Treatment of underfed pigs with GH throughout the second quarter of pregnancy increases fetal growth

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Abstract

Circulating growth hormone (GH) concentrations increase in pregnancy and administration of GH during early-mid pregnancy increases fetal growth in well-fed pigs. To determine whether increased maternal GH could promote fetal growth when feed availability is restricted, fifteen cross-bred primiparous sows (gilts) were fed at approximately 30% of *ad libitum* intake, from mating onwards and were injected daily i.m. with recombinant porcine GH (pGH) at doses of 0, 13.4 ± 0.3 and 25.6 ± 0.5 µg/kg live weight from day 25 to day 51 of pregnancy (term ~115 days). Treatment with pGH increased maternal backfat loss between day 25 and day 51 of pregnancy, and increased maternal plasma IGF-I concentrations measured at day 51 of pregnancy. Fetal body weight, length and skull width at day 51 of pregnancy

were increased by maternal treatment with pGH. Fetal plasma glucose concentrations were increased and maternal/fetal plasma glucose concentration gradients were decreased by maternal pGH treatment at 13.4, but not 25.6 µg/kg.day. Fetal plasma concentrations of urea were decreased by both levels of pGH treatment. Overall, fetal weight was negatively correlated with fetal plasma concentrations of urea, positively correlated with maternal plasma α -amino nitrogen concentrations and unrelated to glucose concentrations in either maternal or fetal plasma. This suggests that the availability of amino acids, not glucose, limits fetal growth in the first half of pregnancy in underfed gilts, and that maternal GH treatment may improve amino acid delivery to the fetus.

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Introduction

Fetal growth rate is sensitive to the rates of placental delivery of nutrients and oxygen from maternal to fetal blood (Milner & Gluckman 1996). This supply varies with the availability of circulating nutrients in the mother and with the efficiency of their transfer by the placenta to the fetus. Severe maternal undernutrition, particularly in early-mid pregnancy (Stein *et al.* 1995), or low protein intake in late pregnancy (Godfrey *et al.* 1996) reduces birth weight in humans. Conversely, maternal hyperglycaemia increases placental glucose transfer and birth weight in humans (Allen 1939, Cardell 1953, Hauguel *et al.* 1986) and in rats (Herrera *et al.* 1985). Conditions which reduce the availability of substrates (Godfrey *et al.* 1996, Noblet *et al.* 1985, Stein *et al.* 1995), or those which reduce nutrient delivery across the placenta (Owens *et al.* 1989), can retard fetal growth. However, increased feed supply throughout pregnancy in adolescent sheep promotes growth of maternal tissues, restricts growth of placenta and fetus and reduces birth weight (Wallace *et al.* 1996, 1997).

In humans, high carbohydrate intake in early pregnancy and low protein intake in late pregnancy are both associated with lower weights of placenta and infant at birth (Godfrey *et al.* 1996). Treatments that increase substrate concentrations in maternal plasma, or their transfer across the placenta may therefore increase fetal growth rate (Gluckman & Harding 1997), whereas those that promote growth of maternal tissues may reduce placental and fetal growth (Wallace *et al.* 1996, 1997).

The maternal somatotrophic axis may play a significant role in enabling the mother to adapt to and sustain pregnancy by coordinating nutrient use between maternal, placental and fetal tissues. Maternal blood concentrations of GH, IGF-I and/or IGF-II are increased during early or throughout all of pregnancy in a number of mammalian species (Donovan *et al.* 1991, Gargosky *et al.* 1990, Hills *et al.* 1996, Kelly *et al.* 1976, Mirlesse *et al.* 1993, Nason *et al.* 1996, Owens 1991, Sinha *et al.* 1990). Intrauterine growth retardation is associated with lower than normal concentrations of GH and IGF-I in maternal plasma in humans (Chowden *et al.* 1996, Lassarre *et al.* 1991,

Mirlesse *et al.* 1993), whilst GH-deficiency in dams impairs fetal growth in rats (Spencer *et al.* 1994). This indicates that maternal GH and/or IGF-I are required for normal fetal growth. GH is not able to cross the placenta (Fohlenhag *et al.* 1994), but elevated GH in pregnancy may promote fetal growth either by increasing maternal plasma glucose concentrations through its antagonistic effects on insulin action (Herrera *et al.* 1994) or by increasing placental capacity to transfer nutrients (Jenkinson *et al.* 1999). In growing non-pregnant pigs, GH treatment increases muscle growth and circulating levels of sugars, fatty acids, insulin and IGF-I, and reduces adipose deposition, even when feed is restricted by 20% to 40% below *ad libitum* rates (Buonomo *et al.* 1995, Campbell *et al.* 1988, Etherton *et al.* 1987, Gopinath & Etherton 1989). *In vitro*, GH antagonises insulin-stimulated glucose uptake and lipogenesis in porcine adipose tissue (Magri *et al.* 1990, Walton & Etherton 1986). However, the effects of GH administration on circulating metabolites and insulin sensitivity in pregnant pigs are unknown. Maternal GH may affect the placenta directly, or indirectly via increased circulating IGF-I, since the placenta expresses growth hormone receptors (Barnard *et al.* 1993, Frankenke *et al.* 1992, Scott *et al.* 1992) and type-1 IGF receptors (Chastant *et al.* 1994). Maternal GH treatment increases placental growth in early pregnant, well-fed pigs (Sterle *et al.* 1995) and in mid to late pregnant sheep (Stelwagen *et al.* 1994), and increases placental capacity for simple diffusion in late pregnant sheep (Harding *et al.* 1997).

Previous studies in pigs have observed different effects of maternal GH administration on fetal growth. This may reflect differences in the stage of pregnancy at which treatment occurred, the increase in maternal plasma GH achieved, the age and parity of the dam and the amount and type of feed provided. Birth weight of pigs was not responsive to an approximate three-fold increase in maternal GH concentrations during administration of GH from day 97 of pregnancy until term (~115 days) in gilts and sows (Kveragas *et al.* 1986), nor to doubling of circulating GH following twice-daily injections with GH-releasing factor from day 102 of pregnancy until term in sows (Etienne *et al.* 1992). Treatment of primiparous sows with GH at 32 or 64 µg/kg.day during the first quarter of pregnancy was originally reported to have no effect on fetal growth (Kirkwood *et al.* 1993), but others have since observed increased fetal and placental growth in gilts following maternal GH treatment at lower or similar doses commencing slightly later in early pregnancy (Kelley *et al.* 1995, Rehfeldt *et al.* 1996, Sterle *et al.* 1995). Daily administration of 6 mg pGH to sows later in pregnancy, from day 80 to 94, increased birth weight of progeny (Rehfeldt *et al.* 1993). The aim of the present study was to determine whether GH treatment of pigs during the second quarter of pregnancy could promote fetal growth whilst the maternal anabolic response to GH was restrained using feed restriction.

Materials and Methods

Animals

The study was approved by the Animal Experimentation Ethics Committee of Bunge Meat Industries, Corowa. Fifteen crossbred (Large White × Landrace) gilts were bred at first oestrus to crossbred (Large White × Duroc) boars. They were fed 1.8 kg/day of a ration containing 13.5 MJ digestible energy per kg dry matter and 15.05% protein throughout pregnancy. This level of nutrition in the pregnant gilt corresponds to approximately 30% of *ad libitum* feed intake and permits maintenance of pregnancy and limited maternal live weight gain during pregnancy, but restricts fetal growth (Noblet *et al.* 1985). Live weight and backfat depth (110 mm from the midline over the 13th rib, using B-mode live ultrasound) were measured at mating, day 25 of pregnancy and day 51 of pregnancy. Gilts were allocated to three treatment groups such that live weight and backfat depth at mating were not significantly different between treatment groups. The live weights and backfat depths of gilts were, respectively, 141.8 ± 1.5 kg (mean ± S.E.M.) and 18.7 ± 2.4 mm at mating and 145.3 ± 1.7 kg and 19.6 ± 3.0 mm at day 25 of pregnancy. From day 25 to 50 of pregnancy, gilts received daily i.m. injections of 1 ml of sterile water in the neck region, containing 0, 2, or 4 mg pGH (recombinant porcine growth hormone, Southern Cross Biotech Pty. Ltd, Melbourne, Australia), which delivered average dose rates of pGH across the treatment period of 0, 13.4 ± 0.3 and 25.6 ± 0.5 µg/kg.day respectively. Gilts were bled via jugular venipuncture on day 51 of pregnancy, and then immediately sacrificed by injection of 20 ml of 325 mg/ml pentobarbitone sodium (Lethabarb, Virbac (Aust.) Pty. Ltd, Peakhurst, NSW) into an ear vein. The uterine tract was immediately removed and weighed, and the fetuses removed. Blood was collected from the umbilical cord of each fetus. The weight, nose-tail length and skull width of each fetus was measured, and the fetal liver was dissected and weighed. Ponderal index of each fetus was calculated as weight/nose-tail length³ (kg/mm³).

Circulating metabolite and hormone analyses

Blood samples were collected into EDTA tubes on ice and plasma was obtained by centrifugation at 1800 g for 10 min at 4 °C. Plasma was stored at -20 °C. IGFs were extracted from plasma by size exclusion high performance liquid chromatography at pH 2.5, using a modification of the original procedure (Scott & Baxter 1986), as described previously (Owens *et al.* 1990). IGF-I and IGF-II concentrations were determined by RIA of neutralised chromatography fractions devoid of IGF-binding proteins (Carr *et al.* 1995, Francis *et al.* 1989). Insulin was measured by radioimmunoassay (Insulin-CT kit, CIS bio international, Gif-sur-Yvette Cedex, France).

Glucose (GLUC HK, cat. no. 07 3672 4, Roche Diagnostic Systems, Germany), urea (UREA, cat. no. 07 3685 6, Roche Diagnostic Systems, Germany), free fatty acids (NEFA C, cat. no. 279-75401, Wako Pure Chemical Industries, Osaka, Japan), triglycerides (TRIG, cat. no. 07 3679 1, Roche Diagnostic Systems, Basel, Switzerland), and cholesterol (CHOL, cat. no. 07 3663 5, Roche Diagnostic Systems, Germany) were also measured in maternal and fetal plasma. Amino nitrogen concentrations in maternal and fetal plasma were measured by colorimetric assay (Evans *et al.* 1993). Maternal/fetal transplacental concentration gradients for glucose and urea were calculated by dividing the concentration in maternal plasma by that in fetal plasma.

Western ligand blot analysis of circulating IGF-binding proteins

IGF-binding proteins in plasma collected from all gilts were individually measured by Western ligand blot analysis following incubation with ^{125}I -labelled IGF-II as described previously (Owens *et al.* 1991). Densitometric analysis was performed using the software package MacBas 2.2 (Fuji Film Corporation, Tokyo, Japan), and density values for each binding protein were expressed relative to the density of the 50 kDa binding protein in a reference sample included in each analysis. The density value for IGFBP-3 was obtained by summing the density values for the 45 and 50 kDa binding proteins, which have been identified by immunoblotting and sequencing as isoforms of pIGFBP-3 (Walton & Etherton 1989). Other binding proteins on these blots have been previously identified as IGFBP-1 (29 kDa) and IGFBP-2 (34.5 kDa), based on their co-migration during electrophoresis with binding proteins purified from other species (Owens *et al.* 1991).

Statistics

The effects of treatment on dams were analysed by one-way ANOVA (Program 7D, BMDP Statistical Software, Inc., USA). Treatment group means were compared with the control group by *t*-test using Bonferroni's correction for multiple comparisons. Measurements of individual fetuses were treated as repeated measures on each dam. Fetal measurements were analysed using an unbalanced repeated measures method (Program 5 V, BMDP Statistical Software, Inc., LA, USA), for treatment effects using litter size as a covariate. Where treatment effects were significant, data was further analysed to determine the relationship between pGH dose and responses, using the unbalanced repeated measures method with linear and quadratic contrasts for the dose of pGH administered, with litter size as a covariate. This model was progressively reduced to exclude non-significant factors. Associations were analysed by linear regression.

Figure 1 Effect of maternal pGH treatment on change in maternal live weight and backfat depth. Data are presented as mean $\pm$ S.E.M. Change in live weight and back fat depth are from days 25 to 51 of pregnancy. Treatment group means were compared with the control group by *t*-test using Bonferroni's correction for multiple comparisons. Change in maternal live weight did not differ between treatment groups ($P>0.1$). The loss of maternal backfat was increased by treatment with either dose of pGH ($P<0.05$).

Results

Maternal live weight gain over the treatment period did not differ between treatment groups ($P>0.1$, Fig. 1), but was weakly positively correlated with pGH dose ($r=+0.52$, $n=15$, $P=0.045$). Loss of maternal backfat over the treatment period was more than doubled by either dose of pGH ($P<0.01$, Fig. 1). The change in maternal backfat depth per unit live weight was negatively correlated with fractional (%) weight gain of dams ($r=-0.69$, $n=15$, $P<0.005$).

Administration of pGH to pregnant gilts did not alter the concentrations of glucose (5.23 ± 0.16 mmol/l), urea (4.65 ± 0.23 mmol/l), triglycerides (0.81 ± 0.07 mmol/l), α -amino nitrogen (4.03 ± 0.17 mmol/l) or cholesterol (2.24 ± 0.07 mmol/l) in maternal plasma. Maternal plasma concentrations of urea and cholesterol were strongly positively correlated with each other ($r=+0.74$, $n=15$, $P=0.0015$). Concentrations of free fatty acids in maternal plasma were 60–75% lower in dams treated with pGH (Fig. 2), and overall were strongly negatively correlated with both fractional (%) weight gain of dams ($r=-0.68$, $n=15$, $P=0.005$) and maternal plasma α -amino nitrogen ($r=-0.85$, $n=15$, $P<0.0002$), and strongly

Figure 2 Effect of maternal pGH treatment on maternal plasma concentrations of free fatty acids and IGF-I at day 51 of pregnancy. Data are presented as mean $\pm$ S.E.M. Treatment group means were compared with the control group by *t*-test using Bonferroni's correction for multiple comparisons. Maternal plasma free fatty acid concentrations were decreased by treatment at either pGH dose ($P < 0.05$). Maternal plasma IGF-I concentrations were increased in gilts treated with the higher dose of pGH ($P < 0.001$), but not in gilts treated with the lower pGH dose ($P > 0.1$).

positively correlated with the change in maternal backfat depth per unit live weight ($r = +0.73$, $n = 15$, $P < 0.002$). Maternal plasma α -amino nitrogen was also strongly positively correlated with fractional (%) weight gain of dams ($r = +0.78$, $n = 15$, $P < 0.001$). Maternal plasma concentrations of IGF-I were increased three-fold (Fig. 2) by pGH treatment at $25.6 \mu\text{g/kg/day}$. Maternal plasma IGF-II ($65.3 \pm 2.8 \text{ nmol/l}$) and insulin ($22.9 \pm 4.0 \text{ IU/ml}$) concentrations were not affected by pGH administration. Maternal insulin and glucose concentrations were strongly positively correlated ($r = +0.77$, $n = 15$, $P < 0.001$). Administration of pGH had no effect on maternal plasma concentrations of IGF-binding proteins as assessed by Western ligand blot analysis of plasma from individual pregnant gilts (data not shown). A Western blot of IGF-binding proteins in maternal plasma pooled from each treatment group is presented in Fig. 3. Fractional (%) weight gain of pregnant gilts was not related to either IGF-I or IGF-II ($P > 0.1$) but was positively correlated with the total IGF concentration (sum of IGF-I plus IGF-II) in maternal plasma ($r = +0.63$, $n = 15$, $P = 0.012$). The total plasma IGF concentration in mothers was also

Figure 3 Effect of maternal pGH treatment on the IGF-BP profile at day 51 of pregnancy in maternal plasma pooled from gilts treated with (Lane a) 0, (Lane b) 13.4 or (Lane c) $25.6 \mu\text{g/kg/day}$. Lane C_{14} shows molecular weight markers, with the visible band corresponding to a molecular weight of 30 kilodaltons.

negatively correlated with the change in maternal backfat depth per unit live weight during treatment ($r = -0.59$, $n = 15$, $P = 0.02$) and the concentration of free fatty acids in maternal plasma ($r = -0.65$, $n = 15$, $P = 0.009$), and was strongly positively correlated with the concentration of α -amino nitrogen in maternal plasma ($r = +0.86$, $n = 15$, $P < 0.001$). Maternal plasma IGF-I ($r = +0.65$, $n = 15$, $P = 0.008$) and total IGF concentrations ($r = +0.64$, $n = 15$, $P = 0.009$) were both positively correlated with maternal plasma insulin concentrations. Maternal plasma IGF-II concentrations were correlated positively with maternal plasma α -amino nitrogen ($r = +0.74$, $n = 15$, $P < 0.002$) and negatively with maternal plasma free fatty acids ($r = -0.53$, $n = 15$, $P < 0.05$).

Weight of the uterus and contents ($8.1 \pm 0.6 \text{ kg}$), and litter size (11.1 ± 0.6 fetuses/dam) were not altered by maternal pGH administration. Fetal body weight, liver weight, and skull width increased ($P < 0.0001$) with increasing dose of pGH administered to the mother (Fig. 4). Fetal weight, liver weight, and skull width were increased by 18%, 26% and 6% respectively in pregnant gilts treated with $25.6 \mu\text{g/kg/day}$. When adjusted for litter size, fetal length was increased by 5% in gilts treated with $25.6 \mu\text{g/kg/day}$ (Fig. 4). Maternal GH dose was positively correlated with fetal liver weight in absolute terms ($r = +0.45$, $n = 166$, $P < 0.0001$), and as a proportion of fetal body weight ($r = +0.25$, $n = 166$, $P < 0.0001$). Average fetal weight per litter was negatively correlated ($r = -0.60$, $n = 15$, $P = 0.017$) with the change in maternal backfat depth during the treatment period (Fig. 5). Both litter size ($r = -0.54$, $n = 15$, $P < 0.05$) and total body weight of fetuses per dam ($r = -0.55$, $n = 15$, $P < 0.05$), but not body weights of individual fetuses were negatively correlated with glucose concentrations in maternal plasma. Overall, fetal weight was also weakly positively correlated with the concentration of α -amino nitrogen in maternal plasma ($r = +0.169$, $n = 166$, $P = 0.03$).

Fetal plasma glucose concentrations changed in a quadratic fashion ($P = 0.015$) with the dose of pGH

Figure 4 Effect of maternal pGH treatment on fetal weight and size at day 51 of gestation. Data are presented as mean $\pm$ S.E.M. Means for fetal length are adjusted to a litter size of 11.1 fetuses. Fetal weight, liver weight and skull width increased linearly with increasing dose of pGH administered to the mother ($P < 0.0001$). Fetal length was positively related to maternal pGH dose ($P = 0.0003$) and negatively related to litter size ($P = 0.038$).

administered to the pregnant gilt (Fig. 6). This change in fetal plasma glucose concentrations was accompanied by a change in the maternal/fetal plasma glucose concentration gradient in gilts treated with $13.4 \mu\text{g pGH/kg.day}$ ($P = 0.036$). Fetal plasma urea concentrations decreased linearly with increasing dose of pGH ($P = 0.0002$), accompanied by a linear increase in the maternal/fetal plasma urea concentration gradient ($P = 0.003$). The maternal/fetal plasma urea concentration gradient also increased with litter size ($P = 0.005$). Fetal plasma concentrations of urea were negatively correlated with fetal weight ($r = -0.34$, $n = 138$, $P < 0.0001$) and with the concentration of glucose in maternal plasma ($r = -0.26$, $n = 139$, $P = 0.0018$), and were positively correlated with maternal plasma concentrations of urea ($r = +0.52$, $n = 139$,

Figure 5 Average fetal weight per litter at day 51 of gestation increases with increasing loss of maternal backfat during GH treatment period. Average fetal weight at day 51 of gestation was negatively correlated with change in maternal backfat depth during the treatment period ($r = -0.60$, $n = 15$, $P = 0.017$).

Figure 6 Effect of maternal pGH treatment on concentrations of glucose and urea in fetal plasma, and on fetal:maternal glucose and urea concentration gradients at day 51 of gestation. Data are presented as mean $\pm$ S.E.M. Means for maternal/fetal plasma urea concentration gradient are adjusted to a litter size of 11.1 fetuses. Maternal plasma glucose and urea concentrations were not affected by pGH treatment ($P > 0.01$). Fetal plasma glucose concentrations ($P = 0.015$) and maternal/fetal plasma glucose concentration gradient ($P = 0.036$) changed in a quadratic fashion with the dose of pGH administered to the pregnant gilt. Fetal plasma urea concentrations decreased ($P = 0.0002$) and maternal/fetal plasma urea concentration gradient increased ($P = 0.003$) linearly with increasing dose of pGH. The maternal/fetal plasma urea concentration gradient also increased linearly with increasing litter size ($P = 0.005$).

$P < 0.0001$) and cholesterol ($r = +0.29$, $n = 139$, $P < 0.001$), and with fetal plasma glucose concentrations ($r = +0.36$, $n = 139$, $P < 0.0001$). No significant associations were observed between fetal plasma glucose and either fetal weight or maternal plasma glucose concentrations.

Administration of pGH to the pregnant gilt did not affect fetal plasma concentrations of IGF-I (1.79 ± 0.06 mmol/l) or IGF-II (20.2 ± 0.7 mmol/l). Fetal weight at day 51 of gestation was not related to fetal plasma IGF-I concentration overall ($P > 0.8$) or within treatment groups. Overall, there was a negative association between fetal plasma IGF-II and fetal weight at day 51 of gestation ($r = -0.29$, $n = 118$, $P = 0.002$). Fetal plasma concentrations of IGF-I and IGF-II were positively correlated with each other ($r = +0.47$, $n = 118$, $P < 0.0001$).

Discussion

Administration of GH to feed restricted pigs throughout the second quarter of their first pregnancy increased maternal backfat loss and plasma IGF-I concentrations, and decreased maternal plasma FFA concentrations. Maternal weight gain and plasma amino acid concentrations were positively correlated with GH dose. The dose of GH required to affect changes in maternal weight and fat

depth is lower in the pregnant gilt than in the non-pregnant growing pig. In this study, treatment with GH more than doubled the rate of loss of backfat at an average dose of either 13.4 or 25.6 µg pGH/kg.day. In contrast, doses of 30 µg pGH/kg.day failed to decrease carcass lipid content in non-pregnant growing pigs, and a dose of 70 µg pGH/kg.day decreased carcass lipid by 25% (Etherton *et al.* 1987). This suggests that pregnancy enhances the sensitivity of pigs to the antilipogenic effects of GH. In contrast, pregnancy induces GH resistance in rats (Chiang & Nicoll 1991, Gargosky *et al.* 1991).

Maternal anabolic and catabolic responses to GH were strongly correlated. As maternal weight gain increased, fat loss and plasma amino acid concentrations increased, and plasma FFA concentrations decreased. Maternal responses to exogenous GH are probably direct responses to GH, and not due to actions of IGF-I, since treatment of pigs with IGF-I only promotes growth during the neonatal period (Schoknecht *et al.* 1997, Walton *et al.* 1995). However, maternal anabolism (weight gain, plasma α -amino nitrogen concentration) and antilipogenesis (loss of maternal backfat, plasma FFA concentrations) were related to the total IGF concentrations in maternal blood. The major site of increased IGF-I gene expression in GH-treated pregnant pigs is the liver (Sterle *et al.* 1998), suggesting that increased maternal plasma concentrations of IGF-I in the present study reflect increased hepatic IGF-I synthesis.

Fetal weight (body weight, liver weight) and size (length, biparietal diameter) were increased by pGH treatment of mothers in the present study. Maternal GH treatment does not affect fetal growth in well-nourished rats (Gargosky *et al.* 1991), and decreases (Chiang & Nicoll 1991) or fails to restore fetal growth (Woodall *et al.* 1999) in undernourished pregnant rats. Previous studies of the effects of administration of pGH in well-fed pregnant pigs at similar doses to those used in the present study and at similar stages of gestation have also demonstrated increases in fetal growth. Sterle *et al.* (1995) reported a 10% increase in fetal weight following GH treatment of well-fed gilts with 32 µg pGH/kg.day from day 30 to day 43 of pregnancy, whilst Kelley *et al.* (1995) reported a 20% increase in fetal length, but no change in fetal weight, following treatment of *ad libitum*-fed gilts with 15 or 30 µg pGH/kg.day between days 28 and 40 of pregnancy. Greater fetal growth responses to maternal GH administration during early-mid gestation in our study than those previously reported (Kelley *et al.* 1995, Sterle *et al.* 1995) may partially reflect the greater degree of maternal undernutrition in our study. This may have produced slower rates of fetal growth and constrained maternal anabolic responses to GH. In the present study, gilts were fed at 1.8 kg/day of a ration containing 13.5 MJ digestible energy/kg and 15.05% protein, whilst Kelley *et al.* (1995) fed gilts a similar diet at 3.3 kg/day, and Sterle *et al.* (1995) fed gilts at 2.3 to 2.7 kg/day.

Maternal GH treatment probably increases fetal growth via changes in maternal metabolism, rather than by acting at the placenta. Porcine placenta contains few GH receptors at this stage of pregnancy, and administration of GH to pigs from day 30 to 43 of pregnancy does not increase IGF-I mRNA in uterus or placenta (Sterle *et al.* 1998). However, maternal GH treatment also stimulates hepatic IGF-I synthesis, and this IGF-I may affect placental growth or function via placental IGF-I receptors (Chastant *et al.* 1994). Sterle *et al.* (1995) showed increases in placental as well as fetal weight in response to maternal GH treatment at this stage of pregnancy in pigs, and suggested that fetal growth was increased in part by enhancing placental size and transport capacity. It is possible that placental function was enhanced by maternal GH treatment in this study, at least at the lower dose of GH used, since fetal plasma glucose concentrations were increased, and maternal/fetal glucose gradient was decreased in gilts treated with 13.4 µg pGH/kg.day. However, fetal weight was not correlated with fetal plasma glucose or the maternal/fetal glucose gradient, implying that glucose is not the nutrient which limits fetal growth in the undernourished pregnant pig, and that improved placental glucose transport was not the major mechanism for increased fetal growth in response to maternal GH treatment. We therefore suggest that maternal GH treatment increased fetal growth by increasing availability to the fetus of an alternative growth-limiting nutrient, probably via increased maternal plasma concentrations. Fetal growth was increased and plasma concentrations of urea were decreased in fetuses from sows treated with both doses of pGH, suggesting that protein catabolism may be decreased in these fetuses, and that the supply of alternative fuels for oxidation is increased by both doses of pGH. The concentrations of free fatty acids in maternal plasma at day 51 of pregnancy were decreased, and maternal plasma concentrations of glucose, urea and triglycerides were unaltered by GH treatment. Maternal plasma α -amino nitrogen concentrations were positively correlated with GH dose and with fetal weight, suggesting that GH treatment may increase fetal growth by increasing the supply of one or more amino acids which limit fetal growth.

We did not observe changes in fetal plasma IGF-I or IGF-II in response to maternal pGH treatment, despite increases in fetal growth. This suggests that maternal GH administration at low doses increased fetal growth through improved nutrient availability to the fetus, without nutritional modulation of the fetal endocrine IGF axis. At day 51 of gestation, fetal weight was not correlated with fetal plasma IGF-I, but was negatively correlated with fetal plasma IGF-II across all treatment groups. Later in gestation (from day 70), fetal serum concentrations of both IGF-I and IGF-II have been shown to correlate positively with fetal weight in pigs (Hausman *et al.* 1991), but relationships between fetal plasma IGFs and growth have not been reported during early gestation in the pig.

In summary, treating the underfed gilt with 13.4 or 25.6 µg pGH/kg.day during the second quarter of pregnancy increased fetal weight, whilst also increasing maternal backfat loss. This suggests that pGH is able to at least partially overcome any maternal constraints to fetal growth brought about by restricted feeding of the sow during pregnancy. We hypothesise that maternal GH treatment increases fetal growth through changes in maternal metabolism which increase the availability of amino acids to the fetus.

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