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EFFECT OF BOVINE SOMATOTROPIN AND BOVINE GROWTH HORMONE-RELEASING FACTOR ON THE SOMATOTROPIC CASCADE IN LACTATING DAIRY CATTLE

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William Kenneth VanderKooi

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Michael J. VanderHaar
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By

William Kenneth VanderKooi

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ABSTRACT

EFFECT OF BOVINE SOMATOTROPIN AND BOVINE GROWTH HORMONE-RELEASING FACTOR ON THE SOMATOTROPIC CASCADE IN LACTATING DAIRY CATTLE

By

William Kenneth VanderKooi

Primiparous Holstein cows received 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) by continuous i.v. (jugular) infusion or no infusion (controls, n=10) from 118 to 181 d postpartum. Cows treated with rbGRF tended to have more (P=.13) serum ST at d 57 than cows treated with rbST. Despite this fact, rbGRF-treated cows tended to have less (P=.15) serum insulin-like growth factor-I (IGF-I), lower (P<.01) liver IGF-I mRNA expression, less (P=.03) serum IGF-I binding protein-3 and less (P<.01) serum insulin at d 57 than rbST-treated cows. Also, rbST and rbGRF increased (P=.02) mammary IGF-I receptor mRNA expression.

Results are consistent with the idea that rbST and rbGRF stimulate the somatotropic cascade to increase milk yield. However, rbST stimulated the cascade more than rbGRF. Perhaps, differences in ST variant profile and/or ST pulsatility caused the different effects of rbST and rbGRF on the somatotropic cascade. Furthermore, both treatments increased milk yield similarly even though rbGRF did not stimulate the cascade as much as did rbST. Perhaps, the galactopoietic effects

of rbGRF are not mediated exclusively through the somatotropic cascade.

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LIST OF ABBREVIATIONS

BW	Body weight
DMI	DMI
DNA	Deoxyribonucleic acid
EB	Energy balance
GRF	Growth hormone-releasing factor
IGFBP	Insulin-like growth factor binding protein
IGF-I	Insulin-like growth factor-I
mRNA	Messenger ribonucleic acid
rbGRF	Recombinant bovine growth hormone-releasing factor
rbST	Recombinant bovine somatotropin
SCM	Solids-corrected milk
ST	Somatotropin

INTRODUCTION

The agricultural industry is developing new methods to meet the increasing demand for food in response to the world's growing human population. As the year 2000 approaches, current methods for promoting productivity in agriculture will be enhanced by the employment of biotechnology.

One of the first products of biotechnology developed for the dairy industry is recombinant bovine somatotropin (rbST). The advances of recombinant deoxyribonucleic acid (DNA) technology have made rbST both abundant and affordable. Daily administration of rbST to dairy cattle during lactation substantially increases milk yield and the efficiency of milk production (Peel et al., 1983), resulting in greater economic return and efficient utilization of resources.

Scientists throughout the world have conducted over 1,000 studies examining the effects of rbST on over 20,000 cows (Bauman, 1992). Scientists from the U.S. Food and Drug Administration (FDA), after reviewing the scientific literature, have concluded that the use of rbST in dairy cattle presents no increased health risk to consumers (Juskevich and Guyer, 1990). Nevertheless, public perception is of paramount importance if rbST or any new

technology is to be effectively implemented (Bauman, 1992) and utilized as a means for enhancing productivity in the dairy industry.

An elaborate regulatory system controls the secretion of somatotropin (ST) *in vivo*. Two neuroendocrine hormones, namely growth hormone-releasing factor (GRF) and somatostatin, are primary controllers of ST release (Frohman et al., 1992). *In vivo*, GRF stimulates and somatostatin inhibits the release of ST from somatotrops in the anterior pituitary.

Administration of rbST to dairy cattle induces a series of coordinated changes in whole-body metabolism that subsequently increase milk synthesis. Current dogma suggests that insulin-like growth factor-I (IGF-I) in serum partially mediates ST-induced increases in milk production. The IGF-I peptide is predominantly synthesized and secreted from the liver in response to ST binding with the hepatic ST receptor. The biological effects of IGF-I are further regulated by specific high-affinity binding proteins (IGFBPs) which control access of IGF-I to target tissues. For the purpose of this thesis, I will refer to the interactions of ST, IGF-I, and IGFBPs as the ST/IGF/BP cascade (Figure 1).

As one might speculate, administration of recombinant bovine growth hormone-releasing factor (rbGRF) stimulates milk yield in lactating dairy cattle similar to that of rbST (Baile et al., 1985). Dahl et al. (1993), however, found that cows treated with rbGRF produced more milk than cows treated with rbST even though concentration of ST and IGF-I were similar between the two

groups. Thus, we formulated the hypothesis that rbGRF stimulates the ST/IGF-I/BP cascade more than does rbST in lactating dairy cattle.

The objective of this thesis is to quantify and compare the effects of 63-d infusion of exogenous rbGRF and rbST on the endocrine ST/IGF/BP cascade in lactating dairy cows. Certainly other facets, such as mammary triiodothyronine, plasminogen, blood flow, and local autocrine and/or paracrine action of IGF-I might also be important in mediating the galactopoietic effects of rbST. Nevertheless, this thesis will focus specifically on the regulation and interaction of the components of the endocrine ST/IGF/BP cascade in response to rbGRF and rbST administration.

For the purpose of this thesis, I will refer to endogenous growth hormone as ST, exogenous recombinant bovine growth hormone as rbST, endogenous growth hormone-releasing factor as GRF, and exogenous recombinant bovine growth hormone-releasing factor as rbGRF.

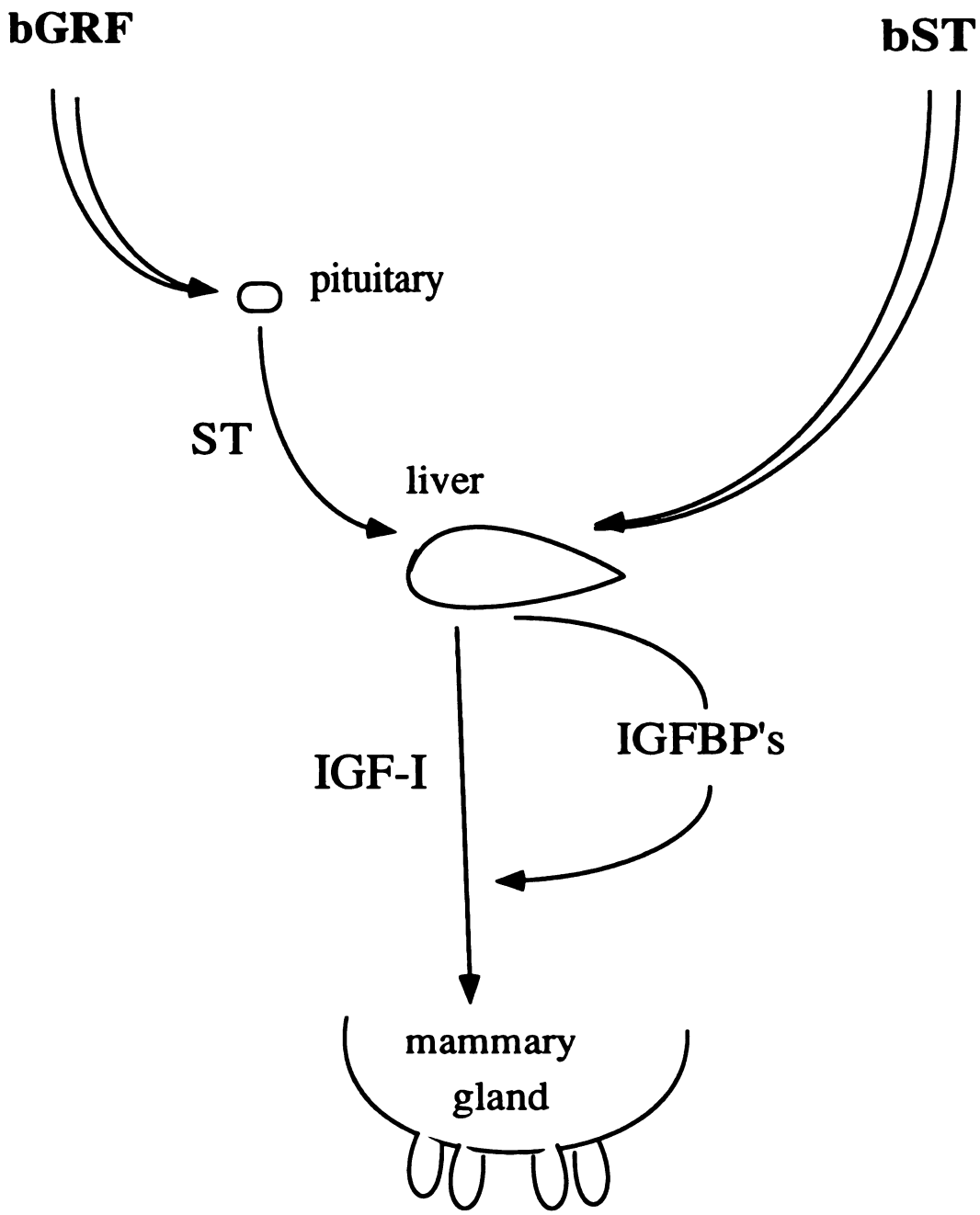

Figure 1. Schematic diagram of the endocrine somatotropic cascade.

LITERATURE REVIEW

Effects of rbST on milk production

History

The ability of ox pituitary gland extracts to increase milk yield of the cow was first shown by Gruter and Stricker (1929) more than 60 years ago. Since then, the specific hormone responsible for this action has been identified as ST, and the genetic sequence has been elucidated to enable the synthesis of rbST (Seeburg et al., 1983). When cows are treated with rbST, typically milk yield gradually increases within the first few days of treatment but no effect on milk composition and feed intake occurs in the short-term (Peel and Bauman, 1987). Also, an increase in the concentration of IGF-I in serum is characteristic within the first few days of rbST administration to dairy cows (Davis et al., 1984). Although the effects of rbST on lactation have been extensively studied during the past several years, the precise mechanisms by which rbST stimulates milk synthesis have not been established. Several theories, however, have been proposed.

Mammary cell numbers

Brumby and Hancock (1955) proposed that ST stimulates growth of the mammary gland by increasing mammary epithelial cell numbers or decreasing

mammary cell loss. The IGF-I peptide presumably mediates the effect of ST on mammary cell numbers. Baumrucker (1989) observed that IGF-I stimulated [^{3}H]thymidine uptake into DNA of lactating bovine mammary tissue *in vitro*. Their study, however, revealed that ST had no direct effect on DNA synthesis. Capuco et al. (1989) found that rbST treatment did not increase total mammary DNA in lactating cows, thus rbST presumably does not increase mammary cell numbers directly *in vivo*.

However, ST may prevent mammary cell loss. This idea is supported by the low levels of milk plasminogen observed in milk of cows treated with rbST (Politis et al 1990). Plasminogen is a serine-protease that is partially responsible for mammary gland involution. Thus, low levels of plasminogen in milk during rbST treatment suggest a reduction in mammary cell loss.

Nevertheless, increased cell numbers or decreased cell loss cannot explain the rapidity of the rise in milk yield in response to rbST injections. Milk yield increases substantially within the first few days of rbST treatment to dairy cows (Peel and Bauman, 1987). Thus, the galactopoietic effects of rbST must be at least partly the result of increased productivity of existing secretory cells.

Cellular metabolic activity

Hutton (1957) proposed that the galactopoietic effects of ST are due mainly to increased metabolic activity of alveolar epithelial cells. Specifically, these changes may result from an increase in mammary cell metabolism

resulting in a greater extraction rate of milk precursors by the mammary gland.

An increase in metabolic activity of mammary epithelial cells in response to rbST is supported by the observation that thyroxine-5'-monodeiodinase activity increases in mammary tissue of cows treated with rbST (Capuco et al., 1989). Thyroxine-5'-monodeiodinase catalyzes the conversion of thyroxine to the more active triiodothyronine in the mammary gland; thus, an increase in thyroxine-5'-monodeiodinase is associated with enhanced cellular metabolism. Enhanced mammary epithelial cell metabolism involving triiodothyronine may be a mechanism whereby rbST elicits galactopoietic effects in dairy cows.

Mammary blood flow is an important determinant of the galactopoietic response to the physiological concentration of ST (Hart et al., 1980). An increase in mammary blood flow results in greater availability of metabolites to the mammary gland. Although rbST administration to cows increases blood flow and subsequent nutrient supply, the blood flow response likely is caused through other hormonal stimulation (Davis et al., 1983). Presumably, local production of vasodilator substances decreases mammary vascular resistance which subsequently increases blood flow. This vasodilation is likely linked to mammary metabolism, because similar increases in udder blood flow and milk yield were obtained in thyroxine-treated cows (Davis et al., 1983). Nevertheless, it remains to be elucidated whether or not the increase in mammary gland blood flow is a cause of, rather than a response to, the increase in synthesis of milk during rbST and/or thyroxine administration

(Gluckman et al., 1987).

Insulin may be indirectly involved in mediating the effects of rbST on activity of the mammary gland. Insulin coordination of glucose homeostasis to alter activity of the mammary gland is evident with rbST treatment. Hepatic glucose production increases and whole body glucose oxidation decreases in response to rbST administration (Bauman, 1989). This coordination results in greater availability of glucose to the mammary gland. These effects are presumably mediated by insulin. Furthermore, Gallo and Block (1990) found that rbST stimulates glucose uptake in the bovine mammary gland.

An increase in lipolysis and/or a decrease in lipogenesis appears to mediate the galactopoietic effects of rbST. Cows in early lactation (negative energy balance) increase mobilization of free fatty acids (FFA) in response to rbST treatment (Peel et al., 1981). This may simply be a result of the increased energy deficit evoked by rbST treatment (Gluckman et al., 1987). Barber et al. (1992) reported that ST clearly regulates lipid metabolism in adipose tissue in a manner which should favor nutrient utilization by the mammary gland. Insulin resistance (decrease in lipogenesis) and increased lipolysis provide a mechanism for preferential utilization of glucose and fatty acids (FA) by the mammary gland of cows treated with rbST. These effects, however, are probably too small to account for the effects of ST on milk production (Barber et al., 1992).

Direct action of ST on the mammary gland

The rbST compound may act directly on bovine mammary tissue to stimulate milk yield. *In situ* hybridization results indicate that a low level of ST-receptor mRNA is evenly distributed throughout the bovine mammary gland (Hauser et al., 1990). In a similar study conducted by Glimm et al. (1990), ST receptor mRNA was identified and characterized in mammary tissue from normal and rbST-treated lactating cows using northern and *in situ* hybridization analyses. *In situ* hybridization revealed that the ST receptor gene is primarily expressed in alveolar epithelial cells of mammary tissue (Glimm et al., 1990).

Despite these observations, the theory suggesting direct action of ST at the mammary gland has been challenged because of several findings. McDowel et al. (1987) demonstrated that local arterial infusion of ST into the udder of lactating ewes did not increase milk production of the infused udder half. Also, mammary tissue seems devoid of functional ST receptor protein (Keys and Djiane, 1988) and no direct effect of ST on milk synthesis has been demonstrated in lactating mammary tissue in culture (Gertler et al., 1983).

Summary

Somatotropin is a homeorhetic regulator that partitions nutrients towards the mammary gland for increased milk synthesis (Bauman, 1992). The primary factors mediating the ability of rbST to coordinate whole-body metabolism and stimulate nutrient uptake and metabolism of mammary cells remain to be elucidated.

Physiological effects of rbST

ST characteristics

The ST peptide, synthesized and secreted by the pituitary, is primarily transported through the circulation to target tissues to cause a biological effect through an endocrine pathway. Endogenous ST, produced by the anterior pituitary, can either be 190 or 191 amino acids long, and can have either leucine or valine at position number 126 in the protein sequence (Wood et al., 1989). Therefore, four different variants of ST are produced naturally. Typically, pituitary production of ST involves approximately equal amounts of the 190 and 191 amino acid proteins, and about two-thirds of the total produced has leucine at position 126, while the remaining one-third has the amino acid valine at position 126 (Wood et al., 1989). Consequently, the ST in serum of cows treated with rbGRF presumably would consist of four native variants in substantial amounts, although the profile may be different than untreated cows. In contrast, the serum ST of rbST-treated cows would be overwhelmingly the recombinant variant which is the 191 amino acid variant with leucine at position number 126.

Binding of pituitary and recombinant bST to receptors in bovine liver membranes was investigated by Wood et al. (1989). Both sources of rbST have equivalent specific activities. Nevertheless, these results do not provide evidence that either compound is more biologically active with regard to the

somatotropic cascade *in vivo*.

Although exogenous ST had a slightly longer half-life than endogenous ST in rats (12.7 vs 10.5 min), this difference was not significant (Chapman et al., 1990). Hence, ST secreted in response to rbGRF infusion and exogenous rbST presumably have similar half-lives *in vivo* in cattle.

ST pulsatility

The secretion of ST in adult mammals is pulsatile in nature, but the physiological significance of such pulsatility is incompletely understood. Recent studies in animals and man suggest a cooperative interaction of hypothalamic somatostatin and GRF on somatotropic pituitary cells in the generation of ST pulses (Hindmarsh et al., 1991). Differences in pulsatility of ST in serum may moderate the different *in vivo* effects of rbST and rbGRF on the ST/IGF/BP cascade when both are continuously infused.

Administration of continuous ST in rats is more effective than pulsatile administration for inducing ST-receptor in liver (Maiter et al., 1992). In contrast, Mathews et al. (1989) observed no effect of pulsatility on ST receptor expression in rat hepatocytes. Hence, they suggested that the effects of ST on its receptor abundance are manifested at a post-transcriptional level, because ST receptor mRNA is not significantly affected by either hypophysectomy or rbST treatment.

Maiter et al. (1992) demonstrated that pulsatile infusion of ST is more effective than continuous infusion in stimulating rat liver IGF-I mRNA

expression, and that this effect is not mediated by an increase in ST-receptor mRNA. In contrast, Bick et al. (1992) observed that rats have greater concentration of serum IGF-I after continuous ST treatment than after pulsatile injections of ST. However, the pulsatile treatment induced a greater body weight gain than the same dose of ST infused continuously (Bick et al., 1992). The reason for the discrepancy is not known, but local production of IGF-I in the target tissue is likely involved. In another study, the levels of hepatic IGF-I mRNA were similar in rats given continuous or pulsatile ST treatment, whereas the IGF-I mRNA levels in skeletal muscle and cartilage were higher after pulsatile than after continuous ST treatment (Isgaard et al., 1988). In light of the effects of ST pulsatility on growth, the effects of serum ST pattern on galactopoiesis may also be important.

Endocrine IGF-I action

Salmon and Daughaday (1957) initially reported that a serum factor, now termed IGF-I, mediates the action of ST on bone cartilage. The IGFs are a family of polypeptides, related structurally to proinsulin, which have growth promoting effects both *in vitro* and *in vivo*. At physiological concentrations, IGF-I is a potent mitogen of undifferentiated bovine mammary epithelial cells cultured on collagen in serum-free media. Despite the information available, significant gaps in our knowledge exist regarding the mechanisms by which these peptides stimulate cell replication (Clemmons et al., 1989).

In cows, the expression of ST receptor and IGF-I mRNA are found in

several tissues, but predominately in liver tissue (Hauser et al., 1990). The level of IGF-I mRNA is regulated by ST and nuclear run-on assays were used to show that ST regulation of IGF-I is manifested at the transcriptional level (Mathews et al., 1986). Thus, transcriptional mechanisms are predominately responsible for enhanced IGF-I expression elicited by rbST (Mathews et al., 1986; Bichell et al., 1992).

During lactation, IGF-I may coordinate the metabolism of various organs and tissues to support increased milk yield (Bauman, 1992). Treatment with rbST increases concentration of ST and IGF-I in lactating animals (Vicini et al., 1991), whereas concentration of serum IGF-I during normal lactation is inversely correlated with milk production and serum ST concentration. This observation has perplexed scientists investigating the role of IGF-I on lactation, however some theories have been postulated to explain this observation.

The IGF-I peptide exerts its biological effects, like other peptides, by binding to cell surface receptors (Zapf et al., 1978). Polypeptide hormones and growth factors are internalized by receptor-mediated endocytosis after binding to cell surface receptors (De Diego et al., 1991). Onset of lactation is associated with structural changes in the IGF-I receptor and the onset of lactation is associated with increases in the abundance of IGF-I receptors (DeHoff et al., 1988). These changes in the receptor could play a role in modulating the physiological effects of IGFs on mammary tissue and possibly enhance clearance of serum IGF-I. The binding of IGF-I declines during the

prepartum period, increases 75% with the onset of lactation, and then declines during the postpartum period (Hadsell et al., 1990).

Stelwagen et al. (1992) suggested that low concentration of IGF-I in serum during early lactation is important for mediating preferential nutrient utilization by the mammary gland. The IGF-I peptide plays a role in several different tissues throughout the body (D'Ercole et al., 1984). If serum IGF-I increased during early lactation, the use of metabolites would increase in several other tissues throughout the body and the mammary gland would have to compete for these metabolites (Stelwagen et al., 1992). Low blood concentration of IGF-I during this part of lactation, however, combined with a selective increase of mammary receptors or mammary receptor affinity would give the mammary gland an advantage over other tissues in utilization of the available metabolite pool (Stelwagen et al., 1992).

Regardless of inconsistencies, considerable attention has been focused on IGF-I as an important factor mediating the galactopoietic actions of ST. The contribution of hepatic to total IGF-I varies markedly between tissues and provides evidence that hepatic IGF-I may have specific endocrine functions in selected tissues (Hodgkinson et al., 1991). Overall, similarity in the temporal pattern between milk yield response and serum concentration of IGF-I is consistent with a role for serum IGF-I in mediating a portion of the effects of rbST in lactating cows through an endocrine fashion (Cohick et al., 1989).

Lavandero et al. (1990) demonstrated IGF-I binding in rat mammary

tissue. In goats, infusion of IGF-I into the mammary arterial supply of a hemi-gland enhances milk secretion and mammary blood flow of the infused hemi-gland in intact conscious goats (Prosser et al., 1990). The more pronounced effect in the infused compared with non-infused hemi-glands suggest that IGF-I acts directly on the mammary gland (Prosser et al., 1990). In contrast, Shamay et al. (1988) concluded that galactopoiesis is not affected by IGF-I in organ culture of bovine mammary tissue. Furthermore, the IGF-I peptide stimulates induction of carrier-mediated glucose transport activity in mouse mammary epithelial cells (Prosser et al., 1987). However, no subsequent increase in α-lactalbumin activity is observed. Despite the lack of information, it seems likely that, during lactation, IGF-I plays a specific role to enhance mammary epithelial cell function *in vivo* in response to rbST treatment. Nevertheless, the mechanism by which IGF-I mediates the galactopoietic actions of ST remains to be elucidated.

Autocrine and/ or paracrine IGF-I action

Dai et al. (1992) demonstrated that the introduction and expression of a IGF-I transgene in FRTL-5 cells create an autocrine and/or paracrine loop with a non-transformed phenotype and that tropic hormones can synergize with endogenous transgene IGF-I to regulate cell proliferation. Schlechter et al. (1986) reported that ST stimulates long bone growth by inducing local production of IGF-I, which in turn stimulates cell proliferation in an autocrine and/or paracrine fashion. The autocrine and/or paracrine pathway also may

be important for IGF-I action in the mammary gland. IGF-I mRNA is present and is localized in the stromal component of mammary tissue (Hauser et al., 1990). Exogenous ST increases mammary IGF-I mRNA abundance in rats (Kleinberg et al., 1990). Furthermore, bovine mammary explants were found to synthesize and secrete IGF-I in lactating non-pregnant dairy cows (Campbell et al., 1991).

IGFBPs

The IGF peptides circulate bound to large molecular weight binding proteins, which make measurement of the IGFs and assessment of their biological role complicated (Hintz, 1984). Apart from their function as IGF-I carriers, the binding proteins also play a modulating role in the interaction between IGFs and their target cells (Hardouin et al., 1987; McCusker et al., 1991).

As transporters of IGF-I, IGFBPs regulate the rate of efflux of IGF-I from vascular space (Camacho-Hubner et al., 1991). Furthermore, Cohick and Clemmons (1991) suggested that IGFBPs may act to coordinately regulate IGF-I transport from mesenchymal cells to epithelial cells within tissues.

At the tissue level, Campbell et al. (1991) reported that bovine mammary explants from pregnant, non-lactating cows and lactating cows synthesized and secreted IGFBPs. Similarly, McGrath (1991) reported that normal bovine mammary cells secrete IGFBP-2 and IGFBP-3. In normal mouse mammary epithelial cells, Fielder et al. (1992) demonstrated that

IGFBPs are expressed and released into medium in response to both lactogenic hormones and IGFs.

IGFBP-3

The major circulating binding protein is IGFBP-3, to which most of the circulating IGF-I is bound (Vicini et al., 1991). Administration of rbST increases serum levels of IGFBP-3 in dairy cows (Cohick et al., 1992). Zapf et al. (1989) reported that IGF-I mediates much of the ST-associated increase in IGFBP-3 levels *in vivo*. A similar study reported that insulin and IGF-I are stimulators of IGFBP secretion and most likely mediate the change in serum concentration of IGFBPs in response to rbST administration (McCusker and Clemmons, 1988) .

Blum et al. (1989) concluded that IGFBP-3 acts as a reservoir, releasing continuously small amounts of IGF-I and thereby creating a steady state situation of receptor occupancy. This slow-release of the IGF-I may be more mitogenic than temporarily large concentrations of free IGF-I (Blum et al., 1989). Binoux and Hossenlopp (1988) concluded that IGFBP-3 complexes do not cross the capillary barrier. This observation provides further evidence that IGFBP-3 acts as a reservoir and as a moderator of IGF-I action.

IGFBP-3 can be either soluble in serum or associated with a cell surface. Soluble IGFBP-3 inhibits IGF-I action by sequestering and preventing IGF-I receptor binding, whereas surface-associated IGFBP-3 enhances the growth-promoting effects of IGF-I in bovine fibroblasts (Conover et al., 1990). Cell-

associated IGFBP-3 enhances the presentation of IGF-I to its cellular receptor and this heightens cellular receptor activity to IGF-I and related peptides (Conover, 1992). The IGFBP-3 can also prevent IGF-I-induced IGF-I receptor down-regulation (Conover and Powell, 1991), thereby maintaining IGF-I effectiveness despite elevated concentration of serum IGF-I in response to rbST administration.

Several tissues express the IGFBP-3 gene (Albiston and Herington, 1992). It has been suggested by Ernst and Rodan (1990) that locally produced IGFBP-3 may function by keeping IGF within tissue compartments, thereby increasing the bioactivity of IGF-I. Furthermore, cell-derived IGFBP-3 may function in a buffering capacity to restrict IGF-I and target cell interaction, thereby modulating the biological response to changes in local IGF-I levels (Conover and Powell, 1991).

In addition to synthesis of IGFBP-3, its degradation may also play an important role for regulating IGFBP-3 concentration as well as concentration of unbound IGF-I in serum (Davenport et al., 1992). Evidence suggests that there is a specific protease present in serum which can degrade IGFBP-3 to enhance IGF-1 clearance as well as increase the amount of free IGF-I in serum (Davies et al., 1991; Davenport et al., 1992). Davies et al. (1991) concluded that the presence of a circulatory IGFBP-3 protease may be an adaptive response to increase the bioavailability of the IGFs.

IGFBP-2

Administration of rbST decreases concentration of serum and lymph IGFBP-2 in dairy cows (Cohick et al., 1992). Binoux and Hossenlopp (1988) concluded that IGF-I complexed with IGFBP-2 can cross the capillary barrier. The IGFBP-2 can leave the microcirculation and distribute to subendothelial tissues (Booth et al., 1990). Plasma IGFBP-2 levels are relatively stable, and therefore may serve as a reservoir of IGF-I that is available for IGF-I transport across the capillary barrier to tissues (Clemmons et al. 1991). The IGFBP-2 can bind with IGF-I and act as an inhibitor or a potentiator of IGF-I mediated DNA synthesis (Bourner et al., 1992). Thus, IGFBP-2 may transport IGF-I from circulation to their target cells as well as modulate IGF-I activity.

Insulin

Effects of exogenous ST on circulating insulin concentration have been puzzling. Treatment of dairy cows with rbST chronically increased circulating insulin concentration in some, but not all studies (Peel and Bauman, 1987). The reasons for this inconsistency are not identified, but rbST-induced increases in milk yield occur regardless of whether a chronic hyperinsulinemia is observed (McGuire et al., 1992).

Nevertheless, Vicini et al. (1991) speculated that an insulin response to rbST treatment is probably due to coordinated metabolic changes. The rbST treatment has three main effects on metabolism: 1) increased glucose irreversible loss, which reflects increased liver gluconeogenesis (Cohick et al., 1989); 2) decreased whole body glucose oxidation (Bauman et al., 1988); and

3) reduced glucose response to insulin (Sechen et al., 1989).

Exogenous insulin can also increase IGF-I mRNA expression, indicating that insulin can act as a regulator of the IGF-I system *in vivo* (Salamon et al., 1989). Furthermore, in hepatocyte primary culture, insulin appears to regulate IGF-I synthesis at the mRNA level (Phillips et al., 1991).

Effects of rbGRF on milk production

It was not until 1982 that GRF was isolated and characterized from extracts of pancreatic tumors of two acromegalic humans (Rivier et al., 1982). Following its isolation, the GRF peptide was described for several species including cattle (Esch et al., 1983), sheep and goats (Brazeau et al. 1984). The galactopoietic activity of exogenous GRF administration were first reported in lactating ewes (Hart et al., 1985) and lactating dairy cows (Baile, 1985) less than ten years ago. The administration of exogenous GRF to lactating dairy cows stimulates an increase in concentration of serum ST and a subsequent increase in concentration of serum IGF-I (Enright et al., 1986; Lapierre et al., 1990; Dahl et al., 1993). Recently, rbGRF has been expressed in E. coli (Kirschner et al., 1989), and this 1-45 (Leu^{27}, Hse^{45}) rbGRF was used in the experimental trial conducted for this thesis.

Physiological effects of rbGRF

The GRF peptide binds to the plasma membrane of the somatotrops in

the anterior pituitary (Morel, 1991). After GRF binding, the area occupied by secretory granules in the somatotrops decreases, reflecting a release of ST (Morel, 1991). *In vitro*, secretion of ST induced by GRF occurs immediately and continues for duration of the exposure (Cronin et al., 1983). In somatotrops, the GRF peptide increases total immunoreactive ST concentration (Fukata et al., 1984), ST mRNA (Gick et al., 1984; Tanner et al., 1990), transcription rate of the ST gene (Baringa et al., 1983), and translocation of ST mRNA from the nucleus to the cytoplasm (Morel, 1991).

Studies investigating the effects of rbGRF administration on subsequent synthesis and secretion of IGF-I have been limited. The increases in concentration of serum IGF-I following rbGRF administration are presumably a result of the increase in concentration of serum ST that, in turn, stimulates IGF-I synthesis and secretion predominantly from the liver.

No studies, to date, have been published which investigated the effect of rbGRF administration on concentration of serum IGFBPs, expression of liver ST receptor, liver IGF-I, or mammary IGF-I receptor. However, GRF likely effects these components of the ST/IGF/BP cascade in a manner similar to rbST.

MATERIALS AND METHODS

Design and Management

Thirty primiparous Holstein cows were used in a randomized complete block design. Ten blocks of three cows were formed based on calving date. Within each block, cows were assigned randomly to treatment (10 cows/treatment).

Treatments began 118 d postpartum and were: 1) continuous i.v. (jugular) infusion of 29 mg/d rbST; 2) continuous i.v. (jugular) infusion of 12 mg/d of rbGRF (1-45) homoserine lactone; and 3) no infusion (controls) for 63 d.

Eight days before treatment began, VETport® (Thermedics, Worburn, MA) infusion catheters were implanted surgically into cows assigned to rbST and rbGRF treatments. In preparation for surgery, hair was clipped over the neck and shoulder areas, these areas were washed, and the cow was given a sedative i.m. (Rompun, Mobay Co., Shawnee, KN). A vertical path approximately 6 cm wide along the neck, and areas approximately 12 cm in diameter at the top of the shoulder and at the base of the neck over the right jugular were anesthetized by s.c. injections of lidocaine (American Veterinary Products Inc., Fort Collins, CO). The cow was immobilized on an operating

table, drapes were laid around the anesthetized areas, and the anesthetized areas were scrubbed with aseptic soap. A 3 to 5 cm incision was made at the top of the shoulder and over the right jugular vein. Catheter tubing was inserted aseptically at the shoulder, routed s.c. using a stainless steel rod and inserted into a jugular vein using a needle. Upon completion both incisions were stapled closed. The neck of the cow was wrapped with stretch wrap after final cleansing with hydrogen peroxide.

Infusion pumps (AS-2BH, Autosyringe, Inc., Hooksett, NH) were connected to the catheters with a .20-μm pore Sterile Acrodisc syringe filter (Gelman Sciences, Ann Arbor, MI) placed between the syringe, containing either rbST or rbGRF solutions, and the infusion catheter. The infusion pumps were fixed in back-packs that were mounted and secured with adjustable straps on the withers of a cow. Control cows did not wear a backpack and were not infused with any solution.

Bottles of rbGRF and rbST were stored at 4°C and solubilized on the day of use with 21 ml of sterile distilled water. Syringes were changed daily at 0700 h. Infusion volume was 12 ml/d for both rbGRF or rbST. Infusion catheters were coated initially with 1% bovine serum albumin dissolved in sterile water. Cows received rbGRF or rbST in pulses every 3.75 min. Treatments began at 1700 h on day 0.

Cows were housed in tie stalls and exposed to 24 h of light per day. Cows receiving rbGRF were milked at 0700, 1600, and 2330 h, and cows

receiving rbST or no infusion were milked at 0545, 1430, and 2200 h. Milk production was recorded daily, and milk samples were collected weekly beginning at 2 wk before treatment began. Fat, protein, and lactose concentration in milk were measured using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Yield of solids-corrected milk (SCM) and output of energy in milk were calculated: SCM (kg) = [27.1 (kg fat) + 14.4 (kg solids-not-fat) - .166 (kg milk)] (Tyrrell and Reid, 1965); milk energy (Mcal) = [milk yield x (.0921 x % fat + .0490 x % solids-not-fat - .0563], (Tyrrell and Reid, 1965).

A total mixed ration (TMR) was fed ad libitum. The TMR was formulated (Appendix A) to provide adequate nutrition for a cow (590 kg BW) producing 38.5 kg/d of milk containing 3.5% fat and assuming an intake of 22.7 kg/d dry matter (DM) (NRC, 1989). Feed was offered daily at 0330 and 1630 h. The amount of feed offered and refused was recorded daily. Samples of feed offered were collected weekly and chemically analyzed for DM, crude protein (CP) and neutral detergent fiber (NDF). Net energy of lactation (NE_l) value of feed was calculated (NRC, 1989). DM content was determined gravimetrically after drying samples at 105°C for a minimum of 12 h. The percentage of NDF in the TMR samples was determined in duplicate according to Goering and Van Soest (1970) with omission of decahydronapthalene, sodium sulfite, the substitution of triethylene glycol for 2-ethoxyethanol, and inclusion of α-amylase (Robertson and Van Soest, 1977).

Nitrogen content of fractions was determined using a modified Kjeldal procedure (Watkins et al. 1987). The CP content of samples was determined by multiplying the nitrogen content of the fractions by 6.25. All laboratory analyses were performed by a single technician. Standard samples were carried through all laboratory analyses to ensure consistent measurements.

Body weight (BW) of cows was recorded at 1030 h on two consecutive days at 2-week intervals beginning on experimental wk -1. NE_l requirement for maintenance of cows was calculated based on body weight (.08 x $BW^{.75}$, NRC 1989).

Energy balance (EB) was calculated: EB (Mcal NE_l/d) = [feed energy input (Mcal NE_l/d, NRC, 1989) - maintenance energy (Mcal NE_l/d, NRC, 1989) - milk energy output (Mcal NE_l/d, Tyrrell and Reid, 1965)].

Blood Collection and Analysis

An indwelling jugular catheter (16 gauge; Ico-Rally, Palo Alto, CA) was inserted into the left jugular vein contralateral to the rbGRF- or rbST-infused vein on d 55 of the experiment. The catheters were coated with heparin (TDMAC Heparin, Polysciences Inc., Warrington, PA) to reduce fibrin accumulation and sterilized with ethylene oxide. Catheter patency was maintained by flushing with 3.5% Na citrate and 50% sucrose in sterile water after collection of each sample.

Blood samples were collected from cows at 20-min intervals for 6 h

(0900 to 1500 h) at d 57. Blood samples were stored at 4 ° C for approximately 24 h, and then serum was harvested by centrifugation for 30 min at 1550 x g and frozen at -20 ° C until assayed. Serum ST (Moseley et al., 1982) and IGF-I (Dahl et al., 1990), insulin (Villa-Godoy et al., 1990) and IGFBPs (Hossenlop et al., 1986) were quantified in blood collected at d 57.

Briefly, concentration of ST in serum was assayed by the Upjohn Co. using a double antibody radioimmunoassay procedure described by Niswender et al. (1969). It was modified using ^{125}I in place of ^{131}I and 1% bovine serum albumin in phosphate buffer was used instead of egg whites. The reference standard was NIH-B17-GH. Antisera against purified GH (NIH-B17) was prepared in guinea pigs. The second antibody was raised in sheep against guinea pigs gamma globulin.

Concentration of IGF-I in serum was assayed by Upjohn using a glycl-glysine HCl extraction and a double antibody radioimmunoassay. Recombinant DNA-derived human IGF-I (Amgen Corp., Thousand Oaks, CA) was used for standards and radioiodination. Concentration serum IGF-I was measured by radioimmunoassay using rabbit anti-hIGF-I (IMC, Terre Haute, IN). After overnight incubation of samples and standards with the first antibody, labeled IGF-I (15,000 cpm/tube) was added and samples were incubated for an additional 48 h. The IGF-I complexed with the antibody was precipitated with goat anti-rabbit gamma globulin (Antibodies Inc., Davis, CA).

Concentration of insulin in serum was assayed using a double antibody radioimmunoassay procedure as described by Villa-Godoy et al. (1990). Bovine insulin (5 ug) was iodinated using the chloramine T method. An insulin standard solution (.02564 ng/ul in preparation was diluted with .05 phosphate buffer, .85% NaCl, pH 8.6/B1 buffer in a 1:1 ratio) was used to quantitate measurements. Concentration of serum insulin was measured by radioimmunoassay using guinea pig anti-bovine insulin (diluted to 1:30,000). After overnight incubation of samples and standards with the first antibody, labeled insulin (20,000 cpm/tube) was added and samples were incubated for an additional 48 h. The insulin complexed with the antibody was precipitated with sheep anti-guinea pig gamma globulin.

Serum IGFBP-2 and -3 were quantified in blood collected at d 57 using western ligand blotting (Hossenlop et al., 1986). Briefly, serum (2 ul) diluted at 1:8 using .05M Tris buffer pH 7.4 was mixed with sample buffer containing .42M tris-chloride and 13.3% sodium-dodecyl-sulfate-polyacrylamide (SDS). After heating at 60°C for 30 min, samples were electrophoresed on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE was performed in a 12.5% resolving, 4% stacking gel using constant current (approximately 20 volts applied overnight) in a Vertical Gel System (Bethesda Research Laboratories, Gaithersburg, MD). Proteins were transferred to nitrocellulose filters (Schleicher and Schuell Inc., Keene, NH) in a wet electroblot system (Hoeffer Scientific Instruments, San Francisco, CA).

Hybridization of membranes was as described by Davenport et al. (1990) using ^{125}I-IGF-I iodinated by the chloramine T method. Following overnight hybridization, blots were washed and exposed to autoradiography film (X-OMAT AR film; Eastman Kodak Co., Rochester, NY). After 24 to 48 h, film was developed and the relative concentration of serum IGFBP-3, -2, -1 and -4 were determined by densitometry (Model FB934 densitometer; Fischer Biotech, Fischer Scientific) with software from Biomed Instruments Inc., Fullerton, CA of the 36 and 43, 33, 27 and 23 kd bands, respectively.

After ligand blotting analysis, nitrocellulose filters were incubated for 90 min with a dilution of 1:1000 of bovine IGFBP-3 antiserum and then for 2 h with alkaline phosphatase-conjugate goat antirabbit immuoglobin G (GAR-AP; Bio-Rad Chemical Division, Richmond, CA). Color development substrates from a GAR-AP Bio-Rad kit were used to detect alkaline phosphatase activity to depict the presence of the IGFBP-3 bands in the membrane.

Tissue Collection and Analysis

At d 63 of the treatment period, liver and mammary tissue were removed from a cow within 5 min of slaughter. A 10 g piece of liver and several 10 g pieces of mammary tissue from the left hemi-gland were removed and immediately placed in liquid nitrogen. After freezing, tissue was stored at -70°C.

Total RNA was isolated from liver and mammary tissue (Chirgwin et al.,

1979). Briefly, 1 g of tissue was homogenized in 24 ml guanidinium thiocyanate (4 M) and then layered over 5.7 M cesium chloride in an ultracentrifuge tube. The tubes were centrifuged at 100,000 x g for 16 h. The RNA pellet was recovered and purified further by chloroform-phenol extraction.

After isolation of total RNA, expression of mRNA for ST-receptor, IGF-I and β-actin in liver and IGF-I receptor and β-actin in mammary tissue were determined by northern blot hybridization. Briefly, total RNA (20 ug/sample for liver and 40 ug/sample for mammary tissue) was denatured in 50% formamide, 2.2 M formaldehyde at 55 C for 15 min. The sample mixture was electrophoresed in an agarose-formaldehyde gel (1.2% agarose) made with 1x MOPS buffer (.04M 3-N-morpholino-propane-sulfonic acid, 10mM sodium acetate, 1mM ethylenediamine tetraacetic acid) pH 7.0 and 4.45% v/v formaldehyde. Following electrophoresis, RNA was transferred onto a GeneScreen Plus membrane (NEN Research Products, Boston, MA) using an electroblot system. The membrane was then baked 2 h at 80° C to reverse the formaldehyde reaction. Nucleic acids in the membrane were hybridized at 42° C in a solution containing 50% formamide, 10% dextran sulfate and 1% SDS in 1 M NaCl. Overnight hybridization was completed with a ^{32}P-labeled cDNA probe (500,000 cpm/ml). For IGF-I, the probe was made by random-priming of a human IGF-I cDNA clone (Bell et al., 1984); homology of bovine and human IGF-I mRNA is 93% (Fotsis et al., 1990). For ST receptor, a

rabbit cDNA clone obtained from Dr. William Wood was used (Genetech Inc., San Francisco, Ca; Leung et al., 1987). For IGF-I receptor, a cDNA clone was used (American Type Culture Collection, #59294). For β-actin, a cDNA clone obtained from Dr. William Helfrich was used (Michigan State University, East Lansing, MI). Following hybridization, membranes were washed at medium stringency and then exposed to autoradiography film for 2 to 5 d. Band intensities of developed film were subjected to densitometry for quantification. Membranes were rehybridized with the respective probes. Between hybridization with each probe, the membranes were stripped by soaking in boiling solution (.01% SDS, .01% SSC) for 2 h.

Statistical Analysis

Characteristics of ST in serum (baseline, number of peaks, peak amplitude, peak length and mean) during the 6-h sampling period at d 57 were determined using a pulse analysis program (PULSAR; Merriam and Watcher, 1982). To minimize heterogeneous variance; number of peaks, peak amplitude and peak length were transformed to natural logarithms for analysis (Gill, 1978).

All data were subjected to randomized complete block ANOVA. Mean comparisons were made using orthogonal contrast (C1= Control vs rbST + rbGRF and C2= rbST vs rbGRF).

When significant (P>.10), initial BW, DMI, SCM and EB were included

in the model as a covariate for analysis of BW, DMI, SCM and EB.

RESULTS

BW, DMI, SCM and EB

Initial measures of BW, DMI, SCM and EB, which were measured 7 d before treatment began, were significant when tested as a covariate; therefore BW, DMI, SCM and EB reported for d 50 to 62 were adjusted by covariance (Table 1). Average covariate-adjusted BW, DMI, SCM and EB are reported for the 13 d period before slaughter (d 50 to 62). Body weight was not different among treatment groups. Dry matter intake for rbGRF-treated cows (23.4 kg/d) tended to be greater (P=.08) compared with rbST-treated cows (22.2 kg/d). There was no difference in SCM between rbST- (34.1 kg/d) and rbGRF-treated cows (33.1 kg/d). Hormone treatments (rbST and rbGRF) increased (P<.01) SCM by 18% compared with controls (28.5 kg/d). Calculated EB was positive for all treatments. However, the rbST-treated cows (3.8 Mcal/d) tended to have lower (P=.07) EB compared with rbGRF treated cows (6.3 Mcal/d). Also, average EB for rbST-treated and rbGRF-treated cows was lower (P<.01) than controls (8.6 Mcal/d).

Serum ST

Results for serum hormone concentrations are from d 57 of the

Table 1. Least squares means for BW, DMI, SCM and EB between d 50 and 62 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum).

					Pr > F	
	Con	rbST	rbGRF	SEM	C1[a]	C2[b]
BW, kg	544	543	548	6	.82	.54
DMI, kg/d	22.7	22.2	23.4	.9	.92	.08
SCM, kg/d	28.5	34.1	33.1	1.2	.01	.52
EB[c], Mcal/d	8.6	3.8	6.3	.7	.01	.07

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

[c] Energy balance, calculated as {feed energy input [NE], NRC, 1989] - maintenance energy [.08 x $BW^{.75}$, NRC, 1989] - milk energy output [milk yield x (.0921 x % fat + .0490 x % solids-not-fat - .0563),Tyrrell and Reid, 1965]}

treatment period. Concentration of ST in serum increased from 3.3 ng/ml for controls to 14.2 and 18.3 ng/ml for rbST- and rbGRF-treated cows, respectively (Table 2). The pooled effect of rbST and rbGRF treatments increased ($P<.01$) concentration of ST in serum compared with controls. Also, cows treated with rbGRF tended to have greater ($P=.13$) concentration of ST in serum than cows treated with rbST at d 57.

Parameters of ST pulsatility were adjusted by log-transformation to minimize heterogeneous variance for statistical analyses. Infusion of rbGRF increased ($P=.05$) number of peaks/d and amplitude (ng/ml) and tended to increase ($P=.10$) peak length (min) compared with rbST-infused cows (Table 2). Figure 2 illustrates the pulsatility profile of a representative cow from each treatment for d 57.

Serum IGF-I

Concentration of IGF-I in serum at d 57 increased ($P<.01$) from 110 ng/ml for controls to 257 and 211 ng/ml for rbGRF- and rbST-treated cows, respectively (Figure 3). Cows treated with rbGRF tended to have greater ($P=.15$) concentration of IGF-I in serum compared to cows treated with rbST. The greater concentration of IGF-I in serum for rbST-treated cows occurred despite the fact that concentration of ST in serum was slightly lower, not higher, in rbST- treated cows compared with rbGRF-treated cows.

Table 2. Least squares means for baseline ST, number of peaks, amplitude, peak length and mean ST for d 57 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum).

	Con	rbST	rbGRF	SEM	Pr > F	
					C1[a]	C2[b]
Baseline ST, ng/ml	2.7	13.1	16.0	1.9	.01	.28
Number of peaks, 6 h (natural log)	.61	.25	.72	.16	.54	.05
Amplitude[c],ng/ml (natural log)	1.0	.8	1.8	.4	.50	.05
Peak length, min (natural log)	.71	.44	.99	.22	.98	.10
Mean ST, ng/ml	3.3	14.2	18.3	1.8	.01	.13

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

[c] Calculated relative to baseline ST concentrations.

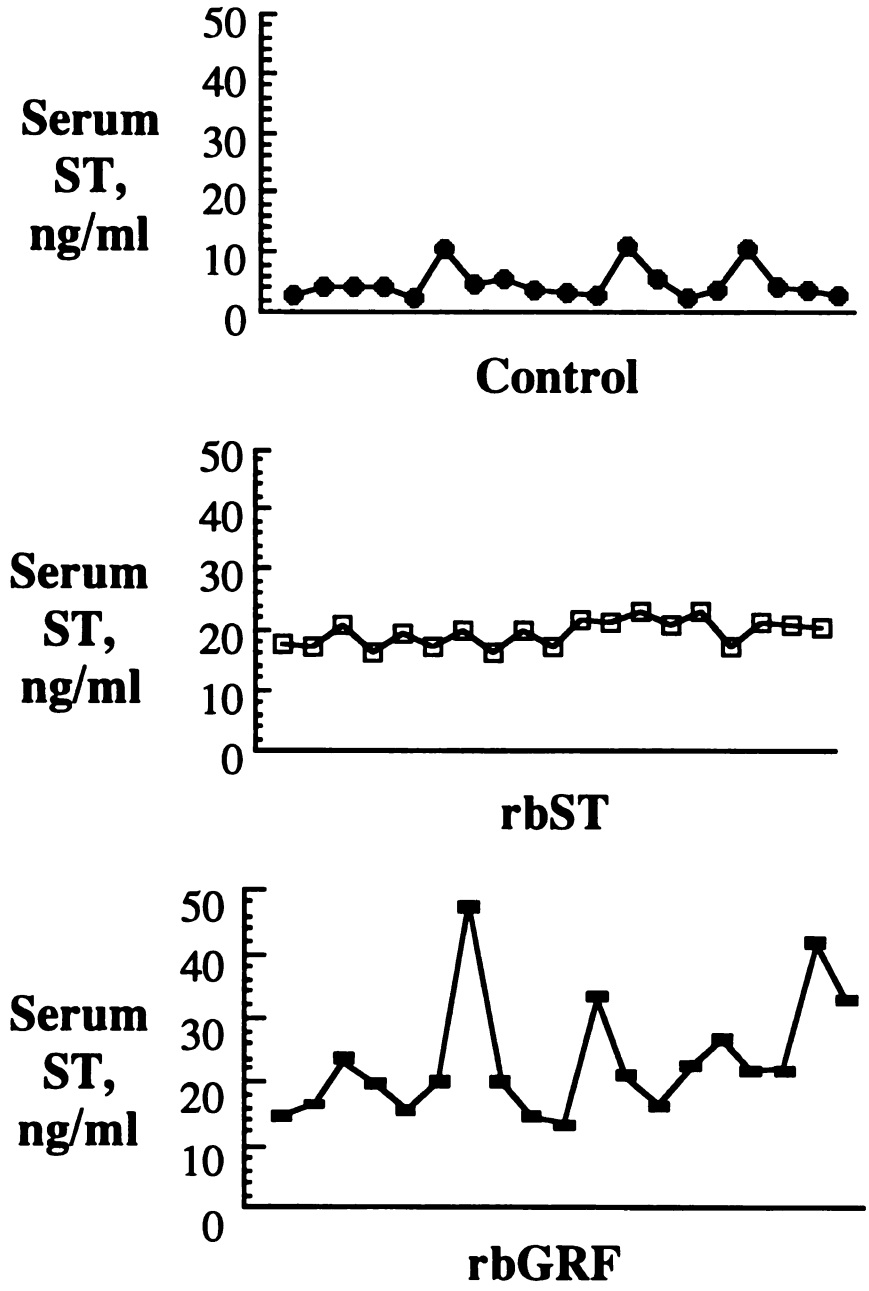

Figure 2. Concentration of ST in serum for d 57 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum). Each graph depicts the pulsatility profile of a representative cow from each treatment. Each point represents one of ninteen samples taken at 20 min intervals over a 6 h period.

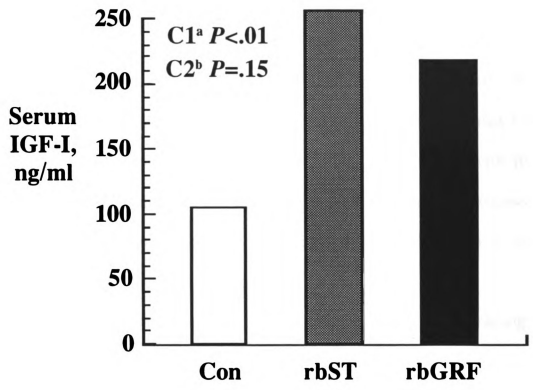

Figure 3. Least squares mean concentration of IGF-I in serum for d 57 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum). Pooled standard error of the mean among treatments was 22 ng/ml.

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

Serum IGFBPs

Serum IGFBP-3 had two forms at 43 and 36 kd as shown by autoradiographs of western ligand blots in Figure 5. Immunoblots using bovine IGFBP-3 antiserum indicated that these two bands were indeed IGFBP-3. At d 57 IGFBP-3 in serum increased ($P<.01$) from 6.0 densitometric units for controls to about 16 densitometric units hormone-treated cows, respectively (Figure 4). Also, cows treated with rbST had greater ($P=.03$) concentration of IGFBP-3 in serum compared to cows treated with rbGRF. Furthermore, the ratio of IGFBP3:IGF-I was not difference among groups.

Serum IGFBP-2 had one band at 33 kd as shown by autoradiographs of western ligand blots in Figure 5. IGFBP-2 in serum at d 57 decreased from 11.1 densitometric units for controls to 8.9 densitometric units for rbST treatment and 7.2 densitometric units for rbGRF treatment (Figure 4). Both treatments together decreased ($P=.03$) IGFBP-2 in serum compared with controls. There was no difference ($P>.27$) between rbST and rbGRF treatment on serum IGFBP-2.

Serum insulin

Concentration of insulin in serum on d 57 increased from .41 ng/ml for controls to .72 and .46 ng/ml for rbST- and rbGRF-treated cows, respectively (Figure 6). Hormone-treated cows tended to increase ($P=.08$) concentration

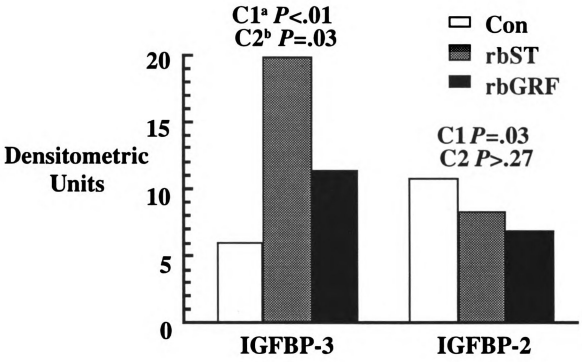

Figure 4. Least squares means of IGFBP-3 and IGFBP-2 in serum for d 57 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum). Pooled standard error of the mean among treatments was 2.7 and 1.1 densitometric units for IGFBP-3 and IGFBP-2, respectively.

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

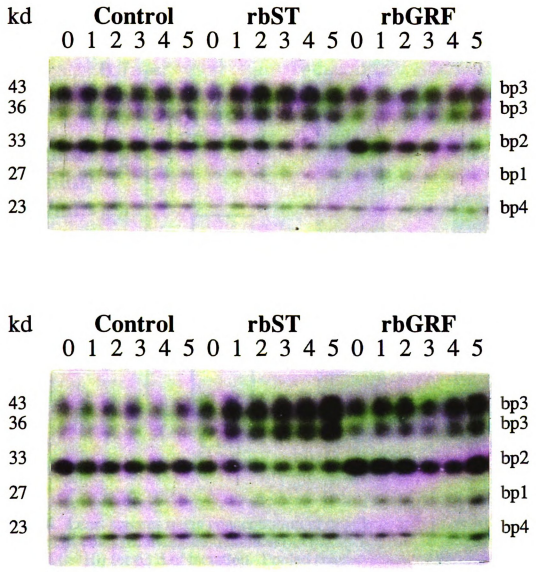

Figure 5. Representative autoradiographs of western ligand blots for IGFBP's (blocks 8 and 9). Numbers 0 through 5 represent d 0, 1, 2, 3, 29, and 57 of treatment, respectively.

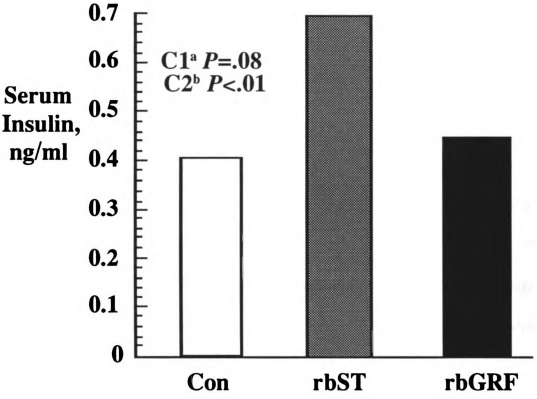

Figure 6. Least squares mean concentration of insulin in serum for d 57 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation. Pooled standard error of the mean among treatments was .08 ng/ml.

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

of insulin in serum compared with controls. Also, concentration of insulin in serum was greater ($P<.01$) for rbST- compared to rbGRF-treated cows.

Cows treated with rbST had heavier ($P<.01$) livers (wet-basis) compared with cows treated with rbGRF. Perhaps, greater insulin concentration in serum resulted in increased glycogen storage in the liver of rbST- compared with rbGRF-treated cows.

Liver ST receptor and IGF-I mRNA

Results for tissue mRNA are for d 63 of the treatment period, and have been adjusted for the amount of β-actin mRNA expression in liver. Abundance of mRNA for ST receptor was measured from autoradiographs of northern blots using densitometry (Figure 8 and 9). The band which hybridized to the ST receptor probe was measured at 4.5 kb. Expression of mRNA for ST receptor in liver was not different among treatments (Figure 7).

Expression of mRNA for IGF-I in liver, measured at 7.5 kb (Figure 8 and 9), was 290% and 191% of control for rbST- and rbGRF-treated cows, respectively (Figure 7). Hormone treatments increased ($P<.01$) expression of IGF-I mRNA in liver compared to controls. Also, rbST-treated cows increased ($P<.01$) expression of IGF-I mRNA in liver more than did rbGRF-treated cows. The results for expression of IGF-I mRNA in liver are consistent with concentration of IGF-I in serum for the three treatment groups.

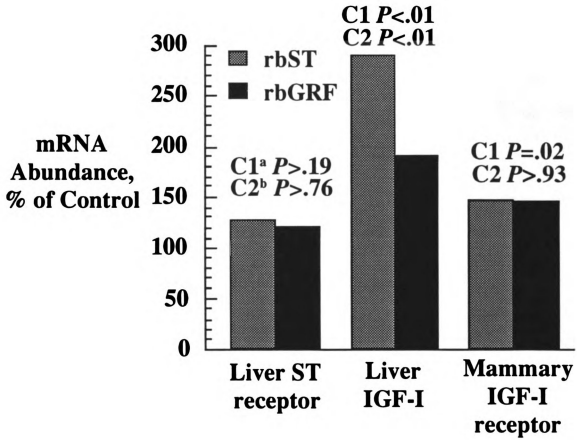

Figure 7. Abundance of mRNA for ST receptor and IGF-I in liver and IGF-I receptor in mammary tissue for d 63 of treatment of cows receiving 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum). Each bar represents the average of abundance of mRNA (least squares means) as a percent of the abundance of the respective mRNA for control cows (n=10).

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

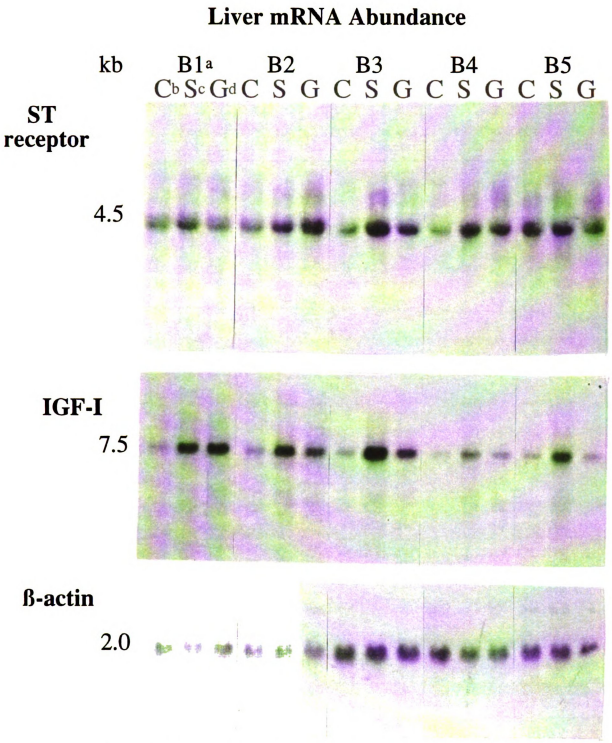

Figure 8. Autoradioraphs of northern blots for liver ST receptor, IGF-I and ß-actin mRNA for blocks 1 through 5.

[a] Block [b] Control [c] rbST treatment [d] rbGRF treatment

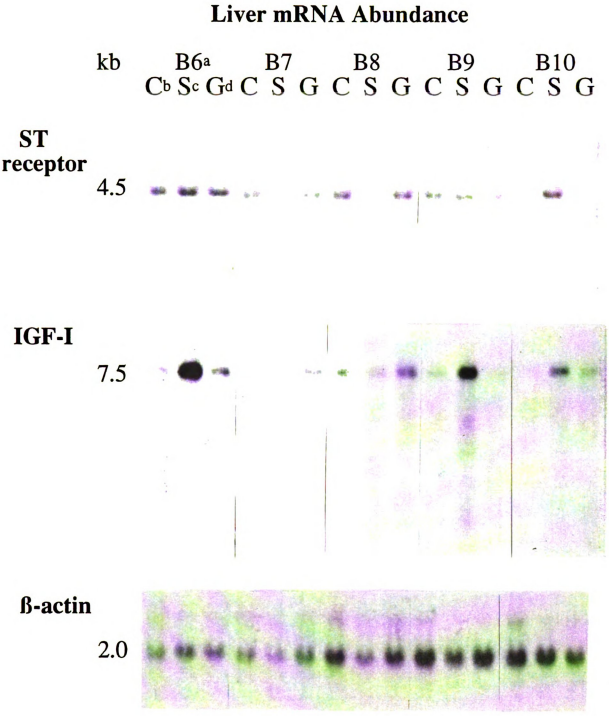

Figure 9. Autoradioraphs of northern blots for liver ST receptor, IGF-I and ß-actin mRNA for blocks 6 through 10.

[a] Block [b] Control [c] rbST treatment [d] rbGRF treatment

Mammary IGF-I receptor mRNA

The predominant type-I IGF receptor mRNA species in mammary tissue was 11.3 kb (Figure 10). Expression of β-actin mRNA in mammary tissue was used to adjust the measurement of IGF-I receptor mRNA in mammary tissue. The IGF-I receptor mRNA expression was 146% and 144% (P=.02) of control for rbST and rbGRF treatments, respectively (Figure 7). Expression of IGF-I receptor mRNA in mammary tissue was not different between rbST- and rbGRF-treated cows.

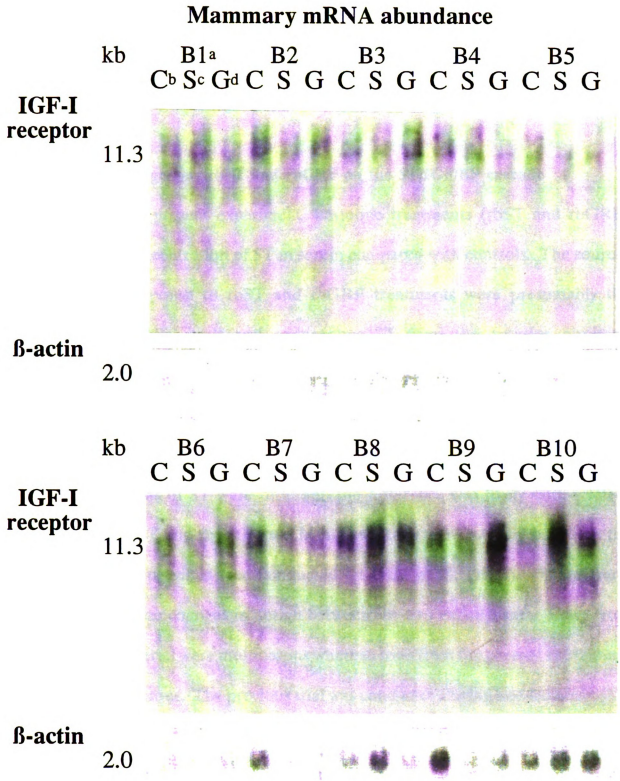

Figure 10. Autoradioraphs of northern blots for mammary IGF-I receptor and ß-actin mRNA for blocks 1 through 10.

[a] Block [b] Control [c] rbST treatment [d] rbGRF treatment

DISCUSSION

Effects of exogenous rbST and rbGRF on the ST/IGF/BP cascade

In the present experiment, hormone treatments (rbST and rbGRF) increased concentration of ST in serum compared with controls. The sources of ST in response to rbST and rbGRF treatments were presumably the exogenous rbST itself and endogenous ST from the anterior pituitary, respectively. Although our goal was to have equal elevations of ST in serum for the two hormone-treated groups, concentration of ST in serum tended (P=.13) to be greater for rbGRF- compared with rbST-treated cows. If concentrations of ST were different, the difference was likely dose-related. Doses of rbST and rbGRF used in current study were selected based on the results of a previous trial conducted by Dahl et al. (1993). There were, however, some changes in design between the two experiments. Cows from the previous trial were multiparous whereas cows from the present experiment were primiparous. The previous trial was conducted during late-lactation (175 to 235 d postpartum) whereas the present experiment was conducted during mid-lactation (118 to 181 d postpartum). Despite these differences in parity and stage of lactation between the two trials, the responses in serum ST were similar with only a trend for greater response with rbGRF in the present study.

Similar to the findings of Dahl et al. (1993), hormone treatments increased concentration of IGF-I in serum compared with controls. However, concentration of IGF-I in serum tended (P=.15) to be greater for rbST- compared to rbGRF-treated cows. The greater concentration of IGF-I in serum of rbST-treated cows occurred despite the fact that concentration of ST in serum tended to be lower, not higher, for rbST- compared to rbGRF-treated cows. Possible explanations for the IGF-I response to rbST and rbGRF treatment will be discussed in the next section of this discussion. Negative EB has been associated with low concentration of IGF-I in serum for lactating dairy cows (McGuire et al., 1992). Even though hormone treatments decreased EB compared to controls, and rbST-treated cows tended (P=.07) to have lower EB compared to rbGRF-treated cows, all cows were in positive EB. Thus, EB probably did not play a role in regulating concentration of IGF-I in serum.

Administration of rbST increases serum concentration of IGFBP-3 in dairy cows (Cohick et al., 1992). Cows treated with rbST increased (P=.03) concentration of IGFBP-3 in serum compared with rbGRF-treated cows. Zapf et al. (1989) reported that IGF-I mediates much of the ST-associated increase in IGFBP-3 levels *in vivo*. Consequently, greater concentration of IGFBP-3 in serum for rbST- compared to rbGRF-treated cows was likely related to the trend for greater concentration of IGF-I in serum of rbST- compared with

rbGRF-treated cows.

The exact role for IGFBP-3 has not yet been defined. However, IGFBP-3 can prevent IGF-I-induced IGF receptor down regulation (Conover and Powell, 1991), thereby maintaining the effectiveness of the elevated concentration of serum IGF-I during rbST administration. Blum et al. (1989) suggested that IGFBP-3 acts as a reservoir for IGF-I, continuously releasing low amounts of IGF-I and thereby creating a steady state of receptor occupancy. Binoux and Hossenlopp (1988) concluded that IGFBP-3 complexes do not cross the capillary barrier, which provides further evidence that IGFBP-3 acts as a reservoir and as a moderator of IGF-I action.

In the present experiment, exogenous hormone treatments decreased concentration of IGFBP-2 in serum. Cohick et al. (1992) found that rbST administration decreases IGFBP-2 concentration in serum. The physiological implications of this decrease in IGFBP-2 is not well understood. Binoux and Hossenlopp (1988) conclude that IGFBP-2 complexes cross the capillary barrier. Thus, IGFBP-2 may be involved in the transport of the IGFs from circulation to their target cells. The IGFBP-2 can leave the microcirculation and distribute to subendothelial tissues (Booth et al., 1990). Furthermore, plasma IGFBP-2 levels are relatively stable, and therefore may serve as a reservoir of IGF-I available for transport (Clemmons et al. 1991). Bourner et al. (1992) found that the IGFBP-2 can bind with IGF-I and act as an inhibitor or a potentiator of IGF-I-mediated DNA synthesis.

Although expression of ST receptor mRNA in liver was not different for the three groups, the results do not necessarily suggest that the activity or abundance of ST receptors in the liver was affected. Mathews et al. (1989) suggested that, in rat hepatocytes, any effect of ST on receptor expression is manifested at a post-transcriptional level, because ST receptor mRNA was not significantly affected by either hypophysectomy or rbST treatment. It is also possible that the hormone treatments alter the affinity or turnover of the receptors to increase their biological activity and abundance. Somatotropin binding studies utilizing liver from cows from the present experiment are currently being conducted in another laboratory (R. Michael Akers, Virginia Polytechnic Institute and State University). Results from these binding studies will reveal whether or not there is increased activity or abundance of the ST receptor despite the fact that mRNA expression was not altered.

Expression of mRNA for IGF-I in liver was substantially increased by both treatments and to a greater extent for rbST treatment. In hypophysectomized rats, exogenous ST increases abundance of hepatic IGF-I mRNA (Bichell et al., 1992). Furthermore, nuclear run-on assays were used to show that ST regulation of IGF-I is manifested in part at the transcriptional level (Mathews et al., 1986). Thus, transcriptional mechanisms are predominately responsible for enhanced IGF-I expression elicited by rbST (Roberts et al., 1986; Bichell et al., 1992).

Expression of mRNA for IGF-I receptor in mammary tissue increased

similarly for hormone treatments compared to controls. These results are different from those of Glimm et al., (1992) in which mammary IGF-I receptor mRNA was decreased in two out of three rbST-treated cows, but increased for the third cow. The third cow was in negative EB because her feed intake dropped significantly during the experiment. Glimm et al. (1992) speculated that the response for the third cow may reflect a high nutrient priority afforded to mammary tissue function during lactation. Stelwagen et al. (1992) suggested that cows in early lactation (usually in negative energy balance) have a selective increase of mammary IGF-I receptors or IGF-I receptor affinity, giving the mammary gland an advantage over other tissues with regard to IGF-I action and subsequent utilization of the available metabolite pool. Cows in the present experiment were in lower EB compared with controls. Consequently, the increase in expression of IGF-I receptor mRNA in mammary tissue may be a mechanism by which the mammary gland can maintain maximum functional capacity in early lactation or during rbST or rbGRF treatment.

The ST/IGF/BP cascade is stimulated more by rbST than rbGRF

Results of the present experiment are consistent with the hypothesis that rbST and rbGRF treatments stimulate the ST/IGF/BP cascade. However, the extent to which the hormone treatments stimulated the cascade was different. Even though concentration of ST in serum tended to be higher for cows

treated with rbGRF compared with cows treated with rbST, concentration of IGF-I in serum tended to be lower, not higher, for rbGRF treatment compared with rbST treatment. Cows treated with rbST also had greater expression of IGF-I mRNA in liver than cows treated with rbGRF. In agreement with concentration of IGF-I in serum, increased IGF-I mRNA expression indicates that IGF-I protein synthesis in the liver was greater for rbST- than rbGRF-treated cows. Furthermore, rbST treatment increased IGFBP-3 to a greater extent than rbGRF treatment. Thus, compared with rbST, rbGRF resulted in greater ($P<.01$) liver IGF-I mRNA expression, a trend for greater ($P=.15$) serum IGF-I and greater ($P=.03$) serum IGFBP-3. Perhaps the difference in serum IGF-I was less significant than the other two measures because IGFBPs may interfere with IGF-I radioimmunoassay, and because the IGFBP profiles were different for the two hormone treatments. Nevertheless, these results indicate that the endocrine ST/IGF/BP cascade was less responsive to endogenous ST secreted in response to rbGRF than it was to exogenous rbST. Possible reasons why the ST/IGF/BP cascade was less responsive to rbGRF than to rbST include their differential effects on ST variants, pulsatility profiles and/or concentration of insulin in serum.

Endogenous ST, produced in the anterior pituitary, can be either 190 or 191 amino acids long, and can have either leucine or valine at position number 126 in the protein sequence (Wood et al., 1989). Typically, pituitary production of ST involves approximately equal amounts of the 190 and 191

amino acid proteins, and about two-thirds of the total produced has leucine at position 126, while the remaining one-third have the amino acid valine at position 126 (Wood et al., 1989). Treatment with rbST leads to a predominant variant in circulation which is similar to the 191 amino acid native variant with leucine at position 126 in the protein sequence. Cows treated with rbGRF will likely stimulate release of all variants of ST from the anterior pituitary. It is possible that the exogenous rbST variant stimulates IGF-I mRNA expression to a greater extent than the combination of variants secreted in response to rbGRF. Thus, the increase in IGF-I mRNA in the liver and subsequent serum IGF-I concentration in the present experiment may be a result of differences in circulating ST variant profiles in response to rbST vs rbGRF treatment.

Pulsatility of ST in circulation may also be an important determinant of ST action *in vivo*. We found that cows treated with rbGRF had greater pulsatility (number of peaks/d, amplitude and peak length) of ST in serum than rbST-treated cows. In fact the rbST-treated cows exhibited little pulsatility of ST in serum. Perhaps, ST at steady concentrations is a more effective secretagogue for liver IGF-I than ST at pulsatile concentrations. Bick et al. (1992) found that rats have greater concentration of IGF-I in serum after continuous (similar to continuous rbST) than after pulsatile (similar to continuous rbGRF) administration of the same daily dose of ST. The mechanism to explain increased concentration of IGF-I in serum for continuous verses pulsatile infusion is unclear. However, it is possible that

differences in pulsatility may alter the binding characteristics or abundance of the ST receptor in the liver and subsequently increase synthesis and secretion of IGF-I.

In the present experiment, cows treated with rbST tended (P=.10) to have greater concentration of insulin in serum than cows treated with rbGRF. Perhaps this greater concentration of insulin in serum is the reason that IGF-I mRNA was stimulated to a greater extent in rbST- than rbGRF-treated cows. Exogenous insulin increases IGF-I mRNA expression in rats, thus insulin regulates the IGF-I system *in vivo* (Salamon et al., 1989). In hepatocyte primary culture, insulin also appears to regulate IGF-I release at the mRNA level (Phillips et al., 1991). Thus, it is possible that insulin was a factor in stimulating IGF-I mRNA expression and subsequent synthesis and secretion of IGF-I and IGFBP-3 in liver tissue to a greater extent in rbST- compared with rbGRF-treated cows.

Similar milk yield despite different effects on the ST/IGF/BP cascade

Despite the fact that rbST stimulated the endocrine ST/IGF/BP cascade to a greater extent compared to rbGRF, both treatments stimulated milk yield similarly. Thus, it is possible that the galactopoietic effects of rbGRF are not mediated exclusively through the endocrine ST/IGF/BP cascade.

One possible reason to support this speculation is that the four different ST variants produced in response to rbGRF stimulate galactopoiesis through

an autocrine/paracrine pathway as well as the endocrine ST/IGF/BP pathway. The combination of these variants may stimulate production of IGF-I and its binding proteins in target tissues such as the mammary gland to a greater extent than the single rbST variant. Thus, less stimulation of the endocrine cascade by rbGRF treatment may be supplemented by an autocrine and/or paracrine pathway resulting in similar milk yield for rbST- and rbGRF-treated cows. In further support, Krivi et al., (1989) reported differences in galactopoietic activity of ST variants in lactating cows.

Pulsatility of ST in circulation may also be an important determinant of the galactopoietic effects for the hormone treatments. As mentioned previously, cows treated with rbGRF had greater pulsatility (number of peaks/d, amplitude and peak length) of ST in serum than rbST-treated cows. Perhaps the greater pulsatility with rbGRF treatment stimulated galactopoiesis at an autocrine/paracrine level more than did rbST treatment. Bick et al. (1992) found that pulsatile infusion (similar to rbGRF treatment) induced a greater body weight gain than the continuous infusion (similar to rbST treatment). In another study the levels of hepatic IGF-I mRNA were similar in rats given continuous and pulsatile ST treatment, whereas the IGF-I mRNA levels in skeletal muscle and cartilage were higher after pulsatile than after continuous ST treatment (Isgaard et al., 1988). Thus, it is possible that more pulsatile secretion of ST, which was a result of rbGRF treatment, may enhance the galactopoietic effects of ST either directly or by stimulating autocrine

and/or paracrine action of IGF-I at target tissue even though circulating concentration of IGF-I tended to be lower in rbGRF-treated compared to rbST-treated cows.

It may also be possible that the increased immunoassayable IGF-I was not reflected in increased biological activity of IGF-I for rbST- compared with rbGRF-treated cows. Although immunoassayable IGF-I concentration in serum was greater with rbST treatment, IGFBP-3 was also greater; thus, more of the serum IGF-I was likely bound to IGFBP-3 in serum of rbST-treated cows. Consequently, the two treatments may have had a similar pool size of unbound biologically active IGF-I even though the pool size of bound IGF-I and IGFBP-3 was greater for rbST than rbGRF treatments. Nevertheless, it is difficult to determine whether this was the case or not because current methods do not allow us to measure the amount of unbound verses bound IGF-I in serum.

Lastly, it is possible that the reason why milk yield was similar, despite less stimulation of the endocrine ST/IGF/BP cascade by rbGRF, is that the rbGRF compound may have direct effects at tissues which support increased milk yield.

SUMMARY AND CONCLUSIONS

The main objective of this thesis was to quantify and compare the long-term effects of exogenous rbST and rbGRF on the endocrine ST/IGF/BP cascade in lactating dairy cows. Results from the present experiment are consistent with the idea that treatment with rbST and rbGRF stimulate the endocrine ST/IGF/BP cascade to increase milk yield. However, rbST treatment stimulated the cascade to a greater extent compared with rbGRF treatment. It is likely that differences in the profile of ST variants, pulsatility of ST and/or concentration of insulin in serum may have facilitated the differences in the effects of rbST compared to rbGRF on the somatotropic cascade.

However, both rbST and rbGRF treatments increased milk yield to the same extent compared to controls. Possibly, the galactopoietic effects of rbGRF are not mediated exclusively through the endocrine ST/IGF/BP cascade.

Lastly, results from the present experiment indicate the complexity of the coordination of physiology in response to rbST and rbGRF treatment. The precise mechanisms by which these hormone treatments enhance galactopoiesis remains to be elucidated. In the future, whether or not IGF-I is the sole

mediator of the galactopoietic actions of rbST and rbGRF needs to be investigated. One possible experiment would be to investigate the effects of rbST or rbGRF with concurrent administration of an IGF-I antibody.

In addition to the investigation of the endocrine actions of IGF-I, the effects of rbST or rbGRF treatment on autocrine and/or paracrine action of IGF-I and its binding proteins in various tissues need to be investigated. This may be achieved by quantifying the expression of ST receptor, IGF-I and IGFBP mRNA in tissues (such as mammary gland, liver and adipose) which support enhanced milk yield in response to hormone treatments.

APPENDICES

APPENDIX A

Table 3. Ingredient composition of diets fed during the treatment period.

Ingredient	percentage, %DM
Alfalfa haylage	21.5
Corn silage	20.5
High moisture shell corn	19.0
Ground shell corn	10.5
Soybean meal	11.7
Whole cotton seed	10
Mega-Lac	.3
Energy balancer	1.5
Bypass protein	2.0
Mineral/Vitamin mix	2.0

Table 4. Chemical composition of diets fed during the treatment period.[ab]

Dry matter, %	59
Crude protein, %	18
Neutral detergent fiber, %	31
Acid detergent fiber, %	19
Net energy lactation, Mcal/d	.79

[a] Calculated values from NRC book values and forage report values.

[b] Average percentage of CP and NDF analyzed from weekly TMR samples were 17.4 and 33.5, respectively.

APPENDIX B

Introduction

A second objective of this experiment was to investigate the short-term effects of rbST verses rbGRF treatment on the endocrine ST/IGF/BP cascade.

Materials and Methods

Additional blood samples were collected for analysis of the short-term effects. Blood samples were collected from cows at 6 h intervals for 2 d (800 h on d 0 to 2000 h on d 1). Blood samples were also collected at 24 h intervals for 2 d (1400 h on d 2 to 1400 h on d 3). Samples of blood were stored at 4°C for approximately 24 h and then serum was harvested by centrifugation for 30 min at 1550 x g and frozen at -20°C until assayed. Blood samples within a day were pooled into one sample for each day.

Concentration of serum ST (Moseley et al., 1982) was quantified in blood collected at d 1. Concentration of IGF-I, IGFBP-2 and IGFBP-3 in serum were quantified in blood collected at d 0, 1, 2 and 3 using the same methods as described in the previous materials and methods section.

All data was subjected to randomized complete block ANOVA. Short-term effects of the treatments on ST was analyzed with mean comparisons using orthogonal contrast (C1= Control vs rbST + rbGRF and C2= rbST vs

rbGRF). Short-term responses for concentration of IGF-I, IGFBP-2 and IGFBP-3 in serum were analyzed using linear orthogonal contrast (CL1= Linear; Control vs rbST + rbGRF and CL2= Linear; rbST vs rbGRF).

Results and Discussion

Concentration of ST in serum was increased (P<.01) for hormone treatments compared with controls (Figure 11). Treatment with rbST increased (P<.01) concentration of ST to a greater than did rbST treatment at d 1. Since the long-term effects of rbST increased ST to a lesser extent than rbGRF treatment, the acute increase of ST for rbST compared to rbGRF is not likely dose related. The reason for the greater increase in concentration of ST in serum at d 1 is likely that the ST system *in vivo* had not yet equilibrated in response to rbST infusion.

Hormone treatments increased (P<.01) concentration of IGF-I from d 0 to 3 compared with controls (Figure 12). These result are consistent with the literature since an increase in the concentration of IGF-I in serum is characteristic within the first few days of rbST administration to dairy cows (Davis et al., 1987). The linear response for serum IGF-I between rbST and rbGRF treatment was different (P<.01). The slope for concentration of IGF-I in serum at d 1 rose more rapidly for rbST- compared to rbGRF-treated cows. The faster response by rbST treatment can likely be attributed to the greater concentration of ST in serum at d 1.

Hormone treatments increased (P<.01) concentration of IGFBP-3 from d 0 to 3 compared to controls (Figure 13). Consequently, these greater concentration of IGFBP-3 in serum can be attributed to the elevated concentration of IGF-I in serum since it has been reported that IGF-I mediates much of the ST-associated increase in IGFBP-3 levels (Zapf et al., 1989. Also, similar to the IGF-I response, concentration of IGFBP-3 rose faster in rbST-compared to rbGRF-treated cows. The linear response for IGFBP-3 between rbST and rbGRF treatment was different (P=.03). Thus, the more rapid increase in concentration of IGF-I may, in turn, be attributed to the rapid rise in concentration of IGF-I from d 0 to 3. The linear response for the concentration of IGFBP-2 between rbST and brGRF treatment was not different (P>.65) (Figure 14).

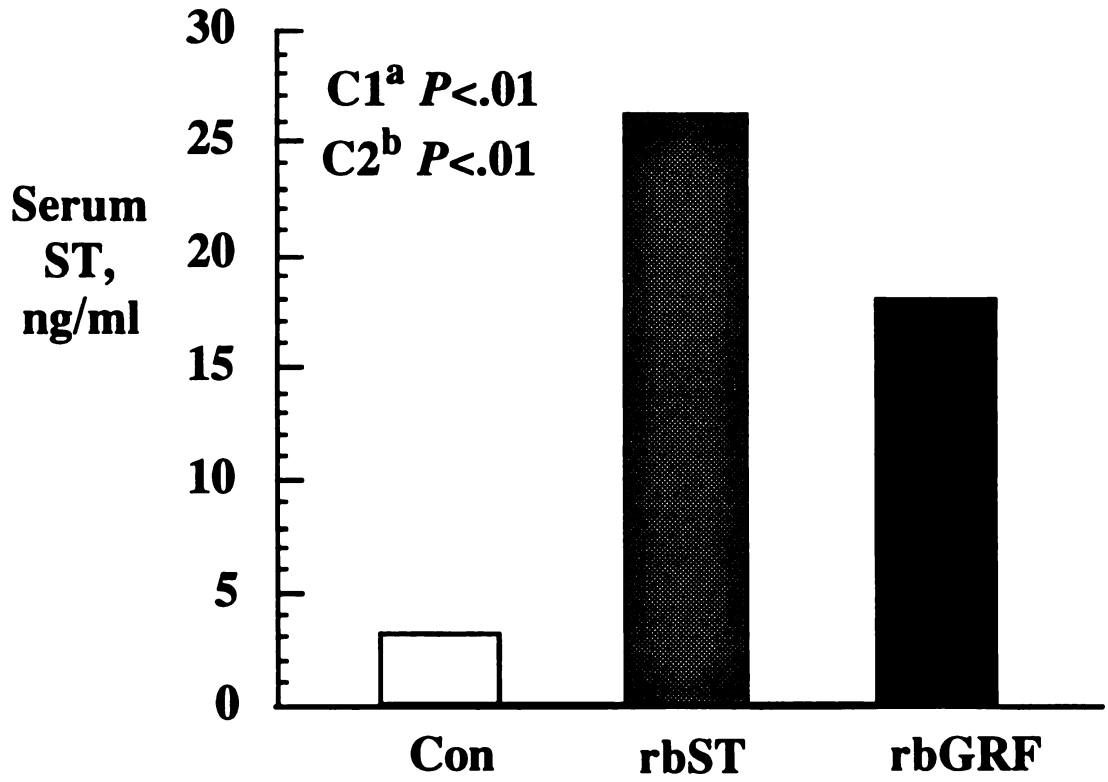

Figure 11. Least squares mean concentration of ST in serum for d 1 of treatment of cows receiving no treatment (n=10), 29 mg rbST/d (n=10) or 12 mg rbGRF/d (n=10) for 63 d of lactation (d 118 to 181 postpartum). Pooled standard error of the mean among treatments was 1.6 ng/ml.

[a] Orthogonal contrast of means for control vs. average of rbST- and rbGRF-treated cows.

[b] Orthogonal contrast of means for rbST treatment vs. rbGRF treatment.

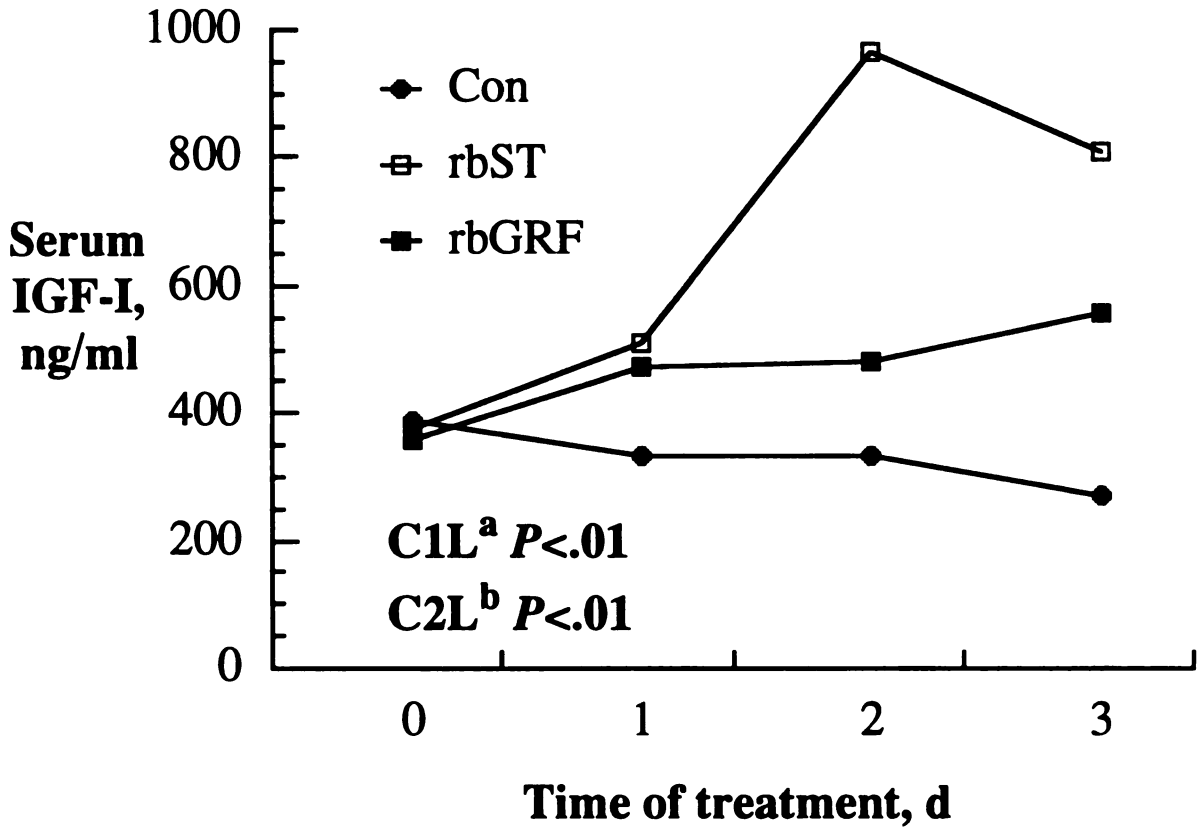

Figure 12. Least squares mean concentration of IGF-I in serum of cows receiveing no treatment (n=10), 29 mg rbST/d (n=10) of 12 mg rbGRF/d (n-10). Each point represents the average of a treatment group at d 0, 1, 2 and 3. Standard error of the mean among treatments was 49 ng/ml.

[a] Orthogonal contrast of control vs. average of rbST- and rbGRF- treated cows for linear contrast of means at d 0 to 3.

[b] Orthogonal contrast of rbST treatment vs. rbGRF treatment for linear contrast of means at d 0 to 3.

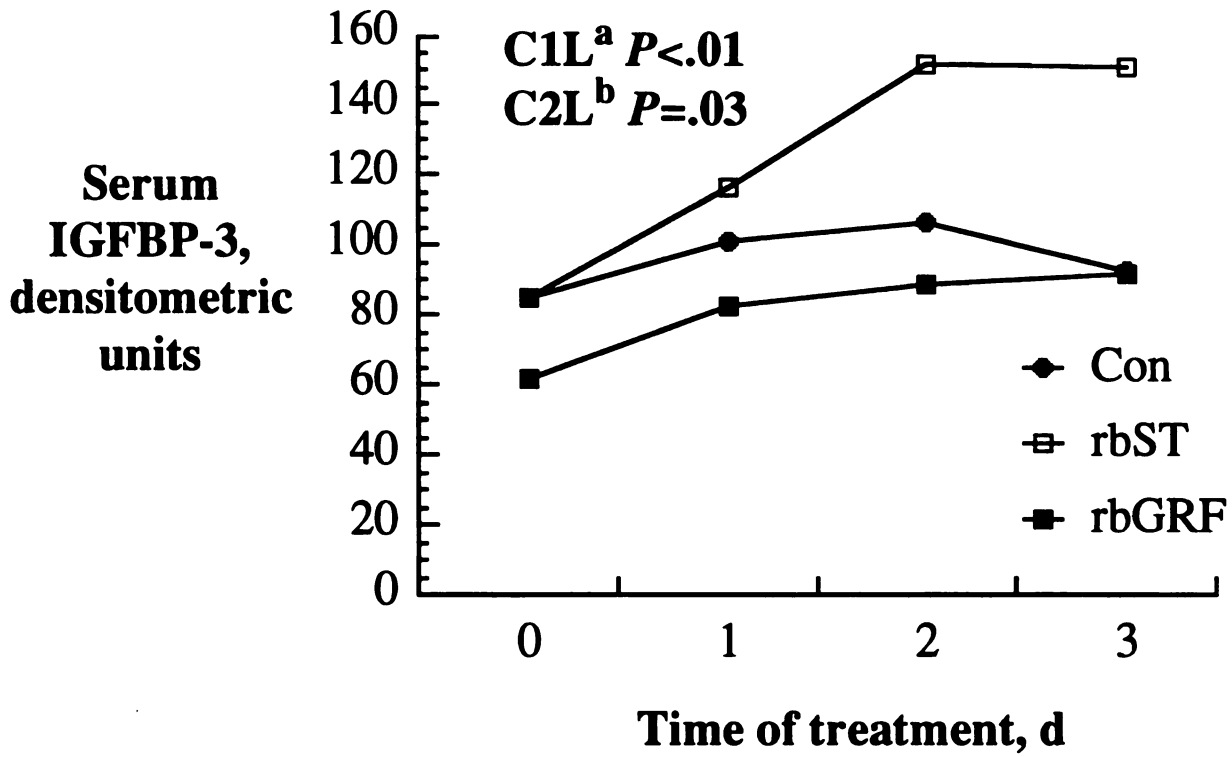

Figure 13. Least squares mean concentration of IGFBP-3 in serum of cows receiveing no treatment (n=10), 29 mg rbST/d (n=10) of 12 mg rbGRF/d (n-10). Each point represents the average of a treatment group at d 0, 1, 2 and 3. Standard error of the mean among treatments was 9.6 densitometric units.

[a] Orthogonal contrast of control vs. average of rbST- and rbGRF- treated cows for linear contrast of means at d 0 to 3.

[b] Orthogonal contrast of rbST treatment vs. rbGRF treatment for linear contrast of means at d 0 to 3.

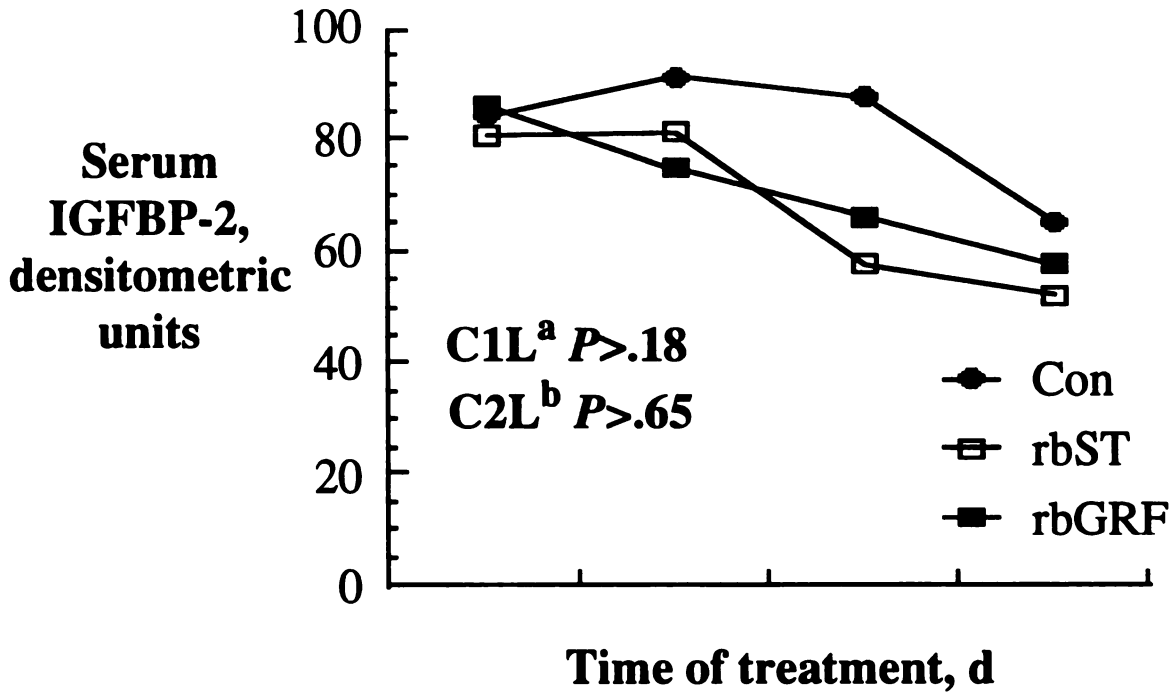

Figure 14. Least squares concentration of IGFBP-2 in serum of cows receiveing no treatment (n=10), 29 mg rbST/d (n=10) of 12 mg rbGRF/d (n-10). Each point represents the average of a treatment group at d 0, 1, 2 and 3. Standard error of the mean among treatments was 5.5 densitometric units

[a] Orthogonal contrast of control vs. average of rbST- and rbGRF- treated cows for linear contrast of means at d 0 to 3.

[b] Orthogonal contrast of rbST treatment vs. rbGRF treatment for linear contrast of means at d 0 to 3.

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