THE UNIVERSITY OF MANITOBA

EFFECT OF EXOGENOUS GROWTH HORMONE ON GROWTH AND INSULIN PHYSIOLOGY IN INTACT RAM LAMBS AND STEERS

BY

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ABSTRACT

Average daily gain, feed efficiency, carcass composition and endocrine parameters were studied in intact ram lambs treated from nine to 22 weeks of age with daily subcutaneous injections of 0.1 mg/kg bovine pituitary growth hormone (bpGH) or sterile saline. Endocrine parameters were also studied in beef steers treated with daily subcutaneous injections of 25 mg/kg recombinant bovine growth hormone (rbGH). The effect of insulin infusion at five dose rates (.2, .5, 1.0, 6.0 and 30.0 mU/kg/min) at 11 and 20 weeks of age on plasma glucose response was studied in the lambs. Mononuclear leukocyte insulin binding was studied in lambs and steers. GH had no effect ($P \geq .05$) on average daily gain, feed efficiency or carcass composition in lambs but increased gain by 20% ($P < .05$) and improved feed efficiency by 20% in steers ($P < .05$). Lambs at 11 weeks of age were generally more sensitive but less responsive to infused insulin than at 20 weeks of age. At 11 weeks, the effect of bpGH was to reduce ($P = .059$) plasma glucose response to insulin infused at 30 mU/kg/min which was the highest dose studied. At 20 weeks of age the plasma glucose response to insulin in lambs generally increased with increasing dose. Thus a maximum response was not observed. At 20 weeks of age the sensitivity to insulin was greater in the bpGH treated lambs at doses .5, 6,and 30, but not 1.0 mU/kg/min. In ram lambs bpGH increased percent binding of insulin to mononuclear leukocytes at high insulin concentrations but in steers rbGH decreased percent binding of insulin to mononuclear leukocytes at low insulin concentrations.

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INTRODUCTION

The control of growth in young ruminants (defining growth as an increase in muscle and bone but not necessarily fat) is coordinated by the complex interaction of several hormones with nutrient supply, genetic potential, and the environment surrounding the animal. Although several of these interactions are poorly understood, it is well known that the pituitary secreted hormone 'growth hormone' holds a major role in the coordination and control of growth.

The existence of a hormone responsible for overall somatic growth was first suggested by the classic work of Evans and Long (1922) (cited in Hadley 1984), in which hypophysectomised young rats failed to grow to adult size. In subsequent experiments the injection of crude pituitary extracts stimulated normal growth and thus suggested the existence of a growth hormone. It was not until 1959 however, 19 years after the isolation of bovine growth hormone that Brumby (1959) demonstrated similar somatotrophic effects of growth hormone in the ruminant.

Subsequent to the discovery that growth hormone was able to promote anabolic effects in ruminants, there have been few experiments which have adequately evaluated the anabolic effects of growth hormone for use as a growth promotant. One of the major reasons for this lack of experimentation was the relative scarcity of growth hormone, which, until the recent development of recombinant DNA technology, was only availiable via pituitary extraction. To quote researchers at Cornell University involved in growth hormone and lactation research, " it would require the pituitaries of some

200 cows to obtain enough growth hormone to treat one cow for one day " (Harding 1987). Thus it was extremely difficult and expensive to obtain adequate amounts of growth hormone for experimentation purposes. Using recent technology, it is now possible to economically produce unlimited amounts of recombinant growth hormone and thus we can expect to see much more work being done with it in the near future.

There are several reasons which make growth hormone so potentially attractive as a growth promotant. The first is in relation to the growth promotants which are in use today. The awareness of the consumer and the governing forces of agriculture to the potential dangers of growth promoting substances used by the agricultural industry are increasing. These fears are not without substantiation. Consider the growth promoting substance diethylstilbestrol (DES): a great breakthrough in its time; the dangers were soon discovered and DES was banned for use as a growth promotant. Concerning several of the growth enhancing implants in use today, the hormones within these implants present certain risks to the consumer, and the government has in some cases chosen to impose withdrawal periods before the animals can go to slaughter for human consumption. In addition, the European Economic Community has banned the use of all growth promotant implants in meat producing animals (Phelps 1986). It is possible that similar restrictions will be placed on Canadian agriculture.

It appears then that it is the responsibility of the agricultural scientific community to develop a safe growth promotant. Growth hormone is a polypeptide and it is therefore readily digested

(inactivated) by normal human digestive processes. Bennet et al. (1950) determined that the ruminant growth hormones are biologically inactive in humans. Thus, even if growth hormone were not digested it would pose no danger to human health.

Another attractive feature of growth hormone as a growth promotant may be its physiological effectiveness in inhibiting carcass fat accumulation as well as stimulating muscle and bone growth. The general public has been warned of the dangers encountered upon the consumption of their favourite red meat; cholesterol, artherosclerosis, and a myriad of other diseases are all deemed the responsibility of one factor: 'animal fat'. There has consequently been a trend towards the consumption of leaner meats. To remain competitive with other sectors of agriculture, the producers of red meat must follow consumer trends as well. The use of growth hormone as a growth promotant may be a tool which will enable the red meat industry to create 'designer beef', a product which can respond quickly to a changing market.

The purpose of this thesis was to investigate the effect of exogenous growth hormone on growth rate, feed efficiency, carcass composition, and selected parameters of insulin physiology in growing intact ram lambs. In addition, the effect of exogenous growth hormone on selected parameters of insulin physiology in beef steers was studied.

LITERATURE REVIEW

Pituitary growth hormone

Pituitary growth hormone (pGH) is a polypeptide hormone secreted from the anterior pituitary gland into the circulation. It consists of 191 amino acid residues with two intramolecular disulphide bonds (Hadley 1984). The structure is highly similar to that of prolactin.

The release of pGH is under the control of two hypothalamic hormones: somatostatin, an inhibitory hormone, and somatocrinin or growth hormone releasing factor (Tannenbaum 1980). The effects of breed, sex, and selection on pGH, and the effects of exogenous pGH and recombinant growth hormone (rGH) on various metabolic and growth processes have been studied.

Effects of pituitary growth hormone at the cellular level

Adipose Tissue

Studies of the actions of pGH at the cellular level, both *in vitro* and *in vivo*, are few. pGH is known to have a direct lipolytic effect on rat adipose tissue *in vitro* (Fain *et al.* 1965, Weiser *et al.* 1974). The effects of pGH on adipocytes in the ruminant, however, have not been thoroughly investigated. Vernon (1978) studied the effect of pGH on adipocyte metabolism of ovine adipose tissue in the presence of insulin. The results suggested that pGH inhibited the lipogenic effects of insulin, but did not alter lipolysis in ovine adipose tissue. Vernon (1982) reported similar results, indicating that pGH acted to inhibit lipogenesis in ovine

adipose tissue. Duquette (1984) reported that ovine GH had no direct lipolytic effect on ovine adipose tissue. The available data thus suggest an anti-lipogenic role of pGH with no evidence to suggest a lipolytic role in ruminants.

Skeletal muscle

pGH is known to stimulate muscle protein synthesis in the monogastric (Manchester et al. 1959, Cheek and Graystone 1978). However, only one published study is available on the effects of pGH on ruminant skeletal muscle. Eisemann et al. (1986) reported that protein synthesis rates in biceps and triceps muscle groups were increased over that of controls in steers injected with pGH. These data must be interpreted with some caution, however, as the whole animal was subjected to pGH treatment and the observed effects may have been mediated by some pGH induced factor and not directly by pGH.

Endogenous growth hormone and growth

In general the relationship between levels of endogenous growth hormone in the blood and specific growth parameters is poor (Trenkle and Topel 1978, Davis et al. 1984). This in itself is not surprising given the complexity of factors which coordinate the development of tissues involved in overall animal growth.

A major hindrance in understanding the relationship of endogenous growth hormone levels with growth is the mode of secretion of growth hormone. Initial studies assumed that the secretion of growth hormone was constant and thus assumed that single daily blood samples would be representative of the growth hormone profile of the

animal. Davis et al. (1977) observed that growth hormone secretion in ram lambs was not constant but rather that it was dynamic and episodic over time. Growth hormone secretory spikes were observed to be at random with no uniformity or rhythm in sheep.

This characteristic of growth hormone secretion has made growth hormone data extremely difficult to interpret and to ultimately relate to growth parameters. Most recent growth hormone results are compared on the basis of four serum profile parameters: overall serum concentration, baseline concentration, peak frequency, and peak amplitude (Davis et al. 1977, Grigsby and Trenkle 1986, Ohlson et al. 1981, Dodson et al. 1983).

Sex and endogenous growth hormone

It is well known that with adequate nutrition, intact males grow at a faster rate, more efficiently, and produce a leaner carcass than do either castrates or females of the same species. Davis et al. (1977) conducted a study measuring growth hormone serum profiles in both ram and wether lambs on the basis of four growth hormone profile parameters. Significant sex differences were noted for all observed growth hormone profile parameters (baseline, overall, frequency and amplitude), suggesting that castration somehow resulted in decreased growth hormone secretion. Similar though nonsignificant differences have been observed in growth hormone secretory patterns in ram and ewe lambs (Davis et al. 1984). Overall, a trend toward greater growth hormone concentrations is exhibited by intact males in comparison to both castrates and females. These results suggest that the male has an inherent ability which allows it to secrete greater amounts of growth hormone than either castrates or females of the

same species. Perhaps the testicular steroids in the male are more stimulatory to growth hormone secretion than ovarian steroids.

It is well known that sex steroids in the form of implants are capable of improving growth rates in farm animals (Schanbacher 1984). Davis et al. (1977) compared growth hormone parameters in implanted wether lambs (either androgenic or estrogenic implant), intact lambs, and wethers. The results indicated that both androgenic and estrogenic implants significantly increased overall and baseline concentrations of growth hormone and increased the frequency but not the peak amplitude of growth hormone. This further supports the suggestion that differences observed between castrate and intact animals may be the result of gonadal steroid removal.

Breed and endogenous growth hormone

Breeds of cattle differ in genetic potential, which results in breed differences in growth rate and final body weight. As well, breeds differ in propensity to fatten. The exact means by which these genetic differences are attained are not yet understood, but there appears to be a relationship between growth hormone and breed.

Grigsby and Trenkle (1986) studied growth hormone secretory patterns in Simmental, Limousin, and Angus steers. Simmental cattle were characterised as a fast growing breed with a large mature size, the Limousin as a slower growing breed with a delayed propensity to deposit fat, and the Angus as a slow growing animal which fattens at a smaller size. The only difference in growth hormone observed was a significantly higher overall growth hormone concentration of the Simmental as compared to both the Angus and the Limousin.

In a similar study, Ohlson et al. (1981) evaluated growth

hormone secretory patterns in Hereford and Simmental bull calves. The Simmental calves exhibited greater average daily gains and body weights at sampling. Only two growth hormone parameters (overall and baseline concentration) were significantly greater in Simmental as compared to Herefords, although there appeared to be a trend towards higher growth hormone peak amplitude in the faster growing Simmentals. No statistically significant correlations were observed between average daily gains, body weight and any of the growth hormone parameters.

These studies of growth hormone parameters in different breeds indicate a trend towards higher growth hormone levels in faster growing, more efficient, leaner animals. However, there are no definite statistical correlations linking growth hormone and growth parameters.

Selection and endogenous growth hormone

Assuming that growth hormone secretion may be the key factor which influences breed expression in terms of growth performance selection pressure exerted upon a population of animals in the direction of better growth performance should concurrently change growth hormone levels. Dodson et al. (1983) compared growth hormone parameters in two lines of Targhee rams, which were or were not selected on the basis of growth rate. The unselected had received no selection pressure for 20 years, whereas the selected line had been selected on the basis of growth rate and efficiency of gain for 1.5 generations (4 years). Selected rams exhibited significantly higher birth weight and weight at 270 days. Growth hormone differences were only observed in terms of overall concentration,

with selected rams exhibiting significantly higher overall concentration than unselected. Though no other statistically significant differences were observed, there appears to be a general trend towards higher values for all growth hormone profile parameters in the selected line. Davis et al. (1983) compared a selected line of Hereford bulls with an unselected line on the basis of performance and growth hormone profile parameters. Selected animals had recieved selection pressure for 5 generations (20 years) on the basis of weight and muscling score. Results indicated that selected animals had significantly higher average daily gains and higher overall growth hormone concentrations. Though none of the other growth hormone profile parameters were shown to be of significance, there again appeared to be a trend of increase in all growth hormone profile parameters in selected animals.

Exogenous pituitary growth hormone

The practicality of pGH as an anabolic agent for use as a growth promotant has not yet been adequately assessed for agricultural use. However, several studies have been conducted with ruminants, although nothing very conclusive exists.

Anabolic effects in sheep

Initial studies assessing the anabolic effects of pGH in lambs were promising. Wagner and Veenhuizen (1978) reported impressive improvements in gain (20%) and feed efficiency (14%) in lambs injected daily with pGH. In addition, pGH treated animals exhibited a greater propensity to deposit lean tissue while concurrently depositing less fat (+25% lean and -37% fat compared to controls). However, the above results were only presented in abstract form. The

results of a subsequent study (Muir et al. 1983) which also involved the injection of growing lambs with pGH differed in some respects to those previously reported by Wagner and Veenhuizen (1978). No significant effects of pGH on average daily gain or lean deposition were noted. Statistically significant differences were found, however, for feed conversion efficiency and fat deposition. Johnsson et al. (1985) conducted a study with virtually the same hypothesis and noted significant increases in gain, feed conversions, and deposition of lean in pGH treated lambs as compared to controls in agreement with the results reported by Wagner and Veenhuizen (1978).

Anabolic effects in cattle

Only two studies to date have been published assessing the anabolic effects of pGH in cattle (Brumby 1959, Fabry et al. 1987). No statistical analyses were presented by Brumby, but the overall effect appeared to be a stimulated rate of gain. Fabry et al. (1987) reported a 24% increase in average daily gain in pGH treated heifers.

Recombinant growth hormone

Recombinant growth hormone (rGH) is growth hormone produced by E.coli incorporated with growth hormone protein strands (Hart et al. 1984). The production of recombinant growth hormone is a relatively new technique which has potential for thew production of large quantities of rGH. Leung et al. (1985) reported that rGH had biological and immunological properties similar to pGH. Olsen et al. (1986) reported that rGH produced from E.coli had an amino acid composition identical to the 191 amino acid pGH. Hart et al. (1984)

reported that rGH demonstrated short term diabetogenic activities similar to those seen in response to pGH in sheep. Assessment of rGH in three biological test systems, the lactating cow, the hypophysectomised rat, and a somatotropin receptor system indicated that bovine rGH generally had higher biological activity than pGH (Olsen et al. 1986).

Effects at the tissue level

Investigations into the effects of rGH on ruminant tissues, namely adipose tissue and skeletal muscle, are not abundant due to the youth of this substance.

Injection of lambs with rGH resulted in an increase in perirenal adipose tissue lipolytic activity (Pullar et al. 1986). Peterla et al. (1987) reported that rGH did not affect lipolysis in ovine adipose tissue, but was effective in reducing insulin induced lipogenesis. The effects of rGH at the tissue level appear to be similar to those of pGH, but rGH may have a lipolytic effect not as yet observed for pGH.

No studies have been published to date as to the effects of rGH on skeletal muscle in vitro, but it is logical to assume the actions to be similar to that of pGH.

Anabolic effects of rGH

As previously stated, exogenous pGH has been noted to induce anabolic actions in growing ruminants. The studies evaluating the effectiveness of exogenous rGH as a growth promotant at present are limited to two.

Pullar et al. (1986) administered daily subcutaneous injections of rGH to ram lambs between 12 and 18 weeks of age.

Treatment with rGH increased average daily gains by 30% and, although the rGH treated animals had a greater carcass weight at slaughter, the treated and untreated lambs did not differ in carcass composition. The authors suggested that the lack of carcass composition response may have reflected an effect of diet or been the influence of endogenous steroid hormones, as, in this study, intact male lambs were used whereas in other studies either female or wether lambs were used.

Johnsson et al. (1987) administered three doses of rGH to both ewe and wether lambs. Treatment with rGH had no effect on live weight gains, feed intake, or feed conversion efficiency. Lambs given the highest dose of rGH had less visceral fat, proportionally less total fat, and more muscle and bone in the shoulder joint than control lambs. As well, the weights of the major fat depots in the abdominal cavity and of fat dissected from the shoulder joint showed a negative linear relationship to the dose of rGH. The investigators in this study suggest that the absence of growth rate and feed efficiency effects of rGH may have been due to a low sodium concentration in the diet.

Insulin

Biochemical characteristics

Insulin is one of the major hormones required for the normal growth and development in farm animals. Insulin is a polypeptide hormone consisting of an A and B chain of 21 and 30 amino acid residues respectively, linked via two disulphide bonds. Insulin has a molecular weight of about 6000 (Dickson 1982). Insulin structure

varies among species, the differences being restricted to the 8,9 and 10 positions of the A chain and to position 30 of the B chain in mammals (Hadley 1984).

Insulin is secreted from the B-cells of the pancreatic islets of Langerhans (Weekes 1986). The secretion of insulin is dependent upon several stimuli, most of which are consistent with the homeostatic function of insulin in the promotion of storage and uptake of nutrients.

It is generally understood that insulin i) stimulates lipogenesis and inhibits lipolysis ii) stimulates the uptake and incorporation of amino acids into protein while inhibiting proteolysis iii) stimulates the uptake and utilization of glucose by many tissues and iv) inhibits gluconeogenesis and glucose release from the liver (Prior and Smith 1982). These insulin actions are generally accepted for the monogastric; in the ruminant the role of insulin is not as well defined.

Function of insulin in the ruminant

In contrast to monogastrics, which use glucose as a major substrate for energy storage, ruminants, as a result of microbial fermentation in the rumen, absorb little or no glucose from the gastrointestinal tract (Leng 1970). Instead, acetate is utilized as a major substrate for energy storage and ruminants are almost exclusively dependent upon gluconeogenesis for provision of glucose both when fed and fasted (Leng 1970). Some earlier researchers assumed that since glucose absorption is limited in the ruminant, insulin is of lesser importance in the ruminant than in the monogastric (Bassett 1975). This view has since been considered

simplistic and it is now understood that insulin plays an important role in the ruminant. However, the major function of insulin in the ruminant does not appear to be the removal of exogenous glucose loads as in the monogastric. The importance of insulin in the ruminant appears to be in relation to protein synthesis and partitioning of nutrients to tissues.

Insulin and adipose tissue

The effects of insulin in the monogastric are well established (Hadley 1984). Lipolysis of adipose tissue is inhibited by insulin in vitro and lipogenesis is stimulated in the presence of insulin (Lane 1981).

Early experiments assessing the effects of insulin on ruminant adipose tissue indicated a low responsiveness of adipose tissue to the lipogenic effects of insulin (Vernon 1978). These findings were interpreted to mean that the role of insulin with respect to regulating lipogenesis in the ruminant was minimal or nonexistent (Prior and Smith 1982). Recently, however, it has been found that ruminant adipose tissue becomes refractory upon removal from the animal and longer in vitro incubation periods are required to observe maximum responsiveness of the tissue. Etherton and Evock (1986) recently reported that bovine adipocytes were quite sensitive to the lipogenic effects of insulin in vitro. Thus insulin may play an important role in adipose metabolism in the ruminant.

Insulin and skeletal muscle

Insulin is known to be a major anabolic hormone involved in muscle growth in the monogastric. In diabetes, there is a marked loss of muscle protein which can be reversed by the administration of

insulin (Pozefsky et al. 1969). Insulin is known to stimulate protein deposition (Jefferson 1980) and inhibit protein degradation (Long et al. 1984) in monogastrics.

The effects of insulin on ruminant muscle is less well documented. Intravenous infusions of high doses of insulin have been shown to decrease plasma concentrations of specific amino acids in sheep (Call et al. 1972, Prior and Christenson 1978). Prior and Smith (1983) conducted an experiment involving alloxan diabetic cattle to determine the role of insulin in protein and amino acid metabolism. Alloxan treatment caused a marked increase in serum urea nitrogen. Insulin treatment of the alloxanised animal resulted in a decrease in serum urea nitrogen. In addition, alloxan treatment caused a two to three fold increase in plasma concentrations of specific amino acids. Insulin treatment maintained normal concentrations of these specific amino acids. These data suggest insulin may promote tissue anabolism by stimulating tissue uptake of amino acids and protein synthesis rates.

Relationship between endogenous insulin levels and growth rate

The importance of insulin to growth is clearly indicated by the effects of diabetes as stated previously. How insulin levels relate to growth rates in ruminants is less well understood.

Trenkle and Topel (1978) reported a positive correlation between plasma insulin concentration and carcass adipose tissue, and a negative correlation with carcass muscle. Eversole et al. (1981) found a positive correlation between serum insulin concentrations and average daily gain in cattle. Carcass analysis determined a positive relationship between both protein and fat gain and serum insulin

levels.

Gregory et al. (1982) measured the secretory response of insulin to tolbutamide in cattle, as the secretory response in monogastrics has been shown to relate to body fatness (Wood et al. 1977). Insulin secretory response was higher in older animals but no relationship was determined between secretory response and the proportion of dissectable fat in the empty carcass. In addition, insulin secretory response was shown to be lower in Hereford than Friesan steers despite the fact that the Hereford steers were fatter.

The relationship between insulin levels and growth is not clear. The fact that insulin concentration and secretion are not highly correlated with growth is not unusual if one takes into account the within day variation of plasma insulin concentration (Trenkle 1978) as well as the changes that can occur in metabolic clearance rate and receptor dynamics (Etherton 1982).

Alteration of insulin action by receptor and post-receptor modifications

The response of a tissue to serum insulin is dependent on insulin secretion rate, insulin receptor number and affinity, and post-receptor defects (Gliemann et al. 1980). Secretion rates and circulating plasma levels are fairly simple to evaluate whereas receptor number and affinity and post receptor defects are more difficult to quantify.

The insulin receptor serves two important functions: it recognises and distinguishes insulin from all other hormones through specific binding to insulin, and, the hormone receptor complex stimulates a transmembrane message which results in the production of

the intracellular biological effects of insulin (Etherton 1982). Any alteration or change at either the receptor or post-receptor level can mediate a change in the effectiveness of insulin.

The biological response of an animal to insulin is normally determined using a dose response curve, by plotting the magnitude of glucose removal from the plasma against increasing doses of injected insulin. Whole body dose response curves must be treated with caution, however, as the heterogeneity of tissue response to insulin within the animal must be taken into account.

Changes in receptor and post-receptor dynamics and their resultant effect on insulin action have been characterised. A change in the number of insulin receptors on target cells or receptor affinity changes results in an alteration of the insulin dose response curve by either a shift to the right or left without any change in maximal response. This shift has been termed a change in insulin 'sensitivity' (Kolterman et al. 1982), whereas an alteration in post-receptor action, which is reflected in an upward or downward shift in the dose response curve at high insulin concentrations, is termed a change in insulin 'responsiveness' (Kolterman et al. 1982).

It is thus evident that the biological actions of insulin are dependent not only on the plasma levels of the hormone but also on receptor dynamics. To understand the mechanisms of insulin action on growth, all aspects in insulin physiology must be taken into account.

Relationship between growth parameters and insulin receptor parameters

Knowledge of insulin receptors and their relation to growth in farm animals is very limited. Obesity in swine is associated with decreased insulin binding to liver microsomes suggesting a lower

population of receptors (Meserole and Etherton 1984). Similar findings have been reported for humans and rats (Felig and Soman 1979, Gliemann et al. 1980). In the case of human obesity, where a reduction in number of insulin receptors is observed, a change in insulin sensitivity at physiological insulin levels is also observed but there is no change in the responsiveness to insulin (Kolterman et al. 1982).

In ruminants no evidence exists as of yet to suggest such a relationship between insulin binding and obesity. McCann and Reimer (1985) measured glucose response to exogenous insulin in obese and lean heifers. The obese heifers were found to have reduced sensitivity to insulin, but normal responsiveness to the glucoregulatory effects of insulin. These responses suggest that obesity caused a reduction in the number of receptors or a decrease in affinity as found in the monogastric. However, McCann and Reimer (1985) performed no receptor analysis in this study.

Perhaps more relevant to farm animal production than the kinetics of insulin metabolism in obese and other abnormal animal states is a study linking body composition and insulin sensitivity. Yki-Jarvinen and Koivisto (1983) selected three separate groups of atheletes (runners, weight lifters, and controls which represented increasing percent body fat, respectively) and subjected them to both body composition and insulin sensitivity measurements. Results indicated that whole body sensitivity to insulin was directly proportional to percent body muscle and was inversely proportional to percent body fat. This study supports the results reported in obese subjects with respect to changes in receptor concentrations as

sensitivity to insulin and insulin receptor concentration are both inversely proportional to the percent body fat present in the subject. This suggests that body composition may be an important factor in determining insulin sensitivity in normal subjects.

Growth hormone-insulin interaction

Metabolically, both insulin and growth hormone play important roles in protein and adipose deposition. In the whole animal, however, the relationship between these two hormones of major importance in growth is not well understood.

Insulin is generally thought of as being lipogenic in nature (Etherton and Evock 1986), promoting fat deposition and in ruminants. Growth hormone is thought to be lipolytic and anabolic with regards to muscle (Eisemann et al. 1986). The first evidence of some interaction between growth hormone and insulin was observed in an experiment where growth hormone administration in sheep was noted to have diabetogenic effects (Bassett and Wallace 1966). Later experiments using both pGH and rGH confirmed that the diabetogenic actions of growth hormone were indeed the actions of the hormone and not the result of biologically active contaminants in pGH (Hart et al. 1984). Boyd et al. (1987) observed similar effects of exogenous growth hormone administration in pigs, where exogenous GH antagonised the ability of insulin to stimulate glucose disposal. In humans this phenomenon of growth hormone has been attributed to a post-receptor defect in insulin action (Bratusch-Marrain et al. 1984). However, studies conducted with acromegalic patients, a condition characterised by hyperinsulinemia and insulin resistance have indicated a good correlation between decreased monocyte insulin

receptor concentration, increase in monocyte insulin receptor affinity and severity of insulin resistance with the magnitude of plasma growth hormone elevation (Muggeo et al. 1979).

In vitro studies on the actions of growth hormone at the adipose tissue level in ruminants are limited to one study reported by Vernon (1982) in which growth hormone antagonised the stimulatory effect of insulin on lipogenesis in ovine adipose tissue.

Whole animal studies involving the administration of exogenous growth hormone have indicated the ability of growth hormone to increase serum insulin concentrations (Johnsson et al. 1985, Johnsson et al. 1987, Wagner and Veenhuizen 1978). Johnsson et al. (1985) observed that the increase in baseline insulin due to growth hormone did not appear to be an immediate direct response to daily injections of growth hormone, but rather that the baseline insulin concentration was increased over the entire day.

Pooling the knowledge that has been disclosed with respect to the individual actions of both insulin and growth hormone and the overall effects of the administration of exogenous growth hormone, it appears that high levels of growth hormone increase serum insulin levels and impair the lipogenic actions of insulin on adipose tissue while leaving muscle and bone relatively unaffected such that nutrients normally utilised by adipose tissue are partitioned to muscle and bone.

MATERIALS AND METHODS

Animals and management

Purebred Suffolk lambs (14 intact males) were weaned at six weeks of age. Due to the University sheep flock being on an eight month breeding cycle, average date of birth was July 1, 1986. All lambs were treated with 1.5 mg selenium, 69 I.U. vitamin E (Distocel, BTI products INC. Montreal Que.) and five ml Covexin-8 (Burroughs Wellcome Inc. Kirkland, Que.) prior to weaning. At seven weeks of age the lambs were introduced to a Westfalia Separator automatic feeder system (Westfalia Systemat, Centrico Inc., Elk Grove, IL) modified to accommodate sheep (Appendix 1). The feeder allowed the recording of daily feed intake on an individual animal basis. The lambs were maintained in a single pen with one automatic feed dispenser and two watering stations. The diet fed was a pelleted ration consisting of 49% barley, 40% alfalfa meal, 10% canola meal, and 1% premix containing Vitamins A and D each at 50,000 I.U./kg (Appendix 2). Lambs had free access to cobalt iodised salt and water throughout the trial. Between seven and nine weeks of age the lambs were allowed to adjust to the use of the automatic feeder. Live weight was measured weekly following overnight withdrawal of water. Balanced by weight, the lambs were allocated to control and growth hormone treatment groups at nine weeks of age. From the age of nine to twenty-two weeks the lambs received daily single subcutaneous injections of either sterile saline, pH 10.3 (control) or bovine pituitary extracted growth hormone (bpGH, 0.1mg/kg/day). Injections were administered in the axillary region at 1500h daylight savings time (DST) and the solutions for injection were freshly prepared each week and stored at 4C. The bpGH (lot#AFP-961413, The

Research and Education Institute Inc., Harbor UCLA Medical Centre, CA) was dissolved in sterile saline which had been adjusted to a pH of 10.3 by the addition of NaOH (Johnsson et al. 1985). The saline or bpGH was administered in a volume of 0.5ml and the dose of bpGH was calculated on the basis of the treatment group mean live weight measured three days prior to the beginning of each treatment week. The lambs were subjected to insulin infusion tests at eleven and 20 weeks of age, with the duration of the test periods being seven and nine days, respectively. For these tests, indwelling jugular catheters were inserted into the right and left jugular veins of each lamb. Catheters were again inserted (one per lamb) at 17 weeks of age and the lambs were repeatedly blood sampled for twelve hours. The lambs were slaughtered at 22 weeks of age. A single blood sample was collected from each lamb at slaughter for insulin receptor analysis.

Four lambs died during this trial due to heart valve infection. The first lamb died at 11 weeks of age, two at 12 weeks, and a fourth at 17 weeks of age. A fifth lamb was euthanised four days prior to the proposed date of slaughter due to refusal of feed and poor performance.

Insulin infusions and blood sampling

All blood samples, excluding the sample for insulin receptor analysis, were collected via the indwelling jugular catheters. Whole blood collected for serum (3ml) was withdrawn from the catheter and allowed to clot at room temperature. Upon retraction of the clot, the sample was centrifuged and the serum aspirated and subsequently stored at -20C for later analysis. Whole blood collected for plasma

(2ml) was withdrawn into syringes which contained heparin (0.5 U), inverted several times and transferred into ice chilled 10x75cm test tubes. Following centrifugation, the plasma was aspirated and stored at -20C for later analysis. At the time of ensanguination and slaughter, blood for insulin receptor analysis was collected into a 250 ml plastic bottle containing 10 ml of chilled anticoagulant (4.5% EDTA pH 7.4) and was stored at 4C until analysis the same day.

i)11th week insulin infusion

Indwelling jugular catheters were inserted into both the right and left jugular veins 24h prior to insulin infusion. Lambs were placed in individual holding crates 12h prior to infusion with free access to water until 2h prior to infusion. Access to feed was withheld from 12h prior to each infusion. Solutions were continuously infused into the left catheter at a rate of 0.37 ml/min using a Technicon Autoanalyser pump. Infusion duration was three hours, consisting of one hour of sterile saline infusion followed by two hours of insulin infusion. Blood samples for both serum and plasma were taken at times -40, -20, 0, 20, 40, 60, 80, 100, 120min, with time zero being the beginning of the insulin infusion. Blood samples obtained were analysed for insulin and glucose concentration. For this infusion series, six of the seven lambs from each treatment group were studied. Lambs were randomly assigned to either AM or PM infusion times, such that six lambs could be infused simultaneously and all twelve infusions could occur on the same day. Initially, lambs were to be infused with five doses of insulin over ten days of infusion with one recovery day scheduled between successive infusion days. On a given infusion day, all

animals, both AM and PM, received the same dose of insulin and doses were randomised across infusion days. Due to unforeseen difficulties, only 4 doses of insulin were administered. The doses of insulin administered were 0.2, 1.0, 6.0,and 30.0 mU/kg/min. Insulin (Sigma Chemical Co., St. Louis, MO, Cat# 1-550, lot no. 95F-0078) infusion solutions were freshly prepared in sterile saline each morning of infusion.

ii) 12 hour sampling period

The 12 hour sampling period occurred when the lambs were 17 weeks of age. Blood samples were taken for serum collection every 20 minutes for 12 hours, starting at 1300h DST. Samples for plasma were collected every two hours also commencing at 1300h. Due to catheterisation difficulties, only five bpGH treated and three control lambs were catheterised. Lambs were free roaming within the pen during sampling and were allowed normal access to water and feed during the sampling period.

iii)20th week insulin infusion

All procedures were the same as for the 11 week infusion series although the randomisation design was altered due to animal loss and design changes. All five lambs from each treatment group were used. Lambs were randomly assigned to one of two latin squares for infusion on alternate days. In order to randomise administration of insulin doses, a design of two independent 5x5 latin squares was used to administer five doses of insulin to five animals per day. Thus, animals from the two treatment groups were randomised into two groups, and insulin infusions were administered to each group on mornings of alternate days. The doses of insulin administered were

0.2, 0.5, 1.0, 6.0 and 30.0 mU/kg/min.

Slaughter and carcass evaluation

Lambs were separated randomly into two groups and one group was slaughtered on each of two consecutive days. Lambs were anaesthetised with Somnotol (M.T.C. Pharmaceuticals, Mississauga, Canada) at a dose of 0.2ml per kg live weight prior to ensanguination. Carcasses were dressed and weights of skin plus fleece, head, feet, full and empty gut, major organs, and selected fat depots were measured. Cold carcass weights were measured after hanging the carcass overnight at 4C. The 10th to 12th rib section from each carcass was removed following chilling and a trace of fat, lean and bone at the 10th rib was made onto acetate. Area (cm2) was later calculated for bone, ribeye, and fat using a digital planimeter (Tamaya Technics Inc. Tokyo, Japan). The rib section was then frozen at -20C until later thawing when a total dissection into fat, lean, and bone was made.

Hormone analysis

Serum samples were analysed in duplicate for insulin and growth hormone concentration according to the methods of Kennedy et al. (1988). Serum insulin was measured using a double antibody method, using iodinated porcine insulin, guinea pig-antibovine insulin as the first antibody and goat-anti-guinea pig serum as the second antibody. Serum growth hormone was measured using a double antibody method using iodinated ovine growth hormone (oGH), rabbit-anti-ovine growth hormone antiserum as the first antibody and sheep-anti-rabbit serum as the second antibody.

Plasma glucose analysis

Plasma glucose concentration was analysed in duplicate using the Technicon Autoanalyser neocuproine method.

Mononuclear leukocyte insulin receptor analysis

At slaughter, a 250ml blood sample was collected from all lambs into a plastic bottle containing 10ml of chilled anticoagulant (4.5% EDTA, pH 7.4). Mononuclear leukocyte insulin binding analysis was performed according to the methods described by Kennedy et al. (1987a) with the modification that ovine insulin standards were used at the final concentrations of .4, 1.1, 5.1, 10.1, 50.1, 100.1, 200.1, and 500.1 in addition to the total binding tube (tracer insulin only, .1 ng/ml) (Kennedy et al. 1988).

The bound to free ratio of iodinated insulin was calculated according to Scatchard (1949) and the analysis of the scatchard plots was computerised using the unconstrained fit method of Kahn et al. (1978) where the affinity of receptors when empty (Ke) and full (Kf) and number of receptor sites per cell were calculated assuming that there exists only one population of insulin receptors and that binding affinity decreases with increasing concentration of insulin (negative cooperativity).

Guelph beef trial

The Guelph beef trial was conducted in cooperation with Dr. Brian McBride, Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada. Twenty purebred Hereford steers were injected daily with either 25mg recombinant bovine growth hormone (rbGH, Cyanamid, 95% pure) or saline. Duration of the trial was 112 days. Animals were slaughtered as yearlings (as determined by

dentition). At slaughter, approximately 250ml of whole blood was collected in a bottle containing 10ml of chilled anticoagulant (4.5% EDTA, pH 7.4). Each sample was inverted prior to storage at 4C. On the day of collection, the blood samples were packed into insulated chests containing frozen ice packs for overnight shipment via courier to the University of Manitoba. Blood sample analysis commenced at 0700h the day following collection. Two samples were sent each working day over a period of two weeks. Isolation of mononuclear leukocytes and insulin receptor analysis were performed as described above.

Statistical analysis

Unless otherwise stated, the effect of treatment was analysed using the t-test procedure (Steel and Torrie 1980).

Individual lamb average daily gain was calculated by regressing weekly weight gain from day zero on time. Average daily gain was equal to the slope of this line. The difference between treatments was analysed using the t-test procedure. Initial and final live weights as well as total feed consumption and feed conversion efficiency were compared between treatments using the t-test. On several days the feeder malfunctioned and sheep were group fed. Thus, several days of feed intake data were missing. The missing feed data for these days were estimated by averaging individual feed consumption values for two days before and after the day for which feed data were missing. In addition, the feed intake for week seven was also unknown because of feeder malfunction. Thus, calculation of feed conversion (total feed (kg)/total gain (kg)) efficiency was based on all weeks except week seven. The effect of

treatment on carcass characteristics was evaluated using the t-test. Statistical comparisons between treatment groups were also made using component weights as a percent of carcass weight.

Treatment effect on serum GH concentrations was assessed by calculating an overall mean GH concentration and a mean pre-injection GH concentration. The individual animal means were subjected to the t-test procedure. The effect of treatment on overall plasma glucose concentration during the 12hr sampling period and the pre-infusion serum insulin concentrations for the 20th week insulin infusion series was tested using the t-test.

Basal glucose levels were calculated by averaging the pre-infusion glucose values. Response was calculated as the change from basal glucose level. Plasma glucose in response to insulin infusion did not uniformly reach an asymptotic level in all lambs. Therefore glucose results, both untransformed and log transformed for each animal dose combination, were subjected to a stepwise regression to find best-fit equations for the glucose response. Predicted glucose values, calculated for 60, 80, 100, 120 minutes insulin infusion at the five dose rates, were compared in saline and pbGH treated lambs using a General Linear Model procedure (SAS Institute Inc.) following a split plot design. As the high insulin doses (6.0 and 30.0 mU/kg/min) were included to assess maximal glucose response to insulin (responsiveness) and lower doses to assess submaximum response (sensitivity), results for the low (.2, .5, 1.0 mU/kg/min) and high doses of insulin were statistically analysed separately. Means for time were compared using the Newman Keul's test of mean differences (Steel and Torrie 1980). The low insulin dose of .5

mU/kg/min was not included at the 11th week infusion period.

Insulin binding to mononuclear leukocytes was compared in saline and bpGH treated lambs using a General Linear Model (SAS Institute Inc.) following a split plot design. Concentrations of insulin less than 5.1 ng/ml were analysed separately from concentrations greater than or equal to 5.1 ng/ml according to the methods of Kennedy et al. (1988).

Best fit values for Ke, Kf, and number of receptor sites per cell (negative cooperativity) were computed using the NLIN program (SAS Institute, Inc. 1985). Log transformations were performed on Ke, Kf, and number of receptor sites per cell in an attempt to reduce the differences in variation noted between groups.

Results

Hormone concentrations

The effect of daily pbGH injections on serum levels of immunoreactive GH is shown in Figure 1. Overall serum GH concentration in pbGH treated lambs was 16-fold greater ($P<0.01$) than that of saline treated lambs. Pre-injection serum GH concentration was also significantly ($P<.01$) higher in pbGH treated lambs (Table 1). Serum insulin concentration (Table 2) did not differ ($P\geq.05$) between treatment groups at 20 weeks of age.

Plasma glucose concentrations

Overall plasma glucose concentration did not differ significantly ($P\geq.05$) between treatment groups (Table 1).

Growth and performance

Performance results are shown in Table 3. Initial and final live weights did not differ ($P\geq.05$) between treatment groups. Average daily gain and feed conversion efficiency was similar in the two treatment groups ($P\geq.05$).

Carcass characteristics

Carcass characteristic means for bpGH and saline treated lambs are presented in Table 4. None of the carcass characteristics were significantly affected by treatment ($P\geq.05$). Mean organ weights are shown in Table 5. None of the organ weights were significantly affected by treatment ($P\geq.05$).

Glucose response to insulin infusions

Results of the analysis of variance for glucose response to four

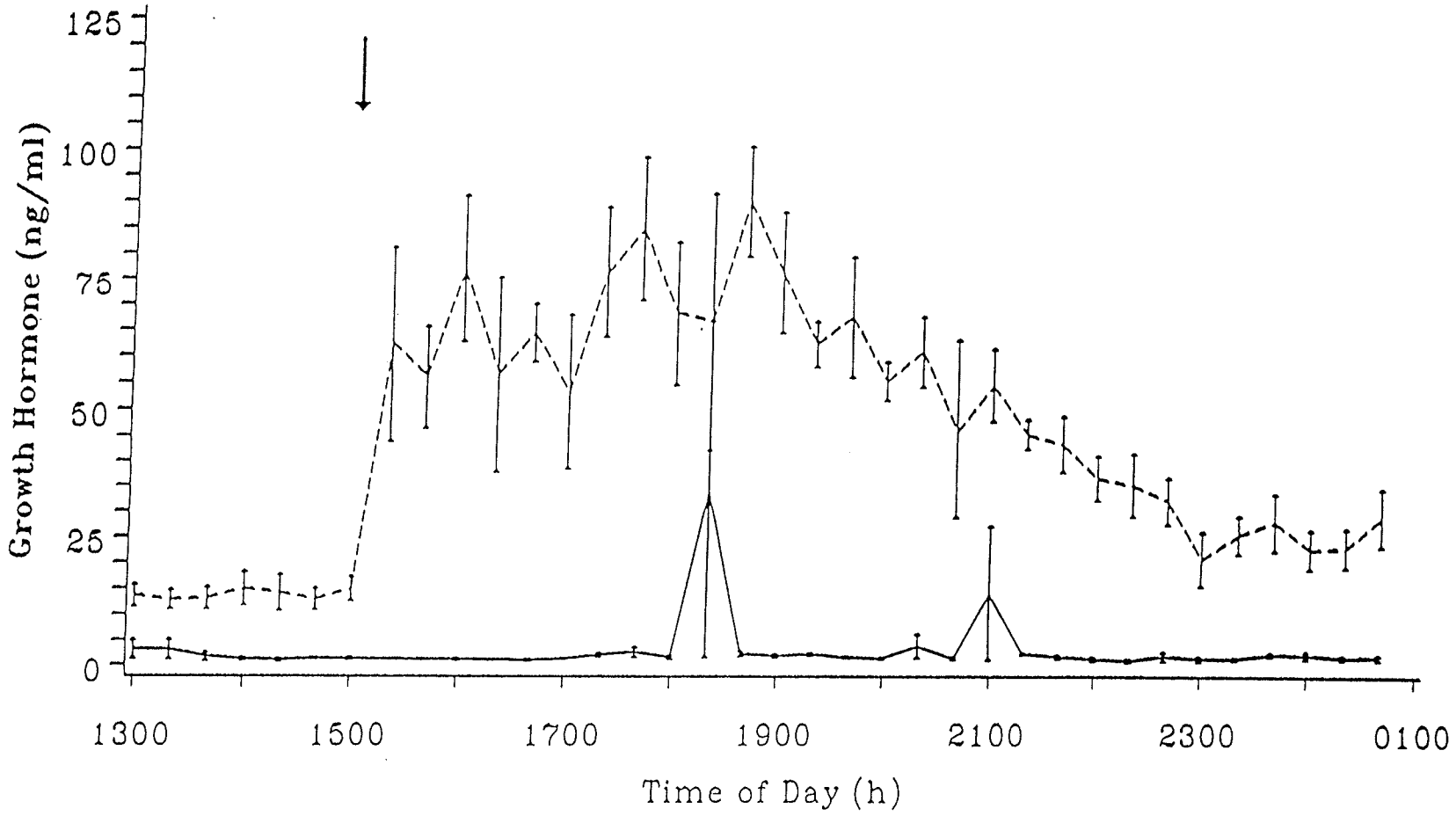

Figure 1. Circadian variation in serum concentrations of growth hormone at 17 weeks of age in intact male lambs receiving daily injections (↓) of pbGH (·---) at 1500h, compared with saline injected control animals (——). Values are the mean for five pbGH and three saline treated lambs

Table 1.
The effects of daily bpGH injections on serum levels of immunoreactive growth hormone and plasma glucose levels during a 12 hour period at 17 weeks of age.

	treatment		level of significance
	saline	bpGH	
GH preinjection (ng/ml)*	2.1±.7	13.6±2.2	P<0.01
GH overall (ng/ml)	2.8±1.3	49.3±3.3	P<0.01
glucose (mmol/dl)	4.47±.2	4.73±.2	ns

* preinjection means were calculated from samples collected at 20,40,60 80,100 and 120 minutes prior to injection of GH or saline

Table 2.
The effects of daily pbGH injections on serum concentration of immunoreactive insulin at 20 weeks of age in intact ram lambs.

	treatment		level of significance
	saline	pbGH	
insulin (ng/ml)	1.73±.2	1.95±.4	.64

Table 3.
The effects of daily bpGH injections on growth and feed intake in intact ram lambs.

	treatment *	
	saline	bpGH
initial live weight (kg)	14.8±1.6	14.0±1.3
final live weight (kg)	32.9±3.1	34.4±2.9
average daily gain (g/day)	266±27	268±25
total feed consumption (kg)	62.2±2.0	64.9±1.7
total gain (kg)	18.4±2.5	20.3±2.4
feed conversion efficiency ** (kg feed/kg gain)	4.0±.6	3.8±.6

* treatment means were nsignificantly different (P>.05)
** feed conversion results do not include week 7 data

Table 4.
The effect of daily bpGH injections on carcass characteristics of ram lambs at 22 weeks of age.

	treatment *	
	saline	bpGH
hot carcass weight (kg)	15.0±1.6	15.3±3.5
cold carcass weight (kg)	14.5±1.6	14.7±1.5
dressing percentage (hot weight/live weight)	44.8±1.4	43.8±.8
omental fat (g)	344±86	252±41
kidney fat (g)	105±39	166±28
channel fat (g)	50±11	43±10
10th to 12th rib section dissection		
total weight (g)	263±36	314±44
bone (g)	83±10	96±10
ribeye (g)	66±6	75±8
other lean (g)	59±10	63±10
fat (g)	55±12	80±22
area of 10th rib side		
total (cm2)	32.7±2.8	35.2±2.2
ribeye (cm2)	9.9±.9	11.3±.7
bone (cm2)	1.4±.6	1.2±.2
fat (cm2)	21.4±2.4	22.7±1.7

* treatment means were not significantly different (P>.05)

Table 5.
The effects of daily bpGH injections on selected organ weights of ram lambs at 22 weeks of age

	treatment *	
	saline	bpGH
full gut (kg)	7.4±.7	7.9±.5
full rumen (kg)	4.8±.6	4.9±.4
full intestine (kg)	2.7±.2	3.1±.2
empty rumen (kg)	0.9±.1	1.0±.1
empty intestine (kg)	1.2±.1	1.4±.1
liver (kg)	.8±.1	.9±.7
spleen (g)	105±15	161±29
heart (g)	194±21	196±13
lung (g)	442±36	458±50
testis (g)	104±25	87±30
kidney (g)	136±11	134±11
pancreas (g)	42±7	42±4
adrenal (g)	2.8±.3	2.8±.1
head (kg)	1.5±.2	1.3±.1
blood (kg)	1.2±.1	1.5±.1
greasy fleece (kg)	4.1±.5	4.4±.5
feet (kg)	.9±.1	.8±.2

* treatment means were not significantly different (P>.05)

insulin doses at the 11th week infusion period are given in Table 6. Animal within treament effect was significant (P<.01)for both the low and high doses of insulin. For low doses of insulin the glucose response was markedly influenced by dose (P<.001) but the effect of treatment and the dose x treatment interaction was not significant. At high insulin doses the effects of dose were significant (P<.01) with the glucose response to the 30.0 mU/kg/min dose less than the response to the 6.0 mU/kg/min dose. However, the dose x treatment interaction approached significance (P=.059). Figure 2 shows the plasma glucose LS means +SE for treatment x dose interaction at both high and low doses. At the high doses of insulin the control lambs exhibited a greater response to insulin than bpGH treated lambs but only when the dose rate was 30.0 mU/kg/min. Thus the response to 30.0 mU/kg/min was less than the response to 6.0 mU/kg/min in bpGH but not saline treated lambs. Time was a significant factor at the low doses of insulin (P<.01) but not at the high doses of insulin (P≥.05). For low doses the response increased with increased time.

Table 7 shows the results of the analysis of variance for glucose response to insulin at the 20th week infusion period. Treatment had no effect (P≥.05) on glucose response to insulin. Animal within treatment was significant at both the low doses (P<.01) and the high doses of insulin (P<.01). Dose significantly affected glucose response to insulin at both low and high insulin infusion rates in that response increased with dose (P<.001). Treatment x dose interaction was significant at the low doses of insulin (P<.01) and the high doses of insulin (P=.052). Figure 3 shows the plasma glucose LS means +SE for treatment x dose interaction at both low and high doses of insulin. Within the low insulin dose range, the glucose

Table 6.
LS means ±SE for plasma glucose response* to four insulin doses grouped by insulin dose: low doses(.2, 1.0 mU/kg/min) and high doses (6.0, 30.0 mU/kg/min) at the 11 week infusion period.

	low doses of insulin	high doses of insulin
treatment **		
saline	-1.4±.3	-2.6±.3
bpGH	-1.5±.3	-2.2±.3
dose (mU/kg/min)		
.2	-1.2±.1	-
1.0	-1.7±.1	-
6.0	-	-2.5±.1
30.0	-	-2.2±.1
time (min)		
60	-1.1±.1a	-2.3±.1a
80	-1.3±.1ab	-2.5±.1a
100	-1.5±.1bc	-2.5±.1a
120	-1.8±.1c	-2.3±.1a
level of significance		
treatment	.71	.37
animal(treatment)	.0001	.0001
dose	.0001	.0007
treatment x dose	.63	.059
time	.0001	.3
treatment x time	.78	.96
dose x time	.97	.27
treatment x dose x time	.99	.48

* response was calculated as preinfusion plasma glucose -plasma glucose at specific times during insulin infusion (mmol/dl plasma) (see materials and methods).
** seven lambs per treatment were infused
a,b,c: means within a column followed by similar letters were not significantly different.

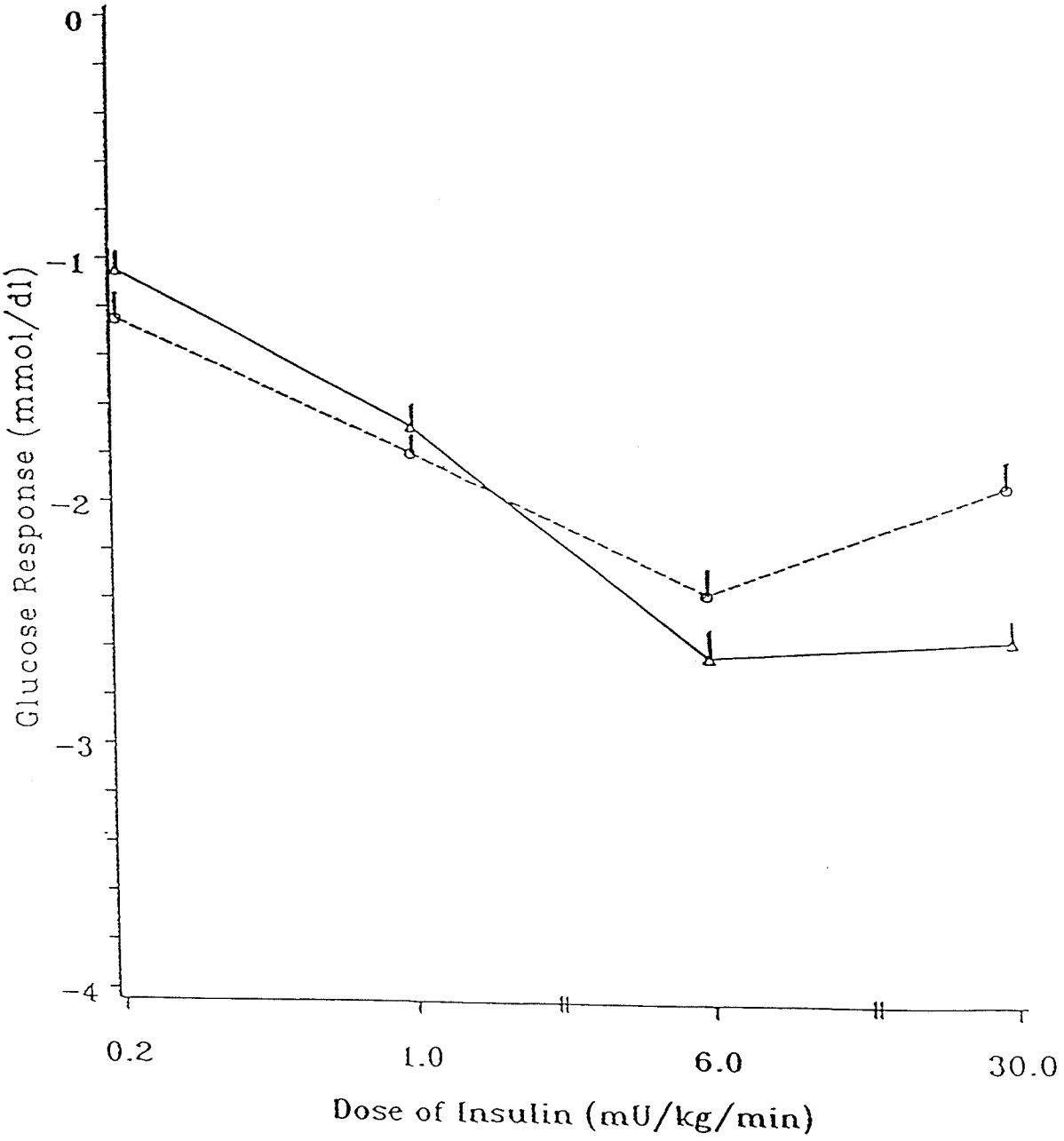

Figure 2. Treatment x dose of insulin interaction LSmeans +SE for glucose response to insulin infusion in intact male lambs at 11 weeks of age. bpGH (-o-) saline (-△-)

Table 7.
LS means ±SE for plasma glucose response* to five insulin doses grouped by insulin dose: low doses(.2, .5, 1.0 mU/kg/min) and high doses(6.0, 30.0 mU/kg/min) at the 20 week infusion period.

	Low doses of insulin	High doses of insulin
treatment **		
saline	-.9±.2	-2.8±.2
bpGH	-.9±.2	-3.1±.2
dose (mU/kg/min)		
.2	-.5±.1	-
.5	-1.0±.1	-
1.0	-1.2±.1	-
6.0	-	-2.7±.1
30.0	-	-3.3±.1
time (min)		
60	-.6±.1a	-2.7±.1a
80	-.8±.1ab	-3.1±.1b
100	-1.0±.1b	-3.2±.1b
120	-1.1±.1b	-3.1±.1b
level of significance		
treatment	.97	.16
animal(treatment)	.0002	.0001
dose	.0001	.0001
treatment x dose	.0001	.052
time	.0065	.003
treatment x time	.94	.89
dose x time	.91	.75
treatment x dose x time	.99	.73

* response was calculated as preinfusion plasma glucose - plasma glucose at specific times during insulin infusion (mmol/dl plasma) (see materials and methods)
** five lambs per treatment were infused
a,b,c: means within a column followed by similar letters were not significantly different.

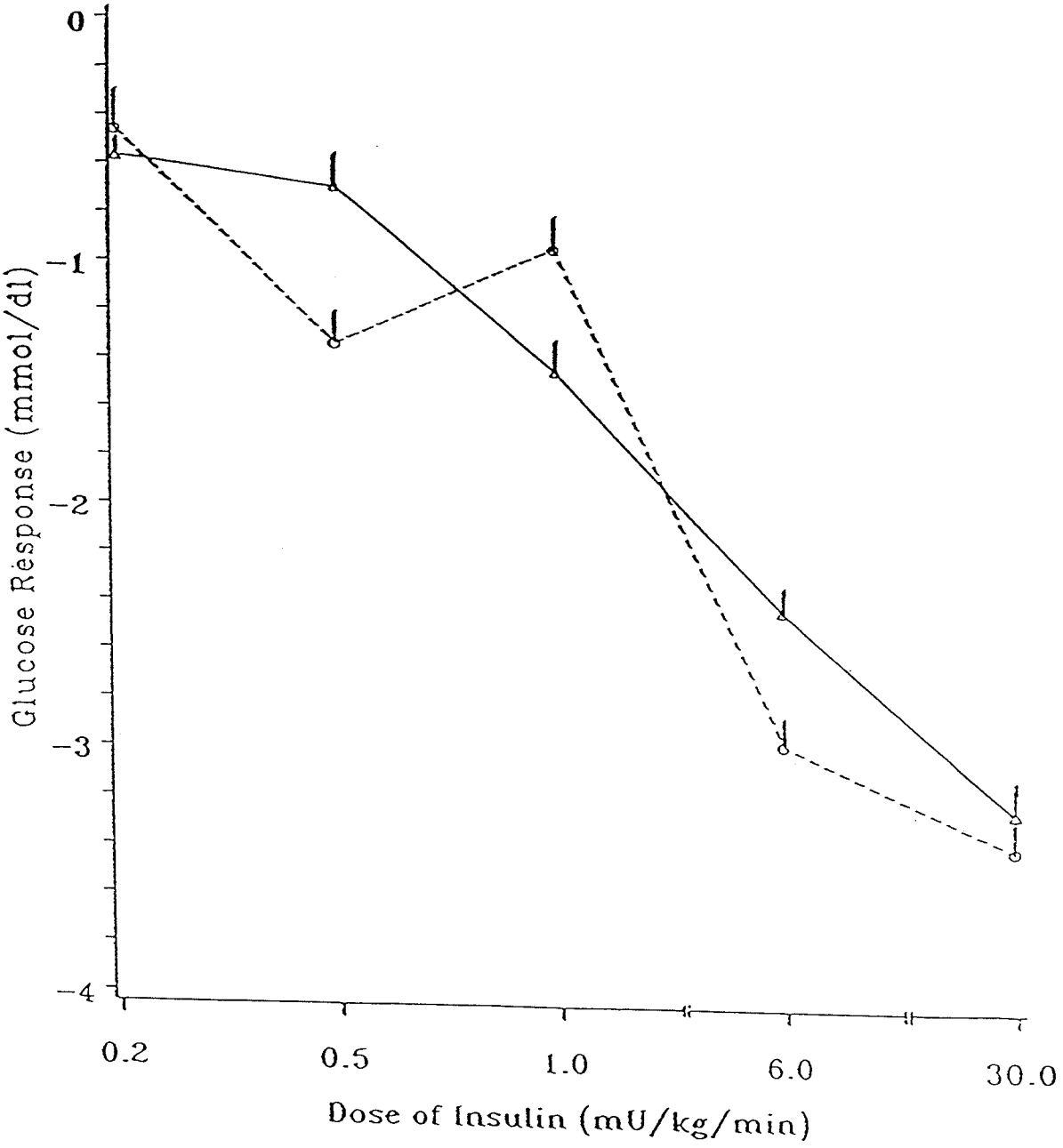

Figure 3. Treatment x dose of insulin interaction LSmeans+SE for glucose response to insulin infusion in intact male lambs at 20 weeks of age. bpGH (-o-) saline (-o-)

response to insulin at dose 0.2 and 1.0 mU/kg/min was greater for controls than for bpGH injected lambs, but not at the dose rate of .5 mU/kg/min. For the higher insulin doses (6.0 and 30.0 mU/kg/min), bpGH injected lambs exhibited greater glucose response to insulin than controls but only at the dose rate of 6.0 mU/kg/min. Time significantly affected glucose response to insulin at both low ($P<.01$) and high ($P<.01$) doses of insulin. The magnitude of the response increased steadily with time at low insulin dose rates but remained constant after 80 minutes of infusion at the high dose rates.

Mononuclear leukocyte insulin receptor analysis

Insulin binding curves for control and bpGH treated lambs are shown in Figure 4. At insulin concentrations $\geq$ 5.1 ng/ml ($\geq$.73 in log10) binding decreased with increasing concentration of insulin as was indicated by a significant ($P<.01$) concentration effect. At insulin concentrations $\geq$ 5.1 ng/ml the effect of bpGH treatment on percent binding approached significance ($P\leq.059$) but treatment x dose effect was nonsignificant. Thus the overall binding for bpGH treated lambs tended to be greater than that of saline treated lambs at concentrations insulin greater or equal to 5.1 ng/ml. At insulin concentrations less than 5.1 ng/ml (.1, .4, 1.1 ng/ml), treatment, concentration of insulin and the interaction of treatment x concentration had no significant ($P\geq.05$) effect on percent binding.

Results for the negative cooperativity analysis of lamb insulin binding data are shown on Table 8. There appears to be a trend toward a higher number of receptor sites in lambs treated with bpGH. However, the variation was large and no significant treatment

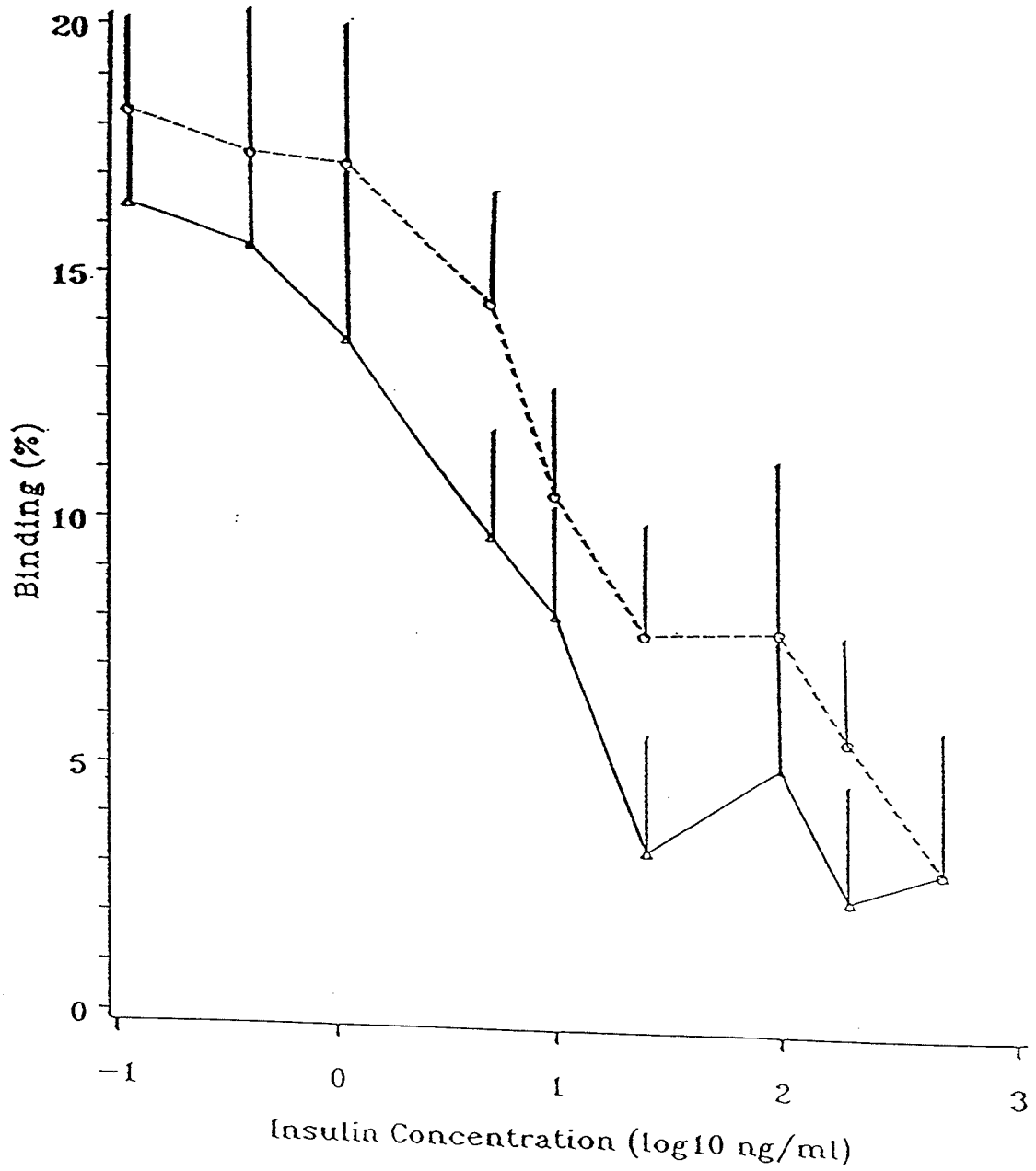

Figure 4. LSmeans ±SE for insulin binding to mononuclear leukocytes at selected insulin concentrations in intact male lambs receiving daily injections of bpGH (-o-) or saline (-Δ-)

Table 8.
LS means ±SE mononuclear leukocyte insulin receptor binding characteristics in ram lambs at 22 weeks of age as derived by negative cooperativity analysis.

	treatment *		
	saline	bpGH	level of significance
sites/cell (x10E3)	58.4±34.8	97.7±59.4	.58
binding affinity			
Ke (x10E8 Mol-1)	1.1±.6	1.4±1.1	.78
Kf (x10E6 Mol-1)	12.5±12.2	15.2±14.9	.89
Kf/Ke	.04±.03	.03±.02	.91
log sites/cell	4.5±.2	4.7±.2	.59
logKe	-2.0±.2	-2.1±.3	.82
logKf	-4.1±.5	-3.9±.6	.90
logKf/Ke	-2.1±.3	-1.9±.3	.65

* five lambs per treatment

effect was observed ($P \geq .05$). Other parameters from the negative cooperativity analysis were not significantly ($P \geq .05$) affected by bpGH treatment.

Guelph beef trial

Although no individual animal data are presented, growth effects of rbGH were obtained via personal communication with Dr. B. McBride. Treatment of steers with rbGH significantly ($P \geq .05$) increased rate of gain and feed efficiency by 20% and reduced the carcass fat/lean ratio.

Insulin binding curves for steers treated with either GH or saline are given in Figure 5. At insulin concentrations less than 5.1 ng/ml (.1, .4, 1.1 ng/ml) treatment with rbGH significantly ($P < .05$) reduced percent binding and the effect of insulin concentration on binding approached significance ($P \leq .06$). At insulin concentrations greater or equal to 5.1 ng/ml there was no treatment effect ($P \geq .05$), although binding did decrease ($P < .01$) with an increase in insulin concentration.

Results for the negative cooperativity analysis of binding data for steers are given on Table 9. There appears to be a trend towards higher number of receptor sites in steers treated with rbGH, although the variation in rbGH treated animals was large and no significant treatment effect was observed ($P \geq .05$). Other parameters from the negative cooperativity analysis were also not significantly ($P \geq .05$)affected by rbGH treatment. However, affinity of receptors when full (Kf) tended ($P = .13$) to be reduced, and sites per cell tended ($P = .16$) to be elevated in rbGH treated steers.

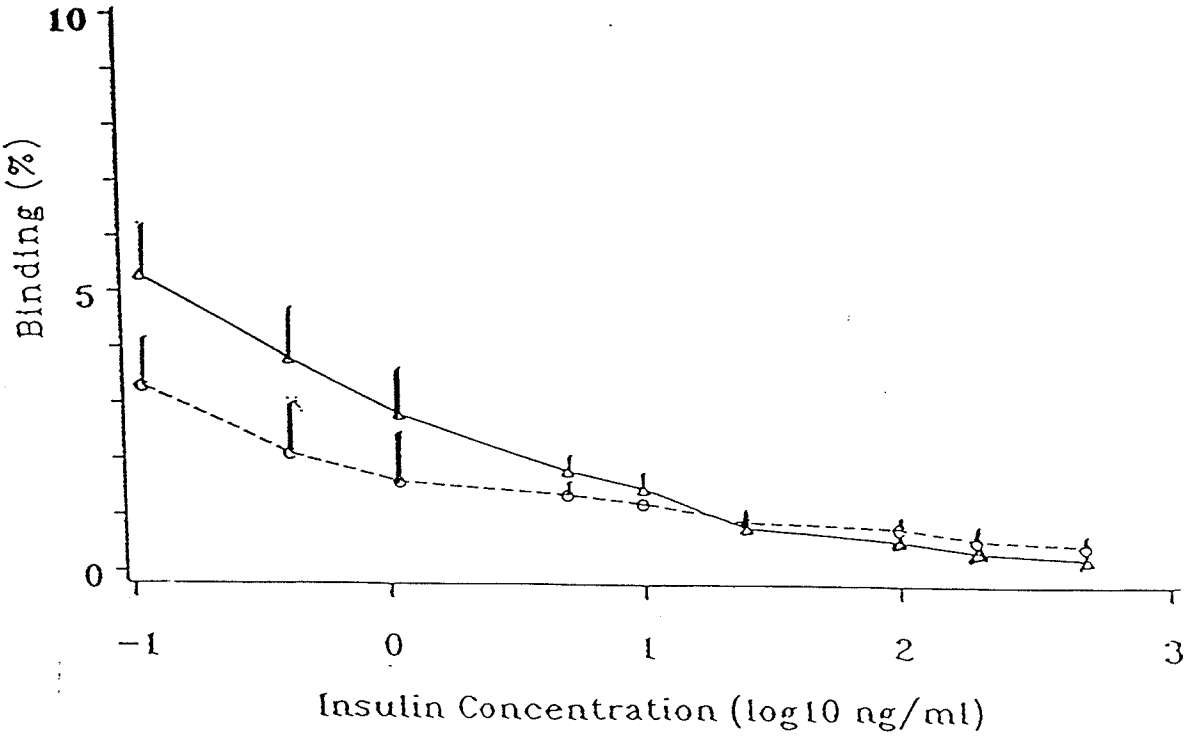

Figure 5. LSmeans+SE for insulin binding to mononuclear leukocytes at selected insulin concentrations in beef steers receiving daily injections of rbGH (-o-) or saline (—o—)

Table 9.
LS means ±SE mononuclear leukocyte insulin receptor binding characteristics in beef steers as derived by negative cooperativity analysis.

	treatment *		
	saline	rbGH	level of significance
sites/cell (x10E3)	15.9±2.5	45.9±20.2	.16
binding affinity			
Ke (x10E8 mol-1)	.71±.27	.48±.22	.51
Kf (x10E6 mol-1)	.35±.12	.13±.06	.13
Kf/Ke	.006±.001	.003±.001	.15
log sites/cell	4.15±.08	4.36±.31	.32
logKe	-2.27±.21	-2.62±.25	.32
logKf	-4.58±.22	-5.21±.31	.12
logKf/Ke	-2.33±.08	-2.59±.13	.08

* ten steers per treatment

DISCUSSION

Serum hormone and glucose concentrations.

Dose of bGH (.1mg/kg/day), methods of administration and dilution procedures used in this experiment were similar to those described by Johnsson et al. (1985). All GH radioimmunoassays (RIA) were conducted using RIA grade ovine growth hormone (oGH) for standard curve construction. Available information (NIADD) reports cross reactivities to be 100% for bpGH in the ovine RIA used in this study. Thus it was concluded that the ovine RIA would be appropriate for the determination of both exogenous and endogenous bpGH in lamb serum.

Serum levels of immunoreactive growth hormone found in bpGH treated lambs are in agreement with those reported in other studies where bpGH has been administered to growing lambs. Muir et al. (1983) administered once daily injections of bpGH (7mg) and reported serum GH levels to be elevated by 1700% compared to controls for 24h post injection. Johnsson et al. (1985) reported 5-fold elevations of serum GH when calculated for 28h post injection. The seemingly lower GH elevation reported by Johnsson et al. (1985) compared to the present study was likely due to the fact that GH levels were reported for a longer period than in the present study. Johnsson et al. (1987) using rbGH at a dose the same as used in the present study (.1mg/kg/day) reported 24 hour post injection mean GH concentration to be 8 times that of controls. The elevation in serum GH level found in the present study (6 fold increase pre-injection, 17 fold increase over 10hr post injection) appears similar to that found in comparable studies. Mean GH concentrations of control lambs in the present trial were similar to the concentration of serum GH in

growing intact male Suffolk lambs (Kennedy et al. 1988).

As found in the present trial, Johnsson et al. (1987) found no effect of exogenous GH at a dose of .1 mg/kg/day on plasma glucose. However at a dose of GH (.25 mg/kg/day) larger than that used in the present trial Johnsson et al. (1987) found GH treated lambs had significantly higher glucose concentrations than that of controls. Wagner and Veenhuizen (1978) also reported a glucose elevating effect of exogenous GH. Hyperglycemia was also found in growing barrows treated with GH (Boyd et al. 1987). In two of the trials where an increase in plasma glucose concentrations was observed, GH also resulted in concurrent elevations in serum insulin levels (Johnsson et al. 1987, Wagner and Veenhuizen 1978) suggesting that growth hormone exerted a negative influence on the ability of insulin to stimulate glucose removal from the plasma. Also Johnsson et al. (1987) found that rGH at .1 mg/kg/day caused a doubling of serum insulin in lambs. However, in the present study, neither plasma glucose nor serum insulin concentrations was affected by exogenous GH administration.

Growth and performance

Growth responses to exogenous GH in ruminants have been varied. Exogenous GH has been reported to improve average daily gains (Pullar et al. 1986) and feed conversion efficiency (Wagner and Veenhuizen 1978, Muir et al. 1983, Johnsson et al. 1985). Exogenous GH has also been reported to have no effect on growth rate (Muir et al. 1983, Johnsson et al. 1987) or feed efficiency (Johnsson et al. 1987). This variability in response may be due in part to inconsistencies

in the experimental designs used in the study of the effects of exogenous GH.

In this study, initial live weights of the treatment groups were similar as were final live weights. Average daily gains for the two treatment groups were similar as well. Thus there was a lack of effect of exogenous growth hormone on the growth of the lambs. The lack of effect of GH in this trial may have been due to protocol differences between this study and others where growth effects of growth hormone have been observed (Johnsson et al. 1985, Wagner and Veenhuizen 1978).

Kennedy and Belluk (1987) and Johnsson et al. (1985) reported ad libitum feed intakes for Suffolk ram lambs and female crossbred lambs, respectively, to be higher than those reported in this study. The energy in the ration was determined to be limiting (90% of NRC recommendations for lambs with high growth potentials (appendix 2). It appears that feed consumption in this study was limited due to the use of computer feeding. In other growth trials assessing the effect of exogenous growth hormone there has been ad libitum access to feed (Johnsson et al. 1985, Johnsson et al. 1987, Muir et al. 1983) and feed intake was not affected by exogenous growth hormone administration. However, a feeding limitation imposed by use of the automatic feeder in this trial may have masked some of the effects of exogenous GH administration. This may also have been the case in the study of Johnsson et al. (1987) where intake of all crossbred female lambs studied was less than that found in a previous similar trial (Johnsson et al. 1985). Another difference between this study and others involving exogenous growth hormone that may have had an effect on growth responses is the sex of animal that was used. In recent

studies the experimental animals have either been females or castrated males (Fabry et al. 1987, Johnsson et al. 1985, Johnsson et al. 1987, Muir et al. 1983, Wagner and Veenhuizen, 1978) . In one study where intact male lambs were used, a 30% increase was reported on growth rate but no effect on carcass composition was observed (Pullar et al. 1986). Intact male lambs have been observed to exhibit higher serum growth hormone concentrations than either females or castrated males (Davis et al. 1977, Davis et al. 1984). The fact that intact males have higher serum growth hormone concentrations and exhibit superior growth rates as compared to females and castrated males suggests that intact males may be growing at a maximal rate. It is possible that the higher levels of growth hormone present in the intact male allows a growth rate and composition of tissue deposition which can only be moderately altered with administration of additional growth hormone. It has been shown in studies involving growing swine that the optimum doses of GH to maximise growth, feed intake, and feed to gain ratios are not identical (Boyd et al. 1987).

The levels of immunoreactive bpGH as measured over the 12 hour sampling period indicate that injection of GH elevated immunoreactive serum GH levels. However, immunoreactivity does not necessarily indicates biological activity (Hadley 1984), thus the lack of effect of the bpGH could be due to a lack of biological activity. Whether the bpGH as received had no activity, whether activity was affected by weekly storage of the diluted hormone, or whether immunologic responses to the bpGH altered the effectiveness of bpGH are unknown, but these possibilities must be considered.

Carcass characteristics

Carcass responses to exogenous GH in ruminants have been relatively uniform. Wagner and Veenhuizen (1978) found daily injections of bpGH increased protein gain and decreased fat gain. Muir et al. (1983) reported daily injections of GH decreased carcass fat content. Daily injections of GH increased carcass lean (Johnsson et al. 1985) and in another study increased carcass lean and decreased carcass fat (Johnsson et al. 1987). However, Pullar et al. (1986) using intact males reported exogenous GH had no effect on carcass lean or fat. In this study, using intact ram lambs as did Pullar et al. (1986), there was also found to be no effect of treatment on carcass characteristics studied. The apparent lack of effect of bpGH in this study may be due to the feed restriction imposed by the automatic feeder as previously discussed, or more likely may be the effect of testicular hormones secreted by the intact ram lambs. The lambs may already have been depositing lean and fat in proportions unalterable by administration of exogenous bpGH.

Glucose response to insulin infusion

Changes in insulin sensitivity in terms of insulin dose response curves are characterised by a change in response to insulin at physiological levels of the dose response curve with no change in maximal insulin action. Changes in sensitivity are thought to be due to change in numbers of insulin receptors (Kolterman et al. 1982). The numbers of receptors is thought not to affect maximum response because of the existence of spare receptors. Hoffman et al. (1980)

reported maximal response to insulin in hepatoma cells at less than 1% receptor occupancy; sugesting that 99% of the insulin receptors were spare. Changes in responsiveness to insulin are thought to be due to post receptor defects in insulin action resulting in an upward or downward shift in the dose response curve at high insulin concentrations (Kolterman et al. 1982).

At both the 11th and 20th week infusion periods, and at both high and low doses of insulin, glucose response to insulin increased with increasing dose of insulin in all cases but the 11th week infusion period where response to the insulin dose of 6.0 mU/kg/min (-2.5±.1 mmol/dl) was greater than response to the dose of 30.0 mU/kg/min (-2.2±.1 mmol/dl). This dose effect was more pronounced in the control lambs. Brockman (1983) reported that insulin normally supresses the rate of appearance of endogenous glucose in sheep and that this effect is reduced during insulin induced hypoglycemia. In monogastrics (Ganong 1981) as well as in young ruminants (Edwards 1969) hypoglycemia increases adrenocortical secretion resulting in increased adrenal medullary adrenalin secretion. Both adrenalin and glucocorticoids act to alleviate the hypoglycemic state, the adrenalin initiates glycogenolysis, and the glucocorticoids increase gluconeogenesis. It is possible that at the highest dose of insulin (30.0 mU/kg/min) a hypoglycemic threshold was attained for bpGH lambs and resistance to insulin induced hypoglycemia was exhibited by the lambs through an elevation of endogenous glucose output.

Based on published results (Weekes et al. 1983) demonstrating insulin dose response curves of sheep, it was expected that the

maximal response of glucose would be observed at the two highest doses of insulin studied. At 11 weeks of age the dose of 6.0 mU/kg/min gave a response equal to 30 mU/kg/min in control lambs but greater than 30 mU/kg/min in bpGH lambs. At 20 weeks of age the response to 30 mU/kg/min insulin was greater than the response to 6.0 mU/kg/min insulin infusion, especially in control lambs. The dose yielding maximum response is thus age and treatment group dependent. Time of infusion may also have been important in this respect in that at 11 weeks of age, maximum response was achieved at 60 minutes of infusion but not until 80 minutes of infusion at 20 weeks of age.

Time also had a significant effect on glucose response at low insulin doses at the 11th and 20th week infusion periods. Initially, the experimental design had not considered viewing the time intervals as separate entities, but the failure of glucose response to reach asymptotic levels necessitated such analysis. Over increasing time of insulin infusion, glucose response to low infusion doses increased as well such that response at 100 minutes of infusion was greater than at 60 minutes of infusion. However, response at 120 minutes of infusion equalled that at 100 minutes. The failure of glucose response to continue in a decreasing manner after 100 minutes of infusion may well be explained by the hypoglycemia threshold theory presented earlier, such that minimal physiological permissible levels of glucose were reached and the lamb was acting in a manner to counteract hypoglycemia. The overall significance of time as a factor in increasing glucose response to insulin infusion is physiologically sound as the infused insulin requires time to mediate its homeostatic effects on glucose.

The overall effect of treatment on glucose response to insulin

infusion was not significant for either infusion period. However, the treatment x dose interaction was significant for the low doses of insulin at week 20 and approached significance at high insulin doses for both week 11 and week 20.

The treatment differences between low and high doses of insulin relate to bpGH induced changes in insulin sensitivity and responsiveness. For low dose rates in the 11th week infusion period the effect of treatment and the treatment x dose interaction were not significant. Thus there was no effect of bpGH on sensitivity to insulin at 11 weeks of age. For the high dose rates in the 11th week infusion period, the treatment x dose interaction was significant indicating bpGH caused a decrease in responsiveness to insulin. In other studies involving growth hormone in monogastrics (Bratusch-Marrain et al. 1984), it appeared that growth hormone acted to cause post-receptor defects, and to thus reduce responsiveness to insulin. On the basis of this theory, growth hormone may exert diabetogenic effects and thus halt glucose uptake in some tissues in lambs. However, this interpretation must be considered with considerable caution, as the treatment x dose effect only approached significance.

Treatment x dose interaction with respect to glucose response to insulin infusion at the 20th week infusion period was different from that found at the 11th week infusion period. In control lambs the glucose response increased with increasing dose. However, in bpGH treated lambs the response to 1.0 mU/kg/min was less than the response to .5 mU/kg/min. It is possible that counterregulatory effects of adrenalin or glucocorticoids were exaggerated in bpGH treated lambs receiving insulin at the dose of 1.0 mU/kg/min. At the

dose of 6.0 mU/kg/min insulin, the bpGH treated lambs exhibited greater responsiveness than controls but this was not seen at 30.0 mU/kg/min. Counterregulatory effects of adrenalin or glucocorticoids may have masked an effect of bpGH on responsiveness as measured using the 30.0 mU/kg/min dose rate. For both control and bpGH treated lambs plasma glucose continued to fall with doses up to 30.0 mU/kg/min. Thus it cannot be concluded that the dose of 30.0 mU/kg/min yielded maximum response. Therefore, over the dose rates selected, responsiveness may not have been observed. In examining the entire dose response curves at 20 weeks of age it appears that bpGH treated lambs tended to be more sensitive than control lambs, with the exception found at the dose rate of 1.0 mU/kg/min. It is possible that bpGH treated lambs at 20 weeks of age are more sensitive to insulin (as indicated for the dose of 0.5 mU/kg/min) but that counterregulatory mechanisms mask differences in sensitivity and/or responsiveness at higher dose rates. The use of glucose clamps (Weekes et al. 1983) to study insulin sensitivity and responsiveness would be more informative, as counterregulatory mechanisms are not induced with this experimental technique.

Glucose response to insulin at 11 and 20 weeks of age could not be compared statistically because different experimental protocols were used for these two periods. However, several differences seem evident when visually comparing the response curves for these two periods (Figures 2 and 3). Younger lambs were generally more sensitive but less responsive than older lambs. In the younger lambs bpGH reduced the responsiveness and it may be that higher endogenous GH levels of young lambs (Davis et al. 1984) cause reduced responsiveness. It could also be interpreted that young

lambs (due to endogenous GH) have more active counterregulatory mechanisms as has been suggested by Edwards (1969). bpGH appeared to increase sensitivity in the older lambs and the older lambs appeared less sensitive than young lambs. The fact that the lambs may not have exhibited maximal response to insulin at the high doses of insulin at 20 weeks of age suggests that only sensitivity was studied and doses higher than 30 mU/kg/min would be needed to study responsiveness in older lambs. This in itself suggests that the maximum response (responsiveness) of older lambs must have been greater than younger lambs.

Mononuclear leukocyte insulin binding

Analysis of insulin binding was conducted using the negative cooperativity model, as there is biological evidence for the existence of insulin receptor negative cooperativity (DeMeyts et al. 1973). Specific binding data were also analysed as predicted values resulting from the use of models such as the negative cooperativity model have been criticised (Klotz 1982) for increasing variability in insulin receptor characteristics.

Maximum mononuclear leukocyte insulin binding for control lambs in the present study was comparable to that reported by Kennedy et al. (1988) for intact Suffolk ram lambs. Insulin binding to many tissues of monogastrics has been reported to decrease with increases in body fat content (Etherton 1982). Considering the negative effect that growth hormone has been found to exhibit with respect to carcass fat content (Wagner and Veenhuizen 1978, Johnsson et al. 1985), it would be expected that exogenous GH would increase insulin receptor binding. Kennedy and Belluk (1987) found intact Suffolk ram lambs

had less percent kidney fat than Strain2 (50% Finnish Landrace, 25% Suffolk, 25% Shropshire) lambs. The Suffolk lambs had higher overall serum GH concentrations and a higher percent insulin binding to mononuclear leukocytes than Strain2 lambs at low physiological concentrations of insulin with breed differences becoming less evident at higher insulin concentrations (Kennedy et al. 1988). In the present study, treatment of lambs with bpGH did not affect percent insulin binding to mononuclear leukocytes at physiological concentrations of insulin, but did significantly increase percent binding at insulin concentrations greater than 5.0 ng/ml. Negative cooperativity analysis results suggest that receptor number and insulin binding affinity were not significantly affected by treatment, but there was a trend towards increased receptor number in bpGH treated lambs, which could partially account for increased insulin binding in bpGH treated lambs. Kennedy et al. (1988) also were unable to explain mononuclear leukocyte binding differences on the basis of negative cooperativity analysis results.

The effects of rbGH injections on receptor parameters in beef steers was different than the response to bpGH in lambs. Percent binding in beef steers at the lowest concentration of insulin was about one third that of dairy cows (Kennedy et al. 1987) and Suffolk lambs but similar to that of the Strain2 lambs studied by Kennedy et al. (1988). At physiological concentrations of insulin, rbGH treatment resulted in decreased insulin binding to mononuclear leukocytes. Negative cooperativity analysis suggested a trend towards increased receptor number in rbGH animals but also suggested that decreased receptor affinity may account for lower percent

insulin binding to mononuclear leukocytes in rbGH treated steers. Studies with monogastrics have reported a good correlation between elevated serum GH levels and a decrease in monocyte receptor numbers and an increase in receptor affinity. In the present study, between animal variation was large and although there was a trend towards an increase in receptor number in both steers and lambs due to GH, the effects were not significant.

SUMMARY AND CONCLUSION

Daily injections of bpGH in intact ram lambs were effective in raising serum levels of immunoreactive GH. However, the bpGH had no effect on growth rate, feed efficiency or carcass composition of intact ram lambs. It appears that the proper explanation for the apparent lack of effect of exogenous bpGH administration in growing ram lambs is that intact male ruminants have higher endogenous serum concentrations of GH than castrates. Considering that all but one of the studies which report anabolic effects of growth hormone use either castrate or female animals, exogenous GH may not be capable of such dramatic alterations in intact males. Perhaps as well, the feed restriction imposed by the automatic feeder limited fat deposition and masked any fat altering effects of the exogenous GH.

Insulin physiology was affected in that bpGH injected lambs exhibited differences in insulin responsiveness and sensitivity as compared with controls; however, the results were not consistent between infusion periods. At the 11th week infusion period the effect of bpGH was to reduce response to insulin at the dose of 30 mU/kg/min which was the highest dose studied. Lambs at 11 weeks of age appeared more sensitive and less responsive to insulin compared to lambs at 20 weeks of age. At the 20 week infusion period, response generally fell with increasing dose of insulin thus a maximum response was not observed. Sensitivity at doses studied appeared greater in GH animals at doses .5, 6, and 30 mU/kg/min but not at 1.0 mU/kg/min. Percent insulin binding to mononuclear leukocytes was also affected by GH treatment in both intact ram lambs and steers. Steers had percent binding one third that of the lambs. Treatment with GH increased percent binding in lambs but decreased

percent binding in steers. Negative cooperativity analysis indicated no effect of GH treatment.

Further studies evaluating response to exogenous growth hormone in both intact and castrate males as well as further studies into the aspects of insulin physiology affected by exogenous GH are required, both to determine the future of GH as a ruminant growth promotant and to assess the mode of action of growth hormone.

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APPENDICES

APPENDIX 1

Automatic feeding system

Several alterations were made to the Westfalia feeder system in order to accommodate growing lambs. The portion dispenser volume was decreased so as to dispense 25g of pelleted feed. Feeder bowl height was lowered to 30cm and feeder bowl depth was decreased to a depth of 12cm. A chute to allow for single lamb feeding was designed for the access portion of the feeder. The dimensions of the chute were 60cm in height and 105cm in length. In addition, protective plywood panels were attached to all sides of the feeder and also near the bowl feeding area to prevent lambs from damaging feeder electronics.

The Codatron Interval Program supplied with the Westfalia automatic feeder was used as the feeding regimen. This program divided each 24 hour day into 24 one hour intervals commencing at 0900hr DST. During each of the first 20 hours, individual lambs could enter the feeding station and receive five percent of their daily feed allotment. The daily feed allotment was set in excess of that which could be consumed by a lamb if fed ad libitum. At the end of the 20 hour period a four hour catch-up period allowed the lamb to consume the remaining feed alloted to it. The program drop interval was set at 99 seconds. Thus a lamb would have to remain at the feeding bowl longer than 99 seconds to receive a second 25g drop.

APPENDIX 2

Ration composition

diet ingredients	
ingredient	(% as fed)
barley	49
alfalfa meal	40
canola meal	10
vitamin-mineral premix	1

diet composition (calculated values)	
component (100% DM basis)*	
TDN	72.6
protein	18.3
Ca	0.91
P	0.46

* components were calculated according to NRC guidelines