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These studies were conducted to examine the influence of several variables on the growth hormone binding protein (GHBP) in serum of pigs. Continuous long term porcine somatotropin (pST) injections (daily for 6 - 7 wk) increased GHBP activity ($p < .05$). However, periodic short term pST injections (daily, every second d or every fourth d for 2 wk) did not cause significant change in GHBP levels ($p > .40$). No difference was observed between fed animals and animals fasted for 5 days ($p > .3$). Between 0 and 6 mo of age boar and gilt serum GHBP activity were not significantly different from each other, but increased significantly with age in both sexes ($p < .0001$). There was no significant correlation between serum GHBP and body weight in this study ($p > .30$). In pregnant sows, GHBP concentrations were highest at the beginning (day 72) of the third trimester (p

< .05). These values were compared with information in the literature on serum growth hormone (GH) concentrations and GH receptor activity under similar conditions. Growth hormone receptor activity reported by other researchers and GHBP activity appear to vary concurrently except during fasting which may indicate alternate regulation of either the GHBP or the GH receptor.

Assessment of Factors Regulating Growth Hormone Binding Protein in Pigs

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TABLE OF CONTENTS

INTRODUCTION 1

LITERATURE REVIEW 3
Growth Hormone Structure 3
Secretion of Growth Hormone 4
Growth Hormone Action 6
Growth Hormone Receptor 8
Discovery and Characterization of Growth Hormone Binding Protein 11
Amino Acid Sequence of Growth Hormone Binding Protein 12
Molecular Origin of Growth Hormone Binding Protein 13
Physiological Role of Growth Hormone Binding Protein 14
Serum Growth Hormone Binding Protein and Stature 14
Growth Hormone Binding Protein in Relation to Sex and Age 16
Growth Hormone Binding Protein During Gestation 16
Growth Hormone Binding Protein Correlations with Growth Hormone Receptors 17
Growth Hormone Binding Protein Secretion Patterns 17
Effects of Hormones and Ions on Growth Hormone Binding Protein Levels 18
Growth Hormone Binding Protein Assays 19

ASSESSMENT OF FACTORS REGULATING SERUM GROWTH HORMONE BINDING PROTEIN IN PIGS 21

ABSTRACT 22

INTRODUCTION 24

MATERIALS AND METHODS 25
Continuous Long Term pST Treatment 25
Periodic Short Term pST Treatment 25
Fasting 26
Age and Sex 27
Weight 27
Gestation 28
Assay 28

RESULTS AND DISCUSSION 30
Continuous Long Term pST Treatment 30
Periodic Short Term pST Treatment 30
Fasting 31
Age and Sex 31
Weight 32
Gestation 32
Summary 33

IMPLICATIONS 35

LITERATURE CITED 39

BIBLIOGRAPHY 42

APPENDIX 52

LIST OF TABLES

Table		Page
1.	Effects of continuous long term porcine somatotropin treatment on GHBP activity	36
2.	Effects of periodic short term porcine somatotropin treatment on GHBP activity	36
3.	Effects of fasting on GHBP activity	37
4.	Effects of age and sex on GHBP activity	38
5.	Effects of gestation on GHBP activity	38

ASSESSMENT OF FACTORS REGULATING GROWTH HORMONE BINDING PROTEIN IN PIGS

INTRODUCTION

Growth hormone binding protein (GHBP) has been shown to be bound with growth hormone (GH) in the serum and appears to have a structural relationship to the membrane bound GH receptor (Leung et al., 1987; Baumann et al., 1988a). Baumann et al. (1987) and Lim et al. (1990) have suggested that GHBP may have a physiological role to increase GH half-life and reduce the effect of high GH levels immediately after secretion. Alternatively GHBP may exist only as a result of proteolytic cleavage of the membrane bound GH receptor and may have no physiological role. Studying factors which regulate variations in serum GHBP could help to elucidate functional roles for this protein. It has been debated whether GHBP concentrations are regulated solely by receptor cleavage or if additional mechanisms control GHBP levels (Leung et al., 1987; Baumbach et al., 1989; Smith et al., 1989; Sadeghi et al., 1990). How serum GH concentrations, GH receptor activity and possibly serum GHBP activity interact to regulate growth is unknown.

Growth hormone receptor levels tend to be negatively correlated to serum GH concentrations in various metabolic states. For example, during postnatal growth in pigs, GH

levels decrease (Klindt and Stone, 1984). While, receptor levels increase in growing rats (Mathews et al., 1989). In contrast, pregnant sows' serum GH is unchanged while receptor activity increases in pregnant rats (Atinmo et al., 1976; Mathews et al., 1989). Very little receptor work has been done in pigs under these conditions. Animals in severe protein deprivation display a sharp rise in GH and decrease in receptor levels (Machlin et al., 1968; Baxter et al., 1981). Exogenous GH treatment reduces GH secretion and increases receptors (Abe et al., 1983; Chung and Etherton, 1986; Zanelli et al., 1989).

Levels of GHBP have been studied in some of these metabolic states in humans and in laboratory animal species, but no extensive study has been conducted with any single species. More importantly, little work has been done with any of the domestic animals. This work was undertaken to determine whether differing physiological states and ages in the domestic pig are associated with changes in relative serum GHBP activity.

LITERATURE REVIEW

Growth Hormone Structure

Growth hormone (GH) is a growth promoting polypeptide arising from a family of genes that produce GH, prolactin, and placental lactogen. Growth hormone can aggregate and form dimers and polymers, but the monomeric form is a single chain polypeptide approximately 190 amino acids in length with two disulfide bonds (Li et al., 1966). The amino acid sequence and the position of the two disulfide bonds vary slightly between species, and these differences have been summarized by Wallis (1978). Studies of recombinant pig GH (rpGH) show that slightly more than half of the polypeptide is alpha helical, folding into a bundle of four antiparallel helices (Abdel-Meguid et al., 1987). The folded regions appear to be conserved between mammalian species and may exist in other polypeptide hormones of the GH family. Within an individual animal the GH monomer has variable weights (approximately 22kD) and isoelectric points (Ip) with an average Ip of 8.0. Variation in size and charge of GH can result from many processing possibilities, including variable genetic transcription, post translational processing and glycosylation. Reduction of the bonds, or breaking of the molecule to form a two chain peptide held by

disulfide bonds increases the apparent molecular weight by 2 kD (Bennett et al., 1989). These variations in molecular size can occur before or after secretion (Farrington and Hymer, 1990).

Secretion of Growth Hormone

Growth hormone is secreted from the anterior pituitary by cells called somatotrophs. Secretion of GH occurs in a pulsatile fashion. Secretion is regulated by two factors from the hypothalamus; a growth hormone releasing factor (GRF) and somatotropin (GH) release inhibiting factor (SRIF). Growth hormone is also capable of regulating its own secretion via a short-loop negative feedback mechanism. Exogenous GH suppresses GH peak amplitude in rats, possibly by stimulating release of insulin-like growth factors produced by GH sensitive cells, which in turn inhibit GH secretion (Abe et al., 1983).

Growth hormone secretion varies with the metabolic status of the animal. Stage of growth, pregnancy, and nutritional status have potentially differing effects on GH levels.

During fetal pig growth serum GH concentrations increased from 2.2 ng/ml to 100 ng/ml concurrently with fetal weight from 10 g to 200 g until d 75 in utero. From d

75 to birth little variation was observed (Klindt and Stone, 1984).

Scanes et al. (1987) reported that GH levels in piglets decreased between 2 to 10 d of age. Then no change occurred up to weaning. Between 5 and 24 wk of age overall plasma GH mean concentration of boars dropped from 5.8 ng/ml to 2.1 ng/ml of plasma (Klindt and Stone, 1984). Siers and Swiger (1971) and Dubreuil et al. (1987) have reported that GH levels in gilts and barrows decreased from birth to 5 mo ($p < .01$) with no difference between sexes. Siers and Swiger further reported that serum GH level was more negatively correlated with average daily gain than with age, suggesting that the decrease in GH concentration with increasing age may actually be due to the increase in body weight. Animals with lower GH levels also had leaner carcasses, leading the authors to conclude that animals which better utilized GH had lower GH concentrations (Siers and Swiger, 1971).

Several studies have shown that nutrient intake regulates GH secretion. In one study by Pond et al. (1986) sows fed a 16% protein control diet or a 3% protein diet exhibited no change in serum GH levels during pregnancy. However, sows on a .5% protein diet had an increase in GH level between wk 10 and 15 of gestation (Atinmo et al., 1976). Gilts fed 33% of recommended digestible energy did

not have different GH levels from pregnant or nonpregnant control gilts (Pond et al., 1986).

Growing pigs fed a diet containing only .5% protein had an average GH increase of 9 ng/ml above pigs on a control diet at 12 wk of age (Atinmo et al., 1976). In another study four barrows and four gilts weighing 20 - 24 kg, fasted four d, showed an increase from approximately 6 ng/ml at d 0 to a peak at d 2 of 12.2 +1.1 ng/ml ($p < .01$) and then fell to 8.5 + 0.5 ng/ml by d 4 (Machlin et al., 1968). Buonomo and Baile (1991) also reported an increase in serum GH levels in pigs after 48 h of fasting. Fasting is also reported to increase GH levels in sheep (Driver and Forbes, 1981).

Growth Hormone Action

Growth hormone reportedly increases tissue growth through both direct and indirect action (Green et al., 1985). Cell differentiation is promoted directly through GH interaction with cellular GH receptors (Morikawa et al., 1982). Growth hormone indirectly stimulates cell multiplication in tissues which grow by hyperplasia via IGF-I interaction with cellular IGF-I receptors. IGF-I is produced mainly by the liver in direct response to GH stimulation (Holder et al., 1981).

In vivo, GH stimulates both protein deposition and milk production. It also increases plasma concentrations of free fatty acids and glucose, and reduces fat deposition. Lipolysis is induced by the antagonism of insulin by GH, reduced glucose transport, and inhibition of lipogenic enzymes (Vernon and Flint, 1989). Stimulation of cell hyperplasia by IGF-I increases hypertrophy of muscle tissue using energy that would otherwise be deposited as fat (Etherton, 1989).

Treatment of livestock with exogenous GH stimulates the metabolic effects of GH beyond what is normally expected. The stimulation of milk production (reviewed by Peel and Bauman, 1987) may, in part, be supported by the increase in glucose available for lactose synthesis (Kopchick and Cioffi, 1991). Treatment of dairy cows with recombinant bGH increased milk production by 3.9 to 5.6 kg/d depending upon dose (Chilliard, 1988). Johnsson and Hart (1985) reported that female lambs given daily injections of pituitary bovine GH (pbGH) had increased average daily gain (ADG), improved feed conversion efficiency and increased lean tissue deposition. Daily injections of pGH in growing pigs resulted in significantly increased ADG, feed conversion, and lean body mass (Machlin, 1972). Injection of highly purified pituitary pGH into rapidly growing pigs for 30 d

resulted in a 10% increase in growth rate and a 4% increase in feed efficiency (Chung et al., 1985). Growth hormone treatment appears to also affect fatty acid composition. Cooked pork loin from pGH treated animals showed a 33% decrease in total fatty acid content, due nearly entirely to loss of the saturated fatty acid component (Clark, 1991).

Growth Hormone Receptor

Peptide hormones act through receptors in target cell membranes. Grichting et al. (1983), noted that the effects of GH differed at various levels of receptor occupancy, suggesting that different intracellular second messengers may be involved. Growth hormone receptor has a structure similar to those for prolactin, erythropoietin, and several cytokines (Hochberg et al., 1991a). Prolactin and GH compete for the same binding sites although the affinity of each for the other's receptor is much lower than for its own (Yamada and Donner, 1984).

The GH receptor is approximately 620 amino acid residues long with a binding domain, a central hydrophobic transmembrane domain of 24 residues, and a cytoplasmic domain. The weight of the receptor has been estimated at 130 kD after glycosylation and association with ubiquitin and 70 kD before glycosylation (Yamada and Donner, 1984;

Leung et al., 1987). Leung et al. (1987), identified two cDNAs which could possibly be expressed as GH receptors. The first had all three normal domains. The second had a truncated cytoplasmic domain of 8 amino acid residues perhaps analogous to the truncated growth factors which function as oncogenes. Receptors for GH have been identified in liver, adipocytes, monocytes, chondrocytes, muscle, endothelium, epithelium and epidermis (Barnard et al. 1984; Jafari and Khansari, 1990; Lobie et al., 1990).

Growth hormone receptors likely function in a manner similar to many other peptide hormones through a second messenger which so far has yet to be identified. Roupas and Herington (1988) showed that the receptor is rapidly internalized after it is inserted into the membrane. This internalization is apparently not dependent upon GH binding to the receptor. The binding of ^{125}I-GH to the cell membrane is blocked if cellular protein synthesis is stopped, suggesting that the GH receptor is not recycled after it has been internalized. The receptor is thought to be internalized via coated pits and is not dissociated from its ligand (GH) for reuse but is processed along with GH. Internalized GH has a half-life of 20 - 30 min before being released extracellularly as 25% intact and 75% degraded. It is assumed that the receptor is released concomitantly and

this may be a source of serum GHBP (Roupas and Herington, 1987).

The mechanism of regulation of the GH receptor is not well understood. A study of GH receptor mRNA levels in rats showed an increase from birth to adulthood. No differences existed between males and females except for elevated levels during pregnancy (Mathews et al., 1989). Chronic treatment of pigs with increasing doses of pGH caused a linear increase in GH binding to liver membrane (Chung and Etherton, 1986). Long term hGH treatment of rats increased ^{125}I-GH binding in skeletal muscle (Zanelli et al., 1989). The increase in GH binding activity was the result of an increased number of receptors rather than a change in binding affinity. Similarly, in fasted rats a decrease in GH binding activity was the result of a decreased number of receptors rather than a change in binding affinity (Baxter et al., 1981). Level of nutrition also appears to influence GH receptor activity. For example, Breier et al. (1988) observed that high affinity liver GH receptor activity is reduced in poorly fed steers. Similarly, Bass et al. (1991) reported that GH binding to liver membranes was highest in well fed lambs. In rats, it has been reported that 9 d of sever dietary protein restriction also caused a decrease in liver cell binding of GH (Maiter et al., 1989).

Discovery and Characterization of Growth Hormone Binding Protein

A specific, high affinity growth hormone binding protein (GHBP) was first reported in rabbit serum (Ymer and Herington, 1985) and human plasma (Baumann et al., 1986). Since then GHBP has been reported in numerous animal species including the dog and pig (Daughaday et al., 1987b), rat (Amit et al., 1990a), mouse (Sadeghi et al., 1990), sheep, chicken, goose, and horse (Davis et al., 1991). A secondary low affinity GHBP was identified in human plasma (Baumann and Shaw, 1990).

Rabbit GHBP identified by Ymer and Herington (1985) had an estimated MW of 100 kD and had an affinity constant (K_a) for human GH of 1.59×10^9 M^{-1}. Its binding equilibrium was reached in 2 h at 21C, and binding was completely reversible. Growth hormone from various species competed for rabbit GHBP, but prolactin did not compete. Human GHBP had a K_a of 3.2×10^8 M^{-1} and a maximum binding (B_{max}) capacity of 1.8×10^{-9}M for hGH and had a molecular weight, time to binding equilibrium and reversibility similar to that for rabbit GHBP (Herington et al., 1986). Human GHBP was found to be specific for hGH. Prolactin and GH of other species did not compete for binding (Herington et al., 1986). Bauman et al. (1986) found a molecular weight of

approximately 65 kD for human GHBP using a more sensitive gel filtration column. They also found an isoelectric point of 5.1 and a K_a of 2 to 3 x 10^8 M^{-1} as well as the same specificity and binding kinetics reported by Herington et al. (1986). In domestic animals binding affinity is higher for hGH than for the species homologous GH (Shaw and Baumann, 1988; Davis et al., 1991). Davis et al. (1991) reported that the binding affinity of pig GHBP (pGHBP) was 2.2 x 10^9 M^{-1} for hGH and 2.0 x 10^7 M^{-1} for pGH. They also noted that ovine GH and ovine placental lactogen, a GH-like growth promoting hormone, also competed for binding to pGHBP with a greater affinity than pGH. The B_{max} of pGHBP for pGH was 4.1 x 10^{-9}M and for hGH was 4.7 x 10^{-11}M. They observed the B_{max} of ovine GHBP for hGH to be 6.0 x 10^{-10}M, and the B_{max} of chicken GHBP for hGH to be 1.7 x 10^{-10}M and for cGH to be 1.5 x 10^{-10}M.

Amino Acid Sequence of Growth Hormone Binding Protein

Rabbit serum GHBP has been purified and its amino acid sequence compared to liver GH receptor (Leung et al., 1987). The sequence of the N-terminal amino acids of the GHBP was found to correspond identically to the amino terminus (extracellular domain) of the GH receptor. Further study (Smith et al., 1989) using a cDNA which produced GHBP showed

that mouse GHBP had the same sequence as liver GH receptor minus the transmembrane and intracellular domains of the receptor, which were replaced by a small hydrophilic tail. Baumbach et al. (1989) obtained similar results for rat GHBP.

Molecular Origin of Growth Hormone Binding Protein

Only in mice and rats has GHBP been reported to have its own unique mRNA which encodes the hydrophilic tail (Baumbach et al., 1989; Sadeghi et al., 1990). In other species the GHBP may arise from proteolytic cleavage of the receptor or a unique mRNA alternately processed from the mRNA of the receptor.

To show that the majority of GHBP in rat serum was derived from a unique mRNA, Sadeghi et al. (1990) used a monoclonal antibody against the hydrophilic tail which occurred only on the mRNA derived GHBP. This MAb recognized nearly all of the serum GHBP present, suggesting rat GHBP was derived from the unique mRNA and not the receptor. DNA complementary to human GH receptor has been cloned but no cDNA for human GHBP has been identified (Hocquette et al., 1990).

Physiological Role of Growth Hormone Binding Protein

Baumann et al. (1987) measured the metabolic clearance (MCR) and distribution volume (V_d) of hGH complexed with human GHBP and free hGH in the rat and found it to be six times slower than that of free hGH. The V_d of GH-GHBP corresponded to the volume of the intravascular compartment. Free GH had a V_d close to the extracellular volume. *In vitro*, GHBP inhibited GH stimulation of preadipocytes (Lim et al., 1990). These two experiments indicate that GHBP may slow MCR of GH, confining it to the vascular system, and dampening the biological effect of GH at the cellular level.

Serum Growth Hormone Binding Protein and Stature

Human stature has long been of medical interest. Many young dwarf patients respond favorably to GH treatment, showing an increase in IGF-I levels and normal growth (Laron et al., 1966). Laron type dwarfism, however, is a genetic recessive disease. These patients lack GH receptors and IGF-I cannot be stimulated by GH (Eshet et al., 1984). Activity of GHBP in sera of 13 Laron dwarfs was negligible. GHBP was also significantly lower in genetically heterozygous carriers (Laron et al., 1989).

African pygmies had a significantly lower %SB in GHBP assays compared to normal subjects and a correlated decrease

in IGF-I levels (Baumann et al., 1989b). Control and pygmy children had increasing GHBP levels through childhood, but adolescent pygmies did not show the increase that occurred during adolescence in normal individuals. Serum from adult pygmies showed only 30% of the specific binding (GHBP levels) of normal adult serum (Merimee et al., 1990).

Baumann et al. (1991) suggest a further role for GHBP, based on blood samples from the Mountain Ok people of Papua New Guinea. The people have normal growth factor levels including IGF-I. At the same time their stature is comparable to that of African pygmies. Their sera have half of the GHBP activity of normal control sera suggesting a possible role for GHBP in growth regulation (Baumann et al., 1991).

Dwarf poodles and minipig strains were reported to have serum GHBP activities similar to their larger counterparts. The Yucatan micropigs, on the other hand, did show significantly lower GHBP levels when compared to standard Yucatan pigs (Daughaday et al., 1987b). In a recent unpublished study, a breed of dwarf chickens was found to have no detectable GHBP activity (Eric Schneiderman, personal communication).

Growth Hormone Binding Protein in Relation to Sex and Age

Adult female rats were reported to have twice the GHBP activity of adult male rats (Massa et al., 1990). In a comparison of men, women, and children of various ages, only neonates showed a significantly lower GHBP level (Baumann et al., 1989a). A larger study of children (n = 97) and neonates (n = 6) again showed a significantly lower GHBP level for neonates (Silbergeld et al., 1989). These workers also observed that the GHBP levels doubled by the age of six years yet were significantly lower than normal adults. Young rats also showed a significant increase in GHBP levels with age (Carlsson et al., 1990).

In humans, GHBP levels correlated with height before puberty for both sexes (Silbergeld et al., 1989). During puberty, however, body weight correlated with GHBP levels only in males. Growth rates in this study did not appear to be correlated with GHBP at any time.

Growth Hormone Binding Protein During Gestation

GHBP levels in pregnant mice began to increase around day 9 and plateaued at day 14 through 17 of pregnancy (Smith and Talamantes, 1988). Hypophysectomy reduced mid-pregnancy GHBP activity to the nondetectable levels of nonpregnant mice (Sanchez-Jimenez et al., 1990).

Pregnant women in the first trimester have been observed to have significantly higher GHBP than nonpregnant women. Levels then decrease significantly through the third trimester (Maheshwari and Norman, 1990).

Growth Hormone Binding Protein Correlations with Growth Hormone Receptors

Serum GHBP of premature infants was significantly lower than that of full term infants (Daughaday et al., 1987a). The low concentration of GH receptors in fetal liver membranes of domestic animals was suggested to be correlated with low human fetal serum GHBP. It was also suggested that serum GHBP activity might be a useful non-invasive way to assess relative liver GH receptor activity.

Growth Hormone Binding Protein Secretion Patterns

It has been suggested that rat and human GHBP pulses 30 to 60 min after a GH pulse (Bick et al., 1990; Hochberg et al., 1991b). These pulses were identified with the Pulsar program developed by Merriam and Wachter (1982). The pulses appear to rise 1 - 3 %SB above the basal level. Snow et al. (1990) observed GHBP patterns over a 24 h period without investigation of GH levels. While GHBP appeared to cycle, there was little variation outside of a 95% confidence

interval, and therefore there is no cyclic pattern to serum GHBP activity. This appears to disagree with the previous observation by Bick et al. (1990) and Hochberg et al. (1991b).

Effects of Hormones and Ions on Growth Hormone Binding Protein Levels

Barnard and Waters (1988) found that 25 mM Ca^{2+} increased nearly 20 times the affinity for hGH of GHBP isolated from rabbit liver cytosol for. Affinity for other GH's was not similarly affected. The authors suggested that a negative charge on the hGH interacted with Ca^{2+} making it more readily accepted by the GHBP and that other GH's did not have such a negative charge.

Growth hormone treatment elevated GHBP and liver receptor numbers in hypophysectomized mice (Sanchez-Jimenez et al., 1990). Ten male pigs 102 - 112 days old treated with rpGH for 12 days, showed a significant increase in GHBP levels compared to controls treated with diluent (Ambler et al., 1990). As further evidence that GH affects activity of GHBP, it has also been observed that GH deficient children treated with GH show a significant increase in GHBP levels (Postel-Vinay et al., 1990). Boys with pubertal delay

treated with testosterone displayed a decrease in GHBP (Postel-Vinay et al., 1990).

Treatment of male rats with hGH produced a concomitant increase in both serum GHBP and liver membrane GH receptors (Bick et al., 1990).

Carlsson et al. (1990) isolated both GHBP and GH receptor mRNAs from rat liver. A probe for the two mRNAs was used to search other tissues for the mRNAs. Both mRNAs were found in liver, heart, muscle, skin, kidney, adrenal and intestine, but in variable concentrations and ratios. Increases and decreases of the GH receptor and the GHBP mRNA appeared to be positively correlated. The mRNA concentrations were assumed to reflect relative rates of receptor and GHBP transcription.

Ambler et al. (1990) reported that GH treatment increased both serum GHBP and GH liver receptors in pigs. The increase in receptors was reported to account for only 64% of the change observed in GHBP activity.

Growth Hormone Binding Protein Assays

Growth hormone binding protein was initially hard to identify due to the known presence of GH aggregates and the nature of the rapid dissociation of GH from GHBP in assays (Baumann, 1990). The initial method of measuring GHBP in a

serum or blood sample involved incubating it with radio-labeled GH (usually ^{125}I) and then separating the free *GH from the larger and heavier *GH-GHBP complex. This could be done by column chromatography, but the dissociation of the complex began immediately and increased with time and dilution of the sample (Baumann et al., 1988b). Rapid gel filtration columns (Ymer and Herington, 1985; Baumann et al., 1986), HPLC (Tar et al., 1990), or anion exchange columns (Baumann et al., 1988b) allowed reproducible separation of free *GH from the *GH-GHBP complex. All of these techniques were difficult to use for large sample numbers with multiple replications.

A standard radioimmunoassay (RIA) was developed using monoclonal antibodies (MAbs) against the rabbit GH receptor (Barnard et al., 1989). Approximately half of the MAbs recognized the human GHBP.

More recently a rapid competitive protein binding assay was developed for GHBP which separated free GH from GH-GHBP complex using dextran-coated charcoal (Amit et al., 1990b). The rapidity of the separation greatly reduced dissociation and improved precision with large numbers of samples.

ASSESSMENT OF FACTORS REGULATING SERUM GROWTH HORMONE BINDING PROTEIN IN PIGS

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ABSTRACT

These studies were conducted to examine the influence of several variables on the growth hormone binding protein (GHBP) in serum of pigs. Continuous long term porcine somatotropin (pST) injections (daily for 6 - 7 wk) increased GHBP activity ($p < .05$). However, periodic short term pST injections (daily, every second d or every fourth d for 2 wk) did not cause significant change in GHBP levels ($p > .40$). No difference was observed between fed animals and animals fasted for 5 days ($p > .30$). Between 0 and 6 mo of age boar and gilt serum GHBP activity were not significantly different from each other, but increased significantly with age in both sexes ($p < .0001$). There was no significant correlation between serum GHBP and body weight in this study ($p > .30$). In pregnant sows, GHBP concentrations were highest at the beginning (d 72) of the third trimester ($p < .05$). These values were compared with information in the literature on serum growth hormone (GH) concentrations and GH receptor activity under similar conditions. Growth hormone receptor activity reported by other researchers and GHBP activity appear to vary concurrently except during fasting which may indicate alternate regulation of either the GHBP or the GH receptor.

Key Words: Pigs, Growth Hormone Binding Protein, Fasting, Growth, Gestation, Growth Hormone

INTRODUCTION

Growth hormone binding protein (GHBP) has been shown to be bound with growth hormone (GH) in the serum and appears to have a structural relationship to the membrane bound GH receptor (Leung et al., 1987; Baumann et al., 1988). Baumann et al. (1987) and Lim et al. (1990) have suggested that GHBP may have a physiological role to increase GH half-life and buffer the effect of high GH levels immediately after secretion. Alternatively, GHBP may exist only as an artifact of proteolytic cleavage of the membrane bound GH receptor. Studying factors that regulate variations in serum GHBP could result in elucidation of the involvement of this protein in GH action.

Levels of GHBP have been studied in some metabolic states in humans and in laboratory animal species, but no controlled and extensive studies have been conducted with a single species. More importantly, little research has been conducted with any of the domestic animals. This study was undertaken to determine whether differing physiological states and ages in the domestic pig are associated with changes in relative serum GHBP activity.

MATERIALS AND METHODS

Continuous Long Term Porcine Somatotropin Treatment

Fourteen crossbred meat type barrows with an average weight of 50 Kg were randomly assigned control and treatment groups. Animals were housed four to a pen and allowed *ad libitum* access to food and water. Experimental animals were given IM injections of recombinant pST (provided by Pitman-Moore Inc., Terre Haute, IN) 3 $mg \cdot kg^{-1} \cdot d^{-1}$ for 7 wk. Control animals were similarly injected with the buffer used to dilute the pST. Blood was collected at slaughter on the last day of treatment. Serum was collected and frozen until assayed for GHBP.[4] Results were analyzed with a one tailed t-test (Statgraphics, 1991).

Periodic Short Term Porcine Somatotropin Treatment

Fifteen crossbred barrows between 5 to 6 mo of age, were housed one to a pen and limited to 85% of the normal *ad libitum* consumption. Animals were randomly assigned to one of four experimental groups. Four pigs in group 1 served as a control. Three pigs in group 2 received IM injections of recombinant pST (provided by Pitman-Moore, Terre Haute, IN)

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[4]Sera kindly provided by Dr. C. Y. Hu and Dr. R. Wander, OSU, Corvallis, OR, 97330.

60 $\mu g \cdot kg^{-1} \cdot d^{-1}$. Four pigs in group 3 received injections of rpST at 120 $\mu g \cdot kg^{-1}$ every second d and four pigs in group 4 received injections of rpST at 240 $\mu g \cdot kg^{-1}$ every fourth d. Injections were given for 2 wk. Blood was collected from the vena cava and centrifuged to collect serum. Serum was frozen until assayed for GHBP.[5] Results were analyzed by analysis of variance (ANOVA) (Statgraphics, 1991).

Fasting

Twenty Landrace X Yorkshire pigs weighing 80 - 110 kg were assigned randomly to control and treatment groups. Each group consisted of five gilts and five barrows and each group was housed in a separate pen. Control animals had *ad libitum* access to feed and water. Experimental animals had similar access to feed and water for ten d prior to the experiment. On d 0 feed was removed from the experimental group but access to water was continued. Blood samples were drawn from each pig via ear veins on d 0, 1, 3, and 5. Immediately after the final sample was obtained, feed was returned to the experimental group. Serum was collected by centrifugation of blood and frozen until assayed. Results were analyzed by split-plot repeated measures ANOVA with d,

[5]Sera kindly provided by C. Evock and Dr. N. Steel, USDA-ARS, Beltsville, MD, 20705.

treatment and d X treatment as the main effects. Log transformation allowed the univariate hypothesis of equal variation to be accepted (SAS, 1988).

Age and Sex

Three gilts and three boars (1.5 - 2.5 kg) were assigned randomly from each of five litters of Landrace X Yorkshire pigs for a total of 30 pigs. Piglets stayed in their litters until weaning and were then allotted randomly by sex to six pens, three pens of five gilts and three pens of five boars. One ml of blood was collected at 5 d of age from each pig by tail clipping. At 1, 3 and 6 mo, 5 ml of blood was collected from the jugular vein. Animals were allowed *ad libitum* access to feed and water. Collected serum was frozen until assayed. Percentage specific binding in male and female sera was analyzed by split-plot repeated measures ANOVA with age, sex and age X sex as the main effects. A transformation was not required for the univariate hypothesis to be acceptable (SAS, 1988).

Weight

Twenty randomly allotted Landrace X Yorkshire gilts and boars at 6 mo of age between 72 and 125 kg were weighed and 5 ml of blood drawn from the jugular vein. Serum was frozen

until assayed for GHBP. Simple linear regression was performed with weight dependent on %SB (Statgraphics, 1991).

Gestation

Blood was collected from ear veins of Landrace X Yorkshire sows on d 0, 36, 72, and 112 of gestation. Sera were frozen until assayed for GHBP. Ten random samples were chosen for each of the four time periods.[6] Results were analyzed by ANOVA. Log transformation was required to reduce unequal variability (Statgraphics, 1991).

Assay

Human GH (NIDDK-hGH-I-1, 2.2 IU/mg) was radioiodinated (^{125}I purchased from Amersham Corp., Arlington Heights, IL) using a modification of the chloramine-T method originally described by Hunter and Greenwood (1962). Specific activity ranged from 85 - 180 μCi/μg. The ^{125}I-hGH was purified twice on a Biogel P100 (purchased from Biorad, Hercules, CA) column (1 x 50 cm). The assay described by Amit (1990) was modified as follows: Total binding tubes for samples were prepared by incubating for 2 h at room temperature 50 μl of serum with 350 μl of PBS (.14 M NaCl, .01 M $NaPO_4$, pH 7.0)

[6]Sera kindly provided by Dr. W. Kwansa, PIC Inc., Franklin, KY, 40511.

and 100 µl of ^{125}I-hGH containing 20,000 CPM. Then 500 µl of ice cold dextran-coated charcoal [2% activated charcoal and .2% dextran (both purchased from Sigma Chemical Co., St. Louis, MO) in PBS] was added and vigorously vortexed for one s. This mixture was then centrifuged at 1700 x g for 20 min at 4C. The supernatant was counted in a Beckman model 5500 Gamma Counter. Nonspecific binding (NSB) was prepared similarly except that 10 µg of unlabeled hGH was added to the incubation mixture. Specifically bound hormone was calculated as total binding minus NSB. The standard measurement of GHBP in samples was recorded as percentage specifically bound (%SB). It was determined as CPM of ^{125}I-GH specifically bound ÷ CPM of total ^{125}I-GH added. Nonspecific binding ranged from 800 - 6000 CPM. Specific binding ranged from 1000 - 3000 CPM. Intraassay coefficient of variation was 8% to 15%. Interassay coefficient of variation was 13%. The accuracy and sensitivity of the above assay was not defined because known quantities of purified GHBP were not available.

RESULTS AND DISCUSSION

Continuous Long Term Porcine Somatotropin Treatment

Animals treated with 3 mg of rpST per d had a significantly higher ($p < .05$) %SB compared with that of control animals (Table 1). This response confirms the previous report that GH treatment increased GHBP in pigs (Ambler et al., 1990). This response is also similar to the increase reported for pig liver GH receptors (Chung and Etherton, 1986) and rat skeletal muscle GH receptors (Zanelli et al., 1989) under long term GH treatment. The growth stimulating effects of exogenous GH treatment may be due in part, to increased cellular stimulation by increased receptor numbers. Up-regulation of the receptors may in turn yield increased GHBP activity. Exogenous GH increases systemic levels of GH but decreases GH secretion (Abe et al., 1983).

Periodic Short Term Porcine Somatotropin Treatment

Treated groups were not different ($p=.43$) from each other or from the control group in %SB (Table 2). Ambler et al. (1990) reported an increase in GH liver receptors and GHBP for pigs treated with rpST for 12 d at 3 mo of age. The lack of short term response in our study may have

resulted from age differences between our pigs and those of Ambler or low sample numbers because the standard errors were greater than 10% of the mean. Effect of periodic GH treatment on receptor levels has not been reported.

Fasting

No difference ($p > .30$) in GHBP activity was observed between fed and fasted pigs. Percentage SB on each sampling d showed no difference ($p > .31$) between control and experimental groups (Table 3). Machlin et al. (1968) and Buonomo and Baile (1991) reported that fasting caused an increase in serum GH levels in pigs after 48 h while fasted rats had a decrease in liver GH receptor levels (Baxter et al., 1981). In the current study it appears the fasted pigs either did not experience a decrease in GH receptor levels or the GHBP did not reflect that decrease.

Age and Sex

Newborn pigs had an average %SB of 5.8 which increased ($p < .0001$) by age six mo to an average of 10.2 %SB (Table 4). Male and female %SB was not significantly different ($p > .05$) at any age. Klindt and Stone (1984) and Scanes et al. (1987) reported a decrease in serum GH concentration in piglets during the first 2 mo of life. Dubreuil et al.

(1987) reported a further decrease between 7 to 23 wk of age with no difference between male and female pigs. A difference in GHBP activity was reported between male and female rats (Massa et al., 1990). However, no difference was reported between male and female humans (Silbergeld et al., 1989; Baumann et al., 1989). Both groups reported an increase in GHBP activity with age of the experimental subject. Similarly, Ambler et al. (1990) observed higher serum GHBP in pigs older than 100 d than in 20 d old piglets.

Weight

There was no significant ($p=.34$) regression of body weight on %SB for either boars or gilts at 6 mo of age. Decreases in GH serum concentrations of gilts and barrows were reported to be more closely related to weight than age (Siers and Swiger, 1971). GHBP levels do not appear to be related to body weight of individual animals.

Gestation

Pregnant sow serum displayed highly variable binding (SE > 10%). Log transformation of the data was required to remove a funnel-shaped pattern (indicative of unequal variance) from the residuals. Samples from sows collected

on d 72 of gestation displayed significantly ($p < .05$) higher %SB compared with other days of gestation (Table 5). Increases in GHBP levels have also been noted for mice during mid-pregnancy (Smith and Talamantes, 1988) and also in humans during the first trimester (Maheshwari and Norman, 1990). Mathews et al. (1989) reported an increase in GH liver receptors of pregnant rats. No increase in GH levels was reported for pregnant sows under normal feeding conditions (Antinmo et al., 1976).

Summary

This work reports the systemic concentrations of GHBP in a single domestic animal species (pig) under varying physiological conditions. During postnatal growth and during pregnancy GHBP levels increased. Treatment with exogenous GH also increased GHBP levels while fasting had no observable effect on the levels of GHBP.

A review of the literature indicates that concentrations of endogenous GH and GH receptors tend to oppose each other. It has been reported that GHBP originates from the receptor or an alternate transcription of the receptor's mRNA (Leung et al., 1987; Smith et al., 1989; Sadeghi et al., 1990). Whether GHBP levels vary concurrently with receptor levels is currently being debated

(Daughaday et al., 1987; Ambler et al., 1990). GHBP levels reported in the current study generally vary in accord with receptor levels under similar treatment reported by others. Fasting, a fairly severe treatment, however, did not result in the expected decrease in GHBP levels. An absence of a fasting effect may reflect the existence of a further regulatory mechanism for GHBP or the GH receptor.

IMPLICATIONS

Pig GHBP appears to be regulated in a manner similar to the GH receptor, suggesting a common origin of these proteins. This lends further support to the suggestion of Daughaday et al. (1987) that measurement of GHBP concentrations may be a non-invasive way to assess GH receptor activity under normal growing conditions.

TABLE 1. EFFECTS OF CONTINUOUS LONG TERM PORCINE SOMATOTROPIN TREATMENT ON GHBP ACTIVITY

Treatment	n	Dose	GHBP %SB
Control	7	0 mg	10.8 ± 0.5
Experimental	7	3 mg	12.3 ± 0.6[a]

[a]Significantly different from control ($p < .05$).

TABLE 2. EFFECTS OF PERIODIC SHORT TERM PORCINE SOMATOTROPIN TREATMENT ON GHBP ACTIVITY

Treatment	n	Dose	GHBP %SB
Control	4	0 µg/kg	6.9 ± 0.7[a]
Daily	3	60 µg/kg	7.8 ± 1.2[a]
Every 2nd d	4	120 µg/kg	8.3 ± 0.9[a]
Every 4th d	4	240 µg/kg	7.9 ± 0.7[a]

Means (± SE) with similar superscripts do not differ ($p > .40$)

TABLE 3. EFFECTS OF FASTING ON GHBP ACTIVITY

Day	Treatment	n	GHBP %SB
0	Control	10	8.4 ± 0.8[a]
	Fasted	10	8.7 ± 0.9[a]
1	Control	10	9.6 ± 1.1[a]
	Fasted	9	9.0 ± 1.2[a]
3	Control	10	8.6 ± 0.8[a]
	Fasted	10	7.3 ± 1.1[a]
5	Control	10	9.7 ± 0.8[a]
	Fasted	10	8.7 ± 1.5[a]

Means (± SE) with similar superscripts do not differ ($p > .3$).

TABLE 4. EFFECTS OF AGE AND SEX ON GHBP ACTIVITY

Age (Mo)	Sex	n	GHBP %SB
0	Male	9	5.9 ± 0.7[c]
	Female	6	6.4 ± 0.5[c]
1	Male	9	6.7 ± 0.6[c]
	Female	8	6.9 ± 0.5[c]
3	Male	8	8.0 ± 0.6[a]
	Female	7	6.1 ± 0.8[c]
6	Male	8	10.6 ± 0.9[b]
	Female	9	9.9 ± 0.5[b]

[a]Significantly higher than females at 3 mo ($p < .08$).
[b]Significantly different from other superscripted values in the column ($p < .0001$).

TABLE 5. EFFECTS OF GESTATION ON GHBP ACTIVITY

Day	n	GHBP %SB
0	10	8.5 ± 1.5
36	10	6.2 ± 1.8
72	10	11.5 ± 1.1[a]
112	10	8.0 ± 1.9

[a]Significantly different from other values in the column ($p < .05$).

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APPENDIX

RADIOIODINATION OF hGH

Preparation of Hormone for Radioiodination

Purified hGH (NIDDK-hGH-I-1, 2.2 IU/μg)is dissolved in .01 M $NaHCO_3$. Aliquots of 2.5 μl containing 2.5 μg purified hGH are dispensed into 1 ml serum vials and stoppered. Aliquots are flash frozen in isopentane and dry ice and stored at -20 C.

Preparation of Anion Exchange Column

Anion exchange resin (Ag 1-X8 Biorad, Hercules, CA) is packed in a 1.25 x 6 cm column (5 ml plastic pipet tip with glass wool in the tip). Resin is rinsed with O.5 M sodium phosphate buffer (pH 7.5). Next, 5 ml of 5% BSA-PBS solution is added to coat the column with protein. Finally the column is rinsed with .05 M sodium phosphate buffer (pH 7.5) and sealed until used.

Radioiodination of hGH

Radioiodination is conducted in the serum vial (materials are added with a 1 cc disposable syringe with a 22 gauge needle unless otherwise noted) as follows:

1. Add 25 μl of .5 M sodium phosphate, pH 7.5 , tap vial.

2. Drain anion exchange column.

3. Add .5 mCi ^{125}I (in NaOH pH 7-11, Amersham Corp., Arlington Heights, IL) to the vial in 5 μl with a Hamilton syringe.

4. Add 30 μl of .5 μg/μl Chloramine-T (Sigma Chemical Co., St. Louis, MO) in .05 M sodium phosphate, pH 7.5.

5. Tap vial repeatedly for 30 seconds to mix contents.

6. Add 50 μl of 2.5 μg/μl Na metabisulfite in .05 M sodium phosphate, pH 7.5, tap vial.

7. Add 100 μl of .05 M sodium phosphate buffer pH 7.5 and transfer contents of the vial to the disposable column.

8. Add 70 µl of .05 M sodium phosphate buffer pH 7.5 and transfer contents of the vial to the disposable column.

9. The Biorad anion exchange Ag 1-X8 column is eluted with .05 M sodium phosphate, pH 7.5. 1.0 ml/fraction. Five fractions of eluate are collected into tubes containing 1.0 ml of .5% BSA-PBS. The labeled hormone should elute in fraction 3 and the free ^{125}I should remain in the column.