Effect of maternal body condition on placental and fetal growth and the insulin-like growth factor axis in Dorset ewes

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This study investigated the effects of maternal body condition on fetal growth. Fetal and placental parameters from Dorset ewes of body condition score 2.0 (lean, $n=5$), 3.5 (moderate, $n=7$) and 5.0 (fat, $n=4$) at mating were studied on day 65 of gestation. The fetal weight and fetal weight:crown–rump length ratio were greater in fat ewes than in ewes of moderate condition. The raised total and mean placentome weight in fat ewes compared with ewes of moderate condition may have contributed to their increased fetal growth. However, the fetal crown–rump length was not affected. With *in situ* hybridization, insulin-like growth factor II (IGF-II) mRNA and insulin-like growth factor binding protein 2 (IGFBP-2), -3 and -6 were all detected in the placentome capsule; IGF-II mRNA was also found in the mesoderm of the fetal villi and IGFBP-3 and IGFBP-6 were present in the caruncular stroma of the maternal villi. Ewes of moderate condition, which had the smallest placentae, had the greatest placental expression

of IGF-II, IGFBP-2 and IGFBP-3. In the intercotyledonary endometrium, IGFBP-3, IGFBP-5 and uterine milk protein (UTMP) mRNA were all expressed in the glandular epithelium. IGFBP-3 and IGFBP-5 absorbance values were lowest in the lean ewes, whereas UTMP values were highest. Maternal insulin concentrations were greater in fat ewes, whereas plasma glucose and IGF-I concentrations in the fetal compartment were lowest in fat ewes. Therefore, in obese ewes, fetal and placental growth is increased in mid-gestation in association with higher maternal insulin concentrations and lower expression of IGFBPs in the maternal placentomes. Placental and fetal development in lean ewes may be promoted by reduced IGFBP expression in the placentomes and enhanced UTMP production by the endometrial glands. The ewes of moderate condition had the smallest fetuses and placentae coupled with the highest placental expression of IGF-II and IGFBPs.

Introduction

Although the fetal genome undoubtedly influences fetal size, the intrauterine environment and in particular the fetal nutrient supply is also a major determinant of fetal growth (Walton and Hammond, 1938; Carr-Hill *et al.*, 1987; Gluckman *et al.*, 1990; Harding and Johnston, 1995). Various consequences of fetal under-nutrition present at birth, with low-birth weight lambs, for example, having an increased incidence of death from hypothermia, infection and starvation (Alexander, 1974). Complications also arise in later life: human epidemiological studies reveal an increased predisposition to infertility (Ibnaez *et al.*, 2000), cardiovascular disease and non-insulin-dependent diabetes in adults born with a low-birth weight (Barker, 1995). In sheep, unsuitable maternal nutrition during pregnancy can compromise future wool (for review, see Black, 1983; Kelly *et al.*, 1996) and carcass quality (for review, see Bell, 1992) and reproductive performance (Gunn *et al.*, 1995; for review,

see Rhind *et al.*, 2001). The term ‘fetal programming’ is used to describe such permanent alterations in fetal development (for review, see Barker and Clark, 1997; McMillen *et al.*, 2001).

Inappropriate substrate delivery to the fetus may compromise fetal growth by modifying the nutritionally sensitive glucose–insulin–insulin-like growth factor I (IGF-I) axis within the fetal compartment (Fowden, 1995). Although the fetus develops its own endogenous reserves (Fowden, 1997), it depends primarily on the mother for nutritional support. Factors that regulate fetal substrate delivery are thus important determinants of fetal growth. Nutrients provided directly from the maternal compartment, via the placenta, are derived either from products of digestion after ingestion or as mobilized components of the maternal body reserves (McCrabb *et al.*, 1992a). Despite the impact of maternal diet on fetal growth having been addressed (Holst *et al.*, 1992; McCrabb *et al.*, 1992a; Heasman *et al.*, 1999; Osgerby *et al.*, 2002), the effects of maternal body condition are less well understood.

The placenta mediates the exchange of substrate and waste products between the dam and fetus, as well as

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moderating the composition of specific substrate (Chung *et al.*, 1998). The ewe has a cotyledonary placenta, with fetomaternal exchange taking place at 88–100 discrete sites known as placentomes (Latshaw, 1987). The number of placentomes is fixed by day 56 of gestation (Wallace, 1948), whereas placental proliferation peaks between days 50 and 60, and the placenta attains a maximum weight by day 80 (term 150; Ehrhardt and Bell, 1995). Inappropriate maternal nutrition in early–mid-pregnancy can disrupt placental development, with the condition of the mother at mating reportedly affecting this interaction (McCrabb *et al.*, 1992b).

Gene-deletion studies in mice indicate that the IGF system, a nutritionally sensitive group of proteins, has a fundamental role in placental as well as fetal development (Baker *et al.*, 1993; for reviews, see Wathes *et al.*, 1998; Han and Carter, 2000). IGF-I and IGF-II act via the type 1 IGF receptor (IGF-1R) to mediate cellular proliferation, differentiation and metabolism (Jones and Clemmons, 1995). IGF-2R acts as a degradative pathway, removing excess IGF-II from the circulation (Braulke, 1999). IGF binding proteins (IGFBPs), IGFBP-1–6, modulate the biological activities of the IGFs (Ferry *et al.*, 1999).

Endocrine factors within the maternal circulation may also influence fetal substrate availability, regulating nutrient partitioning between the maternal, placental and fetal compartments (Wallace *et al.*, 1997, 2001). Two closely related glucose transporters, GLUT1 and GLUT3, transport glucose across the placenta by facilitated diffusion (Bell *et al.*, 1999). Glucose regulates the expression of these transporters in a time- and concentration-dependent fashion (Das *et al.*, 1998, 2000). Insulin promotes lipogenesis within the maternal compartment, mediating glucose and amino acid uptake by the adipose and muscle tissue (Vernon *et al.*, 1981; McNeill *et al.*, 1997). IGF-I, in contrast, influences the placental transfer of glucose and amino acids (Kniss *et al.*, 1994; Liu *et al.*, 1994).

In addition to placentally derived substrate, factors of uterine origin also support fetal development. The uterine milk proteins (UTMPs) are the most prevalent proteins in the pregnant ovine uterine luminal fluid, and are considered to offer nutritional support to the fetus, in addition to providing other binding, carrier and immunomodulatory actions within the uterine space (Moffatt *et al.*, 1987; Skopets and Hansen, 1993; Hansen, 1998; McFarlane *et al.*, 1999).

This study aimed to determine the effects of maternal body condition on fetal growth and to elucidate the mechanisms involved. The effects of maternal body condition on factors regulating fetal substrate delivery were investigated, including placenta and maternal plasma concentrations of glucose, insulin and IGF-I. The effects of maternal body condition on placental growth were determined by investigating the uterine IGF and IGFBP mRNA axis using *in situ* hybridization on day

65 of gestation, during the highly proliferative phase of placental growth. The effect of body condition on UTMP expression was also studied.

Materials and Methods

Animals

All procedures were performed under the UK Animals (Scientific Procedures) Act 1986 and took place within the normal seasonal breeding cycle of the Dorset ewe (*Ovis aries*). Mature, multiparous Dorset ewes ($n = 19$) were fed to yield three groups of differing body condition scores. These scores were reached 12 weeks before mating to prevent a flushing effect before mating. Body condition scoring assesses the muscle and fat cover of an animal. In sheep, the condition is determined by careful palpation of the spinous and transverse processes in the loin area, immediately behind the last rib and above the kidney. This region provides an accurate indication of the present condition of the ewe, as condition is last to be laid down here but first to be lost (Henderson, 1990). The condition is interpreted on a scale of 1.0–5.0 where 1.0 is emaciated and 5.0 obese (based on the criteria of the Meat and Livestock Commission, 1988). In this study, ewes were 2.0 (lean, group L; $n = 5$), 3.5 (moderate, group M; $n = 8$) or 5.0 (fat, group F; $n = 6$). Maternal body condition and weight were assessed weekly throughout the study.

All animals were individually housed 9 weeks before mating to allow time for acclimatization. Ewes were housed on wheat-straw bedding under conditions of natural light and ambient temperature with free access to water. Ewes were fed a complete diet of pelleted sheep nuts providing 100% of their daily maintenance requirements to sustain condition (based on the criteria of the Meat and Livestock Commission, 1988). The complete diet was fed in two equal rations at 08:00 and 16:00 h daily and supplied 10.8 MJ metabolizable energy and 150 g crude protein per kg dry matter. The rations were reviewed weekly and altered according to changes in maternal body weight and the stage of gestation.

Oestrus was synchronized by withdrawing progestagen impregnated sponges (60 mg medoxyprogesterone acetate, Veramix; Upjohn Ltd, Crawley) 12 days after insertion. At sponge withdrawal, ewes received an injection of a prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) analogue, Estrumate (0.5 ml i.m.; Schering Plough Animal Health, Uxbridge) before being presented to two raddled Texel rams 48 h later. The ewes were checked for raddle marks (an indicator of mating) four times a day and day 0 of gestation was taken as the first date at which an obvious raddle mark was first observed. Pregnancy was confirmed by measuring plasma progesterone concentrations on day 16 of gestation using an enzyme immunoassay kit (Ridgeway Science Ltd, Alvington).

Measurements

On day 65 of gestation the gravid uterus was removed *post mortem* through a midline incision at a consistent point along the length of the cervix and weighed. Fetal blood samples were collected by cardiac puncture immediately before intra-cardiac administration of sodium pentobarbitone to the fetus. The blood was placed on ice for no more than 20 min before being centrifuged at 1500 *g* for 15 min at 4°C and then stored at –20°C until analysis. The fetus was blotted dry, weighed and sexed, and the crown–rump length was measured. From the body of the uterus four placentomes and six pieces of intercotyledonary endometrium (with fetal membranes attached) were dissected. All tissue samples were wrapped in aluminium foil, rapidly frozen in liquid nitrogen-tempered isopentane and stored at –80°C until required for sectioning. The remaining placentomes were dissected from the uterine wall, counted and individually weighed.

Blood sampling and assays

Maternal blood samples were collected weekly by jugular venepuncture from 3 weeks of gestation onwards (after confirmation of pregnancy), 5 h after the morning feed. These and the fetal blood samples were analysed for systemic insulin, glucose and IGF-I. Maternal plasma IGF-I was analysed by radioimmunoassay after ethanol–acetone–acetic acid extraction according to the method of Enright *et al.* (1989) using recombinant human IGF-I (rhIGF-I; Bachem Ltd, Saffron Waldon) as standard, iodinated rhIGF-I (iodogen method) as tracer, and antibody raised against rhIGF-I (Biogenesis Ltd, Poole). The detection limit was 8.6 ng ml^{–1} and the inter- and intra-assay coefficients of variation (CVs) were 8.3% and 3.1%, respectively. Fetal plasma IGF-I concentrations were measured by a two-site immuno-enzymatic assay kit (DRG Diagnostics, Immunodiagnostic Systems Ltd, Tyne and Wear). The detection limit was 6.0 ng ml^{–1} and the inter- and intra-assay coefficients of variation were 1.18% and 2.5%, respectively. Plasma insulin was measured by ELISA (DRG Diagnostics, Immunodiagnostic Systems Ltd, Tyne and Wear). The detection limit was 0.1 ng ml^{–1} and the inter- and intra-assay CVs were 7.1% and 3.2%, respectively. Maternal plasma glucose was measured by an enzymatic colorimetric method according to the method of Werner *et al.* (1971), using a glucose reagent from Roche Diagnostics (Lewes). Colour development was monitored at 420 nm. The detection limit was 1.5 mmol l^{–1} and the inter- and intra-assay CVs were 2.7% and 2.2%, respectively. Fetal plasma glucose was below the limit of detection of this technique and was therefore measured using an automated glucose analyser (Bayer Opera, Business Group Diagnostics, Tarrington, NY).

In situ hybridization

Oligonucleotide probes. All probes used were single-stranded oligonucleotides (Babraham Institute, Cambridge). Sense probes were always included as negative controls as any signal produced on applying this probe could be regarded as non-specific. IGF-II, IGFBP-2, IGFBP-3 and IGFBP-6 mRNA were investigated in the placentomes. IGFBP-3, IGFBP-5 and UTP mRNA were studied in the intercotyledonary tissue (the areas of endometrium between the placentomes). These probes were chosen for each region based on previous studies on the localization of the placental IGF system during ovine pregnancy (Reynolds *et al.*, 1997; Gadd *et al.*, 2000a,b). All probes used in the study are described (Table 1).

Localization of mRNA by in situ hybridization. This procedure was performed as described by Reynolds *et al.* (1997). All chemicals were purchased from Sigma Chemical Co. (Poole) or BDH (Poole), unless otherwise stated. In summary, frozen placentome and intercotyledonary tissue sections were cut (10 µm thick) and thaw-mounted onto 1 mg ml^{–1} poly-L-lysine (*M_r* > 300 000)-coated glass slides. Sections were fixed in 4% (w/v) paraformaldehyde in 0.01 mol PBS l^{–1} at pH 7.0 for 5 min at room temperature (20°C), before three washes in 0.01 mol PBS l^{–1} and sequential dehydration in 70% and 95% ethanol.

The oligonucleotide (5ng) was labelled with [³⁵S] dATP (Amersham International, Aylesbury) at the 3'-end using deoxynucleotidyl transferase (Pharmacia Biotech, St Albans) at 34°C for 60 min. The labelled probe was subsequently diluted to a final concentration of 1:100 000 c.p.m. ml^{–1} in hybridization buffer and 100 µl was added to each section. The sections were incubated in a humidified box overnight at 42.5°C. After incubation, slides were washed in 1 × SSC 0.2% (w/v) sodium thiosulphate pentahydrate solution at room temperature for 30 min, then at a higher stringency in 1 × SSC 0.2% (w/v) sodium thiosulphate pentahydrate solution for 60 min at 57.5°C. Sections were finally rinsed in 1 × SSC, 0.1 × SSC, 70% ethanol, and 95% ethanol, for 1 min each. Slides were then air-dried for at least 2 h and exposed to β-max hyperfilm (Amersham International) for the time indicated (Table 1). Uterine samples shown to be positive for each of the probes (Reynolds *et al.*, 1997; Wathes *et al.*, 1998) were included in each experiment as a positive control.

Photographic emulsions

Slides previously exposed to X-ray film were coated with a photographic emulsion (LM1; Amersham International) according to the manufacturer's instructions and stored at 4°C for the time indicated (Table 1). Slides were then developed in 20% phenisol (v/v), fixed in 1.9 mol sodium thiosulphate-pentahydrate l^{–1},

Table 1. The sequence of the antisense probes and exposure times for *in situ* hybridization, X-ray films and emulsions

Probe	Antisense sequence	Exposure time (days)	
		X-ray film	Emulsion
IGF-II	554–598 of ovine IGF-II gene (O'Mahoney and Adams, 1989) 5'-AAC-TGG-AGG-GTG-TCC-ACC-AGC-TCC-CCG-CCG-CAC-AGA-GTC-TCG-CTG-3'	21	28
IGFBP-2	452–496 of ovine IGFBP-2 gene (Delhanty and Han, 1992) 5'-CAG-AGT-GCT-CCT-CCG-CAT-TGT-CTG-CAA-CCT-GCT-CCG-GGC-TGG-CCG-3'	30	37
IGFBP-3	546–578 of bovine IGFBP-3 gene (Spratt <i>et al.</i> , 1991) 5'-AGC-CTG-GTT-CTC-TGT-GCT-CCC-CAT-GCT-GTG-GTC-3'	30	37
IGFBP-5	291–335 of bovine IGFBP-5 gene (Moser <i>et al.</i> , 1992) 5'-TCG-GAG-ATG-CCG-GTG-TGC-TTG-GGC-CCG-AAG-ATC-TTG-GGC-GAG-TAG-3'	21	28
IGFBP-6	292–336 of bovine IGFBP-6 gene (Moser <i>et al.</i> , 1992) 5'-CCG-CTT-CCG-GTA-GAA-GCC-CCT-ATG-GTC-ACA-ATT-AGG-CAC-GTA-GAG-3'	30	37
UTMP	1210–1254 of ovine UTMP gene (Ing and Roberts, 1989) 5'-GTT-GAA-CTT-AAC-AAC-CAC-CCG-GAC-CTC-CTT-CGT-GTT-CCG-GGG-GAC-3'	10	17

IGF-II: insulin-like growth factor II; IGFBP: insulin-like growth factor binding protein; UTMP: uterine milk protein.

and counterstained with haematoxylin and eosin for microscopic confirmation of the cellular localization of the radioactive signal.

Absorbance measurements

An image analysis system (Seescan plc, Cambridge) was used to quantify the radioactive signal as arbitrary absorbance units using a linear grey scale of 0.0–2.1 (Reynolds *et al.*, 1997). A blank section of film was placed under the image analyser lens and measured to determine a background reading of the autoradiograph under analysis. Measurements were made of the antisense and sense images obtained from four sections per sample. At least six readings per section were taken, giving a minimum of 24 readings per region per sample, for each region where localization had been confirmed. The sense values were subtracted from the antisense values to produce a mean value of specific hybridization for a particular region for that sample. The detection limit was taken as an arbitrary absorbance unit greater than 0.01. The CVs for duplicate measurements of the pairs of slides were as follows: IGF-II, 6.5%; IGFBP-2, 4.2%; IGFBP-3, 11.0%; IGFBP-5, 4.9%; IGFBP-6, 1.8%; and UTMP, 7.7%. All samples for each probe were processed in the same batch to avoid any possible inter-batch variation.

Statistical analysis

All values are given as mean $\pm$ SEM. Statistical analyses were performed using Statistical Package for

the Social Sciences (SPSS) version 9.0. A one-way ANOVA was used to test the effects of body condition on: (i) fetal parameters; (ii) placental parameters; and (iii) IGF or UTMP mRNA expression in a given uterine region. Fisher's LSD tests were used to determine which parameters differed between groups. If the data were non-homogeneous, including after log-transformation, a Kruskal–Wallis non-parametric one-way ANOVA was performed. The data were pooled if no changes were detected. Maternal insulin, glucose and IGF-I concentrations were analysed using a repeated measures design using the MIXED procedures of SAS (version 6.12). Correlation analysis was performed to analyse the relationship between variables.

Results

Maternal parameters

Of the 19 ewes mated, 16 had singleton pregnancies and three had twins (two in group F and one in group M). The data presented in this study concern only ewes that had singleton pregnancies (F: $n = 4$; M: $n = 7$ and L: $n = 5$), to avoid any nutritional effects of the number of fetuses. The weights of the ewes were significantly different at mating (F: 75.8 ± 2.32^a kg versus M: 63.6 ± 1.58^b kg versus L: 57.4 ± 2.01^c kg; $a > b > c$, $P < 0.03$). All ewes maintained their specified body condition score throughout the study.

Placental and fetal data

The mean placentome weight, fetal weight and fetal weight:crown–rump length ratio were significantly

Table 2. The effect of maternal body condition on uterine, placental and fetal parameters in ewes on day 65 of gestation

	Body condition score		
	5.0 (fat, <i>n</i> = 4)	3.5 (moderate, <i>n</i> = 7)	2.0 (lean, <i>n</i> = 5)
Mean placentome weight (g)	8.2 ± 1.17 ^a	5.8 ± 0.62 ^b	6.2 ± 0.30 ^{a,b}
Total placentome number	86 ± 4.0	89 ± 6.2	92 ± 2.9
Fetal weight (g)	110 ± 2.82 ^a	97.3 ± 2.42 ^b	103 ± 4.89 ^{a,b}
Crown-rump length (cm)	15.8 ± 0.22	15.6 ± 0.14	15.6 ± 0.17
Fetal weight:crown-rump length (g cm ⁻¹)	6.9 ± 0.18 ^a	6.2 ± 0.14 ^b	6.6 ± 0.34 ^{a,b}
Total placentome weight (g)	703 ± 94.7 ^c	515 ± 54.8 ^d	576 ± 46.6 ^{c,d}
Total uterine weight (g)	1900 ± 154 ^e	1449 ± 144 ^f	1539 ± 178 ^{e,f}

Values are the mean ± SEM.

Within rows a > b, *P* < 0.05; c > d, *P* < 0.06; e > f, *P* < 0.07.

greater in fat ewes than in ewes of moderate condition (*P* < 0.05; Table 2). A similar trend was observed for the total placentome weight (*P* < 0.06) and total uterine weight (*P* < 0.07; Table 2). All parameters measured in the lean ewes were intermediate between the other two groups (Table 2). When all the groups were considered together, the fetal weight tended to be positively correlated with the total (*r* = 0.469, *P* < 0.07, *n* = 16) and mean (*r* = 0.469, *P* < 0.07, *n* = 16) placentome weight, but there was no significant relationship between fetal weight and the weight of the mother at mating. Factors unaltered by body condition were (*n* = 16 in each case): total number of placentomes (89.7 ± 2.92), fetal crown-rump length (15.7 ± 0.10 cm), placenta weight:fetal weight ratio (5.67 ± 0.35) and ponderal index (0.03 ± 0.0007 g cm⁻³).

In situ hybridization data

IGF-II mRNA was localized in the placentome capsule, the fetal mesoderm in both the fetal villi of the placentome and in the intercaruncular region, and within the glands and luminal epithelium of the intercotyledonary endometrium (Fig. 1). IGF-II expression was highest in the ewes of moderate condition in the fetal mesoderm, placentome capsule and luminal epithelium (Fig. 2), although there was no effect of body condition score on expression in the glands (0.11 ± 0.01 absorbance units, *n* = 15).

IGFBP-2, IGFBP-3 and IGFBP-6 were all expressed in the placentome capsule and IGFBP-3 and IGFBP-6 mRNA were also present in the stroma of the maternal villi (Fig. 3). Within the capsule the absorbance values of these IGFBPs were consistently highest in the ewes of moderate condition, although only that of IGFBP-2 achieved statistical significance (Fig. 4). Expression of IGFBP-3 (0.03 ± 0.007 absorbance units, *n* = 16) and IGFBP-6 (0.13 ± 0.01 absorbance units, *n* = 15) in the maternal villi was unaffected by body condition.

IGFBP-3 and IGFBP-5 were both expressed in both the luminal and glandular epithelium in the intercoty-

ledonary endometrium (Fig. 5). There was no effect of body condition score on expression in the luminal epithelium (IGFBP-3: 0.11 ± 0.01 absorbance units; IGFBP-5: 0.90 ± 0.03 absorbance units, *n* = 16), but in the glands, IGFBP-3 and IGFBP-5 values showed a progressive decrease from the fat ewes, through the ewes of moderate condition, to the lean ewes (Fig. 6).

UTMP mRNA was localized in the luminal epithelium and at significantly higher concentrations in the endometrial glands (Fig. 5). Expression in the glands was greatest in the lean ewes (Fig. 6). Evidence from the photographic emulsions revealed that the UTMP mRNA was not uniformly distributed over the luminal epithelium but was restricted to areas adjacent to the mouths of glands. Concentrations in the luminal epithelium were unaffected by body condition (0.08 ± 0.04 absorbance units, *n* = 15).

Maternal plasma insulin, glucose and IGF-I

Plasma insulin concentrations altered significantly with body condition, with fat ewes having greater insulin concentrations than lean ewes and ewes of moderate condition between day 20 and day 48 of gestation (Fig. 7a; *P* < 0.04). Maternal insulin was positively correlated with the weight of the ewe at mating (*r* = 0.603, *P* < 0.05, *n* = 15). In contrast, maternal insulin was negatively correlated with UTMP expression in the endometrial glands (*r* = 0.509, *P* < 0.05, *n* = 16). Body condition had no significant overall effect on plasma glucose (Fig. 7b) or IGF-I concentrations (Fig. 7c).

Fetal plasma glucose, IGF-I and insulin

Fetal plasma glucose concentrations were highest in ewes of moderate condition (Table 3). Because maternal glucose concentrations were similar in all groups, the fetal:maternal glucose ratio followed a similar (although non-significant) trend with values highest in fetuses in the fat ewes and lowest in those from ewes of moderate condition. Fetal insulin concentrations also followed this

Fig. 1. Localization of insulin-like growth factor II (IGF-II) mRNA in the ovine placentome (a–f) and intercotyledonary tissue (g,h) on day 65 of gestation. Sections were treated with antisense (a,c,e,g) or sense (control; b,d,f,h) oligonucleotide probes. (a) and (b) are autoradiographs; (c–h) are sections coated with photographic emulsion and counterstained with haematoxylin and eosin. In the placentome, IGF-II mRNA is expressed in the placentome capsule (PC; c,d) and mesoderm of the fetal villi (FM; e,f). In the intercotyledonary tissue, IGF-II mRNA is localized in the luminal epithelium (LE) and fetal mesoderm (FM) (g,h). Scale bars represent (a,b) 2 mm and (c–h) 200 μ m.

same pattern and were negatively correlated with the mean placentome weight ($P < 0.05$). Fetal plasma IGF-I concentrations were lowest in the fat ewes (Table 3). Fetal IGF-I was negatively correlated with the weight of the ewe at mating ($r = 0.49$, $P < 0.05$, $n = 16$) and the placentome:fetal weight ratio ($r = 0.49$, $P < 0.05$, $n = 16$).

Discussion

This study investigated the effects of maternal body condition at mating on placental and fetal growth. Previous research in sheep has demonstrated the importance of acquiring an optimum body condition score before mating, as body condition, ovulation rate and subsequent lambing percentages are inter-related (Henderson, 1990). Nutrient availability around mating may influence early-embryo survival, possibly via effects on oocyte quality (Snijders *et al.*, 2000) or progesterone production (Abecia *et al.*, 1997). Sufficient body reserves are also required for utilization during pregnancy and in lactation, when feeding is commonly of poorer quality and more expensive (Henderson, 1990). In lowland ewes, such as the Dorset breed, optimum lambing percentages are achieved at body condition score 3.5, with underfeeding and overfeeding depressing ovulation rates (Henderson, 1990; MAFF, 1996). In the present study, maternal body condition also altered fetal growth, with the ewes of moderate condition having the smallest fetuses in mid-gestation. This result indicates that different mechanisms may have occurred to promote fetal growth in early gestation in the fat and lean ewes, such that fetal weights in the two extreme groups were similar.

The fat ewes were characterized by higher placental and fetal weights, and there was a trend ($P < 0.07$) for these two parameters to be correlated. A similar positive relationship between fetal and placental weights has been documented in previous studies (sheep: Kelly, 1992; Gadd *et al.*, 2000a; humans: Schubring *et al.*, 1997). The body mass index, a measure of maternal fatness (weight/height²), was positively correlated with placental and fetal weight in women (Perry *et al.*, 1995), and Kinare *et al.* (2000) also reported a relationship between the pre-pregnancy weight of the mother and the placental volume in mid-gestation. In contrast, Greenwood *et al.* (2000) found a negative relationship between maternal fatness in early gestation with placental and fetal weight at birth in the ewe and in the present experiment there was no correlation between weight of the ewe at mating and fetal weight at day 65. Therefore, it is clear that the relationships between body condition and placental and fetal development are not straightforward and are also almost certainly influenced by diet and breed effects, which vary between experiments. Furthermore, the relationship between fetal and placental weight may change during the

Fig. 2. Insulin-like growth factor II (IGF-II) mRNA expression measured as arbitrary absorbance units in (a) mesoderm of the fetal villi, (b) placentome capsule and (c) luminal epithelium in the intercotyledonary endometrium in ewes of body condition score 5.0 (fat, $n = 4$), 3.5 (moderate, $n = 7$) or 2.0 (lean, $n = 5$). Expression was consistently highest in the ewes of moderate condition ($a > b$, $P < 0.05$). Values are the mean $\pm$ SEM.

course of the pregnancy (Heasman *et al.*, 1998; Osgerby *et al.*, 2002).

In addition to absolute weight, the fetal programming hypothesis has drawn attention to the importance of fetal proportions as a guide to health in later life (Barker and

Fig. 3. See legend on facing page.

Clarke, 1997). In the fat ewes compared with the ewes of moderate condition, the fetal crown–rump length was unchanged despite the heavier fetal weight, resulting in an increased fetal weight:crown–rump length ratio. Maternal fatness thus altered fetal growth asymmetrically, appearing to promote soft tissue development, whereas skeletal growth remained unchanged. Maternal obesity has been related to an increased incidence of congenital heart defects in humans (Watkins and Botto, 2001). Maternal body condition may therefore influence fetal development such that peri-natal and post-natal health can be compromised.

The increased placental and fetal weights in the obese ewes were associated with increased maternal insulin concentrations, although maternal IGF-I and glucose concentrations were not significantly altered by maternal body condition. Previous studies in sheep fed to achieve a zero energy balance found that insulin, IGF-I and glucose concentrations were all higher in obese animals than in lean animals (McCann *et al.*, 1997). The increase in insulin was attributable to an exaggerated glucose-induced response rather than to decreased insulin removal (McCann *et al.*, 1989). Although the insulin concentrations were higher early in gestation in the present study, the insulin concentrations in all body-condition score groups converged from day 55 of gestation onwards. At this time, placental and fetal demands for glucose are increasing. This may remove glucose from the maternal circulation in an insulin-independent fashion unrelated to the extent of obesity. In a similar way, adolescent ewes with a high intake have exhibited increased concentrations of insulin in comparison with groups of moderate intake (Wallace *et al.*, 1999). However, contrary to the present findings, the high concentrations of insulin promoted maternal tissue deposition at the expense of placental growth; both maternal weight and placental weight were increased in the present study. This finding indicates that the implications of increased maternal insulin may differ between growing and mature ewes.

Contrary to findings in the maternal compartment, plasma glucose, insulin and IGF-I concentrations were lowest in the fetal compartment of fat ewes. As maternal insulin and IGF-I do not cross the placenta in physiologically relevant amounts (Brown and Thorburn, 1989), concentration on the fetal side is regulated independently. Fetal plasma IGF-I was negatively correlated with maternal weight and the placental:fetal weight ratio. As glucose provides energy and carbon for growth,

Fig. 4. Insulin-like growth factor binding protein (IGFBP) mRNA expression measured in arbitrary absorbance units in the placental capsule. There were three groups of ewes of body condition score 5.0 (fat, $n = 4$), 3.5 (moderate, $n = 7$) or 2.0 (lean, $n = 5$). Expression of all three IGFBPs tended to be higher in the ewes of moderate condition ($a > b$, $P < 0.05$; $c > d$, $P < 0.08$). Values are the mean $\pm$ SEM.

Fig. 3. The localization of insulin-like growth factor binding protein 2 (IGFBP-2; a–d), IGFBP-3 (e,g,h) and IGFBP-6 (f,i,j) mRNA in the ovine placenta. (a,b,e,f) are autoradiographs; (c,d,g–j) are sections coated with photographic emulsion and counterstained with haematoxylin and eosin. Sections were hybridized with either (a,c,e–j) antisense or (b,d) sense (control) oligonucleotide probes. (a,c) IGFBP-2, (e,g) IGFBP-3 and (f,i) IGFBP-6 mRNA were localized in the placental capsule (PC) with (h) IGFBP-3 and (j) IGFBP-6 also being expressed in the caruncular stroma (CS) of the maternal villi. Scale bars represent (a,b,e,f) 2 mm and (c,d,g–j) 200 μ m.

Fig. 5. Localization of insulin-like growth factor binding protein 3 (IGFBP-3; a,b), IGFBP-5 (c–e) and uterine milk protein (UTMP; f–h) mRNA in the luminal epithelium (LE) and glandular epithelium (GE) of the ovine intercotyledonary endometrium on day 65 of gestation. UTMP expression was not uniform in the luminal epithelium but was confined to areas adjacent to the mouths of the glands. There was also intermittent expression of IGFBP-5 in the area underlying

Fig. 6. (a) Insulin-like growth factor binding protein 3 (IGFBP-3), (b) IGFBP-5 and (c) uterine milk protein (UTMP) mRNA expression measured in arbitrary absorbance units in the glandular epithelium of the intercotyledonary endometrium. There were three groups of ewes of body condition score 5.0 (fat, $n = 4$), 3.5 (moderate, $n = 7$) or 2.0 (lean, $n = 5$). IGFBP expression was maximal in the fat ewes ($a > b$, $P < 0.05$; $c > d$, $P < 0.08$), whereas UTMP expression was highest in the lean ewes ($a > b$, $P < 0.05$). Values are the mean $\pm$ SEM.

Fig. 7. Maternal metabolite profiles for plasma (a) insulin, (b) glucose and (c) insulin-like growth factor I (IGF-I) in ewes of fat (■, $n = 4$), moderate (▲, $n = 7$) and lean (●, $n = 5$) body condition. Values are the mean. The overall SEM for each group is as follows: insulin (ng ml⁻¹): fat ± 0.05 , moderate ± 0.02 , lean ± 0.03 ; glucose (mmol l⁻¹): fat ± 0.05 , moderate ± 0.03 , lean ± 0.05 ; IGF-I (ng ml⁻¹): fat ± 14.2 , moderate ± 7.01 , lean ± 6.4 . Fat ewes had greater insulin concentrations than lean ewes and ewes of moderate condition (* $P < 0.04$), but glucose and IGF-I values were not significantly different between groups.

the luminal epithelium, although this was not quantified. Sections were hybridized with (a,b,c,e-g) antisense and (d,h) sense (control) oligonucleotide probes. (a,b,e,g,h) Sections coated with photographic emulsion and counterstained with haematoxylin and eosin; (c,d,f) are autoradiographs. Scale bars represent (c,d,f) 2 mm and (a,b,e,g,h) 200 μ m.

Table 3. The effect of maternal body condition on plasma glucose, insulin and insulin-like growth factor I (IGF-I) in the ovine fetus on day 65 of gestation

	Body condition score		
	5.0 (fat, <i>n</i> = 4)	3.5 (moderate, <i>n</i> = 7)	2.0 (lean, <i>n</i> = 5)
Fetal plasma glucose (mmol l ⁻¹)	1.1 ± 0.16 ^b	2.1 ± 0.18 ^a	1.5 ± 0.11 ^b
Fetal:maternal glucose ratio	2.7 ± 0.70	1.7 ± 0.15	2.3 ± 0.14
Fetal plasma insulin (ng ml ⁻¹)	0.10 ± 0.02	0.17 ± 0.03	0.11 ± 0.02
Fetal plasma IGF-I (ng ml ⁻¹)	19.4 ± 1.28 ^b	24.1 ± 0.92 ^a	23.3 ± 1.93 ^{a,b}

Values are the mean ± SEM.

Within rows a > b, *P* < 0.05.

insulin promotes tissue accretion, and IGF-I exerts metabolic, mitogenic and differentiative activities, the factors responsible for promoting the fetal growth observed in fat ewes remain to be elucidated. Although the differences failed to reach statistical significance, the group of fat ewes in the present study had the highest fetal:maternal glucose ratio, whereas this was lowest in the lean ewes. This gradient is one of the key factors regulating glucose transfer across the placenta, which would be expected to increase with the higher ratio (Hay *et al.*, 1990).

A major aim of the present study was to investigate potential mechanisms whereby placental growth may be regulated. Gene-deletion studies in mice have emphasized the significance of the IGF axis to placental and fetal growth. Mice lacking the IGF-II gene exhibit placental hypoplasia and produce progeny that are 60% of their normal birth weight (DeChiara *et al.*, 1990; Baker *et al.*, 1993). In a similar way, deletion from the mouse IGF-II gene of a transcript specifically expressed in the labyrinthine trophoblast resulted in decreased placental growth from day 12 of gestation, which was followed by fetal growth restriction from day 16 onwards (Constancia *et al.*, 2002). IGF-II can potentiate growth by at least four inter-related actions (Gardner *et al.*, 1999). IGF-II is expressed in ewes in the placentome capsule and at higher concentrations in the mesoderm of the fetal villi (Reynolds *et al.*, 1997; Osgerby *et al.*, 1999a). However, in the present study, ewes of moderate condition developed the smallest placentas despite expressing the greatest placental IGF-II mRNA concentrations. Previous work in sheep has not determined any positive relationships between concentrations of placental IGF-II expression and placental growth in adolescent (Gadd *et al.*, 2000a) or adult (Osgerby *et al.*, 2003) ewes on different diets. Circulating IGF-II concentrations in non-pregnant ewes are no different in lean and obese animals (McCann *et al.*, 1997). These data strongly indicate that although IGF-II is clearly a major growth factor in the placenta, the nutritional control of ovine placental growth is not regulated via altered IGF-II expression.

The present study provides further evidence that the regulation is mediated via altered IGFBP expression.

The IGFBPs can modulate IGF action by intercepting receptor interaction and ligand transduction (Wetterau *et al.*, 1999). Maternal body condition altered placental IGFBP expression in the present study, and ewes of moderate condition tended to express the greatest concentrations of IGFBP-2, IGFBP-3 and IGFBP-6 in the placentome capsule. This combined increase in IGFBP concentrations may have contributed to the low placental weights observed in this group by inhibiting IGF-II action. These results are consistent with two previous studies in which expression of IGFBP-3 in the maternal placental villi (Osgerby *et al.*, 2003) and IGFBP-2 expression in the placentome capsule (Gadd *et al.*, 2000a) were higher in sheep with smaller placentae.

In lean ewes, conversely, the low IGFBP expression found in the placentomes in the present study may have enabled these animals to acquire a similar placental weight to the ewes of moderate condition. In addition to the placenta, the fetus acquires support from the UTMPS secreted from the endometrial glands (Stewart *et al.*, 2000). Glandular UTMPS expression was negatively related to both maternal weight and maternal plasma insulin concentrations. IGFBP-3 (*P* < 0.04) and IGFBP-5 (*P* < 0.08) expression in the endometrial glands was positively correlated with maternal weight, with lean ewes expressing lower concentrations than fat ewes. Therefore, the lean ewes were able to compensate for their poor condition by altering IGFBP expression in both the placentomes and glands to promote placental growth and the glandular supply of nutrients. Compensatory placental growth has previously been reported in Welsh Mountain ewes of low-body condition at mating (Osgerby *et al.*, 1999b), in ewes with maternal feed restriction in early pregnancy (Heasman *et al.*, 1998), and in cows in poor body condition (Rasby *et al.*, 1990). However, it is possible that the lean ewes would have been unable to sustain this compensatory mechanism in late gestation when nutrient demands increase.

This study has focused mainly on the IGF system as a possible mediator of placental and fetal growth but other possible modulators should also be considered. For example, Thomas *et al.* (2001) used

immunocytochemistry to detect leptin protein at the maternal and fetal interface of the ovine placenta and proposed that leptin may mediate nutrient partitioning to the fetus via interaction with placental leptin receptors. Leptin synthesis is nutritionally sensitive (Levy and Stevens, 2001), with circulating concentrations varying according to the maternal body condition, dietary intake and plasma insulin concentrations (Thomas *et al.*, 2001). Thus, the effects of body condition on leptin function and fetal growth warrant further investigation.

In summary, maternal body condition altered fetal growth asymmetrically in mid-gestation in the present study. Fetal weight and the fetal weight:crown-rump length ratio were modified, whereas skeletal growth remained unchanged. This finding indicates that aspects of fetal development are sensitive to maternal body condition, potentially producing significant implications on health in post-natal life. These changes in fetal development may be attributed to alterations in placental weight, uterine IGF and UTP expression and systemic insulin associated with differing maternal body conditions.

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