Effect of growth hormone administration on IGF binding protein-3 mRNA levels in porcine tissues

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ABSTRACT

The effect of short-term GH treatment on steady-state insulin-like growth factor binding protein-3 (IGFBP-3) mRNA levels in liver, kidney, longissimus dorsi muscle, stomach and jejunum was examined in pigs. Ten female crossbred pigs were allocated to either saline or GH (70 µg/kg/day) treatment by subcutaneous injection for 4 days. They were allowed to feed *ad libitum*, and were weighed daily. At the end of the treatment period, the pigs were slaughtered and samples of liver, kidney, skeletal muscle, stomach and jejunum were collected and total RNA was extracted. Steady-state levels of IGFBP-3 mRNA were quantified by RNase protection assay and were compared with the level of IGF-I class 1 and class 2

transcripts. IGFBP-3 mRNA increased in response to GH in both liver and kidney, but not in the other tissues sampled. Hepatic IGF-I mRNA responded to short-term GH treatment with a fourfold increase in IGF-I class 1 mRNA and an eightfold increase in IGF-I class 2 mRNA, which was liver specific. IGF-I class 1 mRNA was not responsive to GH treatment in other tissues. The short-term nature of this treatment suggests that the increase in hepatic IGFBP-3 and IGF-I transcripts is a relatively early response to treatment with GH, and that the increase in plasma concentrations of IGFBP-3 in response to GH are derived from the liver, the kidney, or both.

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INTRODUCTION

The developing pig is particularly sensitive to treatment with growth hormone (GH), which increases the average daily weight gain (Etherton *et al.* 1987), enhances protein accretion and reduces fat deposition (Campbell *et al.* 1989). A number of components of the somatotrophic axis are involved in the response to GH, but the way in which each is regulated to provide a co-ordinated response in the pig has not been fully elucidated. Many of the growth promoting actions of GH are mediated via insulin-like growth factor-I (IGF-I), and it is well established that GH treatment increases porcine plasma concentrations of IGF-I (Owens *et al.* 1990, Coleman & Etherton 1991). The primary source of the increased plasma IGF-I concentration is likely to be the liver, although many other tissues are able to produce IGF-I resulting in autocrine

or paracrine, in addition to endocrine, effects of the peptide. It is not known what contribution the production of IGF-I in each tissue makes to the overall plasma IGF-I pool.

Growth hormone and nutritional status are the major regulators of porcine IGF-I gene expression (Brameld *et al.* 1996). Different leader exons (exons 1 and 2) for IGF-I are alternatively spliced to exon 3, which encodes part of the mature IGF-I protein. Initiation of transcription at multiple dispersed start sites upstream of exon 1 or 2 yields two distinct IGF-I mRNAs, class 1 and class 2, which produce different precursor peptides (Weller *et al.* 1993). In the liver, expression of hepatic IGF-I class 2 mRNA is more sensitive to changes that affect optimal growth than is IGF-I class 1 mRNA. IGF-I class 2 mRNA expression is closely related to circulating IGF-I concentrations and growth rates when they are altered by energy

manipulation (Weller *et al.* 1994). By using sensitive, exon-specific ribonuclease (RNase) protection assays, these authors dissected the tissue-specific expression of IGF-I class 1 and class 2 transcripts in response to GH administration in addition to changes in dietary protein intake. Increased plasma IGF-I concentrations were accompanied by increased hepatic IGF-I mRNA expression in response to GH. Increased transcription rates were derived from both exons, although exon 2 expression was found only in the liver of GH-treated pigs. In muscle, the response of IGF-I class 1 transcripts to GH administration was dependent on muscle fibre type. In adipose tissue, there was a significant effect of both GH and dietary manipulation on the level of IGF-I class 1 mRNA expression and an interaction between the treatments (Brameld *et al.* 1996). These studies confirmed and extended the work by Coleman *et al.* (1994) suggesting that GH treatment increased IGF-I expression in the liver and adipose tissue, but not in skeletal muscle, of growing pigs.

Insulin-like growth factor binding protein-3 (IGFBP-3), associated with the acid-labile subunit, is the primary carrier of IGF peptides in the circulation. The rat has been the most extensively studied species (Shimasaki *et al.* 1989). The wide tissue distribution of IGFBP-3 suggests that it has an important role in the autocrine/paracrine action of IGF-I, although no correlation has been observed between IGF-I and IGFBP-3 mRNA expression in different tissues (Albiston & Herington 1992). In the pig, plasma IGF-I and IGFBP-3 levels are increased in GH-treated animals (Dunaiski *et al.* 1997), but regulation of IGFBP-3 mRNA expression by GH has not been studied. In the rat, liver IGFBP-3 mRNA expression is regulated by GH, but appears to be less sensitive than IGF-I mRNA to circulating GH concentrations (Domenè *et al.* 1993). The current study was designed to compare the effect of short-term GH treatment on the expression of IGFBP-3 mRNA with IGF-I class 1 and class 2 mRNA levels in liver, kidney, skeletal muscle (longissimus dorsi), stomach and small intestine of the growing pig which is more sensitive than the rat to treatment with exogenous GH (Walton *et al.* 1995).

MATERIALS AND METHODS

Animals and procedures

Ten female crossbred pigs (55 kg) were randomly allocated to one of two treatment groups. Pigs were individually penned and were fed 110 g/kg body

weight per day divided into three meals given at 0800, 1200 and 1600 h. The diet consisted of 20.4% total crude protein, 14.5 MJ/kg digested energy and 1.28% lysine. Animals were weighed daily during the course of the experiment. Animals received a once-daily s.c. injection of either saline or porcine GH (70 µg/kg/day) (Bresatec Pty Ltd, Adelaide, Australia) for 4 days. On the last day of treatment, the daily dose was given as two injections 12 h apart. One hour after the last injection, the animals were killed and samples were taken from the left anterior lobe of the liver, the left kidney, including sections of the medulla and cortex, skeletal muscle from a point 6.5 cm from the midline over the last rib (longissimus dorsi), the small intestine (jejunum) and the posterior stomach. Tissue samples were snap frozen in liquid nitrogen and stored at -80 °C until they were required for analysis.

Isolation of RNA

Approximately 2 g frozen tissue were homogenised in 1 ml RNazol B (Tel-Test Inc., Friendswood, Texas, USA). An aliquot of 2 ml RNazol B and 0.4 ml chloroform was added to the homogenised sample and extraction of total RNA was then performed according to the manufacturer's instructions. The final RNA pellet was washed with 0.5 ml LiCl (4 mol/l) followed by 0.5 ml ethanol (70% v/v), before the RNA was resuspended in 100 µl diethyl pyrocarbonate (DEP-C)-treated water. RNA was quantified by measuring the absorbance at 260 nm.

Generation of radioactive riboprobes and markers

DNA molecular weight markers BMVII (Boehringer Mannheim Biochemicals, Indianapolis, Indiana, USA) were end labelled using a DNA 5' end labelling kit (Boehringer Mannheim Biochemicals). High specific activity riboprobes were generated using a T7/T3 transcription kit (Promega Corporation, Madison, WI, USA) with 100 ng linearised template DNA and 10 units RNA polymerase.

Plasmids pRP1 and pRP2 were kindly donated by Dr Stewart Gilmour (University of Auckland) and were used to generate antisense riboprobes for the detection of porcine IGF-I class 1 and class 2 mRNA as previously described (Weller *et al.* 1993). Briefly, pRP1 and pRP2 plasmids were linearised with HindIII and transcribed with T7 RNA polymerase in the presence of [α -³²P]UTP (Bresatec Pty Ltd).

FIGURE 1. Subcloning of IGFBP-3. Three separate restriction digests were carried out using pPBP3ES. To yield a fragment containing only exon 1 pPBP3ES was digested with EcoRI and PstI (A); to yield a fragment containing the 3' end of exon 1 and the 5' end of exon 2, pPBP3ES was digested with PstI and SalI (B); and to yield a fragment containing the remainder of exon 2 and the 5' end of exon 3, pPBP3ES was digested with SalI and SmaI (C). These digests generated fragments that were 208 bp, 256 bp and 131 bp in length respectively. These fragments were isolated as described in the text and subcloned into pBluescript II SK⁺.

For detection of IGFBP-3 mRNA, riboprobes were generated from the plasmid pPBP3ES (kindly donated by Dr Shunichi Shimasaki, Whittier Institute, La Jolla, CA, USA; Shimasaki *et al.* 1990). The strategy for developing the probes is outlined in Fig. 1. Plasmid pPBP3ES contained a 595-base pair (bp) fragment encoding the first three exons of porcine IGFBP-3 subcloned into the EcoRI/SmaI site of the multiple cloning region of pBluescript II SK⁺. Analysis of the DNA sequence revealed several unique restriction sites suitable for subcloning. The plasmid pPBP3ES was digested with EcoRI and PstI, PstI and SalI, or SalI and SmaI to yield three separate fragments encoding exon 1, part of exons 1 and 2, or part of exons 2 and 3 of porcine IGFBP-3. These fragments were isolated from agarose gels and ligated unidirectionally into the multiple cloning site of pBluescript II SK⁺. The ligation mixes were transformed into competent DH5 α bacteria and plated onto agar plates containing 100 μ g/ml ampicillin. After incubation at 37 °C overnight, resistant colonies were selected and plasmid DNA was prepared. Successful ligation of the IGFBP-3 fragments into pBluescript II SK⁺ was verified by digestion with the appropriate restriction enzymes and electrophoresis on 1% agarose gels. Each sequence was verified using the dideoxy chain termination method using a kit according to the manufacturer's instructions (Pharmacia-LKB Biotech, Uppsala, Sweden).

To generate antisense probes for detection of porcine IGFBP-3 mRNA, 10 μ g pPBP3ES, p256BP3, p208BP3 or p131BP3 were linearised by overnight digestion with 10 units EcoRI, SalI, PstI and SalI respectively. Completion of digestion was visualised on ethidium bromide-stained 1% agarose gels and the linearised plasmid DNA purified by phenol-chloroform extraction and precipitation with 2.5 vol ethanol in the presence of 0.3 M sodium acetate pH 5.2. The DNA template was resuspended in buffer at a concentration of 100 ng/ μ l. Riboprobes were generated using a T7/T3 transcription kit (Promega Corporation). The transcription reaction was initiated by adding, in order: 2 μ l 5 $\times$ transcription buffer (200 mM Tris-HCl, pH 7.5, 30 mM MgCl₂, 10 mM spermidine, 50 mM NaCl); 1 μ l 100 mM dithiothreitol; 0.5 μ l RNasin; 2 μ l ATP, CTP, GTP mix (7.5 mM each); 1.2 μ l 100 μ M UTP; 0.5 μ l linearised template DNA; 25 μ Ci [α -³²P]UTP and 10 units T3 RNA polymerase; it was then incubated at 37 °C for 60 min. The DNA template was degraded by the addition of 1 unit RNase free DNase and incubation at 37 °C for 15 min. To the reaction was added 80 μ l DEP-C H₂O and 42 μ l phenol-chloroform-isoamyl alcohol (25:24:1), followed by thorough mixing and centrifugation at 12 500 *g* for 5 min. The upper aqueous phase (90 μ l) was removed to a fresh tube and the riboprobe precipitated by the addition of 10 μ l 10 mg/ml tRNA (Sigma, St Louis, Missouri,

USA), 4 µl 5 M NaCl and 250 µl ethanol and centrifuged at 12 500 g for 15 min. The RNA pellet was washed with 500 µl 70% ethanol and resuspended in 40 µl DEP-C H₂O. To determine percentage incorporation of the radioactive nucleotide, 1 µl riboprobe mix was aliquoted into 2 ml scintillant (Beckmann, Fullerton, California, USA) immediately after the completion of the transcription reaction and after the RNA pellet had been precipitated with ethanol and resuspended in DEP-C water. Radioactivity was measured using a β counter and the activity of the probe adjusted to 5×10^5 c.p.m./µl.

The plasmid pT7 RNA 18S (Amnion Inc., Austin, Texas, USA) was used to generate low specific activity riboprobes for detection of 18S ribosomal RNA.

Ribonuclease protection assays

RNase protection assays were carried out as described (Saunders *et al.* 1991). For detection of IGF-I class 1, class 2 and IGFBP-3 mRNA, 50 µg total RNA was added to each hybridisation reaction. For detection of 18S ribosomal RNA, 10 µg total RNA was used to ensure that quantitation was within the linear range of the assay. Each individual sample was analysed in duplicate. Hybridisation was carried out at 45 °C overnight. RNase digestion was performed at 37 °C for 1 h, except for pRP1, with which digestion was carried out at room temperature.

Statistical analysis

All data were analysed using SigmaStat statistical software version 1.0 (Jandel Scientific Software, CA, USA). Average daily weight gain and feed : gain ratio were analysed using Student's *t*-test. Gels were developed using a phosphorimager (Model No. 425E, Molecular Dynamics, CA, USA) and quantified by measuring the intensities (integrated volumes) of each band using Image Quant version 3.2 software (Molecular Dynamics). The intensities of protected fragments corresponding to IGF-I class 1, class 2 and IGFBP-3 mRNA were normalised and expressed as a percentage of the corresponding intensities for 18S ribosomal RNA protected fragments. To avoid interassay variation, all samples from the same tissue type were analysed on one gel. Data were analysed for statistically significant differences using Student's *t*-test and are expressed as the mean $\pm$ S.E.M. with $n=5$. It should be noted that values expressed as a percentage of control relate only to each individual

FIGURE 2. Effect of GH treatment (70 µg/kg/day) on (A) average daily weight gain and (B) feed : gain ratio over 4 days of treatment. Data were analysed using Student's *t*-test and values are expressed as the mean $\pm$ S.E.M. ($n=5$). *Significantly different from the saline group ($P<0.05$).

gel and comparison between them should not be made.

RESULTS

Administration of GH for 4 days significantly increased the average daily weight gain and decreased feed : gain ratio (Fig. 2).

IGFBP-3 detection

An antisense RNA probe complementary to the first three exons of porcine IGFBP-3 mRNA was generated from pBFP3ES. This riboprobe was used in an RNase protection assay and hybridised to

FIGURE 3. Analysis of liver and kidney IGFBP-3 mRNA expression from pigs treated with GH. (A) Autoradiograph of RNase protection assay using an IGFBP-3 probe hybridised with 50 µg total liver RNA from vehicle- (samples 1–5) or GH- (samples 6–10) treated pigs. Each sample was analysed in duplicate. The band at 139 bp represents protection of IGFBP-3 mRNA. (B) Autoradiograph of RNase protection assay using a IGFBP-3 probe hybridised with 50 µg total kidney RNA from saline- (samples 1–5) or GH- (samples 6–10) treated pigs. (C) Autoradiograph of RNase protection assay using a ribosomal 18S RNA probe hybridised with 10 µg total kidney RNA from vehicle- (samples 1–5) or GH- (samples 6–10) treated pigs. Each sample was analysed in duplicate. The band at 80 bp is derived from protection of ribosomal 18S RNA and is used to control for variations in RNA quantitation. The corresponding RNase protection assay representing expression of 18S RNA in porcine liver is shown in Fig. 5C. Graphical representations of liver IGFBP-3 (D) and kidney IGFBP-3 (E) mRNA levels are shown. Band intensities were quantified using Image Quant software and are expressed as a percentage of the intensity obtained from hybridising the same RNA samples with an 18S RNA probe. Results are expressed as the mean $\pm$ s.e.m. ($n=5$). Significant differences from the vehicle group: * $P<0.03$, ** $P<0.05$.

FIGURE 4. Representative autoradiographs of RNase protection assays for IGFBP-3 mRNA expression in muscle (A), stomach (C) and jejunum (E) of saline- and GH-treated pigs. Graphical representations of muscle (B), stomach (D) and jejunum (F) mRNA are also shown. Band intensities were quantified using Image Quant software and are expressed as a percentage of the intensity obtained from hybridising the same RNA samples with an 18S RNA probe. Results are expressed as the mean $\pm$ S.E.M. ($n=5$).

porcine liver RNA. The results revealed that, although a 665-nucleotide riboprobe of the expected size was generated, no protected fragments were detected at the expected size of 595 bases, although

fragments were identified at approximately 250 and 238 bases (data not shown). Investigation of the DNA sequence revealed a region rich in G and C residues, and generation of the two protected

FIGURE 5. Analysis of hepatic IGF-I class 1 and class 2 mRNA expression in pigs treated with GH. (A) Autoradiograph of RNase protection assay using a IGF-I class 1 probe hybridised with 50 μ g total liver RNA from saline- (samples 1–5) or GH- (samples 6–10) treated pigs. Each sample was analysed in duplicate. The major band at 200 bp results from the protection of exon 1–3 class 1 IGF-I mRNA species. The band at 175 bp is a result of RNase sensitivity under these assay conditions (Weller *et al.* 1993). The band at 147 bp is derived from protection of mRNAs comprising exon 3 alone and represents all transcripts other than class 1. (B) Autoradiograph of RNase protection assay using a IGF-I class 2 probe hybridised with 50 μ g total RNA from saline- (samples 1–5) or GH- (samples 6–10) treated pigs. Each sample was analysed in duplicate. The band at 190 bp represents protection of exon 2–3 class 2 IGF-I mRNA species. The 147-bp protected fragment is also evident using this riboprobe. (C) Autoradiograph of RNase protection assay using a ribosomal 18S RNA probe hybridised with 10 μ g total liver RNA from saline- (samples 1–5) or GH- (samples 6–10) treated pigs. Each sample was analysed in duplicate. The band at 80 bp is derived from protection of ribosomal 18S RNA and is a control for variations in RNA quantitation. Graphical representation of IGF-I class 1 (D) and class 2 (E) liver mRNA levels are also shown. Band intensities were quantified using Image Quant software and are expressed as a percentage of the intensity obtained from hybridising the same RNA samples with an 18S RNA probe. Results are expressed as the mean $\pm$ s.e.m. ($n=5$). Significant differences from the saline group: * $P<0.05$, ** $P<0.01$.

fragments was consistent with an RNase-sensitive region at the 3' end of exon 1. This required subcloning of the IGFBP-3 fragment in order to remove the GC-rich region. The strategy used is outlined in Fig. 1. Digestion with EcoRI and PstI produced a 208-bp exon 1-specific fragment that encompassed the GC-rich region. Digestion with PstI and SalI produced a 256-bp fragment encoding the 3' end of exon 1 and the 5' end of exon 2, and digestion with SalI and SmaI produced a 131-bp fragment that encoded the remainder of exon 2 and the 5' end of exon 3. These fragments were subcloned into pBluescript II SK+ and linearised with EcoRI, PstI and SalI respectively, and used as templates for the production of antisense riboprobes for different regions of IGFBP-3.

The exon 1-specific riboprobe derived from plasmid p208BP3 was the correct size (278 nucleotides), but it failed to resolve protected fragments when hybridised with porcine liver RNA (data not shown). Using plasmid p256BP3 as a template for generating an exon 1/2-specific riboprobe generated the expected 289-nucleotide probe. This RNA species, however, hybridised only partly to IGFBP-3 mRNA, yielding a protected fragment of 158 bases in length (data not shown). This analysis was not taken further.

An exon 2/3-specific riboprobe was generated with plasmid p131BP3. A 192-nucleotide riboprobe of the expected size protected a fragment of 139 bases in length when hybridised with porcine liver RNA. This result indicated that p131BP3 was suitable for generating a porcine IGFBP-3-specific riboprobe for use in an RNase protection assay to detect steady-state levels of IGFBP-3 mRNA.

The steady-state level of IGFBP-3 mRNA was measured in a number of tissues using this riboprobe and the results are illustrated in Figs 3 and 4. The data show that IGFBP-3 mRNA was significantly increased by GH treatment in liver ($P<0.03$) and kidney ($P<0.05$) (Fig. 3), but not in longissimus dorsi muscle, stomach and jejunum (Fig. 4).

IGF-I mRNA detection

IGF-I class 1 mRNA was detected in all tissues examined, whereas IGF-I class 2 mRNA tran-

scripts could only be detected in the liver. Additional IGF-I mRNA transcripts were also detected and are represented as a 147-nucleotide fragment. This fragment is generated from protection of exon 3 alone and represents non-class 1 and non-class 2 IGF-I mRNA transcripts (Weller *et al.* 1993). These exon 3 transcripts were also detected in all tissues examined. GH administration increased the level of IGF-I class 1 ($P<0.02$) and class 2 mRNA in liver ($P<0.01$) (Fig. 5). In kidney, longissimus dorsi muscle, stomach and jejunum, the IGF-I class 1 mRNA level was not changed by GH treatment (Fig. 6). Expression of the exon 3-only IGF-I transcript was also increased by GH treatment in liver (Fig. 5).

DISCUSSION

The effect of administration of exogenous GH to the pig is well established in terms of growth performance, improvement in food conversion efficiency and increased average daily weight gain (Etherton *et al.* 1986, Campbell *et al.* 1988, Klindt *et al.* 1992). In addition, plasma concentrations of IGF-I and IGFBP-3 are increased in pigs in response to growth hormone, consistent with their known actions as key components of the somatotropic axis, co-ordinating the regulation of growth (Owens *et al.* 1990, Dunaïski *et al.* 1997). Expression of IGF-I mRNA is also increased in some porcine tissues in response to GH, but there is no information on the expression of IGFBP-3 in pigs; most information on IGFBP-3 mRNA is derived from studies in the rat. IGFBP-3 is widely expressed, suggesting it has an important role in mediating the action of IGF peptides (Shimasaki *et al.* 1989), although there is little correlation between the expression of IGF-I and IGFBP-3 (Albiston & Herington 1992). Mechanistically, this is of little relevance in the pig, which is more sensitive to GH administration than is the rat (Walton *et al.* 1995). The main objective of this study was to examine the effect of short-term GH administration on the steady-state levels of IGFBP-3 in a number of tissues in the pig, and compare it with the known expression patterns of IGF-I mRNA.

To accomplish this, we had to demonstrate the established effects of GH treatment. Accordingly,

FIGURE 6. Representative autoradiographs of RNase protection assays for IGF-I class 1 mRNA expression in kidney (A), muscle (C), stomach (E) and jejunum (G) of saline- and GH-treated pigs. Graphical representations of kidney (B), muscle (D), stomach (F) and jejunum (H) mRNA are shown. Band intensities were quantified using Image Quant software and are expressed as a percentage of the intensity obtained from hybridising the same RNA samples with an 18S RNA probe. Results are expressed as the mean $\pm$ S.E.M. ($n=5$).

the results showed that a 4-day treatment with GH at a dose of 70 µg/kg/day significantly increased average daily weight gain and improved feed efficiency, confirming the efficacy of the treatment and allowing mechanistic conclusions to be drawn from the study.

A porcine IGFBP-3 cDNA was first isolated from a liver and an ovarian cDNA library based on sequence information derived from the purification of a polypeptide from porcine follicular fluid, which was able to inhibit follicle-stimulating hormone-induced oestradiol production by rat ovary granulosa cells (Shimasaki *et al.* 1990). A single mRNA species (2.6 kb) encoding porcine IGFBP-3 was detected in porcine ovary, testis, brain and liver by Northern blotting. The cDNA contained five exons, with the mature protein being encoded in the first four exons (Shimasaki *et al.* 1990). A plasmid derived from these original clones, containing the first three exons of porcine IGFBP-3, was kindly provided by Dr Shimasaki. Northern blotting has been the method of choice for the detection of IGFBP-3 mRNA in porcine tissues. Lee *et al.* (1993) measured IGFBP-3 mRNA using a human cDNA probe in liver, kidney, muscle and lung in samples taken from pigs throughout development. Although IGFBP-3 was identified in all tissues, there was a preponderance in the kidney. These authors noted that this was consistent with high expression of IGFBP-3 mRNA in the rat in one study (Albiston & Herington 1992), but differed from the findings of a study of juvenile rats, which demonstrated greatest IGFBP-3 mRNA levels in the liver (Shimasaki *et al.* 1989). Other studies have detected IGFBP-3 mRNA in the porcine ovary – primarily in the corpus luteum – sertoli cells and pancreas (Samaras *et al.* 1992, Besset *et al.* 1996, Gadsby *et al.* 1996, Zhou *et al.* 1996, Peng *et al.* 1998).

We attempted to quantify steady-state IGFBP-3 mRNA in a number of porcine tissues, using the more sensitive RNase protection assay. Initial experiments using the full-length insert from the plasmid pPBP3ES as a template to produce a riboprobe proved unsuccessful, as it generated two protected fragments of smaller size (250 and 238 bases), instead of the expected protected fragment of 595 bases. In order to correct this, the cDNA sequence containing the first three exons of porcine IGFBP-3 was digested with suitable restriction enzymes and subcloned to generate three probes complementary to different regions of porcine IGFBP-3 mRNA (see Fig. 1). However, a probe generated from p208BP3 did not protect any RNA species in porcine liver. A second probe generated from p256BP3 protected a fragment that was

smaller than the expected 256 bases and was not used. An exon 2- and 3-specific probe of 192 bases was generated from p131BP3. In a preliminary series of RNase protection assays, this IGFBP-3 probe gave rise to one protected fragment, 139 bases in length, that was specific to porcine IGFBP-3 mRNA, with a linear response over a range of RNA concentrations from 20 to 100 µg. This cDNA clone was used in all subsequent assays to detect changes in steady-state IGFBP-3 mRNA levels.

Using this riboprobe, we were able to demonstrate the expression of IGFBP-3 mRNA in all the tissues sampled, confirming the results of Lee *et al.* (1993). In addition, the results demonstrated that IGFBP-3 mRNA was increased by a short-term administration of GH in the liver and kidney, but not in other tissues. This suggests that the liver and kidney are the major source of the increase in circulating IGFBP-3 in the pig (Walton & Etherton 1989), at least at the early stage of GH administration. Whether this is a direct effect of GH or a result of increased IGF-I is not clear. Type 1 IGF and GH receptors are present in both liver and kidney (Lee *et al.* 1993), therefore either hormone may stimulate IGFBP-3.

Further confirmation of the effects of GH in this study were provided by the increase in IGF-I class 1 and class 2 mRNA in response to GH treatment in porcine liver. These findings support previous studies in which GH administration increased IGF-I class 1 and class 2 mRNA expression in liver (Brameld *et al.* 1996). In comparison, a decrease in hepatic IGF-I class 1 and class 2 mRNA is associated with a reduction in growth rate (Weller *et al.* 1994). Non-class 1 and non-class 2 IGF-I transcripts, as indicated by a 147-nucleotide protected fragment, were also sensitive to energy status (Weller *et al.* 1994), and increased with GH treatment in the present study.

IGF-I class 2 transcripts are more sensitive to changes in energy status than are class 1 transcripts, leading to a positive correlation between IGF-I class 2 gene expression and plasma IGF-I levels (Weller *et al.* 1994). In the present study, GH treatment resulted in a fourfold increase in liver IGF-I class 1 mRNA expression, whereas IGF-I class 2 transcripts were increased eightfold. This agrees with previous data suggesting that IGF-I class 2 transcripts are the primary source of endocrine-regulated IGF-I (Weller *et al.* 1994). The absence of IGF-I class 2 transcripts in muscle (Weller *et al.* 1994) and the absence of any detectable IGF-I class 2 transcripts in adipose tissue (Brameld *et al.* 1996), and in kidney, stomach and jejunum in our study confirms that the liver is the major source of endocrine-regulated IGF-I.

Our study confirmed that the growth-promoting effect of GH on longissimus dorsi muscle is not associated with local expression of IGF-I. In longissimus dorsi, IGF-I expression was not regulated by GH or energy status (Grant *et al.* 1991, Coleman *et al.* 1994, Weller *et al.* 1994). In contrast, in semitendinosus muscle GH treatment increased IGF-I class 1 mRNA expression (Brameld *et al.* 1996).

It is clear that GH has an important role in IGF-I and IGFBP-3 gene expression. In the pig, the major source of endocrine-regulated IGF-I appears to be the liver, although GH also regulates IGF-I expression in adipose tissue, which may contribute to circulating concentrations (Wolverton *et al.* 1992). Whether this is due to an increase in IGF-I class 1 or class 2 expression has not been determined. In the liver, IGF-I class 2 transcripts are more responsive to endocrine growth control, although a subpopulation of transcripts termed non-class 1 and non-class 2 also appears to be regulated by GH. We have shown that IGFBP-3 gene expression in liver and kidney is also sensitive to exogenous GH. Whether this is a direct effect of GH or an indirect one through IGF-I is not clear. In the pig, IGF-I and IGFBP-3 mRNA expression are not affected by GH treatment in longissimus dorsi muscle, stomach or jejunum.

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