

## ARTICLES

# Altered Placental and Fetal Expression of IGFs and IGF-Binding Proteins Associated With Intrauterine Growth Restriction in Fetal Sheep During Early and Mid-Pregnancy

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**ABSTRACT:** The insulin-like growth factors (IGFs) are postulated to be altered in association with the development of intrauterine growth restriction (IUGR). The present studies examined placental and fetal hepatic mRNA concentration of components of the IGF system at two time points (55 and 90 d gestational age, dGA; Term 147 dGA) in a hyperthermia (HT)-induced sheep model of placental insufficiency-IUGR. Maternal plasma insulin and IGF-I were constant at 55 and 90 dGA and were unaffected by treatment. Umbilical vein insulin concentrations tended to be reduced at 90 dGA following HT exposure. Caruncle IGF-I mRNA was increased at 90 dGA in HT placentae ( $p < 0.05$ ), while cotyledon concentrations were constant over gestation and unaltered by treatment. In control cotyledons, IGF-II mRNA concentration increased ( $p < 0.01$ ) and IGFBP-3 decreased between 55 and 90 dGA ( $p < 0.01$ ). Cotyledon IGF-II and caruncle IGFBP-4 mRNA were elevated at 55 dGA in HT placentae compared with control ( $p < 0.01$  and  $p < 0.05$  respectively). Fetal hepatic IGF-I, IGFBP-2, -3 and -4 concentrations rose over gestation ( $p < 0.05$ ), but there were no treatment effects. These data suggest that changes in placental IGF expression in early and mid gestation may predispose the pregnancy to placental insufficiency, resulting in inadequate substrate supply to the developing fetus later in gestation. (*Pediatr Res* 60: 507–512, 2006)

The insulin-like growth factors, IGF-I and -II, are mitogenic polypeptides that play essential roles in conceptus cell proliferation, differentiation and metabolism. The IGF-binding proteins (IGFBP-1–IGFBP-6) regulate IGF bioavailability, modulating the biologic effects of the IGFs, in addition to acting as growth factors independent of the IGFs (1). The importance of these growth factors and their associated binding proteins in the etiology of fetal growth restriction (IUGR) has been highlighted through gene deletion models in mice (2).

The fetal liver is the primary source of IGFs and IGFBPs in fetal circulation (3). IGF-I concentrations in fetal circulation have been found to be reduced in several animal models of IUGR, suggesting that alterations in IGF-I production and/or availability may contribute to disorders of fetal growth (4). In humans, fetal plasma IGF-I concentration is positively correlated with birth weight (5) and is reduced in IUGR (6). Significant increases in fetal hepatic IGFBP-1 and -2 have been observed in animal models of IUGR, late in pregnancy, and these increases are postulated to exert an inhibitory effect on the growth promoting action of IGF-I during fetal life (7). Additionally, several studies report increases in IGFBP-4, and decreases in IGFBP-3 in IUGR fetal circulation (8,9).

A rapid phase of placental growth precedes fetal growth, and in the sheep, maximal placental growth occurs between 40 and 80 d gestational age (dGA), peaking at approximately 55 dGA (term 147 dGA), with the majority of fetal growth occurring after 100 dGA (10). The IGF/IGFBP axis has been described for the sheep placenta (11) and fetus (12), although placental studies have concentrated on localization rather than quantification during early pregnancy (11). Additionally, studies concerning nutritional restriction-induced sheep IUGR have reported changes in the IGF axis in mid- to late gestation (13,14). Pregnant sheep subjected to elevated environmental temperatures (hyperthermia, HT) for the period of early and mid-gestation (40–120 dGA), produce a fetus whose placental, body and liver weights are significantly reduced at term, 45%, 50%, and 45%, respectively (15,16). This asymmetrically restricted IUGR fetus is the result of placental insufficiency and shares many characteristics with the severe human

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**Abbreviations:** bp, basepair; CBT, core body temperature; dGA, days gestational age; HT, hyperthermia; IGFBP, insulin-like growth factor binding protein; RPA, ribonuclease protection assay; TN, thermoneutral control

IUGR fetus, including hypoglycemia, hypoxia and an increased brain-liver ratio (17).

While IGFs and their binding proteins have been extensively studied in fetal and placental growth, studies investigating interactions of IGFs in disorders of fetal growth have concentrated on late gestation IUGR. Considering the chronic developmental nature of this condition, and the role the IGFs and the binding proteins play in placental and fetal development, it is important to understand the ontogenic changes that may occur in the IGFs and IGFFBPs throughout the development of IUGR. The present studies examine the ontogeny of the IGFs, and their alterations during the development of placental insufficiency induced IUGR.

## MATERIALS AND METHODS

**Biologic preparation.** Twenty-one time-mated 2- to 3-y-old Columbia-Rambouillet ewes pregnant with a single fetus were used. Animal care was in strict compliance with National Institutes of Health guidelines within an American Association for Accreditation of Laboratory Animal Care certified facility, and the University of Colorado Health Sciences Center Animal Care and Use Committee approved the study. Surgery was performed at 32–37 dGA to insert core body temperature (CBT) transmitters (18). Approximately 2–4 d after surgery, once core body temperature was 39.2°C, ewes were allocated to either the control (thermoneutral; TN) or HT treatment. A total of 10 ewes were maintained in the TN regime, and a further 11 ewes were moved to individual pens in a HT chamber (Bainbridge, Marcellus, MI). The relevant temperature, lighting and feeding regimes used in this treatment are as previously described (18).

**Experimental design and tissue preparation.** Ewes were killed following one of the designated time points; at 55 dGA (early gestation,  $n = 5$ ; TN and  $n = 6$ ; HT) or at 90 dGA (mid gestation,  $n = 5$ ; TN and  $n = 5$ ; HT). Maternal arterial blood samples were collected approximately 2 d before autopsy, pre-feeding. At autopsy of the 90 dGA animals, 2 mL of blood was drawn from the fetal umbilical vein, immediately after opening the uterus. The gravid uterus was removed and dissected into placental and fetal components, as previously described (19). With selected B type placentomes (20), we carefully separated the caruncle and cotyledon by squeezing the caruncle with gentle traction on the cotyledon (21). Fetal liver and brain was collected, weighed and the liver was snap frozen. Approximately 6–10 g of the frozen cotyledon, caruncle, and liver tissue was later ground under liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until total cellular (tc) RNA extraction.

**Plasma insulin and IGF-I concentrations.** Insulin concentrations were determined using a Mercodia sheep insulin ELISA (Alpco, Windham, NH) (22). The interassay coefficients of variation were 2.1% and 9.7% for high- and low-quality control samples, respectively, while the intra-assay coefficients of variation were 2.7% and 8.5%, respectively. Plasma IGF-I was measured by immunoradiometric assay after extraction (Diagnostics Systems Laboratories, Inc, Webster, TX) as previously described (23). The interassay coefficients of variation were 8.3% and 7.2% for a high and low quality control sample respectively, while the intra-assay coefficients of variation were 1.5% and 1.3%, respectively.

**Construction and preparation of sheep cDNAs.** Sheep IGF-I, -II and IGFBP-1, -2, -3 and -4 cDNAs were amplified by RT-PCR that corresponded to IGF-I; basepair (bp) 508 to 698 of Genbank Accession number M30653), IGF-II; (bp 342-839 of MN\_001009311), IGFBP-1; (bp 556-735 of X54979), IGFBP-2 (bp 645-413 of S44612), IGFBP-3 (bp 1029-693 of M76478), IGFBP-4 (bp 855-1083 of S77394). Reverse Transcriptase reactions were conducted using 1  $\mu\text{g}$  of 135 dGA fetal hepatic tcRNA, 1  $\mu\text{L}$  (0.5  $\mu\text{g}$ ) of oligo (dT)<sub>12-18</sub> and Superscript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA). Thirty-five cycles of amplification were performed in the associated PCR, 1 min at 94°C, 1 min at 55°C (IGF-I), 58°C (IGF-II), 53°C (IGFBP-1), 60°C (IGFBP-2), 55°C (IGFBP-3) and 54°C (IGFBP-4), 1 min at 72°C, and finally 10 min at 72°C. *Taq* polymerase amplified PCR products were ligated into pCR II and transformed into Inv $\alpha$ F<sup>+</sup> *E. coli* cells (Invitrogen Corporation, Carlsbad, CA). Plasmids were purified as described and the insert was sequenced (Davis Sequencing, University of California, Davis, CA) in both directions to verify authenticity. All cDNA sequence verification was performed using basic local alignment search tool (BLAST NCBI, M.D.).  $\beta$ -actin cDNA (bp 637-1135 of U39357) was generated as previously described (19).

**RNA extraction, RNA probe synthesis and RNase protection assay.** Fetal liver, caruncular and cotyledonary tcRNA was isolated using Tri-Reagent® (Molecular Research Center Inc) (19). The plasmid containing the cDNA fragment of interest was linearized with the appropriate restriction endonucleases (BamHI or *EcoRV*). IGF-II, IGFBP-4 and  $\beta$ -actin were restricted with internal restriction endonucleases to yield shorter and more stable probes for RPA. The IGF-II cDNA was restricted with *Fsp*-1 (New England BioLabs, Beverly, MA), which when used in RPAs, displayed a protected fragment of 177 bp. IGFBP-4 was restricted with *Sty*I (New England BioLabs) to yield a protected fragment of 179 bp, whilst  $\beta$ -actin was restricted with *Ava*II (New England BioLabs) to yield a protected fragment of 141 bp. Antisense [ $\alpha$ -<sup>32</sup>P]CTP radiolabeled cRNAs ( $2 \times 10^5$  cpm) were synthesized by *in vitro* transcription using T3, Sp6 or T7 RNA polymerases (Ambion, Austin, TX) in the presence of unlabeled dNTPs. RNase protection assays were performed as previously described (19).

**Statistical analysis.** Maternal CBT analysis has been previously reported (18). Placental and fetal data were analyzed using Student's *t*-test. Tissue IGF-I, -II, IGFBP-1, -2, -3, and -4 mRNA levels were normalized to  $\beta$ -actin mRNA, and analyzed using two-way ANOVA and Tukey's means-separation test.

## RESULTS

Control TN ewes had a mean CBT throughout the study of  $39.2 \pm 0.1^{\circ}\text{C}$  and 55 dGA HT ewes had elevated CBTs similar to those previously published for 90 dGA HT ewes (Mean CBT  $39.9^{\circ}\text{C}$ ) (18). Placental weights were not significantly different at 55 dGA between treatments, though at 90 dGA, placental weights were approximately 70% of normal (Table 1). Fetal weights were similar at 55 dGA, but were significantly reduced at 90 dGA (Table 1). Fetuses at both gestational ages displayed signs of hepatic growth restriction, resulting in a significant increase in brain/liver ratio at 55 dGA (Table 1), and tending to be increased at 90 dGA. While fetal weight was unaltered at 55 dGA, placental weight was reduced to 74% of control, resulting in a significantly increased fetal/placental ratio at 55 dGA (Table 1). There were however no significant differences in placental weight or fetal/placental ratio at 90 dGA.

**Table 1.** Placental and fetal weights, fetal CRL and associated ratios of TN and HT fetuses at 55 and 90 dGA, following 15 and 55 days in treatment, respectively

	TN	HT	<i>p</i> -value
55 dGA	$n = 5$	$n = 6$	
Gestational age (d)	$55.60 \pm 0.40$	$55.33 \pm 0.49$	ns
Placental weight (g)	$177.31 \pm 17.28$	$131.76 \pm 19.30$	ns
Fetal weight (g)	$29.92 \pm 1.59$	$30.15 \pm 2.55$	ns
Fetal/placental ratio	$0.17 \pm 0.01$	$0.24 \pm 0.01$	< 0.01
Brain weight (g)	$1.30 \pm 0.07$	$1.33 \pm 0.13$	ns
Liver weight (g)	$2.27 \pm 0.17$	$1.96 \pm 0.20$	ns
Brain/liver ratio	$0.58 \pm 0.03$	$0.68 \pm 0.03$	< 0.05
CRL (cm)	$9.66 \pm 0.51$	$9.48 \pm 0.47$	ns
Ponderal index ( $\text{g}/\text{cm}^3$ )	$3.54 \pm 0.59$	$3.61 \pm 0.34$	ns
90 dGA	$n = 5$	$n = 5$	
Gestational age (d)	$93.67 \pm 0.92$	$92.50 \pm 1.03$	ns
Placental weight (g)	$531.94 \pm 87.58$	$354.90 \pm 52.63$	ns
Fetal weight (g)	$676.23 \pm 45.58$	$514.22 \pm 39.72$	< 0.05
Fetal/placental ratio	$1.42 \pm 0.21$	$1.63 \pm 0.26$	ns
Brain weight (g)	$15.92 \pm 0.91$	$13.18 \pm 1.10$	ns
Liver weight (g)	$42.43 \pm 5.42$	$27.28 \pm 3.86$	< 0.05
Brain/liver ratio	$0.39 \pm 0.03$	$0.52 \pm 0.08$	ns
CRL (cm)	$27.17 \pm 0.53$	$25.75 \pm 0.67$	ns
Ponderal index ( $\text{g}/\text{cm}^3$ )	$3.38 \pm 0.22$	$2.99 \pm 0.11$	ns

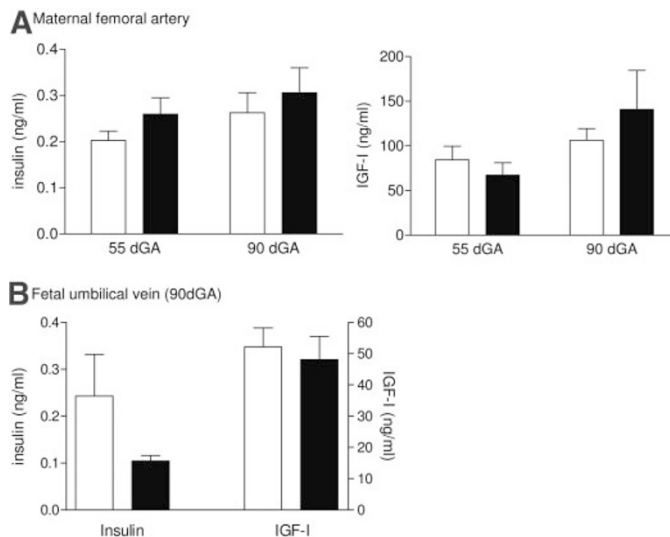
Values are means  $\pm$  SEM.

Maternal arterial plasma concentrations for insulin and IGF-I concentrations remained unaltered across early and mid gestation and there were no significant treatment effects (Fig. 1). Although umbilical venous concentrations of insulin in the 90 dGA HT group were reduced to 43% of control (Fig. 1), due to large variance in the data, statistical significance was not obtained ( $p = 0.15$ ).

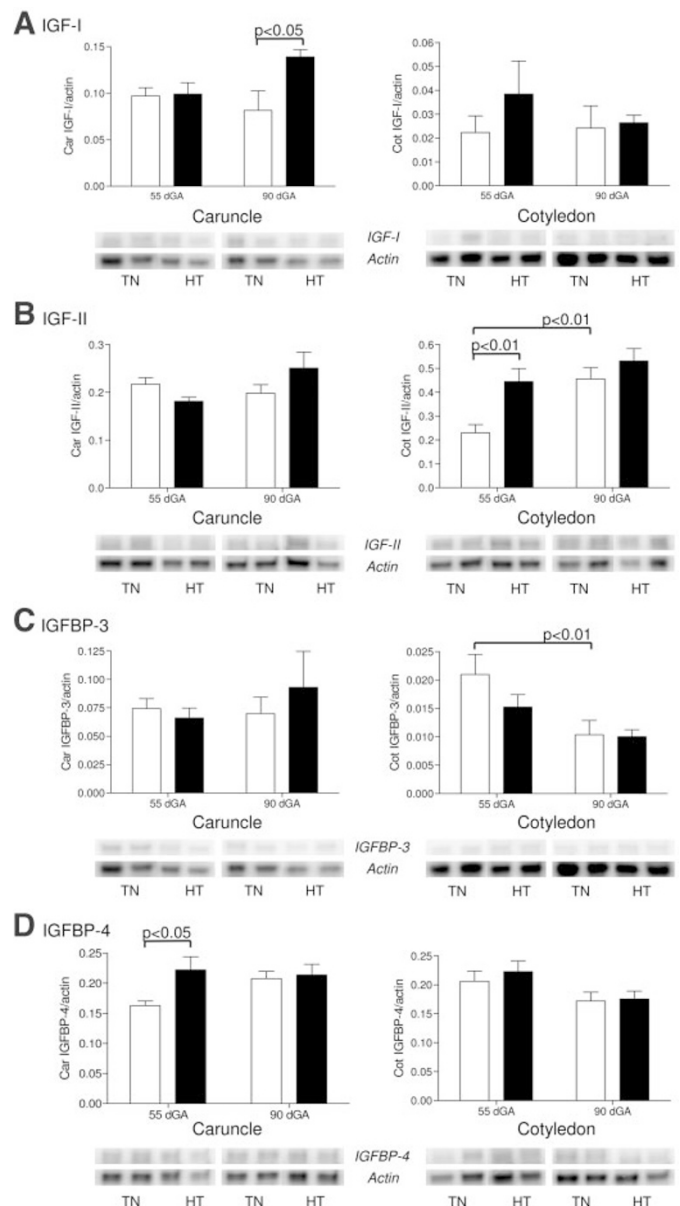
Figure 2 displays the relative mRNA concentrations of IGF-I, IGF-II, IGFBP-3 and IGFBP-4, in caruncle and cotyledon tissue. Caruncular IGF-I mRNA expression did not display an effect of gestational age; however, due to increased IGF-I mRNA expression in HT placentae at 90 dGA there was a significant day by treatment interaction for caruncular IGF-I mRNA expression (Fig. 2A). No effect of gestational age or treatment was observed in cotyledon tissue. IGF-II expression in TN cotyledonary tissue increased significantly with gestational age (Fig. 2B). As a result of increased IGF-II mRNA in HT placentae at 55 dGA (Fig. 2B), there was a significant day by treatment interaction ( $p < 0.01$ ) for cotyledonary IGF-II mRNA concentration. In caruncular tissue, no effect of gestational age or treatment was observed.

IGFBP-1 was not detected in caruncle or cotyledon using 50  $\mu\text{g}$  of tcRNA. IGFBP-2 was detected in both caruncle and cotyledon tissue, however, due to inconsistent banding patterns, results were not suitable for analysis. Caruncular IGFBP-3 concentrations were consistent across gestation and were unaffected by treatment. However, cotyledonary IGFBP-3 declined with advancing gestation (Fig. 2C), though there were no treatment effects. IGFBP-4 mRNA concentration did not display gestational age effects in caruncular or cotyledon tissue. IGFBP-4 mRNA concentration was significantly elevated in HT caruncle tissue at 55 dGA, compared with control (Fig. 2D).

TN fetal hepatic IGF-I mRNA concentrations increased with gestational age (Fig. 3A). There were no treatment effects on IGF-I mRNA concentration at either 55 or 90 dGA. IGF-II



**Figure 1.** Maternal arterial (A) and fetal venous umbilical (B) concentrations of insulin and IGF-I in TN (□) and HT (■). Maternal plasma was obtained at 55 and 90 dGA, while fetal umbilical blood was sampled only in the 90 dGA groups.



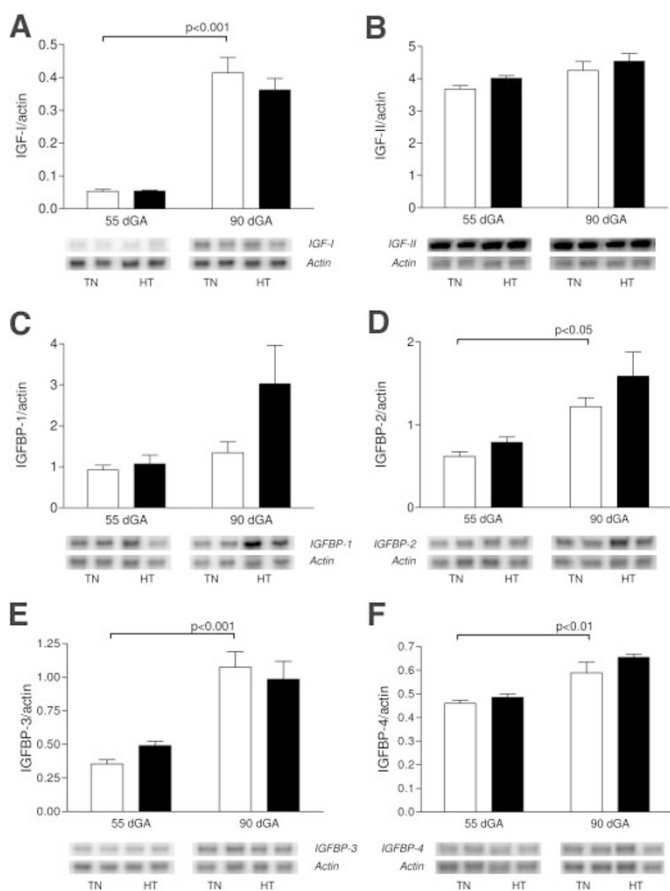
**Figure 2.** Concentration at 55 and 90 dGA of ovine IGF-I (A), IGF-II (B), IGFBP-3 (C) and IGFBP-4 (D) mRNA (IGF(BP)/actin) densitometry units in TN (□) and HT (■) maternal caruncle and fetal cotyledon tissue.

mRNA concentrations did not differ across early pregnancy, were not affected by treatment and were considerably greater than IGF-I mRNA concentrations (Fig. 3B). The concentration of IGFBP-2 (Fig. 3D), IGFBP-3 (Fig. 3E) and IGFBP-4 (Fig. 3F) mRNA increased significantly with gestational age, while IGFBP-1 remained unaltered (Fig. 3C). The IGFBPs studied were unaffected by treatment, though IGFBP-1 exhibited a trend toward increased levels at 90 dGA (Fig. 3C).

## DISCUSSION

This study demonstrates that in a situation of early-onset placental and fetal growth restriction, circulating fetal and maternal plasma insulin and IGF-I levels remain unaltered, though fetal insulin concentrations tend to be reduced by mid gestation. Alterations do occur in placental tissue mRNA





**Figure 3.** Fetal hepatic concentrations of IGF-I (A), IGF-II (B), IGFBP-1 (C), IGFBP-2 (D), IGFBP-3 (E) and IGFBP-4 (F) mRNA (IGF(BP)/actin densitometry units) at 55 and 90 dGA in TN (□) and HT (■). Representative RNase protection assay images are displayed in lower panels.

concentration for certain members of the IGF axis in early and mid gestation, though no evidence is found of changes in fetal hepatic expression of IGFs and IGFBPs. These changes occur concurrently with an increased fetal/placental ratio and brain/liver ratio at 55 dGA in HT pregnancies, and significant reductions in fetal and liver weights by 90 dGA.

**Placental IGFs.** During early and mid gestation, the placental expression of IGF-I mRNA is greater than in late pregnancy (24,25), suggesting that IGF-I plays an important role in early placental development as a placental growth factor (25). Placental IGF-I expression is mainly localized to the maternal stroma (12), and although statistical comparison between the two tissue types is not possible, our data support previous localization observations. Aberrant placental IGF-I production has been implicated in IUGR (6,26), where reduced fetal growth may have resulted from an inability of the placenta to increase placental nutrient transfer capacity (27). In the studies presented here, we observed a significant increase in caruncle IGF-I mRNA concentration of the HT placenta during mid gestation, while cotyledonary IGF-I mRNA was unaltered. Studies reporting the placental expression of IGF-I have focused on near term collection points and have reported conflicting data, with reports of no differences in placental IGF-I mRNA concentrations using PCR (28), while other reports document significant increases in placental

IGF-I mRNA through ISH and RNA dot blot techniques (24). Up-regulation of IGF-I mRNA in the HT caruncle at 90 dGA may be an attempt to increase trophoblast differentiation, placental angiogenesis and nutrient transport, at a stage of pregnancy when fetal demands on the transport capacity of the placenta are normally increasing.

Placental vascular development is a particularly important aspect of placental development, setting the stage for later nutrient and gaseous exchange. The IGFs and IGFBPs play a role in implantation, early pregnancy and throughout gestation promoting vascular development (11,29). The increased cotyledon IGF-II mRNA at 55 dGA in the current study could play a role in altered placental angiogenesis. IGF-II mRNA is observed from 50 dGA onwards in the sheep placenta, predominantly in the fetal mesenchyme tissues with lower concentrations in the caruncular stroma (11,30). Coinciding with the increased IGF-II mRNA concentration, we have previously reported increased VEGF, Ang-1, Ang-2 and Tie-2 mRNA concentration in 55 dGA HT cotyledon (19,31). IGF-II has a direct stimulatory effect on migration and proliferation of endothelial cells through binding to the IGF-II/Mannose-6-phosphate receptor (32). Specifically, IGF-II appears to stimulate VEGF mRNA expression in monkey granulosa and keratinocyte cells (33,34), through the MAP kinase pathway, by increasing HIF-1 $\alpha$ , a potent inducer of VEGF mRNA transcription (33). Therefore, both growth factors, IGF-II and VEGF, may act together to stimulate tissue-specific cotyledonary angiogenesis during exposure to hyperthermic conditions, an effect that is most likely acute in nature, as by 90 dGA, both cotyledon VEGF (19) and IGF-II mRNA concentrations are no longer elevated. It is important to remember that while growth factor concentrations may be similar at the later stages of pregnancy, alterations in vascular development occurring earlier in pregnancy have potentially set the stage for later placental functional impairment through possible reductions in the total nutrient exchange surface area.

**Placental IGFBPs.** IGF binding protein 1 is the most prevalent IGFBP in the human uterus during implantation and facilitates trophoblast penetration at the maternal interface (29). However, in the less invasive sheep placenta, where a syncytialtrophoblast forms, expression of IGFBP-1 mRNA is localized to the luminal epithelium at 13 dGA, before attachment of the embryo, and becomes undetectable by 21 dGA (35). The failure to detect IGFBP-1 in cotyledon or caruncle in the present study is in agreement with this previous report and may reflect differences in trophoblast invasion mechanisms between different species (35). Additionally, there may be a redundancy between the various types of IGFBP, and different species may use different IGFBPs for similar functions (12). The concentration of IGFBP-3 mRNA decreased in cotyledons with increasing gestational age. These findings are in concordance with an earlier ontogenetic study in control sheep placenta, where IGFBP-3 is expressed in high concentrations in the luminal epithelium in early gestation (13–15 dGA), suggesting a role in implantation, after which concentrations remain low (11). In the caruncle, IGFBP-3 levels are reportedly higher and localized to maternal vessel walls, where it is

postulated to play a role in the transfer of IGFs from circulation to target tissues (11).

Concomitantly with the up-regulation of IGF-II in cotyledon, IGFBP-4 mRNA expression is increased in the 55 dGA HT caruncle. IGFBP-4 has been localized to the caruncular capsule and stroma in ovine pregnancy and in stroma underlying the luminal epithelium. Similarly, IGFBP-4 has been localized adjacent to implantation sites in murine pregnancy (11), and has been suggested to play a physiologic role in the implantation process. IGFBP-4 has been demonstrated to inhibit the actions of the IGFs in bone and neuronal cells (1,36), and the increased co-expression of IGF-II in cotyledon and IGFBP-4 in caruncle at early gestation suggests that IGFBP-4, rather than IGFBP-1 may play an important role in regulating IGF-II effects at the maternal-fetal interface of the sheep placenta, possibly limiting trophoblast epithelium interaction and exchange surface area development.

**Fetal hepatic IGF and IGFBP concentration.** Hepatic expression of IGF-I and IGFBP-2, -3 and -4 are developmentally regulated and in control TN tissues show a significant increase with gestational age, similar to changes observed in other fetal organ studies (3,30,37), while concentrations of IGFBP-1 tend to be increased. In agreement with other models of growth restriction, hepatic IGF-II expression is more abundant than that of IGF-I, remains unchanged throughout the early and mid stages of pregnancy and lacks a significant relationship with placental restriction (38,39). Furthermore, this study demonstrates no significant effect of treatment on fetal hepatic IGF and IGFBP concentration during early or mid-gestation.

In this report no change in hepatic IGFBP-1 mRNA concentration was observed during early fetal development, though at 90 dGA, before maximal fetal growth, IGFBP-1 mRNA exhibited a trend to be up-regulated, suggestive of increased expression as seen later in pregnancy in other models of placental insufficiency (40). IGFBP-1 responds to chronic changes in nutritional status or oxygenation (7), therefore, the failure to demonstrate an increase in fetal hepatic IGFBP-1 suggests that the HT fetuses are likely not chronically hypoxic or hypoglycemic at this early stage of gestation. Studies performed in late gestation human IUGR demonstrate increases in plasma IGFBP-1 and hepatic IGFBP-1 mRNA concentrations, and decreases in IGF-I and IGFBP-3 in fetal plasma and liver (5,6,41). Similar alterations are not apparent in early and mid-gestation IUGR sheep, suggesting that the changes in the IGF axis during late gestation may be secondary to the near term, hypoxic, hypoglycemic and hypoinsulinemic stage of fetal growth restriction.

## CONCLUSIONS

This study describes fetal and placental changes in expression of the IGFs and four of its binding proteins in early and mid-gestation in a model of IUGR, caused by a placental insult in early gestation. The observed greater fetal/placenta ratio at 55 dGA that was attenuated by 90 dGA suggests a number of developmental processes occur in response to the insult. Firstly, the increased ratio is supportive of an impaired

IGF-II/IGFBP-4 driven placental development process early in gestation that later in pregnancy manifests itself through reduced nutrient delivery to the developing fetus. Secondly, the maintenance of fetal weight at 55 dGA suggests that an improved functional placenta actually results, perhaps as an acute compensatory response to stress. Although by 90 dGA, while fetal IGFs are unaltered, fetal weight and liver weight have become significantly decreased, suggesting that compensatory effects that occurred early in gestation have now failed. These reductions in fetal and liver weight support the concept that by 90 dGA, before maximal fetal growth, placental nutrient supply is already impaired. This reduced nutrient supply could be manifested by reductions in trophoblast epithelium development and subsequent nutrient exchange surface area such that fetal growth is now reduced. From other studies in this model we know that 45 d later, by 135 dGA (15), fetuses are hypoglycemic and display reduced IGF levels. This implies that at 90 dGA, the impact of the *in utero* nutritional environment upon fetal IGF production and subsequent growth is just emerging. Combined, these data suggest that subtle changes in placental IGF expression in early gestation may predispose the developing placenta to functional insufficiency, resulting in inadequate substrate supply to the developing fetus.

## REFERENCES

- Mohan S, Baylink DJ 2002 IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. *J Endocrinol* 175:19-31
- Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type I IGF receptor (Igf1r). *Cell* 75:59-72
- Carr JM, Owens JA, Grant PA, Walton PE, Owens PC, Wallace JC 1995 Circulating insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and tissue mRNA levels of IGFBP-2 and IGFBP-4 in the ovine fetus. *J Endocrinol* 145:545-557
- 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. *J Endocrinol* 146:23-34
- Ong K, Kratzsch J, Kiess W, Costello M, Scott C, Dunger D 2000 Size at Birth and Cord Blood Levels of Insulin, Insulin-Like Growth Factor I (IGF-I), IGF-II, IGF-Binding Protein-1 (IGFBP-1), IGFBP-3, and the Soluble IGF-II/Mannose-6-Phosphate Receptor in Term Human Infants. *J Clin Endocrinol Metab* 85:4266-4269
- Larsen T, Main K, Andersson AM, Juul A, Greisen G, Skakkebaek NE 1996 Growth hormone, insulin-like growth factor I and its binding proteins 1 and 3 in last trimester intrauterine growth retardation with increased pulsatility index in the umbilical artery. *Clin Endocrinol* 45:315-319
- McLellan KC, Hooper SB, Bocking AD, Delhanty PJ, Phillips ID, Hill DJ, Han VK 1992 Prolonged hypoxia induced by the reduction of maternal uterine blood flow alters insulin-like growth factor-binding protein-1 (IGFBP-1) and IGFBP-2 gene expression in the ovine fetus. *Endocrinology* 131:1619-1628
- Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, Hintz RL, Rosenfeld RG 1995 Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab* 80:1548-1555
- Tapanainen PJ, Bang P, Wilson K, Unterman TG, Vreman HJ, Rosenfeld RG 1994 Maternal hypoxia as a model for intrauterine growth retardation: effects on insulin-like growth factors and their binding proteins. *Pediatr Res* 36:152-158
- Ehrhardt RA, Bell AW 1995 Growth and metabolism of the ovine placenta during mid-gestation. *Placenta* 16:727-741
- Reynolds TS, Stevenson KR, Wathes DC 1997 Pregnancy-specific alterations in the expression of the insulin-like growth factor system during early placental development in the ewe. *Endocrinology* 138:886-897
- Han VK, Carter AM 2000 Spatial and temporal patterns of expression of messenger RNA for insulin-like growth factors and their binding proteins in the placenta of man and laboratory animals. *Placenta* 21:289-305
- Gallaher BW, Breier BH, Keven CL, Harding JE, Gluckman PD 1998 Fetal programming of insulin-like growth factor (IGF)-I and IGF-binding protein-3: evidence for an altered response to undernutrition in late gestation following exposure to periconceptual undernutrition in the sheep. *J Endocrinol* 159:501-508
- McMullen S, Osgerby JC, Milne JS, Wallace JM, Wathes DC 2005 The effects of acute nutrient restriction in the mid-gestational ewe on maternal and fetal nutrient status, the expression of placental growth factors and fetal growth. *Placenta* 26:25-33

15. Thureen PJ, Trembler KA, Meschia G, Makowski EL, Wilkening RB 1992 Placental glucose transport in heat-induced fetal growth retardation. *Am J Physiol* 263:R578–R585
16. Regnault TR, de Vrijer B, Galan HL, Davidsen ML, Trembler KA, Battaglia FC, Wilkening RB, Anthony RV 2003 The relationship between transplacental O<sub>2</sub> diffusion and placental expression of PlGF, VEGF and their receptors in a placental insufficiency model of fetal growth restriction. *J Physiol* 550:641–656
17. Regnault TR, Galan HL, Parker TA, Anthony RV 2002 Placental development in normal and compromised pregnancies - A review. *Placenta* 23:S119–S129
18. Regnault TR, Orbus RJ, Battaglia FC, Wilkening RB, Anthony RV 1999 Altered arterial concentrations of placental hormones during maximal placental growth in a model of placental insufficiency. *J Endocrinol* 162:433–442
19. Regnault TR, Orbus RJ, de Vrijer B, Davidsen ML, Galan HL, Wilkening RB, Anthony RV 2002 Placental expression of VEGF, PlGF and their receptors in a model of placental insufficiency-intrauterine growth restriction (PI-IUGR). *Placenta* 23:132–144
20. Vatnick I, Bell AW 1992 Ontogeny of fetal hepatic and placental growth and metabolism in sheep. *Am J Physiol* 263:R619–R623
21. Stegmann JH 1974 Placental development in the sheep and its relation to fetal development. *Bijdragen tot de Dierkunde* 44:4–72
22. Limesand SW, Hay WW Jr 2003 Adaptation of ovine fetal pancreatic insulin secretion to chronic hypoglycaemia and euglycaemic correction. *J Physiol* 547:95–105.
23. Nagatani S, Zeng Y, Keisler DH, Foster DL, Jaffe CA 2000 Leptin regulates pulsatile luteinizing hormone and growth hormone secretion in the sheep. *Endocrinology* 141:3965–3975
24. Sheikh S, Satoskar P, Bhartiya D 2001 Expression of insulin-like growth factor-I and placental growth hormone mRNA in placentae: a comparison between normal and intrauterine growth retardation pregnancies. *Mol Hum Reprod* 7:287–292
25. Wang CY, Daimon M, Shen SJ, Engelmann GL, Ilan J 1988 Insulin-like growth factor-I messenger ribonucleic acid in the developing human placenta and in term placenta of diabetics. *Mol Endocrinol* 2:217–229
26. Nieto-Diaz A, Villar J, Matorras-Weinig R, Valenzuela-Ruiz P 1996 Intrauterine growth retardation at term: association between anthropometric and endocrine parameters. *Acta Obstet Gynecol Scand* 75:127–131
27. Sorem KA, Siler-Khodr TM 1998 Placental IGF-I in severe intrauterine growth retardation. *J Matern Fetal Med* 7:1–7
28. Abu-Amero SN, Ali Z, Bennett P, Vaughan JJ, Moore GE 1998 Expression of the insulin-like growth factors and their receptors in term placentas: a comparison between normal and IUGR births. *Mol Reprod Dev* 49:229–235
29. Han VK, Bassett N, Walton J, Challis JR 1996 The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes: evidence for IGF-IGFBP interactions at the fetomaternal interface. *J Clin Endocrinol Metab* 81:2680–2693
30. Delhanty PJ, Han VK 1993 The expression of insulin-like growth factor (IGF)-binding protein-2 and IGF-II genes in the tissues of the developing ovine fetus. *Endocrinology* 132:41–52
31. Hagen AS, Orbus RJ, Wilkening RB, Regnault TR, Anthony RV 2005 Placental expression of angiopoietin-1, angiopoietin-2 and tie-2 during placental development in an ovine model of placental insufficiency-fetal growth restriction. *Pediatr Res* 58:1228–1232
32. Volpert O, Jackson D, Bouck N, Linzer DI 1996 The insulin-like growth factor II/mannose 6-phosphate receptor is required for proliferin-induced angiogenesis. *Endocrinology* 137:3871–3876
33. Kwon YW, Kwon KS, Moon HE, Park JA, Choi KS, Kim YS, Jang HS, Oh CK, Lee YM, Kwon YG, Lee YS, Kim KW 2004 Insulin-like growth factor-II regulates the expression of vascular endothelial growth factor by the human keratinocyte cell line HaCaT. *J Invest Dermatol* 123:152–158
34. Martinez-Chequer JC, Stouffer RL, Hazzard TM, Patton PE, Molskness TA 2003 Insulin-like growth factors-1 and -2, but not hypoxia, synergize with gonadotropin hormone to promote vascular endothelial growth factor-A secretion by monkey granulosa cells from preovulatory follicles. *Biol Reprod* 68:1112–1118
35. Wathes DC, Reynolds TS, Robinson RS, Stevenson KR 1998 Role of the insulin-like growth factor system in uterine function and placental development in ruminants. *J Dairy Sci* 81:1778–1789
36. Cheung PT, Smith EP, Shimasaki S, Ling N, Chernausk SD 1991 Characterization of an insulin-like growth factor binding protein (IGFBP-4) produced by the B104 rat neuronal cell line: chemical and biological properties and differential synthesis by sublines. *Endocrinology* 129:1006–1015
37. Langford K, Nicolaidis K, Miell JP 1998 Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. *Hum Reprod* 13:1389–1393
38. Owens JA, Kind KL, Carbone F, Robinson JS, Owens PC 1994 Circulating insulin-like growth factors-I and -II and substrates in fetal sheep following restriction of placental growth. *J Endocrinol* 140:5–13
39. Jensen EC, Harding JE, Bauer MK, Gluckman PD 1999 Metabolic effects of IGF-I in the growth retarded fetal sheep. *J Endocrinol* 161:485–494
40. Unterman T, Lascon R, Gotway MB, Oehler D, Gounis A, Simmons RA, Ogata ES 1990 Circulating levels of insulin-like growth factor binding protein-1 (IGFBP-1) and hepatic mRNA are increased in the small for gestational age (SGA) fetal rat. *Endocrinology* 127:2035–2037
41. Fant M, Salafia C, Baxter RC, Schwander J, Vogel C, Pezzullo J, Moya F 1993 Circulating levels of IGFs and IGF binding proteins in human cord serum: relationships to intrauterine growth. *Regul Pept* 48:29–39