GROWTH HORMONE, INSULIN AND GLUCOSE IN YOUNG GILTS SELECTED ON THE BASIS OF RAPID VERSUS SLOW GROWTH RATE

By

SHARON ANN NORTON

Bachelor of Science
Virginia Polytechnic Institute & State University
Blacksburg, Virginia
1981

Master of Science
Virginia Polytechnic Institute & State University
Blacksburg, Virginia
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Thesis Approved:

Charles V. Maxwell
Thesis Adviser

Donald S. Buchanan

Michael T. Zavy

J. E. Breazile

Robert Teeter

Norman N. Durham
Dean of the Graduate College

1256212

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CHAPTER I

INTRODUCTION

Progress in the efficiency of livestock growth and production has evolved through improvements in herd health, management, genetics, reproduction and nutrition More recently, the potential for increasing productive efficiency through the manipulation of the endocrine system has been recognized. If this potential is to be realized, it is necessary to determine the extent and if possible, the mechanisms whereby our current production practices impact the endocrine system in order to produce significant advances in growth and feed efficiency. The current developments in biotechnology offer the promise of more rapid improvements in growth and feed efficiency More thorough understanding of endocrine control as it relates to current production practices, such as selection for growth rate, is a prerequisite to the application of this technology in the swine industry.

The following study was undertaken to more precisely examine how selection pressure aimed at increased growth rate was related to the level and pattern of two growth-related hormones and a metabolic nutrient. Temporal and challenged secretory profiles of growth hormone, insulin and

glucose in two lines of young gilts selected for rapid versus slow growth were evaluated to more clearly define the influence of selection pressure on the endocrine system of the pig relative to a selected performance parameter.

CHAPTER II

REVIEW OF LITERATURE

Insulin

History

The first evidence of a pancreatic islet organ appeared in evolution as early as the cyclostomes (hagfish, lamprey) Present day invertebrates produce an insulin like peptide (Faukmer, Edmin, Havu, Lundgren, Narques, Ostberg, Steiner & Thomas, 1973) The initial extraction of the insulin polypeptide and its therapeutic use in alleviating diabetes in dogs is credited to Banting and Best (Banting, Best, Collip, Campbell & Fletcher, 1922), although prior to that time several other investigators had extracted what was described as an active pancreatic polypeptide which exhibited a hypoglycemic action. Since the time of the discovery of insulin and its use to alleviate diabetes mellitus, a normally fatal metabolic disorder, the complex role of insulin in protein and fat metabolism has received much attention. Technologic developments have allowed the extraction and preparation of insulin in a crystalline form (Abel, 1926), the elucidation of the amino acid sequence (Sanger, 1960) and finally the synthesis of the molecule

(Meienhofer, Schnabel, Brinkoff, Zabel, Sralen, Klostermeyer, Brandenburg, Okuda & Zahn, 1963, Katsoyannis, Tometsko & Fukata, 1963) and has led to increased progress in the elucidation of the role of insulin and its interaction with other metabolic hormones.

Chemistry

The gene for preproinsulin which is in the islet cell nucleus is transcribed into a specific mRNA (Lomedico, Rosenthal, Efstratiadis, Gilbert, Kolodner & Tizard, 1979). This mRNA is translated into preproinsulin (Chan & Steiner, 1977) which is cleaved into proinsulin and ultimately to insulin. Initial processing results in the removal of a hydrophobic 23 amino acid pre-segment that serves as a signal sequence for the transfer of the polypeptide chain through the microsomal membranes of the rough endoplasmic reticulum (Blobel and Dobberstein, 1975). Proinsulin is generated by the proteolytic removal of the signal peptide. Proinsulin contains two polypeptide regions, that ultimately become insulin alpha and beta chains, connected by an intermediate connecting or c-peptide, by way of basic dipeptides (Chan, Kiem & Steiner, 1976) The c-peptide is variable in length between animal species Interchain disulfide bridges between the beta chain components of proinsulin create a loop in the molecule (Busse & Carpenter, 1976). During the last stage of production of the active peptide, proteolytic enzymes in the Golgi apparatus cleave

the molecule at the basic amino acids between the c-peptide and the alpha and beta chains, resulting in both active insulin and the c-peptide as secretory products. Active insulin is a peptide hormone with a molecular weight of approximately 60 kd consisting of an alpha chain of 21 amino acids and a beta chain of 30 residues. After its conversion from the prohormone, insulin is concentrated and stored with c-peptide in secretory vesicles. From x-ray crystallographic study (Hodgkin & Mercola, 1972; Pitts, Wood, Horuk, Bedarkar & Blundell, 1980) the three dimensional, electron dense, granular structure of stored insulin has been established. Normally, insulin exists as a two Zn^{++} hexamer. Although a four Zn^{++} molecule has been reported to be formed under high chloride ion concentrations (1.2 M), this form probably does not exist in vivo (Bentley, Dodson, Dodson, Hodgkin & Mercola, 1978). The insulin concentration in mature granules is approximately 27 mg/ul, favoring a crystalline formation of the hormone It has been postulated that the oligomeric forms and the inclusion of the metal ions Zn++ and Ca++ may assist the granular formation (Adams, Blundell, Dodson, Dodson, Vijayun, Baker & Harding, 1969; Blundell, Cutfield, Cutfield, Dodson, Dodson, Hodgkin & Mercola, 1972). It is thought that the granular formation of insulin may provide a method whereby the hormone can be more efficiently concentrated, thereby providing thermodynamic stability and greater resistance to proteolytic cleavage (Pitts et al,

1980). The Ca^{++} and Zn^{++} may also aid in reducing the hormone's solubility at physiological pH. Without these elements a pH of approximately 6.3 is necessary for crystallization to occur (Pitts et al., 1980).

The composition of the insulin molecule varies between species. Porcine insulin is the most similar to human and differs only at the alanine residue at the carboxy-terminus of the beta chain (Steiner, Kemmler, Clark, Oyer & Rubenstein, 1972). The specific biological activity of mammalian insulin also varies, ranging from 22 to 27 U/mg Proinsulin has a low biological activity whereas the unassociated alpha and beta chains are essentially inactive.

Pancreatic Secretion

The islet of Langerhans has been described by Orci (1977) as a multihormonal micro-organ. The endocrine tissue of the pancreas consists of various cell types which display tight junctions with homologous and heterologous cells (Friend & Gilula, 1972; Andrew, MacVicoar, Dudek & Halton, 1981). The mammaliam pancreatic islet contains recognizable cell types which have been identified and localized within the islet through the use of indirect immunofluorescence techniques specific for insulin, glucagon and somatostatin (Dubois, 1975; Orci, Bactens, Rufener, Amherdt, Ravazolla, Stedner, Malaisse-Lagae & Under, 1976; Bactens, Malaisse-Lagae, Perrelet & Orci, 1979) Insulin synthesis occurs in

the beta-cell (Orci, 1976, Orci et al., 1976), glucagon production and synthesis in the alpha cells, and somatostatin in D cells. The latter two cell types are in close proximity to each other and are found in the periphery of the islet, while insulin synthesizing beta-cells are concentrated within the center of the islet and are in contact mainly with other beta-cells.

Due to the close anatomical relationship of these cells within the pancreas, it is not suprising that they are involved with each other to provide hormonal regulation. Unger & Orci (1973) and Samols, Morri & Marks (1965) have suggested that a specialized communication exists between the islet cell types via paracrine secretion, and/or tight junctions. In support of the latter, Unger & Orci (1981) observed the presence of tight junctions between islet cells. The secretion of glucagon by alpha cells was found to be inhibited by insulin secreted by the beta-cell population. In addition, isolated beta-cells were less responsive to glucose, suggesting that glucagon stimulates insulin release while insulin inhibits the secretion of glucagon. Recent studies examining this interaction have identified the presence of glucagon receptors on pancreatic beta-cells, which provides a molecular basis for the regulatory role of this hormone in insulin synthesis and/or secretion (Van Schraniendizk, Foriers, Hooghe-Peters, Rogiers, DeMeyts, Sodvyez & Pipeleers, 1985). Somatostatin from the D-cell population inhibits both insulin and

glucagon, whereas glucagon stimulates the synthesis and secretion of somatostatin (Koerker, Ruch, Chideckel, Palmer, Goodner, Esnick & Gale, 1974; Arimura & Fishback, 1981) The effect of insulin on somatostatin synthesis and secretion is unclear (Larner, 1982).

Pipeleers, In'T Veld, Maes & Van de Winkel (1982) hypothesized that glucose homeostasis is dependent on the number and integrity of beta cells and also with the nature of their interconnections with alpha and D-cells. This work has provided the basis for the concept that the microanatomy of the islet creates the anatomical basis for the functional cooperativity between islet cells in the control of insulin secretion. Consistent with this idea is the observation of aberrations in the pattern of islet cell distribution which has been associated with cases of diabetes mellitus.

Malaisse, Sener, Herchnelz & Hutton (1979) have suggested that the rate of insulin release from intact isolated cells is primarily determined by the fuel capacity of the stimulus. However, recent in vivo work of Pipeleers, Schurt, In'T Veld, Maes, Hooghe-Peters, Van de WInkel & Gepts (1985) has suggested that insulin releasing ability of glucose and amino acids is dependent upon cytoplasmic cyclic 3'-5', adenosine monophosphate (cAMP) levels, in addition to the fuel capacity of the stimulus. With a depletion of intracellular cAMP concentrations, only a fraction of insulin stored in a cell can be released by glucose or amino acids. From these findings it appears that the normal

endocrine pancreas has the capacity to effectively mobilize insulin only if the beta-cell contains adequate cAMP levels Glucagon appears to be responsible for maintaining beta-cell cAMP at sufficiently high levels to allow glucose and amino acids to exert their insulin releasing action. Somatostatin exerts an inhibitory effect by reducing intracellular cAMP within beta cells. Insulin release, therefore, appears to be controlled by hormones through two different cellular mechanisms which have the potential of operating in series to amplify an extracellular stimulatory effect. This type of interaction has been termed by Rasmussen (1981) as synarchtic regulation of cell function.

In isolated islets, glucagon regulates the cAMP production of beta cells with somatostatin and epinephrine exerting a suppressive effect via independent receptors that influence cAMP production. The level of insulin in the blood is rigorously controlled and in a normal state, to a great extent, reflects the energy status of the animal. All inputs of a fuel nature, including amino acids, glucose and fatty acids will stimulate the release of insulin, although glucose is probably the most potent secretagogue (Larner, 1981) Oral glucose administration, compared to an intravenous route, is a more potent stimulator of insulin release (MacEntyre, Holdsworth & Turner, 1964). This observation suggests that gastrointestinal factors may play a role in insulin synthesis and release from the pancreas Penold, Mintz, Muller & Cahill (1978) investigated this

possibility and found that several gastrointestinal hormones such as CCK-PZ, gastrin and gut glucagon would stimulate insulin release. Brown & Otte (1978) also found gastric inhibitory peptide (GIP), a structural homolog to glucagon and secretin, to be a potent stimulator of the beta-cell. Therefore, the upper gastrointestinal tract may form an endocrine enteropancreatic axis, which serves to control the utilization of nutrients released by the digestive process (Bloom & Grossman, 1978; Hedeskov, 1980).

Both epinephrine and norepinephrine inhibit insulin secretion via alpha-adrenergic receptors, whereas beta-2-adrenergic receptors stimulate its secretion. Smith, Pork & Robertson (1979) noted that various external stimuli interact with alpha-adrenergic receptors to inhibit insulin release. These stimuli include exercise, hypoxia, surgery, hypothermia and severe burns. Vagal stimulation and a variety of cholinergic agents have been observed to increase insulin secretion. The interplay of both adrenergic and cholinergic receptor types controls tonic and phasic secretion of insulin.

Through the use of light microscopic autoradiography, van Houten, Posner, Kopriwa & Brawer (1979) have localized insulin binding sites in rat brain tissue. These sites were located in the circumventricular organs, medial basal hypothalamus, paravagal region and areas associated with glucose monitoring, satiety and gastrointestinal regulation This study suggested that insulin binding in these areas may

influence the release of hypophysiotropic substances as well as feed intake. Using a rabbit model, Ishikawa (1981) demonstrated hypothalamic regulation of both parasympathetic and sympathetic input of the pancreatic islet cell as indicated by electrical stimulation of the ventromedial hypothalamus producing a decrease in insulin secretion.

Wollheim & Sharp (1981) described the kinetics of glucose induced insulin release from the beta-cell, in vivo and in vitro following islet stimulation, and found that insulin peaks sharply and declines within several minutes, afterwhich a second peak of longer duration usually occurs at approximately 1 h post stimulus (Cerase & Luft, 1967, Curry, Bennett & Grodsky, 1968). This biphasic response is proposed to reflect the presence of two intracellular pools of insulin which exhibit differences in stimulatory sensitivity, level of priming, and/or proximity to the plasma membrane (Lacy, Howell, Yound & Fink, 1968, Grodsky, 1972; Malaisse, Van Obberghan, Davis, Somers & Ravazzola, 1974) More recent work has shown the initial insulin release to be associated with the release of stored intracellular Ca^{++} and decreased Ca^{++} efflux. The secondary release occurs with the influx of extracelluar Ca^{++} and a secondary release of intracellular, stored Ca^{++}. Colca (1983) has suggested that the secondary release is related to an increased activity or concentration of calmodulin-Ca^{++} dependent, sensitive protein kinase and(or) changes in the cytoarchitecture

Target Cell Interaction

Insulin Receptor. Czech (1981) has described a native receptor that exists in a variety of target tissues for insulin which is a large, disulfide-linked complex consisting of two alpha subunits ranging in size between 250 and 350 kd, and two 90 kd beta subunits. The beta subunit of the receptor is sensitive to proteolysis which results in the formation of a $beta_1$ subspecies of 45 to 50 kd (Jacobs, Hazum & Cuatrecasas, 1980). Two important structural features of the insulin receptor have been noted. First, the general structure of the receptor protein is very similar to that of the immunoglobulins, in that two large and two relatively smaller subunits are joined by disulfide linkages (Czech, 1981). A second interesting feature is the divalency of the receptor for binding to insulin. Previous experiments have shown that receptor bound to $^{125-}$I-insulin could be precipitated with antibodies against the binding site on the receptor, indicating other binding sites which were unoccupied (Kahn, Baird, Flier & Jarrett, 1977)

The role of the insulin receptor is two-fold in that it recognizes and binds insulin among all other substances in the blood (Roth, Lesniak, Bur, Muggeo, Megyesi, Harrison, Flier, Wachslight-Robard & Gordon, 1979). Following binding, a signal is generated to result in the production of a characteristic insulin response (Catt, Harwood, Aquilera & Defore, 1979, Kahn, 1979).

Insulin binding data subjected to Scatchard (1945) analysis demonstrates a curvilinear function which has been ascribed to receptor site heterogeneity. Two receptor populations are recognized which are characterized as high affinity, low capacity and low affinity, high capacity sites which differ by one order of magnitude (De Meyts, 1976). In addition, Olefsky & Chang (1979) have suggested the possibility that the insulin receptor may exist in interconvertible states. It has been known for some time that insulin levels fluctuate in response to physiological conditions (Blackard & Nelson, 1970), however, more recently, it has been recognized that receptor concentrations and affinity also fluctuate (Forgue & Freychet, 1975). Fasting produces increases in adipocyte receptor affinity without a change in receptor number (Olefsky, 1976).

In an adipose cell there are estimated to be 50,000 to 160,000 potential receptors (Kono & Barham, 1971; Gavin, Roth, Neville, DeMeyts & Buell, 1972), however, only 2 % of the binding sites needed to be filled to elicit a maximal metabolic response. These investigators concluded that the apparent biological advantage of these "spare" receptors is an increased sensitivity to low insulin concentrations This conclusion was supported by the findings of Kono & Barham (1971) and Cuatrecasas (1972) in which trypsin treatment reduced both the maximum insulin binding capacity

of cells and the sensitivity of cells to insulin-induced glucose oxidation.

Insulin binding to its receptor sites also has the effect of altering the affinity of neighboring receptors for insulin. Increasing receptor saturation which results in a decreased affinity of the population of receptor sites is termed negative cooperativity. This phenomenon occurs through what has been demonstrated to be a ligand-induced acceleration in dissociation rate of the insulin receptor complex. (DeMeyts, Neville, Gavin & Lesniak, 1973; DeMeyts, 1976; De Meyts, Beavo & Roth, 1976). These investigations have suggested that negative cooperativity is a fundamental feature of the insulin receptor and is inducible in all species studied. DeMeyts (1978) has further suggested that negative cooperativity is the only property of insulin that has been as tightly conserved as the structure of the molecule. Hence, negative cooperativity was present in both the hormone and receptor even in species where other properties are altered such as affinity for binding (non-mammalian and hystricomorph insulins), and the ability to dimerise (guinea pig insulin), Piron, Micheals-Place, Wailbroeck & DeMeyts (1975). This suggests that the amino acid residues involved in triggering negative cooperativity were among the invariant residues in the receptor binding region. Studies of chemically modified insulins demonstrate an ability to bind and saturate the receptor, despite a reduced affinity Therefore the regions involved in

negative cooperativity constitute a distinct site from the binding region which is termed the cooperative site (DeMeyts, Van Obberghan, Roth, Wollmer & Brandenburg, 1978).

Receptor Kinetics. Based on biochemical theory, it has been accepted that large, hydrophilic, polypeptide hormones, such as insulin, bind and act at the cell surface to direct subsequent biological functions. Conversely, smaller, hydrophobic molecules such a steroid or thyroid hormones, readily enter the intracellular environment and act at the level of cytosolic or nuclear receptors to produce cellular changes (Roth, 1979).

With the use of electron microscope autoradiography and other related techniques, the internalization of insulin, in vitro and in vivo has been documented (Stein & Gross, 1959, Goldfine, Kriz & Wong, 1981). In IM-9 lymphocyte cultures, treatment of lymphocytes with trypsin to remove the plasma membrane insulin receptor, blocked the internalization of ^{125}-I-insulin, providing evidence that the cell surface receptor is involved in the internalization process Further studies conducted by Carpentier, Gorden, Amherdt, Van Obberghan, Kahn & Orci (1978) also demonstrated insulin uptake by IM-9 lymphocytes, but only Goldfine et al. (1981) were able to demonstrate an insulin interaction with intracellular organelles. The same phenomenon was also observed with hepatocytes in vivo (Renston, Jones, Hradek, Wong & Goldfine, 1980). Immediately following injection of $^{125-}$I-insulin into the portal vein, radioactivity was

detected primarily at the cell surface. Ten minutes later, however, the majority of radioactivity was present in the cell interior. As was the case with the IM-9 cultures, intracellular insulin was associated with the Golgi and vesicles. It was concluded that the vesicular association may suggest the internalization process occurs via an endocytotic process.

Post-Receptor Events. Approximately 40 years ago Stadie, Haugaard & Vaughn (1949) suggested that the intial step in insulin action at the target site was a firm binding to the cell. Some time later, work by Narahara (1972) clearly demonstrated the binding of insulin to frog sartorious muscle preceeded glucose transport. Binding activity was observed very soon after exposure to the system, however, a substantial lag was noted before the initiation of glucose transport. Freychet (1971) found that mono-iodinated insulin and derivatives of varying biological potency, bound to rat liver membrane fractions and adipocytes resulting in decreases in insulin binding directly proportional to the rate of glucose oxidation capacity.

It is now recognized that the biological actions of insulin are initiated after the hormone binds to its receptor on the plasma membrane at the target site (Cuatrecasas & Hollenberg, 1976). There are, however, differences which exist in the rate at which cellular changes take place in response to insulin (Goldstein, 1978).

At the level of the cell membrane, rapid effects are noted in terms of the uptake of glucose and amino acids Intermediate modes of action are observed in the cytosol, endoplasmic reticulum, ribosomes, mitochondria and lysosomes which are involved in processes associated with enzyme activites and concentrations and protein synthetic rates. Lastly, long-term effects best describe those events associated with the cell nucleus in terms of the modulation of DNA and RNA synthesis (Young, 1981).

Early studies concerning the cellular mechanisms involved in insulin action suggested that as long as insulin was linked to its receptor, a signal was transmitted into the cell and throughout the plasma membrane. This preliminary model, a second messenger model, was analogous to the mechanism whereby cAMP activated protein kinase (Krebs, 1972). Binding of cAMP to the receptor protein results in the dissociation of the receptor unit from a catalytic unit. When the catalytic unit is not bound to the receptor unit, it is completely active. Thus, it was assumed that insulin action occurred via a release from inhibition of the catalytic unit by binding to its receptor protein which subsequently altered the conformation of the receptor. The model did not, however, explain the subsequent steps which were associated with changes in cellular dynamics as a result of insulin binding Concentrated efforts to elucidate the event(s) following insulin binding to its receptor have been undertaken,

however, many aspects of the cellular changes are still unclear (Czech, 1982). Seal & Czech (1982) have suggested that the regulation of many of the intracellular activities of insulin are the result of phosphorylation and dephosphorylation states of key metabolic enzymes such as glycogen synthetase, glycogen phosphorylase, pyruvate dehydrogenase, hydroxymethylglutaryl CoA reductase, ATP citrate and ribosomal protein S6. Understanding of the intervening steps between the initial stimulus and the ultimate effect have only become available in the past several years due to conceptual and experimental difficulties in analyzing these changes in the intact cell. Initial confusion arose from the observation that cAMP, (Jefferson, Exton, Butcher, Sutherland & Park, 1968), cGMP (Fain & Butcher, 1976) Ca^{++} (Sansui & Rubin, 1978), Mg++ (Frazer & Russell, 1975), H_2O_2 (Little & deHaun, 1980), membrane hyperpolarization (Zierler & Rogers, 1980) and intracellular fluid pH (Sonnenberg & Schneider, 1977) all demonstrated a regulatory function. Seal and Czech (1982), investigating chemical mediators of insulin action, isolated a component from plasma membranes in response to insulin that possessed the ability to activate in vitro, two enzymes involved in glucose-related dephosphorylation reactions. The substance was characterized as small, approximately 20 kd, and hydrophobic, possessing a peptide component necessary for its activity. In addition, the substance exhibited a net negative charge at physiological pH

Similarity to the low molecular weight insulin-dependent substance described by Larner, Galasko. Cheng, DePaoli, Huang, Daggy & Kellogg (1979) following acid extraction of muscle, and by Kiechle, Jarett, Kotagal & Popp (1981) by extraction of adipocytes was noted. A similar insulin-induced, low molecular weight factor was also isolated from hepatocytes following incubation with insulin (Horvat, 1980). In all cases, proteolytic cleavage was requisite to mediator production as pretreatment of isolated membranes with serine protease inhibitors, traysol or arginine, specifically blocked the responsiveness of cells to insulin. The results of these studies led to the theory that insulin binding to target membrane receptors results in proteolysis of a membrane component which has arginine and serine specificity. The cleavage results in the release of a peptide fragment that acts as a mediator of one of more enzymes. These findings were confirmed by a similar study conducted independently by Jarett, Kiechle & Parker (1982) demonstrating that the interaction of insulin with the adipocyte plasma membrane produced a 10 to 15 kd chemical mediator which activated pyruvate dehydrogenase. Subsequent experiments isolated the factor from skeletal muscle, heptoma and IM-9 lymphocytes From this, it was concluded that the mediator may act by altering protein kinase and phosphoprotein phosphatases that modulate the state of phosphorylation and subseqent activity of these enzyme systems. The possibility of two distinct mediators was also

recognized--one which influenced phosphorylation of substrate and the other dephosphorylation activities.

More recently, Simpson and Cushman (1986) have described the insulin-induced glucose transport mechanism in the adipose cell. Using a combination of 3-o-methylglucose uptake and cell fractionation techniques of McEkll and Jarett (1970), insulin was observed to induce a 20- to 40-fold increase in glucose transport within 15 minutes of binding. The half-life of the response was 3 to 4 minutes with complete reversibility attained with insulin antiserum. In this study, using a non-insulin stimulated, intact cell, levels of glucose transporters in cytosol and high density microsomal membranes were relatively low (7 pmol/mg membrane protein), whereas a relatively high concentration of glucose transporters were located in the low density microsomal membrane fraction (82 pmol/mg membrane protein). Once stimulated by insulin, however, there was a significant shift in the concentration of transporters from the low to the high fractions and plasma membrane. These results agreed with the earlier observations of Crowford and Renold (1965 a,b) in that the increase in glucose transport in response to insulin was the result of a change in the maximum transport velocity (V_{max}) and not a change in the affinity (K_m) of the transporter for glucose. These experiments demonstrated that the principle action of insulin was the induction of the translocation of glucose transporters from the intracellular pool to the

extracellular pool. This mechanism has been named the Translocation Hypothesis and is envisioned as an exocytotic process in which vesicles become associated with the plasma membrane and fuse. When insulin is dissociated from its receptor, glucose transporters migrate back to the intracellular pool in an endocytotic fashion. In addition to the insulin-stimulated redistribution of transporters, Kono, Robinson, Blevins & Ezaki (1982) found that insulinomimetic agents such as H_2O_2, trypsin and p-chlormercuriphenylsulfonate also produced a redistribution phenomenon indistinguishable from that produced by insulin. It is probable, however, that these agents do not act by first binding to the insulin receptor, but rather, due to their cellular permeability, most likely induce translocation via an intracellular mechanism.

A final level of regulation of glucose transport involves the actual number of glucose transporters. Altered metabolic states have been associated with the inability of the adipose cell to respond to insulin. Insulin resistant states have been accounted for by reductions in intracellular transporter numbers rather than an impairment in redistribution or sensitivity as observed by Kahn & Cushman (1984) in the fasted rat. Karnieli, Hissin, Simpson, Salans & Cushman (1981) observed streptozotocin-induced diabetes to be the result of a deficit in the intracellular receptor pool. Conversely, in the hyperinsulin-sensitive state the cell exhibits enhanced

glucose transport. A comparable model of hyperinsulin-responsiveness has also been reported by Guerre-Milo, Lavau, Horne & Wardzala (1985) in the obese Zucker rat. The increased rate of maximally-induced glucose transport activity was correlated with an enlarged pool of potentially mobilizable glucose transporters in the basal state in comparison to lean controls. Therefore, the redistribution potential, sensitivity and number of glucose transporters all appear to modulate glucose homeostasis.

Although the redistribution of glucose transporters is believed to be a major mechanism in glucose transport, other evidence suggests that the influence of other hormones may be involved in the regulation of glucose transporter activity (Kashiwagi & Foly, 1982). For example, Green (1983) and Kuroda, Simpson, Hanner, Londos & Cushman (1984) demonstrated an inhibitory action of several known lipolytic substances on both basal and insulin-stimulated glucose transport. Investigations by Simpson and Cushman (1986) of the steady state distribution of transporters, noted that isoproterenol, a known inhibitor, together with adenosine deaminase, elicited a 60% decrease in insulin-induced glucose transport. However, there did not appear to be any further modulation by lipolytic and antilipolytic agents in terms of glucose transport. It was therefore concluded that these hormones may alter glucose transport activity by altering the intrinsic activity of glucose transporters rather than at the level of transporter redistribution.

Further experiments failed to demonstrate a clear relationship between the level of cAMP as reflected by A-kinase (cAMP dependent protein kinase) and glucose transport activity and sensitivity. Therefore the action of lipolytic and lipogenic agents appears to be mediated independently of the adenylate cyclase mechanism.

Insulin and Insulin-Like Growth Factors

In 1963, Froesch and coworkers (Froesch, Burgi, Muller, Humbel, Jacob & Lambert, 1963) demonstrated that most insulin-like activity in human serum is different from insulin and only 5 to 10 % can be suppressed by anti-insulin antiserum Non-suppressible insulin like activity (NSILA) has been purified from human serum and two polypeptides with molecular weights of approximately 75 kd have been isolated (Rinderknecht & Humbel, 1976). The extracts have been shown to have insulin-like activity both in vivo and in vitro (Froesch, Zapf, Audhya, Ben-Porath, Segan & Gibson, 1975). Activity is expressed both through the insulin receptor and through a separate receptor in vitro which exhibits a lower affinity for insulin. Rinderknecht & Humbel (1976) determined the complete sequence of these two peptides and identified them as insulin like growth factor (IGF) I and II. Both show remarkable structural homology with insulin Relaxin, a polypeptide hormone produced and stored in the corpus luteum also demonstrates considerable structural homology (Schwabe, McDonald & Steinetz, 1976; Schwabe,

McDonald & Steinetz, 1977), but has not been reported to demonstrate any insulin-like effects. Sequence homology of the IGF's with insulin suggest a common ancestry (Blundell & Humbel, 1980). Within vertebrate classes, variation between IGF-1, or somatomedin C, and insulin have been recognized indicating divergence occurred before the appearence of vertebrates. The divergence of IGF-1 and IGF-2 is thought to have coincided with the appearence of the first mammals (Zapf, Froesch & Humbel, 1981). A portion of the receptor binding region is identical to insulin in the IGF's which explains why it exhibits some affinity for the insulin receptor and associated insulin-like activity (Blundell, Bedarkar, Rinderknecht & Humbel, 1978). Residues that recognize the insulin receptor are markedly different in relaxin (Schwabe et al., 1976). A more complete description of the IGF's in relationship to growth are addressed further in the growth hormone section of this review.

Degradation

In the pig, the half life of insulin is approximately 9 min The main sites of degradation are the liver and kidney with approximatley 50% of the insulin reaching the liver via the portal circulation being destroyed in a single passage (Terris and Steiner, 1978). Insulin is filtered by the renal glomeruli and is reabsorbed by the tubules where it is degraded into its component amino acids. Muscle and adipose tissue also bind and inactivate insulin, but their combined

contribution is relatively insignificant in comparison to renal and hepatic routes (Orci, 1977)

In vitro Actions

In addition to its in vivo actions, insulin regulates a variety of metabolic processes in vitro. Its effects on growth and proliferation of cells in culture have been recognized for sometime (Gey and Thalheiemer, 1924) Since then, insulin has been shown to stimulate the proliferation of many cell types under a variety of experimental conditions (Straus, 1981). Insulin also acts synergistically in vitro with other hormones and growth factors to stimulate the cell cycle of cells that have been arrested in G_1 by deprivation of serum (Massague, Blinderman & Czech, 1982). The high affinity insulin receptor mediates growth stimulation in rat heptoma cells. By limiting an essential element such as phosphate (Kamely & Rudland, 1976) Rachler, Podskalny, Goldfine & Wells (1974), proposed that stimulation of the growth of fibroblasts by insulin is mediated by weak binding to receptors for the IGF's. Investigations that followed (Rinderknecht & Humbel, 1978; Svoboda, Van Wyk, Klapper, Fellows, Grissom & Schluetter, 1980; Marquardt, Todaro, Henderson & Oroszlan, 1981) confirmed this observation However, in all these systems, the level of insulin used was supraphysiological. Gospodarowicz & Moran (1976) suggested that at high levels, insulin is able to serve as a somatomedin (IGF) analog.

King, Kahn, Rechler & Nissey (1980) demonstrated that there was a significant interaction of insulin at high concentrations with the IGF I receptor in adipose and fibroblast cells. Ewton & Florini (1980) concluded that IGF I is the most active hormonal stimulator of anabolic processes in cultured muscle cell and that many of the anabolic actions of insulin results from its structural homology to IGF I, as evidenced primarily by the stimulation of myoblast differentiation and proliferation (Ewton and Florini, 1981).

Insulin in Growth and Development

From numerous sources, evidence has established a critical role for insulin in normal growth and development (Bergen, 1974; Martin, Ramsey & Harris, 1984). Investigations which have spanned nearly 30 years have identified the liver, muscle and adipose tissue as primary sites of insulin action, and have elucidated mechanisms involving carbohydrate, protein and fat metabolism. Information obtained has indicated that the primary action of insulin is anabolic in nature; promoting glucose and amino acid uptake, glycogen synthesis, lipogenesis and protein synthesis (Fritz, 1972; Czech, 1981). Insulin is thought to exert these effects via an activation of transport systems for nutrients, ions and intracellular enzymes which ultimately regulates the metabolic fate of carbon derived from major nutrients such as carbohydrates,

glycerides and amino acids. The effects are both short and long-term, affecting acute changes associated with feeding behavior (Lemagen, 1983) and metabolic reactions governing homeorhesis, (Prior & Smith, 1982).

Insulin has been considered a fetal growth hormone and several groups have defined the relationship between insulin and fetal development. Studies in humans (Liggins, 1972; Driscoll, 1965) have shown babies from diabetic mothers are longer and heavier than those from non-diabetic mothers. In rats, decreased fetal development was demonstrated as a result of decreased insulin exposure to the uterus (Girard, Kervran, Sougglet & Assam, 1974). Picon (1967) injecting insulin during the last trimester of gestation produced heavier, fatter feti with a greater nitrogen retention than normal untreated rats. In pigs, alloxan-induced diabetes in gilts during late gestation resulted in elevated maternal glucose and fetal insulin, however, this group reported no changes in body composition or development compared with controls (Esweke & Martin, 1978).

During the postnatal period, insulin deficiency profoundly limits growth (Pond, 1970; Canolty & Martin 1982) Explanations for observed growth depression are related to the metabolic actions of the hormone in vivo In terms of protein metabolism, stimulation of protein synthesis by insulin occurs independently of glucose or amino acid uptake (Fain, 1974). Wool, Stirewalt, Kurihara, Low, Bailey & Oyer (1968) observed insulin therapy

following alloxan-induced diabetes in rats increased protein synthesis via an enhancement in the translation of mRNA. This was postulated to occur through a more favorable assembly of the polyribosomes.

In vitro, insulin stimulates the incorporation of all naturally occurring amino acids into protein, including those from intracellular sources, suggesting that increased synthesis is not completely attributable to an increased uptake (Manchester, 1959). More recently, Fraymond and Maycock (1979) reported that insulin inhibited the degradation of muscle protein both in vivo and in vitro.

The chief effect of insulin on protein metabolism in the liver is anticatabolic. An examination of the synthetic effect of insulin in perfused rat livers demonstrated that the presence of insulin decreased urea nitrogen production but induced no enhancement in the synthesis of acid glycoprotein, fibrinogen or haptoglobin. The incorporation of amino acids was mainly into structural protein (John & Miller, 1969). Pilkis & Korner (1971) observed that purified polysomal preparations from the livers of diabetic animals incorporate protein less efficiently than normal controls. From these findings it was hypothesized that insulin deficiency is related to defects in polysomal aggregation. Steiner (1966) has reviewed the effects of insulin on hepatic enzyme biosynthesis and has demonstrated that insulin administration to diabetic rats resulted in a shift in concentrations of gluconeogenic and glycolytic

enzymes. The concentrations of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1-6-diphosphatase and glucose-6-phosphatase were decreased while levels of hexokinase, phosphofructokinase and pyruvate kinase were increased. This group also observed an increase in total liver RNA content. Enhanced RNA polymerase activity has been associated with insulin administration by others (Pilkis & Salmon, 1972).

The importance of insulin in livestock production is indicated by the observation that alloxan-induced diabetic pigs are 50% lighter than normal control animals (Ramos, Leveille & Alee, 1971), with normal growth restored by insulin therapy. Although it is apparent that abnormal growth can be associated with a deficiency of insulin, the relationship of growth to circulating insulin levels is slight (Etherton, 1982). This is best illustrated by observations of Wangsness, Martin & Gahagan (1977) in which obese, slow-growing, Ossabaw pigs were observed to have higher insulin concentrations than lean, more rapidly growing, Yorkshire pigs. Etherton (1982) has suggested that the overall concentrations of insulin cannot adequately be related to growth performance due to unknown differences in parameters such as metabolic clearance rate, secretion rate and tissue sensitivity. Furthermore, insulin levels fluctuate substantially during the day, making assessments of continuous concentrations difficult to determine (Vasilalos & Wangsness,1981).

In addition to these considerations, which are thought to contribute to the lack of association between insulin and livestock growth performance, the enhancement of the anabolic effects of insulin in vivo through an insulin-mediated hepatic production of IGF-1 has been recognized (Daughaday, Phillips & Mueller, 1976), but remains controversial. Conflicting reports have been made by Gahagan (1980) in which serum levels of IGF-1 were depressed in obese rats in conjunction with elevated serum insulin concentrations, suggesting perhaps an optimal level of insulin is required for IGF-1 production. The mechanism of hepatic IGF-1 output has been described by Baxter, Bryson & Turtle (1980) who suggested that insulin regulates the number of hepatic somatogenic receptors. Hence, low IGF-1 concentrations and depressed growth in the diabetic state may be the result of a deficiency in the action of insulin at this site. Recently, Oka, Mottola, Oppenheimer & Czech (1984) reported a similar effect of insulin on adipocyte IGF-2 receptors. Therefore, in addition to its possible involvement in potentiating the release of IGF-1 by increased hepatic growth hormone receptor numbers, insulin may also serve to regulate IGF-2 action via an interaction with the IGF-2 receptor.

At the present time, much emphasis is being focused on the relationship between receptor-related events associated with insulin binding in meat animals. Tissue sensitivity to insulin, especially in the adipocyte, appears to change with

the level of maturity and in this way affects growth performance (Etherton & Kensinger, 1984). Insulin has also been observed to regulate lipogenic capacity, as evidenced by the sharp decline in lipid synthesis in the absence of insulin, with restoration by the addition of insulin (Vernon, 1982). This suggests that insulin acts as a mediator of lipogenic enzyme concentrations or activity by either increasing synthesis, decreasing the rate of degradation or enzyme activation by covalent modifications or allosteric effects.

The efficacy of exogenous insulin as a viable method to improve growth performance of meat animals has met with little success. Steele & Etherton (1983) were unable to elicit significant growth changes in pigs fed high or low protein diets and receiving 1 $U \cdot kg\ BW^{-1} \cdot d^{-1}$ of insulin. Since plasma insulin concentration responded to the administration of insulin, the lack of response suggests either the level of insulin was not rate-limiting in these pigs or insulin administration produced receptor down-regulation occurred.

Clearly, further research is necessary to elucidate the synergistic and antagonistic actions of insulin with other hormones as well as its receptor mediated effects in order to fully understand and to optimally coordinate and direct efficient and economical animal growth performance.

Growth Hormone

Discovery, Chemistry and Localization

Pituitary growth hormone or somatotropin, has been recognized as essential for normal growth, development and maintenance from the neonatal through the postnatal period. Growth hormone is the most abundant of the adenohypophyseal hormones, comprising 10% of the dry weight of the anterior pituitary, approximately 8 mg per gland in humans (Laron, 1982). Somatic growth is controlled by growth hormone, and a continual, regulated secretion is necessary for normal growth and development to occur. The role of growth hormone during the postnatal period is not totally clear, but the metabolic effects are presumably important to maintain homeostasis, as evidenced by conditions resulting from abnormal secretion rates or patterns. For example, hypopituitarism, as a result of inadequate growth hormone secretion, manifests itself in short stature, growth and reproductive abnormalities. On the other hand, excessive, uncontrolled secretion results in acromegaly, which is characterized by an abnormal increase in size of the internal organs and extremities and a thickening of the skin (Daughaday, 1977).

Growth hormone was first extracted from purified granules originating from hypothalamic proteins, and fractinated by DEAE-chromatographic methods (LaBella, Krass, Fritz, Vivian, Shin & Queen, 1971) Human growth hormone

consists of 191 amino acids crosslinked by two disulfide bridges and has a molecular weight of 22 kd. (Laron, Pertzelan, Kwity, Livach-Zrinsky & Keret, 1976; Li, Liu & Dixon, 1971, Niall, Hogan, Sauer, Rosenblum & Greenwood, 1971). Ultrastructural and immunocytochemical localization studies conducted by Duello & Halmi (1979) identified and characterized the site of hGH synthesis. These cells, called somatotropes, were described as abundant, round to ovoid in shape and densely granulated with a mean diameter of 368 nm. Lactotropes, which are the site of the synthesis and secretion of the related peptide prolactin, were much different and were described as less numerous, small (185 mn), angular cells with relatively fewer granules in comparison to somatotropes.

Determinations of the molecular size of the hormone in blood by gel filtration of plasma on Sephadex G-75 and G-100 followed by radio-immuno and receptor assays of the effluent fractions revealed the presence of three substances (Goodman, Tannenbaum & Robenowitz, 1972; Gordon, Lesniak, Hendricks & Roth, 1974; Lewis, Singh, Tutweiler, Siegel & Vanderlaan, 1980) which have been identified as variants within a family of growth hormones rather than a single hormone The one found in highest concentration was noted to be the same size of pituitary GH (22 kd) and has been called "little growth hormone" The second was determined to be approximately two times as large and has been called "big growth hormone". Both forms are readily detected by

radioimmunoassay (RIA) and radioreceptor assay (RRA) techniques with the latter normally providing a better assessment of biological activity The 22 kd form may represent the form of biological importance as it exhibits a greater activity in RRA. Gordon, Lesniak, Eastman, Hendricks and Roth (1976) have suggested that the larger form is converted, enzymatically, (Yadley & Chrambach, 1973 to the smaller peptide and therefore represents a precursor or pro hormone with seventy to ninety percent of the immunoreactive growth hormone in plasma represented by the smaller peptide. A third variant is a 20 kd molecule which differs from the 22 kd GH by an internal deletion of 15 amino acids and comprises 10% to 15% of the total pituitary GH content of all species studied (Lewis, Pence, Singh & Vanderlaan, 1975). Analysis of the mRNA of both the 20 and 22 kd forms are identical except for the 45 nucleotide deletion which results in the absence of amino acid residues between 32 and 46 present in the 22 kd hormone (DeNoto, Moore & Goodman, 1981). This variant has been shown by Lewis, Dunn, Bonewald, Seavy & Vanderlaan (1978) to possess growth-promoting activity with potency similar to the 22 kd GH The variant does, however, lack the insulin-antagonistic, transient insulin-like activity and suggested lipolytic activity of the 22 kd GH The lack of these functions has been suggested to arise from this peptide being the result of active divergent splicing of a single

mRNA at the two potential intervening sequence sites (Freisan, 1980).

Many multigene families such as the GH family have been identified in eukaryotic genomes. The growth hormone family encompasses at least seven genes (Moore, Conkling & Goodman, 1982) Human GH is 85% structurally homologous to human chorionic somatomammotropin (hCS) which is synthesized in the placenta (Niall et al., 1971). In light of this structural homology, it is not surprizing that the mRNA's of hGH and hCS are also very similar (Martial, Hallewell Baxter & Goodman, 1975). In addition, both of these hormones are related to prolactin, a peptide hormone synthesized and secreted from the adenohypophysis. These phylogenetically related hormones forming the GH family probably resulted from gene duplication which occurred 350 million years ago (Miller & Eberhardt, 1985). Several lines of evidence have suggested that hGH and hCS, which are the most closely related of all the sequences, divergered only 50 to 60 million years ago at a time that is consistant with the emergence of placental mammals (Moore et al , 1982, Hung, Hover & Moore, 1985).

Growth Hormone Secretion

Hypothalamic Control of Secretion. Initial endeavors to identify the possible central nervous system control of GH secretion logically focused on the hypothalamus, a major site of neural outflow The hypothalamus has been observed

to exert a predominantly stimulatory effect on GH secretion as evidenced by ablation experiments, which resulted in the cessation of growth and a defective GH response to hypoglycemia, L-DOPA and arginine. Studies producing lesions of specific hypothalamic nuclei have resulted in abnormal GH secretory responses, indicating that these sites are associated with the regulation of GH secretion. Lesions of the median eminence and medial basal hypothalamus blocks both the insulin-induced (Abrams, Parker, Blanko, Reichlin & Daughaday, 1966) and stress-mediated (Brown, Schlach & Reichlin, 1971) GH release in the primate. Lesions of the ventromedial nuclei (VMN) of young female rats has been reported to result in depressed growth and a decrease in plasma and pituitary GH (Frohman and Bernardis, 1968, Frohman, Bernardis, Burck, Moran & Dhariwal, 1972).

Growth hormone secretion is characterized predominantly by regularly recurring episodic bursts (Martin, 1976). Individual secretory peaks in humans have been reported to reach 20 to 50 ng/ml, and usually occur during the first and last few hours of sleep (Finklestein, Roffwarg, Boyar, Kream & Hellman, 1972). The frequency and magnitude of GH secretory episodes are age dependent and can be modulated by such factors as sleep patterns, exercise and stress. Reichlin (1974) has reported that episodes of GH secretion are not accounted for by variations in glucose, amino acids or free fatty acids, which suggests that episodic surges are primarily the result of neural control mechanisms. This

does not exclude, however, that the surges are unrelated to the metabolic needs of the animal.

Autoregulation of Secretion. The ability of GH to regulate its own secretion at the level of the central nervous system has been examined in rats (Tannenbaum, 1980). This study demonstrated that GH can regulate its own secretion via a negative feedback system. Administration of rGH to the cerebral spinal fluid resulted in a significant suppression of GH secretory burst amplitude. The specific site of this regulation was not clear from since previous investigations (Martin, 1979) have implied a number of plausible sites of GH secretory control. One potential site, as previously mentioned, is the median eminence since the results of Katz, Molitch & McCann (1969) and Vooght, Clemens, Negro-Vilar, Welsch & Meites (1971) indicated that injection of GH into this region caused marked alterations in rat pituitary growth hormone (rpGH) secretion. Oliver, Mical & Porter (1977) have suggested that GH may reach the brain to modify its own secretion via a retrograde transport route via the hypothalamo-hypophyseal portal system which has been reported as a transport phenomenon utilized by many of the pituitary hormones at this level. An alternative hypothesis of the feedback mechanism has been suggested by Weindl and Joynt (1972) in which GH from the peripheral circulation is transported directly to the median eminence which possesses a greater permeability.

<u>Hypophysiotropic Factors.</u> The existence of neurohumoral control of the anterior pituitary gland by the hypothalamus was proposed for the first time by Green and Harris (1947). The first attempts to establish this connection by these investigators failed to demonstrate a direct innervation between the anterior pituitary and the central nervous system. Harris (1955) published the portal vessel chemotransmitter hypothesis of anterior pituitary regulation which outlined the concept that hypophysiotropic hormones are synthesized by neurons in the hypothalamus, transported to nerve endings in the pituitary stalk-median eminence region, released into the interstitial space within the primary portal capillary plexus, and finally distributed to the anterior pituitary through the portal vessels. Halasz (1962), using electrical stimulation and lesioning techniques, identified a hypophysiotropic area of the hypothalamus which was believed responsible for the synthesis and regulation of secretion of releasing factors and sustaining pituitary function It is now recognized that the secretion of GH is controlled by both a hypothalamic inhibitory and a hypothalamic releasing factor from this region. These substances are growth hormone releasing factor (GRF) and somatotropin release inhibitory factor (SRIF) more commonly known as somatostatin (SS).

Growth hormone releasing factor (GRF) was first isolated, purified and characterized from human subjects who presented signs of acromegaly and elevated GH levels that

abated with removal of a pancreatic tumor (Rivier, Spiess, Thorner & Vale, 1982). Investigations by other groups at the same time (Guillemin, Brazeau, Bohlen, Esch, Ling & Wehrenberg, 1982; Brazeau, Ling, Bohlen, Esch, Ying & Guilleman, 1982) isolated a 44 residue peptide subsequently termed human pancreatic growth hormone releasing factor (hpGRF). A 37 and 40-residue form has also been recognized, however, all evidence indicates that hp(1-44)-NH_2 is the most potent form in vitro (Spiess, Rivier, Thorner, & Vale, 1982; Arimura, Culler, Turkelson, Luciano, Thomas, Obhara, Groot, Rivier & Vale, 1983), and exists as an amidated COOH-terminal peptide (Bohlen, Esch, Brazeau, Ling & Guillemin, 1983). Potency studies have indicated that the 29 N-terminal residues possess all the information required for full in vitro activity, and further suggests that the C-terminal region is not critical for receptor recognition (Rivier et al., 1982). Amino acid sequencing studies have shown the hpGRF-37 and hpGRF-40 could feasibly be generated from hpGRF-44 by cleavage of arginine residues from the N-terminal side of residues 38 and 41 (Brazeau et al. 1982). Other evidence which would indicate that hpGRF-44 is the primary circulating form in the hypothalamic-hypophysiotropic axis is that monoclonal antibodies raised against the 44-residue form inhibits most of the activity of endogenous hpGRF-44. The primary structure of human GRF as established by Edman degradation is TYR-ALA-ASP-ALA-ILE-PHE-THR-ASN-SER-TYR-ARG-LYS-VAL-LEU-GLY-GLN-LEU-SER-ALA-ARG-LYS-

LEU-LEU-GLN-ASP-ILE-MET-SER-ARG-GLN-GLN-GLY-GLU-SER-ASN-GLN-GLU-ARG-GLY-ALA-AGR-VAL-ARG-LEU-NH_2. Normally, GRF-44 is synthesized and secreted from the ventromedial hypothalamus where it is transported via axons to the hypothalo-hypophyseal portal system.

The porcine form of the molecule was isolated from 2500 porcine hypothalami by acid extraction, immunoaffinity chromatography, gel filtration and a two step reverse phase HPLC procedure. The final structure was characterized by gas phase sequence analysis. The porcine molecule is very similar to the human structure with the exception of the substitution of three different amino acids between residue 34 and 44 (Bohlen et al., 1983).

All forms have been observed to elicit the secretion of immunoreactive GH in vitro and in vivo in rats (Wehrenberg & Ling, 1983), humans (Borges, Blizzard, Gelato, Furlanetto, Rogol, Cronin, Kaiser, MacLeod, Merriam, Loriaux, Speiss, Rivier, Vale & Thorner, 1983), primates (Almeida, Pittmaster & Merriam, 1983), sheep (Baile, Della-Fera & Buomono, 1983), cattle (Moseley, Krabill, Friedman & Olsen, 1984) and chickens (Leung & Taylor, 1983) with the 40 and 44 amino acid factors possessing equal potency in vivo. It is also interesting to note that hpGRF belongs to the glucagon-secretin family and is closely related structurally to the peptide PHI-27, a relatively new member of the glucagon family (Spiess, Rivier & Vale, 1983)

The discovery and elucidation of the structure and function of somatostatin (SS) was a major achievement in the study of hypothalamic peptides (Burgus, Ling, Butcher & Guillemin, 1973; Brazeau, 1973). Similar to GRF, SS is produced in the medial preoptic nuclei of the hypothalamus and is transported via axons to the median eminence and into the hypothalamo-hypophyseal portal system (Rice & Critchow, 1975; Martin, 1981). It was isolated from ovine hypothalamic extracts were subsequently purified and chemically characterized as being a cyclic tetradecapeptide. The primary amino acid structure of SS is H-ALA-GLY-CYS-LYS-ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS-OH with a disulfide bridge between residues 3 and 14. The biosynthetic mechanism of SS is still unclear, however, Arimura, Sato, Dupont, Hishi & Schally (1975) reported the isolation of a larger compound of similar structure from tissue extracts which may indicate a prohormone form exists.

Since its initial discovery, SS has been found to have a wide variety of effects and anatomical distribution. It is found not only in the central nervous system, but in the gastrointestinal tract as well, especially in the pancreas as was stated previously. In all animals tested, it is a potent inhibitor of GH secretion stimulators including exercise, arginine, L-DOPA, and hypoglycemia (Hall, Schally, Evered, Kastin, Mortimer, Tunbridge, Besser, McCoy, Goldie, McNeilly, Phenekos & Weightman, 1973; Hansen, Orskov, Seyer-

Hansen & Lundback, 1973; Parker, 1974; Siler, Vanden Berg & Yen, 1974).

The onset of SS action is rapid and the duration of its effect brief (Martin, Brazeau, Tannenbaum, Willoughby, Epelbaum, Terry & Durand, 1978). Under the influence of SS in vitro, GH secretion from isolated pituitary cells, declines with a mean half-life of 24 to 34 min which corresponds closely to the disappearance of GH in vivo. Following the cessation of SS infusion, GH immediately rebounds.

The balance of SS and GRF necessary to ultimately control GH release is still not well understood, however, several tenable hypotheses have been presented. The administration of monoclonal antibodies against rGRF completely abolished the pulsatile release of GH. In a subsequent experiment, the administration of GRF-44 or 40 to rats resulted in inconsistant responses in GH secretion. However, pretreatment of rats with anti-rat somatostatin antiserum followed by administration of GRF-44 and 40 consistantly stimulated GH secretion. From this series of experiments it was concluded that GRF is the primary effector of GH release, however, somatostatin in part determines the responsiveness of somatotropes to GRF (Wehrenberg, Ling, Bohlen, Esch, Brazeau & Guillemin, 1982). Evidence for control of GH release by SS has also been demonstrated from experiments examining the effects of passive immunization against SS on GH release (Spencer &

Garssen, 1983, Spencer, 1986). Administration of anti-somatostatin antisera in several species tends to increase GH concentrations and enhance growth rate. The specific effects on SS are not known, as SS levels were not estimated in the experiments.

Martin (1979) has described a mechanism whereby SS is released from nerve terminals, the cell bodies of which arise from the medial preoptic area (MPOA) of the hypothalamus. Therefore, if neural interconnections exist between the MPOA and the VMH, it is plausible that SS acts as a neurotransmitter to regulate the secretion of GRF at the level of the hypothalamus in addition to its pituitary effects. It has been suggested that hypothalamic SS secretion is controlled by preoptic and adenohypopyseal pathways that serve to regulate SS-containing neurons in the hypophysiotropic area (Reichlin, Saperstein, Jackson, Boyd & Patel, 1976) More recently, Baile (1983) has also suggested that the effects of GRF are primarily inhibited by SS Cronin, Rogol & Thorner (1982) have reported that in vitro, hpGRF increases intracellular cAMP in somatotropes and SS attenuates this rise, thus providing a plausible mechanism of GRF and SS-mediated pulsatile GH release In addition, the GH-releasing activity of GRF by this group was observed to be strongly potentiated by pretreatment of cells with glucocorticoids and the thyroid hormones Tannenbaum (1984) has also described a possible mechanism whereby GRF and SS interact to effect GH release, utilizing a passive

immunization technique with somatostatin antiserum and administration of hpGRF into the cerebral ventricles. The hpGRF produced a dose dependent inhibition of GH. Immunoneutralization with SS antiserum failed to restore the amplitude of GH surges over suppressed GH profiles. It was concluded that a GRF-mediated release of somatostatin was therefore not the mechanism whereby hpGRF mediated the the suppression of GH secretion. Rather, it was suggested that hypothalamic GRF inhibits its own neurosecretion through an ultrashort loop negative feedback mechanism which removes GRF induced stimulaton of GH release, or by the release by GRF of another yet to be recognized substance. Renaud (1967) has previously described a mechanism of recurrent inhibition whereby axon collateral terminals of peptidergic neurons terminate directly or indirectly on their cell origin to inhibit firing. The mechanism was previously proposed by Motta, Franschini & Martin (1969) for the pathway of ultra-short loop feedback of GRF and recently for the release of somatostatin (Limpkin & Negro-Vilar, 1981) This mechanism provides a possible explanation of the phasic nature of GH release in which bursts of GH are evident at distinct intervals Fukata, Kasting & Martin (1985) utilized a push-pull perfusion technique to investigate the role of SS in the suppression of GH secretion Injection of GH into the median eminence decreased GH levels while exerting no effect on SS These authors concluded that SS release was of minor importance in

GH release and suggested that GRF was the major modulator of GH secretion.

Berelowitz, Szabo, Frohman, Firestone, Chu & Hintz (1981) have examined the effect of somatomedin C (SMC) on GH release Using an in vitro technique, it was reported that SMC stimulated a 390% increase in release of SS over basal levels within 20 min of its addition to the incubation medium. After 24 h of incubation, SMC had produced 90% inhibition of the release of GH from adenohypophyseal cells. These results suggest that SMC maybe involved in the GH negative feedback loop both at the level of the hypothalamus in the short term, and adenohypophysis in the long-term The delayed response in the adenohypophysis was postulated to be the result of changes associated with cellular metabolism rather than a simple block of activity.

Growth Hormone Receptors

Distribution. The majority of the work published in the past several years is in general agreement that GH exerts much of its growth-promoting effects via the stimulation of release of IGF-1 from the liver and probably other organs.(Daughaday, 1981; Etherton & Kensinger, 1985)

From the initial observations concerning the IGF-1 releasing action of GH, it was postulated that since the liver was the primary site of IGF-1 secretion, receptors for GH were exclusively restricted to this location Since then, however, using radioiodinated GH, the distribution of

GH binding sites have been identified in a number of other tissue , both in vivo and in vitro. Studies of this nature have demonstrated GH binding to adipocytes and cultured preadipocytes (Nixon & Green, 1983), lymphocytes (Lesniak, Gordon, Roth & Gavin, 1974), pancreas, lung, spleen, heart (Cicia-Torres, Turyn & Dellacha, 1983; Kostyo, 1985), in addition to hepatocytes (Messina, Eden & Kostyo, 1985).

Receptor Stucture and Chemistry. The chemical nature of the GH receptor has been studied in a variety of the now recognized tissue binding sites. In hepatocyte, lymphocyte and adipocyte cultures, the native receptor appears to be a glycoprotein complex of 200 to 300 kd (Walker & Friesen, 1979; Donner, 1983; Hughes, Simpson & Friesen, 1983; Carter-Su, Schwartz & Kikuchi, 1984). A smaller, 130 kd structure has also been isolated which may represent a subunit of the GH receptor, but its significance is not yet clear (Donner, 1983; Carter-Su et al., 1984).

Due to the diverse metabolic actions of GH, it has been suggested that a heterogenous population of GH receptors exists rather than a single receptor type (Kostyo, 1985). In many species thus studied, attachment of GH to its receptor results in a diabetogenic state characterized by hyperinsulinemia and hyperglucosemia. In addition, GH produces transient insulin-like effects characterized by hypoglycemia produced by increased cellular glucose uptake and decreased hepatic gluconeogenesis. These effects have been ascribed to intrinsic properties of the GH receptor and

possibly GH itself, rather than artifacts resulting from impurities. This has been demonstrated through the use of recombinantly derived methionyl hGH which exhibits all of the aforementioned characteristics (Kostyo, Gennick & Sauder, 1984).

An alternative suggestion which has been made concerning the multiplicity of action of GH is the GH molecule may possess more than one active site. Structure-function studies of various GH derivatives of hGH and pGH fragments have demonstrated that structural modifications can produce changes in the mode of activity of GH (Goodman & Kostyo, 1981, Gennick, Kostyo, Mills & Eden, 1983), suggesting several active sites per molecule and possibly different receptors specific to each site which ultimately are responsible for the seemingly diverse metabolic actions of GH.

Growth Hormone-Receptor Interaction. As with insulin and many other peptide hormones, the initial step is an interaction with the surface tissue receptor With the advent of techniques to label polypeptide hormones, without loss of activity (Hunter & Greenwood, 1962), it has been possible to examine the binding characteristics of GH The binding of GH to its receptor is both time and temperature dependent with binding equilibrium declining as temperature decreases from 37 to 15 C. Subsequent experiments also demonstrated a temperature dependency for binding and conclusively demonstrated the presence of surface membrane

receptors for GH (Lesniak et al., 1974). It has also been demonstrated that pretreatment of membrane preparations with trypsin results in a significant decline in GH binding capacity, suggesting GH binding is dependent upon protein elements associated with the plasma membrane (Lesniak et al , 1974).

Another interesting feature of GH binding is the length of time necessary for GH occupation of the receptor to elicit its anabolic action. Observations by Isaksson, Reagen & Kostyo (1976) have shown that only a short encounter of GH with its receptor is necessary to produce a sustained metabolic function.

The responsivness of the GH receptor to GH also varies with age. In the rat, GH responsiveness does not occur until approximately 10 d of age after which normal growth and development are totally dependent upon GH (Albertsson-Wikland & Isakksson, 1976). Responsiveness then declines with age. This trend has been associated with changes in the GH receptor. Studies in the rat (Maes, deHertogh, Watrin-Granger & Ketelslegers, 1983) and sheep (Gluckman, Butler & Elliot, 1983) have shown a reduced number of hepatic GH receptors in the fetus and early neonate which increase with age and then decline.

Other physiological manipulations also alter GH responsiveness via receptor related phenomena. Hypophysectomized rats display an increased sensitivity to GH which has been correlated with an increase in receptor

numbers, but no change in receptor affinity (Pickard & Postel-Vinay, 1984).

Binding of GH is also altered following exogenous administration of the hormone. Kostyo (1985) has postulated that under these conditions, the GH receptor down-regulates and suggested this experimentally inducible phenomenon may explain the response of the target cell to the episodic nature of GH secretion. Immediately, following an episode of GH release, the tissue receptor down regulates, making the cell less responsive to further GH stimulation, and then up-regulates during the nadir and prepares the cell for the next GH surge.

Metabolic Actions of Growth Hormone.

The regulation of cellular nutrient supply and utilization by GH has been problematic due to a combination of extreme physiological complexity, experimental variation, lack of suitable measurement techniques, and impurities in the hormone preparations being used. These concerns and questions have been reflected in the GH-related literature which has spanned over 50 years. Taking all of this into consideration, however, there is little doubt that GH is intimately associated with processes related to protein, lipid and carbohydrate metabolism.

Protein Metabolism

The overall actions of GH related to protein metabolism results in a positive nitrogen balance due to a stimulation of protein synthesis in muscle, liver and other organs. Therefore, GH has long been recognized as an anabolic hormone in terms of its effects on protein metabolism.

Early work by Evans & Simpson (1931) demonstrated this property by increasing the bodyweight of rats by the administration of bovine growth hormone (bGH) Simpson (1949) established that the administration of GH to hypophysectomized rats alleviated the depressed growth and associated synthesis of liver protein and RNA. Kostyo & Knobil (1959) and Manchester & Young (1959) observed the in vitro GH-mediated incorporation of amino acids into isolated diaphragm tissue of rats. These effects were argued to be the result of GH stimulating the release of insulin from the pancreas (Young, 1945). In later experiments, GH appeared to modulate rat liver incorporation of amino acids into protein in vitro (Korner, 1961), and also to assist tRNA translation (Korner & Grumbley, 1966, Korner, 1967). Pegg & Korner (1965) reported the RNA-polymerization activity of rat liver nuclei was stimulated in GH treated rats. Jefferson & Korner (1967), infused rat livers in situ with GH and observed an accentuated incorporation of labelled amino acids into protein and precursors into nucleic acids by GH within 30 min of its addition The concentrations of amino acids to effect this enhancement, however, were three

times higher than normal physiological levels The higher requirement suggested that an amino acid had become limiting during the perfusion. In addition, GH also significantly increased the labelling of nucleic acids by tritiated orotic acid in both normal and hypophysectomized rats. This was not thought to be an insulin-mediated effect as Exton, Jefferson, Butcher & Park (1966) had previously indicated that insulin suppressed approximately 50% of net hepatic glucose production. In this experiment, the addition of insulin produced a substantial inhibition of hepatic glucose output, indicating insulin was not mediating protein and nucleic acid anabolic effects, but was rather a consequence of GH action. The proposition that insulin mediated GH effects was based upon the theory of Randle, Garland, Hales, Newsholn (1963) which stated that GH evokes rapid increases in plasma free fatty acids (FFA) which produces their preferential use as opposed to glucose. This situation leads to increased peripheral glucose concentrations and the subsequent secretion of insulin which in turn stimulates the uptake of amino acids.

Kostyo (1968) conducted a series of experiments to determine the mechanism whereby GH stimulates RNA and protein synthesis utilizing the liver perfusion technique of Jefferson & Korner (1967). Using hypophysectomized rats, the addition of GH perfusion medium produced an increase in hepatic RNA synthesis and ribosome activity, whereas no changes were noted in controls. It was concluded that GH

was an anabolic hormone. In these studies, amino acid transport was not stimulated immediately, but lagged 10 to 20 min following GH addition to isolated diaphragm tissue of hypophysectomized rats. Secondly, when protein synthesis was blocked with cyclohexamide or puromycin, the effect on amino acid uptake was inhibited, indicating that GH mediated uptake of amino acids was dependent upon protein synthesis. Talwar, Gupta & Gros (1964) had previously noted the incorporation of radioactive precursors into liver protein was increased by GH administration. This group concluded that the effect of the hormone was associated with the rate of RNA synthesis. In addition to the increased synthetic rate due to enhanced ribosomal attachment to mRNA, Korner (1970) also noted the 40S subunit of the ribosomes in hypophysectomized rats were deficient in their incorporation of amino acids. Complete function was restored with GH. More recently, Albertsson-Wikland, Eden & Isaksson (1980), using a rat model, reported GH stimulated protein synthesis. Chung and coworkers (1985) injecting highly purified porcine growth hormone (pGH) to growing pigs observed an increase in muscle mass which was explained on the basis of a stimulatory action of the hormone on protein synthesis In these experiments plasma blood urea nitrogen (BUN) was also depressed which was postulated be a pGH-mediated decrease in hepatic amino acid oxidation

Growth Hormone and the Somatomedins

Salmon & Daughaday (1957) first demonstrated the in vitro incorporation of radioactive sulfate into chondroitin sulfate of cartilage from hypophysectomized rats was depressed. However, when serum from normal rats was added to the medium or rats were pre-treated with GH, the rate of sulfate incorporation was restored. On the other hand, the direct addition of GH resulted in no stimulatory effect. From these experiments, the existence of serum growth factors was recognized. In 1978 Rinderknecht & Humbel successfully isolated and sequenced what was called insulin like growth factor-1 (IGF-1) from serum (Rinderknecht & Humbel, 1978). Insulin like growth factor-I is a member of a family of peptides which display insulin-like and growth-promoting properties. Differential actions within this family has been recognized, namely multiplication stimulating activity (MSA), insulin like activity (ILA) and sulfation factor activity (SFA). The family is collectively referred to as the somatomedins. The prefix, 'somato' indicates the relationship to somatotropin (GH) and to growth of the soma, while "medin" denotes their action in mediation of the effects of GH (Daughaday, Hall, Raben, Salmon, Van den Brande & Van Wyk, 1972)

With the advent of RIA, it became apparent that there was a substantial amount of ILA that was not due to immunoreactive insulin Froesch et al (1963) designated this unbound insulin like compound, non-suppressible insulin

like activity (NSILA). Two futher subclasses of NSILA are recognized, an acid ethanol soluble form, NSILA-S (Froesch et al , 1967), and an acid stable form, NSILA-P (Poffenbarger, 1975). Furthermore, NSILA-S contains the two biologically active peptides, IGF-1 and IGF-2 (Rinderknect & Humbel, 1976). Associated with NSILA, which became evident upon further purification, were three distinguishable compounds, which differ in their charge and chemical character, somatomedin-A, B and C.

Somatomedin-A (SMA) is a neutral peptide which stimulates the incorporation of sulfate into chick cartilage. Recent evidence has suggested that SMA and IGF-2 are identical (Daughaday, 1982). Somatomedin-B (SMB) is an acidic peptide which has been observed to stimulate the incorpororation of thymidine into DNA glial cells. As of 1978, however, SMB was no longer considered a part of the somatomedin family due to other considerations (Fryklund & Sievertsson, 1978). Somatomedin-C (SMC) is a basic peptide which stimulates both sulfate and thymidine uptake into rat cartilage (Hall, 1975). SMC is nearly identical in structure to IGF-1 (Van Wyk, Svoboda & Underwood, 1980; Klapper, Svoboda & Van Wyk, 1983).

It is remiss to discuss GH without consideration of the somatomedins as it is clear that many of the growth-promoting effects of GH such as amino acid uptake, protein synthesis, RNA and DNA synthesis are heavily associated with the action of the somatomedins (Daughaday, 1972) Many

excellent reviews are available concerning the role of the somatomedins in mediating the effects of GH (Phillip & Vassilopoulou-Sellin, 1980; Daughaday, 1982, Hall & Sara, 1983). Recent attempts have been made concerning the relationship between SMC and different rates of growth and body composition in livestock The results are limited and the accuracy of conclusions based on these experiments are questionable due to experimental design and analytical techniques. Keeping this in mind, however, Lunde-Larssen & Bakke (1975) reported SMC levels to be higher in fast versus slow growing pigs. Conversely, Gahagan, Martin & Leach (1980) were not able to demonstrate a difference in SMC concentrations in older fast and slow growing swine populations. Lunde-Larssen (1977) reported higher SMC levels in fast versus slow growing bulls. Wangsness, Olsen & Martin (1981) found higher SMC levels in fast as compared to slow growing sheep. Chung, Etherton & Wiggins (1985) reported higher SMC concentrations in young swine receiving chronic GH therapy versus control pigs.

There is some indication that not all metabolic actions of GH are due to the direct action of SMC. Isaksson, Jansson & Gause (1982) noted the local administration of GH to the proximal cartilage plate of rat tibia directly stimulated bone growth. Several other related experiments conducted in a number of independent laboratories (Eden, Isaksson & Martin, 1983, Madsen, Friberg, Roos, Eden & Isaksson, 1983) have reported similar direct actions of GH.

It is clear that further elucidation of the direct and indirect actions of GH and the relationship to the somatomedins will remain an active area of investigation.

Carbohydrate and Lipid Metabolism

It has been established that GH plays a role in the regulation of carbohydrate and lipid metabolism. As with its other metabolic effects, its modulation of carbohydrate and lipid utilization are complex and demonstrate considerable diversity in action. Debodo and Altzuler (1957) observed GH to both increase and decrease glucose concentrations in the blood. Similarly, Swiskocki & Szego (1965) noted a dual role of GH in both increasing and decreasing plasma free fatty acid (FFA), while Goodman (1965) reported GH both stimulated and inhibited the synthesis of long chain fatty acids in adipose tissue.

These seemingly opposing effects of the same hormone have been suggested to be the result of GH possessing both early and late effect (Goodman, 1968). In general, the early effects of GH are described as insulin-like and favor glucose and fat storage. The time frame of these effects is generally within an hour. Other factors can, however, enhance and lengthen the duration of these early effects For example, Goodman (1965) has reported high carbohydrate containing diets, as well as hypophysectomy can lengthen the duration of the early effects The late effects of GH which are more commonly recognized as the normal or true

physiological action of the hormone are anti-insulin like in that the utilization of glucose is reduced at the expense of mobilizing stored fat for energy. Concomitantly with increased lipid mobilization, fatty acid synthesis is depressed (Goodman, 1963; Goodman, 1965, Goodman, 1966) These effects generally occur at least 2 h following the administration of GH in vivo or in vitro. Experiments elucidating the long and short term effects of GH by Goodman (1968) demonstrated the acute effects of GH on glucose metabolism were the result of GH producing a transient increased permeability to glucose. It was further concluded that this insulin-like effect lasted approximately 2 h and was reversed by cellular processes that were dependent upon RNA and protein synthesis. An examination of the late effects of GH were also conducted to assess the extent and mechanism whereby the net utilization of glucose and a resultant decrease in fatty acid synthesis and CO_2 production occurred. It was found that the inhibitory effect of a single injection of GH in adipose tissue excised 3 h post injection from hypophysectomized rats resulted in depressed metabolism of glucose, pyruvate and fructose. The decrease in pyruvate utilization indicated GH exerted its influence on steps other than glucose transport and also late in the metabolic scheme. The enzyme pyruvate decarboxylase was chosen as a probable site of GH inhibition Since pyruvate metabolism is known to be sensitive to available CoA, and GH was proposed to increase

lipolysis and fatty acid oxidation, CoA would become limiting Furthermore, the re-esterification of FFA would tend to drive pyruvate to glycerol thereby decreasing the amount of pyruvate available for decarboxylation. Unfortunately, these experiments failed to demonstrate increaed lipolysis associated with GH treatment. Following these initial endeavors, other investigators have reported inconsistent findings concerning the lipolytic effects of GH. Results of experiments conducted with pituitary intact rats revealed a GH-mediated mobilization of fats from adipose which was reflected in increased plasma levels of non-esterified free fatty acids (Goodman & Knobil, 1961), it was suggested, however, that glucocorticoids played an important role in this lipolytic action. Machlin, Horino, Hertelendy & Kipnis (1968) produced increased FFA concentrations in pGH injected swine which indicated the possibility of a lipolytic action. In 1972, however, using a more purified source of pGH and injecting swine daily for 8 weeks, an examination of several carcass indices showed decreased lipid accretion. This was not attributed to lipolysis, and it was concluded that the purification of the pGH preparation resulted in the loss of this specific intrinsic activity. Chung et al (1985) administering a highly purified source of pGH to growing barrows observed a 50% increase in intramuscular lipid of the longissimus muscle and tended to increase plasma FFA levels but not significantly Bauman, Eisemann & Currie (1982) indicated

that specific GH preparations contain GH variants in addition to the 22 kd form. Upon purification, in vitro effects are lost. Both pGH and bGH following purification failed to stimulate lipid mobilization (Assa & Laron, 1977; Frigeri, 1980). Lewis, Singh, Tutweiler, Siegel & Vanderlaan (1980) has also demonstrated purified sources of GH are devoid of SFA, and the characteristic insulin-antagonistic effect. It is interesting to note, however, that the enzymatic cleavage of some of these preparations has rendered the hormone active and may therefore represent an important requisite in initiating physiological effects. More recently Goodman & Gritching (1983) and Goodman (1984) investigated the possibility that the lipolytic actions of the hormone were attributable to a contaminant of GH preparations. The same hormone preparation used in earlier experiments that was concluded to contain an acidic contaminant (Frigeri, 1980) which, once isolated from the GH preparation, left GH devoid of lipolytic activity. Upon isolation and subsequent tests of lipolytic potential of hGH and this acidic compound from a single hGH preparation revealed both to increase lipolysis in the presence of .1 ug/ml of dexamethazone after 1 h The acidic component was characterized and determined to be a variant of GH which was able to bind the GH receptor and exert lipolytic effects. Therefore the experiments did not provide information contrary to the lipolytic role of GH. The physiological significance, it was concluded that in normal conditions,

there is little physiological demand for lipid mobilization. In fact, in many species, situations requiring lipid mobilization are associated with stress and, under these conditions, GH secretion is depressed. Therefore, it is possible that the physiological role of GH as it relates to lipid metabolism is to maintain the functional integrity of the lipolytic machinery rather that signaling the lipolytic process. Recently, Chung et al. (1985) has hypothesized that GH potentiates the response of the adipose to other agents such as the glucocorticoids and epinephrine, but is not lipolytic in itself.

In summary, since GH seem to effect metabolism at many levels, there would appear to be substantial potential for it use in the livestock industry as a growth promotant to ultimately improve growth performance. There are yet some questions remaining concerning its direct mode of action and also with its indirect action in mediating the release of the somatomedins. More information addressing these questions will have to be made available before this technology can be the most efficiently applied in the livestock industry.

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CHAPTER III

GROWTH HORMONE, INSULIN AND GLUCOSE PLASMA PATTERNS IN GILTS SELECTED FOR RAPID VERSUS SLOW GROWTH RATE

Summary

Fifteen gilts from two lines of swine developed from inter se matings of a composite of purebred Duroc, Landrace, Spot, Hampshire and Yorkshire breeds selected for rapid versus slow growth through five generations during the growing-finishing period were examined for differences in their twelve hour plasma profiles of growth hormone (GH), insulin and glucose Growth hormone patterns in rapid growth line gilts (RGL) was best described by an 8th order polynomial regression equation (P<.05) while the pattern of GH secretion in slow growth line gilts (SGL) was best described by a 5th order polynomial equation. Mean GH concentrations in SGL and RGL gilts were 4.06 and 3 17 ng/ml, respectively (P<.07). Differences in mean GH concentration were paralleled by plasma profile area (PPA), with greater (P= 08) area for SGL than RGL gilts Mean maximum GH concentration and mean peak amplitude also tended to be greater for SGL than RGL gilts (P< 10) Insulin

plasma profiles in RGL and SGL gilts were described by 10th and 5th order polynomial regression equations, respectively ($P<.05$) Insulin concentrations were higher ($P<.0001$) for RGL as compared to SGL gilts, however, no differences were observed in PPA between the two lines. Glucose plasma profiles were different between the two lines and were linear (SGL) and cubic (RGL) with RGL gilts exhibiting higher ($P<.0001$) plasma glucose concentrations and greater PPA ($P<.05$) than SGL gilts. The number of glucose secretory events tended to be higher for RGL than SGL gilts ($P<.10$). These results indicate the suggested diabetogenic effect of GH may be evident in rapidly growing pigs which differ in their secretory patterns of GH but which exhibit lower overall mean GH concentrations.

Introduction

For many years the focus of the livestock industry has been the optimization of genetic potential and environmental factors which impact production and ultimately affect the rate and composition of postweaning growth Through improvements in herd health, reproduction, selection programs, nutrition and environmental physiology, considerable progress in production efficiency has been attained (Harbison, Goll, Parrish Wangard & Kline, 1978) Recent development of techniques with the potential of producing unlimited quantities of specific regulatory proteins has shifted emphasis toward the investigation of

new methods to produce more rapid and physiologically integrated improvements in growth and lean tissue feed conversion.

The endocrine system has been recognized as a key regulator of the growth process, and is therefore an important link between the genetic and ultimate phenotypic expression of economically important traits (Siers & Hazel, 1970, Buhlinger, Wangsness, Martin & Ziegler, 1978, Ohlson, Davis, Ferrell & Jenkins, 1981; Etherton & Kensinger, 1984, Chung, Etherton & Wiggins, 1985). The manipulation of the endocrine system, therefore, may be a viable means whereby more rapid improvements in production efficiency can be achieved. Hence, a clear understanding of the impact of current production practices, such as selection for growth rate, on the endocrine system is requisite to the application of this technology to the livestock industry.

This study was undertaken to more precisely examine how selection for increased growth rate is related to the level and pattern of two growth-related hormones and a metabolic nutrient. The plasma profiles of porcine growth hormone, porcine insulin and glucose in young gilts from two lines selected for rapid versus slow growth were assessed to more clearly define the influence of selection for growth on the endocrine system of the pig.

Materials and Methods

Animals and Management

Two lines of pigs developed from inter se matings of a composite of purebred Duroc, Landrace, Hampshire, Spot, and Yorkshire breeds were selected for five generations during the growing-finishing period for rapid (RGL) versus slow growth (SGL). Litters were kept with their dams until weaning at 8 wks of age and then grouped with genetic contemporaries to yield a density of 16 to 18 pigs per pen per genetic group. Creep rations were formulated to contain 18% crude protein. The growing-finishing rations which were fed beginning at 12 wks of age was formulated to contain 16% and 14% crude protein, respectively.

Data obtained from previous generations of these lines include individual gain, pen feed efficiency as well as feed efficiency and gain of full versus limit fed (90%) pairs, and ultrasonic backfat measurements.

Experimental Method and Procedure

For this experiment, eight gilts from the RGL with an average bodyweight of 52.6 kg and average daily gain of .69 kg/d, and seven gilts from the SGL with an average bodyweight of 51 7 kg and an average daily gain of .56 kg/d were utilized A summary of growth and carcass characteristics of the 1985 October-September farrowing population of barrows and gilts from which pigs were

selected for study is shown in Table 1. Pigs from the RGL grew 25 percent faster, had 6 percent greater backfat and produced carcasses that were 17 percent fatter compared to SGL pigs. Conversely, SGL barrows and gilts produced carcasses that were approximately 11% leaner compared to RGL pigs. Pigs most closely performing at the average of each selected growth line were chosen for the experiment. Characteristics of the sample population are listed in Table 2.

A tygon, microbore tubing catheter (1.27 mm ID x .51 mm OD), coated with 7% TDMAC-heparin[1] was placed intravenously in the proximity of the cranial vena cava of each animal. Ketamine-sulfate anesthesia, using the prescribed dosages for intramuscular use in 50 kg swine was used in this procedure. The catheter was anchored by a suture at the point of exteriorization, and the distal end was brought to a dorsal midpoint on each animal caudal to the ears, fitted with a luer stub adapter (16 guage), capped, housed in a Whirl-Pac bag and securely taped in place. Following catheterization, pigs were allowed to recover for 72 h, to minimize effects of surgical stress on hormonal profiles. During this adaptation period several blood samples were collected through the catheters to insure their patency and to serve as part of the adaptation procedure. For the duration of the adaptation period and experiment, pigs were maintained in individual steel metabolism crates, at an

[1] Polysciences, Inc , Warrington, PA , Ctlg No. 3813

TABLE 1

SUMMARY OF SELECTED GROWTH AND CARCASS CHARACTERISTICS OF BARROWS AND GILTS FROM THE OCTOBER-SEPTEMBER FARROWING

		GROWING PHASE			100 KG		
LINE	N	ADG (kg/d)	F/G[a]	FI[b]	PROBE BF(mm)	%LEAN	%FAT
RGL	285	.740	2.76	3 61	31 44	37.62	19 71
SGL	185	594	2 77	2.05	29.57	41.64	16 81

[a] Feed efficiency, kg feed/kg gain

[b] Feed intake, kg feed/day

TABLE 2

SAMPLE POPULATION CHARACTERISTICS

LINE	n	BW (kg)	ADG (kg/d)	AGF (d)
RGL	8	52.6	69	114
SGL	7	51.7	56	130

average ambient temperature of 25 C. A 14 h light 10 h dark lighting cycle was imposed with an incandescent source to be consistent with the current photoperiod. A total of 2000 g/d of a corn-soybean diet formulated to contain .65 percent lysine was consumed daily (Table 3). Meals were offered from 0600to 0700 h and again at 1700 to 1800 h. Free access to water was provided.

The experiment was designed to examine plasma growth hormone, insulin and glucose plasma profiles, in eight rapid and seven slow growing gilts. At 0600 h on the sampling day, 10 ml blood samples were obtained at 20-min intervals for a 12 h period. Blood samples were collected into pre-chilled (4 C), 12 ml disposable syringes which contained 400 ul of sterile 3% (w/v) sodium citrated saline (.9%, w/v). After inverting the syringes several times, blood was carefully transferred into chilled (4 C), 15 ml glass Pyrex culture tubes which contained 200 ul (5400 KIU) of aprotinin[2], a protease inhibitor. Samples were centrifuged at 3000 x g for 20 minutes at 4 C, the plasma harvested and eight subsamples of each sample was aliquoted into 1.5 ml conical polypropylene tubes and frozen at -20 C until analyses were performed.

Immunoreactive plasma insulin was estimated by a modification of the radioimmunoassay originally described by Soeldner and Sloane (1965) Radioiodinated porcine insulin was 48 to 52% precipitable by a 1 200,000 dilution in assay

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2 Sigma Chemical Co , St. Louis, MO., Ctlg No A6279.

TABLE 3

COMPOSITION OF DIFT

Item	%
Ingredient Composition[a]	
Corn, yellow (IFN 4-02-931)	81 80
Soybean meal (IFN 5-04-604)	15.65
Dicalcium phosphate	1 15
Calcium carbonate	80
Salt	35
Vitamin-trace mineral premix[b]	.25
Chemical Composition[a]	
Calcium	62
Phosphorous	.54
Crude Protein	14 00
Lysine	65

[a] Dry matter basis.

[b] Supplied 4,000,000 IU vitamin A, 3,000,000 IU vitamin D, 4 g riboflavin, 20 g pantothenic acid, 30 g niacin, 800 g of choline chloride, 15 mg vitamin B12, 10,000 IU vitamin E, 2 g menidione, 200 mg iodine, 90 g iron, 20 mg manganese, 10 g copper, 90 g zinc and 100 mg selenium per ton of feed

buffer (Appendix) of guinea pig anti-porcine insulin antiserum[3] used as the primary antibody. Porcine ^{125}I-insulin with a specific activity of 87 uCi/ug was utilized as the radioactive competitor[4]. Each assay tube contained 100 ul of assay buffer, 100 ul sample or standard, 200 ul primary antibody, and 200 ul ^{125}I-porcine insulin adjusted to 10,000 CPM. The reaction mixture was incubated for 18 hours at 4 C. Separation of bound and free ^{125}I-porcine insulin was achieved by the addition of 200 ul of a 1 10 dilution of goat anti-guinea pig IgG antiserum (heavy and light chain specific)[5] followed by a 2 h incubation at room temperature with subsequent centrifugation at 3000 x g at 4 C for 30 min. With the exception of the total counts tube, the supernatant was discarded and the precipitate was counted in a Packard Model 5330 Auto-Gamma Scintillation Spectrometer for 1 min. Standards containing 2.5, 5, 10, 25, 50, 100, high and low concentration internal control samples and 200 uU/ml porcine insulin were assayed in triplicate with each batch of unknowns Unknowns were assayed in duplicate and concentration estimates were calculated using a logit-log transformation. Reassay was employed on all sample replicates with a coefficient of variation greater than 10%

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[3] Generously provided by Dr William Buhi, University of Florida, College of Medicine.

[4] New England Nuclear Products, Boston, MA., Ctlg No. NEX -104.

[5] Cooper Biomedical, Malvern, PA , Ctlg No 0107-0081, Lot 14066

Stepwise dilution of a 50 50 (v/v) pool of charcoal stripped plasma (4 uU/ml) and 200 uU/ml porcine insulin standard and substitution of 50 ul of each standard with 50 ul of charcoal stripped plasma decreased binding in a dose dependent, parallel fashion relative to the standard curve in assay buffer alone (Appendix). Sensitivity of the assay was .75 uU porcine insulin/tube with a mean ED_{50} of 35 uU/ml. Inter-assay and intraassay coefficients of variation were 10.8% (N=11) and 6.1% (N=25), respectively.

Plasma porcine growth hormone (pGH) concentrations were quantified by radioimmunoassay as originally described by Marple & Aberle (1972) with the following modifications. Standards were prepared from dilutions of a pGH[6] stock with assay buffer (.01 M PBS plus 1% bovine serum albumin), to yield concentrations of 1, 2, 4, 6, 8, 10, 15 and 20 ng/ml. The chloramine-T method for the radioiodination of pGH (Greenwood & Hunter, 1963) as modified by Klindt (personal communication) utilized 5 ug pGH, 3 ug chloramine-T, 10 ug sodium metabisulfite and 1 mCi ^{125}I with a reaction time of 60 seconds. The radioiodinated product was chromatographed on AG 1-X8, 200-400 mesh, chloride form anion exchange resin[7], eluted with .05 M phosphate buffer, and 1 ml fractions collected. A titer of 1 40,000 of the primary antibody[8] was used to achieve total binding between 45 and

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[6] USDA pGH B-1 provided by Dr. Doug Bolt, USDA, Beltsville, MD

[7] BIO-RAD Laboratory, Richmond, CA.

[8] Generously provided by Dr Dennis Marple, Auburn University, guinea pig anti pGH serum 202-7

50%. Total binding non-specific binding ratio was 90 1. Goat anti-guinea pig IgG (heavy and light chain specific)[9] was diluted 1 10 and combined with a 6% (w/v) solution of polyethylene glycol (MW~8000 daltons) in assay buffer containing 1% normal guinea pig serum. Intra-assay and inter-assay coefficients of variation were 4.2% (N=25) and 11.2% (N=7) ng/ml, respectively. Dose response curves for dilutions of unstripped porcine plasma (2.0 ng/ml) were parallel to the standard curve between 1 and 25 ng/ml (Appendix). Plasma samples were assayed in duplicate and standards in triplicate for each batch of unknowns. The sensitivity of the assay was .3 ng/tube with an ED_{50} of 11.5 ng/ml

Plasma glucose concentrations were assayed colorimetrically by the glucose oxidase method with the quantity of o-dianiside-H_2O_2 catalyzable by peroxidase indicating the quantity of H_2O_2 formed from the reaction of glucose, H_2O and O_2 catalyzed by glucose oxidase[10].

Statistical Analysis

Data were analyzed as a split plot in time design using least squares analysis of variance procedures. The model for glucose, insulin and GH included the main effects of selection line, replication and the appropriate

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9 Cooper Biomedical, Malvern, PA. Ctlg No 0107-0081, Lot 15687.

10 Sigma Chemical Co., St Louis, MO., Diagnostic Kit No 520

interactions. Main plot effects were tested with pig within replication by time as the appropriate error term Subplot effects including sampling time and interactions including sampling time were tested with the residual.

Polynomial regression equations were fit to growth hormone, glucose and insulin concentrations with time as a continuous independent variable. The overall degree of polynomial selected was at the first point the highest order polynomial equation was significant ($P<.05$)

The area under each plasma profile curve for each pig was calculated by defining and applying to array data the trapezoid summation algorithm·

$$\text{TOTAL AREA} = I*(2*T_0 + 2*T_1 +,...,2*T_{36})*.5$$

$$\text{BASELINE AREA} = [P] * TN$$

$$\text{ADJUSTED AREA} = \text{TOTAL AREA} - \text{BASELINE AREA}$$

where

I = the sampling interval,

T0, T1, ...,T36 = sampling times,

[P] = the mean concentration of each plasma parameter,

NT = the total number of sampling times and

ADJUSTED AREA = area under the profile curve without baseline.

The PULSAR program of Merriam & Watcher (1982) was employed to identify and describe secretory events for GH, insulin and glucose for each pig. Estimated parameters

included overall mean concentration, baseline concentration, peak number, peak amplitude, maximum amplitude, minimum amplitude, inter-peak interval and peak duration. Estimated parameters generated from PULSAR and adjusted area under each profile curve for each plasma parameter were examined using least squares procedures to determine differences between the two selection lines. Linear correlation was used to examine the relationships among plasma parameters within and between GH, insulin and glucose profiles for the two selection lines.

Results

A comparison of PPA between SGL and RGL gilts for GH, insulin and glucose is presented in Table 4. Glucose PPA was greater for RGL compared to SGL gilts (P<.05). Differences in insulin concentration tended to be higher for the RGL, however, differences between selection lines were non-significant. Conversely, plasma GH PPA was greater for the SGL (P=.08).

Comparisons of glucose plasma parameters calculated from the PULSAR program are presented in Table 5. Overall glucose concentrations, which are the arithmetic average of all temporal concentrations, were higher (P<.05) for RGL than SGL gilts. Likewise, smoothed mean baseline concentrations, the mean of all temporal concentrations not contributing to pulse events, were higher (P<.05) for RGL versus SGL gilts Number of peaks over the 12 h period were

greater (P<.10) for RGL compared to SGL gilts, 8.25 versus 6 50, respectively. There were no differences in maximum, minimum, average peak amplitude, peak duration or interpeak interval for glucose between selection lines. Differences in the overall and smoothed mean concentrations of glucose were associated with higher (P<.0001) overall insulin concentrations in RGL versus SGL gilts (Table 6). However, smoothed baseline insulin concentrations were not significantly different between lines. Maximum insulin levels and mean peak amplitude both tended to be higher for the RGL, but were not significantly different. Likewise, pulse duration and interpeak intervals both tended to be longer in RGL gilts, however, no significant differences were observed.

Comparisons of GH plasma profile parameters are presented in Table 7. Overall mean GH concentrations were higher for the SGL (P=.06) while smoothed mean GH concentrations were not significantly different. The higher overall GH levels were paralleled by higher mean peak amplitude and maximum concentrations (P<.10) for SGL compared to RGL gilts.

Correlations of GH plasma profile parameters with GH, insulin and glucose paramters in RGL gilts are tabulated in Table 8. As anticipated, the number of GH pulses (NGH) was negatively correlated (P<.001) with the average interpeak interval for GH (GHINT). In addition, NGH was positively correlated with the duration of glucose peaks (P<.05) In

TABLE 4

PLASMA PROFILE AREAS FOR GROWTH HORMONE, INSULIN AND GLUCOSE IN RAPID AND SLOW GROWTH LINE GILTS

PLASMA PROFILE	SELECTION LINE	AREA[a]	SEM
Glucose	SGL	62139.8[b]	1053.7
	RGL	66062.8[c]	1178.1
Insulin	SGL	17829.2	526.9
	RGL	18333.2	471.3
Growth Hormone	SGL	2506.2[d]	104.6
	RGL	2219.7[e]	93.6

[a] Area = Total Area - Baseline Area.

[bc] Means are different ($P<.05$).

[de] Means are different ($P=.08$).

TABLE 5

GLUCOSE PLASMA PROFILE PARAMETERS IN RAPID AND SLOW GROWTH LINE GILTS

	PLASMA GLUCOSE[a]	
	SGL	RGL
Overall [Glucose] (mg/dl)	84.97 (1.42)[b]	90.50 (1.59)[c]
Smoothed [Glucose] (mg/dl)	80.41 (1.53)[b]	86.52 (1.70)[c]
Maximum [Glucose] (mg/dl)	110.40 (4.22)	100.20 (3 77)
Minimum [Glucose] (mg/dl)	72.12 (3.79)	67.82 (3.39)
Number of Peaks	6 50 (67)[d]	8.25 (.50)[e]
Peak Amplitude (mg/dl)	14.90 (6 81)	19 45 (6.09)
Peak Duration (min)	68 66 (6.22)	64 12 (5 56)
Inter-Peak Interval (min)	170 69 (10 49)	151.04 (9 39)

[a] Values in parenthesis denote SEM

[bc] Means differ (P< 05)

[de] Means differ (P< 10).

TABLE 6

INSULIN PLASMA PROFILF PARAMFTFRS IN RAPID AND SLOW GROWTH LINF GILTS

	PLASMA INSULIN[a]			
	SGL		RGL	
Overall [Insulin] (uU/ml)	22.27	(.72)[b]	25.76	(.64)[c]
Smoothed [Insulin] (uU/ml)	21.55	(89)	21.51	(79)
Maximum [Insulin] (uU/ml)	39.89	(2.44)	42.86	(2.19)
Minimum [Insulin] (uU/ml)	15.04	(.93)	15.18	(.83)
Number of Peaks	6.75	(.55)	6.62	(.49)
Peak Amplitude (uU/ml)	9.57	(1.22)	12 39	(1.09)
Peak Duration (min)	55.76	(7.64)	63.57	(6 83)
Inter-Peak Interval (min)	185.31	(25.40)	195 15	(22.72)

[a] Values in parenthesis denote SFM.

[bc] Means differ (P<.0001)

TABLE 7

GH PLASMA PROFILE PARAMETERS IN RAPID AND SLOW GROWTH LINE GILTS

	PLASMA GH[a]			
	SGL		RGL	
Overall [GH] (ng/ml)	4.06	(.14)[b]	3.17	(.13)[c]
Smoothed [GH] (ng/ml)	2.56	(.23)	2.32	(.20)
Maximum [GH] (ng/ml)	11.13	(.98)[d]	6.88	(.74)[e]
Minimum [GH] (ng/ml)	1.43	(.05)	1.37	(.04)
Number of Peaks	6.50	(.83)	6.88	(.74)
Peak Amplitude (ng/ml)	3.60	(.39)[d]	2.60	(.34)[e]
Peak Duration (min)	62.27	(6.71)	56.36	(6.00)
Inter-Peak Interval (min)	160.89	(20.56)	171.35	(18.34)

[a] Values in parenthesis denote SEM.

[bc] Means differ (P=.06).

[de] Means differ (P<.10)

TABLE 8

CORRELATIONS OF GH PLASMA PROFILE PARAMETERS WITH GH, INSULIN AND GLUCOSE PARAMETERS[a] IN RGL GILTS

	NGH	CGH	GHINT	GHDUR	NIN	CIN	ININT	INDUR	NGL	CGL	GLINT	GLDUR
NGH		-.29	- 91[b]	33	.60	-.14	-.25	.21	-.45	-.25	.54	.80[c]
CGH			45	02	-.43	10	.45	.24	.41	-.14	-.35	.02
GHINT				29	.28	-.05	.28	-.19	.39	0	-.62	-.62
GHDUR					.43	-.12	-.45	-.29	.07	-.40	.29	.41

[a] NGH, No GH peaks, CGH, [GH], GHINT, GH peak interval, GHDUR, GH peak duration; NIN, No insulin peaks, CIN,[insulin]; ININT, insulin peak interval; INDUR, insulin peak duration; NGL, No. glucose peaks, CGL, [glucose]; GLINT, glucose peak interval, GLDUR, glucose peak duration

[b] P< 001.

[c] P< 05.

SGL gilts (Table 9), frequent GH pulsing was negatively associated with GH peak interval (P<.05), while brief GH pulses (GHDUR) were associated (P<.05) with short GHINT.

Correlations of insulin with GH, glucose and insulin plasma parameters in RGL gilts (Table 10) indicated the number of insulin peaks (NIN) was negatively associated with the length of the interpeak interval (P<.01), and negatively associated with the number of glucose peaks (P<.05). However, NIN was positively correlated with the glucose peak interval (P<.01). In relation to glucose, as NGL decreased, the NIN increased (P<.01), and longer glucose interpeak intervals were positively associated with a greater number of insulin pulses (P<.05). Conversely, an increased frequency of glucose pulsing was positively associated (P<.01) with amplitude of insulin pulses (CIN). A longer insulin interpeak interval (ININT) was associated with insulin pulses of longer duration (P<.05). In the SGL (Table 11), only the insulin pulse amplitude (CIN) was associated (r=.79) with a greater insulin peak duration of (P<.05)

In addition to those relationships of glucose with insulin, GH and glucose plasma events previously described in the RGL, a negative association between the number of glucose pulses (NGL) and the glucose interpeak interval (P<.01) was evident, such that as the number of glucose pulses increased, the shorter the interval between peaks (Table 12). In the SGL (Table 13), the number of

TABLE 9

CORRELATIONS OF GH PLASMA PROFILE PARAMETERS WITH GH, INSULIN AND GLUCOSE PARAMETERS[a] IN SGL GILTS

	NGH	CGH	GHINT	GHDUR	NIN	CIN	ININT	INDUR	NGL	CGL	GLINT	GLDUR
NGH		0	-.79[b]	-.56	56	-.52	-.63	-.04	-.49	.22	.30	.19
CGH			.16	07	-.72	.32	.28	-.71	06	.69	-.32	07
GHINT				.77[b]	.69	.50	.29	.11	-.11	-.32	-.34	-.31
GHDUR					-.59	.18	-.14	-.04	-.21	- 46	-.68	.71

[a] NGH, No GH peaks, CGH, [GH]; GHINT, GH peak interval, GHDUR, GH peak duration; NIN, No insulin peaks; CIN,[insulin]; ININT, insulin peak interval, INDUR, insulin peak duration, NGL, No glucose peaks, CGL, [glucose]; GLINT, glucose peak interval, GLDUR, glucose peak duration.

[b] P< 05

TABLE 10

CORRELATIONS OF INSULIN PLASMA PROFILE PARAMETERS WITH INSULIN, GH AND GLUCOSE PARAMETERS[a] IN RGL GILTS

	NIN	CIN	ININT	INDUR	NGH	CGH	GHINT	GHDUR	NGL	CGL	GLINT	GLDUR
NIN		-.64	-.79[b]	.69[c]	.60	-.43	-.64	.43	-.72[b]	.09	.86[c]	.17
CIN			-.05	.14	-.15	.10	-.05	-.12	.69[b]	.14	-.48	.14
ININT				.73[c]	-.25	.48	.29	-.45	.59	.17	-.52	.11
INDUR					.22	.24	-.19	.38	.23	-.02	-.29	.38

[a] NIN, No of insulin peaks; CIN, [insulin]; ININT, insulin peak interval; INDUR, insulin peak duration; NGH, No. of GH peaks; CGH, [GH]; GHINT, GH peak interval GHDUR, GH peak duration; NGL, no. glucose peaks; CGL, [glucose]; GLINT, glucose peak interval; GLDUR, glucose peak duration.

[b] P<.01

[c] P<.05.

TABLE 11

CORRELATIONS OF INSULIN PLASMA PROFILE PARAMETERS WITH INSULIN, GH AND GLUCOSE PARAMETERS[a] IN SGL GILTS

	NIN	CIN	ININT	INDUR	NGH	CGH	GHINT	GHDUR	NGL	CGL	GLINT	GLDUR
NIN		-.63	-.45	.63	.56	-.72	-.69	-.59	-.16	-.23	.58	.13
CIN			.79[b]	-.63	-.52	.32	.50	.18	.19	.43	.18	.18
ININT				-.32	-.63	.29	.29	-.14	.67	.50	.21	.57
INDUR					-.04	-.71	.11	-.04	-.36	-.61	.43	-.18

[a] NIN, No of insulin peaks; CIN, [insulin]; ININT, insulin peak interval; INDUR, insulin peak duration; NGH, No. of GH peaks; CGH, [GH]; GHINT, GH peak interval GHDUR, GH peak duration; NGL, no. glucose peaks; CGL, [glucose]; GLINT, glucose peak interval, GLDUR, glucose peak duration.

[b] $P < .05$.

TABLE 12

CORRELATIONS OF GLUCOSE PLASMA PROFILE PARAMETERS WITH GLUCOSE, INSULIN AND GH PARAMETERS[a] IN RGL GILTS

	NGL	CGL	GLINT	GLDUR	NIN	CIN	ININT	INDUR	NGH	CGH	GHINT	GHDUR
NGL		-.11	-.76[b]	.07	-.72[c]	.69[c]	.59	.23	-.45	.41	.40	.04
CGL			.48	-.40	.09	.14	.17	.69[c]	-.25	-.14	0	-.21
GLINT				-.62	.86[b]	-.48	-.52	.74[c]	.55	-.36	-.62	.29
GLDUR					.17	.14	.11	.38	.80[b]	.02	-.62	.41

[a] NGL, No of glucose peaks, CGL, [glucose]; GLINT, glucose peak interval; GHDUR, glucose peak duration; NIN, no. insulin peaks; CIN, [insulin]; ININT, insulin peak interval; INDUR, insulin peak duration; NGH, no. GH peaks, CGH, [GH], GHINT, GH peak interval, GHDUR, GH peak duration.

[b] P<.01.

[c] P< 05.

TABLE 13

CORRELATIONS OF GLUCOSE PLASMA PROFILE PARAMETFRS WITH GLUCOSE, INSULIN AND GH PARAMETERS[a] IN SGL GILTS

	NGL	CGL	GLINT	GLDUR	NIN	CIN	ININT	INDUR	NGH	CGH	GHINT	GHDUR
NGL		22	-.17	.17	-.16	.19	.67	-.36	.22	.68	-.32	-.46
CGL			.32	.61	-.23	.43	.50	-.60	.22	.68	-.32	-.46
GLINT				.75[b]	.58	.18	.21	.43	.30	-.32	-.34	-.69
GLDUR					.13	.54	.57	.43	.19	.07	-.31	-.71

[a] NGL, No of glucose peaks; CGL, [glucose]; GLINT, glucose peak interval; GHDUR, glucose peak duration, NIN, no. insulin peaks; CIN, [insulin]; ININT, insulin peak interval, INDUR, insulin peak duration; NGH, no. GH peaks; CGH, [GH], GHINT, GH peak interval, GHDUR, GH peak duration

[b] P< 05.

significant correlations were not as numerous compared to the RGL, with only longer glucose pulse duration (GLDUR) correlated with a longer glucose interpeak interval (P<.05).

In order to determine if time of day at which GH, insulin and glucose plasma events occurred was different between selection lines, comparisons of the number of events of each parameter in the AM (0600 to 1200), and PM (1220 to 1800) were performed (Table 14). The number of secretory events between selection lines were not different for GH or insulin, however, RGL gilts displayed a greater number of glucose pulses in the AM compared to SGL gilts (P<.10). For both selection groups the number of pulse events tended to be greater in the AM versus the PM.

Polynomial regression analysis revealed differences in the shape of the GH, glucose and insulin plasma profiles in RGL versus SGL gilts. The relationship of plasma glucose concentrations to time over the 12 h period was best described by a 3rd order polynomial regression equation, while the response was different in the SGL, being described by a linear equation (P<.05). The relationship of plasma insulin concentrations to time were more complex for both lines with insulin plasma responses in RGL and SGL gilts best described by 10th and 5th order polynomial regression equations, respectively (P<.05) Similar to the insulin response, the relationship of GH in the RGL was complex, and was best described by an 8th order polynomial regression equation The regressed pattern of GH in the RGL was

TABLE 14

COMPARISON OF THE NUMBER OF GH, INSULIN AND GLUCOSE EVENTS[a] IN THE AM AND PM BETWEEN RGL AND SGL GILTS

PARAMETER	AM		PM	
	RGL	SGL	RGL	SGL
GH	3.75	3.62	3.25	2.92
INSULIN	3.75	3.79	3.00	2.20
GLUCOSE	4.50[b]	3.17[c]	3.63	3.25

[a] Peaking events detected by PULSAR

[b] Means differ (P< 10).

characterized by well-defined nadir and peaks, with an even interpeak interval The relationship of GH to time in the SGL demonstrated considerably more scatter (R^2 model=.15) and was best described by a 5th order polynomial equation. The illustration of the relationship between the regressed profiles of insulin and GH in RGL and SGL gilts, is shown in Figures 1 and 2, respectively. Comparing the figures, a reciprocal relationship between GH and insulin is evident in both lines, however, the pattern is more dynamic in the RGL than the SGL gilts.

Discussion

The results of this experiment have demonstrated that selection for growth rate results in marked differences in the plasma profile characteristics of plasma GH, insulin and glucose in young, growing swine.

At equal bodyweights, RGL carcasses exhibited more backfat and greater lipid to lean ratio than the SGL. These carcass characteristics are consistent with reports of swine populations in which selection for growth rate was imposed (Buhlinger et al., 1978).

In this study overall plasma GH concentrations were slightly lower in RGL gilts which is consistent with observations from studies examining GH levels in more extreme populations of lean and obese pigs, such as the Yorkshire versus the Ossabaw breed, respectively (Wangsness, Martin & Gatchel, 1980). In these and other similar

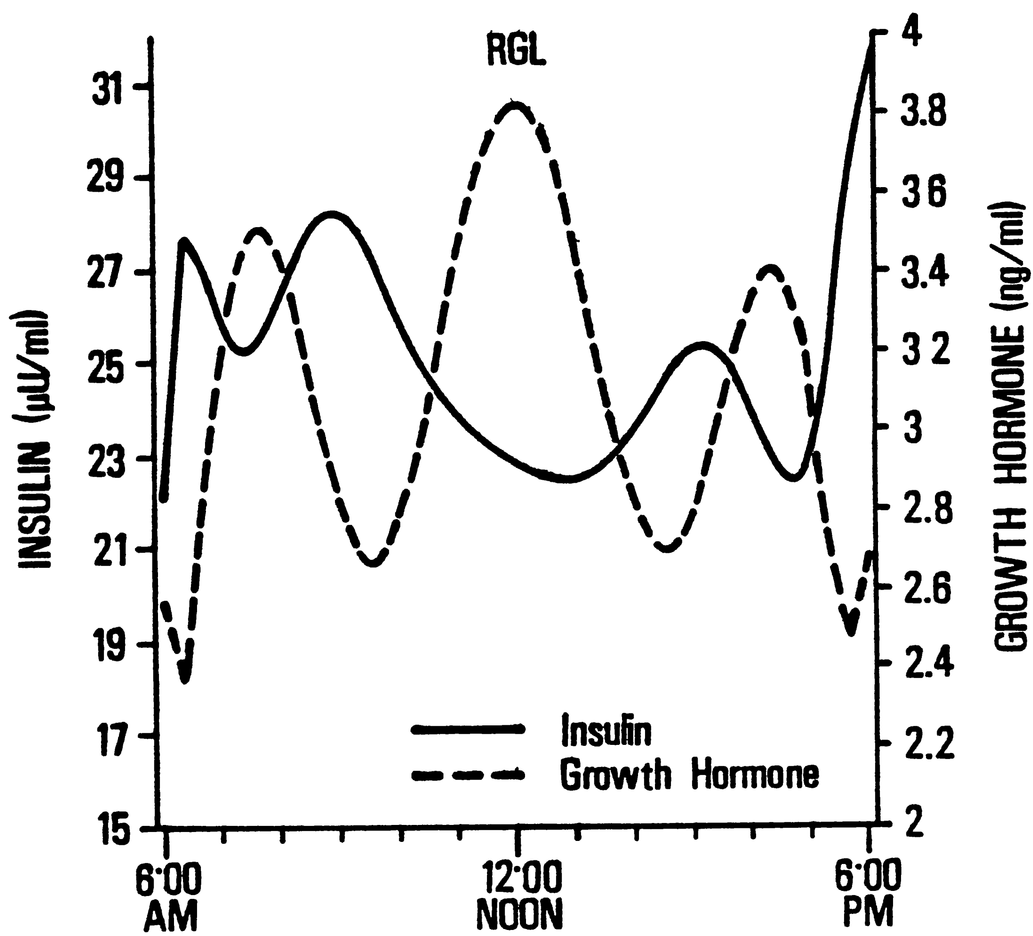

Figure 1. Illustration of the reciprocal relationship of regressed GH and insulin profiles in RGL gilts

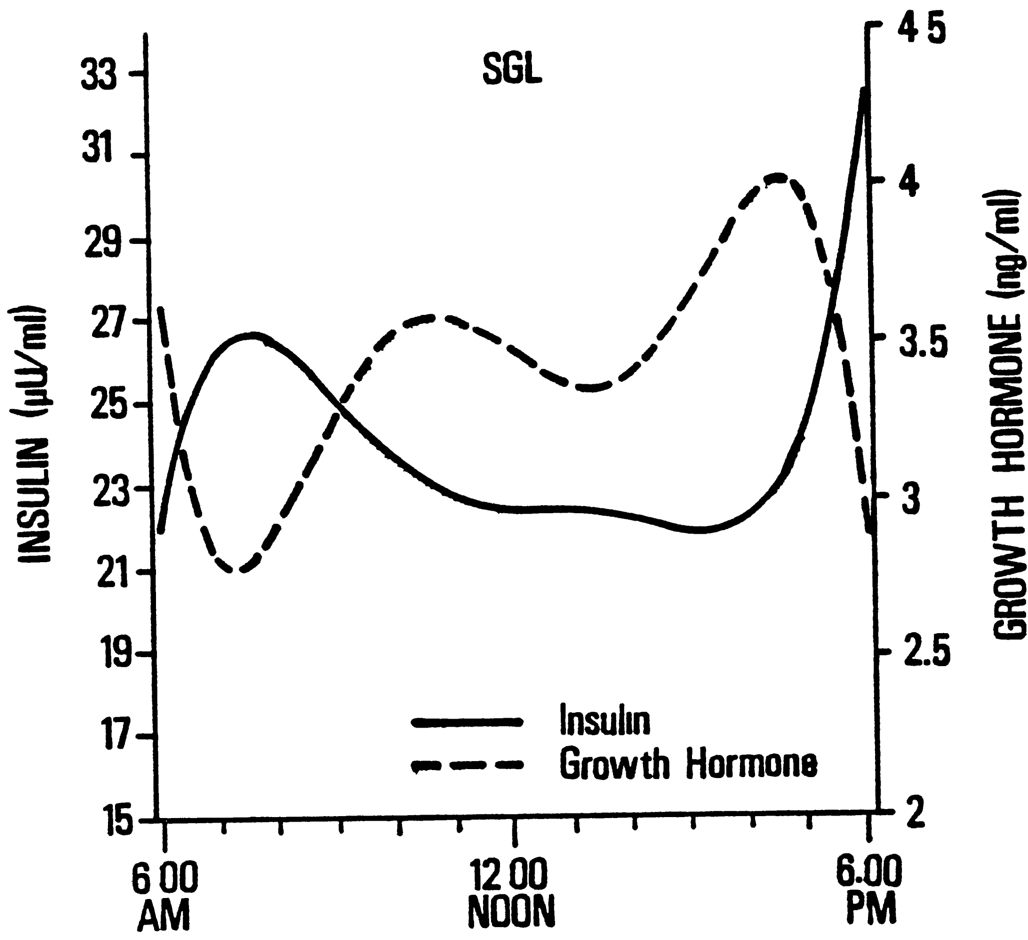

Figure 2. Illustration of the reciprocal relationship of regressed GH and insulin profiles in SGL gilts

experiments conducted with rats (Martin & Gahagan, 1977), and humans (Copinschi, Wegienka, Hane & Forsham, 1967), lower circulating GH concentrations have been noted in genetically obese individuals. Therefore, the slightly lower GH levels of the RGL in the present experiment may be partially explained by their relatively greater proportion of adipose to lean tissue. Furthermore, recognizing the possible lipolytic action of GH in adipose tissue, both in vivo (Wagner & Veenhuizen, 1970) and in vitro (Sengupta, Long & Allen, 1981, Goodman & Gritching, 1985), the relatively lower carcass lipid of the SGL versus the RGL may be related to higher levels of circulating GH in the SGL.

Given the stimulatory effect of GH on growth rate, the significantly lower GH concentrations of the RGL is incongruent with their more rapid growth pattern. These observations suggest the possibility that factors other than overall plasma concentrations of somatogenic hormones and nutrients may be biologically important in accurately relating plasma profile characteristics such as these with differential rates of growth. This possibility has been recently suggested based on similar findings by other groups (Klindt, Jenkins & Leymaster 1986, Etherton & Kensinger, 1984, Trenkle & Topel, 1978).

Due to the lipogenic nature of insulin on adipose tissue, it was not surprising that RGL gilts exhibited significantly higher circulating insulin concentrations given their greater percentage of backfat and carcass lipid

These observations are consistent with those of similar studies examining the role of insulin in growth and development. Hoffman, Wangsness, Hagen & Etherton (1983) showed selection for backfat thickness in swine was accompanied by elevated fetal plasma insulin in the high backfat line Conversely, Hetzer & Miller (1973) clearly demonstrated that selection against backfat in swine resulted in decreased adipose tissue lipogenic capacity. In addition, in studies also examining the relation to feed intake in hyperinsulinemic individuals, hyperphagia and excessive body weight gain were also observed (Martin, Sheahan, Ramsay, Gahagan, Campion 1982; Bray & York, 1979; Zucker & Antoniades, 1972). Consistent with these findings, the RGL population from which gilts in this study were selected, exhibited higher feed intake in relation to SGL pigs. Hence, differences in growth rate of the RGL may be partially attributable not only to greater levels of lipid accretion, but also higher levels of feed intake.

Results of previous studies have suggested an exogenous GH-mediated antagonism of insulin action (Chung et al., 1985). There has been much controversy as to whether these diabetogenic, as well as lipolytic actions of GH are mediated by a non-GH contaminant of the hormone preparations (Goodman & Gritching, 1985, Frigeri, 1983, Bauman, Eisemann & Currie, 1982). In this study, although plasma insulin levels were higher in RGL gilts, glucose concentrations were also significantly higher, possibly suggesting a mild tissue

insensitivity to insulin in this line. This effect, however, was apparent with lower overall plasma GH concentrations in the RGL. This apparent inconsistency with previous reports of the suggested diabetogenic effect of GH may indicate that insulin antagonistic effects are displayed by endogenous GH. In addition, lower levels of GH in the RGL may indicate a greater tissue sensitivity to GH, requiring less total GH to elicit insulin antagonistic effects that are evident in the more rapidly growing line. Factors other that simple, independent concentrations of GH and insulin may be of physiological significance in explaining apparent differences in nutrient uptake, utilization and growth. A similar lack of correlation between serum concentrations of hormones and various growth indices have been reported in cattle (Hafs, Purchas & Pearson, 1975) and swine (Siers & Hazel, 1970). In both studies, high serum GH levels were associated with slow growth rate and low ratios of carcass lean to fat. These observations together with the results of this experiment further support the contention that the simple assessment of overall concentrations of metabolic hormones and nutrients such as GH, insulin and glucose ignores the possible physiological importance in the complex integration and dynamics associated with these plasma factors This latter conclusion is even more tenable considering the interrelationships between GH and insulin in RGL and SGL gilts which are apparent from Figs 1 and 2 From these

expressions of the best fit multiple regression equations, the plasma relationship between GH and insulin appear to be strongly related, in a reciprocal fashion in both lines. The notible difference, however, between the reciprocal profiles is a more dynamic relationship which is evident in RGL gilts, thereby suggesting the endocrine system of the RGL may be more responsive to endocrine and metabolic stimuli than in SGL gilts.

A comparison of the results of correlations among the plasma parameters for GH, insulin and glucose provide further support that both the mode of secretion and integration of parameters are important in defining the mechanism(s) whereby differences in growth rate in the two lines are mediated. The greater number of significant correlations among GH, insulin and glucose plasma parameters in RGL gilts may indicate greater metabolic and endocrine efficiency due to a higher level of integration among various growth parameters.

The results of this experiment support the hypothesis that the mode of secretion of somatotropic hormones and the response of metabolic nutrients to changes in hormonal flux govern the rate of growth in young swine selected on the basis of growth. The nature of the interrelationhsip among plasma parameters is complex and highly integrated. Analyzing simple circulating concentrations of metabolic hormones and nutrients without recognizing modes and interrelationships of secretion may not adequately provide

a basis for relating differences in the genetic potential of growth to growth-related factors. It is evident therefore that more integrated and less conventional types of sampling and analyses are warranted to more conclusively relate differences in these and other key growth factors to genetically-induced differences in growth performance.

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CHAPTER IV

PLASMA pGH, INSULIN AND GLUCOSE RESPONSES TO hGRF (1-44-NH_2), ARGININE, GLUCOSE AND SALINE INFUSIONS IN RAPID AND SLOW GROWING GILTS

Summary

Plasma growth hormone (GH), insulin and glucose concentrations were estimated in fifteen Duroc, Hampshire, Landrace, Spot and Yorkshire crossbred gilts selected on the basis of rapid versus slow growth rate. Gilts were challenged with sterile solutions of human growth hormone releasing factor (hGRF), arginine (ARG), glucose (GLU) and saline (SAL), with blood samples collected at -10, 2.5, 5, 10, 30, 60, 120, 240 and 480 min relative to infusion time. Plasma GH secretory profile area (SPA) was greater ($P<.05$) for slow growth line (SGL) versus rapid growth line (RGL) gilts infused with SAL, however, insulin and glucose SPA did not differ as a result of SAL infusion The response of plasma glucose, GH, and insulin, as assessed by SPA, were not different between selection lines following GLU infusion, however, insulin concentrations were higher (P< 05) in RGL as compared to SGL gilts at 2.5 and 5 min Likewise, peak glucose concentrations following GLU challenge were higher

(P< 05) for RGL than for SGL gilts. There were no differences in glucose and insulin clearance rates between lines. A strong, reciprocal relationship between plasma GH and insulin was apparent in the SGL as a result of each infusion, while the same reciprocity was not as clear in RGL gilts. Insulin SPA resulting from ARG infusion was greater for RGL than for SGL gilts (P<.005), however, there were no differences in plasma glucose or GH SPA as a result of ARG challenge. Plasma glucose SPA was greater (P<.05) while insulin and GH SPA did not differ as a result of hGRF infusion. Both ARG and hGRF produced increases in GH in RGL and SGL gilts from 2.5 to 30 min, but the magnitude of response did not differ between lines. These results indicate the existence of differences in response to provocative stimuli which may be reflected in the levels of performance of young swine selected on the basis of growth rate.

Introduction

Rate of growth has been associated with differences in the response of endocrine and metabolic nutrient parameters to a variety of provocative stimuli. Previous experiments have demonstrated depressed GH secretory capacity and tissue insensitivity to insulin in slow-growing, obese versus more rapidly-growing, lean individuals (Copinschi, Wegienka, Hane & Forsham, 1967, Wangsness, Martin & Gahagan, 1977) In pigs, comparisons of hormonal status have been conducted with

populations characterized by extreme differences in lean to fat ratios and rate of gain as a result of selection for backfat and gain. Little information is available, however, examining differences in the endocrine response of less extreme populations which more closely typify the type of pig found in the swine industry. The objectives of the present study were to examine possible differences in the responsiveness of GH, insulin and glucose to provocative stimuli which may exist in young swine which have been selected on the basis of rapid versus slow growth rate.

Materials and Methods

Two lines of pigs developed from inter se matings of a composite of purebred Duroc, Landrace, Hampshire, Spot, and Yorkshire breeds selected for rapid (ADG=.69 kg/d) versus slow growth (ADG=.56 kg/d) for five generations during the growing-finishing period were used as the source of swine for this study. A description of animal management and feeding, sample population performance and carcass characteristics, and the surgical and analytical techniques have been previously described (Norton, 1986). At the time of the experiment, RGL gilts weighed an average of 52.6 kg and were 114 d of age, while SGL gilts weighed 51.7 kg and were 130 d of age

Glucose Infusions, hGRF, and Saline Infusions

Pigs were infused with 1 g/kg $BW^{.75}$ alpha-D glucose[1], .1 ug/kg human growth hormone releasing hormone $(1\text{-}44\text{-}NH_2)$[2], and .9% w/v sterile saline, following a latin square pattern over an 8-d period. A 1 d stabilization period between infusions was allowed. On the day of sampling, one preinfusion sample (-10 min) was collected followed by the respective infusions via intravenous cannula over a 2 min period and were completed at time = 0 min. Each cannula was rinsed with 10 ml of 3% w/v citrated saline to purge the cannulae of any residual infusion solution. Subsequent blood samples were collected at 2 5, 5, 10, 30, 60, 120, 240 and 480 min relative to infusion.

Arginine Infusions

After withdrawing one pre-infusion blood sample at -10 min, .5 g/kg $BW^{.75}$ neutralized arginine hydrochloride[3], was infused over a 1 h period with a double syringe infusion pump. The rate of infusion was .833 ml/min such that the termination of the arginine infusion coincided with that of glucose, hGRF and saline infusions. Since there was only a single catheter in each pig, blood samples could be withdrawn after the 1 h arginine infusion. As with the other challenges, blood samples were collected at 2.5, 5, 10, 130, 60, 120, 240 and 480 min relative to infusion.

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[1] Sigma Chemical Co., St. Louis MO., Ctlg. No G5000
[2] Sigma Chemical Co., St Louis, MO , Ctlg No. G0138
[3] Sigma Chemical Co., St Louis, MO., Ctlg. No A6279.

Calculations and Statistical Analyses

Plasma insulin, glucose and GH responses to each challenge were calculated by two methods. The first involved estimation of area under each response curve using a trapezoid summation algorithm which has been previously described (Norton, 1986). The estimation of secretory area has been employed by others (French, Baum & Porter, 1970, Wangsness et al., 1977; Pellerier, Dubreuil, Couture, Petitclerc, Lapierre, Gaudreau, Brazeau & Morisset, 1986), and is thought to provide a better assessment of overall secretory response than mean peak or overall concentrations This is especially true in cases of less distinct responses, such as those observed in this experiment in response to ARG and hGRF .

A second method was employed to evaluate the response of plasma insulin and glucose to the GLU infusion. This process involved transformation of plasma glucose and insulin concentrations to logarithmic concentrations and subsequently fitting each to a linear regression with time as an independent variable. The slopes of each equation were then subjected to an analysis of variance to determine significant line effects on glucose and insulin clearance rates

The experiment was analyzed as a split plot in time using a least squares analysis of variance procedure Main plot effects included replication, line and their interaction, and were tested with pig within replication by

line Subplot effects included infusion, time and all appropriate interactions and was tested with the residual.

Results

Differences in the temporal secretory levels and profiles of secretion in plasma glucose, insulin and GH in these two lines of gilts has been previously described (Norton, 1986). In the present experiment, the SAL infusion served as a control. As a result of SAL infusion, plasma GH SPA was greater (P<.05) for SGL compared to RGL gilts whereas both insulin and glucose SPA were not different between lines (Table 15).

Glucose Infusion

Plasma glucose response to GLU infusion consistantly peaked in both selection lines at 2.5 min, and was greater (P<.05) for RGL compared to SGL gilts (Figure 3). Glucose clearance rates were not different between lines, while glucose SPA were slightly, but not significantly higher in the RGL (Table 16). Plasma insulin levels peaked by 10 min in both lines with insulin concentrations higher (P<.05) by 2 5 and 5 min for RGL versus SGL gilts (Figure 4). Insulin SPA was slightly greater for RGL gilts, but not significantly different from the SGL (Table 16) Plasma GH concentrations decreased as a result of GLU infusion in both selection lines by 2 5 min, with a greater decline displayed by the SGL, but this was primarily the result of the relatively higher

TABLE 15

SECRETORY PROFILE AREAS (SPA) FOR GH, INSULIN AND GLUCOSE FOLLOWING SALINE[a] INFUSION IN RGL AND SGL GILTS

PLASMA PARAMETER	SELECTION LINE[b]	
	RGL	SGL
GROWTH HORMONE	2068.25[c] (396.28)	3325.47[d] (400.99)
INSULIN	17402.73 (806.16)	16101.31 (800.42)
GLUCOSE	65517 63 (1598.63)	64712.95 (1664.04)

[a] [Salıne] = 9 g/l.

[b] Means - (S.E.M.).

[cd] Areas are dıfferent (P<.05)

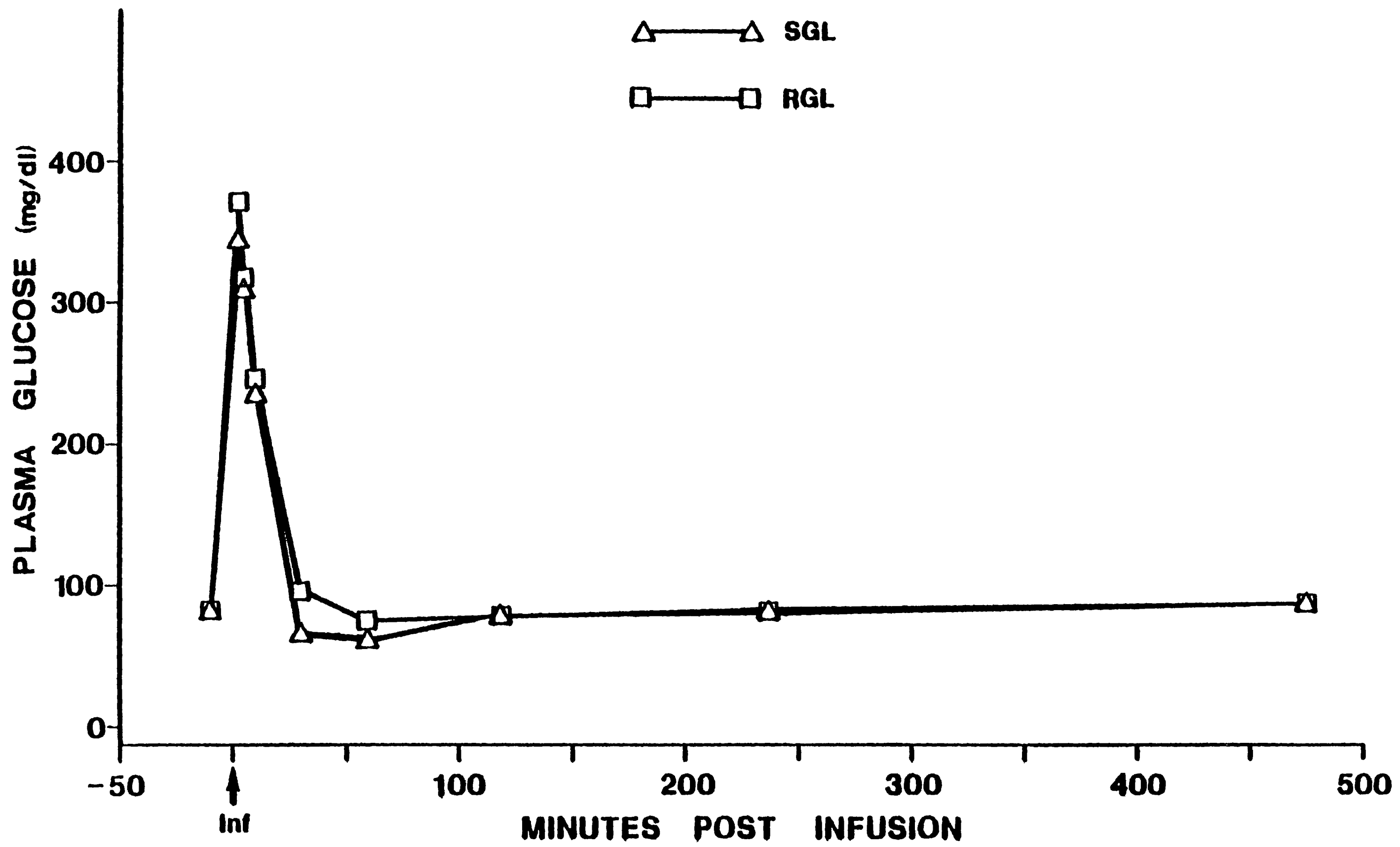

Figure 3 Effect of Intravenous Infusion of 1 g/kg BW 75 Glucose on Plasma Glucose in RGL and SGL Gilts

TABLE 16

SECRETORY PROFILE AREAS (SPA) FOR GH, INSULIN AND GLUCOSE FOLLOWING GLUCOSE CHALLENGE (1 g/kg $BW^{.75}$) IN RGL AND SGL GILTS

PLASMA PARAMETER	SELECTION LINE[a]			
	RGL		SGL	
GROWTH HORMONE	2681.01	(470.16)	3151 47	(507.83)
INSULIN	17755.06	(994.52)	17322.91	(1089.45)
GLUCOSE	69785.10	(2533.79)	67300.36	(2325 17)

[a] Means - (S.E.M.)

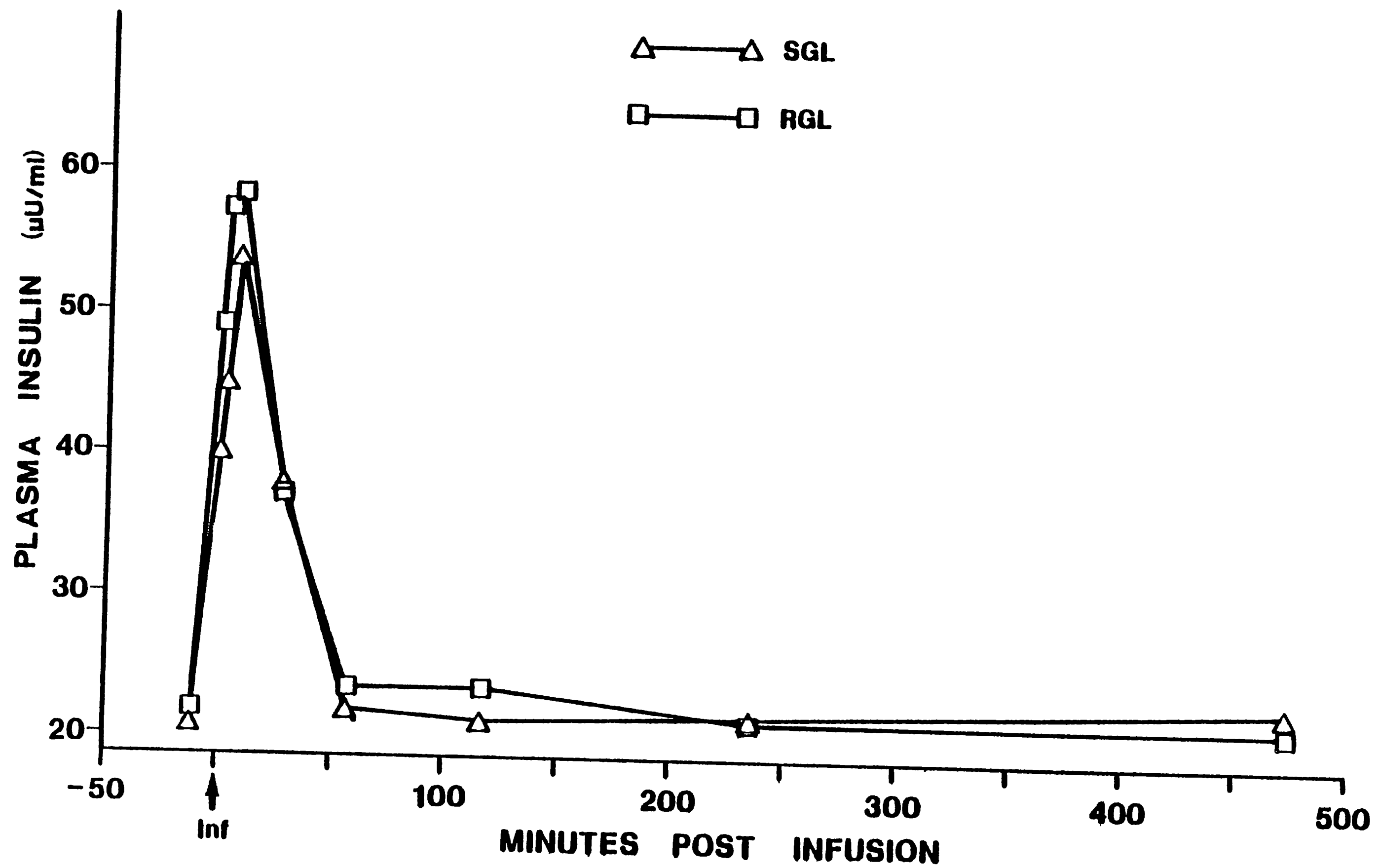

Figure 4 Effect of Intravenous Infusion on 1 g/kg BW^{75} Glucose on Plasma Insulin in RGL and SGL Gilts

initial GH concentration at -10 min. Plasma GH levels rebounded by 10 and 30 min in SGL and RGL gilts, respectively, however, due to large variation, no difference in response between lines was observed Insulin and GH exhibited a reciprocal relationship between -10 and 60 min in both lines with plasma insulin elevated and GH concentrations depressed as a result of GLU infusion (Figures 5 and 6).

<u>Arginine Infusion</u>

The responses to ARG in plasma glucose, glucose and GH are presented in Table 17. Glucose and GH SPA were not different between lines as a result of ARG infusion, however, insulin SPA was greater (P<.005) in RGL gilts (Figure 7). Plasma glucose concentrations peaked in SGL gilts between 2 5 and 10 min while the response was more latent and attenuated in the RGL (figure not shown). Plasma insulin concentrations paralleled and slightly lagged the elevation in glucose in both lines. The response of GH to ARG is presented in Figure 8 A biphasic response between -10 and 60 min was evident in the RGL, while two distinct pulse events were noted in the SGL from -10 to 60 min Peak GH concentrations in SGL gilts tended to be greater, but were not different from that of RGL gilts (6 2 ng/ml versus 5.0 ng/ml, respectively). Following the peak event(s), GH response in both lines paralleled each other from 60 to 480 min. The relationship of response between GH and insulin as a result of ARG infusion are presented in Figures 9 and 10. Insulin and GH demonstrated a

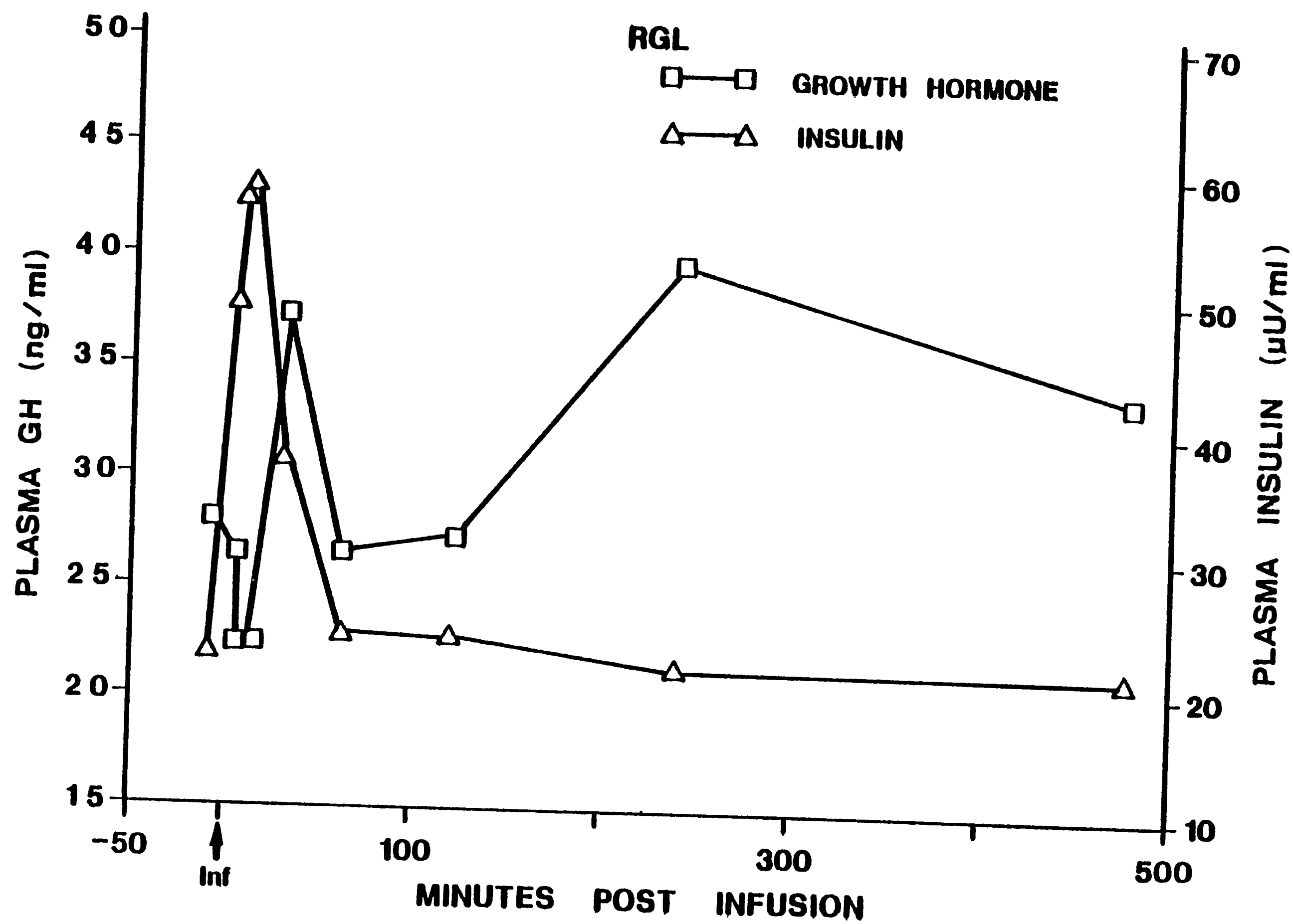

Figure 5 Response of Plasma GH and Insulin in RGL Gilts Following Glucose (1 g/kg BW $^{.75}$) Challenge

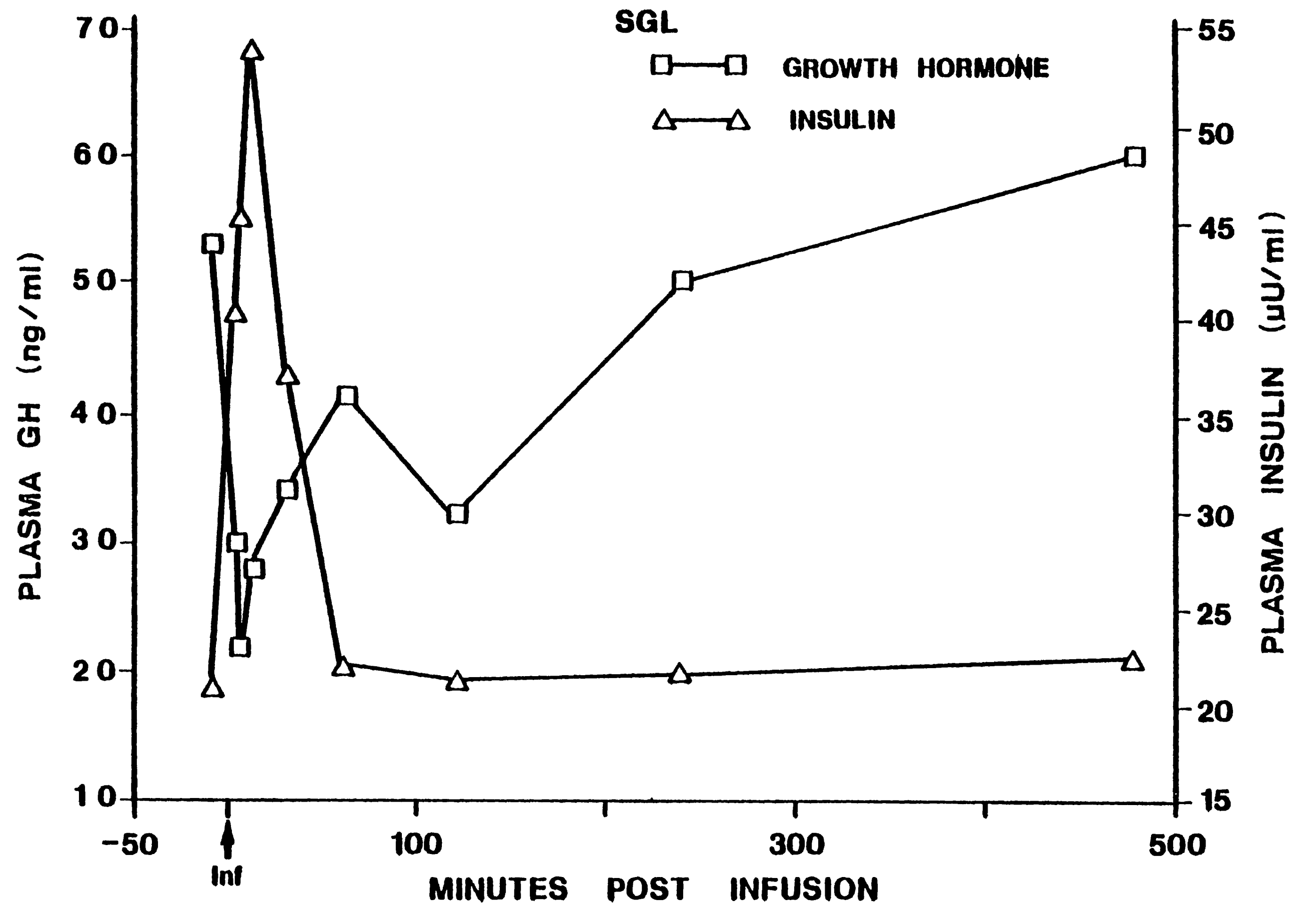

Figure 6 Response of Plasma GH and Insulin in SGL Gilts Following Glucose (1 g/kg ·75) Challenge

TABLE 17

SECRETORY PROFILE AREAS (SPA) FOR GH, INSULIN AND GLUCOSE FOLLOWING ARGININF CHALLENGE (.5 g/kg $BW^{.75}$) IN RGL AND SGL GILTS

PLASMA PARAMETER	SELECTION LINE[a]	
	RGL	SGL
GROWTH HORMONE	2620.89 (303.25)	2679.92 (262.62)
INSULIN	19128.11[b] (493.67)	15865.82[c] (567.14)
GLUCOSE	63262.76 (1039.04)	62135.79 (1229.41)

[a] Means - (S.E.M.)

[bc] Areas are different (P<.005).

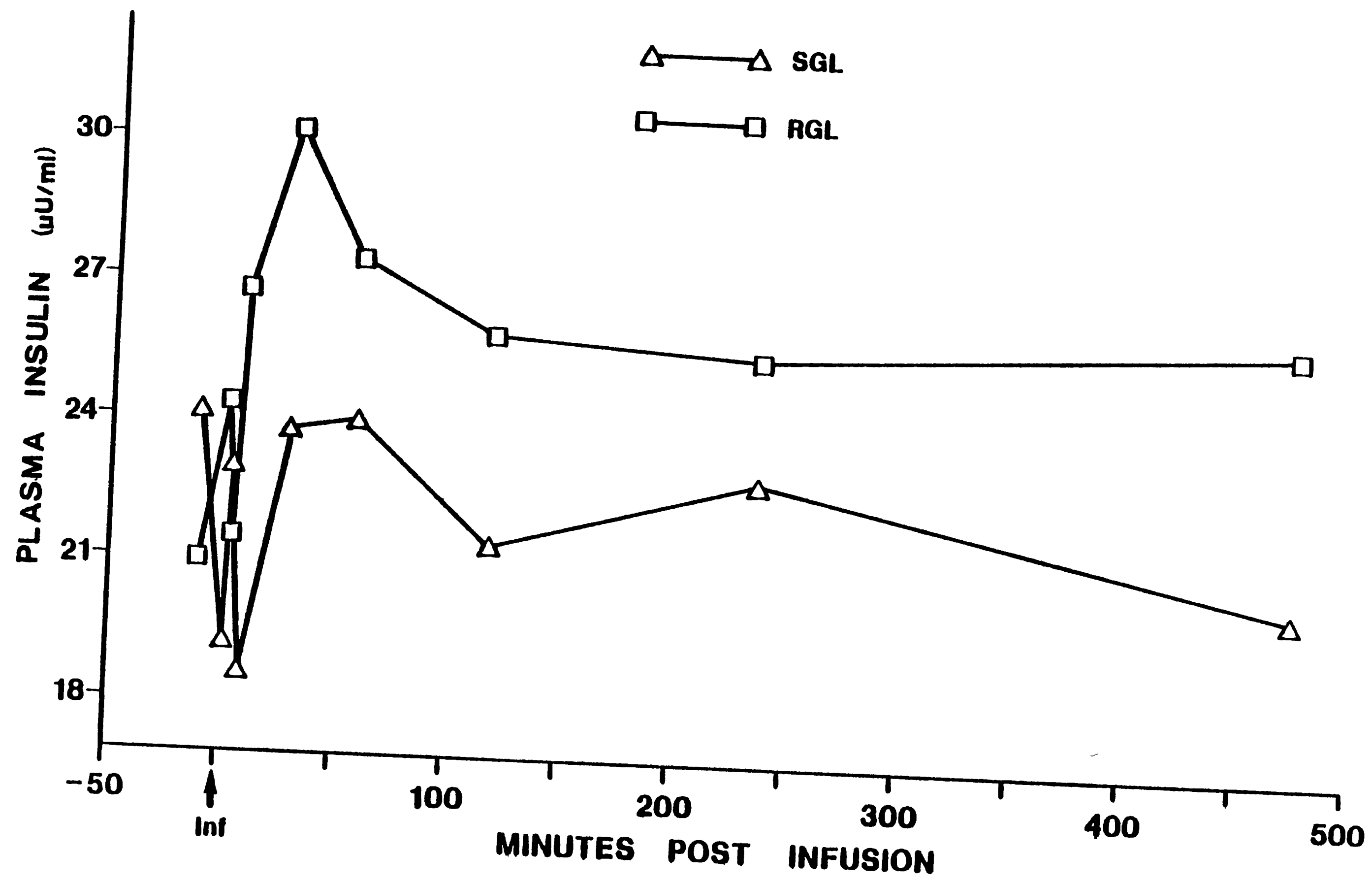

Figure 7 Effect of Intravenous Infusion of 5 g/kg BW ^{75} Arginine Hydrochloride on Plasma Insulin in RGL and SGL Gilts

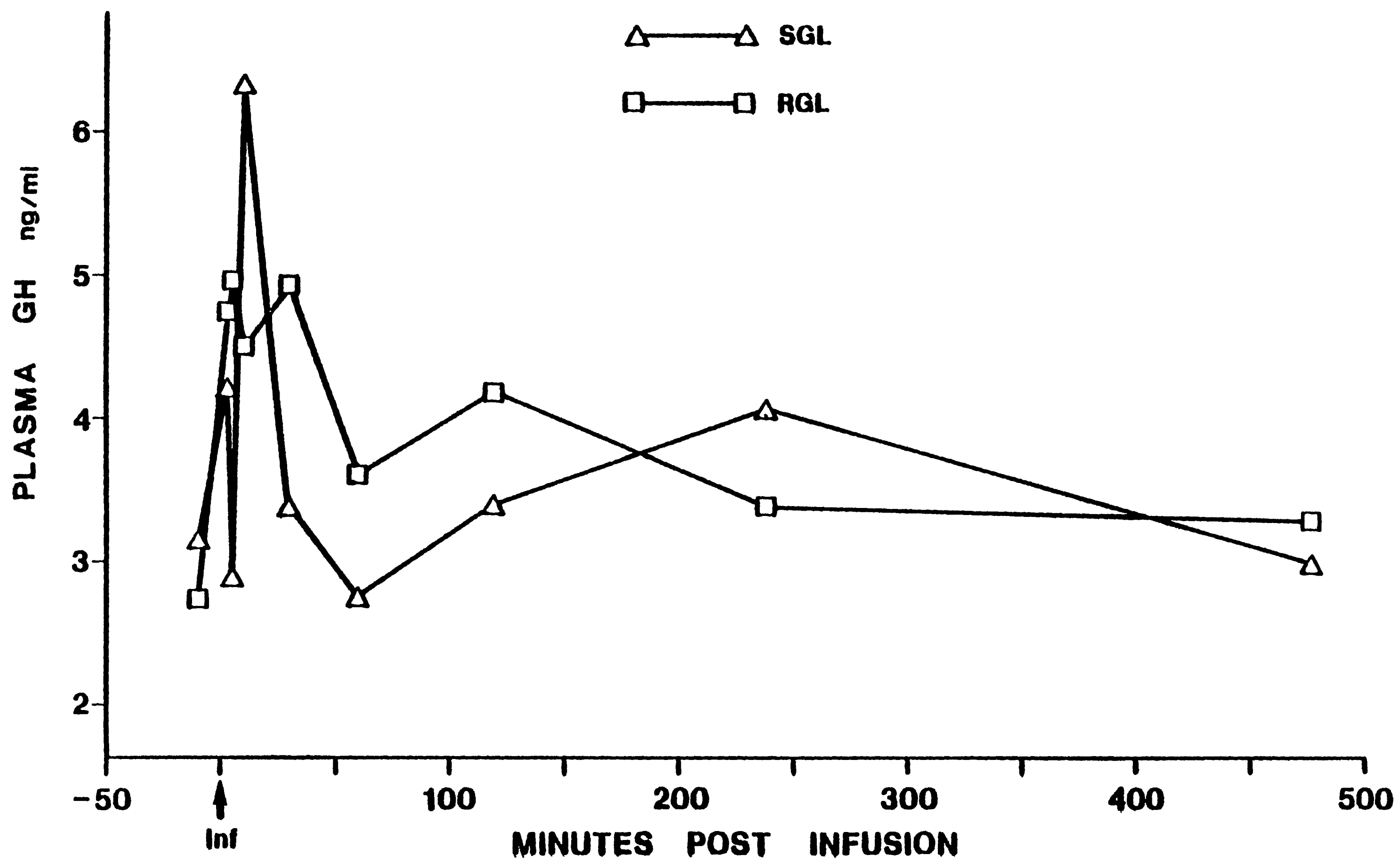

Figure 8 Effect of Intravenous Infusion of .5 g/kg BW ^{75} Arginine Hydrochloride on Plasma GH in RGL and SGL Gilts

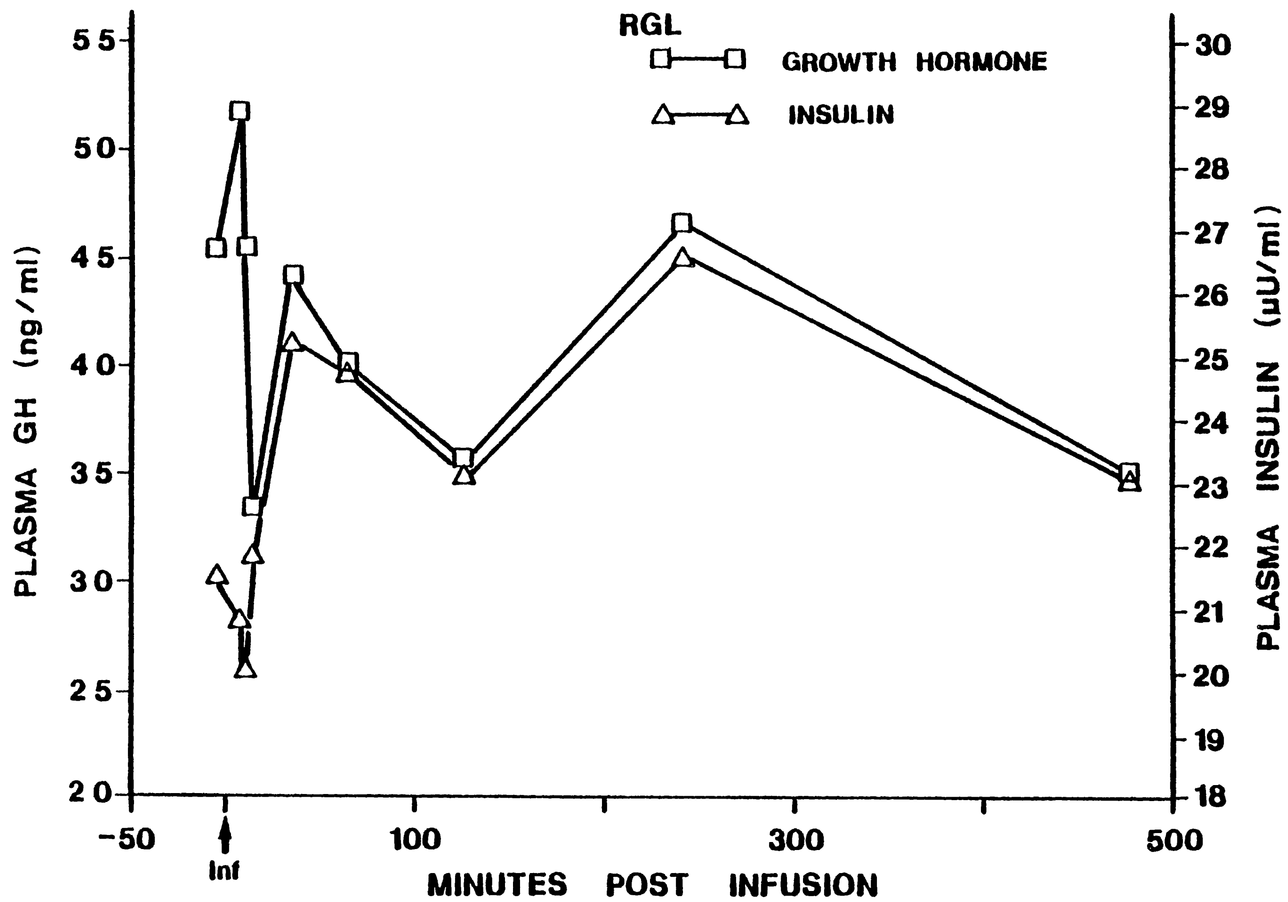

Figure 9. Response of Plasma GH and Insulin in RGL Gilts Following Arginine Hydrochloride (.5 g/kg BW$^{.75}$) Challenge

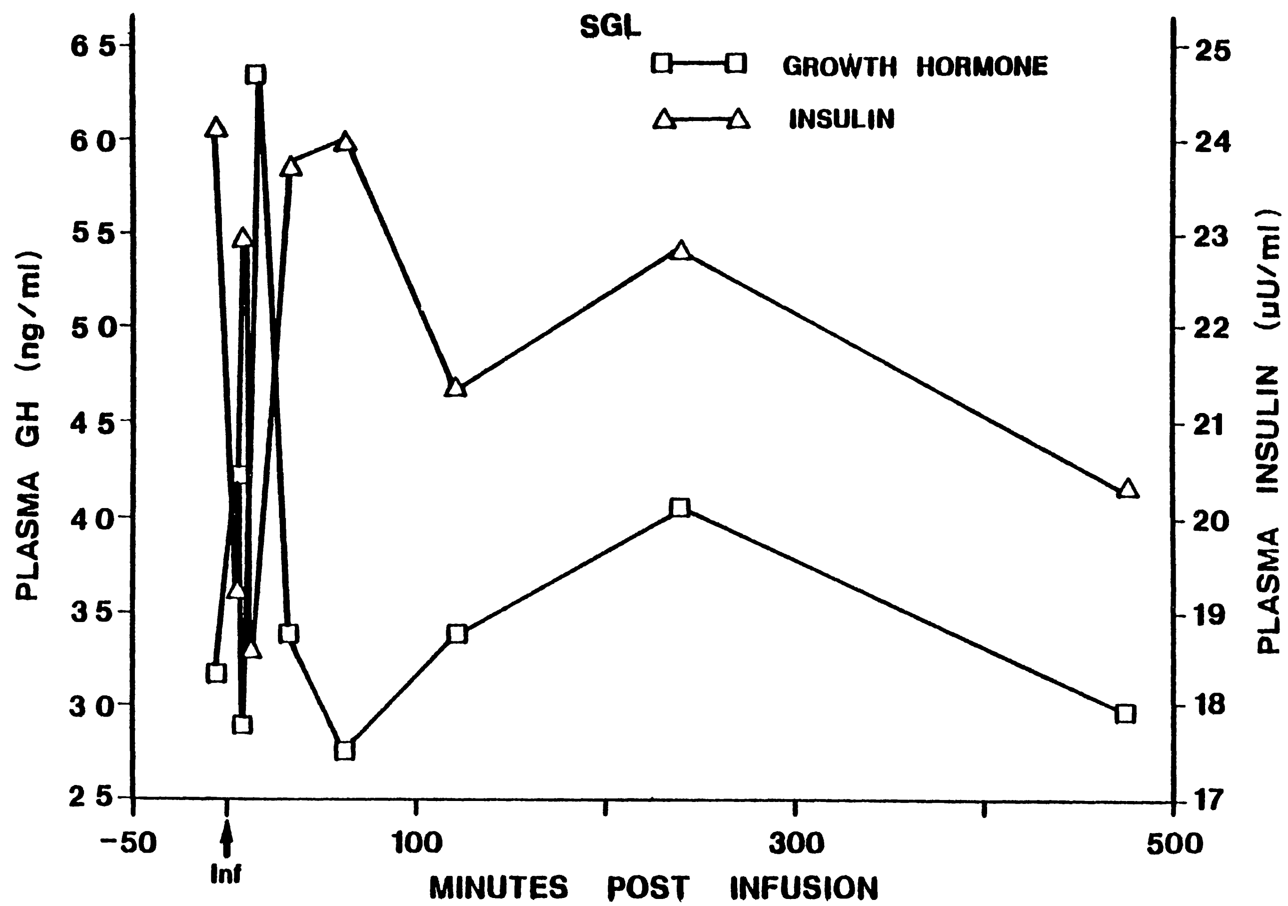

Figure 10 Response of Plasma GH and Insulin in SGL Gilts Following Arginine Hydrochloride (5 g/kg BW ^{75}) Challenge

distinct reciprocal relationship, while the response in the RGL was not as pronounced with insulin tending to slightly lag GH

hGRF Infusion

Plasma glucose, insulin and GH responses as evaluated by SPA are summarized in Table 18 Examination of overall GH and insulin responses by a comparison of the area under each response curve revealed no selection line differences in response, however, area under the plasma glucose response curve was larger (P<.05) for the SGL. In addition, plasma glucose concentrations were higher (P<.05) at 30 min post infusion for SGL gilts (90.5 mg/dl compared to 83 2 mg/dl in RGL pigs). Insulin response in both lines was essentially identical which reflected the similarity in insulin SPA between lines In both RGL and SGL gilts, hGRF resulted in an initial decline in insulin concentrations followed by a subsequent increase by 30 and 60 min (Figure 11). Plasma GH response to hGRF is shown in Figure 12. Plasma GH peaked in both lines by 2.5 min with no difference in concentration between lines. The pattern of response differed between lines with a biphasic response observed in the SGL with a secondary peak occurring at 10 min post-infusion.

Similar to the response resulting from ARG infusion, GH and insulin profiles in SGL gilts demonstrated a reciprocal relationship between GH and insulin from -10 to 30 min post infusion (Figure 13). Conversely, in the RGL, the same level

TABLE 18

SECRETORY PROFILE AREAS (SPA) FOR GH, INSULIN AND GLUCOSE FOLLOWING hGRF(1-44-NH_2) CHALLENGE (1 ug/kg BW) IN RGL AND SGL GILTS

PLASMA PARAMETER	SELECTION LINE[a]			
	RGL		SGL	
GROWTH HORMONE	2972.33	(255 64)	2449.90	(273.29)
INSULIN	17962.56	(421.22)	17293.19	(470.94)
GLUCOSE	60358.45[b]	(1039.04)	64634.36[c]	(1229.50)

[a] Means - (S.E.M.)

[bc] Areas are different (P<.05).

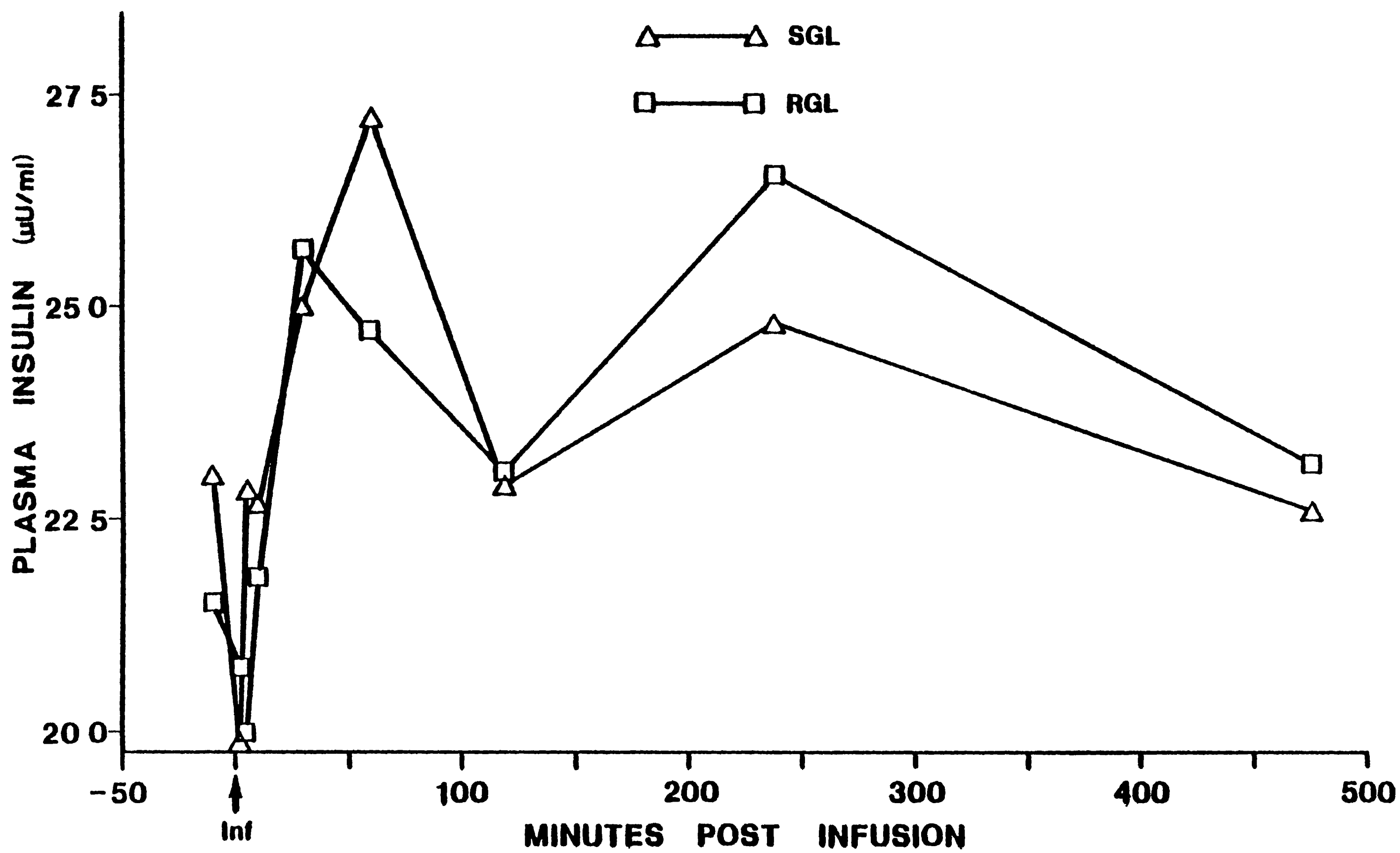

Figure 11 Effect of Intravenous Infusion of 1 ug/kg BW hGRF on Plasma Insulin in RGL and SGL Gilts

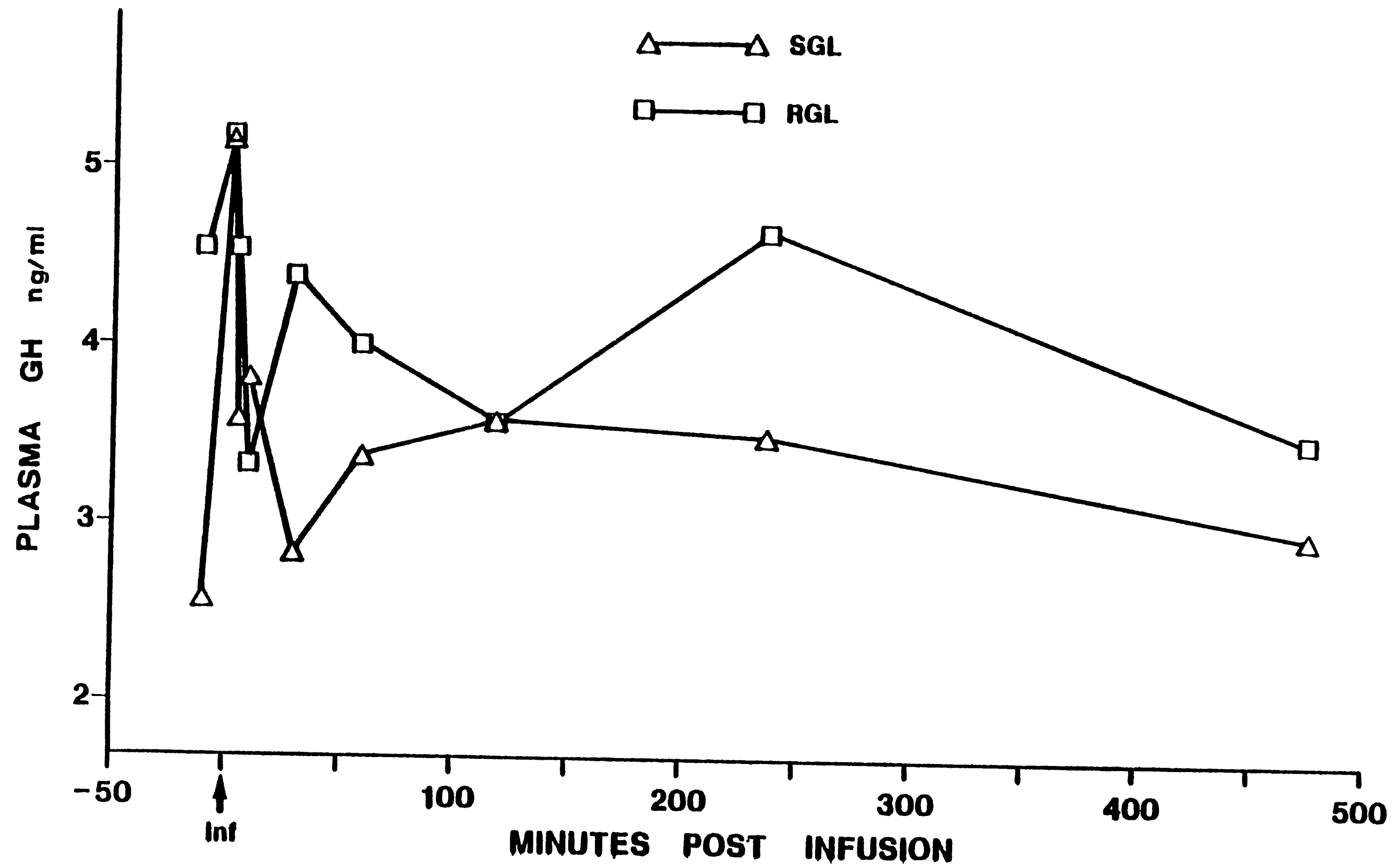

Figure 12 Effect of Intravenous Infusion of 1 ug/kg BW hGRF on Plasma GH in RGL and SGL Gilts

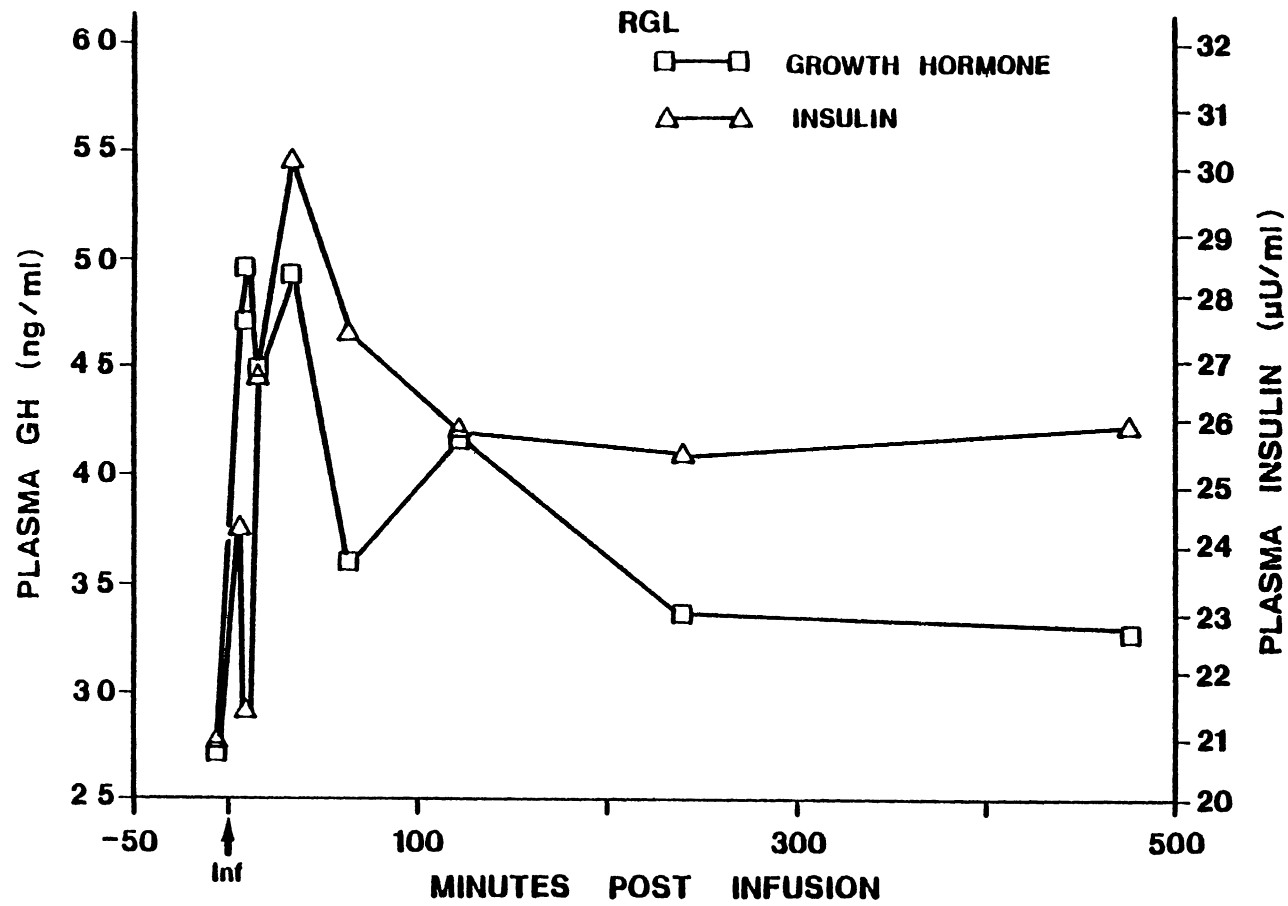

Figure 13. Response of Plasma GH and Insulin in RGL Gilts Following hGRF (.1 ug/kg BW) Challenge

of reciprocity was not apparent with insulin tending to lag slightly in the first 10 min following hGRF infusion (Figure 14)

A comparison of the response to hGRF and ARG in SGL and RGL gilts is presented in Figures 15 and 16, respectively. Comparing the responses to ARG and hGRF within each line, there were no differences in GH SPA although a more consistent response to ARG was observed in both lines.

Discussion

The results of this experiment have provided evidence that selection for growth rate produces differences in GH, insulin and glucose secretory capacity in swine as assessed by provocative challenge.

The assessment of basal GH secretion (SAL infusion) revealed a smaller (P<.05) SPA for RGL compared with SGL gilts. This trend is consistent with the results of the previous experiment in which temporal, unchallenged SPA and overall concentration of GH were lower in RGL gilts (Norton, 1986). Although insulin and glucose SPA did not differ between lines as a result of the SAL infusion, a tendency for both insulin and glucose to be higher in the RGL was observed which is also consistent with our earlier reports

The infusion of GLU produced increases in both insulin and glucose in both selection lines Although peak insulin and glucose concentrations were higher in the RGL group, only peak glucose concentrations were significantly different

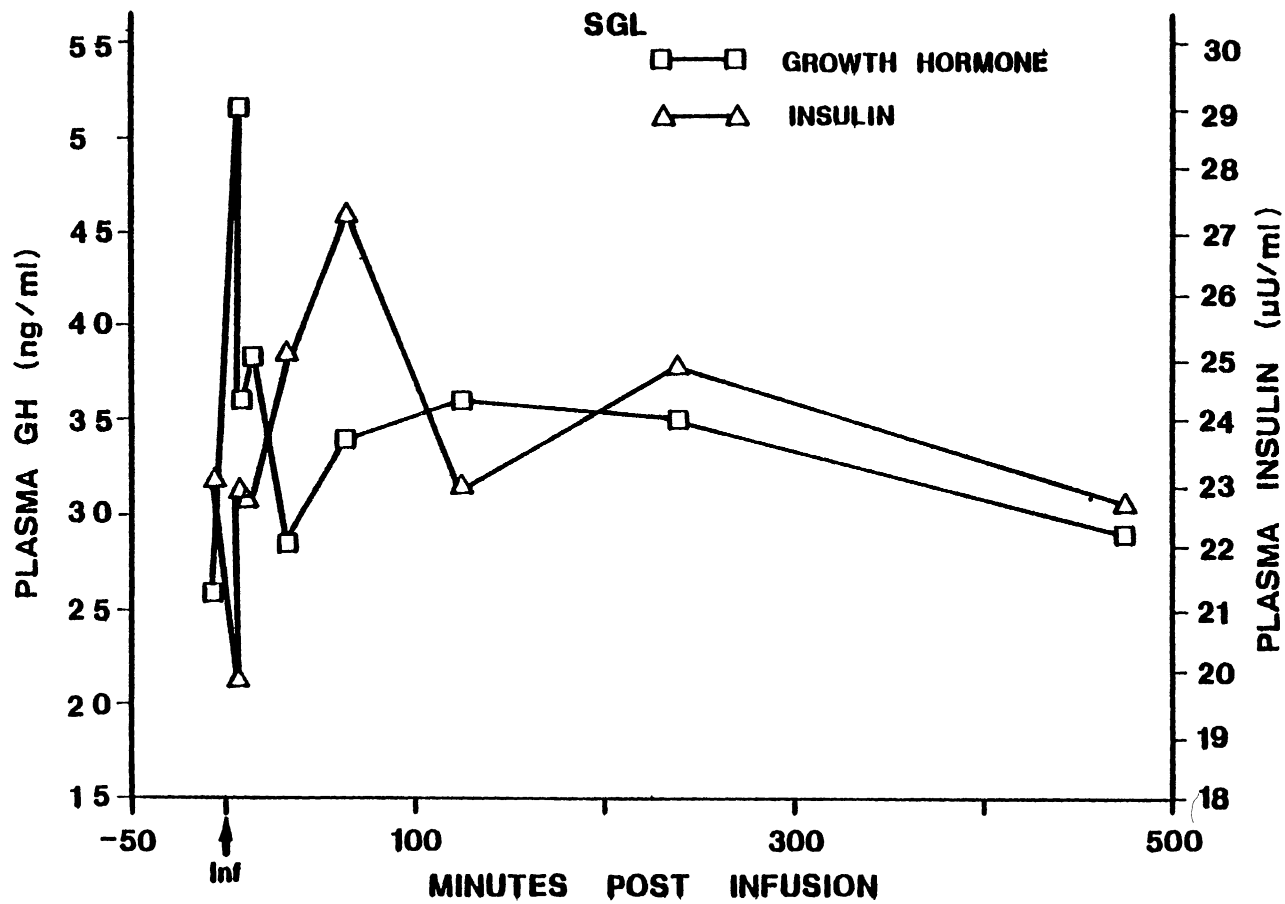

Figure 14 Response of Plasma GH and Insulin in SGL Gilts Following hGRF (1 ug/kg BW) Challenge

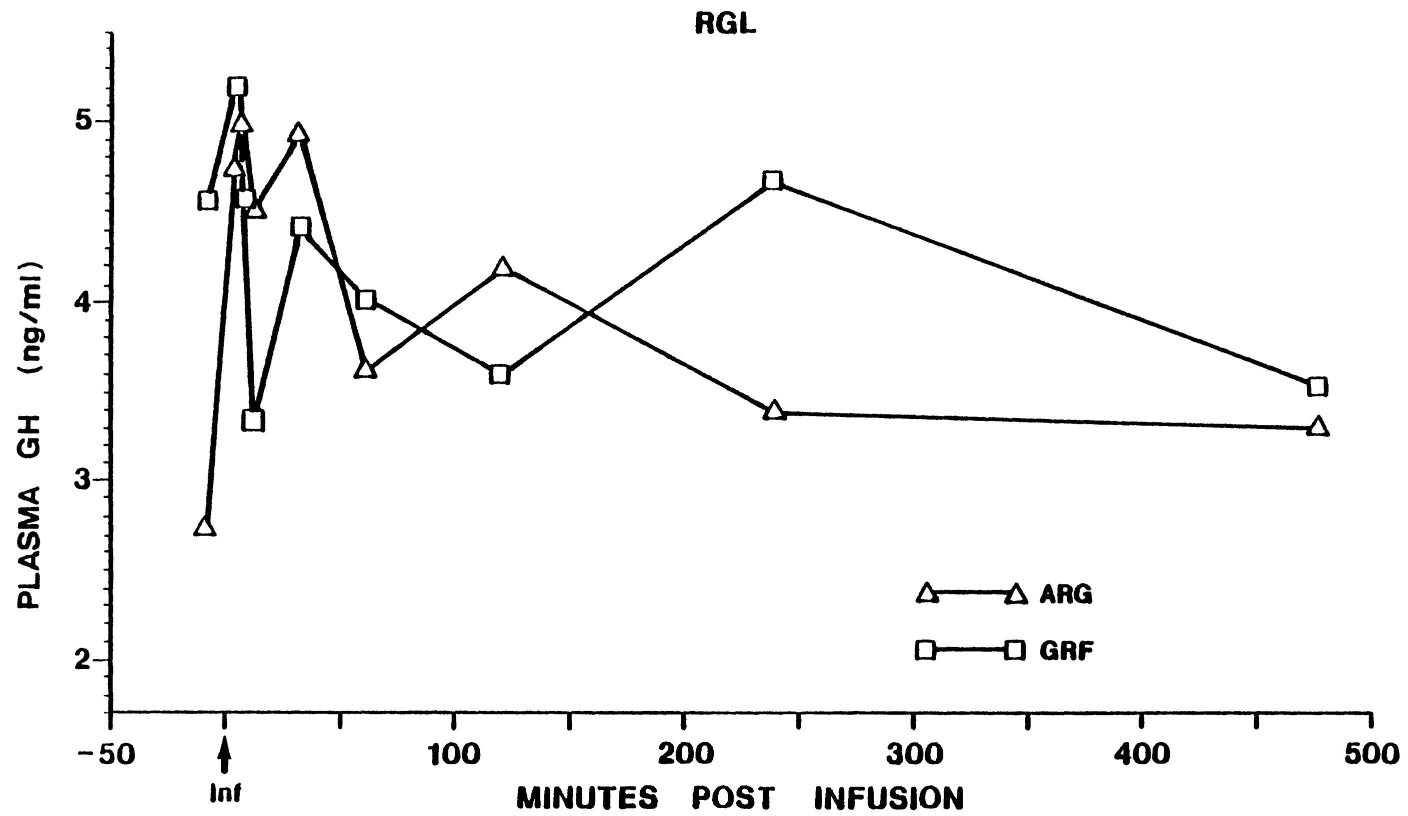

Figure 15. Comparison of the Response of Plasma GH to Arginine Hydrochloride Versus hGRF Challenge in RGL Gilts

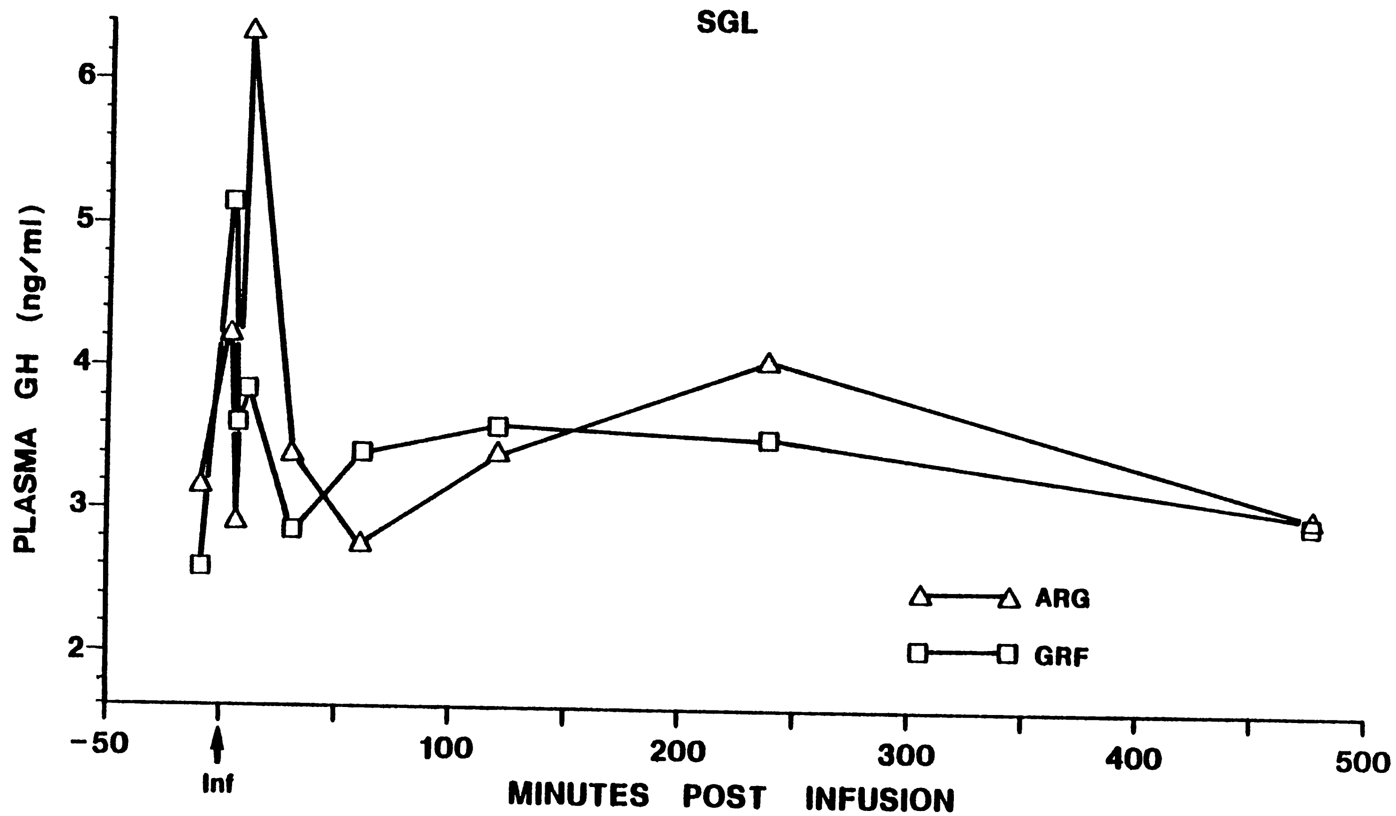

Figure 16. Comparison of the Response of Plasma GH to Arginine Hydrochloride Versus hGRF in SGL Gilts

between lines. In addition, ARG produced a greater response in insulin in the RGL compared with the SGL between 10 and 60 min post infusion. These results concur with other experiments in which notable differences in plasma insulin and glucose as the result of provocative stimuli between populations of swine differing greatly in performance and carcass composition (Bray & York, 1971; Gregory, Lovell, Wood & Lester, 1977; Wangsness, et al., 1977; Wangsness, Martin & Gatchell, 1980). These results, together with observations from this study of slight differences in glucose and insulin SPA and the exaggerated response to arginine challenge in conjunction with modest elevations in blood glucose, may suggest the occurrence of a very mild tissue insensitivity to insulin in the RGL.

The lack of difference in insulin and glucose clearance rate in the RGL and SGL is not in agreement with previous reports of glucose tolerance tests in swine (Wangsness et al., 1977) and humans (Hollander, Asplin & Palmer, 1982). In light of the other differences, the reasons for this are not clear, but may be the result of the less extreme differences in body composition and growth rate between selection lines which were more pronounced in previous studies.

Plasma GH response to ARG and hGRF were similar, however, peak levels following ARG or hGRF administration were of lessened magnitude than those reported from earlier experiments (Atinmo, Baldijao, Houpt, Pond & Barnes, 1978, Kraft, Baker, Ricks, Lance, Murphy & Coy, 1985) A

comparatively diminished response to ARG was observed in the present experiment relative to other similar experiments This apparent discrepancy may be partially explained by length of infusion. Other investigators using shorter infusion periods (15-30 min) in conjunction with intermittent sampling from a second catheter have observed significant elevations in GH within 20 to 30 min post infusion Therefore, in this study, using a 60 min infusion period with no blood sampling during the infusion, the major GH release may already have occurred by 2.5 min post-infusion. Furthermore, GH concentrations observed between 2.5 and 10 min in our experiment were similar to those reported by Atinmo et al. (1978) at 60 min within a similar time frame post-infusion.

Although hGRF produced elevations in GH in all pigs, concentrations of GH were considerably lower in magnitude than results obtained in other similar experiments. In those experiments, however, the dose of GRF was higher (1-8 ug/kg BW) than that administered in our study. In addition to a comparatively smaller response, the time of response was highly variable. Previous studies examining the administration of human pancreatic growth hormone releasing hormone (hpGRF) in pigs have shown extremely variable responses to a single 1 ug/kg hpGRF treatment that were not observed with sheep and cattle (Baile, Della-Fera & Buonomo, 1983, Plouzek, Anderson, Hard, Molina & Trenkle, 1983, Moseley, Krabill, Friedman & Olsen, 1984; Pellelier et al

1986) The variability in response in these studies together with those of this experiment suggest that animal to animal variation in evoking a GH response may be affected by specie, physiological state of the animal or differences in the synchrony of the administration of secretogogues such as hGRF and arginine with the temporal rhythm of secretion (Elsasser & Rumsey, 1986).

Due to the less extreme nature of the two selection lines, if differences in the secretory capacity of GH, insulin and glucose exist, perhaps more concentrated, pharmacological doses of each infusion may be necessary to elicit significantly different responses in these secretory parameters. Finally, evaluation of other growth-related factors such as non-esterified free fatty acids, IGF-1, T_3 and T_4 and the glucocorticoids will be required to more adequately determine the nature of the interaction of endocrine function with selected performance parameters.

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CHAPTER V

SUMMARY AND CONCLUSIONS

The central role of the endocrine system in growth and development has been well recognized. Based upon these observations, growth physiologists and endocrinologists have examined numerous methods, most involving the exogenous administration of growth-promoting substances, to achieve substantial and rapid improvements in livestock productivity. Although these studies have demonstrated that the administration of growth-promoting agents such as GH, insulin, anabolic steroids, T_3/T_4, or GRF, or the suppression of anti-growth factors such as somatostatin, can enhance animal growth, these investigations for the most part have not adequately accounted for or reflected the complexities and interactions associated with animal growth. Furthermore, many of the populations selected for study have included one or more exotic or novel genotypes and/or the experimentation conducted under unusual environmental or nutritional conditions which differ greatly from those encountered in the livestock industry. Hence, the relevancy of these results and the application of specific practices stemming from these experiments in the swine industry is questionable

To more accurately evaluate the role of the endocrine system in animals which more closely typify those found in the industry, pigs raised in a conventional farrow to finish operation and selected on the basis of growth rate were used in this study. The research objectives of the experiments were to (1) examine differences in both the levels and secretory profiles of growth hormone, insulin and glucose between two lines of pigs selected on the basis of rapid versus slow growth rate, (2) determine correlations within these selection lines between plasma secretory parameters in order to assess differences in the degree of synchrony of hormonal and metabolic events, and (3) to compare differences in the response of plasma growth hormone, insulin and glucose to human growth hormone releasing hormone, arginine hydrochloride and glucose to evaluate the short term secretory capacity and responsiveness to provocative stimuli between the two selection lines.

The sampling duration and frequency of the first phase of the experiment allowed for the establishment of plasma profiles The sampling design was necessary to accurately evaluate endocrine and metabolic events as biological systems are inherently variable. Therefore, frequent and substantial durations of measure are necessary to account for the portion of this variability that is of significant physiological importance. This is especially true when evaluating GH since its episodic pattern of secretion is thought to be a major determinant of the growth response

The utilization of PULSAR to detect and define plasma profile characteristics of insulin and glucose was based upon the rationale that although glucose and insulin are not thought of as being 'released' per se, in the same fashion as GH, each is responsive to numerous metabolic and endocrine cues. Hence, both in this context are driven or controlled. Therefore, a portion of the variability during the 12-h period, just as in the case of GH, may be biologically important variation that can and should be measured and evaluated.

From the delineation of a number of the characteristics associated with modulation or control of GH, insulin and glucose secretion, the organization of these events was evaluated for their level of spatial integration and further related to differences in growth performance. The necessity for this type of integrated analysis was evident from results of the first phase of the experiment. The evaluation of each plasma parameter independent of the others (especially in the case of GH) would have provided inconsistent and inconclusive results with respect to growth rate.

It should be mentioned that only three of many important growth regulators were assessed in the present experiment. Therefore, the descriptive scope of the present model is still somewhat limited. Certainly other plasma factors should be examined in future experiments to provide a more accurate description of the physiology of these two

lines Several candidates for examination would include somatomedin-C, a GH-dependent, liver synthesized and secreted peptide, which has been found in higher concentrations in lambs and pigs demonstrating rapid growth and leaner carcasses. Thyroid hormone and thyroid releasing hormone status should also be considered as these are involved in the control of GH secretion as well as associated with both catabolic and anabolic events. Glucocorticoid levels and patterns should be defined as stress responses have been observed to alter the episodic release and sensitivity to many hormones including GH In addition, the glucocorticoids appear to play a permissive role on the effects several other endocrines Similarly, little investigation has been focused on the role of extrahypothalamic brain centers in the control of endocrine release and nutrient utilization.

Beyond the measure of hormone and nutrient patterns and concentrations, questions of how growth processes are regulated at the cellular level, specifically at the level of the receptor are still largely unresolved and should be addressed. A clearer knowledge of the animal's capability to respond through an understanding of ligand-receptor events is requisite to the application of growth enhancement related technology in the swine industry

Lastly, the second phase of the experiment raises the issue of the utility of the application of pharmacological challenge in defining differences in metabolic and endocrine

events The application of these methods is useful as a clinical tool identifing the presence or extent of metabolic pathology or abnormality, such as diabetes or obesity. However, the usefulness of applying these pharmacological methods to answer questions concerning physiological mechanisms associated with relatively slight differences in growth characteristics within normal populations such as those selected for this study is unclear. Perhaps when initially defining physiological mechanisms, more meaningful conclusions can be drawn from integrated analysis of data obtained under temporal conditions as in the first phase of this experiment.

Only when the synergism of the growth-related factors is understood will consistent improvements in growth efficiency be attained. Without a broad base of knowledge, aimed at elucidating the mechanism whereby physioloical events govern growth and development, indiscrimiate application of growth promotants will provide only partial solutions to the question of how to most efficently apply biotechnological techniques in the livestock industry. We must therefore continue to compile information regarding the interactions among growth related events to subsequently interface the use of growth promotants with commonly encountered production practices and environments in the most economically and physiologically advantageous fashion.

APPENDIX

I. Insulin Radioimmunoassay

A. Assay Buffer

Stock A 01 M Phosphate Buffered Saline.

.16 g Sodium Phosphate Monobasic (NaH_2PO_4).

1.25 g Sodium Phosphate Dibasic Heptahydrate (Na_2HPO_4).

9.00 g Sodium Chloride (NaCl).

1.00 g Sodium Azide (NaN_3).

In a 1 liter volumetric flask dissolve all the reagents in 600 ml of distilled deionized water (ddH_2O), then qs with ddH_2O. Adjust the pH to 7 5.

Stock B .1 M EDTA Solution

3.72 g Disodium Ethylene diaminetetraacetate Dihydrate.

.40 g Sodium Hydroxide (NaOH).

Dissolve all reagents in a 100 ml volumetric flask with ddH_2O, then qs with ddH_2O Adjust the pH to 7.5.

Assay Buffer (AB)

89 ml Stock A

10 ml stock B

1 ml Normal Guinea Pig Serum[1]

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[1] Sigma Chemical Co., St Louis, MO , Ctlg No S3634, Lot 64F-9300

B Porcine Insulin Standard[2]

1. 50 mg * 25.2 U/mg = 1260 U
2. Add to 25 ml volumetric flask, qs with assay buffer. Therefore, 1260 U/25 ml = 50.4 U/ml = STOCK A.
3. Transfer 1000 ul of STOCK A to a 100 ml volumetric flask and qs with assay buffer Therefore

   50.4 U/ml/100 ml = .504 U/ml
   = 50,400 mU/100 ml
   = 504 mU/ml
   = 504,000 uU/ml = STOCK B.

4. Transfer 500,000 uU into a 50 ml volumetric flask. Therefore

   504,000 uU/500,000 uU = 1000 ul/X
   X = 992 ul[3]

   Therefore

   500,000 uU/50 ml = 10,000 uU/ml = STOCK C.

5. Standard Dilutions

STND (uU/ml)	Amt of Stock C (25 ml)	ul Stock C
2.5	2.5 * 25 = 62 5 uU	6.25

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2 Sigma Chemical Co., St. Louis, MO., Ctlg. No I3505, from porcine pancreas, 25 2 U/mg, crystalline

3 Contains 499,686 uU.

STND (uU/ml)	Amt of Stock C (25 ml)	ul Stock C
5 0	5.0 * 25 = 125.0 uU	12.50
10.0	10.0 * 25 = 250.0 uU	25.00
25 0	25.0 * 25 = 625.0 uU	62.50
50.0	50.0 * 25 = 1250.0 uU	125.00
100.0	100.0 * 25 = 2500.0 uU	250.00

C. Primary Antibody Dilution and Titer Determination

1. One ml of 1·50 guinea pig anti-pGH antiserum was aliquoted into 10, 100 ul quantities in 1% gelatinized vials with teflon caps, and stored at -80oC. This procedure was performed at 4 C.
2. For titering purposes, the remaining 200 ul of 1 50 Guinea Pig anti-pGH was diluted with 39.8 ml of cold assay buffer to yield 40 ml of a 1 10,000 dilution.
3. Using a autopipette, 250 ul aliquots of the 1 10,000 dilution were delivered into 160, 10 x 75 mm polypropylene tubes, parafilmed and capped. Aliquots were frozen at -80 C until used. This step was also performed at 4 C.
4. Dilutions made initially for determination of antibody titer were 1 100,000. 1 200,000, 1 400,000, 1 600,000, 1 800,000, and 1 1,000,000

D Assay Protocol

1. Assay was performed in glass 10 x 75 mm borosilicate glass tubes.
2. All reagents were brought to room temperature prior to use.
3. Proper volume of buffer was pipetted into all tubes (600 ul, TCT, 400 ul, NSB, 200 ul, TB, 100 ul, standards and unknowns)
4. A volume of 100 ul of insulin working standard or sample was pipetted into the appropriate tubes in triplicate and duplicate, respectively.
5. A volume of 200 ul of the primary Ab (1·200,000) was added to all tubes except the TCT and NSB tubes.
6. A volume of 200 ul of ^{125}I-p-insulin (10,000 CPM) was pipetted into all tubes. Tubes were vortexed and incubated at 4 C for 18 h.
7. Following incubation, 200 ul of second Ab was added to the appropriate tubes. Tubes were vortexed and incubated at room temperature for 2 h. Total reaction volume was 800 ul/tube.
8. Tubes were centrifuged at 4 C for 30 min at 3000 x g.
9. The supernatant was poured off from all tubes except the TCT and the pellet was counted for 1 min/tube.

E Tracer Chromatography

Reagents

1. Assay Buffer (less normal guinea pig serum) .01 M PBS/EDTA, pH 7.5.
2. .01 M PBS/EDTA, pH 7.5 + 25 mg BSA/25 ml.
3. .01 M PBS (8 ml), pH 7.5, 2 ml acetone, 100 mg BSA/10 ml (RIA Grade).

Column Preparation

1. Use a 5 cc disposable syringe for the column.
2. Pack the syringe with 1-2 ml of dry CF-11[4] celluose with a disc of Whatman 40 filter paper in the bottom of the syringe before packing.
3. Rinse the column with 4 ml of the Assay Buffer. Let buffer run to just above the packing material and clamp.

Elution Sequence

1. Apply the isotope to be chromatographed on the column.
2. Apply 3 volumes, 4 ml each of Assay Buffer to yield 12 ml Pool eluent together as fraction I.
3. Apply 6 volumes, 2 ml each of Assay Buffer and collect 2 ml fractions in tubes

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4 Whatman Inc., Clifton, NJ.

labelled 1-6.

4. Apply 3 volumes, 4 ml each of assay buffer + .1% BSA to yield 12 ml. Pool eluent together as fraction II.

5 Apply 6 volumes, 2 ml each of the same buffer and collect 2 ml fractions in tubes labelled 7-12.

6. Apply 2 volumes, 2 ml each of assay buffer + 20 % acetone + 1% BSA.

7. Collect first two ml as fraction III.

8. Collect second 2 ml as fraction IV.

9. Count 10 ul aliquots for 1 min and plot counts versus fraction number to determine radioactive peaks.

II. Growth Hormone Radioimmunoassay

A. Buffers

1. .01 M Phosphate Buffered Saline (PBS)

<u>Stock A</u> 69.01 g Sodium Phosphate Monobasic ($Na_2H_2PO_4*H_2O$).

Dilute to 1 l with ddH_2O to yield a .5 M stock reagent

<u>Stock B</u> 134.04 g Sodium Phosphate Dibasic Heptahydrate ($Na_2HPO_4*7H_2O$)

Dilute to 1 l with ddH_2O to yield a 5 M stock reagent

PBS 5.71 ml Stock A

14.86 ml Stock B

8.17 g Sodium Chloride (NaCl)

10.00 g Thimerasol ($C_9H_9HgNaO_2S$).

Add all reagents to 1 l volumetric flask, qs with ddH_2O, adjust pH 7 and store at 4 C.

2. Assay Buffer (.01 M PBS + 1 % BSA)

Bovine Serum Albumin (BSA)

a. Add 15 g RIA grade BSA in 50 ml ddH_2O to make a 30 % solution (takes approximately 12 h).

b. Add 33 ml of 30 % BSA to 1 l of .01 M PBS.

c. Adjust pH to 7.4 and store at 4 C.

3. Iodination Buffers

.5 M Phosphate Buffer 80 ml Stock A

20 ml Stock B

a. Add to a 1 l volumetric flask and qs with ddH_2O Adjust to pH 7.5, filter through a .45 u millipore filter. Store at 4 C

05 M Phosphate Buffer 4 ml Stock A

21 ml Stock B

a. Add to a 50 ml volumetric flask and qs with ddH2O. Adjust to pH 7.5 and store a 4 C.

B. Porcine Growth Hormone Standard

1. Weigh out 125 ug pGH (USDA pGH-B-1).
2. Transfer to a 50 ml volumetric flask and qs with assay buffer.
3. Prepare standards as follows

Stnd Conc ng/ml	Stock Volume (ul)	Buffer Volume (ml)
1	20	49.98
2	40	49.96
4	80	49.92
6	120	49.88
8	160	49.84
10	200	49.80
15	300	49.70
20	400	49.60

C. pGH Iodination

Reagents

1. Chloramine-T

a. Weigh and transfer .015 g chloramine-T to a 25 ml volumetric flask and qs with 05 M phosphate buffer

2 Sodium Metabisulfite ($Na_2S_2O_5$)

a. Weigh and transfer .025 g sodium metabisulfite to a 25 ml volumetric and qs with .05 M phosphate buffer.

3. Sodium Hydroxide (NaOH)

a. Prepare with dd H2O to yield a solution of pH 9.

4. pGH

a. Weigh and transfer 50 ug pGH and dilute with 50 ul .5 M phosphate buffer to yield a 1 ug/ul preparation.

Column Preparation

a. Use a 5 cc disposable syringe for the column
b. Place a glass bead in the bottom of the syringe.
c. Lightly pack column with BIO-RAD AG 1-X8 anion exchange resin to approximately the 2 cc mark.
d Attach a 16 gu Clay Adams luer stub adapter to the end of the syringe over which attach slide a 2 cm piece of teflon tubing.
e. Rinse column with 1-2 ml of .5 M phosphate buffer followed by 1 ml of assay buffer and finally 2-3 ml of 05 M phosphate buffer

f. Let the last buffer run to just above the resin and clamp

Procedure

a. Add 5 ul of pGH to reaction vial (5 ug).

b. Add 16 ul NaOH (pH 9) to ^{125}I[5] vial to yield a solution of 5 mCi/25 ul or 1 mCi/5 ul

c. Add 25 ul of .5 M phosphate buffer to reaction vial.

d. Add 5 ul of ^{125}I (1 mCi/ul).

e. Add 5 ul of chloramine-T. Mix for 60 sec.

f. Add 20 ul sodium metabisulfite and mix for 2-5 sec.

g. Add 200 ul .05 M phosphate buffer and layer on the column with a pasteur pipette.

h. Rinse the reaction vial with 100 ul of .05 M phosphate buffer and apply to the column.

i. Elute with 6 ml of .05 M phosphate buffer and collect 1 ml fractions

D. Primary Antibody Preparation

a. Gelatinize 10 x 75 mm borosilicate glass tubes with .1 % (w/v) gelatin solution.

b. Antibody, supplied 1 400, lyophilized was reconstituted with 12.5 ml of assay buffer to

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[5] Amersham, 350-600 mCi/ml, Ctlg No IMS 300, Arlington Heights, IL

yield a 1 1000 dilution.

c. Diluted antibody was stored in 200 ul aliquots at -80 C in gelatinized tubes

E. Assay Procedure

a. Standard curve was prepared in triplicate using 300 ul of each standard and 200 ul of assay buffer.

b. TCT, NSB and TB were prepared in tripicate.

c. For unknowns, 300 ul of the sample and 200 ul of assay buffer were assayed in duplicate.

d. 200 ul of the primary antibody (1 40,000) was added to all tubes except the TCT and NSB tubes.

5. 200 ul of assay buffer with 1% normal guinea pig serum was pipetted into the NSB tubes and 800 ul of the assay buffer was added to the TCT tubes.

6. Tubes were vortexed and incubated at 4 C for 24 h.

7. Following incubation, 100 ul of ^{125}I-pGH was added to all tubes. Tubes were vortexed and incubated for 24 h at 4 C.

8. Following incubation 200 ul of the second antibody (1·10) mixed with 500 ml of 6 % (w/v) polyethylene glycol (PEG)[6] was added to all

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[6] Should be prepared 24 h in advance of addition and stored at 4 C

tubes except the TCT. Total reaction volume was 1500 ul/tube.

9. Centifuge tubes for 30 min at 3000 x g, pour off supernatant from all tubes except the TCT. Invert tubes and dry overnight, count for 1 min/tube.

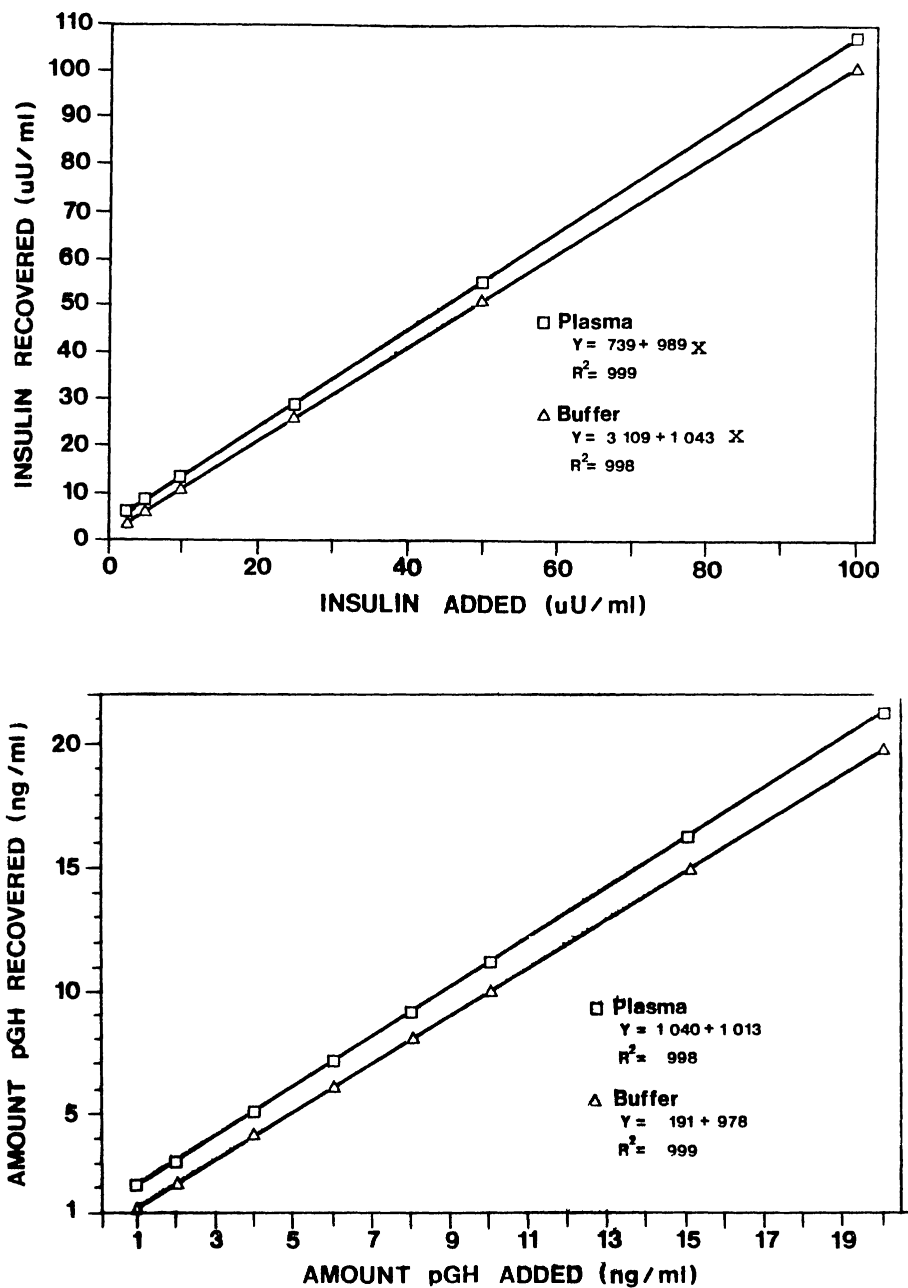

Figure 17 Demonstration of Parallelism and Recovery in Insulin and GH RIA

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VITA

Sharon Ann Norton

Candidate for the Degree of

Doctor of Philosophy

Thesis GROWTH HORMONE, INSULIN AND GLUCOSE IN YOUNG GILTS SELECTED ON THE BASIS OF RAPID VERSUS SLOW GROWTH RATE.

Major Field Animal Science

Biographical·

Personal Data Born in Columbus, Ohio, June 3, 1959, the daughter of Thomas Peter and Rosemary Jean Norton.

Education Graduated from Kecoughtan High School, Hampton, Virginia, in June, 1977, earned Bachelor of Science Degree from Virginia Polytechnic Institute and State Univerisity in June, 1981; earned Master of Science degree from Virginia Polytechnic Institute and State University in July, 1983, completed requirements for the Doctor of Philosophy degree at the Oklahoma State University in December, 1986.

Professional Experience Worked in the capacity of a Graduate Teaching Assistant, Department of Animal Science, Virginia Polytechnic Institute and State University, August, 1982 to July 1983, worked in the capacity of a Graduate Teaching Assistantship, Department of Animal Science, Oklahoma State University, August, 1983 to December, 1986.

Professional Organizations The American Society of Animal Science, Gamma Sigma Delta Honor Society, Sigma Xi Honor Society Golden Key Honor Society and Phi Kappa Phi Honor Society