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GROWTH HORMONE-RELEASING FACTOR IS GALACTOPOIETIC IN DAIRY COWS

By

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I dedicate this dissertation to my mother and father for their encouragement, understanding and love.

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ABSTRACT

GROWTH HORMONE-RELEASING FACTOR IS GALACTOPOIETIC IN DAIRY COWS

By

William John Enright

Three studies were conducted in dairy cattle to examine effects of intravenously administered growth hormone (GH)-releasing factor (GRF) on: (1) yield and composition of milk, (2) efficiency of milk production, and (3) concentrations of hormones and metabolites in blood.

Peak GH responses of bull calves to 0, 2.5, 10 and 40 μg GRF/100 kg body weight (BW) were 7, 8, 18 and 107 ng/ml serum. Although considerable variation was observed, in calves injected with GRF (20 μg/100 kg BW) at 6-h intervals for 48 h peak GH increased from 3.1 to 70 ng/ml serum. Calves infused with 0 or 200 μg GRF/h for 6 h averaged 7.4 and 36.5 ng GH/ml serum, respectively.

Peak height of serum GH increased 10-fold after 10, 20 or 40 μg GRF/100 kg BW administered every 4-h for 24 h to cows. Administration of 20 μg GRF/100 kg BW every 4 h for 10 d increased milk yield from 25.4 to 27.7 kg/d. GRF did not affect milk composition or feed intake, but increased feed to milk conversion efficiency. Increased GH after GRF was similar on d 1 and 10.

Serum GH concentrations increased 5-fold after infusion

of 3.125, 6.25, 12.5, 25.0 or 50.0 mg GRF/cow for 24 h. Infusions of 1 and 3 mg GRF/cow/24 h for 20 d increased milk yield 11 and 23%, respectively. GRF had only small effects on milk composition, feed intake, BW and energy efficiency of milk production. Blood was sampled on d 1, 10 and 19. One mg GRF increased blood concentrations of GH (d 10) and glucose (d 19) but had no effect on prolactin, insulin, cortisol, triiodothyronine (T_3), thyroxine and non-esterified fatty acids (NEFA). In contrast, 3 mg GRF increased GH (d 1, 10 and 19), T_3 (d 10 and 19), insulin (d 19), NEFA (d 1, 10 and 19) and glucose (d 19).

GRF is galactopoietic. Differences in serum GH response to GRF and enhanced pulsatile GH release during GRF infusion may be related to synchrony among exogenous GRF and secretion of endogenous GRF and somatostatin. The anterior pituitary gland does not become refractory to GRF. Changes in milk yield and composition, and blood hormones and metabolites parallel changes previously reported for exogenous GH.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	acid detergent fiber
BW	body weight
cAMP	cyclic adenosine 3',5'-monophosphate
CP	crude protein
DM	dry matter
DNA	deoxyribonucleic acid
EE	energy efficiency
GH	growth hormone
GRF	growth hormone-releasing factor
im	intramuscular
iv	intravenous
mRNA	messenger ribonucleic acid
n	number
NE_l	net energy for lactation
NRC	National Research Council
NS	nonsignificant
SCC	somatic cell count
SCM	solids-corrected milk
SE	standard error of the means
SED	standard error of the differences
sc	subcutaneous
SRIF	somatostatin
TRH	thyrotropin-releasing hormone
vs	versus

INTRODUCTION

Total milk production in the United States by 1990 is forecast at 66.3 million metric tons with a present day value of approximately $16.5 billion (Mix, 1987). Thus, the dairy industry is an important segment of the agricultural economy. Because of continued reductions in profit margins, increasing efficiency of milk production is critical to future success of the dairy industry.

With this in mind, the Intersociety Research Committee (Anonymous, 1978) and the Conference on "Animal Agriculture - Research to Meet Human Needs in the 21st Century" (Pond et al., 1980) identified several high priority areas that needed additional research. Areas included: (1) control of hormonal and metabolic mechanisms, and (2) adoption of principles of genetic engineering technology in order to improve animal efficiency. In response to these needs, recently developed techniques of genetic engineering have now made possible the production of several agents that stimulate milk production and increase efficiency. Growth hormone (GH) is a product of these biotechnologies. Milk yield of cows was increased as much as 40% with daily injections of GH and gross feed efficiency was also improved (Bauman et al., 1985). In addition, recombinantly-derived

GH is at least as potent as pituitary-derived GH (Bauman et al., 1985) and large quantities of GH can now be produced for potential commercial use. Genetical, nutritional and managerial improvements are forecast to increase average annual milk yield per dairy cow in the United States from 5680 kg in 1984 to 7425 kg by the year 2000 (Mix, 1987). However, if GH is used by all dairy farmers on all cows, average milk yield could be increased further to 9281 kg/cow/year (Mix, 1987).

Purification and characterization of human pancreatic GH-releasing factor (GRF; Guillemin et al., 1982) and more recently hypothalamic bovine GRF (Esch et al., 1983) provides a means of specifically stimulating endogenous GH secretion in cattle. Obviously, increasing endogenous GH in blood (via GRF) may improve lactational efficiency similar to that observed with GH. Indeed, GRF may have several advantages relative to GH as a galactopoietic agent. GRF is a smaller peptide [circa 44 amino acids (aa)] than bovine GH (191 aa), and the biologically active portion of GRF is only 29 aa in length. Because of its smaller size, GRF should be easier and more economical to produce and deliver, and less quantity (by weight) of hormone would be required to stimulate milk yield, relative to GH. In addition, GRF likely increases all variants of endogenous GH from the pituitary gland, whereas recombinant GH is only one form of the hormone. A potential disadvantage of GRF, based on

previous research with other hypothalamic releasing factors, is the possibility that the pituitary gland may become refractory in terms of GH release to continued GRF administration.

The primary objective of this research was to determine effects of exogenous GRF on lactational performance of dairy cows. In addition, I characterized changes in blood concentrations of GH, prolactin, insulin, cortisol, triiodothyronine, thyroxine, glucose and non-esterified fatty acids in response to GRF.

REVIEW OF LITERATURE

In this review of literature I will consider the involvement of growth hormone (GH) and growth hormone-releasing factor (GRF) in lactation, especially in ruminants.

Specifically, Section A will deal with the effects and potential mechanisms of action of GH on milk production in ruminants. Section B will deal with hypothalamic regulation of GH secretion, physiology of GRF, and effects and potential mechanisms of action of GRF on GH secretion and milk production in ruminants.

Chapter 1 (Enright et al., 1987) and Chapter 2 (Enright et al., 1986) of this thesis are published and pertinent to certain areas of the review of literature but are not discussed in detail in the review of literature.

A. Growth Hormone (GH) and Lactation

1. Introduction

GH in mammals affects many postnatal bodily processes such as cell division, skeletal growth, and protein, lipid and carbohydrate metabolism. The precise role of GH in the

control of metabolism during growth and lactation is not fully understood in farm species.

Much evidence exists to support the importance of GH in lactation. For example, GH is essential for maintenance of lactation in hypophysectomized goats and sheep (Cowie, 1969). Furthermore, basal concentrations and pulsatile activity of serum GH, and TRH-induced increases in serum GH are greatest in early lactation of cows, the stage of lactation when milk production is greatest (Koprowski and Tucker, 1973; Bourne et al., 1977; Vasilatos and Wangsness, 1981). In addition, increased concentrations of GH in serum are associated with increased milk production in superior vs good producing cows (Kensinger et al., 1984).

In recent years much evidence has become available to support a role for GH in the homeorhetic control of nutrient partitioning during growth and lactation in the ruminant (Bauman and McCutcheon, 1986). Bauman and Currie (1980) originally defined homeorhesis as "the orchestrated changes for the priorities of a physiological state, i.e. coordination of metabolism in various tissues to support a physiological state". Much of the evidence supporting a critical role for GH in homeorhesis during the ruminant lactation has been generated from investigations involving administration of exogenous GH to lactating cows. It appears that GH coordinates the partitioning of nutrients among body tissues for preferential use by the mammary gland

in addition to enhancing the ability of mammary tissue to synthesize milk components (Peel et al., 1981).

In addition to the knowledge gained using exogenous GH, recent research with GH has clearly shown that GH is a potent galactopoietic agent with immense commercial potential to increase efficiency of milk production of dairy cows (Mix, 1987; Peel and Bauman, 1987).

2. Yield and Composition of Milk

Asimov and Krouze (1937) increased milk production in dairy cows by administration of crude extracts from bovine pituitary glands. It was later discovered that GH was the principal galactopoietic component (Young, 1947). Because of the low yield of extraction of GH from pituitary glands and because of the cost and difficulty of the extraction and purification procedures, use of exogenous GH to increase milk production commercially has not been viable. Recently, however, recombinant deoxyribonucleic acid (DNA) technology was used to produce methionyl bovine (b) GH from Escherichia coli bacteria (Seeburg et al., 1983). This technology permits large scale production of GH. Bauman et al. (1982) were first to report that recombinantly-derived methionyl bGH was equipotent to purified pituitary-derived bGH in stimulating milk yield. Exogenous GH is also galactopoietic in goats (Mepham et al., 1984) and sheep (Hart et al., 1985c).

Most studies investigating effects of GH on milk production in dairy cows were of short duration (10 to 21 d), used a daily sc injection of pituitary-derived GH and increased milk yield approximately 5.0 kg/d (Bullis et al., 1965; Peel et al., 1981, 1983; Fronk et al., 1983; Eppard et al., 1985b; Hart et al., 1985a; Richards et al., 1985; McCutcheon and Bauman, 1986a).

Of the four longer-term (10 to 27 wk) studies reported, three utilized pituitary-derived GH (Brumby and Hancock, 1955; Machlin, 1973; Peel et al., 1985) while the fourth employed one dose of pituitary-derived GH and three doses of recombinantly-derived GH (Bauman et al., 1985). In the latter study, recombinant GH at the same dose as pituitary GH was more efficacious in stimulating milk yield. The reason for the difference in potency between the two sources of GH is unknown. In these longer-term studies GH administered im or sc every 1 or 2 d increased milk yield by 6.5 kg/d.

A curvilinear relationship occurs between dose of exogenous GH and increases in milk yield (Eppard et al., 1985b; Bauman et al., 1985). It appears that the optimal dose of pituitary or recombinant GH as galactopoietic agents is 25 to 50 mg/d. Provided mean daily concentration of serum GH is elevated 3 to 4-fold above controls, there is no effect of pattern of administration of GH on milk yield in dairy cows (Fronk et al., 1983; McCutcheon and Bauman,

1986a). Serum concentrations of GH are maximal about 3 h after a sc injection of GH and then decrease gradually so that by 18 to 24 h concentrations of GH approach control values (Fronk et al., 1983; McCutcheon and Bauman, 1986a). The actual increase in milk yield (kg/d) due to exogenous GH was similar in early, mid and late lactation (Peel et al., 1983; Richards et al., 1985). In 24 cows, Eppard et al. (1987) did not observe any adverse health or reproductive problems either during or after exogenous GH for 188 d.

In short-term experiments exogenous GH does not affect percentage of milk fat or protein when cows are in positive energy and nitrogen balance (Peel et al., 1983; Fronk et al., 1983; Eppard et al., 1985b). However, when cows are in negative energy and nitrogen balance the percentage of milk protein decreases slightly in response to GH (Peel et al., 1981, 1983). In contrast, percentage of milk fat is increased by GH when cows are in negative energy balance (Peel et al., 1983; Bitman et al., 1984). Increased yield of milk fat induced by GH is disproportionately greater for long chain fatty acids as compared with increments in short or medium chain fatty acids (Bitman et al., 1984; Eppard et al., 1985a). This effect is most marked when cows are in severe negative energy balance (Bitman et al., 1984) and likely reflects increased mobilization of adipose tissue reserves. GH treatment does not alter percentage of milk lactose (Bauman and McCutcheon, 1986). GH treatment increases concentration of α-lactalbumin but does not alter

concentrations of major mineral elements in milk (Eppard et al., 1985a). In longer-term trials GH administration does not affect milk composition (Peel et al., 1985; Bauman et al., 1985).

3. Lipid Metabolism

When dairy cows are in positive energy balance there is no effect of GH on concentrations of non-esterified fatty acids (NEFA) in blood (Peel et al., 1982a; Eppard et al., 1985b). However, when cows are in negative energy balance during GH treatment, blood NEFA are elevated and indeed rates of irreversible loss and oxidation of NEFA are increased (Peel et al., 1982b; Bitman et al., 1984; Peel and Bauman, 1987). Similarly, GH treatment increases blood NEFA in sheep (Hart et al., 1984b; Hart et al., 1985c) and growing beef heifers (Eisemann et al., 1986a), and increases irreversible loss and oxidation of NEFA in growing beef heifers (Eisemann et al., 1986a).

Whether GH is lipolytic in ruminants is controversial (Peel and Bauman, 1987). Ovine GH does not have a direct lipolytic effect on ovine adipose tissue in vitro (Duquette et al., 1984). The lipolytic effect of chronic GH administration in cattle appears to be due to increased sensitivity of adipose tissue to the lipolytic effect of epinephrine (McCutcheon and Bauman, 1986b; Peters, 1986).

GH may affect the number and (or) affinity of receptors for epinephrine or other lipolytic hormones. Indeed, several pieces of evidence suggest that there are GH receptors in adipose tissue of ruminants. Firstly, there are GH receptors in adipose tissue of other species (e.g., rats; Gavin et al., 1982) and secondly, an in vitro study with ovine adipose tissue demonstrated that GH antagonizes the responsiveness of adipose tissue to insulin (Vernon, 1982).

Several researchers (Chawla et al., 1983; Bauman and McCutcheon, 1986) suggest that the observed acute lipolytic effect of GH (Kronfeld, 1965) is due to impurities in the GH preparation. Indeed, in studies using highly purified pituitary-derived GH or recombinant GH in dairy cows an acute effect of GH on blood NEFA has not been observed (Bauman and McCutcheon, 1986). In contrast, Hart et al. (1984b) reported that pituitary and recombinant GH were acutely lipolytic in vivo in sheep. But, the recombinant GH used by Hart et al. (1984b) was not lipolytic in rat adipose tissue in vitro, while pituitary GH was. These workers suggested that recombinant GH must be altered (e.g., by proteolytic cleavage) in the body before recombinant GH gains the lipolytic property. Bell et al. (1985) believe that caution must be exercised in interpreting the study of Hart et al. (1984b) because the pH extremes used to solubilize the GH preparations may have produced conformational artifacts in the GH molecule. In another study, Hart et al. (1984a) found that bovine pituitary GH,

as commonly extracted, is composed of four distinct fractions. All four were lipolytic (using rat adipose tissue in vitro) while the two middle fractions were the most immunoreactive, and were diabetogenic (using rat adipose tissue in vitro and insulin-tolerance tests in goats) and growth promoting (rat tibia test). Thus, it appears that extracted bovine pituitary GH is composed of various molecular forms with different metabolic properties.

In addition to effects on lipolysis, GH may decrease lipogenesis. For example, GH antagonizes the response of porcine, bovine and ovine adipose tissue in vitro to insulin (Vernon, 1982; Etherton and Walton, 1986). Indeed, there is evidence in rats that GH decreases total activities of lipoprotein lipase, acetyl-coenzyme A carboxylase and palmitate synthetase, while increasing activity of hormone sensitive lipase (Goodman, 1963; Bunyan and Greenbaum, 1965; Toshio et al., 1981).

Bitman et al. (1984) reported that GH treatment of dairy cows in severe negative energy balance did not affect major plasma lipid classes, with the exception of NEFA. Pocius and Herbein (1986) found no effect of GH on blood concentrations of ketones, β-hydroxybutyrate and acetoacetate, or on ketone and CO_2 production from butyrate or palmitate in liver slices taken from GH-treated dairy cows in positive energy balance. Surprisingly, they saw no effect of GH on fatty acid oxidation in the liver, although

lipolysis was increased.

4. Carbohydrate Metabolism

GH treatment of dairy cows generally does not alter blood concentrations of insulin or glucose (Peel et al., 1981, 1982a, 1983; Eppard et al., 1985b; Pocius and Herbien, 1986). Similarly, GH does not affect blood glucose in beef heifers (Eisemann et al., 1986a), although blood insulin is increased in beef heifers and steers (Eisemann et al., 1986a; Peters, 1986). In contrast to cattle, exogenous GH increased blood glucose in sheep and goats (Hart et al., 1984b; Mepham et al., 1985; Hart et al., 1985c). Also, GH treatment increased blood insulin of sheep in one study (Hart et al., 1985c), but not in another (Hart et al., 1984b). In addition, exogenous GH for 2 d impaired the ability of insulin to lower blood glucose concentrations (Hart et al., 1984b). In contrast, 6-h iv infusion of GH in sheep had no effect on glucose metabolism, and there was no evidence for insulin resistance (Laarveld et al., 1986). Thus, it seems that several days of exposure to GH is necessary for insulin resistance in ruminants to develop (Hart, 1983).

Because of increased yield of milk and lactose after GH treatment of dairy cows, mammary uptake of glucose must be increased. Indeed, GH treatment of dairy cows in negative energy balance increases the rate of irreversible loss of

glucose and reduces glucose oxidation to CO_2 (Bauman and McCutcheon, 1986). In addition, there is evidence that GH increases hepatic capacity for propionate and alanine conversion to glucose and CO_2 (Pocius and Herbein, 1986). These additional sources of glucose may become important when milk and component yields are markedly increased by GH.

Thus, in short-term GH trials, changes in irreversible loss and oxidation rates of glucose and NEFA can account in large part for increased secretion of milk, lactose and fat. During negative energy balance, increased mobilization and utilization of adipose tissue as energy substrate (with subsequent sparing of glucose from oxidation) appears to be a very significant part of the process. Changes in lipid and carbohydrate metabolism that occur in response to GH treatment during positive energy balance are less well understood. However, it is likely that glucose synthesis is increased and lipid synthesis is decreased by exogenous GH during positive energy balance (Bauman and McCutcheon, 1986).

5. Protein Metabolism

Effects of GH on amino acid metabolism in lactating dairy cows are unknown. However, Eisemann et al. (1986a, 1986b) found that GH treatment of beef heifers increased nitrogen retention, decreased blood leucine and leucine

oxidation, but had no effect on irreversible loss of leucine, urea nitrogen and urinary excretion of 3-methylhistidine and hydroxyproline.

6. Mammary Gland Function

It is unlikely that GH has a direct effect on mammary function because receptors for GH have not been detected in mammary gland tissue of ruminants (Gertler et al., 1983, 1984; Keys, 1984). In addition, infusion of GH directly into mammary arteries of lactating sheep (so that elevated GH concentrations in peripheral blood were avoided) did not affect milk yield (McDowell and Hart, 1984).

GH may affect the mammary gland indirectly via insulin-like growth factors (IGFs). For example, GH administered to dairy cows for 22 wk increases blood IGFs 3-fold (Peel et al., 1985). However, this effect was not apparent at 8 wk of GH treatment, although milk yield was increased at that time. In the study of Peel et al. (1985), IGF concentrations declined in control cows with advancing lactation, implying a possible role of IGFs in maintenance of lactation. In addition, Davis et al. (1987) reported that GH treatment of dairy cows increased milk yield and IGF-1 but did not affect IGF-2. Sejrsen et al. (1986) reported that blood IGF-1 was elevated in peripubertal dairy heifers treated with GH for 16 wk, and treated heifers had more mammary parenchymal tissue than control heifers.

Indeed, IGF-1 receptors have been identified in mammary tissue of dairy cows (Campbell and Baumrucker, 1986). Specific binding of IGF-1 to these receptors did not change during lactation, although it decreased as pregnancy advanced in non-lactating cows. IGF-1 enhanced DNA synthesis and lactose production in cultured mammary explants and acini from lactating cows (Baumrucker, 1986), suggesting that IGF-1 may increase mammary tissue growth and (or) activity and thereby stimulate milk production.

The effect of GH treatment on rates of transcription and translation for key mammary enzymes has not been examined extensively. However, Eppard et al. (1985a) noted increased α-lactalbumin (an enzyme subunit involved in lactose synthesis) concentration in milk from GH-treated cows.

It has been suggested that increased milk synthesis in GH-treated cows may be due to additional nutrients being supplied to mammary glands. Supplying glucose and(or) casein postruminally to lactating cows increased milk yield approximately 1 to 3 kg/day (Clark, 1975), but these increases are smaller than changes observed with exogenous GH. Indeed, in one study (Peel et al., 1982a), glucose-sodium caseinate administered postruminally did not increase milk yield, either alone or in combination with GH treatment. GH treatment alone increased milk yield 4.3 kg/d while the combination of GH and additional nutrients

increased milk yield 4.8 kg/d. Thus, the galactopoietic action of GH is only mediated in part by increased supply of nutrients to the mammary glands.

7. Blood Flow

GH treatment in dairy cows increased cardiac output 10% and mammary blood flow 35% when milk yield was increased 21% (Davis et al., 1983). Indeed, GH treatment tended to increase heart rate of dairy cows (Tyrrell et al., 1982). In addition, GH-treated goats had an 8% increase in milk yield while mammary blood flow increased 18% (Mepham et al., 1984). But, some goats had increased mammary blood flow during GH treatment without a concurrent increase in milk yield, implying that GH per se may increase mammary blood flow. The time course of increased mammary blood flow after GH treatment is unknown. Generally, increased metabolic activity of an organ will subsequently increase blood flow to that organ. It is likely that this is the case in GH-treated animals. Indeed, thyroxine treatment of dairy cows increases milk yield (and thus metabolic activity of the mammary gland) with a concomitant increase in mammary blood flow (Davis et al., 1983). Thus, GH-induced increases in mammary blood flow are probably due to increased metabolic activity of the mammary gland and not GH per se.

8. Body Weight, and Intake and Utilization of Feed

Body weight (BW) has not been reported for most short-term (10 d) GH studies. In longer-term (22-27 wk) studies (Peel et al., 1985; Bauman et al., 1985) BW of GH-treated cows tended to decrease relative to controls during the first few weeks of treatment. During this time GH-treated cows utilize adipose tissue reserves to support increased milk yield. However, after approximately 4 wk of treatment, feed intake of GH-treated cows increased to supply extra nutrients for higher milk yields and to restore body adipose stores. Thus, by the end of treatment there are no differences in BW between GH-treated and control cows.

In short-term trials, feed intake is generally unchanged or slightly decreased by GH treatment. Increased efficiency of feed utilization for milk production (kg feed/kg milk) is primarily due to increased milk yield with no change in feed intake.

Increased efficiency of feed utilization in longer-term studies is due probably to dilution of fixed feed requirements for maintenance with greater production of milk. GH treatment does not affect digestibility of feed or the partial efficiency of energy utilization for maintenance or milk secretion (Peel et al., 1981; Tyrrell et al., 1982).

9. Other Hormones

GH treatment of dairy cows, beef heifers and steers has no detected effect on blood concentrations of glucagon, prolactin, cortisol, triiodothyronine or thyroxine (Peel et al., 1983; Bitman et al., 1984; Eisemann et al., 1986a; Peters, 1986).

The effect of long-term GH treatment on serum metabolites and hormones in dairy cows is unknown. The only piece of data available is that after 22 wk of GH treatment serum IGFs were elevated approximately 3-fold above controls (Peel et al., 1985).

B. Growth Hormone-Releasing Factor (GRF) and Lactation

1. Introduction

Deuben and Meites (1964) were first to suggest the existence of a specific hypothalamic GH-releasing factor (GRF). They showed that crude hypothalamic extracts stimulate GH release in rats. However, attempts to isolate GRF were unsuccessful until the early 1980's. Identification and sequencing of GRF in 1982 was made possible by the isolation of several GH-releasing peptides from pancreatic islet tumors in two acromegalic patients (Guillemin et al., 1982; Rivier et al., 1982). Because of this, in the last 6 yr GRF has been researched actively.

Most of this research has utilized rats and humans. However, some research with domestic farm species suggests that exogenous GRF is anabolic and galactopoietic.

2. Hypothalamic Regulation of GH Secretion

GH is secreted in a pulsatile manner by somatotropes of the anterior pituitary gland of all mammals studied (Martin et al., 1978), including ruminants (Vasilatos and Wangsness, 1981; Moseley et al., 1982). GH secretion is controlled primarily by peptide releasing and inhibiting neurohormones of hypothalamic origin (Martin et al., 1978). In mammals in general, the primary stimulator of GH secretion is GRF (Martin et al., 1978), while in cattle another significant hypothalamic stimulator of GH secretion is thyrotropin releasing hormone (TRH; Bourne et al., 1977). It is likely that GRF and TRH stimulate GH secretion in cattle by two different second messenger pathways because they synergistically stimulate GH secretion in vitro (Ingram and Bicknell, 1986). The primary hypothalamic inhibitor of GH secretion is GH-release-inhibiting factor (somatostatin; SRIF).

The precise mechanism of the control involved in GH secretion is not completely understood, especially in ruminants. However, GH secretion from bovine anterior pituitary cells in vitro is acutely regulated by

interactions between GRF and SRIF, and an inverse dose-dependent relationship exists between GRF and SRIF (Padmanabhan et al., 1987). In addition, in rats, GRF injected during a predicted nadir of GH in blood is without effect on GH concentrations; however, when GRF treatment is repeated similarly in rats immunized against SRIF, GH concentrations are markedly increased, implying that concentrations of SRIF are high during these nadir periods (Tannenbaum and Ling, 1984). Thus, when concentrations of SRIF in hypophyseal portal blood are likely high, GRF has no effect on GH release and vice versa. Indeed, Plotsky and Vale (1985) found that GRF secretion increases coincident with or immediately precedes a reduction in secretion of SRIF into the hypophyseal portal blood. Thus, GRF and SRIF act in concert to cause episodic release of GH. That is, GRF is responsible primarily for GH pulses (Wehrenberg et al., 1982) but GRF will only be efficacious in the absence of SRIF domination.

It also appears that SRIF has an important role in preventing GRF-induced desensitization of somatotropes. For example, when rat pituitary cells are pretreated with GRF, the response to a subsequent GRF challenge is reduced relative to non-pretreated cells; however, when SRIF is included in the incubation media, the GRF-induced desensitization of somatotropes is partially reversed (Clayton and Bailey, 1987). Similarly, when rats were treated with anti-SRIF, GRF infusion induced GH

refractoriness to a second GRF stimulus (Wehrenberg et al., 1984). In contrast, no evidence of refractoriness exists during infusion of GRF for 24 h in untreated humans where biologically active SRIF is likely present (Vance et al., 1985).

3. Physiology of GRF

Several GH-releasing peptides were isolated from pancreatic tumors in two acromegalic patients in 1982. Guillemin et al. (1982) reported the most abundant peptide to be a 44-amino acid amide [human pancreatic (hp) GRF 1-44-NH_2], while Rivier et al. (1982) reported the most abundant peptide was a 40-amino acid free acid (hpGRF 1-40-OH). It was later determined that the structure of human hypothalamic GRF was identical to the tumor-derived hpGRF 1-44-NH_2 (Ling et al., 1984b).

GRF peptides have now been identified and sequenced for several species, including rats (Bohlen et al., 1984), sheep and goats (Brazeau et al., 1984), pigs (Bohlen et al., 1983) and cattle (Esch et al., 1983). Most of these GRFs are 44 amino acid peptides with an amide group at the carboxy terminus. The exception is rat GRF, which has 43 amino acids and lacks carboxy terminal amidation. There is considerable homology in structure of GRF between the species; e.g., bovine GRF differs from human GRF at only

five residue sites. With the exception of rat GRF, there is near perfect homology among species between residues 1 to 27. The amino terminal region of GRF contains the active site of the molecule and the minimal sequence with full intrinsic GH-releasing activity lies in the 1 to 29 fragment (Spiess et al., 1982; Rivier et al., 1982). Some synthetic analogs based upon the human GRF 1-29-NH_2 sequence, with substitutions in the 1 to 3 region, are several times more potent than hGRF 1-29-NH_2 in stimulating GH release in rats (Lance et al., 1984; Ling et al., 1984a) and cattle (Hodate et al., 1986). In addition, a GRF 1-44 analog has been produced by recombinant DNA technology, which was equipotent to hpGRF 1-44-NH_2 in stimulating GH release in sheep (Kempe et al., 1986). Because of the substantial cost of producing useful quantities of hypothalamic GRFs using a peptide synthesizer, the ability to produce potent GRF analogs and recombinantly-derived GRFs is significant, both for research and potential commercial use.

Each native GRF is active across all species tested; e.g., Baird et al. (1986) found that human, rat, bovine, porcine and ovine GRFs increased GH secretion from rat pituitary cells in vitro. Similar dose-dependent increases and maximum stimulation of GH was observed with each GRF tested. However, rat GRF was 3 to 6 times more potent than other GRFs possibly because of the homologous test system used or possibly due to lack of carboxy-terminal amidation. It is thought that species specificity and potency reside in

the carboxy terminal region.

In the hypothalamus, GRF immunoreactive cell bodies tend to be localized in the arcuate and ventromedial nuclei (Lechan et al., 1984; Merchenthaler et al., 1984). In addition, high concentrations of immunoreactive GRF are in the infundibular nucleus and upper pituitary stalk (Werner et al., 1986). Thus, it appears that GRF fibers project from their cell bodies in the hypothalamus to the median eminence where they impinge upon the long portal vessels. GRF is released into the portal blood vessels and carried to the anterior pituitary gland. In addition, GRF or material with GRF-like immunoreactivity has been identified in many areas of the body including brain (Shibasaki et al., 1984a), gastric antrum, pancreatic islet cells (Bosman et al., 1984), epithelial mucosa of the upper intestine (Christofides et al., 1984), duodenum (Bruhn et al., 1985) and placenta (Baird et al., 1985). Because of these extra-hypothalamic sources of GRF (particularly intestinal) speculation exists as to whether GRF may be a local gut peptide as well as a hypothalamic hormone. Indeed, GRF is structurally a member of the secretin-glucagon family of peptides (Spiess et al., 1982), a group of pancreatic-intestinal peptides that includes vasoactive intestinal polypeptide (VIP), glucagon and secretin.

Specific anterior pituitary receptors for GRF have been identified for rats and cattle (Seifert et al., 1985a;

Velicelebi et al., 1986). In addition, GRF binds to VIP receptors on intestinal epithelial cell membranes and thereby activates adenylate cyclase (Laburthe et al., 1983). The affinity of GRF for these VIP receptors is, however, approximately 800 times lower than that of VIP. Binding of GRF to its anterior pituitary receptor stimulates formation of intracellular cyclic adenosine 3',5'-monophosphate (cAMP; Labrie et al., 1983). Indeed, increases in GH release due to GRF parallel those seen in response to increased cAMP (Brazeau et al., 1982b). It is believed that GRF stimulates cAMP-dependent protein kinase which causes exocytosis of GH secretory granules and acute release of GH (Lewin et al., 1983). In addition, GH release due to GRF is dependent on calcium uptake (Brazeau et al., 1982b).

Not only does GRF cause GH release, but it also stimulates GH synthesis by stimulating transcription rate and concentrations of GH messenger RNA (Barinaga et al., 1983; Gick et al., 1984). More recently, Barinaga et al. (1985) reported that GRF increases GH transcription rate independent of GH release.

GRF is specific for GH release in all species tested including rat (Brazeau et al., 1982a), human (Gelato et al., 1983), goat (Hart et al., 1984c), sheep (Hart et al., 1985b) and cattle (Moseley et al., 1984).

GRF 1-40-OH administered iv to humans had an equilibration half-life of 8 min and an elimination half-life of 52 min based on a specific and sensitive

radioimmunoassay (Frohman et al., 1984). Subsequent work by the same group (Frohman et al., 1986), using high performance liquid chromatography (HPLC) to assay plasma for GRF showed that GRF 1-44-NH_2 in plasma is degraded (within 1 min) to GRF 3-44-NH_2 which was at least 1000 times less biologically active than GRF 1-44-NH_2. Based on HPLC, the half-life of GRF 1-44-NH_2 in this study was 6.8 min. Indeed, the short half-life of biologically active GRF may be a source of the blood GH profile seen after GRF injection, namely, a rapid rise to peak followed by a rapid decline.

4. Factors Affecting GRF-Induced GH Release

Increased concentrations of GH in blood in response to GRF administration decreases with age in several species (Sonntag et al., 1983; Shibasaki et al., 1984b), including cattle (Johke et al., 1984). This is not surprising because basal concentrations of GH also decline with age (Keller et al., 1979; Zadik et al., 1985). Indeed, pituitary sensitivity to somatostatin increases with age (Cuttler et al., 1986). However, using young and old rat pituitaries cultured in vitro, the decline in GRF-induced GH release associated with age does not occur (Sonntag et al., 1983), suggesting that the hypothalamus is involved in the age-related change.

GH response to GRF is greater in males than females (Johke et al., 1984; Clark and Robinson, 1985a), and this effect is not seen in prepubertal animals (Wehrenberg et al., 1985). Gonadal steroids may mediate this gender effect since testosterone enhances GRF-induced GH release, while estrogen either has no effect or is inhibitory (Evans et al., 1985; Wehrenberg et al., 1985). In addition to sex steroids, glucocorticoids modulate GRF-induced GH release. Dexamethasone enhanced GH response to GRF in rats (Wehrenberg et al., 1983). Subsequently, Seifert et al. (1985b) reported that glucocorticoids increase GRF binding capacity of rat pituitary cells, which may explain the increased sensitivity noted by Wehrenberg et al. (1983).

Maximal GH release in response to GRF is reduced in hypothyroid animals in vivo (Dieguez et al., 1986) and in vitro (Dieguez et al., 1985), and the effect can be partially reversed by addition of triiodothyronine in vitro (Dieguez et al., 1985). However, thyroid hormone deprivation in vivo or in vitro did not alter the half-maximal effective dose of GRF (Dieguez et al., 1985).

IGFs administered intraventricularly suppress GH release, an effect thought to be mediated through decreased hypothalamic GRF release (Abe et al., 1983). Indeed, Shibasaki et al. (1986) reported that IGF-1 decreased release of GRF from rat hypothalamic cultures. In addition, IGFs decrease GRF-induced GH release at the level of the pituitary cell (Perez et al., 1985; Yamashita and Melmed,

1986). IGFs also inhibit GRF-induced increases in GH messenger RNA in rat pituitary cultures (Yamashita and Melmed, 1986). These effects of IGF at the level of the anterior pituitary gland are probably mediated by IGF receptors which have been identified (Rosenfeld et al., 1984).

Elevated glucose in blood suppresses GRF-induced GH release in humans (Sharp et al., 1984), but not in rats (Imaki et al., 1986). In addition, decreased GH response to GRF after glucose loading in humans is not associated with increased blood insulin (Sharp et al., 1984). Indeed, insulin, at physiological concentrations, has no effect on GRF-induced GH release from rat pituitary cells in vitro (Ceda et al., 1987). Elevated concentrations of NEFA in plasma reduced GRF-induced GH release in rats, probably due to increased SRIF secretion (Imaki et al., 1986). Also, serum GH response to iv GRF administration was more persistent in sheep fed a restricted diet compared with sheep fed ad libitum (Hart et al., 1985b).

5. Effect of GRF on GH Secretion in Ruminants

A variety of GRFs, including hGRF 1-44-NH_2, hGRF 1-29-NH_2, hGRF 1-24-NH_2, hGRF 1-40-OH, bGRF 1-44-NH_2, , rat GRF 1-29-NH_2 and analogs of hGRF 1-29-NH_2 stimulate GH release in sheep, goats and cattle, including male and female calves,

beef and dairy steers, and "dry" and lactating dairy cows (Hodate et al., 1984, 1986; Moseley et al., 1984; Johke et al., 1984; McCutcheon et al., 1984; Baile et al., 1985; Hart et al., 1985b; Al-Raheem et al., 1986; Lapierre et al., 1987).

Peak concentrations of GH in serum after iv injection of GRF occur within 5 to 20 min and GH returns to baseline concentrations within 90 to 180 minutes (Hodate et al., 1984; Moseley et al., 1984; Petitclerc et al., 1985; Al-Raheem et al., 1986). In some studies high doses (100 to 400 μg/100 kg BW) of GRF induced a biphasic serum GH response to injection with the second peak occurring 100 to 120 min after injection (Moseley et al., 1984; Petitclerc et al., 1985). Serum GH response (peak or area under response curve) to sc injection of GRF is about one third that of the same dose of GRF injected iv (McCutcheon et al., 1984; Johke et al., 1984). Intramuscular and sc injections of GRF cause similar responses in concentrations of GH in serum (McCutcheon et al., 1984).

Generally, serum GH response to injection or infusion of GRF occurs in a dose-dependent manner (Johke et al., 1984; Moseley et al., 1985). However, in some studies no differences can be detected over a range of doses (Moseley et al., 1984; Petitclerc et al., 1985; Al-Raheem et al., 1986). The minimum significantly effective (GH-releasing) dose of GRF administered as a single iv injection to cattle is 10 to 25 μg/100 kg BW (Johke et al., 1984; Moseley et

al., 1984; Al-Raheem et al., 1986). The minimum effective dose of GRF administered as an iv infusion to steers was 10 to 15 $\mu g \cdot h^{-1} \cdot 100$ kg BW^{-1} (Moseley et al., 1985; Al-Raheem et al., 1986).

In dairy steers, serum GH response to hGRF 1-40-OH was the same as that induced by hGRF 1-44-OH (Moseley et al., 1984). Al-Raheem et al. (1986) found that rat GRF 1-29-NH_2 had only 40% of the potency of hGRF 1-40-OH in stimulating serum GH in beef steers. In contrast, Hart et al., (1985b) showed that hGRF 1-44-NH_2, hGRF 1-40-OH, hGRF 1-29-NH_2 and rat GRF 1-29-NH_2 were equipotent in stimulating GH release in sheep. Petitclerc and coworkers (Petitclerc et al; 1985; Lapierre et al., 1987) reported that hGRF 1-44-NH_2 and hGRF 1-29-NH_2 are equipotent for GH release. However, these authors found that the analog, (desamino-Tyr^1, D-Ala^2, Ala^{15}) hGRF 1-29-NH_2, was three times as potent as hGRF 1-29-NH_2 in stimulating GH release in cattle. In contrast, hGRF 1-24-NH_2 was only 5% as potent as hGRF 1-29-NH_2 in lactating cows (McCutcheon et al., 1984). Hodate et al. (1986) noted in calves that analogs of hGRF 1-29-NH_2 exhibited prolonged GH-releasing activity compared with hGRF 1-44-NH_2 or hGRF 1-29-NH_2.

GRF-induced GH release in serum is similar in magnitude and pattern among ruminant species (Hodate et al., 1984). A phenomenon common to most species is the variability of GH response to GRF between animals within a species (McCutcheon

et al., 1984; Moseley et al., 1984; Kensinger et al., 1987).

Peak GH responses in serum to single iv injections of GRF in cattle range from 3 to 20-fold above controls (Moseley et al., 1984; Johke et al., 1984; Hodate et al., 1984). Continuous iv infusion of GRF (3.6 mg/d) into bull calves for 20 d increased mean and baseline concentrations of GH in serum, and frequency and amplitude of GH pulses 2- to 3-fold (Moseley et al., 1987). Similar results were obtained with steers, except that frequency of GH pulses was not affected by GRF infusion (Moseley et al., 1985). Mean serum GH response to GRF administered to ewes every 2 h for 4 d was consistent and repeatable (Hart et al., 1985c). Sheep administered sc with the same daily dose of GRF but at different frequencies (2, 4 or 8 times/d or continuous) had different mean GH responses to GRF (Kensinger et al., 1987). GRF administered at 4 or 8 times/d were equipotent and greater than the other two treatments.

GRF infused iv for 5 d in steers (Moseley et al., 1985) or 20 d in bull calves (Moseley et al., 1987) maintained elevated concentrations of GH in serum. In addition, GH response to GRF is maintained following daily sc administration of GRF for 57 d in lactating cows (Lapierre et al., 1986a) or at 2-h intervals for 4 d in lactating ewes (Hart et al., 1985c). Indeed, in several studies (Lapierre et al., 1986a; Moseley et al., 1987) GH response to GRF was greater on the last day of treatment than on the first. Thus, the anterior pituitary gland apparently does not

become refractory to GRF in cattle and sheep, at least when refractoriness is measured in terms of serum GH response. However, using this measure to indicate refractoriness has limitations. For example, the somatotrope could become refractory to GRF via a receptor and (or) a post-receptor process, and refractoriness could be in terms of GH release and (or) synthesis. Using serum GH response to GRF as an indicator of pituitary responsiveness can not differentiate between these or other possibilities. During the rest of this thesis the term refractoriness is used with this in mind.

6. Effect of GRF on Milk Production

Hart et al., (1985c) were first to demonstrate a galactopoietic effect of GRF. Ewes receiving .6 μg hpGRF 1-44-NH_2/kg BW iv every 2 h for 4 d produced 26.7% more milk than control ewes. In the same experiment, bGH (15 $\mu g \cdot kg^{-1} \cdot 2\ h^{-1}$) increased milk yield 30.7%. Both GRF and GH increased percentage of milk fat. Serum GH concentrations were increased approximately 2-fold above controls in GRF- and GH-treated ewes. Increased milk production was observed within 24 h after initiation of GRF treatment and yield was still increasing at the end of the 4-d period. Posttreatment decline in milk production was slower in GRF- than in GH-treated ewes.

Evidence that GRF is galactopoietic in dairy cows includes one published paper (Enright et al., 1986), several abstracts and proceedings of a symposium. Details of these reports are summarized in Table 1. Data on the effects of GRF in lactating cows on variables other than milk yield and serum GH are limited to one report (Enright et al., 1986). In general, trials have involved .4 to 10 mg of hGRF 1-44-NH_2 or 1-29-NH_2/d administered iv (2 or 6 times/d) or sc (1 or 2 times/d) to mid- to late-lactation cows for 10 d. Under all these circumstances GRF stimulates milk production 4 to 20%. In addition, Lapierre et al. (1986a) reported that GRF increased milk yield 10% during 57 d in late lactation.

7. Mechanism of Action of GRF

The most likely mechanism whereby GRF stimulates milk yield is through increased serum GH. Acutely, GRF appears to be specific for GH release in ruminants. For example, GRF does not affect prolactin, insulin, urea, glucose and NEFA in sheep and goats (Hart et al., 1984c, 1985b), or prolactin, thyrotropin and luteinizing hormone in cattle (Moseley et al., 1984; Hodate et al., 1985).

In longer term studies with GRF, changes in metabolites and hormones parallel those seen with GH administration. For example, at the doses tested, GH and GRF were similar in their ability to increase milk yield, percentage of milk

Table 1. Summary of research on the effects of growth hormone-releasing factor (GRF) on milk yield of dairy cows.

Reference	No. of cows per trt.[1]	Stage of lactation	Type of GRF	Dose of GRF/ admin.[5]	Frequency of admin.	Route of admin.	Duration of trt.	Period of trt. analyzed	Increase in milk yield	Other Comments
Lapierre et al., 1985	5-10	186 d	hGRF[3] 1-44-NH_2	1 µg/kg[6]	2/d	iv	10 d	d 6-10	4.3%	
Baile et al., 1985	10	ND[2]	bGRF[4]	.33 µg/kg	2/d	sc	14 d	ND	4.8%	GH (13.5 mg/inj) increased milk yield 29%.
Petitclerc et al., 1985	6	201 d	hGRF 1-44-NH_2	1 µg/kg	6/d	iv	10 d	d 6-10	19.6%	
			hGRF 1-29-NH_2						16.1%	
Enright et al., 1986	16	178 d	hGRF 1-44-NH_2	.2 µg/kg	6/d	iv	10 d	d 8-10	11%	Milk composition and feed intake not affected. BW increased.
Lapierre et al., 1986a	8-9	254 d	hGRF 1-29-NH_2	10 µg/kg	1/d	sc	57 d	d 1-57	10%	
Lapierre et al., 1986b	6	209 d	hGRF 1-29-NH_2	16 µg/kg	1/d	sc	10 d	d 6-10	14.3%	Milk composition, DM intake, BW not affected.
Petitclerc et al., 1987	5-6	204 d	hGRF 1-29-NH_2	10 µg/kg	1/d	sc	10 d	d 6-10	11%	Feed intake not affected.
			hGRF 1-29-NH_2 analog	.6 µg/kg					11%	
				1.8 µg/kg					16%	

[1]trt. = treatment
[2]ND = not determined
[3]h = human
[4]b = bovine
[5]admin. = administration
[6]kg = kg body weight

fat, and serum GH, insulin, glucose and NEFA in lactating ewes (Hart et al., 1985c). Similarly, growing pigs treated with GRF had the same increases in serum insulin and glucose as did GH-treated pigs (Etherton et al., 1986). In addition, GRF increased nitrogen retention of bull calves (Moseley et al., 1987) to the same degree as that seen in steers treated with GH (Moseley et al., 1982). Thus, based on the available data, it is probable that galactopoietic effects of GRF are mediated by increased serum GH.

An alternative mechanism of action for GRF is the possibility that it directly affects extrapituitary tissues. As mentioned earlier GRF is structurally a member of the secretin-glucagon family of peptides and can bind with low affinity to the VIP-preferring receptor. Thus, GRF may affect tissues and organs associated with the digestive system. The first evidence for such an effect was found by Pandol et al. (1984) using cultured pancreatic acini from guinea pigs. In this system GRF stimulated amylase secretion by interacting with the VIP receptor and increasing cAMP. Rat GRF was approximately half as potent as VIP and was 1000 times more potent than hpGRF in stimulating amylase secretion. Similarly, rat GRF, at supraphysiological levels, increased pancreatic endocrine secretions in the rat (Goffette et al., 1986). In addition, using the perfused dog pancreas, hpGRF 1-40-OH stimulated insulin, glucagon and SRIF release in a dose and glucose-

dependent manner (Hermansen et al., 1986). However, hpGRF 1-44-NH_2 was without effect in this system, although both GRF peptides stimulated GH equally.

GRF administered hourly to rats for 4 h increased epithelial cell proliferation in the digestive tract within 2 h after final GRF administration (Lehy et al., 1986). In this study gastrin secretion was increased but GH was unaffected by GRF. Subsequently, the same research group showed that sc administration of small doses of hGRF 1-44-NH_2 increased gastrin release within 10 min, although serum GH was not yet elevated (Accary et al., 1986). These data and the fact that GRF and gastrin are present in the same cells of human pancreas (Bosman et al., 1984) indicate that GRF per se may have a role in digestive tract function. Indeed, Dubreuil and Morisset (1986a, 1986b, 1986c) showed that GRF administration to rats affects growth and development of organs and tissues associated with the digestive tract. Although these effects may be explained (in whole or in part) by increased serum GH, one can not rule out the possible effects of GRF per se.

GRF may also affect lipid metabolism directly. For example, GRF 1-44-NH_2 increased total extractable lipids in isolated rat adipocytes, probably due to increased glucose transport (Hauner et al., 1985). However, the biological importance of this effect is unknown because supraphysiological levels of GRF were required for activity.

C. Summary

A considerable amount of research has been performed in dairy cows over the last fifty years showing that exogenous GH increases milk yield. Coordinated changes in metabolism in response to GH support the increase in synthesis of all milk components. In short-term experiments, changes in irreversible loss and oxidation rates of NEFA and glucose can account for the increases in yield of milk lactose and fat. In long-term experiments, changes in feed intake and BW (due to turnover of body tissues) account for increased milk production. Increased gross efficiency of milk production (kg feed/kg milk) due to GH appears to be due to a dilution of feed requirements for maintenance over the higher level of milk production.

Preliminary evidence indicates that exogenous GRF increases milk production of dairy cows. Little or no data exists on the effects of GRF in dairy cows on lactational efficiency, milk composition, feed intake, BW and blood concentrations of GH, other hormones and metabolites. Because GRF markedly increases GH secretion it is likely that GH mediates productive responses to GRF. However, the possibility exists that GRF per se may be involved.

Chapter 1

The Effect of GRF on GH Release in Holstein Bull Calves

A. Introduction

Human pancreatic growth hormone (GH)-releasing factor (hp GRF; Rivier et al., 1982) and some of its fragments specifically increase blood concentrations of GH in several species (Gelato and Merriam, 1986), including cattle (Johke et al., 1984; McCutcheon et al., 1984; Moseley et al., 1984, 1985; Al-Raheem et al., 1986, Chapters 2 and 3). Considerable differences in sensitivity and magnitude of GH response are observed between animals given a single intravenous (iv) injection of a given dose of hpGRF (Johke et al., 1984; Moseley et al., 1984). It appears, therefore, that a single injection of hpGRF is not a reliable indicator of the GH response capacity of an animal. In addition, the single injection approach fails to determine capacity of the pituitary to respond to subsequent GRF stimulation. Indeed, work in humans (Losa et al., 1984) and rats (Wehrenberg et al., 1984) demonstrated a decline in GH responsiveness to repeated injections or continuous infusions of GRF. Results of clinical tests in humans and experimental studies with animals on GH responsiveness to a hpGRF challenge may be misleading if based solely on a single iv injection. Thus, alternative approaches are needed to evaluate GH responses to exogenous GRF. Two such approaches are: (a) multiple injections and (b) continuous infusions of GRF. GRF studies using cattle have primarily utilized steers (Moseley et al., 1984, 1985; Al-Raheem et al., 1986) and dairy cows

(McCutcheon et al., 1984; Chapters 2 and 3). GH response to hpGRF is affected by gender (Johke et al., 1984; Clark and Robinson, 1985a), age (Johke et al., 1984) and gonadal status (Wehrenberg et al., 1985). GH response to hpGRF in prepubertal male calves is unknown and may differ from steers and lactating cows.

The overall objective of the present study was to determine the serum GH response and persistency of that response to administration of repeated injections and continuous infusions of hpGRF in bull calves.

B. Materials and Methods

1. General. Holstein bull calves weighing 66 ± 4, 81 ± 3 and 92 ± 4 kg were utilized in Experiments 1, 2 and 3, respectively. Calves were housed indoors in adjacent individual pens and exposed to approximately 15 h of natural daylight daily and ambient temperatures of 12 to 30 C. Human pancreatic growth hormone-releasing factor 1-40-OH (hpGRF) was obtained from Bachem, Inc. (Torrance, CA). The placebo and vehicle for hpGRF was distilled sterile water. Blood samples were collected from each calf via an indwelling jugular cannula. Serum was decanted after centrifugation of blood samples and stored at -20 C until assayed for concentrations of GH (Purchas et al., 1970) and prolactin (Koprowski and Tucker, 1971). Calves were fed

calf starter at 0600 h and high quality alfalfa hay at either 1500 h (Experiments 1 and 2) or 1730 h (Experiment 3). Feeding did not overlap with blood sampling, injections or infusions. GH peak height (Experiments 1 and 2), mean GH (Experiment 3) and area under the GH response curve (Experiments 1, 2 and 3) were used as measures of hpGRF effects. Peak height was defined as the maximum concentration of GH achieved between 0 and 60 min (Experiment 1) or 0 and 30 min (Experiment 2) after injection of hpGRF. Area was defined as the integrated area under the GH response curve for 1 h (Experiment 1) or 30 min (Experiment 2) after injection of hpGRF, and for the 6 h infusion of hpGRF (Experiment 3). Pre-injection and pre-infusion means of GH were calculated from all samples collected before and including the 0-min sample.

Data were examined by analyses of variance (Gill, 1978). Data expressed either as area under the response curve or peak height provided the same statistical inferences; therefore, data expressed as area will be presented but not discussed.

2. Experiment 1. To determine a dose-response relationship between hpGRF and serum GH, a 4 (calf) x 4 (day) Latin square design balanced for residual effects was used. Treatments consisted of a single dose (volume of hpGRF solutions ranged from 5.9 to 7.6 ml) injected via an indwelling jugular cannula at 0915 h on a given day. Doses

of hpGRF were 0 (placebo), 2.5, 10.0 and 40.0 μg/100 kg BW. Treatments were given on consecutive days. Blood samples were collected at -15, -10, -5, 0, 4, 6, 8, 10, 15, 20, 30, 60, 120 and 240 min relative to each injection. Comparisons between mean GH response to dose of hpGRF and placebo were made using Dunnett's t-test (Gill, 1978).

3. Experiment 2. To determine the serum GH response to repeated injections of hpGRF, a complete-block design was used with four calves (blocks) given hpGRF iv at 6-h intervals (i.e., treatments) for 48 h starting at 2000 h. Dose of hpGRF was 20 μg/100 kg BW (injection volume ranged from 3.8 to 4.5 ml). Based on data from Experiment 1, 20 μg was a sub-maximal but effective dose of hpGRF. Blood samples were collected at -15, -10, -5, 0, 4, 6, 8, 10, 15, 20 and 30 min relative to each injection.

4. Experiment 3. To determine the serum GH response to continuous infusion of hpGRF, a single crossover design was used where four calves received continuous iv infusions of 0 and 200 μg hpGRF/h for 6 h. Treatments were given 2 d apart and started at 0930 h each day. Two calves received the 0 μg dose and two received the 200 μg dose on each day. A Harvard infusion/withdrawal pump (Multi-Speed Transmission, Harvard Apparatus Co., Millis, Mass.; model 954) was used. Infusion rate was 4.2 ml/h. Blood samples were collected at

-75, -60, -45, -30, -15, 0, 5, 10, 15, 20, 25, 30, 45 and 60 min relative to the start of infusion, at 15-min intervals for the remaining 5 h of infusion and at 5, 10, 15, 20, 25, 30, 45, 60, 75 and 90 min following completion of infusion.

C. Results

1. Experiment 1. Mean serum GH responses to hpGRF are presented in Table 2. The 40 μg dose of hpGRF increased ($P<.05$) peak height of GH 15-fold over that of the placebo. Although not significantly different, the 10 μg dose increased peak height of GH 2.5-fold above the placebo value. The 2.5 μg dose had no effect on serum GH. Pre-injection mean GH did not differ between treatments and averaged 7.3 ng/ml serum. Peak height of GH in response to the 40 μg dose occurred approximately 10 min post-injection. Mean concentrations of prolactin in serum were not affected by hpGRF administration (37.8 ± 2.9 vs 41.0 ± 3.3 ng/ml; mean prolactin before and after the 40 μg hpGRF dose, respectively).

2. Experiment 2. Pre-injection mean GH did not differ ($P>.05$) between times of hpGRF injections and averaged 3.1 ng/ml serum across all calves and injections (Table 3). Similarly, peak height of GH did not differ ($P>.05$) between times of hpGRF injections and averaged 70 ng/ml serum across all calves and injections (Table 3). Based on mean GH of

Table 2. Serum growth hormone in calves injected with various doses of human pancreatic growth hormone-releasing factor (hpGRF 1-40-OH).

	Dose (μg) of hpGRF/100 kg BW				
GH trait[1]	Placebo	2.5	10	40	SED[3]
Pre-injection mean (ng/ml serum)	5.8	7.2	4.8	11.3	3.2
Peak height (ng/ml serum)	7.2	8.3	18.3	107.3[a]	33.3
Area[2] ($ng \cdot min \cdot ml^{-1}$ serum)	313	327	682	3,534[a]	1,134

[a]Different (P<.05) from placebo.

[1]Values are means of four calves.

[2]Area under 1-h response curve.

[3]Standard error of differences of means.

Table 3. Sequential responses of serum growth hormone in calves to repeated injections of 20 μg human pancreatic growth hormone-releasing factor (hpGRF 1-40-OH)/100 kg BW.

	Time of injection (h)								
GH trait[1]	2000	0200	0800	1400	2000	0200	0800	1400	SED[3]
Pre-injection mean (ng/ml serum)	3.8	3.2	2.3	3.4	4.6	2.5	2.0	3.1	1.1
Peak height (ng/ml serum)	61.7	83.9	55.3	49.3	66.6	95.4	62.4	84.5	34.2
Area[2] ($ng \cdot min \cdot ml^{-1}$ serum)	1,362	1,670	914	1,128	1,347	1,959	1,233	1,821	637

[1]Values are means of four calves.

[2]Area under 30-min response curve.

[3]Standard error of differences of means.

all calves, no obvious diurnal pattern of response to sequential hpGRF injections was observed, and there was no evidence of reduced GH response following the eight consecutive hpGRF injections. However, considerable variation in GH response was observed between times of injection (up to 18-fold differences) within individual calves (Figure 1).

3. Experiment 3. Pre-infusion mean GH did not differ between hpGRF and placebo treatments. Mean GH concentration during the 6 h infusion was 5-fold greater ($P<.05$) for hpGRF-infused calves than for placebo-infused calves (Table 4). There was no evidence of reduced GH response as the infusion continued for 6 h and GH remained at elevated concentrations (3.3-fold above mean GH of controls) 90 min after infusion ceased. In three of the four hpGRF-infused calves GH pulsed at 1 to 3 h intervals and pulses were asynchronous among calves. The fourth calf (7837; Figure 2) showed little pulsatile activity. The serum GH response profiles for all calves depicting the markedly different patterns of response to hpGRF infusion are shown in Figure 2.

Figure 1. Serum concentrations of growth hormone (expressed as area under the 30-min response curve) of individual calves given iv injections of 20 μg hpGRF 1-40-OH/100 kg BW at 6-h intervals (Chapter 1, Experiment 2).

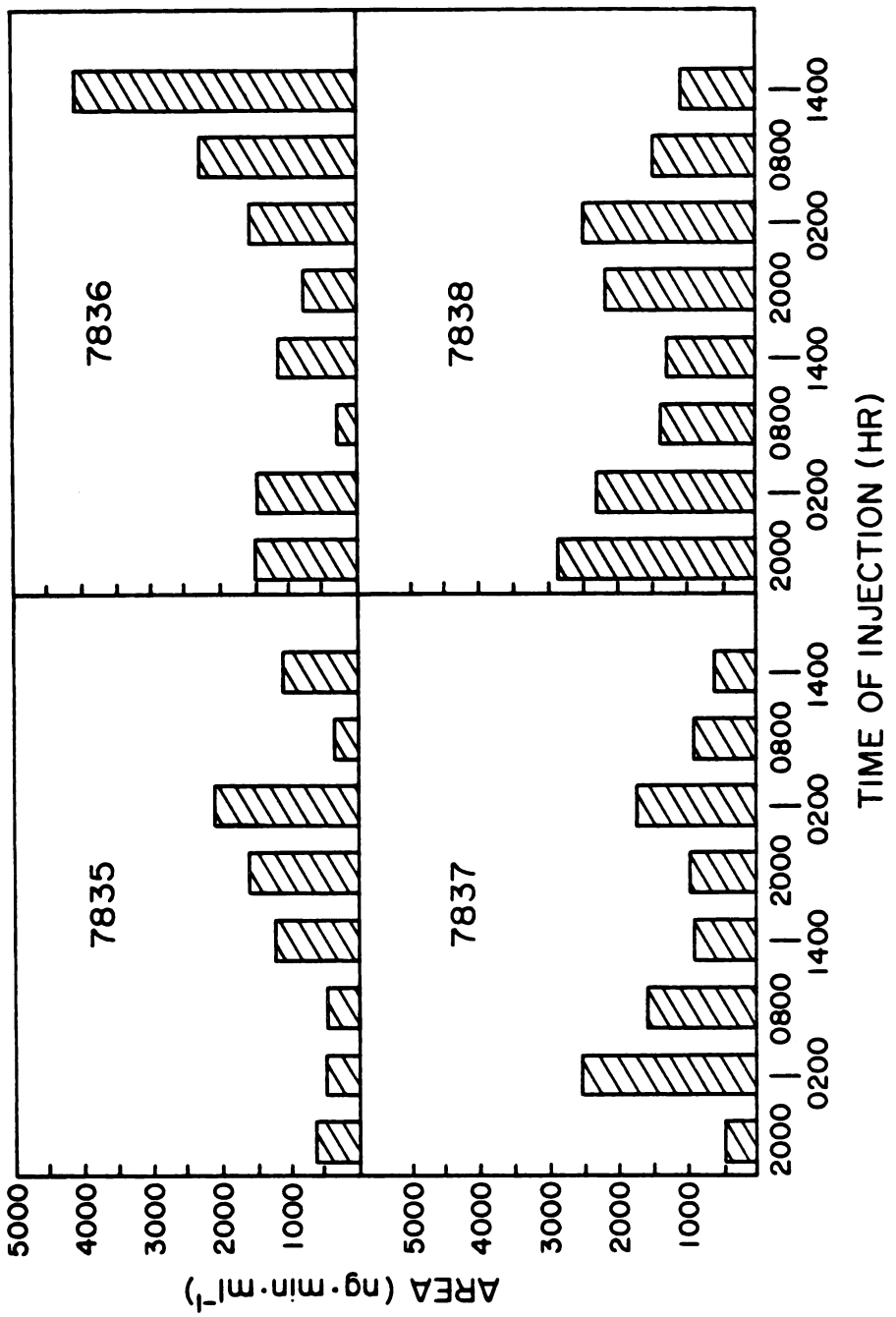

7835
7836
7837
7838
5000
4000
3000
2000
1000
AREA (ng·min·ml⁻¹)
2000 | 0800 | 2000 | 0800 | 1400
0200 | 1400 | 0200 | 1400 | 0200
TIME OF INJECTION (HR)

Table 4. Serum growth hormone response of calves given a 6-h continuous infusion of human pancreatic growth hormone-releasing factor (hpGRF 1-40-OH; 200 μg/h) or placebo.

GH trait[1]	Placebo	hpGRF	SED[3]
Pre-infusion mean (ng/ml serum)	5.1	4.3	1.6
Infusion mean (ng/ml serum)	7.4	36.5[a]	8.4
Area[2] ($ng \cdot min \cdot ml^{-1}$ serum)	2,622	13,451[a]	2,804

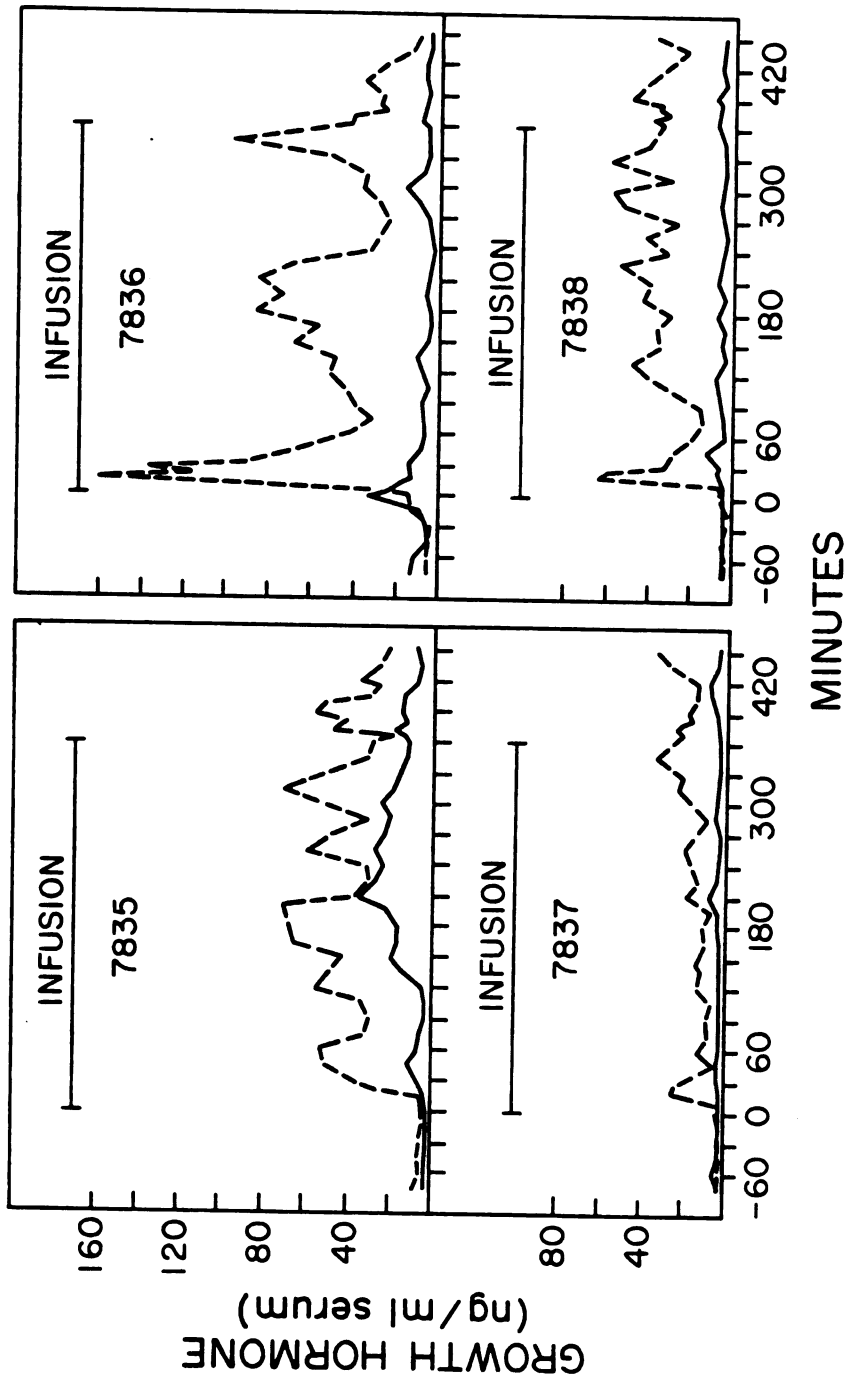
[a]Different ($P<.05$) from placebo.

[1]Values are means of four calves.

[2]Area under 6-h response curve.

[3]Standard error of differences of means.

Figure 2. Serum concentrations of growth hormone of individual calves given continuous iv infusions of 0 (———) or 200 (- - -) μg hpGRF 1-40-OH/h for 6 h (Chapter 1, Experiment 3).


GROWTH HORMONE
(ng/ml serum)
160
120
80
40
80
40
INFUSION
7835
INFUSION
7837
INFUSION
7836
INFUSION
7838
-60
0
60
180
300
420
MINUTES

D. Discussion

Serum GH response to single iv hpGRF injections in bull calves of these experiments fall within the range of mean responses reported for heifer calves (Johke et al., 1984) steers (Moseley et al., 1984; Al-Raheem et al., 1986) and lactating cows (Chapter 2). In agreement with Al-Raheem et al. (1986) and Moseley et al. (1984), my lowest dose of hpGRF (2.5 μg/100 kg BW) did not alter concentrations of GH. In contrast to my study using lactating cows (Chapter 2), GH response of prepubertal bull calves in the present study to the 10 μg dose was markedly less than GH response to the 40 μg dose.

Similar to sheep (Hart et al., 1985c) and cows (Chapter 2), multiple injections of hpGRF consistently increase serum GH in bull calves. However, within individual bull calves the magnitude of GH response to multiple injections of hpGRF is variable. Indeed, individual lactating cows also show a variable GH response when hpGRF is administered at 4-h intervals (Chapter 2). The difference in GH response between hpGRF injections may be due to the timing of exogenous hpGRF administration relative to the animals endogenous GRF and somatostatin (SRIF) status. The single-injection protocol can not take into account the endogenous GRF/SRIF milieu and therefore interpretation of results using this type of protocol is difficult.

In agreement with data in lactating cows (Chapter 3),

infusion of GRF to bull calves resulted in sustained GH response. In addition, GH profiles of GRF-infused animals were more pulsatile than controls (Chapter 3). Thus, it appears that hpGRF infusion induces events which increase episodic secretion of GH. Pulsatile activity of GH during constant infusion of hpGRF has also been reported in humans (Vance et al., 1985). In contrast, hpGRF infusion in steers does not increase pulse frequency, but augments the amplitude of naturally occurring GH pulses (Moseley et al., 1985). Increased pulsatile activity during hpGRF infusion may be due to one or more of the following: episodic SRIF secretion (Plotsky and Vale, 1985); pituitary desensitization to GRF occurring in a cyclical manner (Ceda and Hoffman, 1985); short-term pituitary GRF receptor down-regulation (Catt and Dufau, 1977); GRF and SRIF inhibition of their own neurosecretions via a negative ultrashort loop feedback mechanism (Lumpkin et al., 1985; Richardson and Twente, 1986); direct/indirect feedback of GH on its own secretion (Chihara et al., 1981; Abe et al., 1983; Conway et al., 1985); and(or) feedback of GH-induced somatomedin on the pituitary (Berelowitz et al., 1981; Abe et al., 1983) or hypothalamus (Berelowitz et al., 1981). Possibly, GRF and SRIF regulate their own release at the level of the hypothalamus (Peterfreund and Vale, 1984) or interact at the level of the pituitary (Padmanabhan et al., 1987).

The sustained GH response following multiple injections

or continuous infusion of hpGRF agrees with data in lactating cows (Chapters 2 and 3), steers (Moseley et al., 1985) and sheep (Hart et al., 1985c). Indeed, work in cattle (Moseley et al., 1985; Lapierre et al., 1986a; Chapters 2 and 3) suggests that GRF stimulates synthesis as well as release of GH, as has been demonstrated in rats (Barinaga et al., 1985). Evidence in cattle indicates that pituitary stores of GH do not become depleted and the pituitary does not become refractory to GRF during 19 d of infusion (Chapter 3) or 57 d of consecutive daily injections (Lapierre et al., 1986a). In contrast to results in ruminants, multiple injections (three at 2-h intervals) of hpGRF to humans diminishes the GH response (Losa et al., 1984). However, GH response of humans to long-term treatment with repetitive injections of hpGRF is unknown.

I conclude that GH response of bull calves to hpGRF is dose-dependent and that repeated injections or continuous infusions of hpGRF consistently elicit GH release, although magnitude of response is variable. I hypothesize that differences in GH response to hpGRF within and among calves, and pulsatile secretion in the face of hpGRF infusion, are related to the degree of synchrony among exogenous hpGRF and endogenous GRF and SRIF.

Based on the extensive variability of GH response to single iv injections of hpGRF seen within and among calves of the present study, multiple injections or continuous infusions of hpGRF provide a better indication of GH

responsiveness in an animal.

Chapter 2

The Effect of GRF Administered at Four-Hour Intervals on GH and Lactation in Holstein Cows

A. Introduction

Exogenous growth hormone (GH) to lactating cows increases milk yield (10 to 40%) and milk fat percent (1 to 13%), while percent milk protein decreases (1 to 10%), without a change in percent milk lactose (Peel et al., 1981, 1983; Fronk et al., 1983; Eppard et al., 1985b; Bauman et al., 1985). Most of these studies lasted approximately 10 d and utilized Holstein cows and a single daily subcutaneous injection of pituitary-derived GH. Total increase in milk yield was similar between early and late lactation; thus, the percentage increase was higher in late-lactation cows (Peel et al., 1983). Injection of GH for 10 d did not affect feed intake, which resulted in increased feed efficiency (Peel et al., 1983; Fronk et al., 1983). More recently, Bauman et al. (1985) administered pituitary or recombinant (methionyl bovine somatotropin; MBS) GH daily for 188 d beginning after the peak of lactation. They reported that both GH treatments increased milk yield although pituitary-derived GH was less effective than MBS. Neither treatment altered composition of milk (Bauman et al., 1985).

It seems reasonable to hypothesize that factors which specifically increase endogenous serum GH may also enhance lactational performance. Administration of human pancreatic growth hormone-releasing factor (hpGRF; Guillemin et al., 1982; Rivier et al., 1982) and some fragments of hpGRF

specifically increase serum concentrations of GH in bull calves (Johke et al., 1984; Chapter 1), heifer calves (Johke et al., 1984; Hodate et al., 1984; Plouzek et al., 1984), steers (Moseley et al., 1984, 1985) postpubertal heifers and dry cows (Johke et al., 1984) and lactating cows (McCutcheon et al., 1984). My objectives were to determine concentrations of GH in serum of lactating cows following repeated administration of hpGRF for 1 and 10 d, and to determine effects of hpGRF on lactational performance of cows.

B. MATERIALS AND METHODS

1. Experiment 1. The experimental design was a 4 (cow) x 4 (day) Latin square with repeated measurement. Cows were primiparous Holsteins weighing 562 ± 44 kg and lactating 7 to 11 mo. Three cows were not pregnant, but cow 1932 was pregnant 64 d at the start of the experiment. Milk yield averaged 27 ± 1 kg/d at the start of the experiment. During the experiment the cattle were housed indoors and exposed to continuous fluorescent lighting (and 9 h of natural lighting daily) and ambient temperatures of 0 to 12 C. Treatments consisted of a given dose (volume of hpGRF stock solution varied to meet the required dose and ranged from 2 to 10 ml) injected via an indwelling jugular cannula at 1000, 1400, 1800, 2200, 0200 and 0600 h on a given day. Four doses of

hpGRF 1-44-NH_2 (Peninsula Laboratories, Belmont, CA) were used: 0, 10, 20 and 40 μg/100 kg BW. The placebo and the vehicle for hpGRF were sterile distilled water. Treatments were given on alternate days. Blood samples were collected at -40, -20, 0, 5, 10, 15, 20, 25, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to each injection. Serum was decanted after centrifugation and stored at -20 C until assayed for GH concentration as described by Moseley et al. (1982).

Cows were fed ad libitum a mixed ration [MR; 60% roughage: 40% concentrate on a dry matter (DM) basis] supplemented with 2.7 kg concentrate and 2.3 kg long stemmed alfalfa hay daily. Cows were fed at 0800 h which did not overlap with blood sampling or injections. Cows were milked daily at 0400 and 1600 h.

Peak height of serum GH and area under the GH response curve were used as measures of hpGRF effects. Peak height for the placebo was defined as the mean of all seven serum samples taken between 5 and 40 min after injection of placebo. For hpGRF doses, peak height was the maximum concentration of GH achieved between 5 and 40 min after injection of hpGRF. Area was defined as the integrated area under the GH response curve for 1 h after injection of hpGRF or placebo. Peak heights at least two standard deviations above the pre-injection mean (mean of -40, -20, 0 min samples) defined a GH response. Data were subjected to analysis of variance and contrasts among individual means

were made using Scheffe's interval (Gill, 1978).

2. Experiment 2. Sixteen (8 pairs) mid to late lactation Holstein cows were used in a 2 (cow) x 2 (period) Latin square crossover design with 8 squares. The experiment comprised two 10-d treatment periods in addition to a 10-d pretreatment acclimation period, a 10-d rest period between treatments and a 10-d post-treatment period. Placebo and hpGRF were administered during d 11 to 20 and 31 to 40 (d 1 = beginning of experiment). Cows were paired based primarily on d 4 to 7 milk yield; however, stage of lactation, pregnancy status and parity were also considered. Cows averaged 178 ± 8 d postpartum and 13 cows were pregnant 95 ± 13 d on d 11 of the experiment. BW averaged 626 ± 15 kg at the beginning of the experiment. Seven pairs of cows were multiparous and one pair was primiparous. Cattle were provided with 20 h fluorescent lighting each day which overlapped 15 h of natural lighting. Ambient temperatures ranged from 18 to 29.5 C.

Cows were injected (5.5 to 7.5 ml hpGRF solution as required or 6 ml placebo) via an indwelling jugular cannula with either 20 μg hpGRF/100 kg BW or placebo at 4-h intervals during the two 10-d treatment periods. Thus, during a treatment period, eight cows (one of each pair) received hpGRF while the other eight cows received placebo. Injections were given at 0300, 0700, 1100, 1500, 1900 and

2300 h each day of treatment.

Blood samples were collected at -30, -20, -10, 0, 10, 20, 30, 40, 50 and 60 min relative to the 0700 and 1100 h injections on d 1 and 10 of each treatment period. Within days, data from 0700 and 1100 h were pooled for analyses. Serum was assayed for GH as in Experiment 1. Pre-injection means, peak heights and area under the response curves for concentrations of GH were used as measures of treatment effects. Pre-injection means of GH were calculated as the average of the -30, -20, -10 and 0 min samples. Peak height of serum GH in response to hpGRF or placebo was defined as maximum concentration of GH during the 1-h post-injection period. Area was defined as the integrated area under the 1-h response curve after subtraction of pre-injection mean GH.

Cows were milked at 0530 and 1630 h daily. Daily yields of milk were recorded, and samples of milk were obtained at each milking on d 18 to 20 and 38 to 40 for milk composition analyses. Milk fat, protein and lactose were measured at Michigan DHIA (East Lansing) using an infra-red analyzer (Multispec, Wheldrake, England) and somatic cell counts (SCC) were determined using fluorescence microscopy (Fossomatic, A/S N. Foss Electric, Hillerod, Denmark). Solids-corrected milk (SCM) yield and energy output in milk (Mcal/day) were calculated (Tyrrell and Reid, 1965). Cows were fed ad libitum a MR (60% roughage: 40% concentrate on a DM basis) at 0400 and 1200 h daily, and, in addition,

received 2.3 kg long alfalfa hay at 1200 h. On a DM basis, ingredients (and percentage of ration) in MR were haylage (18%), chopped hay (12%), corn silage (30%), high moisture ear corn (27%), supplement [13%; containing 44% crude protein (CP), phosphorus, calcium, trace minerals, vitamins A and D] and trace mineral salt (.25%). On a DM basis, MR contained 17.6% CP, 20.6% acid detergent fiber, .49% phosphorus and .93% calcium, which meets National Research Council (1978) requirements. Feed intakes were recorded daily. Cows were weighed at 0800 h on d 18 to 20 and 38 to 40.

Milk yield, composition of milk, feed intake and BW data across d 18 to 20 and 38 to 40 were used for statistical analyses. Treatment effects on all variables were tested using analysis of variance (Gill, 1978).

C. Results

1. Experiment 1. Increased release of GH was not associated with time of placebo injections (data not shown). In contrast, the 10, 20 and 40 μg/100 kg BW doses of hpGRF each increased peak height of GH approximately 10-fold ($P<.005$) over that of the placebo (Table 5). Similarly, each dose of hpGRF increased ($P<.005$) area under the

Table 5. Serum growth hormone response to varying doses of human pancreatic growth hormone-releasing factor (hpGRF1-44-NH_2).

	Dose (μg) of hpGRF/100 kg BW				
GH trait[1]	Placebo	10	20	40	Pooled SE
Peak height (ng/ml serum)	1.6[a]	15.6[b]	15.1[b]	16.5[b]	1.2
Area[2] (ng·min·ml^{-1} serum)	277[a]	1134[b]	1166[b]	1412[b]	89.4

[ab]Means in the same row with different superscripts differ ($P<.005$).

[1]Values are means of all four cows and all six injection times.

[2]Area under 1-h response curve.

response curve approximately 4.5-fold above controls. Peak height of GH in response to hpGRF typically occurred at approximately 20 min post hpGRF injection.

A GH response was observed for all four cows, at all six injection times for 10, 20 and 40 μg hpGRF (the 20 μg dose is representative and shown in Figure 3). However, there was considerable variation in response within and between cows, with GH response peaks ranging from 2 to 52 ng/ml of serum. Responses of serum GH to repeated injections of hpGRF were lowest at 1400 and 1800 h, while highest values occurred at 2200 and 0600 h (Table 6).

Experiment 2. Relative to placebo treatment, hpGRF increased yields of milk ($P<.025$), SCM ($P<.005$), fat ($P<.01$), protein ($P<.005$), lactose ($P<.025$) and total solids ($P<.005$) approximately 10%, but, hpGRF did not alter ($P>.05$) composition of milk (Table 7). Milk production throughout the experiment is depicted in Figure 4. The apparent difference in yield of milk between groups of cows on d 9 and 10 was associated with failure to determine milk yield of three of the higher yielding cows in the initial placebo-treated group on these two days. The positive effect of hpGRF on milk yield was apparent within 48 h of initial administration, and effect of hpGRF on milk yield was not detectable by 48 h after final hpGRF administration. SCC in milk did not differ between treatments (153,000 ± 50,000 vs 179,000 ± 53,000; hpGRF vs placebo, respectively).

Figure 3. Responses in serum concentrations of growth hormone to 20 μg hpGRF 1-44-NH_2/100 kg BW administered iv at 4-h intervals to four late lactation cows on four different days. Arrows represent times of injection. Responses to 10 μg and 40 μg doses were similar and are not shown.

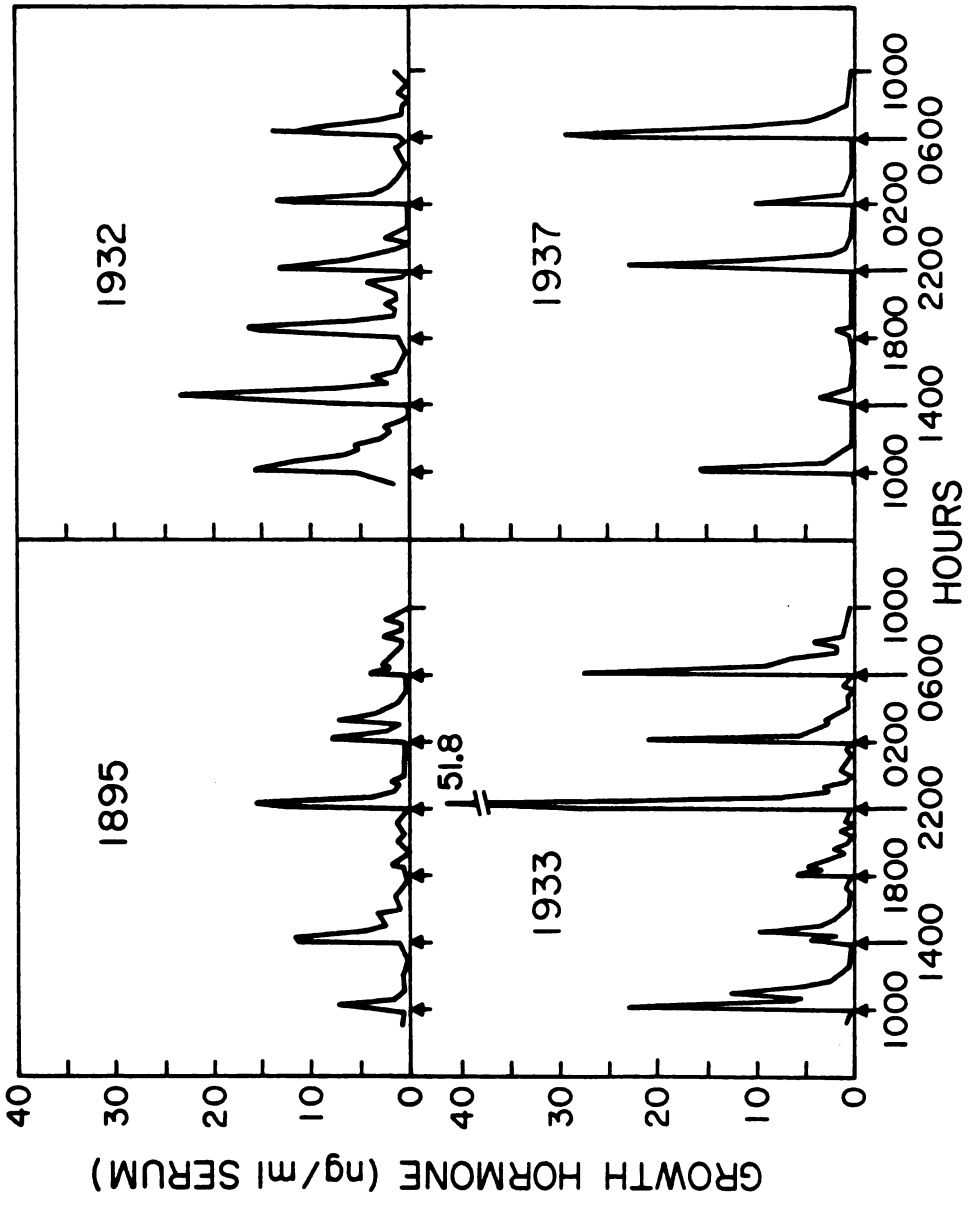

1895
1932
1933
1937
51.8
0
10
20
30
40
1000
1400
1800
2200
0200
0600
1000
HOURS
GROWTH HORMONE (ng/ml SERUM)

Table 6. Sequential response of serum growth hormone to repeated injections of 0, 10, 20 and 40 μg of human pancreatic growth hormone-releasing factor (hpGRF1-44-NH_2) per 100 kg BW.

	Time of injection (h)						
GH trait[1]	1000	1400	1800	2200	0200	0600	Pooled SE
Peak height (ng/ml serum)	11.9^{abc}	7.5^{ab}	6.0^{a}	16.9^{c}	12.0^{bc}	19.0^{c}	1.2
Area[2] ($ng \cdot min \cdot ml^{-1}$) serum	969^{bc}	629^{ab}	532^{a}	1405^{c}	970^{bc}	1544^{c}	89.4

^{abc}Means in the same row with different superscripts differ ($P<.05$).

[1]Values shown are means of all four cows and all four treatments.

[2]Area under 1-h response curve.

Table 7. Mean (±SE) milk, fat, protein, lactose and total solids yields and composition of milk during placebo and hpGRF treatments.[1,2]

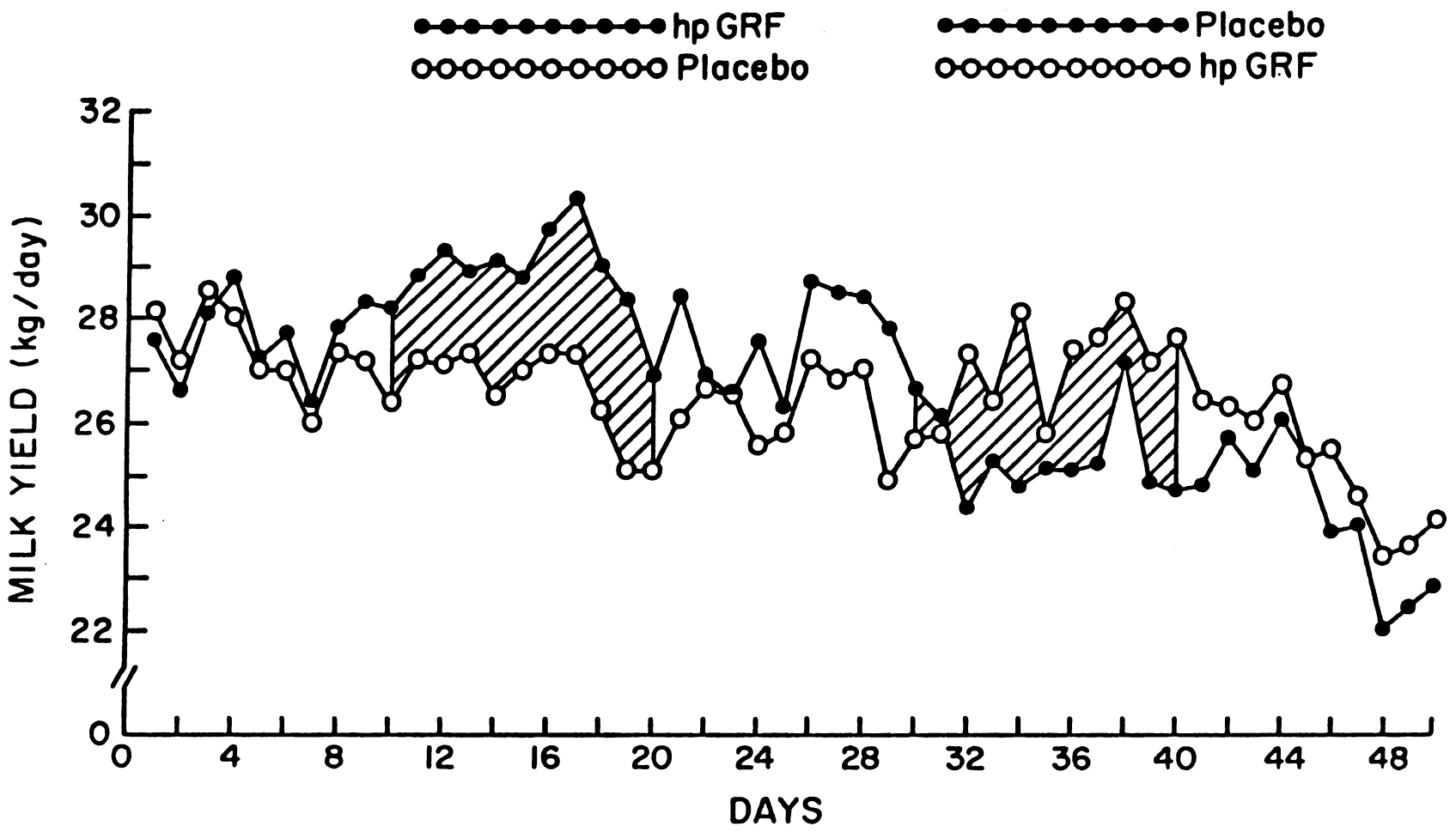
Variables	Placebo	hpGRF	P<
Milk yield, kg/d	25.4 ± 1.1	27.7 ± 1.2	.025
Solids-corrected milk yield, kg/d	25.1 ± 1.2	27.9 ± 1.2	.005
Fat, %	3.7 ± .1	3.9 ± .1	NS[3]
Fat yield, kg/d	.94 ± .05	1.06 ± .05	.01
Protein, %	3.2 ± .1	3.3 ± .1	NS
Protein yield, kg/d	.82 ± .04	.90 ± .04	.005
Lactose, %	5.3 ± .1	5.2 ± .1	NS
Lactose yield, kg/d	1.35 ± .07	1.46 ± .07	.025
Solids, %	13.0 ± .2	13.1 ± .2	NS
Solids yield, kg/d	3.29 ± .16	3.64 ± .15	.005

[1]Mean (±SE) data for cows during the last 3 d of treatment.

[2]Treatments involved injections of placebo or hpGRF 1-44-NH_2 (20μg/100 kg BW/injection) at 4-h intervals during two 10-d periods.

[3]NS = Nonsignificant.

Figure 4. Mean yields of milk, expressed as kg/d, for two groups of cows during the 50-d experimental period. Solid circles represent the mean yield of eight cows throughout the experiment and the open circles represent the mean of their pairmates. Treatments were administered during the two 10-d periods indicated by the hatched areas. The legend indicates the treatment received by each group of cows during a particular treatment period.


hp GRF
Placebo
Placebo
hp GRF
MILK YIELD (kg/day)
32
30
28
26
24
22
0
0
4
8
12
16
20
24
28
32
36
40
44
48
DAYS

Due primarily to increased milk yield, daily energy output in milk was greater (P<.005) in hpGRF- than placebo-treated cows (Table 8). Feed intake did not differ (P>.05) between treatments (Table 8). BW of cows increased 2.4% (P<.005) during hpGRF treatment, and this was due largely to a 13 kg increase in weight of hpGRF-treated cows during the second treatment period.

Mean pre-injection concentrations of GH in serum were similar in placebo- and hpGRF-treated cows on treatment d 1 (Table 9), though by treatment d 10 mean preinjection GH was lower (P<.01) in hpGRF- than in placebo-treated cows. However, whether data were expressed as peak height or area, GH response to hpGRF was greater (P<.001) than response to placebo on d 1 and 10 of treatment. Mean peak height of GH after hpGRF on d 10 was 23% greater than the response on d 1 (24.4 vs 19.9 ng/ml serum, respectively). However, statistical comparisons can not be made due to nature of the design. Time to reach peak height averaged 22 min and was similar on d 1 and 10.

D. Discussion

The 10-fold increase in peak height of GH in lactating cows of the present study in response to all doses of hpGRF agrees with the magnitude of responses reported in bull calves (Johke et al., 1984; Chapter 1), heifer calves (Johke

Table 8. Mean (±SE) energy output in milk, feed intake and BW during placebo and hpGRF treatments.[1,2]

Variable	Placebo	hpGRF	P<
Energy output in milk, Mcal/d	18.8 ± .9	20.9 ± .9	.005
Feed intake, kg DM/d[3]	19 ± 1	19 ± .5	NS[4]
Body weight, kg	629 ± 14	644 ± 16	.005

[1]Mean (±SE) data for cows during the last 3 d of treatment.

[2]Treatments involved injections of placebo or hpGRF 1-44-NH_2 (20μg/100 kg BW/injection) at 4-h intervals during two 10-d periods.

[3]DM = Dry matter.

[4]NS = Nonsignificant.

Table 9. Mean (±SE) serum growth hormone response to placebo and 20 μg hpGRF per 100 kg BW on d 1 and 10 of treatment.[1]

GH Trait	Placebo	hpGRF	P<
Pre-injection mean (ng/ml serum)			
Day 1	.8 ± .1	.7 ± .1	NS[2]
Day 10	.9 ± .1	.5 ± .0	.01
Peak height (ng/ml serum)			
Day 1	1.2 ± .1	19.9 ± 3.1	.001
Day 10	1.7 ± .4	24.4 ± 2.6	.001
Area[3] ($ng \cdot min \cdot ml^{-1}$)			
Day 1	2.0 ± 3.8	611 ± 80	.001
Day 10	2.0 ± 5.0	694 ± 73	.001

[1]Means (± SE) shown represent the mean of 16 cows at two consecutive injections (0700 and 1100 h).

[2]NS = Nonsignificant.

[3]Integrated area under 1-h response curve minus preinjection baseline.

et al., 1984; Hodate et al., 1984; Plouzek et al., 1984), steers (Moseley et al., 1984, 1985), postpubertal heifers and non-lactating cows (Johke et al., 1984). Apparently the doses of hpGRF I chose to administer exceeded the dose response range since I was unable to detect a dose response. In agreement with my data, McCutcheon et al. (1984) did not observe a dose response relationship between hpGRF fragments (1-24 and 1-29) and GH in lactating cows.

The large variation in magnitude of response of GH within lactating cows to repeated injections of hpGRF in this study was also noted in prepubertal bulls (Chapter 1). I speculate that this variance reflects variation in secretion of endogenous somatostatin (SRIF), an inhibitor of GH, and GRF. Thus, the GH response to exogenous hpGRF is likely to be less if secretion of endogenous SRIF in relatively high. Indeed, an inverse dose-dependent relationship between hpGRF and SRIF on release of GH from bovine anterior pituitary cells cultured in vitro has been observed (Padmanabhan et al., 1987).

Moseley et al. (1985) reported that GH response to infusion of hpGRF was sustained for 5 d in steers, and the response of GH to six daily injections of hpGRF in my lactating cows was maintained for 10 d. Thus, there is no suggestion of refractoriness of GH secretion to hpGRF within the intervals tested. Indeed, Jansson et al. (1985) reported that multiple administration of hpGRF enhances GH response to a subsequent hpGRF injection in rats. The

tendency for numerically greater release of GH from lactating cows on d 10 as compared with d 1 may suggest that hpGRF stimulates synthesis as well as release of GH in cows as has been demonstrated in rats (Barinaga et al., 1983, 1985).

Hart et al. (1985c) recently showed that iv injection of hpGRF at 2-h intervals stimulates milk yield in ewes 27% over controls. This response was similar to that achieved with bovine GH (Hart et al., 1985c). To my knowledge, the 11% increase in yield of SCM in my cows is the first report showing a significant positive effect of exogenous hpGRF on milk yield in cattle. The increase in yield was similar in magnitude to several reports (9 to 15% increase; Peel et al., 1981, 1983), but substantially less than other reports (30 to 40% increase; Fronk et al., 1983; Peel et al., 1983; Eppard et al., 1985b; Bauman et al., 1985) which tested the effect of GH on milk production. Smaller percent increases are generally seen in short-term studies where early to mid lactation cows are producing greater than 25 kg milk/d. Larger percent increases tend to be demonstrated in lower yielding or late lactation cows. Although cows in Experiment 2 were at mid to late lactation, average milk production was greater than 25 kg/d and thus the 11% increase in SCM yield achieved compared favorably with GH studies which utilized higher producing cows.

Whether pattern of administration of exogenous GRF

affects milk yield response of dairy cows is not known. The available evidence in cattle suggests that there is no effect of pattern of administration of exogenous GH on milk yield (Fronk et al., 1983) or nitrogen retention (Moseley et al., 1982). In contrast, studies with rats indicate that the pattern of GH or GRF administration affects the growth response (Jansson et al., 1982; Clark and Robinson, 1985b). Because of these conflicting results, a conservative approach was utilized in this study. The frequency and route of administration of hpGRF in Experiment 2 was utilized to attempt to mimic the pulsatile pattern of GH secretion associated with high yielding and early lactation dairy cows (Vasilatos and Wangsness, 1981; Bines et al., 1983).

Cows in negative energy balance may have increased percentage of milk fat and decreased percentage of milk protein in response to exogenous GH administration (Peel et al., 1981, 1983). However, in my study the hpGRF-induced percentage increases in yields of fat, protein and lactose were approximately equal to the increase in milk yield (9%). This is in general agreement with most of the studies involving GH where little or no change in percentages of milk constituents are found. Milk composition tends to be unaltered by GH treatment when cows are in positive energy and nitrogen balance (Bauman and McCutcheon, 1986) as was likely for the mid to late lactation cows utilized in Experiment 2. In short term studies of the effects of GH on

lactational performance of dairy cows feed intake is unchanged and energy output in milk is increased by GH. Thus, feed efficiency (kg milk/kg feed) is increased (Peel et al., 1983; Fronk et al., 1983; Eppard et al., 1985b). The data from Experiment 2 are in agreement with these observations.

In addition to increased feed efficiency, cows in Experiment 2 which received hpGRF treatment for 10 d were heavier than controls. This response was unexpected. Short term effects of GH on BW of lactating dairy cows are unknown, but in long-term studies GH treatment has no effect on BW of lactating cows (Brumby and Hancock, 1955; Peel et al., 1985; Bauman et al., 1985).

I conclude that repeated injections of hpGRF consistently elicit GH responses in lactating cows, and I found no evidence of refractoriness to hpGRF when administered at 4-h intervals for 10 d. Furthermore, hpGRF increases yield of milk and milk components approximately 11% in mid to late lactation Holstein cows without altering percentage of milk constituents. The increased yield was achieved with increased body weight but without increased feed intake. I speculate that GH mediated these responses.

Chapter 3 (Part 1)

The Effect of Infusions of Various Doses of GRF on GH and Lactation in Holstein Cows

A. Introduction

Administration of either pituitary (Bauman et al., 1985; McCutcheon and Bauman, 1986a) or recombinant (Bauman et al., 1985) growth hormone (GH) to dairy cows is galactopoietic in short- (Eppard et al., 1985b; McCutcheon and Bauman, 1986a) and long-term (Peel et al., 1985; Bauman et al., 1985) studies. An alternative approach to stimulate milk production is to administer growth hormone-releasing factor (GRF) which increases endogenous GH secretion in cattle (Johke et al., 1984; Moseley et al., 1985, 1987; Chapters 1 and 2). Indeed, when 20 (Chapter 2) or 100 μg (Petitclerc et al., 1985) of human pancreatic GRF 1-44-NH_2/100 kg body weight (BW) was administered iv every 4 h for 10 d to dairy cows, milk production increased 11 and 20%, respectively.

Increased concentrations of GH in serum in response to sequential (every 4 h) iv administration of GRF are maintained for at least 10 d in dairy cows (Petitclerc et al., 1985; Chapter 2). Similarly, serum GH response to GRF is maintained following sequential (every 6 h for 48 h) iv administration in bull calves (Chapter 1), infusion (microinjections every 3.75 min) for 5 d in steers (Moseley et al., 1985) or infusion (continuous) for 20 d in bull calves (Moseley et al., 1987).

I speculate that increased secretion of GH mediates responses of lactating cows to exogenous GRF. However,

there is no information concerning infusion of various doses of GRF on magnitude and pattern of GH concentrations in serum of lactating dairy cows. Therefore, my first objective was to elucidate serum GH response in lactating dairy cows to 24 h infusion of various doses of GRF.

Milk yield responses to infusions of GRF are unknown, and studies on the galactopoietic effect of GRF have employed only two dose levels (placebo vs GRF). Furthermore, serum GH response of lactating dairy cows to a longer-term continuous GRF stimulus is unknown. Thus, my second objective was to test effects of 20-d infusion of three doses of GRF on lactation and pattern of serum GH response in lactating dairy cows.

B. Materials and Methods

1. Experiment 1. Twenty-nine Holstein cows were used in a randomized complete block design. Cows were blocked based on pretreatment milk yield (5 d average; 28 ± 1 kg/d), stage of lactation (203 ± 15 d after parturition) and parity and, within block, assigned randomly to treatment (four to five/treatment). Six primiparous (one/treatment) and 23 multiparous cows were utilized. Cows weighed 646 ± 14 kg. Eighteen (two to four/treatment) cows were pregnant (mean 143 d; range 84 to 193 d). Cows were housed in adjacent tie-stalls and exposed to continuous fluorescent lighting

(and 13 h of natural lighting daily) and ambient temperatures of 8 to 20 C.

Six doses of bovine GRF 1-44-NH_2 (Peninsula Laboratories, Belmont, CA) were used: 0 (placebo), 3.125, 6.25, 12.5, 25.0, and 50.0 mg/cow/24 h. There were four cows in the 25 mg group and five cows in all other groups. GRF was dissolved in 12.8 ml sterile distilled water and infused via an indwelling jugular cannula for 24 h starting at 1000 h. Cows received pulses of placebo or GRF at 3.75-min intervals via AS-2BH AutoSyringe® infusion pumps (Auto-Syringe, Inc., Hooksett, New Hampshire), a procedure that results in concentrations of GH that are indistinguishable from those obtained with continuous infusion (W.M. Moseley, personal communication). Blood samples were collected via an indwelling jugular cannula at 20-min intervals for 1 h before, during, and for 1 h after the 24 h infusion. Samples were collected from the jugular vein contralateral to the jugular vein being infused. Serum was decanted after centrifugation and stored at -20 C until assayed for GH (Moseley et al., 1982).

Cows were milked daily in their stalls at 0430 and 1530 h. Cows were fed a mixed ration [MR; 50% roughage:50% concentrate on a dry matter (DM) basis] supplemented with 2.3 kg long alfalfa hay daily. Cows were fed ad libitum at 0800 h, 1 h before commencement of blood sampling, and fresh feed was not offered again until blood sampling was completed the next day.

Characteristics of GH in serum (which included mean, baseline, and pulse frequency, amplitude and duration) during the 24 h infusion were determined using a pulse analysis program (PULSAR; Merriam and Wachter, 1982). "G" values (based on peak selection criteria) used were: G (1) = 99999, G (2) = 2.89, G (3) = 1.84, G (4) = 1.27, G (5) = .89. Preinfusion means of serum GH were calculated as the average of the -60, -40, -20 and 0-min samples.

Data were subjected to analyses of variance (Gill, 1978). Characteristics of GH were averaged across the 3.125 to 50.0 mg doses of GRF and compared with placebo using Bonferroni t test (Gill, 1978). Tukey's test (Gill, 1978) was used to compare GH characteristics among doses of GRF (3.125 to 50.0 mg). GH characteristics for each individual dose of GRF were compared with placebo using Dunnett's t test (Gill, 1978).

2. Experiment 2. Fifteen primiparous Holstein cows were used in a randomized complete block design with repeated measurement. The experiment comprised seven 5-d periods as follows: one pretreatment period (d -5 to -2), four treatment periods (d 1 to 5, 6 to 10, 11 to 15, and 16 to 20) and two post treatment periods (d 21 to 25 and 26 to 30). Cows were blocked based on pretreatment milk yield (average of d -19 to -13; 28 ± 1 kg/d), stage of lactation (114 ± 7 d after parturition), and pregnancy status, and,

within block, assigned randomly to treatment (five/treatment). Cows were either not pregnant or in the first trimester of pregnancy on d -5. BW averaged 556 ± 17 kg between d -4 to -2. Cattle were housed in adjacent tie-stalls and exposed to approximately 18 h fluorescent lighting each day (which completely overlapped 11 h of natural lighting). Ambient temperatures ranged from 11 to 20.5 C. Three doses of bovine GRF 1-44-NH_2 (Bachem, Inc., Torrance, CA) were used: 0 (placebo), 1 and 3 mg/cow/24 h. The 3 mg dose of GRF was chosen because it was equipotent in terms of GH response to doses up to 50.0 mg in Experiment 1. The 1 mg dose was chosen because it approximated the effective daily dose used to increase milk production as described in Chapter 2. GRF was dissolved in 12.8 ml sterile distilled water and infused (as described for Experiment 1) for 20 d beginning at 1100 h on d -1 and ending at 1100 h on d 20. Fresh GRF solutions were prepared daily. Cannulas were encased in plastic coiled hoses (Re-Koil® nylon air hose, Milton Industries Inc., Chicago, IL 60639; .625 cm internal diameter, 3.6 m extended length, 8 cm coil) which protected them from damage due to movement of cows. Blood samples were collected at 20-min intervals from 0800 to 2000 h on d 1, 10 and 19. Methods for blood collection and processing, GH assay and serum GH characteristics were as described for Experiment 1.

Cows were milked daily in their stalls at 0400 and 1600 h. Daily yields of milk were recorded, and samples of milk

were obtained at each milking on d 1 to 5 and 16 to 20 for milk composition analyses. Milk fat, protein and lactose were measured using an infrared analyzer (Multispec, Wheldrake, England) and somatic cell counts (SCC) were determined using fluorescence microscopy (Fossomatic, A/S N. Foss Electric, Hillerod, Denmark) at Michigan DHIA (East Lansing). Solids-corrected milk (SCM) yield and energy output in milk (Mcal/day) were calculated (Tyrrell and Reid, 1965).

Cows were fed ad libitum a MR (50% roughage:50% concentrate on a DM basis) at 0400 and 1200 h daily and each cow received 2.3 kg long alfalfa hay daily at 1200 h. In addition, all cows received 1.8 to 5.4 kg of a grain-protein supplement (top dressed) depending on their pre-experiment milk yield. MR averaged 52.6% DM. On a 100% DM basis, ingredients in MR were haylage (15%), chopped hay (10%), corn silage (24.8%), high moisture ear corn (35%), supplement [14.6%; containing 44% crude protein (CP), phosphorus (P), calcium (Ca), trace minerals, vitamins A and D], trace mineral salt (.2%), and sodium bicarbonate (.4%). On a 100% DM basis, MR contained 18.4% CP, 17.3% acid detergent fiber (ADF), .45% P and .95% Ca, which meets National Research Council (NRC; 1978) requirements. The MR had an estimated net energy for lactation (NE_1) of 1.58 Mcal/kg DM. The grain-protein supplement averaged 88% DM. On a 100% DM basis, the supplement contained 22.2% CP, .67%

P and .86% Ca, with an estimated NE_1 of 1.89 Mcal/kg DM. The long alfalfa hay averaged 89% DM. On a 100% DM basis, the hay contained 16% CP, 40% ADF, .22% P, and 1.35% Ca, with an estimated NE_1 of 1.25 Mcal/kg DM. Intakes of the MR were recorded daily. All hay and supplement were consumed.

Cows were weighed at 0700 h on d -4 to -2, 7 to 9, 17 to 19 and 28 to 30. BW, net energy (NE) balance, observed and corrected energy efficiency (EE) of milk production were calculated for d 1 to 5 and 16 to 20. NE balance was calculated as NE intake minus energy output in milk minus NE for maintenance. NE for maintenance was calculated according to NRC (1978); however, the growth allowance for primiparous cows was not utilized because cows did not gain weight during the infusion period. Observed EE was calculated as energy output in milk (Mcal/day) divided by NE intake (Mcal/day). To calculate corrected EE, individual observed EE values were adjusted for daily BW change using 4.92 and 5.12 Mcal NE for each kilogram of body tissue loss and gain, respectively (NRC, 1978). Average daily gain or loss for d 1 to 5 was extrapolated linearly from BW changes between d -4 to -2 and 7 to 9. Average daily gain or loss for d 16 to 20 was extrapolated linearly from BW changes between d 7 to 9 and 17 to 19. NE for tissue gain was subtracted from, or NE for tissue loss was added to, NE intake. Corrected EE was computed using this adjusted NE intake.

All data were subjected to analyses of variance (Gill,

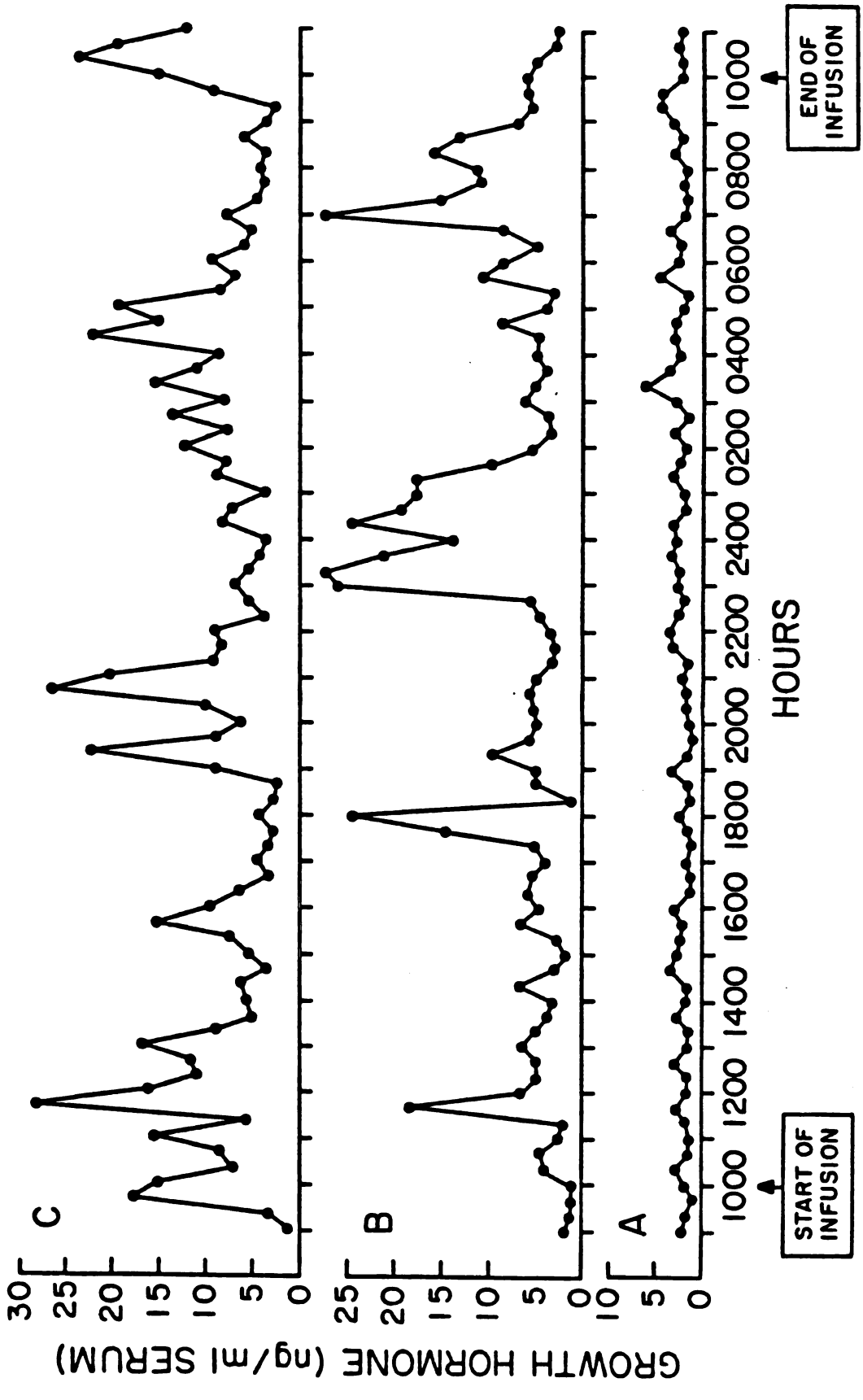
1978). Tests for comparisons of treatment means within periods (d 1 to 5 and 16 to 20) or period means within treatments were used (Gill, 1986). Average milk yield for 1 and 3 mg GRF were compared with placebo within each period using Dunnett's t test (Gill, 1978, 1986). Average SCM yield, percentage and yield of milk components, SCC, DM and NE intake, BW and energy utilization data were compared between periods within each treatment using Student's t test (Gill, 1978, 1986). Dunnett's t test (Gill, 1978, 1986) was used to compare GH characteristics for 1 and 3 mg GRF with placebo across and within sampling days.

C. Results

1. Experiment 1. Preinfusion serum GH was not different between treatments and averaged 2.0 ±.5 ng/ml. In placebo-infused cows serum GH mean (ng/ml) and baseline (ng/ml), and frequency (number/24 h), amplitude (ng/ml) and duration (min) of GH pulses averaged 1.8, 1.5, 3.6, 1.5 and 45, respectively. Serum GH pattern for a representative placebo-infused cow is in Figure 5A.

Across 3.125 to 50.0 mg doses, GRF infusion increased ($P<.005$) serum GH mean (5-fold) and baseline (3-fold), and frequency (2-fold), amplitude (10-fold) and duration (2.5-fold) of GH pulses relative to similar measures in placebo-infused cows. Each of the 3.125 to 50.0 mg doses of GRF

Figure 5. Serum concentrations of GH in representative cows receiving iv infusion of (A) placebo, (B) 3.125 and (C) 50.0 mg bovine GRF/24 h. Individual responses to 6.25, 12.5 and 25.0 mg doses were similar to (B) and (C) but are not shown.


GROWTH HORMONE (ng/ml SERUM)
C
B
A
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1000 1200 1400 1600 1800 2000 2200 2400 0200 0400 0600 0800 1000
HOURS
START OF INFUSION
END OF INFUSION

increased (P<.05) GH mean and pulse amplitude relative to placebo (Table 10). With exception of 6.25 mg, all other doses of GRF increased (P<.05) GH pulse frequency relative to placebo (Table 10). The 12.5 and 50.0 mg doses of GRF increased (P<.05) GH baseline relative to placebo (Table 10). The 6.25 and 25.0 mg doses of GRF increased (P<.05) duration of GH pulses relative to placebo (Table 10). Characteristics of serum GH did not differ among the 3.125 to 50.0 mg doses of GRF (Table 10). Serum GH patterns for representative cows infused with 3.125 and 50.0 mg GRF/24 h are in Figures 5B and 5C, respectively. Across doses of GRF (3.125 to 50.0 mg) serum GH mean was greater (P<.05) during the second 12 h of infusion (9.9 ± .7 ng/ml) compared with the first 12 h (8.3 ± .7 ng/ml), and there was no interaction (P>.05) between dose and time.

2. Experiment 2. Mean daily milk yields (d -5 to 30) for cows infused with placebo, 1 and 3 mg GRF/24 h are in Figure 6. Milk yield during d 6 to 20 averaged 26.5 ± 1.7 kg/day in placebo-infused cows and increased to 29.4 ± 2.0 (11%) and 32.7 ± 2.0 (23%) kg/day in cows infused with 1 and 3 mg GRF/24 h, respectively (Figure 6). The 1 mg GRF dose increased (15%; P<.05) milk yield above that of placebo-infused cows only during d 11 to 15. The 3 mg GRF dose increased milk yield above that of placebo-infused cows during d 6 to 10 (19%; P<.01), d 11 to 15 (29%; P<.01), d 16 to 20 (23%; P<.01) and d 21 to 25 [15%; P<.05; (Figure 6)].

Table 10. Characteristics of serum growth hormone (GH) during 24-h infusions of varying doses of bovine growth hormone-releasing factor (GRF).

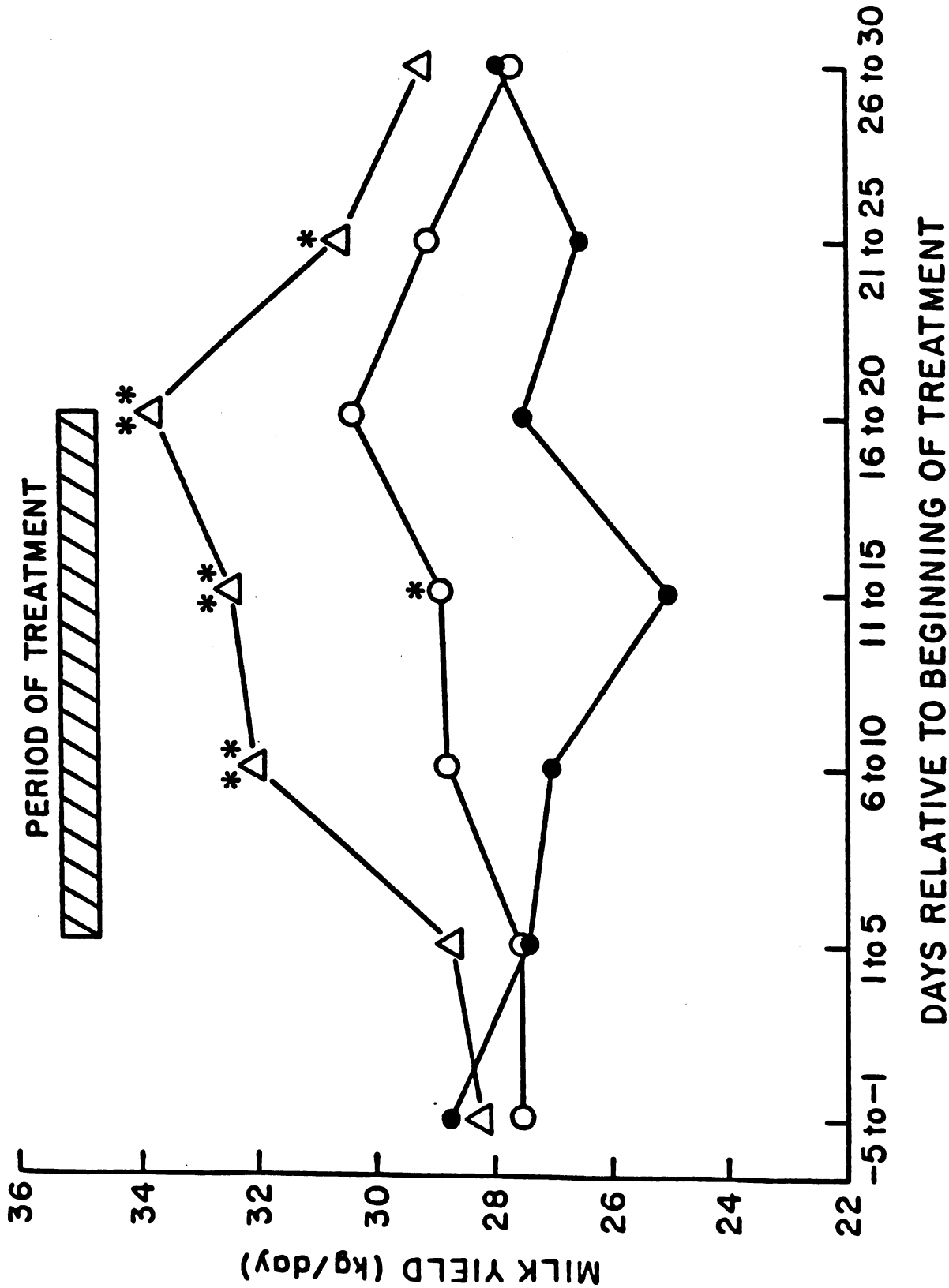
Dose of GRF (mg/24 h)	Mean GH (ng/ml)	Baseline GH[1] (ng/ml)	Number of pulses/24 h	Amplitude of pulses (ng/ml)	Duration of pulses (min)
Placebo	1.8	1.5	3.6	1.5	45
3.125	9.3[a]	3.8	9.4[a]	16.4[a]	96
6.25	6.8[b]	3.4	6.2	12.2[b]	123[a]
12.5	10.8[a]	5.3[b]	8.6[b]	15.2[a]	99
25.0	7.5[b]	3.0	7.3[b]	15.9[a]	142[b]
50.0	11.0[a]	6.0[a]	9.6[a]	14.9[a]	98
SED[2]	1.8	1.1	1.4	3.2	26

[a,b] Differ from placebo (Dunnett's t test).
[a] $P < .01$
[b] $P < .05$

[1]Equal to the smoothed mean GH generated by a pulse analysis program (Merriam and Wachter, 1982).

[2]SED = Standard error of difference of means.

Figure 6. Mean yields of milk (kg/d) for three groups of cows (n = five cows/treatment) during seven 5-d experimental periods. Treatments were intravenous infusions of 1) placebo (●——●), 2) 1 mg GRF/24 h (O——O) and 3) 3 mg GRF/24 h (Δ——Δ). Each point represents the mean of five cows and 5 d. Means of GRF treatments denoted by ** ($P < .01$) and * ($P < .05$) are different from the placebo mean during that period.


PERIOD OF TREATMENT
MILK YIELD (kg/day)
36
34
32
30
28
26
24
22
-5 to -1
1 to 5
6 to 10
11 to 15
16 to 20
21 to 25
26 to 30
DAYS RELATIVE TO BEGINNING OF TREATMENT

Although not analyzed statistically, effects of 1 and 3 mg GRF on milk yield were first observed by 8 and 4 d after start of infusion, respectively. Milk yields of 1 and 3 mg GRF cows merged with milk yield of placebo-infused cows by 6 and 8 d after cessation of infusion, respectively. During the last few days of infusion, milk yields of GRF-infused cows were still increasing. On several days between d 8 and 14, 2 to 3 cows in each group experienced reduced feed intakes and milk yields. The reason for this occurrence is unknown, but it was not limited to a specific treatment group.

Between d 1 to 5 and 16 to 20, daily SCM yield decreased 2% in placebo-infused cows but increased ($P<.01$) 12 and 21% in 1 and 3 mg GRF-infused cows, respectively (Table 11). Over time within each treatment, percent fat in milk did not change whereas percent protein in milk increased ($P<.01$). Over time, percent lactose in milk decreased ($P<.01$) in placebo and 1 mg GRF groups, but was unchanged in the 3 mg group. Percent milk solids increased ($P<.05$) over time in the 3 mg GRF group, but was unchanged in the other groups. Yields of fat, protein, lactose and milk solids increased ($P<.01$) between d 1 to 5 and 16 to 20 in GRF-infused cows proportionately to SCM yield (Table 11). SCC in milk was not changed ($P>.05$) by any treatment (Table 11). The high SCC value (951,000) for the 3 mg GRF group during d 1 to 5 is largely due to one cow who had an average

TABLE 11. Mean milk, fat, protein, lactose and total solids yields, and composition of milk during d 1 to 5 and 16 to 20 of bovine growth hormone-releasing factor (GRF) infusions.

Variables	Placebo		1 mg GRF/24 h		3 mg GRF/24 h		
	D[1] 1 to 5	D 16 to 20	D 1 to 5	D 16 to 20	D 1 to 5	D 16 to 20	SED[2]
Solids-corrected milk yield, kg/d	25.0	24.5	26.4	29.5[a]	27.6	33.3[a]	.39
Fat, %	3.35	3.25	3.71	3.79	3.71	3.76	.06
Fat yield, kg/d	.92	.89	1.02	1.15[a]	1.06	1.27[a]	.02
Protein, %	3.07	3.19[a]	3.05	3.19[a]	3.10	3.29[a]	.02
Protein yield, kg/d	.85	.87	.84	.97[a]	.88	1.11[a]	.02
Lactose, %	4.95	4.83[a]	5.09	5.00[a]	5.20	5.18	.02
Lactose yield, kg/d	1.37	1.33	1.40	1.51[a]	1.49	1.75[a]	.02
Milk solids, %	12.04	11.93	12.52	12.64	12.66	12.90[b]	.05
Milk solids yield, kg/d	3.33	3.27	3.44	3.84[a]	3.61	4.35[a]	.05
SCC, '000	243	90	472	165	951	403	178

[a,b] Periods differ within a treatment (Student's t test).
[a] $P < .01$
[b] $P < .05$

[1] D = Day

[2] SED = Standard error of difference between periods within any treatment.

SCC of 4,302,000. This cow had high SCC values throughout the experiment. However, her milk yield, feed intake and rectal temperature were not adversely affected by the high SCC. In addition, she responded (in terms of increased milk yield) to GRF treatment.

In placebo-infused cows DM and NE intakes decreased ($P<.05$) 6 and 9% between d 1 to 5 and 16 to 20, respectively, whereas GRF-infused cows maintained DM and NE intakes during these times (Table 12). GRF (1 and 3 mg doses) increased ($P<.01$) energy output in milk over time. BW of cows in the 3 mg GRF group decreased ($P=.05$) between d 1 to 5 and 16 to 20 but did not change in the placebo and 1 mg GRF groups (Table 12). Over time, NE balance decreased ($P<.05$) in all groups, although the difference over time became more pronounced as dose of GRF increased (Table 12). Over time, GRF (1 and 3 mg doses) increased ($P<.01$) observed EE but EE corrected for BW was similar within each treatment (Table 12).

Characteristics of serum GH on d 1, 10, and 19 are in Table 13. Across all sampling days serum GH mean (ng/ml), and frequency (number/12 h) and amplitude (ng/ml) of GH pulses increased from 2.1, 2.5 and 3.2 in placebo-infused cows to 4.1, 4.2 and 6.2 ($P<.05$), and 6.8, 5.0 and 8.6 ($P<.01$) in the 1 and 3 mg GRF-infused cows, respectively. In addition, across all sampling days serum GH baseline increased ($P<.01$) from 1.4 ng/ml in placebo-infused cows to 4.2 ng/ml in the cows infused with 3 mg GRF.

TABLE 12. Mean intake, BW and energy utilization during d 1 to 5 and 16 to 20 of bovine growth hormone-releasing factor (GRF) infusions.

Variables	Placebo		1 mg GRF/24 h		3 mg GRF/24h		
	D[1] 1 to 5	D 16 to 20	D 1 to 5	D 16 to 20	D 1 to 5	D 16 to 20	SED[2]
DM intake, kg	17.1	16.1[b]	17.8	17.6	18.4	18.5	.3
Net energy (NE) intake, Mcal/d	27.9	25.5[b]	29.0	27.9	30.0	29.2	.5
Energy output in milk, Mcal/d	18.8	18.3	19.8	22.1[a]	20.7	24.9[a]	.3
BW[3], kg	508.2	507.8	581.7	579.8	583.9	580.6[b]	1.1
NE balance, Mcal/d	+ .5	- 1.4[b]	- .3	- 3.7[a]	- .2	- 5.2[a]	.6
Energy efficiency[4]							
Energy output in milk, Mcal/d / NE intake, Mcal/d							
Observed	.67	.72	.68	.79[a]	.69	.85[a]	.02
Corrected[5]	.70	.73	.73	.70	.79	.77	.04

[a,b] Periods differ within a treatment (Student's t test).
[a] $P < .01$
[b] $P \leq .05$

[1] D = Day

[2] SED = Standard error of difference between periods within any treatment.

[3] BW for d 1 to 5 were extrapolated linearly using data collected on d -4 to -2 and 7 to 9, and for d 16 to 20 using data collected on d 17 to 19.

[4] All energy efficiency values were calculated on an individual cow basis.

[5] Corrected for BW changes.

TABLE 13. Characteristics of serum growth hormone (GH) on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Day of experiment	Dose of GRF (mg/24 h)	Mean GH (ng/ml)	Baseline GH[1] (ng/ml)	Number of pulses/12 h	Amplitude of pulses (ng/ml)	Duration of pulses (min)
1	Placebo	2.4	1.8	2.2	3.0	99
	1	2.9	1.9	2.4	5.6	84
	3	7.2[a]	4.1[a]	5.2[a]	11.0[a]	85
10	Placebo	2.8	1.8	3.2	4.1	102
	1	5.9[b]	3.2	4.8	7.9	72
	3	8.0[a]	5.0[a]	5.4[b]	8.7[b]	69
19	Placebo	1.1	.7	2.0	2.5	69
	1	3.3	2.0	5.4[a]	5.2	63
	3	5.2[a]	3.5[a]	4.4[b]	6.1	76
SED[2]		.8	.5	.6	1.1	12

[a,b] Differ from placebo within day of experiment (Dunnett's t test).
[a]P < .01
[b]P < .05

[1]Equal to the smoothed mean GH generated by a pulse analysis program (Merriam and Wachter, 1982).

[2]SED = Standard error of difference between treatments within any day of experiment.

D. Discussion

The 5-fold GRF-induced increase in mean serum GH of cows in Experiment 1 agrees with the magnitude of responses in bull calves (Chapter 1), steers (Moseley et al., 1985; Al-Raheem et al., 1986) and lactating cows (McCutcheon et al., 1984; Chapter 2). The GH response in my lactating cows did not vary with dose of GRF which agrees with other studies using cows (McCutcheon et al., 1984; Chapter 2). Thus, it appears that in lactating cows there may be a relatively narrow range of GRF doses where a GRF-GH dose response relationship exists. Indeed, data from Experiment 2 supports this idea. That is, on d 1 the 3 mg GRF dose increased mean serum GH while the 1 mg dose did not, suggesting that the iv infusion dose response range is probably between 1 and 3 mg GRF/24 h. By d 10, however, the 1 mg dose of GRF increased mean serum GH concentrations above placebo but the response was numerically less than that induced by the 3 mg dose.

In agreement with my previous study using cows in mid to late lactation (Chapter 2), profiles of serum GH in placebo-infused cows of Experiments 1 and 2 had pulses of low frequency, low amplitude and short duration. Similar to data in bull calves (Moseley et al., 1987; Chapter 1), GRF-infused cows in Experiments 1 and 2 had greater GH pulse

frequency, amplitude and duration than placebo-infused cows. In contrast, GRF infusion in steers does not alter GH pulse frequency, but only augments amplitude of GH pulses (Moseley et al., 1985). Based on data in Experiments 1 and 2, I speculate that the decline in serum GH pulsatile activity with advancing lactation (Vasilatos and Wangsness, 1981) may be associated with insufficient hypothalamic GRF stimulation.

Elevated serum GH release was maintained in lactating cows during infusion of GRF for up to 20 d. Similarly, GRF infused for 5 d in steers (Moseley et al., 1985) or 20 d in bull calves (Moseley et al., 1987) maintained elevated serum GH. In addition, GH response to GRF is maintained following daily subcutaneous (sc) administration of GRF for 57 d in lactating cows (Lapierre et al., 1986a). Thus, the pituitary gland does not become refractory to GRF for at least 20 d of infusion (Experiment 2) or 57 d of consecutive daily injections (Lapierre et al., 1986a) in lactating cows. In fact, relative to placebo, mean GH response to 1 and 3 mg of GRF was higher on d 19 (3.0 and 4.7-fold, respectively) than on d 1 (1.2 and 3.0-fold, respectively) of Experiment 2. Similarly, Moseley et al. (1987) and Lapierre et al. (1986a) reported that GH response to GRF tended to be greater on the last day of their studies than on the first day. In addition, the 1 mg dose of GRF in Experiment 2 did not affect mean serum GH on d 1, but by d 10 it increased mean GH 2-fold above placebo. Collectively, these studies

suggest that exogenous GRF may stimulate synthesis as well as release of GH in cattle. Indeed, GRF stimulates synthesis and release of GH in rats (Barinaga et al., 1985).

The 2.9 (1 mg GRF) and 6.2 (3 mg GRF) kg/d GRF-induced increases in milk yield above yields of placebo-infused cows during d 6 to 20 of Experiment 2 are comparable in magnitude to data in other studies with exogenous GRF (Petitclerc et al., 1985; Lapierre et al., 1986a; Chapter 2) or GH (Peel et al., 1981, 1983; Fronk et al., 1983; Eppard et al., 1985b; McCutcheon and Bauman, 1986a). For example, I (Chapter 2) and others (Petitclerc et al., 1985) increased milk yield 11 and 20% above placebo when 20 and 114 μg GRF/100 kg BW, respectively, were administered iv every 4 h for 10 d. Recently, administration of a fragment (1-29-NH_2) of human GRF via daily sc injection (10 μg/kg BW) for 57 d increased milk production 10% above placebo (Lapierre et al., 1986a). In addition, milk yield increases in my cows are similar to those obtained with injections of GH in cows at a similar level of milk production. For example, Peel et al. (1983) and Eppard et al. (1985b) increased milk yield 15 and 28% with GH in cows producing 28 and 26 kg milk/d, respectively. In a 4-d study, Hart et al. (1985c) reported that, at the doses used, exogenous GH and GRF increased milk yield of ewes to the same extent.

The present study shows for the first time a dose response effect of exogenous GRF on milk yield. Similarly,

a dose response relationship exists between exogenous GH and increased milk yield (Bauman et al., 1985; Eppard et al., 1985b). The optimal dose of pituitary or recombinant GH for milk production is between 25 and 50 mg/d (Bauman et al., 1985; Eppard et al., 1985b). The optimal dose of GRF for milk production is still unknown. However, based on the fact that GRF-induced GH is not different between 3.125 and 50.0 mg GRF/24 h (Experiment 1), it is likely that 3 mg GRF/d may induce maximal milk production response.

The effect of pattern of administration of exogenous GRF on milk yield response of dairy cows is not known. I believe this is not a concern because magnitude of milk yield responses to infusions of 1 or 3 mg GRF/d were similar to responses in studies where GRF was administered six times/d via iv injection (Petitclerc et al., 1985; Chapter 2). Daily dose of GRF, level of milk production, and stage of lactation in these studies (Petitclerc et al., 1985; Chapter 2) were similar to those in Experiment 2. This suggests that iv infusion and relatively frequent (i.e., six times/d) pulsatile iv administration of GRF are equivalent in terms of milk production response. However, if iv administration of GRF is less frequent (i.e., twice/d), milk production is not increased (Lapierre et al., 1985). Indeed, provided mean daily concentration of serum GH is elevated similarly, there is no effect of pattern of administration of GH on nitrogen retention in steers (Moseley et al., 1982) or milk yield in dairy cows (Fronk et

al., 1983; McCutcheon and Bauman, 1986a).

Milk fat and protein percent are not affected by exogenous GH when cows are in positive energy and nitrogen balance, respectively (Peel and Bauman, 1987). Conversely, milk fat percent is increased and milk protein percent is decreased in response to exogenous GH when cows are in negative energy and nitrogen balance, respectively (Peel and Bauman, 1987). Milk lactose percent is not altered by GH which is not surprising because lactose is the primary osmotic regulator of milk volume (Linzell and Peaker, 1971). Although GRF-infused cows in Experiment 2 were in negative energy balance, milk fat percent was not altered by GRF. Possibly, severity of negative energy balance was not sufficient in Experiment 2 to permit detection of this effect on milk fat percent. Similarly, milk fat percent was not affected by GRF in my previous study (Chapter 2). Milk protein percent was not affected by GRF in Experiment 2, possibly because cows were most likely in positive nitrogen balance. The increase in milk protein percent in all treatments over time was expected because milk protein percent increases as lactation advances (Bonnier et al., 1946). The decrease in milk lactose percent in placebo and 1 mg GRF groups during the period of infusion was unexpected and is unexplained.

In 10-d studies testing the effect of GH (Peel et al., 1983; Eppard et al., 1985b) or GRF (Chapter 2) on lactation

of dairy cows, feed intake was not changed by any treatment, including placebo, yet GH or GRF increased milk energy secretion above placebo. Thus, observed gross efficiency (kg milk/kg feed) of milk production was increased by GH or GRF. In contrast, DM and NE intake of placebo-infused cows in Experiment 2 declined, while those of GRF-infused cows remained constant during treatment. This agrees with data from long-term (154 to 188 d) GH trials (Bauman et al., 1985; Peel et al., 1985) where intakes of GH-treated cows were maintained or increased while those of placebo-treated cows decreased as the experiment advanced. Because of sustained NE intake during treatment and large increases in energy output in milk, GRF cows in Experiment 2 increased observed EE of milk production. In my previous 10-d study (Chapter 2) BW of cows was unexpectedly increased by GRF treatment. In contrast, cows receiving 3 mg GRF/d in Experiment 2 lost weight during treatment. Hence, GRF had no effect on EE when corrected for BW changes. Therefore, sustained NE intake and energy from body tissue loss supports GRF-induced increases in energy output in milk.

Results of the present study, in conjunction with previous reports showing GRF-induced increases in milk production of cows (Petitclerc et al., 1985; Chapter 2) and ewes (Hart et al., 1985c), nitrogen retention in bull calves (Moseley et al., 1987) and carcass weight of pigs (Etherton et al., 1986), support the idea that GRF may improve domestic animal performance (Tucker and Merkel, 1987).

Chapter 3 (Part 2)

The Effect of Infusions of Various Doses of GRF on Blood Hormones and Metabolites in Holstein Cows

A. Introduction

It is now clearly established that exogenous growth hormone (GH)-releasing factor (GRF) is galactopoietic in cattle [Petitclerc et al., 1985; Chapters 2 and 3 (Part 1)] and sheep (Hart et al., 1985c). GRF-induced increments in milk yield of dairy cows and ewes are similar to those achieved with exogenous GH (Hart et al., 1985c; Peel and Bauman, 1987) and are probably induced by increased concentrations of serum GH [Chapters 2 and 3 (Part 1)]. If this is true, the galactopoietic effects of GRF are likely mediated by coordinated changes in metabolic and endocrine flux, as has been hypothesized for GH (Bauman and McCutcheon, 1986). Indeed, Hart et al. (1985c) reported that exogenous GRF alters blood concentrations of metabolites and hormones in ewes in a manner similar to exogenous GH.

The effects of chronic exogenous GRF on blood concentrations of "metabolic" hormones and metabolites in cattle are unknown. Therefore, my objective was to test effects of GRF on blood concentrations of prolactin, cortisol, triiodothyronine (T_3), thyroxine (T_4), insulin, non-esterified fatty acids (NEFA) and glucose in lactating dairy cows.

Serum GH and lactational responses in the cows used in this study are in Chapter 3 (Part 1).

B. Materials and Methods

Details of animals, feeding, management and infusions are in Chapter 3 (Part 1). Briefly, 15 primiparous Holstein cows were used in a randomized complete block design with repeated measurement. Three doses of bovine GRF 1-44-NH_2 (Bachem, Inc., Torrance, CA) were infused: 0 (placebo), 1 and 3 mg/cow/24 h. GRF was infused for 20 d beginning at 1100 h on d -1 and ending at 1100 h on d 20. Fresh GRF solutions were prepared daily.

Cows were milked daily in their stalls at 0400 and 1600 h. Cows were fed a complete mixed diet ad libitum (50% roughage:50% concentration on a DM basis) at 0400 and 1200 h daily and each cow received 2.3 kg long alfalfa hay daily at 1200 h. In addition, all cows received 1.8 to 5.4 kg of a grain-protein supplement (top dressed).

Blood samples were collected at 20-min intervals from 0800 to 2000 h on d 1, 10 and 19. Methods for blood collection and processing of serum were as described in Chapter 3 (Part 1). In addition, blood samples were collected at 2-h intervals commencing at 0800 h and processed for plasma (using sodium fluoride as the anticoagulant).

All serum samples were assayed for insulin (Villa-Godoy et al., 1987) and prolactin (Koprowski and Tucker, 1971). Characteristics of prolactin and insulin in serum (included

overall, baseline, pulse frequency and pulse amplitude) within each sampling day were determined using a pulse analysis program (PULSAR: Merriam and Wachter, 1982). "G" values (based on peak selection criteria) used were: G (1) = 99999, G (2) = 2.89, G (3) 1.84, G (4) = 1.27, G (5) = .89.

Serum samples collected at 2-h intervals commencing at 0900 h were assayed for cortisol (Purchas et al., 1985), T_3 (Refsal et al., 1984) and T_4 (Gerloff et al., 1986). In addition, serum samples collected at 20-min intervals between 1400 and 2000 h on d 19 were assayed for cortisol. These samples were used to determine characteristics of cortisol in serum, as described for insulin and prolactin.

Serum samples collected at 1100, 1500 and 1900 h were assayed for NEFA (NEFA C kit, Wako Chemicals USA, Inc., 12300 Ford Road, Suite 130, Dallas, TX 75234, as modified by McCutcheon and Bauman, 1986b). Plasma samples were assayed for glucose [glucose (Trinder) procedure no. 315, Sigma Diagnostics, P.O. Box 14508, St. Louis, MO 63178].

All data were subjected to analyses of variance (Gill, 1978). Characteristics of serum cortisol for the 6-h period on d 19 were analyzed as a randomized complete block design. All other data were analyzed as a randomized complete block design with repeated measurement. Data for 1 and 3 mg GRF treatments were each compared with placebo treatment (control) grouped across and within sampling day, and data for d 10 and 19 were each compared with d 1 (control)

grouped across and within treatments using Dunnett's t test (Gill, 1978, 1986).

In addition, NEFA data were analyzed further (with another repeated measurement in time) for effect of sampling time within day. Because differences were detected, NEFA values at 1500 and 1900 h were compared with values at 1100 h (control) within GRF treatment grouped across sampling days using Dunnett's t test (Gill, 1978).

C. Results

Grouped across days, GRF did not affect overall, baseline or pulse frequency means of prolactin relative to controls. However, 1 mg GRF increased ($P<.05$) prolactin pulse amplitude means above controls, and this effect was primarily on d 10 and 19 (Table 14). On d 19, baseline prolactin means were reduced ($P<.05$) in 3 mg GRF-infused cows relative to controls. Grouped across GRF treatments, overall prolactin means were greater ($P<.05$) on d 10 and 19, baseline prolactin means were greater ($P<.01$) and prolactin pulse frequency means were less ($P<.05$) on d 19 than similar measures on d 1. In control cows, overall and baseline prolactin means increased ($P<.01$) and prolactin pulse frequency means decreased ($P<.05$) on d 19 relative to d 1. In 1 mg GRF-infused cows, overall prolactin and prolactin pulse amplitude means were greater ($P<.05$) on d 19 than d 1.

Table 14. Characteristics of mean serum prolactin on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Day of experiment	Dose of GRF (mg/24 h)	Overall (ng/ml)	Baseline (ng/ml)	Number of pulses/12 h	Amplitude of pulses (ng/ml)
1	Placebo	44.2	32.5	3.0	54.5
	1	45.6	29.1	2.8	64.7
	3	31.5	23.4	3.2	41.7
	Mean	40.5	28.3	3.0	53.6
10	Placebo	45.2	32.4	2.0	43.1
	1	57.4	39.6	2.6	93.3[a]
	3	42.0	24.0	3.2	60.1
	Mean	48.2[b]	32.0	2.6	65.5
19	Placebo	65.3[b]	57.2[b]	1.4[b]	48.7
	1	61.1[b]	39.6	2.0	106.7[ab]
	3	47.8[b]	30.6[a]	2.8	67.9
	Mean	58.1[b]	42.5[b]	2.1[b]	74.4
SED 1		5.1	6.8	.6	17.3
SED 2		9.6	10.0	.8	19.5

[a]P<.05: Differ from placebo within day of experiment.

[b]P<.05: Differ from corresponding value for d 1 of experiment.

SED 1 = Standard error of difference between two days of experiment within any treatment.

SED 2 = Standard error of difference between two treatments within any day of experiment.

In 3 mg GRF-infused cows, overall prolactin means were greater (P<.01) on d 19 than on d 1.

Serum cortisol was not affected by either GRF or day and averaged 6.9 ng/ml (data not shown). Similarly, characteristics of serum cortisol during 6 h on d 19 were not affected (P>.20) by GRF (Table 15).

Grouped across days, T_3 tended to be greater (P=.10) in 3 mg GRF-infused cows relative to controls, and this effect was primarily on d 10 and 19 (Table 16). Grouped across GRF treatments, T_3 was less (P<.05) on d 10 than on d 1 and this effect was primarily in controls. Serum T_4 was not affected by either GRF or day and averaged 54.7 ng/ml (data not shown).

Grouped across days, characteristics of serum insulin were not affected by GRF. On d 19, overall, baseline and pulse frequency means of insulin were greater (P<.05) in 3 mg GRF-infused cows than in controls (Table 17). Grouped across and within GRF treatments, overall and baseline insulin means were greater (P<.05) on d 19 than on d 1.

Grouped across and within days, NEFA increased (P<.05) in 3 mg GRF-infused cows relative to controls (Table 18). Grouped across GRF treatments, NEFA were increased on d 10 relative to d 1 and this effect was primarily in 3 mg GRF-infused cows. Grouped across days and GRF treatments, NEFA were greater (P<.01) at 1100 h than at 1500 and 1900h (Table 19). Grouped across days, within control treatment there was no effect of sampling time on NEFA, but within 1 mg GRF

Table 15. Characteristics of serum cortisol on d 19 of bovine growth hormone-releasing factor (GRF) infusions.

Characteristic of cortisol	Dose of GRF (mg/24 h)				
	Placebo	1	3	Mean	SED[1]
Overall (ng/ml)	4.4	4.8	4.3	4.5	.9
Baseline (ng/ml)	2.5	3.5	2.5	2.8	1.1
Number of pulses/6 h	1.6	1.2	1.6	1.5	.5
Amplitude of pulses (ng/ml)	7.8	7.6	10.1	8.5	1.4

[1]SED = Standard error of difference between treatment means.

Table 16. Concentrations (ng/ml) of serum triiodothyronine on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Day of experiment[1]	Dose of GRF (mg/24 h)[2]			Mean
	Placebo	1	3	
1	1.54	1.45	1.51	1.50
10	1.12[b]	1.22	1.58[a]	1.31[b]
19	1.44	1.51	1.85[ab]	1.60
Mean	1.37	1.39	1.65	

[a]P<.05: Differ from placebo within a row.

[b]P<.05: Differ from d 1 of experiment within a column.

[1]Standard error of difference between two days of experiment within any treatment = .13.

[2]Standard error of difference between two treatments within any day of experiment = .16.

Table 17. Characteristics of mean serum insulin on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusons.

Day of experiment	Dose of GRF (mg/24 h)	Overall (ng/ml)	Baseline (ng/ml)	Number of pulses/12 h	Amplitude of pulses (ng/ml)
1	Placebo	.46	.46	.2	.09
	1	.59	.53	.4	.81
	3	.60	.56	.4	.29
	Mean	.55	.52	.3	.40
10	Placebo	.59	.55	.6	.39
	1	.66	.61	.4	.43
	3	.69	.67	.4	.32
	Mean	.65	.61	.5	.38
19	Placebo	.67[b]	.67[b]	0	0
	1	.80[b]	.76[b]	.4	.39
	3	.99[ab]	.89[ab]	1.2[a]	.67
	Mean	.82[b]	.77[b]	.5	.35
SED 1		.08	.07	.4	.44
SED 2		.10	.09	.4	.42

[a]P<.05: Differ from placebo within day of experiment.

[b]P<.05: Differ from corresponding value for d 1 of experiment.

SED 1 = Standard error of difference between two days of experiment within any treatment.

SED 2 = Standard error of difference between two treatments within any day of experiment.

Table 18. Concentrations (μEq/l) of serum non-esterified fatty acids on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Day of experiment[1]	Dose of GRF (mg/24 h)[2]			Mean
	Placebo	1	3	
1	196	221	294[a]	237
10	225	273	380[ab]	293[b]
19	186	231	277[a]	231
Mean	202	242	317[a]	

[a]P<.05: Differ from placebo within a row.

[b]P<.01: Differ from d 1 of experiment within a column.

[1]Standard error of difference between two days of experiment within any treatment = 30.

[2]Standard error of difference between two treatments within any day of experiment = 35.

Table 19. Concentrations (μEq/l) of serum non-esterified fatty acids at 1100, 1500 and 1900 h grouped for d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Sampling[1] time (h)	Dose of GRF (mg/24 h)			Mean
	Placebo	1	3	
1100	208	263	381	284
1500	202	245	291[a]	246[a]
1900	197	217[a]	278[a]	231[a]

[a]P<.05: Differ from 1100 h sampling time within a column.

[1]Standard error of difference between two sampling times within any treatment = 20.

treatment NEFA were greater (P<.05) at 1100 h than at 1900 h and within 3 mg GRF treatment NEFA were greater (P<.01) at 1100 h than at 1500 and 1900 h. Elevated NEFA at 1100 h were apparent by d 1 (data not shown).

Grouped across days, glucose was greater (P=.06) in 3 mg GRF-infused cows than in controls (Table 20). On d 19, glucose increased (P<.05) in 1 and 3 mg GRF-infused cows relative to controls. Grouped across GRF treatments, glucose was greater (P<.01) on d 10 and 19 than on d 1. Glucose was elevated (P<.05) on d 10 in controls, on d 19 in 1 mg GRF-infused cows and on d 10 and 19 in 3 mg GRF-infused cows relative to respective values on d 1.

D. Discussion

In general, I saw no effect of GRF on prolactin concentrations in the present study which agrees with Moseley et al. (1984) and Chapter 1. Similarly, in most short-term trials with cattle, GH treatment does not affect serum prolactin (Peel et al., 1982a, 1983; Eisemann et al., 1986a). The few significant effects of GRF treatments on baseline and pulse amplitude of prolactin are inconsistent and thus difficult to interpret.

GRF treatment did not affect serum cortisol in my study. To my knowledge, no other studies have tested the effect of GRF on serum cortisol. However, GH treatment of

Table 20. Concentrations (mg/dl) of plasma glucose on d 1, 10 and 19 of bovine growth hormone-releasing factor (GRF) infusions.

Day of experiment[1]	Dose of GRF (mg/24 h)[2]			Mean
	Placebo	1	3	
1	59.0	61.0	61.8	60.6
10	62.1[b]	61.9	65.3[b]	63.1[b]
19	60.9	66.1[ab]	65.2[ab]	64.1[b]
Mean	60.7	63.0	64.1[a]	

[a]$P \leq .06$: Differ from placebo within a row.

[b]$P < .05$: Differ from d 1 of experiment within a column.

[1]Standard error of difference between two days of experiment within any treatment = 1.3.

[2]Standard error of difference between two treatments within any day of experiment = 1.9.

cattle does not affect blood cortisol concentrations (Peel et al., 1982a, 1983; Peters, 1986).

Concentrations of serum T_3 and T_4 in my primiparous cows tended to be higher than concentrations reported by others (Peel et al., 1982a; 1983; Bitman et al., 1984; Gerloff et al., 1986). Cows in these other studies were older than my cows and concentrations of T_3 and T_4 in blood decline with age (Walsh et al., 1980; Refsal et al., 1984). Decreased serum T_3 on d 10 was unexpected and can not be explained by differences in ambient temperature, a variable known to affect blood T_3 concentrations (Magdub et al., 1982). The increase in serum T_3 in 3 mg GRF-infused cows on d 10 and 19 was also unexpected. It is unlikely that GRF per se caused this increase because: (1) I would have expected to observe a concomitant increase in serum T_4, and (2) GRF is without effect on thyroid stimulating hormone release in calves (Hodate et al., 1985). In addition, GH administration to dairy cows does not affect serum T_3 (Peel et al., 1982a, 1983; Bitman et al., 1984). The lack of effect of GRF on serum concentrations of T_4 was expected because GH administration to dairy cows does not affect serum T_4 (Peel et al., 1982a, 1983; Bitman et al., 1984).

Increased serum insulin during the period of infusion in my study is consistent with reports that concentrations of serum insulin increase with advancing lactation in dairy cows (Koprowski and Tucker, 1973; Vasilatos and Wangsness, 1981). In my study increased overall insulin over time was

due primarily to increased baseline and not pulsatile release. In contrast, Vasilatos and Wangsness (1981) reported that pulsatile insulin release was increased with advancing lactation. In addition, pulse frequency was considerably greater in cows of Vasilatos and Wangsness (1981) than in my cows. This was possibly due to increased frequency of sampling (every 10 min) or use of a different pulse program in the study of Vasilatos and Wangnness (1981).

Grouped across days, GRF did not affect serum insulin in my study. This agrees with most GH studies in dairy cows (Peel et al., 1982a, 1983; Eppard et al., 1985b; Pocius and Herbein, 1986). In contrast, GRF treatment of lactating ewes for 4 d increased serum insulin (Hart et al., 1985c). In the present study, by d 19 serum insulin in 3 mg GRF-infused cows increased. Similarly, in longer-term GH studies, insulin was increased in beef heifers (Eisemann et al., 1986a) and steers (Peters, 1986). Therefore, it appears that GRF or GH treatment of cattle for greater than 10 d is necessary before increases in serum insulin are observed.

Increased serum NEFA due to GRF treatment agrees with Hart et al. (1985c) who observed increased blood NEFA in lactating ewes treated with either GRF or GH. In general, GH treatment of cows does not affect serum NEFA when cows are in positive energy balance (Peel et al., 1982a, 1983;

Eppard et al., 1985b; McCutcheon and Bauman, 1986b), but GH increases serum NEFA when cows are in negative energy balance (Peel et al., 1983; Eppard et al., 1985b). Thus, my results are consistent with findings in GH trials because my GRF-infused cows were in negative energy balance [Chapter 3 (Part 1)]. Increased serum NEFA in GH-treated cows in negative energy balance are generally associated with increased milk fat percentage and increased proportions of long chain fatty acids in milk fat (Bitman et al., 1984). However, milk fat percentage was not affected by GRF treatment in my study [Chapter 3 (Part 1)] in spite of elevated serum NEFA.

My finding that 3 mg GRF increased serum NEFA within 21 to 33 h of initial administration indicates that endogenous serum GH (as increased by GRF) may be lipolytic as suggested by Hart et al. (1984a, 1984b). In agreement, Pocius and Herbein (1986) found that blood NEFA tended to be increased within 4 h of injection of GH to dairy cows. In contrast to Hart's suggestion, Bauman and coworkers (Bauman and McCutcheon, 1986; Peel and Bauman, 1987) suggest that the acute lipolytic effect of exogenous GH is due to impurities in the GH preparation. However, in my study increased serum GH was of pituitary origin and was apparently lipolytic.

Increased serum NEFA before feeding in my cows is similar to that observed for meal-fed cows (Barnes et al., 1985). Thus, my cows had increased mobilization of adipose tissue before feeding even though cows were fed ad libitum

twice daily. The pre-feeding increase in serum NEFA was most apparent in GRF-infused cows which agrees with Hart et al. (1985c).

The GRF-induced increase in blood glucose in my study is in agreement with Hart et al. (1985c) who reported increased glucose after GRF or GH administration for 4 d to lactating ewes. In contrast, GH treatment of dairy cows for 10 d did not affect blood concentrations of glucose (Peel et al., 1982a, 1983; Pocius and Herbein, 1986). It has been suggested (Hart et al., 1984b) that GH is diabetogenic, and increased blood glucose and insulin on d 19 lends support to this theory.

Mammary gland uptake of glucose is responsible for greater than 60% of glucose turnover during lactation in dairy cows and most (69-98%) of this glucose is used for lactose synthesis (Bickerstaffe et al., 1974). In my study, yield of lactose in milk was markedly increased by GRF [Chapter 3 (Part 1)]. Thus, GRF treatment may stimulate glucose flux toward lactose synthesis in the mammary gland, while preventing a decline in blood concentrations of glucose.

It should be noted that the effects of GRF per se on hormones and metabolites are unavoidably confounded with concurrent changes in milk production, energy intake, energy balance and body weight. Thus, elucidation of direct cause-effect relationships are not possible using the experimental

approach chosen. Nonetheless, results of the present study and those of Chapter 3 (Part 1) indicate that exogenous GRF influences lactation of dairy cows in a manner similar to that observed in studies testing exogenous GH (Peel and Bauman, 1987). This suggests that increased serum GH mediates the galactopoietic effect of GRF. If this is true, then endogenous GH appears to be as effective as exogenous GH (Bauman and McCutcheon, 1986) in the homeorhetic control of metabolism during lactation.

SUMMARY AND CONCLUSIONS

Studies presented in this dissertation examined effects of intravenously administered growth hormone (GH)-releasing factor (GRF) on lactational performance, serum GH and other hormones and metabolites in blood of dairy cattle.

A dose-response relationship between GRF and serum GH was established in bull calves after single injections of 0, 2.5, 10 or 40 μg GRF/100 kg body weight (BW). Based on mean serum GH of all calves, no obvious diurnal pattern of response to sequential (every 6 h for 48 h) GRF injections was observed, and there was no evidence of reduced GH response following the eight consecutive GRF injections. However, variation (up to 18-fold) in GH response was observed between times of injection within individual calves. Continuous infusion of GRF to calves for 6 h increased and maintained mean and pulsatile GH release, but pulses were asynchronous among calves.

Injections of GRF (10, 20 or 40 μg/100 kg BW) to lactating cows at 4-h intervals for 24 h increased peak serum GH 10-fold relative to controls, but no dose-response relationship was observed. GRF consistently increased GH, but there was considerable variation in response within and among cows. GRF injections (20 μg/100 kg BW) at 4-h

intervals for 10 d increased yields of milk, fat, protein and lactose approximately 11% during d 8 to 10. GRF did not affect milk composition or feed intake. GRF increased BW and feed:milk conversion efficiency of cows. GRF-induced increments in milk yield were apparent within 2 d of initial GRF treatment. Increased milk yields were maintained for the remainder of the 10 d GRF treatment period. Two d after final GRF injection milk yields of GRF-treated cows declined to that of control cows. Mean serum GH response to GRF was similar between d 1 and 10.

Infusion (pulses at 3.75 min-intervals) of 3.125, 6.25, 12.5, 25.0 or 50.0 mg GRF/24 h for 24 h to lactating cows increased mean serum GH 5-fold relative to controls, but no dose-response relationship was observed. GRF increased the pulsatile nature of GH release. Infusion of 1 and 3 mg GRF/24 h for 20 d increased milk yield 11 and 23%, respectively, during d 6 to 20. Three mg GRF-induced increments in milk yield were apparent within 4 d of initial GRF treatment. Milk yields continued to increase throughout the 20 d GRF treatment period. However, milk yields of GRF-treated cows declined to that of control cows by 8 d after cessation of infusions. One and 3 mg GRF treatments increased yields of milk fat (13 and 20%), protein (15 and 26%) and lactose (8 and 17%), respectively. GRF had only small effects on milk composition, energy intake and BW. GRF increased observed energy efficiency of milk production

but had no effect on energy efficiency when corrected for BW changes. Averaged across d 1, 10 and 19, GRF increased mean serum GH in a dose-dependent manner. GRF increased pulsatile GH release. Serum GH response to GRF was as great on d 19 as on d 1 of infusion. With the exception of increased blood glucose concentrations on d 19, 1 mg GRF had no effect on prolactin, insulin, cortisol, triiodothyronine (T_3), thyroxine (T_4) and non-esterified fatty acids (NEFA). In contrast, 3 mg GRF increased T_3 (d 10 and 19), insulin (d 19), NEFA (d 1, 10 and 19) and glucose (d 19), but did not affect prolactin, cortisol or T_4.

Results of this research demonstrate that GRF is a potent stimulator of GH release in cattle and there is no evidence of pituitary refractoriness to GRF over time. I hypothesize that variation in GH response to GRF within and among animals and pulsatile secretion of GH in the face of GRF infusion, are related to the degree of synchrony among exogenous GRF and endogenous GRF and somatostatin. Thus, multiple injections or continuous infusions of GRF provide a better indication of GH responsiveness in an animal than a single injection.

GRF may have several advantages as a galactopoietic agent relative to GH. For example, because of its smaller size, GRF should be less expensive to produce and less quantity of hormone (and thus a smaller delivery device) would be required to increase milk production efficiency. In addition, the advent of smaller and more potent analogs

of GRF will amplify these advantages. Another possible advantage of GRF is that it likely causes release of all forms of endogenous GH from the anterior pituitary and, although not yet tested, this may be physiologically important. A third advantage of GRF is that there is evidence in dairy cows (Chapter 3, Part 1) and sheep (Hart et al., 1985c) that increased milk production is maintained for an extended period of time (e.g., 10 d in dairy cows) after cessation of GRF treatment. Unfortunately, GRF has one distinct disadvantage for use in dairy cattle. That is, GRF is biologically active when injected into humans and will require strict control and licensing for use in dairy cows. However, it is possible that analogs may be synthesized that are specific for the bovine.

I believe that several areas of research on GRF need further investigation. Obviously, effects of GRF for longer periods on milk yield and composition, feed intake, BW, health and reproductive variables, and blood concentrations of GH, other hormones and metabolites need to be examined using larger numbers of animals. Of particular interest in future experiments will be the carryover effects of GRF on milk production and serum concentrations of GH. It is possible that GRF may affect the anterior pituitary gland so that blood concentrations of GH are elevated (with coincident increases in milk yield) for extended periods after cessation of GRF treatment. In addition to testing

the effect of GRF alone, future studies could be designed to determine additive effects of GRF with, for example, simultaneous TRH administration, three-times-a-day milking or long-day photoperiods.

In conclusion, GRF is galactopoietic in dairy cows and results of my studies indicate that GRF influences lactation in a manner similar to exogenous GH. Although the possibility exists that GRF per se may have galactopoietic properties, I speculate that milk yield responses of lactating cows to GRF are mediated by increased secretion of GH. Furthermore, GRF is a potential alternative galactopoietic agent to GH which can enhance efficiency of milk production.

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