Exogenous GH infusion to late-gestational fetal sheep does not alter fetal growth and metabolism

M K Bauer, J E Harding, B H Breier and P D Gluckman

Research Centre for Developmental Medicine and Biology, School of Medicine, Private Bag 92019, Auckland, New Zealand

(Requests for offprints should be addressed to J E Harding; Email: j.harding@auckland.ac.nz)

(M K Bauer is now at Novo Nordisk A/S, Pharmacological Research 3, Pharmacology Health Care Discovery and Preclinical Development, Novo Nordisk Park, 2670 Maaloev, Denmark)

Abstract

The role of GH in the regulation of fetal growth and metabolism in late gestation is not well defined. The aim of this study was to determine the effects of exogenous GH infusion on fetal growth and feto-placental metabolism in the normally growing late-gestation fetal sheep. Eleven fetuses received pulsatile GH infusion (3.5 mg/day) for 10 days while 12 control fetuses received vehicle. The GH infusion was given as a continuous infusion (2.5 mg/day) plus an additional pulsatile component (30 pulses equivalent to 1 mg/day) designed to mimic the natural pattern of GH secretion. Fetal GH infusion raised the circulating fetal concentrations of GH threefold, but did not change fetal concentrations of IGF-I, IGF-binding protein-3, insulin or ovine placental lactogen. GH-treated fetuses had blood urea concentrations 15% lower than controls ($P < 0.05$) and glucose uptake 18% lower per kg fetal

weight ($P = 0.06$). There were no other differences attributable to fetal GH infusion in feto-placental metabolism, placental function or placental blood flow. GH-treated fetuses were larger than controls at postmortem (weight + 13%, $P < 0.01$; girth + 5%, $P < 0.01$; crown-rump length + 3%, $P < 0.05$). However, there were no differences between groups in measures of fetal growth (increment in chest girth and hindlimb length). GH-treated fetuses had heavier mothers and when maternal weight was included as a covariate in the analysis, there was no significant difference between treatment groups that could be attributed to GH treatment. GH infusion to normal fetal sheep does not appear to have a significant effect on feto-placental metabolism or fetal growth.

Journal of Endocrinology (2000) **166**, 591–597

Introduction

Growth hormone (GH) has been shown to be a major endocrine regulator of postnatal growth and intermediary metabolism. However, its role in fetal life is less clear. Despite general acceptance of the dogma that GH does not regulate fetal growth *in utero*, there is increasing experimental evidence that GH may have at least some role in fetal growth in late gestation. GH-deficient dwarf rat fetuses have proportionately reduced body size (Kim *et al.* 1993), while human fetuses with congenital GH deficiency are born relatively short (Gluckman *et al.* 1992). In fetal sheep, hypophysectomy results in reduced length of the limbs and long bones which was not reversed by thyroid hormone replacement (Mesiano *et al.* 1987), although the replacement may not have been completely adequate in this study.

There is also indirect evidence that fetal GH may have a role in feto-placental metabolism. Hypophysectomised fetal sheep have markedly increased fat deposition which is reversed by fetal GH replacement using a crude pituitary

preparation (Stevens & Alexander 1986). GH receptor mRNA is present in both hepatic and extrahepatic tissues in the late-gestation fetal sheep, including muscle and kidney (Klempt *et al.* 1993). Circulating GH concentrations in late-gestation fetal sheep are also sensitive to factors similar to those thought to be important for GH regulation in postnatal life, including GH-releasing hormone (Gluckman 1984), somatostatin (Gluckman *et al.* 1979), free fatty acids (Bassett & Gluckman 1987) and undernutrition (Bauer *et al.* 1995). GH in the maternal circulation may also influence placental function and feto-placental substrate distribution (Harding *et al.* 1997).

Despite this indirect evidence for possible roles of GH in fetal life, we are not aware of a study directly investigating the effects of additional fetal GH administration *in utero*. We therefore undertook this study to determine the effects of 10 days of exogenous GH infusion on fetal growth and feto-placental metabolism in normal late-gestation fetal sheep. GH was infused in a pulsatile fashion to mimic the normal pattern of GH secretion in the fetal lamb (Bauer *et al.* 1995).

Materials and Methods

Animals

Twenty-three Romney Dorset cross-bred ewes with singleton pregnancies of known gestational age were brought to the laboratory at approximately 105 days of gestation. They were housed in individual metabolic cages with free access to water and concentrates.

At 115 days (range 113–117 days) ewes underwent surgery as described previously (Harding *et al.* 1997). Briefly, hysterotomy was performed under general anaesthetic. Catheters were placed in the fetal femoral artery and vein via the tarsal vessels and the common umbilical vein via a para-umbilical incision. Two growth-measuring devices (growth catheters) were placed, one around each half of the fetal chest from sternum to spine (Mellor & Murray 1982). Catheters were also placed in the uterine vein via the utero-ovarian vein draining the pregnant horn, and maternal carotid artery and jugular vein and the maternal femoral artery and vein via the tarsal vessels.

Antibiotics (250 ml streptomycin and 250 ml penicillin; Streptopen, Pitman Moore, Upper Hutt, New Zealand) were given intramuscularly to the ewe and 80 mg gentamycin intravenously to the fetus at the time of surgery and thereafter daily for the next 3 days. Catheters were flushed on alternate days with saline containing 10 IU/ml heparin. Growth catheters were measured twice daily after the ewe had been standing quietly for several minutes, and the mean of the two measurements was recorded.

At the end of the experiment (125–128 days) ewes were killed with an overdose of phenobarbitone. The uterus and its contents were dissected, weighed and measured. The position of all catheters was confirmed. All procedures were approved by the Institutional Animal Ethics Committee.

Experimental procedures

As soon as ewes had recovered consciousness after anaesthesia, fetuses in the GH treatment group began a 10-day pulsatile intravenous infusion of recombinant bovine GH (batch no. PR003; American Cyanamid, Princeton, NJ, USA). The GH was freshly prepared in carbonate-buffered saline, pH 9.4 (containing 145 mM NaCl, 31.5 mM NaHCO₃, 10.7 mM Na₂CO₃). The total daily dose was 3.5 mg, given as a continuous infusion of 2.5 mg (1 ml/h) plus an additional 1 mg divided as 30 superimposed pulses (330 µl/pulse). This pulsatile infusion was designed to mimic the natural secretion of GH (Bauer *et al.* 1995). Control animals received an identical volume of the vehicle (carbonate-buffered saline). Blood samples (4 ml) were taken from the fetus and ewe in the morning before feeding on alternate days for metabolic and hormone measurements. On day 7, five samples were taken at 15-min intervals for measurement of mean GH concentration.

On day 10, GH feto-placental substrate uptakes were measured in five pairs of samples taken over an hour starting 2.5 h after cessation of the GH infusion. The GH infusion had to be stopped as fetal venous access was limited. A solution containing 110 mg antipyrine, 750 µCi 3-O-[methyl-³H]glucose and 120 µCi [¹⁴C]urea in normal saline was infused via a 0.45 µm filter to the fetus via the fetal venous catheter at 3 ml/h for 3.5 h after a 5 ml bolus. After 150 min of infusion to establish a steady state, five sets of blood samples were taken at 15-min intervals. Each set consisted of samples taken from the maternal artery (2.7 ml), uterine vein (2.7 ml), umbilical vein (1.3 ml) and fetal artery (1.3 ml). All blood samples for metabolic measurements were aliquoted into Eppendorf tubes on ice and immediately frozen at –70 °C until assay. Blood for hormone and free fatty acid measurements was centrifuged at 4 °C and plasma separated and frozen at –70 °C until assay. Samples for blood gas measurements were kept in capped syringes on ice until measurements were made within 30 min using a blood gas analyser (Chiron M845; Chiron Corporation, Emeryville, CA, USA).

Assays

Glucose (Kunst *et al.* 1984) and urea (Kerscher & Ziegenhorn 1985) were measured by standard enzymatic colorimetric methods modified for assay using a 96-well plate reader (Ashour *et al.* 1987). Lactate was measured in microplates using an enzymatic procedure based on the reduction of NAD. Amino nitrogen was assayed by colorimetric reaction with β-naphthoquinone sulphonate (Evans *et al.* 1993). Free fatty acids were measured by a modification of a commercial kit assay (Oliver *et al.* 1995). Antipyrine was measured in duplicate by HPLC (Pimentel *et al.* 1986). [¹⁴C]Urea and 3-O-[methyl-³H]glucose activities were determined in duplicate aliquots of blood deproteinised with sulphuric acid and sodium tungstate and counted in a dual channel liquid scintillation counter (Rak-Beta model 1219; LKB Wallac, Turku, Finland) with external standard quench correction for 10 min or to a d.p.m. error of less than 3%. Insulin-like growth factor-I (IGF-I) was measured by double-antibody radioimmunoassay (RIA) after acid-ethanol cryoprecipitation extraction validated for fetal sheep plasma (Breier *et al.* 1994a). Insulin (Oliver *et al.* 1993) and ovine placental lactogen (Oliver *et al.* 1992) were measured by RIA. Ovine IGF-binding protein-3 (IGFBP-3) was measured by RIA (Gallaher *et al.* 1998).

Data analysis

Blood oxygen content was calculated from measured haemoglobin, oxygen saturation, and pO₂ (Gull & Charlton 1993). Blood flows were calculated from measuring antipyrine concentrations according to the Fick principle (Meschia *et al.* 1966). Uptakes of oxygen, glucose, and

Figure 1 GH profile of an individual fetus on day 7 of GH infusion. GH was infused at a constant rate of 2.5 mg/fetus per day. GH pulses (arrows) were superimposed at 48-min intervals (1 mg/fetus per day). Samples for GH analysis were taken every 15 min.

lactate were calculated for the uterus and its contents (referred to as uterine uptake = maternal artery – uterine vein concentration difference $\times$ uterine blood flow), fetus (umbilical vein – femoral artery concentration difference $\times$ umbilical blood flow) and placenta (uterine uptake – fetal uptake). Clearances of [^{14}C]urea and 3-O-[methyl- ^3H]glucose were calculated by steady-state diffusion techniques (Kulhanek *et al.* 1974, Stacey *et al.* 1978). Fetal urea production rate was calculated as the product of [^{14}C]urea clearance and the (fetal artery – maternal artery) urea concentration difference (Gresham *et al.* 1972, Battaglia & Meschia 1986). All uptakes are expressed relative to tissue weights, i.e. fetal uptakes per kg fetus, placental uptakes per kg placenta and uterine uptakes per kg uterus and contents.

All baseline measurements and postmortem measurements were compared using unpaired *t*-tests and analysis of covariance as appropriate. Metabolite and hormone concentrations over the period of the study were tested for GH effects by repeated measures analysis of variance. Not all data were available for every animal at each time-point, largely because catheters failed to sample. Differences in growth rates (chest girth increments) were tested using regression analysis as described previously (Harding 1997).

All values are given as the means $\pm$ S.E.M. Statistical analyses were carried out using the SPSS software package (SPSS version 7.0; SPSS Inc., Chicago, IL, USA).

Results

Fetal GH infusion attained a pulsatile plasma GH profile (Fig. 1), and raised mean circulating fetal GH concentrations threefold (Table 1). Maternal GH concentrations were not changed by fetal GH treatment. Fetal and maternal circulating concentrations of IGF-I, IGFBP-3, insulin and placental lactogen were not altered by fetal GH infusion (Table 1).

GH-treated fetuses had lower blood urea concentrations than controls and higher maternal oxygen concentrations.

Table 1 Effect of a 10-day fetal GH infusion on fetal and maternal hormone concentrations. Values are means $\pm$ S.E.M. (*n*) expressed in ng/ml

	GH	Control
Fetal		
GH	694 $\pm$ 47 (6)	201 $\pm$ 42*** (6)
IGF-I	93 $\pm$ 10 (11)	102 $\pm$ 9 (11)
Insulin	0.73 $\pm$ 0.14 (6)	1.00 $\pm$ 0.15 (5)
Placental lactogen	10.5 $\pm$ 2.7 (6)	10.9 $\pm$ 2.4 (7)
IGFBP-3	941 $\pm$ 83 (6)	833 $\pm$ 65 (7)
Maternal		
GH	4.4 $\pm$ 0.5 (6)	7.5 $\pm$ 1.0 (7)
IGF-I	185 $\pm$ 14 (11)	172 $\pm$ 15 (11)
Insulin	1.06 $\pm$ 0.45 (6)	0.83 $\pm$ 0.21 (7)
Placental lactogen	228 $\pm$ 57 (6)	165 $\pm$ 20 (7)
IGFBP-3	1760 $\pm$ 173 (6)	1552 $\pm$ 120 (7)

****P* < 0.001 for comparison between groups.

There were no differences between groups in fetal or maternal blood glucose, lactate, amino nitrogen or fatty acid concentrations (Table 2).

There was no difference between the GH-treated and control group in umbilical blood flow (174 $\pm$ 11 vs 193 $\pm$ 12 ml/min per kg) or uterine blood flow (911 $\pm$ 92 vs 1191 $\pm$ 123 ml/min per kg), nor in placental clearance of [^{14}C]urea (129 $\pm$ 16 vs 116 $\pm$ 17 ml/min per kg) or 3-O-[methyl- ^3H]glucose (168 $\pm$ 22 vs 164 $\pm$ 26 ml/min per kg). Fetal glucose uptake per kg fetal weight tended to be lower (*P* < 0.06) and placental amino nitrogen uptake was reduced in the GH-treated group. There were no other differences between groups in fetal, uterine or placental uptakes of glucose, lactate, amino nitrogen or oxygen (Table 3). Fetal urea production was not altered by GH (12.8 $\pm$ 2.6 vs 13.2 $\pm$ 3.7 $\mu\text{mol/min per kg}$).

Table 2 Effect of a 10-day fetal GH infusion on fetal and maternal blood metabolite concentrations. Values are means $\pm$ S.E.M. (*n*) expressed in mmol/l

	GH	Control
Fetal		
Glucose	0.87 $\pm$ 0.05 (11)	0.82 $\pm$ 0.07 (11)
Lactate	1.45 $\pm$ 0.18 (11)	1.59 $\pm$ 0.12 (11)
Amino nitrogen	6.84 $\pm$ 0.20 (11)	6.80 $\pm$ 0.23 (11)
Urea	5.59 $\pm$ 0.32 (11)	6.59 $\pm$ 0.36* (11)
Free fatty acids	0.043 $\pm$ 0.007 (6)	0.041 $\pm$ 0.005 (7)
Oxygen	3.38 $\pm$ 0.26 (10)	3.36 $\pm$ 0.16 (9)
Maternal		
Glucose	2.48 $\pm$ 0.08 (11)	2.67 $\pm$ 0.09 (11)
Lactate	0.72 $\pm$ 0.07 (11)	0.75 $\pm$ 0.04 (11)
Amino nitrogen	4.74 $\pm$ 0.28 (11)	4.63 $\pm$ 0.30 (11)
Urea	4.80 $\pm$ 0.34 (11)	5.86 $\pm$ 0.44 (11)
Free fatty acids	0.57 $\pm$ 0.08 (6)	0.44 $\pm$ 0.08 (7)
Oxygen	6.40 $\pm$ 0.23 (9)	5.55 $\pm$ 0.28* (6)

**P* < 0.05 for comparison between groups.

Table 3 Effect of a 10-day fetal GH infusion on fetal, uterine and placental substrate uptakes. Values are means ± S.E.M. (n) expressed in μmol/min per kg

	GH	Control
Fetal		
Glucose	24.7 ± 1.6 (11)	30.1 ± 2.2† (11)
Lactate	28.9 ± 4.0 (11)	23.1 ± 2.1 (11)
Amino nitrogen	13.8 ± 17.9 (11)	48.7 ± 17.3 (11)
Oxygen	300 ± 17 (10)	305 ± 16 (9)
Uterine		
Glucose	58.2 ± 4.4 (9)	73.0 ± 8.8 (7)
Lactate	16.0 ± 4.6 (7)	5.3 ± 4.3 (7)
Amino nitrogen	23.9 ± 13.2 (9)	− 0.3 ± 18.9 (7)
Oxygen	434 ± 47 (8)	455 ± 98 (5)
Placental		
Glucose	444 ± 55 (9)	605 ± 105 (7)
Lactate	− 346 ± 60 (7)	− 236 ± 56 (7)
Amino nitrogen	− 204 ± 153 (7)	521 ± 284* (9)
Oxygen	702 ± 117 (8)	780 ± 364 (4)

†P=0.06; *P<0.05 for comparison between groups.

At the end of the experiment, GH-treated fetuses were found to be larger than controls. They were heavier, longer, had greater girths and heavier liver, kidney, gut, thymus and placenta (Table 4). When expressed per kg fetal weight, the GH-treated fetuses had relatively smaller brains, hearts and pituitaries but no differences were found in any other organ weights or body measurements.

Table 4 Effect of a 10-day fetal GH infusion on fetal and placental size. Values are means ± S.E.M. for 10 GH-treated and 12 control animals unless otherwise specified

	GH	Control
Gestational age (days)	126 ± 0.4	126 ± 0.3
Crown–rump length (cm)	41.9 ± 0.3	40.8 ± 0.4*
Hindlimb length (cm)	31.2 ± 1.5	30.7 ± 0.3
Hock–hoof length (cm)	19.3 ± 1.4	17.1 ± 0.2
Girth (cm)	33.0 ± 0.3	31.4 ± 0.5**
Fetus (g)	3842 ± 93	3341 ± 136**
Brain (g)	42.6 ± 1.2	40.9 ± 1.1
Heart (g)	25.4 ± 0.8	24.0 ± 1.0
Pituitary (mg)	84.8 ± 5.6	92.5 ± 3.5
Lungs (g)	103.2 ± 5.1	97.7 ± 4.2
Liver (g)	154.6 ± 10.9	127.0 ± 7.7*
Spleen (g)	10.2 ± 0.7	8.5 ± 0.5
Kidneys (g)	32.9 ± 1.4	28.9 ± 1.4*
Gut (g)	93.9 ± 4.8	80.2 ± 3.9*
Pancreas (g)	3.9 ± 0.3 (n=7)	4.0 ± 0.3 (n=6)
Adrenals (g)	0.40 ± 0.03	0.36 ± 0.03
Thyroid (g)	0.9 ± 0.1	1.0 ± 0.1 (n=11)
Thymus (g)	15.7 ± 1.5	12.0 ± 0.7* (n=11)
Placenta (g)	496 ± 18	416 ± 31*
Uterus (g)	814 ± 60	710 ± 35
Membranes (g)	440 ± 82 (n=9)	427 ± 81

*P<0.05, **P<0.01 for comparison between groups before correction for maternal weight.

Table 5 Measurements made at surgery. Values are means ± S.E.M. for 10 GH-treated and 12 control animals

	GH	Control
Gestational age (days)	115 ± 0.5	115 ± 0.4
Maternal weight (kg)	62.7 ± 1.9	56.4 ± 1.4**
Fetal sex (male:female)	8:2	5:7
Fetal girth (cm)	23.8 ± 2.3	24.9 ± 0.6
Fetal hindlimb length (cm)	29.3 ± 0.3	27.4 ± 0.6**
Fetal hock–hoof length (cm)	15.0 ± 0.2	14.1 ± 0.3*

*P<0.05, **P=0.01 for comparison between groups.

However, we found that GH-treated animals had heavier mothers and the fetuses were larger at surgery, as indicated by fetal limb length measurements at that time (Table 5). There was also a trend towards more male fetuses in the GH-treated group (80 vs 42%, *P*=0.07). Fetal size was directly correlated with maternal weight over the whole group of animals (Table 6). When this was taken into account by including maternal weight as a covariate, fetal size and organ weights were no longer significantly different between groups at postmortem. The effect of fetal sex was not significant when it was added to the model. Furthermore, fetal growth rate as measured by increment in fetal girth was not changed by fetal GH treatment (6.8 ± 0.8 vs 6.2 ± 0.5 mm/day). Increment in fetal hindlimb length between surgery and postmortem was also not affected by GH administration (1.9 ± 1.4 vs 3.3 ± 0.4 cm). Thus the differences between groups at postmortem were most likely due to different initial sizes rather than the effects of GH treatment.

Discussion

The findings of this study illustrate the importance of careful assessment of the equivalence of experimental groups, and the risks of interpreting increased final size as evidence of increased growth. Fetuses in the GH-treated groups were heavier, longer and had increased weight of a number of organs compared with control animals. However, longitudinal measures of fetal growth (increment in girth and limb length) were not affected by GH treatment. Careful data analysis revealed that this paradox was

Table 6 The relationship between fetal growth parameters and maternal weight. Values are correlation coefficients (r) for 10 GH-treated and 12 control animals

	GH	Control	Whole group
Crown–rump length	0.05	0.28	0.51**
Hindlimb length	0.59	0.39	0.46*
Girth	0.06	0.56*	0.53**
Fetal weight	0.5	0.37	0.56**

*P<0.05, **P<0.01 for correlation coefficients.

explained by the observation that the GH-treated fetuses had heavier mothers and longer limbs at the time of surgery. The relationship between fetal size and maternal constraint, which is a function of maternal size, is well recognised (Heinrich 1992, Morel *et al.* 1994). Thus it is most likely, since the groups differed at the beginning of the experiment, that the differences in outcome were not due to fetal GH therapy. The failure of bovine GH treatment to increase growth in fetal sheep cannot be ascribed to lack of biological activity of the bovine GH preparation used, since the same preparation induced the known metabolic and endocrine responses in adult sheep (Hennies *et al.* 1998).

In the rat, responses to GH have been shown to vary depending on the pattern of GH administration. GH injections have a more pronounced effect on total body weight gain, while a constant infusion of GH leads to selective organ growth and reduction in size of fat pads (Clark *et al.* 1995, 1996). The importance of pulsatile GH secretion in the fetal sheep is still unresolved. However, the pattern of circulating GH in our GH-treated fetuses was clearly pulsatile and in good agreement with the GH pattern we have reported in normally growing fetal sheep of similar gestational age (Bauer *et al.* 1995). Thus it is unlikely that the lack of effect in the present study is due to lack of GH pulsatility.

We started GH treatment immediately after surgery in order to maximise duration of treatment. It is possible that post-operative stress meant that the fetuses underwent a period of relative GH resistance, and that this may have contributed to the absence of observed response to GH treatment. However, there were no observed trends over the period of the study in any of the monitored parameters (fetal blood gases, metabolite and hormone concentrations) suggestive of resolving post-operative stress. There was also no suggestion of fetal growth rate, measured by girth increment, diverging between the groups over time, as would be expected if GH did influence fetal growth and a period of hormone resistance was resolving post-operatively.

For practical reasons, placental function was studied over an hour beginning 2.5 h after GH treatment had been stopped. It is theoretically possible that the cessation of GH infusion before the study contributed to the lack of observed differences between groups. However, there were no observable trends over time in any of the parameters measured in the five sets of samples taken during the study. This suggests that there was not an observable effect due to declining GH levels over this period. Furthermore, the aspect of placental function which has been shown to respond to maternal GH treatment (placental diffusion capacity, measured by [^{14}C]urea clearance; Harding *et al.* 1997) is determined by structural characteristics of the placenta such as surface area and barrier thickness. These characteristics are not likely to have changed over the 3.5-h period of withdrawal of GH

treatment in our study. In addition, the lack of effect of GH treatment on fetal growth, metabolic and endocrine parameters monitored throughout the entire 10-day period makes it unlikely that the lack of difference between groups in measured aspects of placental functions was due only to a brief period of GH withdrawal at the time of study.

Consistent with the failure of GH treatment to increase growth in normal fetuses is the failure of GH to markedly alter fetal metabolic profiles. In postnatal life, GH administration reduces glucose uptake and increases lypolysis (Davidson 1987) as well as increasing protein synthesis and decreasing urea production (Salomon *et al.* 1991). In our study, GH-treated fetuses had reduced glucose uptake per kg fetus and decreased blood urea concentrations, both of which are consistent with the postnatal metabolic effects of GH. However, it is difficult to distinguish these possible GH effects from the altered metabolic profiles of larger fetuses of larger mothers with higher arterial oxygen contents secondary to higher haemoglobin concentrations. The lower blood urea concentrations in the GH-treated group were most likely to be secondary to similar lower maternal blood urea concentrations of well-nourished ewes, since urea crosses the placenta by simple diffusion, and fetal blood levels are thus directly related to those of the mother. This is supported by our finding of no difference in urea production per kg fetus between groups, as estimated by the relatively accurate measurement of [^{14}C]urea clearance.

In contrast, fetal glucose uptake per kg fetus is reported to be unchanged with differing fetal size (Owens *et al.* 1987). Thus the difference we observed may reflect true effects of GH on glucose uptake and insulin sensitivity, since GH is reported to decrease insulin sensitivity in fetal (Parkes & Bassett 1985) and postnatal lambs (Bellver *et al.* 1995). The lack of effect of GH treatment on fetal levels of free fatty acids may seem surprising in view of previous evidence of the effects of GH on fetal fat deposition (Stevens & Alexander 1986). However, their studies were conducted on hypophysectomised fetuses with relative obesity. The normal fetus has relatively little white fat at the stage in development which we studied, and brown fat appears resistant to lipolysis *in utero* because of placental factors (Ball *et al.* 1995).

In summary, our study shows that pulsatile GH infusion to normal fetal sheep does not stimulate fetal growth, nor does it lead to substantial changes in fetal endocrine and metabolic profiles. These findings do not exclude a possible role for GH in the regulation of fetal growth and metabolism, since these experiments were only undertaken in normal fetuses. Since circulating GH concentrations are already extremely high in the normally growing fetus, it is possible that any role for GH in fetal growth and metabolism is already maximal in such fetuses, and cannot be further stimulated by additional GH. GH receptor concentrations are relatively low in fetal tissues,

being approximately 30% of adult concentrations in the fetal liver at this age (Klempt *et al.* 1993). Thus it is probable that the receptors are already fully saturated with endogenous GH or with ovine placental lactogen, which can also bind to the ovine GH receptor (Breier *et al.* 1994b), and antagonises GH action *in vitro* (Herman *et al.* 1999).

In addition, since fetal growth in late gestation is largely limited by nutrient availability, it is possible that, in the normal fetus, additional GH will have no effect on fetal growth unless nutrient availability is also altered. Hence it remains possible that in fetuses with reduced circulating GH concentrations or altered fetal nutrient availability, GH supplementation may result in a demonstrable change in fetal growth and metabolism. Studies examining these questions are currently underway in our laboratory.

Acknowledgements

This work is supported by the Auckland Medical Research Foundation and the Health Research Council of New Zealand. Dr W R Baumbach (American Cyanamid, Princeton, NJ, USA) kindly provided the GH. These studies would not have been possible without the assistance and advice of Pandora Evans, Christine Gibson, Ellen Jensen, Toni Smith-Wong and Pierre van Zijl.

References

- Ashour MA, Gee SJ & Hammock BD 1987 Use of a 96-well microplate reader for measuring routine enzyme activities. *Analytical Biochemistry* **166** 353–360.
- Ball KT, Gunn TR, Power GG, Asakura H & Gluckman PD 1995 A potential role for adenosine in the inhibition of nonshivering thermogenesis in the fetal sheep. *Pediatric Research* **37** 303–309.
- Bassett NS & Gluckman PD 1987 The effect of fatty acid infusion on growth hormone secretion in the ovine fetus: evidence for immaturity of pituitary responsiveness *in utero*. *Journal of Developmental Physiology* **9** 301–308.
- Battaglia FC & Meschia G 1986 *An Introduction to Fetal Physiology*, pp 109–111. London: Academic Press.
- 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.
- Bellver SP, Beermann DH, Bell AW, Van Tassell CP, Hogue DE & McLaughlin CL 1995 Effects of exogenous somatotropin on whole-body glycemic response to insulin in young preruminant and ruminant lambs. *Domestic Animal Endocrinology* **12** 143–156.
- Breier BH, Ambler GR, Sauerwein H, Surus A & Gluckman PD 1994a The induction of hepatic somatotrophic receptors after birth in sheep is dependent on parturition-associated mechanisms. *Journal of Endocrinology* **141** 101–108.
- Breier BH, Funk B, Ambler GR, Surus A, Wells CA, Waters MJ & Gluckman PD 1994b Characterization of ovine growth hormone (oGH) and ovine placental lactogen (oPL) binding to fetal and adult hepatic tissue in sheep: evidence that oGH and oPL interact with a common receptor. *Endocrinology* **135** 919–928.
- Clark RG, Mortensen DL, Carlsson LMS, Carmignac D & Robinson ICAF 1995 Growth responses to patterned GH delivery. *Endocrine* **3** 717–723.
- Clark RG, Mortensen DL, Carlsson LMS, Carlsson B, Carmignac D & Robinson ICAF 1996 The obese growth hormone (GH)-deficient dwarf rat: body fat responses to patterned delivery of GH and insulin-like growth factor-I. *Endocrinology* **137** 1904–1912.
- Davidson MB 1987 Effect of growth hormone on carbohydrate and lipid metabolism. *Endocrine Reviews* **8** 115–131.
- Evans PC, Ffolliott-Powell FM & Harding JE 1993 A colorimetric assay for amino nitrogen in small volumes of blood: reaction with B-naphthoquinone sulfonate. *Analytical Biochemistry* **208** 334–337.
- Gallagher 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 under-nutrition in late-gestation following exposure to periconceptual undernutrition in the sheep. *Journal of Endocrinology* **159** 501–508.
- Gluckman PD 1984 Changing responsiveness to growth hormone releasing factor in the perinatal sheep. *Journal of Developmental Physiology* **6** 509–515.
- Gluckman PD, Mueller PL, Kaplan SL, Rudolph AM & Grumbach MM 1979 Hormone ontogeny in the ovine fetus. III. The effect of exogenous somatostatin. *Endocrinology* **104** 974–978.
- Gluckman PD, Gunn AJ, Wray A, Cutfield WS, Chatelain PG, Guillaud O, Ambler GR, Wilton P & Albertsson-Wikland K 1992 Congenital idiopathic growth hormone deficiency associated with prenatal and early postnatal growth failure. The International Board of the Kabi Pharmacia International Growth Study. *Journal of Pediatrics* **121** 920–923.
- Gresham EL, James EJ, Raye JR, Battaglia FC, Makowski EL & Meschia G 1972 Production and excretion of urea by the fetal lamb. *Pediatrics* **50** 372–379.
- Gull I & Charlton V 1993 Effects of antipyrine on umbilical and regional metabolism in late-gestation in the fetal lamb. *American Journal of Obstetrics and Gynecology* **168** 706–713.
- Harding JE 1997 Prior growth rate determines the fetal growth response to acute maternal undernutrition in fetal sheep of late-gestation. *Prenatal and Neonatal Medicine* **2** 300–309.
- Harding JE, Evans PC & Gluckman PD 1997 Maternal growth hormone treatment increases placental diffusion capacity but not fetal or placental growth in sheep. *Endocrinology* **138** 5352–5358.
- Heinrich UE 1992 Intrauterine growth retardation and familial short stature. *Baillieres Clinical Endocrinology and Metabolism* **6** 589–601.
- Hennies M, Breier BH, Gallaher BW, Krishnamurthi RV & Gluckman PD 1998 Growth hormone induces electrolyte and metabolite imbalance in endotoxin challenged sheep. *Proceedings of the 80th Annual Meeting of the Endocrine Society*, p 144, abstract no. P1–108.
- Herman A, Helman D, Livnah O & Gertler A 1999 Ruminant placental lactogens act as antagonists to homologous growth hormone receptors and as agonists to human or rabbit growth hormone receptors. *Journal of Biological Chemistry* **274** 7631–7639.
- Kerscher L & Ziegenhorn J 1985 Urea. In *Methods of Enzymatic Analysis*, vol 3, pp 444–453. Ed. HU Bergmeyer. Weinheim: Verlag Chemie.
- Kim JD, Nanto-Salonen K, Szczepankiewicz JR, Rosenfeld RG & Glasscock GF 1993 Evidence for pituitary regulation of somatic growth, insulin-like growth factors-I and -II, and their binding proteins in the fetal rat. *Pediatric Research* **33** 144–151.
- Klempt M, Bingham B, Breier BH, Baumbach WR & Gluckman PD 1993 Tissue distribution and ontogeny of growth hormone receptor messenger ribonucleic acid and ligand binding to hepatic tissue in the midgestation sheep fetus. *Endocrinology* **132** 1071–1077.
- Kulhanek JF, Meschia G, Makowski EL & Battaglia 1974 Changes in DNA content and urea permeability of the sheep placenta. *American Journal of Physiology* **5** 1257–1263.
- Kunst A, Draeger B & Ziegenhorn J 1984 Colorimetric methods with glucose oxidase and peroxidase. In *Methods of Enzymatic Analysis*, vol 3, pp 178–185. Ed. HU Bergmeyer. Weinheim: Verlag Chemie.

- Mellor DJ & Murray L 1982 Effects on the rate of increase in fetal girth of refeeding ewes after short periods of severe undernutrition during late pregnancy. *Research in Veterinary Science* **32** 377–382.
- Meschia G, Cotter JR, Makowski EL & Barron DH 1966 Simultaneous measurement of uterine and umbilical blood flows and oxygen uptakes. *Quarterly Journal of Experimental Physiology* **52** 1–18.
- Mesiano S, Young IR, Baxter RC, Hintz RL, Browne CA & Thorburn GD 1987 Effect of hypophysectomy with and without thyroxine replacement on growth and circulating concentrations of insulin-like growth factors I and II in the fetal lamb. *Endocrinology* **120** 1821–1830.
- Morel PC, Blair HT, Ormsby JE, Breier BH, McCutcheon SN & Gluckman PD 1994 Influence of fetal and maternal genotype for circulating insulin-like growth factor I on fetal growth in mice. *Journal of Reproduction and Fertility* **101** 9–14.
- Oliver MH, Harding JE, Breier BH, Evans PC & Gluckman PD 1992 The nutritional regulation of circulating placental lactogen in fetal sheep. *Pediatric Research* **31** 520–523.
- Oliver MH, Harding JE, Breier BH, Evans PC & Gluckman PD 1993 Glucose but not a mixed amino acid infusion regulates plasma insulin-like growth factor IGF-I concentrations in fetal sheep. *Pediatric Research* **34** 62–65.
- Oliver MH, Harding JE, Breier BH, Evans PC & Gallaher BW 1995 The effects of ovine placental lactogen infusion on metabolites, insulin-like growth factors and binding proteins in the fetal sheep. *Journal of Endocrinology* **144** 333–338.
- Owens JA, Falconer J & Robinson JS 1987 Effect of restriction of placental growth on fetal and utero-placental metabolism. *Journal of Developmental Physiology* **9** 225–238.
- Parkes MJ & Bassett JM 1985 Antagonism by growth hormone of insulin action in fetal sheep. *Journal of Endocrinology* **105** 379–382.
- Pimentel G, Figueroa JP, Mitchell MD, Massmann A & Nathanielsz PW 1986 Effect of fetal and maternal intravascular antipyrine infusion on maternal plasma prostaglandin concentrations in the pregnant sheep at 104 to 127 days' gestation. *American Journal of Obstetrics and Gynecology* **155** 1181–1185.
- Salomon F, Cuneo R & Sonksen PH 1991 Growth hormone and protein metabolism. *Hormone Research* **36** 41–43.
- Stacey TE, Weedon AP, Haworth C, Ward RHT & Boyd RDH 1978 Fetomaternal transfer of glucose analogs by sheep placenta. *American Journal of Physiology* **234** E32–E37.
- Stevens D & Alexander G 1986 Lipid deposition after hypophysectomy and growth hormone treatment in the sheep fetus. *Journal of Developmental Physiology* **8** 139–145.

Received 24 September 1999

Revised manuscript received 12 May 2000

Accepted 26 May 2000