

# Maternal nutrition alters the expression of insulin-like growth factors in fetal sheep liver and skeletal muscle

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## Abstract

We investigated the influence of maternal dietary restriction between days 28 and 80 of gestation followed by re-feeding to the intake of well-fed ewes up to 140 days of gestation (term is 147 days) in sheep, on expression of mRNA for insulin-like growth factor (IGF)-I, IGF-II and growth hormone receptor (GHR) in fetal liver and skeletal muscle. Singleton bearing ewes either consumed 3.2–3.8 MJ/day of metabolisable energy (ME) (i.e. nutrient restricted – approximately 60% of ME requirements, taking into account requirements for both ewe maintenance and growth of the conceptus) or 8.7–9.9 MJ/day (i.e. well fed – approximately 150% of ME requirements) between days 28 and 80 of gestation. All ewes were then well fed (150% of ME requirements) up to day 140 of gestation and consumed 8–10.9 MJ/day. At days 80 and 140 of gestation, five ewes were sampled from each group and fetal tissues taken. There was no difference in fetal body weight or liver weights between groups at either sampling date, or skeletal muscle (quadriceps) weight at 140 days.

IGF-I mRNA abundance was lower in livers of nutrient-restricted fetuses at day 80 of gestation (nutrient restricted 2.35; well fed 3.70 arbitrary units), but was higher than well-fed fetuses at day 140 of gestation, after 60 days of re-feeding (restricted/re-fed 4.27; well fed 2.83; s.e.d. 0.98 arbitrary units,  $P=0.061$  for diet  $\times$  age interaction). IGF-II mRNA abundance was consistently higher

in livers of nutrient-restricted fetuses (80 days: nutrient restricted 7.78; well fed 5.91; 140 days: restricted/re-fed 7.23; well fed 6.01; s.e.d. 1.09 arbitrary units,  $P=0.061$  for diet). Nutrient restriction had no effect on hepatic GHR mRNA abundance, but re-feeding of previously nutrient-restricted fetuses increased GHR mRNA compared with continuously well-fed fetuses (80 days: nutrient restricted 70.6; well fed 75.1; 140 days: restricted/re-fed 115.7; well fed 89.4; s.e.d. 10.13 arbitrary units,  $P=0.047$  for diet  $\times$  age interaction). In fetal skeletal muscle, IGF-I mRNA abundance was not influenced by maternal nutrition and decreased with gestation age ( $P<0.01$ ). IGF-II mRNA abundance was higher in skeletal muscle of nutrient-restricted fetuses compared with well-fed fetuses at day 80 of gestation (nutrient restricted 16.72; well fed 10.53 arbitrary units), but was lower than well-fed fetuses after 60 days of re-feeding (restricted/re-fed 7.77; well fed 13.72; s.e.d. 1.98 arbitrary units,  $P<0.001$  for diet  $\times$  age interaction). There was no effect of maternal nutrition or gestation age on fetal skeletal muscle GHR expression. In conclusion, maternal nutrient restriction in early to mid gestation with re-feeding thereafter results in alterations in hepatic and skeletal muscle expression of IGF-I, IGF-II and/or GHR in the fetus which may subsequently relate to altered organ and tissue function.

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## Introduction

Maternal nutrition during pregnancy has a primary role in determining both placental and fetal growth. The effect of reducing maternal nutrient intake on fetal development depends upon the timing, duration and severity of the nutritional insult (see Robinson *et al.* 1999) and may be mediated through alterations in the fetal insulin-like growth factor (IGF) axis (Bauer *et al.* 1995). The insulin-

like growth factors (IGF-I and IGF-II) are mitogenic peptides that have a fundamental role in regulating fetal growth due to their ability to stimulate proliferation and differentiation of a number of cell types (Cohick & Clemmons 1993, Brameld *et al.* 1998). They are expressed by most cell types, with the highest expression of IGF-II occurring during fetal development (Han *et al.* 1988). A number of hormones, including exogenous IGF-I, cortisol and thyroid hormones have been shown to regulate fetal

expression of IGF-I or IGF-II in a tissue-specific manner (Li *et al.* 1993, Kind *et al.* 1996, Forhead *et al.* 1998). The effects of diet on the growth hormone-IGF axis appear to be similar at both fetal and postnatal stages of development, but there appears to be no stimulatory effect of dietary-induced changes in growth hormone (GH) on hepatic IGF-I expression in the fetus. Reduction of maternal feed intake to 25% of metabolisable energy (ME) requirements between days 110 and 124 of gestation (Bauer *et al.* 1995) increased plasma GH concentrations and decreased plasma IGF-I concentrations in both the mother and her fetus. Fetal and maternal plasma IGF-II concentrations and hepatic GH binding were unaltered. The lack of effect on hepatic GH binding is in contrast to postnatal effects of undernutrition, where decreased hepatic GH binding is observed (Breier *et al.* 1988). The effects of maternal nutrition on fetal hepatic or skeletal muscle IGF-I, IGF-II or GH receptor (GHR) mRNA abundance are unknown.

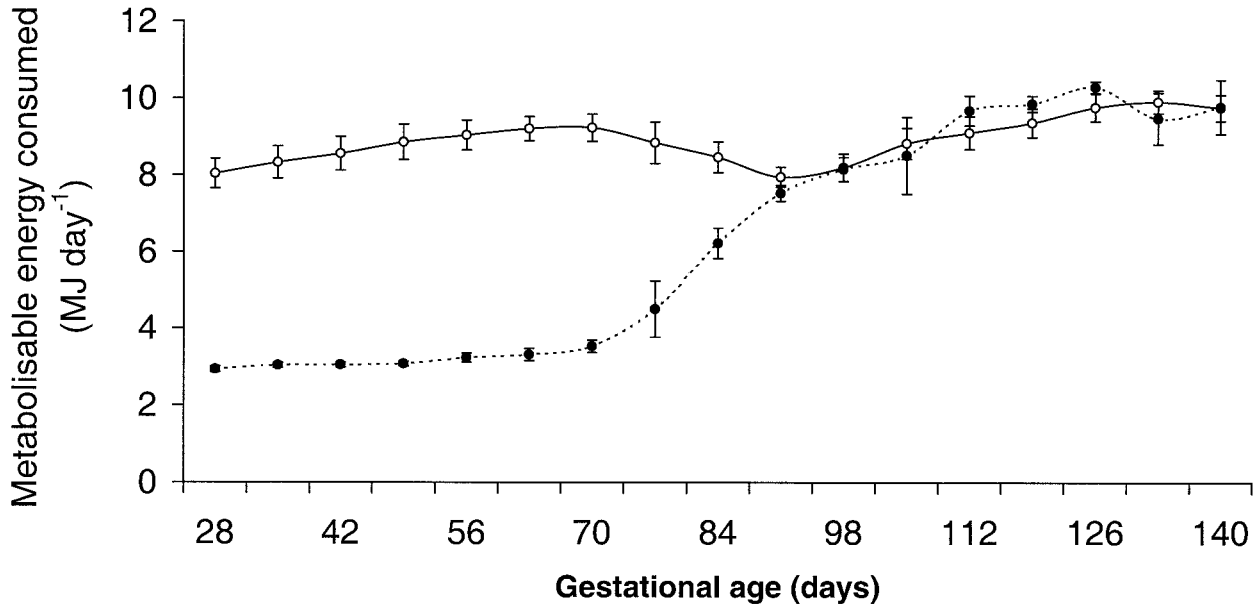
Plasma IGF-I concentrations are positively correlated with fetal body weight at term in infants, calves and lambs (Lassarre *et al.* 1991, Owens *et al.* 1994, Holland *et al.* 1997). This relationship, however, is not observed in fetal lambs whose mothers were nutrient restricted during early to mid gestation and then adequately fed up to term (Heasman *et al.* 1999). These lambs are also characterised as having a longer crown-rump length than fetuses sampled from ewes adequately fed throughout gestation (Heasman *et al.* 1998), indicating that a period of undernutrition in early pregnancy may subsequently alter fetal growth and development even when maternal nutrient intake has been restored. The aim of the following study was to investigate the effects of maternal nutrient restriction in early to mid gestation (i.e. days 28–80 of gestation) followed by a period of re-feeding (i.e. days 80–140 of gestation) on the expression of primary nutritionally responsive genes within the fetus i.e. IGF-I, IGF-II and GHR. Measurements were therefore made of mRNA abundance for each gene in fetal sheep liver and skeletal muscle sampled at the end of the dietary restriction (i.e. day 80 of gestation) and close to term (i.e. day 140 of gestation) after 60 days of re-feeding.

## Materials and Methods

### *Animals and diet*

Forty Welsh Mountain ewes of similar age (median 3 years), weight ( $36.14 \pm 0.85$  kg, means  $\pm$  s.e.m.) and of known mating date were entered into the study. Oestrous activity was determined using a vasectomised ram whose breast was painted to mark ewes. Ewes exhibiting oestrus were then bred with one of two Texel rams and breeding dates were established from the last date of observed coitus. Body condition score was assessed by the physical characteristics in the lumbar region, on and around the backbone

and in the loin area immediately behind the first rib, using a scale of 0–5, with 0 equal to emaciation and 5 being grossly fat, as described by Russel *et al.* (1969) and was  $2.7 \pm 0.2$  arbitrary units at the start of the study. Ewes were individually housed at day 28 of gestation and were fed daily at 0900 h. The ME requirement for each animal was calculated according to its body weight, taking into account requirements for both ewe maintenance and growth of the conceptus on the basis of producing a 4.5 kg lamb at term (Agricultural and Food Research Council 1992). Ewes were allocated to one of two nutritional groups (see below) and either offered 60% (i.e. nutrient restricted), or 225% (i.e. well fed) of their calculated ME requirements (Clarke *et al.* 1998a). Nutrient-restricted ewes consumed all of the feed offered, while those offered 225% of ME requirements only consumed 150% of ME requirements as not all of the hay was eaten. Feed intakes were measured daily and weekly mean ME intakes calculated (Fig. 1). Diets were adjusted fortnightly but in nutrient-restricted ewes, however, the feed was not reduced if maternal body weight decreased. Ewes therefore consumed either 3.2–3.8 MJ/day of ME (i.e. nutrient restricted – 60% of ME requirements) or 8.7–9.9 MJ/day of ME (i.e. well fed – 150% of ME requirements) between days 28 and 80 of gestation. Ewes were allocated into either feeding group, using a stratified randomisation by body weight. At day 42 of gestation, 21 ewes (nutrient restricted  $n=10$ ; well fed  $n=11$ ) were diagnosed as being pregnant with a single fetus using ultrasound scanning. The amount of feed given to each ewe was increased at days 43 and 61 of gestation in order to meet the higher energy requirements associated with growth of the conceptus (Agricultural and Food Research Council 1992). The diet comprised chopped hay that had an estimated ME content of 7.91 MJ/kg dry matter and a crude protein content (nitrogen  $\times 6.25$ ) of 69 g/kg dry matter and a barley-based concentrate that had an estimated ME content of 11.6 MJ/kg dry matter and a crude protein content of 162 g/kg dry matter. The proportion of hay to concentrate fed was approximately 3:1 with respect to dry weight. All diets contained adequate minerals and vitamins. Body condition score was measured every 2 weeks and if any nutrient restricted ewes were found to have a condition score below 1.5 then their level of feeding was increased to meet 100% of ME maintenance requirements, according to current body weight. This occurred in two ewes at day 60 of gestation, but as results obtained from these ewes did not differ significantly from those that remained on the nutrient-restricted diet until day 80 of gestation, they have been included in the results for the nutrient-restricted group. One of these ewes was sampled at day 80 of gestation and one at day 140 of gestation. After day 80 of gestation, all ewes consumed between 8 and 10.9 MJ/day of ME (150% of ME requirements) up to day 140 of gestation (term = 147 days) with the amount of feed increased at days 100 and 120 of



**Figure 1** Mean metabolisable energy (ME) intake for ewes nutrient restricted between days 28 and 80 of gestation and then well fed for the remainder of pregnancy (●) or well fed throughout pregnancy (○). Details of diet and ME contents are given in Materials and Methods. Values are means with their standard errors and  $n=10$  (nutrient restricted) or 11 (well fed) up to day 80 of gestation and  $n=5$  (nutrient restricted/re-fed) or 6 (well fed) from days 80 to 140 of gestation.

gestation. There was no difference in total food intake between groups over this period (Fig. 1).

#### Experimental design

At day 78 of gestation five nutrient-restricted and five well-fed ewes were selected at random and a jugular vein catheter was inserted into each animal. The following day, blood samples were taken into heparinised syringes at hourly intervals between 0800 and 1600 h with the daily time of feeding maintained at 0900 h. Then, at day 80 of gestation, all these animals were killed by intravenous administration of barbiturate (100 mg/kg pentobarbital sodium: Euthatal). The entire uterus was removed and the fetus killed with barbiturate, after which all major organs were dissected and weighed. It was not possible to precisely separate and weigh individual fetal skeletal (i.e. quadriceps) muscles at day 80 of gestation, hence a mixed muscle sample was taken from the hind limb. This blood and tissue sampling procedure was then repeated on five nutrient-restricted and six well-fed ewes between days 138 and 140 of gestation. Cord blood samples taken at the time of euthanasia were obtained from all fetuses at day 140 of gestation, and from four nutrient-restricted and four well-fed fetuses at day 80 of gestation. All operative procedures and experimental protocols had the required Home Office approval as designated by the Animals (Scientific Procedures) Act (1986).

#### Laboratory analysis

Plasma concentrations of glucose, non-esterified fatty acid (NEFA) and cortisol (total) in plasma were determined enzymatically as described by Clarke *et al.* (1998b).

#### RNA preparation and RNase protection assays

Total RNA was isolated from fetal liver and skeletal muscle using the guanidine thiocyanate method (Chomczynski & Sacchi 1987), and quantified by measuring the absorbance at 260 nm.

RNase protection assays were performed on 5 (18S), 50 (IGF-II) or 100 (IGF-I and GHR)  $\mu\text{g}$  total RNA following the methods described previously (Saunders *et al.* 1991). Radiolabelled antisense riboprobes were generated as previously described, corresponding to class 2 transcripts of the sheep IGF-I gene (exon 3 linked to the exon 2 promoter: Pell *et al.* 1993, Li *et al.* 1996), to exon 3 of the sheep IGF-II gene (Li *et al.* 1993), and to the intracellular domain of the sheep GHR gene (Li *et al.* 1996). The IGF-I riboprobe was designed so that when hybridised to total RNA, two bands were possible corresponding to the homologous mRNA transcript class (class 2 transcripts), as well as to any other IGF-I mRNA transcript class (class 1 transcripts), which hybridised to the region of the probe corresponding to exon 3 (Pell *et al.* 1993, Li *et al.* 1996). However, no class 2 transcripts of IGF-I were detected in

this study. The plasmid pT7 RNA 18S (Ambion Inc., Austin, TX, USA) was used to generate low specific activity riboprobes for detection of 18S ribosomal RNA. Protected bands were detected by phosphorimager (Fuji, London, UK) and quantified by image analysis (Multi analyst, BioRad, Hemel Hempstead, UK).

#### Statistical analyses

The results of relative optical density, obtained from the image analysis, were subjected to two-way ANOVA using the Genstat 5 (release 4.1) statistical package (Lawes Agricultural Trust, Rothamsted, Hertfordshire, UK) to test for the effects of diet, age and diet  $\times$  age interactions. Similar statistical analysis was performed on mean plasma glucose, NEFA and cortisol concentrations, using a mean value obtained for each animal taken from the 8-hourly blood samples. Differences of  $P < 0.05$  were considered significant, while differences of  $P < 0.10$  were considered as tending towards significance.

## Results

Maternal dietary restriction (60% of ME requirements) between days 28 and 80 of gestation resulted in nutrient-restricted ewes having a lower body condition score and weighing less than the well-fed group (Table 1). At day 140 of gestation following re-feeding to the level of intake of well-fed ewes (i.e. 150% of ME requirements) from days 80 to 140 of gestation there were no differences in ewe body weight or condition score between groups. Fetal body weights and liver weights were similar between dietary treatment groups at both days 80 and 140 of gestation and there was no effect of nutrient restriction/re-feeding on skeletal muscle (quadriceps) weight at day 140 of gestation.

Nutrient-restricted ewes exhibited a lower ( $P < 0.001$ ) plasma glucose concentration and higher ( $P < 0.001$ ) plasma NEFA concentration than well-fed ewes at day 79 of gestation (Table 2). These differences between groups were, however, modest with all ewes remaining normoglycaemic (i.e. plasma glucose  $> 2.00$  mM) and did not exhibit very high plasma NEFA concentration (i.e.  $> 2.00$  mM). No significant dietary differences were observed between groups at day 139 of gestation, as plasma glucose concentration decreased and plasma NEFA increased with gestational age in continuously well-fed ewes but not previously nutrient-restricted ewes resulting in a significant diet  $\times$  age interaction ( $P < 0.05$ ). Mean plasma cortisol concentrations were similar between groups at both sampling ages. There was no difference in plasma concentration of glucose or cortisol in cord blood between dietary treatment groups but both increased with gestational age (mean glucose: 80 days  $0.50 \pm 0.09$  mM,

$n = 8$ ; 140 days  $1.48 \pm 0.10$  mM,  $n = 11$ ,  $P < 0.05$ ; mean total cortisol: 80 days  $17.9 \pm 2.8$  nM; 140 days  $55.1 \pm 8.2$  nM,  $P < 0.05$ ).

There were no differences between groups in 18S ribosomal RNA (rRNA) abundance in either liver (Fig. 2, Table 3) or skeletal muscle (Fig. 3, Table 4), confirming that a constant amount of total RNA was used in the RNase protection assays. Maternal dietary restriction (60% of ME requirements) between 28 and 80 days of gestation followed by re-feeding to well-fed levels thereafter (i.e. 150% of ME requirements between days 80 and 140), resulted in changes in the expression of IGF-I, IGF-II and GHR in either fetal liver or skeletal muscle when compared with well-fed fetuses from ewes whose intake was maintained at 150% of ME requirements throughout gestation.

For both IGF-I and GHR mRNA abundance in liver there was a significant interaction between gestational age and diet (Fig. 2, Table 3). IGF-I expression was lower in the nutrient-restricted fetuses compared with the well-fed group at day 80 of gestation, but higher in the previously nutrient-restricted group at day 140 of gestation. In contrast, GHR expression was significantly lower ( $P < 0.01$ ) in nutrient-restricted compared with well-fed fetuses at day 80 of gestation and increased with gestational age in both groups, a response that was greater in the previously nutrient-restricted fetuses. For IGF-II there was a tendency ( $P = 0.061$ ) for hepatic mRNA expression to be higher in both nutrient-restricted and nutrient-restricted/re-fed fetuses compared with well-fed fetuses, i.e. an effect of diet, but no effect of gestational age.

IGF-I mRNA abundance in skeletal muscle decreased with gestational age ( $P < 0.001$ ) and was unaffected by maternal nutrition (Fig. 3, Table 4). In contrast, IGF-II abundance was significantly higher ( $P < 0.001$ ) in nutrient-restricted compared with well-fed fetuses at day 80 of gestation, a difference that was reversed by day 140, resulting in a significant diet  $\times$  age interaction ( $P < 0.001$ ). GHR expression in fetal skeletal muscle was unaffected by either maternal nutrition or gestation age.

## Discussion

Previous studies have demonstrated that maternal nutrient restriction (to 60% of ME requirements) in sheep during the period of maximal placental growth (i.e. days 28–80 of gestation) restricts individual placentome growth and results in a smaller placenta at mid-gestation, but has no effect on fetal weight at either mid or late gestation (Clarke *et al.* 1998a, Heasman *et al.* 1998). In previously nutrient-restricted ewes re-fed to 150% of ME requirements up to term, placental and fetal weights were similar to controls (Dandrea *et al.* 1999). In order to reduce fetal growth in late gestation it appears necessary to severely restrict maternal nutrition. Fetal weight was unaffected when

**Table 1** Mean ewe body weight, body condition score (BCS) and fetal body, liver and skeletal muscle (i.e. quadriceps) weights sampled from ewes nutrient restricted between days 28 and 80 of gestation and then well fed, or well fed throughout pregnancy

	Stage of gestation				s.e.d.	Significance		
	80 days		140 days			Diet	Age	Diet × age
	Restricted (60% ME)	Well fed (150% ME)	Restricted/re-fed (60%/150% ME)	Well fed (150% ME)				
Ewe body weight (kg)	35.9	39.8	42.9	43.9	0.99	P<0.001	0.012	NS
Ewe BCS (arbitrary units)	1.8	2.7	1.4	1.8	0.15	P<0.001	0.057	NS
Fetal body weight (g)	235	215	4801	4783	180.5	NS	P<0.001	NS
Fetal skeletal muscle weight (g)	—	—	21.6	21.1	2.46	NS	—	—
Fetal liver weight (g)	14.2	15.1	115.9	110.6	7.44	NS	P<0.001	NS

NS, non-significant ( $P>0.1$ ), s.e.d.=standard error of the difference between means with 17 degrees of freedom, except for skeletal muscle which has eight degrees of freedom. BCS estimated as described by Russel *et al.* (1969). ME, metabolisable energy.



**Table 2** Mean maternal plasma glucose, non-esterified fatty acid (NEFA) and cortisol concentrations sampled from ewes nutrient restricted between days 28 and 80 of gestation and then well fed, or well fed throughout pregnancy

	Stage of gestation				S.E.D.	Significance		
	79 days		139 days			Diet	Age	Diet × age
	Restricted (60% ME)	Well fed (150% ME)	Restricted/re-fed (60%/150% ME)	Well fed (150% ME)				
Glucose (mM)	3.16	3.84	3.24	3.46	0.108	0.006	0.018	0.044
NEFA (mM)	0.52	0.21	0.63	0.56	0.058	0.048	0.001	0.033
Cortisol (nM)	18.6	20.9	26.0	25.0	4.84	NS	NS	NS

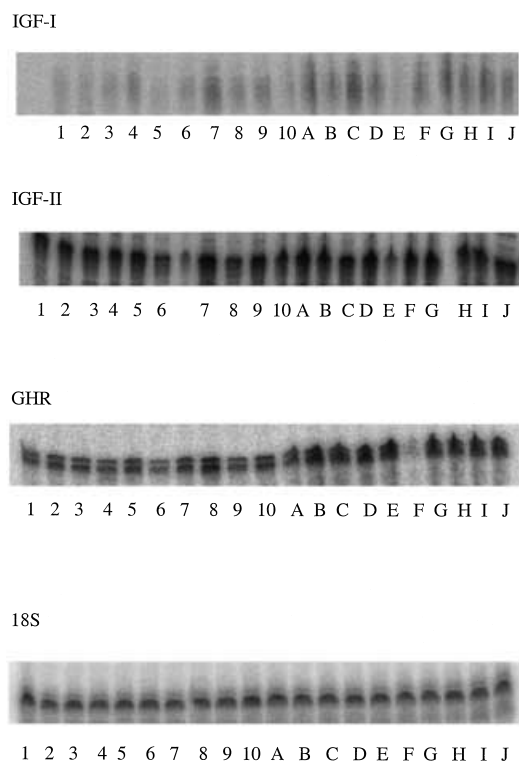
All values are in arbitrary units. NS, non-significant ( $P > 0.1$ ). S.E.D. = standard error of the difference between means with 17 degrees of freedom. ME, metabolisable energy.

ewes were fed 50–60% of ME requirements during late gestation (Symonds *et al.* 1995), but was reduced in another study when maternal feed intake was reduced to 25% of ME requirements between days 110 and 124 of gestation (Bauer *et al.* 1995). Thus placental growth appears sensitive to nutrient supply, but fetal growth

appears to be only affected when nutrient supply is severely reduced.

The major finding of the present study is that maternal nutrient restriction between days 28 and 80 of gestation followed by re-feeding to the level of intake of well-fed ewes had no major effects on maternal glucose or fat metabolism or on fetal body, organ or tissue weights. It did, however, cause alterations in IGF-I, IGF-II and GHR expression in liver and/or skeletal muscle that were dependent on maternal nutrient intake. These may have functional consequences during both fetal and postnatal life. For example, survival of lambs which are of normal birth weight and born by caesarean section following nutrient restriction between early to mid gestation can be reduced to 40% compared with 100% survival rates in lambs born to ewes adequately fed throughout pregnancy (Heasman *et al.* 2000). Maternal nutrient restriction during early to mid gestation also reduces the fetal adrenocorticotrophin and cortisol response to isocapnic hypoxaemia in later gestation (Hawkins *et al.* 2000). Furthermore, maternal undernutrition in late gestation results in enhanced neuropeptide Y mRNA abundance in the fetal hypothalamus near to term (Warnes *et al.* 1998), again in the absence of any effect on fetal body or organ weight. Taken together, all of these findings indicate that maternal undernutrition may alter tissue function in the fetus.

In skeletal muscle the expected decline in IGF-I expression with gestation age (Dickson *et al.* 1991) was observed irrespective of maternal diet. IGF-II expression was higher in skeletal muscle of nutrient-restricted than in well fed-fetuses at day 80 of gestation, but decreased relative to well-fed fetuses following re-feeding. This apparent early rise in IGF-II expression in skeletal muscle of nutrient-restricted fetuses at day 80 of gestation may indicate accelerated muscle cell differentiation and could result in fetuses being born with fewer muscle fibres, although this remains to be established. Previous studies have shown little change in IGF-II expression in ovine skeletal muscle during fetal development (O'Mahoney *et al.* 1991), which supports our findings in well-fed fetuses, although a peak around day 100 of gestation has



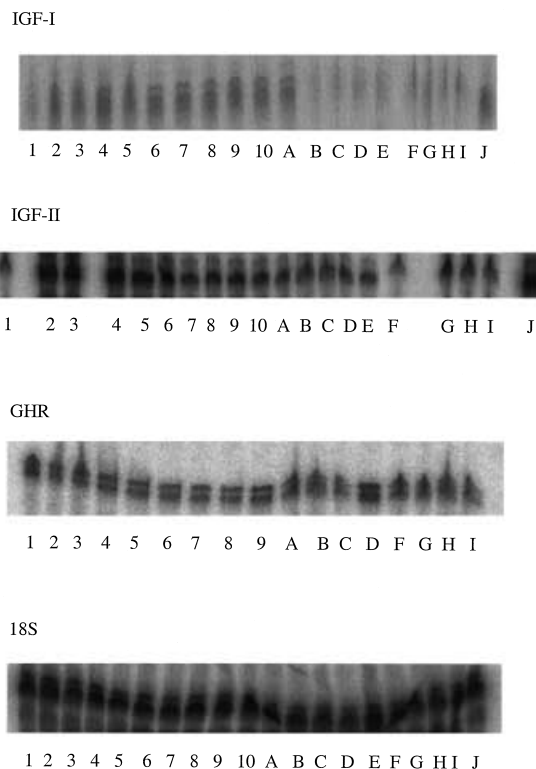
**Figure 2** Effects of maternal nutrient restriction during early to mid gestation on expression of IGF-I and IGF-II, GHR mRNA and 18S rRNA in fetal sheep liver at days 80 and 140 of gestation. Example autoradiographs are shown with lanes 1–5 containing nutrient-restricted fetuses at day 80 of gestation, lanes 6–10 well-fed fetuses at day 80 of gestation, lanes A–E nutrient restricted/re-fed fetuses at day 140 of gestation and lanes F–J well-fed fetuses at day 140 of gestation.

**Table 3** Mean IGF-I, IGF-II and GHR mRNA and 18S rRNA expression in fetal liver sampled from ewes nutrient restricted between days 28 and 80 of gestation and then well fed, or well fed throughout pregnancy

	Stage of gestation				S.E.D.	Significance		
	80 days		140 days			Diet	Age	Diet × age
	Restricted (60% ME)	Well fed (150% ME)	Restricted/re-fed (60%/150% ME)	Well fed (150% ME)				
IGF-I	2.35	3.70	4.27	2.83	0.982	NS	NS	0.061
IGF-II	7.78	5.91	7.23	6.01	1.087	0.061	NS	NS
GHR	70.6	75.1	115.7	89.4	1.46	NS	NS	0.047
18S rRNA	81.3	83.8	83.2	82.3	10.13	NS	NS	NS

All values are in arbitrary units. NS, non-significant ( $P>0.1$ ). S.E.D.=standard error of the difference between means with 17 degrees of freedom. ME, metabolisable energy.

been described in another preliminary study (Dauncey & Gilmour 1996). IGF-II expression in skeletal muscle then normally decreases after birth (Li *et al.* 1993). *In vitro* studies with spontaneously differentiating muscle cell lines that express high levels of IGF-II have shown that



**Figure 3** Effects of maternal nutrient restriction during early to mid gestation on expression of IGF-I and IGF-II, GHR mRNA and 18S rRNA in fetal sheep skeletal muscle at days 80 and 140 of gestation. Example autoradiographs are shown with lanes 1–5 containing nutrient-restricted fetuses at day 80 of gestation, lanes 6–10 well-fed fetuses at day 80 of gestation, lanes A–E nutrient restricted/re-fed fetuses at day 140 of gestation and lanes F–J well-fed fetuses at day 140 of gestation.

differentiation can be delayed if IGF-II expression is inhibited using an antisense oligonucleotide specific to IGF-II (Florini *et al.* 1991). Muscle cells derived from fetal sheep differentiate quicker and to a greater extent and express more IGF-II mRNA than those derived from adult sheep (Brameld *et al.* 1999). Thus, local IGF-II expression may regulate the differentiation of myoblasts and thereby muscle fibre number, as appears to be the case in double muscled animals (Gerrard & Grant 1994). Enhanced fetal muscle development is also associated with increased expression of members of the transforming growth factor- $\beta$  (TGF $\beta$ ) superfamily including myostatin, although it remains to be determined whether this effect involves the IGF axis (Bass *et al.* 1999). In fetal sheep, maternal nutrient restriction over the first 70 days of gestation sufficient to reduce maternal body weight by up to 30% resulted in increased fetal muscle fibre cross-sectional area at 70 but not 140 days of gestation in the absence of any effect on muscle weight or protein and DNA content (Krausgrill *et al.* 1999). The extent to which fetal muscle fibre development may be altered by maternal nutrient restriction between early and mid gestation and the effects on postnatal growth remains to be determined.

The effects of maternal nutrition on IGF-I and IGF-II expression in liver were in marked contrast to those observed in skeletal muscle. Hepatic IGF-II expression was consistently higher in nutrient-restricted compared with well-fed fetuses and not related to changes in hepatic IGF-I expression. This is in contrast to the effects of both IGF-I and cortisol infusion into late gestation fetuses (Kind *et al.* 1996, Li *et al.* 1996), and also to skeletal muscle IGF-II expression in the present study, which exhibited maximal IGF-II mRNA abundance when hepatic IGF-I expression was minimal. It is possible that enhanced hepatic IGF-II expression in nutrient-restricted fetuses at day 80 of gestation is an important adaptive response to reduced placental growth, thereby enabling an increased efficiency of fetal substrate utilisation for tissue growth. As a consequence, fetal body and organ weights are maintained throughout gestation. GHR expression in the liver

**Table 4** Mean IGF-I, IGF-II and GHR mRNA and 18S rRNA expression in fetal skeletal muscle sampled from ewes nutrient restricted between days 28 and 80 of gestation and then well fed, or well fed throughout pregnancy

	Stage of gestation				S.E.D.	Significance		
	80 days		140 days			Diet	Age	Diet × age
	Restricted (60% ME)	Well fed (150% ME)	Restricted/re-fed (60%/150% ME)	Well fed (150% ME)				
IGF-I	3.95	4.39	2.29	1.88	0.502	NS	<0.001	NS
IGF-II	16.72	10.53	7.77	13.72	1.982	NS	0.056	<0.001
GHR	501	522	576	529	52.8	NS	NS	NS
18S rRNA	7414	7292	7482	7129	326.4	NS	NS	NS

All values are in arbitrary units. NS, non-significant ( $P>0.1$ ). S.E.D.=standard error of the difference between means with 17 degrees of freedom. ME, metabolisable energy.

was decreased by nutrient restriction at day 80 and increased with re-feeding in the previously nutrient-restricted fetuses at day 140. Hepatic IGF-I expression was lower in nutrient-restricted fetuses at day 80 of gestation, but higher in restricted/re-fed fetuses at day 140. Plasma concentrations of IGF-I are positively correlated with abundance of IGF-I mRNA in the fetal liver in late gestation (Kind *et al.* 1995), but it remains to be established if a similar relationship occurs at mid-gestation. The reduced hepatic IGF-I expression at day 80 of gestation could contribute to a reduced rate of muscle myoblast proliferation via reduced plasma concentrations of muscle cell mitogens (Gerrard & Judge 1993).

In the present study only class 1 transcripts for IGF-I mRNA in liver were detected. This contrasts with previous studies by others using the same breed of sheep, in which liver specific class 2 transcripts were detectable, although only in very small amounts prior to day 142 of gestation (Li *et al.* 1996). This earlier study did not demonstrate any effect of gestational age or cortisol status on the ratio of class 1 to 2 transcripts for IGF-I, suggesting similar control mechanisms for both. It is therefore unlikely that we have underestimated the magnitude of hepatic differences in IGF-I mRNA between fetuses sampled from nutrient-restricted and well-fed fetuses, particularly at day 80 of gestation when class 2 transcripts for IGF-I mRNA may not be expressed or are below the limits of detection of current assay systems.

In conclusion, maternal nutrient restriction in early to mid gestation followed by re-feeding to the level of intake of well-fed ewes thereafter results in alterations in expression of primary nutritionally responsive genes in fetal hepatic and skeletal muscle. Although these adaptations have no effect on fetal weight close to term it is possible that both hepatic development and skeletal muscle differentiation is markedly altered, which could result in substantial effects for postnatal growth and tissue function. The extent to which such effects may involve either altered myostatin and/or IGF expression remains to be established.

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## References

- Agricultural and Food Research Council 1992 Technical committee on responses to nutrients. Report no. 9, pp 812–815.
- Bass J, Oldham J, Sharma M & Kambadur R 1999 Growth factors controlling muscle development. *Domestic Animal Endocrinology* **17** 191–197.
- Bauer MK, Breier BH, Harding JE, Veldhuis JD & Gluckman PD 1995 The fetal somatotrophic axis during long term maternal undernutrition in sheep: evidence for nutritional regulation *in utero*. *Endocrinology* **136** 1250–1257.
- Brameld JM, Buttery PJ, Dawson JM & Harper JMM 1998 Nutritional and hormonal control of skeletal muscle cell growth and differentiation. *Proceedings of the Nutrition Society* **57** 207–217.
- Brameld JM, Smail H, Imram N, Millard N & Buttery PJ 1999 Changes in expression of IGF-I, IGF-II and GH-receptor (GHR) mRNA during differentiation of cultured muscle cells derived from adult and fetal sheep. *South African Journal of Animal Science* **29** 307–310.
- Breier BH, Gluckman PD & Bass JJ 1988 Influence of nutritional status and estradiol 17 $\beta$  on plasma growth hormone, insulin-like growth factors 1 and 2 and on the response to exogenous growth hormone in young steers. *Journal of Endocrinology* **118** 243–250.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Clarke L, Heasman L, Juniper DT & Symonds ME 1998a Maternal nutrition in early-mid gestation and placental size in sheep. *British Journal of Nutrition* **79** 359–364.
- Clarke L, Heasman L & Symonds ME 1998b Influence of maternal dexamethasone treatment on thermoregulation in lambs delivered by caesarean section. *Journal of Endocrinology* **156** 306–313.



- Cohick WS & Clemmons DR 1993 The insulin-like growth factors. *Annual Review of Physiology* **55** 131–153.
- Dandrea J, Heasman L, Stephenson T & Symonds ME 1999 Maternal nutrition and the manipulation of placental and fetal growth. *Early Human Development* **54** 72–73.
- Daucey MJ & Gilmour RS 1996 Regulatory factors in the control of muscle development. *Proceedings of the Nutrition Society* **55** 543–559.
- Dickson MC, Saunders JC & Gilmour RS 1991 The ovine insulin-like growth factor-I gene: characterization, expression and identification of a putative promoter. *Journal of Molecular Endocrinology* **6** 17–31.
- Florini JR, Magri KA, Ewton DZ, James PL, Grindstaff K & Rotwein PS 1991 'Spontaneous' differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. *Journal of Biological Chemistry* **266** 15917–15923.
- Forhead AJ, Li J, Gilmour RS & Fowden AL 1998 Control of hepatic insulin-like growth factor II gene expression by thyroid hormones in fetal sheep near term. *American Journal of Physiology* **275** E149–E156.
- Gerrard DE & Judge MD 1993 Induction of myoblast proliferation in L6 myoblast cultures by fetal serum of double muscled and normal cattle. *Journal of Animal Science* **71** 1464–1470.
- Gerrard DE & Grant AL 1994 Insulin-like growth factor-II expression in developing skeletal muscle of double muscled and normal cattle. *Domestic Animal Endocrinology* **11** 339–347.
- Han VKM, Lund PK, Lee DC & D'Ercole AJ 1988 Expression of somatomedin insulin-like growth factor messenger ribonucleic acids in the human fetus – identification, characterization, and tissue distribution. *Journal of Clinical Endocrinology and Metabolism* **66** 422–429.
- Hawkins P, Steyn C, McGarrigle HG, Saito T, Ozaki T, Stratford LL, Noakes DE & Hanson MA 2000 Effect of maternal nutrient restriction in early gestation on responses of the hypothalamic–pituitary–adrenal axis to acute isocapnic hypoxaemia in late gestation sheep. *Experimental Physiology* **85** 85–96.
- Heasman L, Clarke L, Firth K, Stephenson T & Symonds ME 1998 Influence of restricted maternal nutrition in early to mid gestation on placental and fetal development at term in sheep. *Pediatric Research* **44** 546–551.
- Heasman L, Stephenson T & Symonds ME 1999 Maternal nutrient restriction during early-to-mid gestation and reprogramming of the fetal IGF-I growth axis at term in sheep. *Proceedings of the Nutrition Society* **57** 124A.
- Heasman L, Clarke L, Stephenson T & Symonds ME 2000 Effect of maternal nutrient restriction in early to mid gestation and thyrotrophin-releasing hormone on lamb survival following Caesarean section delivery near to term. *Canadian Journal of Physiology and Pharmacology* **78** 571–577.
- Holland MD, Hossner KL, Williams SE, Wallace CR, Niswender GD & Odde KG 1997 Serum concentrations of insulin-like growth factors and placental lactogen during gestation in cattle I. Fetal profiles. *Domestic Animal Endocrinology* **14** 231–239.
- Kind KL, Owens JA, Robinson JS, Quinn KJ, Grant PA, Walton PE, Gilmour RS & Owens PC 1995 Effect of restriction of placental growth on expression of IGFs in fetal sheep – relationship to fetal growth, circulating IGFs and binding-proteins. *Journal of Endocrinology* **146** 23–34.
- Kind KL, Owens JA, Lok F, Robinson JS, Quinn KJ, Mundy L, Gilmour RS & Owens PC 1996 Intravenous infusion of insulin-like growth factor I in fetal sheep reduces hepatic IGF-I and IGF-II mRNAs. *American Journal of Physiology* **40** R1632–R1637.
- Krausgrill DI, Tulloh NM, Shorthose WR & Sharpe K 1999 Effects of weight loss in ewes in early pregnancy on muscle and meat quality of lambs. *Journal of Agricultural Science, Cambridge* **132** 103–116.
- Lassarre C, Hardouin S, Daffos F, Forestier F, Franken F & Binoux M 1991 Serum insulin-like growth-factors and insulin-like growth-factor binding-proteins in the human fetus – relationships with growth in normal subjects and in subjects with intrauterine growth-retardation. *Pediatric Research* **29** 219–225.
- Li J, Saunders JC, Gilmour RS, Silver M & Fowden AL 1993 Insulin-like growth factor-II messenger ribonucleic acid expression in fetal tissues of the sheep during late gestation: effects of cortisol. *Endocrinology* **132** 2083–2089.
- Li J, Owens JA, Owens PC, Saunders JC, Fowden AL & Gilmour RS 1996 The ontogeny of hepatic growth hormone receptor and insulin-like growth factor-I gene expression in sheep fetus during late gestation: developmental regulation by cortisol. *Endocrinology* **137** 1650–1657.
- O'Mahoney JV, Brandon MR & Adams TE 1991 Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression. *Molecular and Cellular Endocrinology* **78** 87–96.
- Owens JA, Kind KL, Carbone F, Robinson JS & Owens PC 1994 Circulating insulin-like growth factor-I and factor-II and substrates in fetal sheep following restriction of placental growth. *Journal of Endocrinology* **140** 5–13.
- Pell JM, Saunders JC & Gilmour RS 1993 Differential regulation of transcription initiation from insulin-like growth factor-I (IGF-I) leader exons and of tissue IGF-I expression in response to changed growth hormone and nutritional status in sheep. *Endocrinology* **132** 1797–1807.
- Robinson JJ, Sinclair KD & McEvoy TG 1999 Nutritional effects on foetal growth. *Animal Science* **68** 315–331.
- Russel AJF, Doney JM & Gunn RG 1969 Subjective assessment of body fat in live sheep. *Journal of Agricultural Science, Cambridge* **72** 451–454.
- Saunders JC, Dickson MC, Pell JM & Gilmour RS 1991 Expression of a growth hormone-responsive exon of the ovine insulin-like growth factor-I gene. *Journal of Molecular Endocrinology* **7** 233–240.
- Symonds ME, Bird JA, Clarke L, Gate JJ & Lomax MA 1995 Nutrition, temperature and homeostasis during perinatal-development. *Experimental Physiology* **80** 907–940.
- Warnes KE, Morris MJ, Symonds ME, Phillips ID, Owens JA & McMillen IC 1998 Effects of gestation, cortisol and maternal undernutrition on hypothalamic neuropeptide Y mRNA levels in the sheep fetus. *Journal of Neuroendocrinology* **10** 51–57.

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