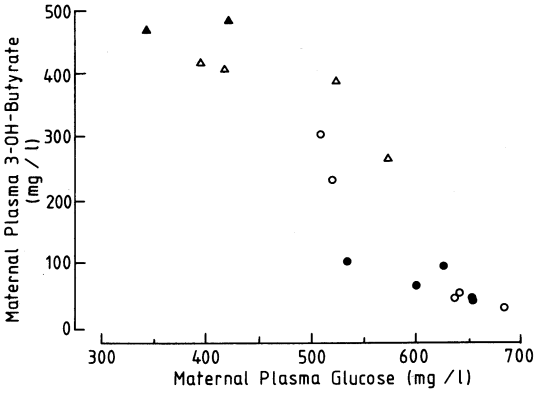

Fig. 4. Relationship between plasma D-3-OH-butyric acid and glucose concentrations in ewes on day 133 of gestation.
○ Group MM.
△ Group MR.
● Group RM.
▲ Group RR.
$r = -0.928$.

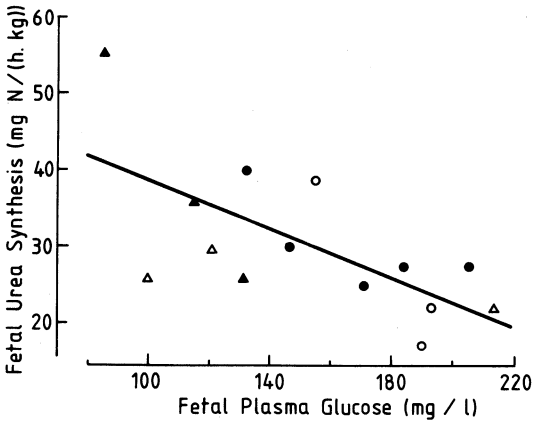

Fig. 5. Relationship between fetal urea synthesis and fetal plasma glucose concentration.
○ Group MM.
△ Group MR.
● Group RM.
▲ Group RR.
The line of the fitted equation $y = 53.5 - 0.158x$, $r^2 = 0.477$ is shown.

Weight Changes

Placental weight in sheep is generally considered to plateau by about day 100 (Alexander 1974; Robinson *et al.* 1977), and can be reduced by underfeeding (Alexander 1978; Mellor 1983), but the dietary restrictions imposed in this experiment appear to have resulted in increased placental size, even when imposed after day 100. We interpret these increases as an attempt by the fetus to compensate for the reduced supply of nutrients in the maternal blood.

This placental compensation appears to have been sufficient to maintain fetal growth in the ewes restricted from day 100 at the same rate as in the unrestricted ewes. Prolonged dietary restriction tended to reduce fetal growth as would be expected (Black 1983; Mellor 1983) but realimentation after restriction between days 50 and 100 resulted in enhanced growth. Thus it appears that the extra placental capacity stimulated by the restriction enhanced the supply of nutrients to the fetus once the restriction was removed. Russell *et al.* (1981) reported enhanced fetal growth under similar conditions in heavier ewes but not in lighter ewes in which fetal growth was reduced. It would seem that whether fetal growth is reduced, unaffected or enhanced by maternal undernutrition depends on the stage of gestation at which it occurs, its duration and its severity; there are not yet sufficient data available to predict the outcome with confidence.

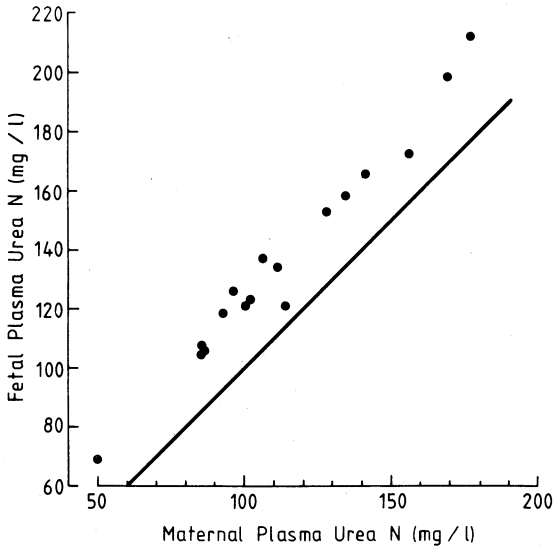

Fig. 6. Relationship between fetal and maternal plasma urea N concentrations. The line of equality is shown.

Treatment MM resulted in the ewes having the same body weight at day 135 as their liveweight at mating. It can be concluded that MM ewes were well nourished because plasma glucose concentrations remained high and D-3-OH-butyrate concentrations remained low (Mellor 1983). The ewes restricted between days 50 and 100 were also well-nourished but had lost some weight and were supporting a higher fetal growth rate. The undernourished ewes (MR, RR) were mobilizing body reserves to meet fetal growth requirements but, whereas the ewes restricted from day 100 were able to support 'normal' fetal growth with a loss of *c.* 3 kg body weight, those restricted from day 50 had lost *c.* 7 kg and were apparently unable to sustain 'normal' fetal growth.

Urea Metabolism

The placental clearance of urea in sheep was estimated by Gresham *et al.* (1972) to be *c.* 15 mg N h^{-1} kg^{-1}, which is somewhat less than the rate of synthesis in fetuses of well-nourished ewes in the present experiment (21.5 mg N h^{-1} kg^{-1}). These results may not be inconsistent because urea probably passes from the fetal fluids to the non-placental uterine circulation (Gresham *et al.* 1972; Faichney *et al.* 1981). The results reported here suggest that as much as a third of fetal oxygen consumption could have been accounted for by amino acid oxidation. This proportion increased with maternal undernutrition, reaching nearly 60% in

the fetuses of the ewes restricted from day 50. In these ewes the fetal drain on maternal tissue resources must have been substantial and was associated with reduced plasma amino acid concentrations. Simmons *et al.* (1974) reported that placental clearance of urea increased to about twice the fed-state values when pregnant ewes were fasted for 4 days, a finding consistent with the 80% increase in urea synthesis observed here after prolonged undernutrition (Table 5). In the fetuses of ewes restricted in mid-gestation and in which growth rate was enhanced, the maximum contribution of amino acid oxidation to fetal oxygen consumption was 40%.

The data obtained in this study suggest that amino acids are the key energy metabolites in the sheep fetus despite the importance of glucose and lactate as fetal fuels (Battaglia and Meschia 1978; Girard *et al.* 1979). Amino acids are normally taken up in excess of fetal requirements for tissue accretion (Lemons *et al.* 1976) by active transport mechanisms (Young 1979), their concentrations are usually two or more times the maternal concentrations (Girard *et al.* 1979; Young 1979; Table 6) and fetal concentrations can be maintained even when maternal concentrations fall (Table 6). By contrast, fetal glucose concentrations decline with maternal concentrations (Fig. 3) in response to undernutrition. The increase in urea synthesis as fetal glucose concentrations decline (Fig. 5) suggests the replacement of glucose as a fuel by amino acids.

Hodgson *et al.* (1982) reported a urea synthesis rate of 40.0 mg N h^{-1} g^{-1} in fetuses of well-nourished ewes. This value is nearly twice the value of 21.5 mg N h^{-1} kg^{-1} obtained in our experiment. Their value for fetuses of undernourished ewes was 16% greater than the present value (group RR). A urea synthesis rate of 40 mg N h^{-1} kg^{-1} suggests that amino acid oxidation could have accounted for up to 59% of fetal oxygen consumption, which is far too high for fetuses of well-nourished ewes. Consideration of the fetal N balance (Faichney 1981) suggests that normal fetal growth could not be maintained in association with such high rates of urea synthesis. It can be seen from Fig. 1 that isotopic equilibrium requires a continuous infusion in excess of 9 h. It is possible that Hodgson *et al.* (1982) underestimated the equilibrium specific radioactivity of urea and hence overestimated production rates at least in their undernourished, monotocous ewes. However, they used a primed infusion in their well-nourished ewes so, as four of their five estimates were in dictocous ewes, the most likely reason for their overestimation of urea production is their method of calculation. As samples were not taken from the uninfused fetus and infusions were not made into each fetus, proper solution of their 3-pool model was not possible and the estimate obtained must include urea produced in both fetuses.

Conclusions

The results of this experiment show that fetal and placental growth and fetal metabolism were affected not only by the current nutritional status of the ewe but also by her nutritional history. Moderate dietary restrictions stimulated placental growth to the extent that fetal growth could be maintained when the restriction occurred during the third trimester but not when applied throughout the second and third trimesters. The extra placental capacity available to fetuses in ewes restricted during the second trimester resulted in enhanced fetal growth. Amino acid oxidation, indicated by fetal urea synthesis, increased as glucose levels declined during maternal dietary restriction. Up to 32% of fetal oxygen consumption could have been accounted for by amino acid oxidation, increasing to 40% in fetuses with enhanced growth rates and nearly 60% in those of chronically undernourished ewes, suggesting that amino acids are key energy substrates for fetal metabolism.

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