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Differences in the somatotropic axis, in blood cortisol, insulin and thyroid hormone concentrations between two pig genotypes with markedly divergent growth rates and the effects of growth hormone treatment

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Abstract

The intention of the current study was to gain more insight into the endocrine and molecular control mechanisms of growth in the pig. For this purpose various growth related parameters were determined in 4-month-old barrows of two extreme pig genotyes, the small, obese Göttingen Miniature (GM) and the large and lean German Landrace (DL). Mean growth hormone (GH) concentration, GH pulse frequency and GH pulse amplitude did not differ between breeds. Likewise, plasma IGF-1, thyroxine, tri-iodothyronine (T_3) concentrations were similar in both breeds. However the plasma GH response (maximum level and area under curve) to a single i.v. injection of GHRH in DL was higher than in GM ($P < 0{\cdot}05$*). Furthermore, basal plasma insulin and in particular plasma cortisol concentrations were higher in GM compared with DL pigs (*$P < 0{\cdot}05$ *and* $<0{\cdot}01$ *respectively). Analysis of cortisol during 4-h frequent blood sampling indicated higher cortisol amplitudes in GM compared with DL (*$P \leq 0{\cdot}01$*). Specific bGH-binding to hepatic membrane preparations was not different between breeds and IGF-1 m RNA concentrations determined by reverse transcription-polymerase chain reaction in liver,* m. semimenbranosus *and* m. longissimus dorsi *were similar in both breeds. I.m. treatment with recombinant porcine somatotropin (rpST; 70 μg/kg live weight) over an 8-day period in contemporary barrows increased without any breed difference, plasma IGF-1, T_3 and insulin concentrations and hepatic specific bGH-binding, but did not affect thyroxine or cortisol concentrations in plasma. IGF-1 gene expression was also elevated in liver and muscle tissues in rpST-treated animals without obvious breed effects. The observations underline the complexity of the hormonal and molecular control of growth and support the notion that differences in growth potential are the consequence of differences at various levels of the somatotropic axis and apparently relate to differences in other control systems of energy metabolism such as the pituitary adrenal axis or the endocrine pancreas as well.*

Keywords: *genotypes, growth, hydrocortisone, pigs, somatotropin.*

Introduction

Undoubtedly the principal hormone involved in stimulating growth in the pig as in other species is pituitary growth hormone (GH). Hypophysectomy arrests somatic growth in the pig, an effect which can be transiently overcome by exogenous GH (Anderson *et al.*, 1981). The growth promoting and anabolic actions of GH are mediated by insulin like growth factors (IGF-1 and IGF-2) acting in both an

endocrine and paracrine manner. During postnatal growth genetically lean and obese, small and large, fast and slow growing pigs do not necessarily differ in their plasma concentrations of GH (Arbona *et al.*, 1988; Buonomo and Klindt, 1993; Clutter *et al.*, 1995) nor that of IGF-1 (Zenobi *et al.*, 1988; Owens *et al.*, 1999). However, pigs selected for fast gain expressed greater concentrations of circulating IGF-I than their contemporaries selected for slow gain (Clutter *et al.*, 1995).

Responsiveness of target tissues may be another factor determining differences in growth potential. For example, the expression of the GH receptor (GHR) in liver and muscles or the response of IGF-1 in these tissues may differ between pig breeds with divergent growth potential. No differences in the expression of the GHR gene were observed between pig breeds with varying growth characteristics, but the observed differences in muscle and adipose tissue IGF-1 expression may relate to the overall growth of the animal (Bramfeld *et al.*, 1996a).

Weiler *et al.* (1998) in their publication stressed the importance of catabolic hormones like cortisol to explain differences in growth potential. According to their data the slow growing wild boar and the Meishan breed displayed higher plasma cortisol concentrations than the fast-growing Large White breed, while the plasma concentrations of IGF-1 and GH in the wild boar were higher than in the Large White breed.

The intention of the present study was to gain more insight into the endocrine and molecular control mechanisms of growth in the pig by determining various growth related parameters. Our experimental approach was the comparison of two extreme genotypes, which differ markedly in their growth potential: The Göttingen Miniature (GM) pig, a small, obese breed and the German Landrace (DL) pig, a conventional breed selected for lean meat production. In these breeds we determined the plasma concentrations of various anabolic and catabolic hormones. Furthermore the concentrations of GHR in the liver and that of IGF-1 mRNA in liver and two types of muscles were measured. In addition the responses of these parameters to a growth hormone treatment (recombinant porcine somatotropin; rpST) were compared.

Material and methods

Animals, treatments and blood sampling

Barrows of the German Landrace (Deutsche Landrasse, DL, no. = 17) and Göttingen Miniature breeds (GM, no. = 16) from the Institut für Tierzucht und Tierverhalten (FAL) experimental farm were used in this study at 4 months of age (range 12 days). Mean body weight at this age for DL and GM was 58 (s.e. 1·9) kg and 24 (s.e. 0·8) kg, respectively. The animals were kept individually in animal crates in a purpose-built environmentally controlled building under constant temperature (20ºto 22ºC). The animals were given food to appetite twice daily. Three to 4 days before the first blood sample was taken animals were fitted surgically with an indwelling jugular catheter. At 08:00 h seven DL barrows and six GM barrows received i.m. injections of 70 µg rpST per kg body weight for 9 days while all other animals received vehicle treatment. rpST was a kind donation by Pitman Moore, USA and was dissolved in carbonate buffer, pH 9·98 (25 mmol/l $NaHCO_3$, 25 mmol/l Na_2CO_3). Daily blood samples (5 ml) were taken at 11:00 h starting 1 day before first treatment with rpST (day 0) until day 9 of treatment. On day 6 of treatment additional blood samples (2·5 ml) were collected at 15-min intervals from 08:30 h to 12:30 h for determination of GH secretion profiles. At 12:30 h control animals were treated i.v. with 7 µg GHRH (Bissendorf Biochemicals/Bachem, Germany) per kg body weight and blood samples were taken 5, 10, 15, 20, 30, 60, 90, 120, 150 and 180 min after GHRH. Heparinized samples were kept on ice and centrifuged within 3 h of collection. Plasma was split into two to three aliquots and stored at –20ºC until analysis.

On day 9 at 06:00 h animals were given one-third of their normal ration and at 08:00 h received rpST or vehicle treatment as before. Starting at 09:00 h control and rpST treated animals were slaughtered in alternate order and standard parameters for carcass evaluation were taken. Tissue samples (two 3 to 5 g, two 1 g) from liver, *m. longissimus dorsi* and *m. semimembranosus* were collected within 15 min after slaughter, sealed in aluminium bags and frozen in liquid nitrogen and stored at –70ºC until analysis of GH receptor and IGF-1 mRNA.

Hormone analysis

Plasma GH levels were determined by a competitive enzyme immunoassay as described previously by Serpek *et al.* (1993) using a rabbit anti pGH antiserum raised by ourselves (K 37 . . .), biotinyl-pGH as a tracer and streptavidin–horseradish peroxidase. The assay has 50% crossreaction with rpST. Intra- and inter-assay variation was 0·11 and 0·16, respectively

Plasma IGF-1 levels were determined by radioimmunoassay after acid ethanol cryoprecipitation as described by Breier *et al.* (1993). The antiserum used has 0·5% crossreactivity with IGF-2 and less than 0·001% with insulin. IGF-1 was labelled by the chloramine-T-method to specific

activities between 40 and 60 µ Ci/µg. Recombinant hIGF-1 (batch 87/518, National Institute for Biological Standards and Controls, Potters Bar, Hertfordshire, UK) was used to standardize a plasma pool which was then used as standard preparation. Intra- and inter-assay variation was 0·10 and 0·14 respectively.

Plasma levels of insulin, thyroxine (T_4) and triiodothyronine (T_3) were determined using commercially established ^{125}I-RIA kits from Behring, Germany (RIA-gnost® Insulin, RIA-gnost® T_4 and RIA-gnost® T_3, respectively) following the instructions of the manufacturer. For the determination of insulin and T_3, 100 µl plasma were used, 20 µl plasma were used for the determination of T_4.

Plasma cortisol levels were determined by a rapid direct EIA as recently described (Marc *et al.*, 2000). The method had been evaluated for direct determination in 20 µl plasma diluted 1 : 40 without prior extraction. The intra- and inter-assay coefficient of variation was 0·09 and 0·13, respectively.

Hepatic growth hormone receptor (*GHR*)

Liver samples were processed in the presence of a proteinase inhibitor. The homogenate was treated with 4 mol/l $MgCl_2$ in order to dissociate and to remove endogenous pGH from the GHR. For the determination of pGHR in the obtained crude membrane preparations, a modified radio receptor assay following the protocol of Sauerwein *et al.* (1991) was performed. Using ^{125}I-labelled bovine GH in GHR measurements for porcine liver membranes yields higher specific binding rates compared with ^{125}I-pGH. Therefore recombinant bGH (rbGH, Eli Lilly, Indianapolis, USA; lot A18-7DT-170B) was used both as unlabelled ligand for displacement studies as well as tracer after iodination by the iodogen method. Equilibrium was reached within 22 h at room temperature; the binding observed was fully reversible by the addition of excess unlabelled rbGH. Repeated analysis of a membrane preparation pool yielded an inter-assay variation of 0·076 (no. = 4).

IGF-1 mRNA quantification

Total RNA was extracted from liver and muscle samples; 500 ng were then applied in a reverse transcription-polymerase chain reaction (RT-PCR) assay using an internal standard as described in detail by Pfaffl *et al.* (1998). The assay had a detection limit of 1600 recombinant IGF-1 RNA molecules per reaction tube, the intra-assay variation was 0·074 (no. = 5) and linearity (r = 0·997) was given between 140 ng to 840 ng total-RNA input into RT-PCR reaction.

Statistics

Data were subjected to two-way analysis of variance using breed and treatment as independent variables. When treatment effects were significant, the Student-Newman-Keuls method was used for pairwise multiple comparisons. The statistical procedures were performed using Sigma Stat™ Statistical Software (Jandel Scientific, Erkrath, Germany). Data are expressed as means ± s.e.

Results

Mean body weight in both breeds was not affected significantly by 9 days of treatment with rpST nor were parameters of carcass quality. Carcass weight for GM and DL was 17·7 (s.e. 1·6) and 44·3 (s.e. 1·5), respectively. Lean : fat ratio (1 : x) in GM was x = 0·4 (s.e. 0·1), for DL this ratio was x = 1·1 (s.e. 0·1).

In control animals mean GH concentrations in daily plasma samples (Figure 1a) did not differ between breeds (1·5 (s.e. 0·1) ng GH per ml plasma in both breeds). Following daily i.m. treatment with rpST

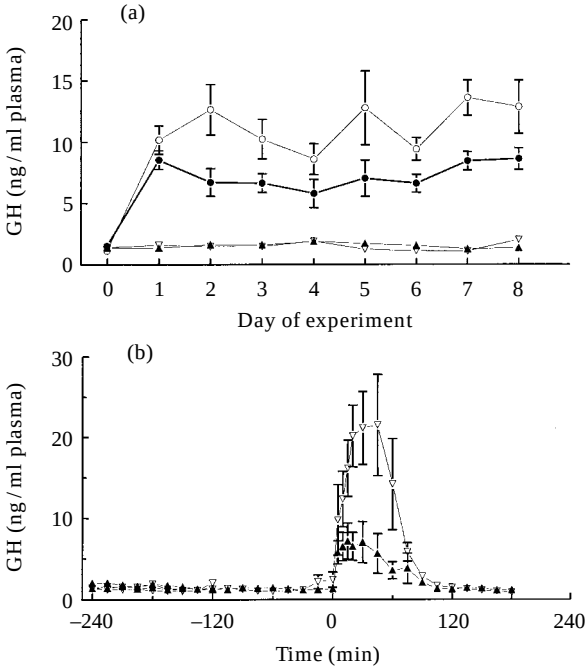

Figure 1 Plasma GH concentrations (mean ± s.e.) in Göttingen Miniature (GM) and German Landrace (DL) pigs. (a) Daily blood samples taken over the treatment period. Animals received once daily i.m. 70 µg rpST per kg body weight (circles) or received vehicle treatment (triangles). Closed symbols: GM, open symbols: DL. (b) Frequent blood samples taken from pigs not treated with rpST, before and after a single i.v. injection of 7 µg GHRH per kg body weight. Closed triangles: GM, open triangles: DL.

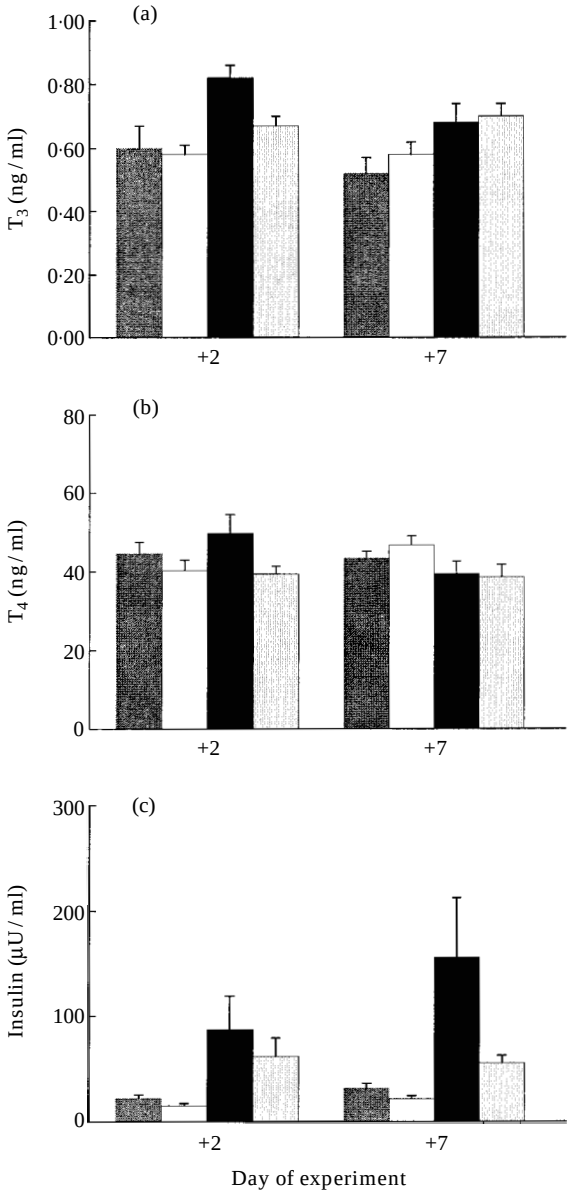

Figure 2 (a) Plasma tri-iodothyronine (T_3), (b) thyroxine (T_4) and (c) insulin concentrations (mean ± s.e.) on days 2 and 7 of vehicle (control) or rpST treatment in Göttingen Miniature (GM) and German Landrace (DL) pigs. ■ GM control, □ DL control, ■ GM rpST, ▒ DL rpST.

GH concentrations rose within 1 day and remained constant throughout the treatment period (7·4 (s.e. 0·5) ng GH per ml plasma and 11·2 (s.e. 1·2) ng GH per ml plasma in GM and DL respectively, $P < 0·001$ between breeds).

During 4-h frequent sampling on day 6 mean plasma GH concentrations (about 1·5 ng GH per ml plasma), GH pulse frequency (about one pulse every 4 h) and GH amplitude (about 2 ng GH per ml plasma) did not differ between breeds in control animals. However, the plasma GH response (maximum level and area under the curve) to GHRH in DL was higher than in GM with maximal GH levels in DL pigs being about 2·5 times higher ($P < 0·05$, Figure 1b).

IGF-1 concentrations were not different between breeds (291 (s.e. 34) and 302 (s.e. 26) ng IGF-1 per ml plasma for DL and GM, respectively); rpST-treatment led to a two-fold increase of IGF-1 plasma levels in both breeds. Neither total plasma T_4 nor T_3 concentration differed between breeds (Figure 2). RpST treatment significantly stimulated T_3 concentrations ($P < 0·05$) without breed difference whereas T_4 levels were not suppressed significantly. Plasma insulin concentrations (Figure 2c) in GM were slightly elevated compared with DL ($P < 0·05$) and were stimulated by rpST treatment ($P < 0·001$) without breed difference.

Plasma cortisol concentrations were significantly higher in GM compared with DL ($P < 0·01$, Figure 3). Analysis of cortisol during 4-h frequent blood sampling in four control GM and four contemporary control DL barrows indicated higher cortisol pulse amplitudes in GM compared with DL (50 (s.e. 6) ng cortisol per ml *v.* 26 (s.e. 3) ng cortisol per ml plasma, respectively, $P < 0·01$) but no breed difference in nadir levels (approx. 10 ng cortisol per ml plasma) or cortisol pulse frequency (approx. one cortisol pulse every 90 min).

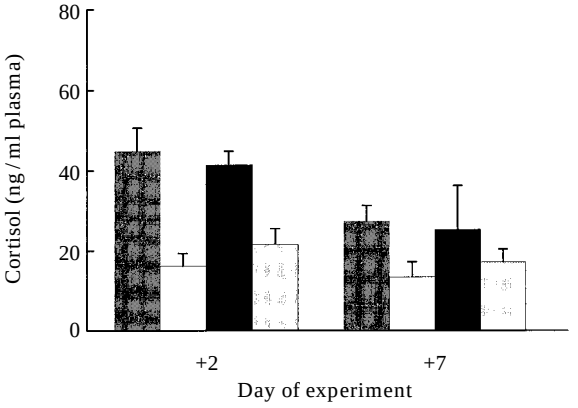

Figure 3 Plasma cortisol concentrations (mean ± s.e.) on days 2 and 7 of vehicle (control) or rpST treatment in Göttingen Miniature (GM) and German Landrace (DL) pigs. ■ GM control, □ DL control, ■ GM rpST, ▒ DL rpST.

Specific bGH-binding to hepatic membrane preparations in control animals was not different between breeds (15·6 (s.e. 2·9)% in DL and 20·7 (s.e. 1·6)% in GM livers). In rpST-treated animals specific bGH-binding was two-fold higher than in controls, without any breed-related differences (DL: 33·6 (s.e. 3·5)%; GM: 39·4 (s.e. 7·3)%; $P < 0·05$ compared with control).

The amount of total RNA extractable per g tissue was neither influenced by breed nor treatment. In control animals, similar expression levels were observed in liver and in the two different skeletal muscles without any breed difference (Figure 4). In rpST-treated pigs, IGF-1 expression was elevated in most tissues compared with control animals ($P < 0·05$); in *m. semimembranosus* of DL and in *m. longissimus dorsi* of GM animals the increase did not reach this level of significance ($P = 0·07$ and $P = 0·12$, respectively). Comparing liver expression rates of rpST-treated animals, GM pigs had lower hepatic expression levels than DL pigs; for muscles, no such difference was observed. When considering the mean expression rates in control animals as baseline, rpST-treated DL pigs had 7·6-fold higher expression rates in liver, for GM pigs the difference was only 3·6-fold. In skeletal muscle, the difference was less pronounced; in *m. semimembranosus* it was 1·5-fold in DL and 2·8 in GM; in *m. longissimus dorsi* it was 1·9 in DL and 2·5 in GM, respectively.

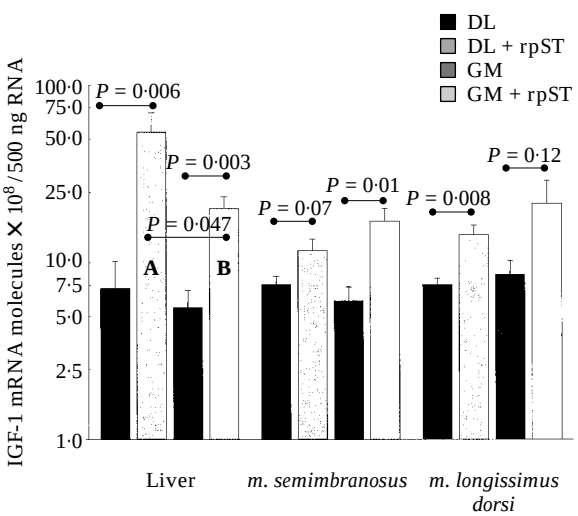

Figure 4 IGF-1 gene expression (mean ± s.e.) in various tissues from Göttingen Miniature (GM) and German Landrace (DL) pigs treated over an 8-day period with either vehcle (control) or rpST.

Discussion

The most striking difference between the two extreme genotypes in this study is the difference in plasma cortisol concentrations. Plasma cortisol levels were significantly higher in GM compared with DL. The crucial rôle of cortisol as a key hormone of catabolic processes in the pig has been stressed before by Weiler *et al.* (1998) who found decreased plasma cortisol concentrations in Large White pigs compared with the European wild boar or the Chinese Meishan boar. Apparently, selection for lean tissue growth is even associated with a lower activity of the HPA axis soon after birth as indicated by lower plasma cortisol concentrations in a composite line (highly selected for high rate of gain) compared with the primitive Chinese Meishan breed (Herpin *et al.*, 1993). Already during foetal life cortisol enhances adipocyte hypertrophy and concurrent action of glucocorticoids and thyroid hormones may be the critical aspect of endocrine regulation of foetal adipogenesis (Hausman, 1999). In the postnatal period cortisol has been shown to potentiate *in vitro* the positive effect of insulin on lipogenesis in swine adipose tissue probably by modulating insulin receptor number and (or) other cellular proteins, e.g. enzymes (Walton *et al.*, 1986). Thus, the elevated plasma cortisol concentrations in GM pigs compared with DL may well account at least in part for the much higher proportion of fat in the carcass of this breed.

The increase in plasma insulin concentrations following rpST treatment without any breed difference is in line with previous reports (Etherton *et al.*, 1986; Johnson *et al.*, 1990). In the present study the slow growing genotype (GM) expressed higher insulin concentrations compared with DL. Likewise, with the exception of Weiler *et al.* (1998) higher plasma concentrations of insulin in obese pigs than in lean pigs have been described before (Wangsness *et al.*, 1981; Mersmann *et al.*, 1982). Studies of Hausman (1989) demonstrate that physiological levels of insulin can stimulate porcine pre-adipocyte differentiation in the neonatal pig. Using older animals, Walton and Etherton (1986) established stimulatory effects of insulin on lipid synthesis in pig adipose tissue *in vitro*. Thus, in addition to elevated plasma cortisol concentrations elevated plasma insulin concentrations in the GM breed may be causally related to the higher fat to lean ratio in this breed.

It has been suggested that thyroid hormones may modulate the perinatal ontogeny of GH receptor gene expression in porcine liver and muscle (Duchamp *et al.*, 1996). In male sheep increased concentrations of T_3 are correlated with increased

growth (Van Kessel *et al.*, 1993) and T_3 is more highly correlated with serum IGF-1 and animal growth rate than GH (Hammond *et al.*, 1990). No such relationship was found in this study either for T_4 or for the more important and bioactive T_3. GH has been implicated not only in the production of IGF but also in the synthesis and metabolism of thyroid hormones. In particular the deiodination of T_4 to T_3 involving the action of a monodeiodinase is believed to be controlled by GH (Jorgensen *et al.*, 1989) which is supported by the present findings in the pig.

In the present study DL barrows had a higher secretory capacity for GH than GM barrows estimated from the concentration of GH after GHRH challenge suggesting that in the genetically small obese GM pig GH release is impaired. The physiological mechanism underlying the differential sensitivity to the same dose of GHRH is not known. In addition to GHRH, GH secretion is under hypothalamic control by somatostatin (SRIF) which counteracts the effects of GHRH by inhibiting GH release from somatotrophs. Hence the low GH response of GM pigs to GHRH challenge may have come about by continued inhibition of GH release due to elevated SRIF levels compared with DL. However, available data in the pig do not support, indeed contradict, this notion. According to recent own findings SRIF may not only be inhibitory to GH secretion but may act in a paradoxically positive manner to sensitize somatotrophs to GHRH, in particular in GM (Elsaesser and Drath, 1995; Torronteras *et al.*, 1996).

The difference in the GH response to GHRH in this study is consistent with the hypothesis that lean and large pigs display higher secretion rates of GH. However, the finding of a breed difference in the responsiveness to GHRH is difficult to reconcile with the lack of any breed difference in unstimulated GH release and thus the differential responsiveness observed in this study might be of no meaning to the control of GH by endogenous GHRH in these two breeds. The lack of any breed difference in unstimulated GH release is in agreement with previous reports in pigs (Arbona *et al.*, 1988; Buonomo and Klindt, 1993; Clutter *et al.*, 1995). It has even been reported that the slow growing wild boar in comparison to a commercial pig breed like the Large White breed displays higher plasma GH concentrations (Weiler *et al.*, 1998), remembering that the function of GH is much more complex than that of a mere growth promoter.

In line with previous work (Sillence and Etherton, 1987) plasma IGF-1 concentrations in this study were increased following rpST treatment. Previous studies (Buonomo and Klindt, 1993; Clutter *et al.*, 1995) have demonstrated possible genetic determinants of variation in plasma IGF-1 for pigs. However a lack of a positive association between mature body size or rates of gain and IGF-1 concentrations has also been reported in pigs (Owens *et al.*, 1990). Our data are in accordance with a previous report from Zenobi *et al.* (1988) demonstrating similar IGF-1 plasma concentrations in GM and a domestic pig breed. The conflicting data underline that IGF-1 is only one factor in the complex control of growth. In agreement with this notion the actions of pGH on growth of pigs were not mimicked by administration of IGF-1 (Klindt *et al.*, 1998).

Because the first step in GH action is binding to a specific cell surface receptor, changes in GHR levels may provide some insight into the importance of GH actions. In accordance with previous findings in the pig (Chung and Etherton, 1986; Combes *et al.*, 1997) the present study indicates, that irrespective of important differences in growth rates of the two extreme genotypes, GH treatment induces a similar increase in GH binding to liver. Furthermore, basal specific binding in liver was similar in both breeds and thus cannot account for the growth differences between GM and DL. Likewise, no difference in GH-specific binding in liver was apparent between the conventional Large White breed and the Chinese Meishan breed which has a reduced rate of muscle growth (Schnoebelen-Combes *et al.*, 1996).

There is still some controversy regarding the relative importance of systemic IGF-1 derived from the liver *versus* local IGF-1 in skeletal muscle. Earlier work in the pig analysing IGF-1 gene expression in the *m. longissimus* suggested that the stimulatory effects of pST on muscle growth are not associated with increased IGF-1 mRNA levels in skeletal muscle and that IGF-1 plays an endocrine rôle in mediating GH-induced muscle hypertrophy (Grant *et al.*, 1991; Coleman *et al.*, 1994). In later studies expression of IGF-1 mRNA was found to be GH responsive in the liver, in adipose tissues and in the *m. semitendinosus*, but in accordance with the previous work not to be GH responsive in *m. longissimus dorsi* (Brameld *et al.*, 1996b). The present study indicates that in another hindlimb muscle, the *m. semimembranosus* IGF-1 gene expression is responsive to GH as well and that IGF-1 in the *m. longissimus dorsi* might be affected by GH too.

The observation in some studies that GH increased IGF-1 mRNA in liver but not in skeletal muscle or only in distinct muscles might indicate, that the effect of GH is tissue specific and suggests that the relative rôles of endocrine *v.* locally synthesized IGF-1 in

mediating the effects of GH may be tissue specific. The findings of Brameld *et al.* (1996b) and the present study describe IGF-1 responsiveness to GH in two types of hindlimb muscles, the *m. semitendinosus* and the *m. semimembranosus*, respectively. Brameld *et al.* (1996b) suggested that the differential responsiveness of IGF-1 expression to GH might be related to differences in fibre type between *m. longissimus* and *m. semitendinosus* described by Ouali and Talmant (1990), or related to differences in the muscle responsiveness to GH as regards increasing muscle fibre area (Ono *et al.*, 1995). However, this notion might not hold true for all breeds and/or ages since in the present study GH increased IGF-1 mRNA in *m. longissimus dorsi* as well and Lewis *et al.* (2000), although using a very high dose of GH also demonstrated increased IGF-1 mRNA in *m. longissimus* of the neonatal pig following GH treatment.

The present study describes in two extreme pig genotypes a broad array of hormonal parameters related to the control of the polygenic trait of growth in the pig. The observations underline the complexity of the hormonal and molecular control of growth and support the notion that differences in growth potential are the consequence of differences at various levels of the somatotropic axis and apparently relate to differences in other control systems of energy metabolism such as the pituitary adrenal axis, or insulin as well.

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References

Anderson, L. L., Bohnker, C. R., Parker, R. O. and Kertiles, L. P. 1981. Metabolic actions of growth hormone in pigs. *Journal of Animal Science* **53:** 363-370.

Arbona, J. R., Marple, D. N., Russell, R. W., Rahe, C. H., Mulvaney, D. R. and Sartin, J. L. 1988. Secretory pattern and metabolic clearance rate of porcine growth hormone in swine selected for growth. *Journal of Animal Science* **66:** 3068-3072.

Brameld, J. M., Atkinson, J. L., Budd, T. J., Saunders, J. C., Pell, J. M., Salter, A. M., Gilmour, R. S. and Buttery, P. J. 1996a. Expression of insulin-like growth factor-1 (IGF-1) and growth hormone-receptor (GHR) mRNA in liver, skeletal muscle and adipose tissue of different breeds of pig. *Animal Science* **62:** 555-559.

Brameld, J. M., Atkinson, J. L., Saunders, J. C., Pell, J. M., Buttery, P. J. and Gilmour, R. S. 1996b. Effects of growth hormone administration and dietary protein intake on insulin-like growth factor I and growth hormone receptor mRNA expression in porcine liver, skeletal muscle, and adipose tissue. *Journal of Animal Science* **74:** 1832-1841.

Breier, B. H., Milsom, S. R., Blum, W. F., Schwander, J., Gallaher, B. W. and Gluckman, P. D. 1993. Insulin-like growth factors and their binding proteins in plasma and milk after growth hormone-stimulated galactopoiesis in normally lactating women. *Acta Endocrinologica* **129:** 427-435.

Buonomo, F. C. and Klindt, J. 1993. Ontogeny of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II) and IGF binding protein-2 (IGFBP-2) in genetically lean and obese swine. *Domestic Animal Endocrinology* **10:** 257-265.

Chung, C. S. and Etherton, T. D. 1986. Characterization of porcine growth hormone (pGH) binding to porcine liver microsomes: chronic administration of pGH induces pGH binding. *Endocrinology* **119:** 780-786.

Clutter, A. C., Spicer, L. J., Woltmann, M. D., Grimes, R. W., Hammond, J. M. and Buchanan, D. S. 1995. Plasma growth hormone, insulin-like growth factor I, and insulin-like growth factor binding proteins in pigs with divergent genetic merit for postweaning average daily gain. *Journal of Animal Science* **73:** 1776-1783.

Coleman, M. E., Russell, L. and Etherton, T. D. 1994. Porcine somatotropin (pST) increases IGF-I mRNA abundance in liver and subcutaneous adipose tissue but not in skeletal muscle of growing pigs. *Journal of Animal Science* **72:** 918-924.

Combes, S., Louveau, I. and Bonneau, M. 1997. Moderate food restriction affects skeletal muscle and liver growth hormone receptors differently in pigs. *Journal of Nutrition* **127:** 1944-1949.

Duchamp, C., Burton, K. A., Herpin, P. and Dauncey, M. J. 1996. Perinatal ontogeny of porcine growth hormone receptor gene expression is modulated by thyroid status. *European Journal of Endocrinology* **134:** 524-531.

Elsaesser, F. and Drath, S. 1995. The potential of immuno-neutralization of somatostatin for improving pig performance. *Livestock Production Science* **42:** 255-263.

Etherton, T. D., Wiggins, J. P., Chung, C. S., Evock, C. M., Rebhun, J. F. and Walton, P. E. 1986. Stimulation of pig growth performance by porcine growth hormone and growth hormone-releasing factor. *Journal of Animal Science* **63:** 1389-1399.

Grant, A. L., Helferich, W. G., Kramer, S. A., Merkel, R. A. and Bergen, W. G. 1991. Administration of growth hormone to pigs alters the relative amount of insulin-like growth factor-I mRNA in liver and skeletal muscle. *Journal of Endocrinology* **130:** 331-338.

Hammond, A. C., Elsasser, T. H., Kunkle, W. E., Rumsey, T. S., Williams, M. J. and Butts, W. T. 1990. Effects of winter nutrition and summer pasture or a feedlot diet on plasma insulin-like growth factor I (IGF-I) and the relationship between circulating concentrations of IGF-I and thyroid hormones in steers. *Domestic Animal Endocrinology* **7:** 465-475.

Hausman, G. J. 1989. The influence of insulin. triiodothyronine (T_3) and insulin-like growth factor-I (IGF-1) on the differentiation of preadipocytes in serum-free cultures of pig stromal-vascular cells. *Journal of Animal Science* **67:** 3136-3143.

Hausman, G. J. 1999. The interaction of hydrocortisone and thyroxine during fetal adipose tissue differentiation:

CCAAT enhancing binding protein expression and capillary cytodifferentiation. *Journal of Animal Science* **77:** 2088-2097.

Herpin, P., LeDividich, J. and Amaral, N. 1993. Effect of selection for lean tissue growth on body composition and physiological state of the pig at birth. *Journal of Animal Science* **71:** 2645-2653.

Johnson, J. L., Coffey, M. T., Esbenshade, K. L., Schricker, B. R. and Pilkington, D. H. 1990. The effect of human growth hormone-releasing factor or porcine somatotropin on serum hormones and metabolites, growth performance and carcass traits in swine. *Journal of Animal Science* **68:** 3204-3211.

Jorgensen, J. O., Pedersen, S. A., Laurberg, P., Weeke, J., Skakkebaek, N. E. and Christiansen, J. S. 1989. Effects of growth hormone therapy on thyroid function of growth hormone-deficient adults with and without concomitant thyroxine-substituted central hypothyroidism. *Journal of Clinical Endocrinology and Metabolism* **69:** 1127-1132.

Klindt, J., Yen, J. T., Buonomo, F. C., Roberts, A. J. and Wise, T. 1998. Growth, body compostition, and endocrine responses to chronic administration of insulin-like growth factor I and (or) porcine growth hormone in pigs. *Journal of Animal Science* **76:** 2368-2381.

Lewis, A. J., Wester, T. J., Burrin, D. G. and Dauncey, M. J. 2000. Exogenous growth hormone induces somatotrophic gene expression in neonatal liver and skeletal muscle. *American Journal of Physiology — Regulatory, Integrative and Comparative Physiology* **278:** R838-R844.

Marc, M., Parvizi, N., Ellendorff, F., Kallweit, E. and Elsaesser, F. 2000. Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. *Journal of Animal Science* **78:** 1936-1946.

Mersmann, H. J., Pond, W. G. and Yen, J. T. 1982. Plasma glucose, insulin and lipids during growth of genetically lean and obese swine. *Growth* **46:** 189-198.

Ono, Y., Solomon, M. B., Evock-Clover, C. M., Steele, N. C. and Maruyama, K. 1995. Effects of porcine somatotropin administration on porcine muscles located within different regions of the body. *Journal of Animal Science* **73:** 2282-2288.

Ouali, A. and Talmant, A. 1990. Calpains and calpastin distribution in bovine, porcine and ovine skeletal muscles. *Meat Science* **28:** 331-335.

Owens, P. C., Gatford, K. L., Walton, P. E., Morley, W. and Campbell, R. G. 1999. The relationship between endogenous insulin-like growth factors and growth in pigs. *Journal of Animal Science* **77:** 2098-2103.

Pfaffl, M., Meyer, H. H. and Sauerwein, H. 1998. Quantification of insulin-like growth factor-1 (IGF-1) mRNA: development and validation of an internally standardised competitive reverse transcription-polymerase chain reaction. *Experimental and Clinical Endocrinology and Diabetes* **106:** 506-513.

Sauerwein, H., Breier, B. H., Bass, J. J. and Gluckman, P. D. 1991. Chronic treatment with bovine growth hormone upregulates high-affinity hepatic somatotropic receptors in sheep. *Acta Endocrinologica* (*Copenh*) **124:** 307-313.

Schnoebelen-Combes, S., Louveau, I., Postel-Vinay, M.-C. and Bonneau, M. 1996. Ontogeny of GH receptor and GH-binding protein in the pig. *Journal of Endocrinology* **148:** 249-255.

Serpek, B., Elsaesser, F. and Meyer, H. H. D. 1993. Development of an enzyme immunoassay for the determination of porcine growth hormone in plasma. *Analytica Chimica Acta* **275:** 183-187.

Sillence, M. N. and Etherton, T. D. 1987. Determination of the temporal relationship between porcine growth hormone, serum IGF-1 and cortisol concentrations in pigs. *Journal of Animal Science* **64:** 1019-1023.

Torronteras, R., Gracia-Navarro, F. and Elsaesser, F. 1996. Different effects of somatostatin on *in vitro* growth hormone release in two porcine breeds with different growth rates. *Journal of Neuroendocrinology* **8:** 891-900.

Van Kessel, A. G., Korchinski, R. S. and Laarveld, B. 1993. Growth and serum thyroid hormone and insulin-like growth factor I (IGF-I) responses to natural passive immunization against somatostatin in the lamb. *Canadian Journal of Animal Science* **73:** 509-516.

Walton, P. E. and Etherton, T. D. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: antagonism by porcine growth hormone. *Journal of Animal Science* **62:** 1584-1595.

Walton, P. E., Etherton, T. D. and Evock, C. M. 1986. Antagonism of insulin action in cultured pig adipose tissue by pituitary and recombinant porcine growth hormone: potentiation by hydrocortisone. *Endocrinology* **118:** 2577-2581.

Wangsness, P. J., Acker, W. A., Burdette, J. H., Krabill, L. F. and Vasilatos, R. 1981. Effect of fasting on hormones and metabolites in plasma of fast-growing, lean and slow-growing obese pigs. *Journal of Animal Science* **52:** 69-74.

Weiler, U., Claus, R., Schnoebelen-Combes, S. and Louveau, I. 1998. Influence of age and genotype on endocrine parameters and growth performance: a comparative study in wild boars, Meishan and Large White boars. *Livestock Production Science* **54:** 21-31.

Zenobi, P. D., Guler, H.-P., Zapf, J. and Froesch, E. R. 1988. Insulin-like growth factors in the Göttinger miniature-pig. *Acta Endocrinologica* **117:** 343-352.

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