Regulation of growth hormone secretion in ruminants

Cathie Anne Plouzek

Iowa State University

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Regulation of growth hormone secretion in ruminants

Plouzek, Cathie Anne, Ph.D.

Iowa State University, 1988
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Regulation of growth hormone secretion in ruminants

by

Cathie Anne Plouzek

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

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For the Major Department
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1988
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GENERAL INTRODUCTION

Pituitary extracts were shown as early as 1931 to be capable of stimulating growth (1). The slow development of adequate technologies for protein purification and amino acid sequencing techniques, however, delayed the identification of the amino acid sequence of ovine and bovine growth hormone (GH) until 1972 (2-3). However, in the mid-1960s, radioimmunoassays were developed to evaluate endocrine concentrations (4-5), which started a rapid acceleration in endocrinology research.

Using these developments, several studies were conducted using purified GH from pituitary glands to stimulate growth in cattle and sheep (6-10). The anabolic potentials of these GH administrations were variable, and were probably due to contaminants in the preparations and the limited quantities of GH actually available. Although the purification techniques today have eliminated many of the previous problems incurred with earlier GH preparations, pituitaries still have to be obtained from the species which will eventually receive the GH preparation.

The development of recombinant deoxyribonucleic acid (DNA) techniques has allowed for the rapid production of large quantities of proteins that have biological activities similar to the naturally produced proteins, including GH (11-16). Because GH produced by recombinant DNA techniques is now available for research, and possibly for administration to livestock in the near future, the ways in which GH has to be effectively administered to animals to enhance growth has
become increasingly important. One concern with the administration of GH has been its susceptibility to enzymatic degradation if administered as a slow release implant. Furthermore, native GH, or that produced by recombinant DNA techniques, has the possibility of inducing antigenic effects with long-term administration. An approach to avoid these side effects of GH during long-term administration has been to introduce GH genes into the genome in order to increase the GH concentration in the plasma of growing animals (17-18). GH and growth were stimulated in some transgenic mice in this way, but this was very expensive and tedious. In addition, the technique worked with very low efficiency, only a small fraction of treated animals responding, and in many, deleterious mutations were produced.

An alternative method of providing effective growth stimulation in animals may be the use of GH-releasing factor (GHRF). GHRF is a small peptide of 44 amino acids, which releases GH from the pituitary. Being a smaller protein than GH, GHRF would have less chance of stimulating an antigenic response in animals. GHRF has the additional benefit of requiring lower doses than GH in order to cause an increase in plasma GH concentrations. Because of the recent isolation and sequencing of GHRF (19-20), very little is known about GHRF as a potential anabolic agent in domestic animals. Since its isolation, GHRF has been shown to increase growth rates in humans and rats (21-22), but only two studies have been conducted in ruminants. In sheep, GHRF administration did not alter growth rate (23). In calves, GHRF administered as a continuous infusion for 20 days increased nitrogen retention between days 9-13.
Based on these contradictory results in ruminants, the relationships between GHRF, GH and growth in ruminants need to be defined more accurately under different physiological conditions in order that the application of GHRF to enhance growth in farm animals may be effective.

The purpose of these works was to increase our understanding of GH secretion in growing ruminants. In a series of experiments, the influences of sex, castration, and aging on GH profiles, GH secretion and clearance rates, GH half-life, and GH response to GHRF were examined as well as the corresponding concentrations of somatomedin, thyroid hormones, insulin, sex steroids, and body composition. In addition, the GH responses to GHRF and somatostatin administration were studied in cattle. GHRF was tested in sheep and cattle for its ability to stimulate a GH response and an anabolic response. The overall objective of these studies was to provide additional information about endogenous GH secretion so that it could be manipulated by GHRF to increase weight gain in ruminants.
REVIEW OF THE LITERATURE

Background

From the earliest times, the existence of a substance controlling growth in animals and humans has been postulated. The Greek physician and scholar Galen (129-199 AD) put forth the view that blood ebbed to and fro in the arteries carrying "vital spirit" to various organs of the body. "Animal spirit" was believed to be formed from "vital spirit" in the brain, with the wasted products of this reaction flowing down the base of the brain and pituitary stalk to the pituitary. The pituitary or "phlegmatic glandule" conducted the waste products by ducts to the nasopharynx where they appeared as nasal mucus or pituita. In the 17th century, Conrad Victor Schneider and Richard Lower argued that anatomical fluids could not pass from the brain to the nose and that the foramina in the bone were used for olfactory nerve transmission. Thus, the fluids passed down the pituitary stalk to the gland which "distilled" them back into the blood (25).

The central role of the pituitary in growth was established when it was shown that removal of the pituitary gland, or hypophysectomy, resulted in dwarfism (26-29). Furthermore, when a growth factor isolated from the pituitary was administered to hypophysectomized rats which had limited growth, growth was restored (26-30). This growth factor, or growth hormone (GH), was believed to act directly on tissues to stimulate growth. However, a GH-dependent factor was found to be present in serum that stimulated in vitro incorporation of sulfate into
cartilage (31). Based on these results, it was postulated that GH stimulated skeletal growth indirectly through generation of a sulfation factor. Plasma extracts containing the sulfation factor were subsequently shown to have a variety of effects on cartilage and to have insulin-like activity in other tissues. Some of these insulin-like properties included the inhibition of lipolysis and the promotion of glucose oxidation in adipose tissue, enhancement of protein synthesis and glucose uptake in muscle, and stimulation of sulfate uptake, amino acid transport and synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), protein and chondroitin sulfate in cartilage (32-33). Due to the diverse actions of sulfation factor on many tissues of the body, and because it appeared to be an intermediate factor in the action of GH on growth, it was later called somatomedin (Sm-C).

Stimuli responsible for regulating the secretion of GH were suggested in 1947. Green and Harris postulated that humoral substances, later termed releasing factors, were liberated from nerve tracts of the hypothalamus. These releasing factors were carried into the primary plexus of capillaries of the hypophysial-portal vessels where they were transported to the adenohypophysis. Within the adenohypophysis, they regulated hormonal release by stimulation or inhibition (34). The first evidence for a GH-releasing-inhibiting hormone, called somatostatin (SRIH), was amassed in 1968 (35) and the peptide was sequenced in 1973 (36). With this discovery of negative GH regulation, a dual controlling system for hypothalamic GH secretion was suggested (35). However, the
positive regulator, GH-releasing factor (GHRF), was not isolated and sequenced until 1982 (19-20).

Initially, GH levels in the plasma were considered to be relatively constant. Later, it was demonstrated that the secretion of GH was pulsatile (37-38). SRIH and GHRF are secreted into the hypophysial-portal circulation (39) and, acting as dual regulators, influenced the pattern of GH secretion (40-45). In rats, the release of GHRF and SRIH generated an ultradian rhythm of GH secretion (45), which varied between the sexes. The male rat had a secretory pattern of high pulses at 3- to 4-hr intervals returning to low concentrations, whereas females had a high baseline of GH with smaller pulses (46). Due to the different growth rates of the sexes, the GH pattern of secretion was thought to influence the growth rate (46). Examining this postulate, Jansson et al. (46-48) demonstrated that administration of GH in the male-type pattern to hypophysectomized rats stimulated growth to a greater extent than the female-type pattern of GH administration.

In ruminants, GH is secreted with peaks occurring at random. The episodic secretion of GH has found to be different between breeds having different growth rates, between various ages of ruminants, between sexes, and between intact and castrated ruminants (37-38, 49-51). The importance of the pattern of GH secretion in ruminants has not been established. Moseley et al. (52) administered GH in various patterns to cattle, but differences in growth rates were not observed. However, it has been found that intact males, which have higher weight gains of less adipose tissue than castrates or females, have more GH in the plasma.
with a higher baseline and higher amplitude of the secretory periods than castrated males (50). Bulls also have a greater secretion rate of GH without a greater metabolic clearance rate (i.e., the rate at which the GH protein is metabolized or broken down in the body), whereas heifers have a greater metabolic clearance rate but no increase in the secretion rate of GH, resulting in lower levels of plasma GH (53). Therefore, it was proposed that GH secretion rates affect plasma GH concentrations to a greater extent than the metabolic clearance rates (54).

GH concentrations in the plasma were influenced by the nutritional status of cattle. When cattle were fed at high levels, GH peaks of secretion were of low amplitude and short duration, whereas, at medium and low planes of nutrition, GH pulses were high with multiple peaks and slow decay (55). These differences in GH secretion may have been caused by a longer half-life of GH and a lower metabolic clearance rate which have been observed in fasting animals (56).

GH concentrations in the plasma have been shown to decline with age in animals (57-59). In cattle, the GH secretion rate per unit of body weight and the pituitary weight per unit of body weight diminished with age (60). The general decrease in GH concentrations as the animals were increasing in body weight resulted in low correlations between GH levels and growth rate (59).

**GHRF and SRIH Regulation of GH**

The hypothalamic releasing factors, GHRF and SRIH, as mentioned previously, act as positive and negative regulators, respectively, of GH
secretion from the hypothalamus. GHRF administration has been shown to induce a release of plasma GH in ruminants (23, 61-64). GH was released in response to intravenous administration of GHRF in a dose-dependent manner which peaked within 5 to 15 min after injection (23, 61-64). It did this by increasing the rate of transcription of GH messenger RNA in somatotrophs (65-66). This messenger RNA was more stable when triiodothyronine was present. When glucocorticoids were also present, GH messenger RNA accumulation by transcriptional and post-transcriptional means increased (67-68).

GHRF has not always been found effective in producing a GH response, probably due to competition with other physiological events, such as elevated levels of SRIH or negative feedback from endogenous levels of GHRF, GH, or Sm-C (22). On the other hand, the response to GHRF may be potentiated by administering the peptide during an endogenous GH secretory period (45, 69).

Although SRIH inhibited GH secretion from the pituitary, SRIH did not inhibit GH gene transcription and GH synthesis (22). Administration of SRIH in all species tested resulted in decreased concentrations of GH in the plasma (70-72). After SRIH administration was halted, GH levels rebounded above normal baseline values which created a peak of GH secretion (73-76). It has also been demonstrated that the magnitude of the GH peak following SRIH administration can be enhanced by the administration of GHRF during the SRIH infusion (76-78).
Endocrine Relationships with GH

Apart from the effects of GHRF and SRIH, several other hormones influence GH during the growth process. In addition to being dependent on GH, the release of Sm-C by the liver seems to require adequate levels of insulin (32, 79). Similarly, the ability of Sm-C to stimulate growth at the tissue level may be dependent upon thyroid hormones (79-80). Thyroid hormones stimulate GH synthesis and secretion (68, 81). In ruminants, GH concentrations have been stimulated by estrogen administration (49, 82). Estrogens also stimulated weight gain. Although estrogens stimulated growth in ruminants, in humans, estrogens depressed Sm-C concentrations (83). Elevated concentrations of Sm-C have been associated with increased growth. GH is required for androgens to stimulate musculature and skeletal growth (80). Androgens may act as estrogens stimulating these growth processes, because testosterone can be aromatized to estradiol in the peripheral tissues (84). In other words, the ability of androgens to enhance GH secretion may be estrogen-mediated (84-86). Androgens inhibited binding of steroids from the adrenal cortex to their receptors in muscle permitting muscle growth (87). In addition, large doses of some adrenal cortex steroids have inhibited GH secretion (80). Elevated levels of adrenal cortex steroids inhibited the effects of insulin on muscle protein synthesis (88).

Insulin action is antagonized by GH in adipose tissue. This results in GH acting to divert nutrients away from lipid synthesis in adipose to other body tissues (89-90). Insulin has been shown to
increase amino acid uptake, protein synthesis, and may be involved
directly in muscle growth (80). GH itself has been shown to be
necessary for tissue growth. Receptors for GH in adipose tissue were
shown to allow GH to affect directly tissue in several ways by
stimulating lipolysis, fatty acid turnover rates in farm animals (80),
and preadipocyte conversion to adipocytes, a process which requires
extensive reorganization of gene expression (91). In muscle tissue, GH
increased protein synthesis and the muscle mass by affecting the rate of
DNA synthesis and uptake of amino acid (80, 92). In vitro, GH
stimulated differentiation of multinucleated muscle cells from cultured
myoblasts, suggesting that it also stimulates cell differentiation in
muscle tissue (91). Additional support for the affects of GH on muscle
growth comes from the positive correlation between GH and total muscle
mass reported in steers (59, 92). GH enhanced bone growth via
somatomedin stimulation (80). It also stimulated the differentiation of
prechondrocytes to chondrocytes in the growth plate which undergo clonal
expansion, a process which is dependent upon Sm-C (91). However,
experiments to show a correlation between baseline GH or GH-stimulated
Sm-C levels and growth rates have not always resulted in significant
relationships (93-94).

Exogenous Endocrine Anabolic Agents

To understand the endocrine regulation of growth, experiments have
been conducted administering different hormones in growing animals.
Because many of these hormones resulted in significant increases in
growth rates in hypophysectomized and intact animals, it resulted in
commercial application of hormones as anabolic agents. Administration of GH to ruminants has been shown to increase nitrogen retention and, in some experiments, to enhance weight gains (6-10, 52, 82, 95-96). Animals receiving GH excreted less nitrogen in the urine, resulting in enhanced nitrogen retention. This demonstrated that GH has a postabsorptive metabolic effect on nitrogen (52, 96).

The sex steroids have been shown to increase GH secretion and enhance weight gain and feed conversion in ruminants (49, 95, 97). The estrogens acted by decreasing urinary nitrogen excretion to enhance nitrogen balance (98). The baseline and spike amplitude of plasma GH were higher with estrogen administration in ruminants (49). Pituitary weight increased with estrogen treatment on an absolute weight basis and in relation to body weight (98). The adrenal glands of ruminants may have produced androgens in response to estrogens to enhance growth (98). Exogenous androgens, however, have not been widely used to increase growth of ruminants (99-101), however. Nevertheless, there was an additive effect of estrogen and androgens on growth (99, 101).

Although Sm-C has been considered to be responsible for increased growth, few experiments administering Sm-C to animals have been conducted (102-106). When Sm-C was administered to rats as a continuous infusion, increases in epiphyseal cartilage width and in the DNA-synthesizing activity of rib cartilage were found to be similar to those caused by GH infusion. When a Sm-C, produced in bacteria from recombinant DNA procedures (15, 105), was administered to hypophysectomized rats in a variety of regimens and doses for 6 or 8
days, body weight gain was increased when a high dose was administered continuously (105). Longitudinal bone growth was also increased with subcutaneous injections of Sm-C. However, GH was much more effective in stimulating weight gain than Sm-C even at 50-fold lower doses (105).

As mentioned before, GHRF has been shown to stimulate GH secretion in ruminants. GHRF administered to cattle for 5 days increased GH without altering the GH secretory patterns, and the GH response did not diminish over this period (107). When GHRF was administered to female rats as pulses which reproduced the male's secretion pattern of GH, growth increased. However, when GHRF was administered as a continuous infusion which reproduced the female's secretory pattern of GH secretion, GHRF was ineffective as a growth stimulus (21). In bull calves administered GHRF as a continuous infusion for 20 days, basal GHRF was increased and there was a rise in the number and amplitude of GH pulses. On days 9 to 14 of the study, GHRF increased urinary nitrogen, increasing nitrogen balance without affecting nitrogen intake, fecal nitrogen, or nitrogen digestibility (24). This type of anabolic response, stimulated by administration of GHRF, is similar to that observed with GH. In sheep, GHRF administration did not stimulate growth rate, feed intake or feed efficiency (23). In a novel attempt to enhance endogenous GHRF secretion, the human GHRF gene was incorporated into the mouse genome. Transgenic mice that expressed the GHRF gene increased their somatic growth considerably (18).

Antibodies to SRIH have also been used to enhance growth. In one study, immunization of sheep with SRIH antibodies resulted in enhanced
GH secretion without affecting growth rate (108). In another, growth was enhanced 15%, but GH levels were unaltered (109-110). These sheep had larger carcasses, but no changes in the proportion of muscle, fat and bone were observed in the carcasses.
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100. Van der Wal P. General aspects of the effectiveness of anabolic agents in increasing protein production in farm animals, in particular bull calves. Environ Qual Saf V:60-78, 1976.


SECTION I. EFFECT OF PULSATILE INTRAVENOUS INJECTIONS OF GROWTH HORMONE-RELEASING FACTOR ON NITROGEN METABOLISM IN SHEEP
Effect of Pulsatile Intravenous Injections of Growth Hormone-Releasing Factor on Nitrogen Metabolism in Sheep$^{1,2,3}$

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$^3$Department of Animal Science.
ABSTRACT

The effect of growth hormone-releasing factor (GHRF) on nitrogen retention and weight gain was measured in eight crossbred wethers, each of which was given six intravenous (iv) injections of GHRF per day. Wethers averaging 42 kg were used in a crossover design for the experiment. Nitrogen balance was evaluated over 5-day periods during which injections of a buffer solution with or without 0.067 ug human pancreatic (hp) (1-40) GHRF/kg body weight was administered at 4-hr intervals. Sheep administered GHRF differed from controls with a 7% decrease in urinary nitrogen (P<0.03). Wethers treated with GHRF did not differ from controls in weight gain, nitrogen retention, apparent nitrogen digestibility, dry-matter intake, or apparent biological value. Blood samples were taken before and after one of the six daily injections. Of the 40 GHRF pulse injections sampled, the animals responded 36 times with increased plasma growth hormone (GH) concentrations. The pattern of GH response was a spike 5-10 min after injection, which returned to baseline within 20 min. Plasma urea nitrogen, somatomedin-C, insulin, triiodothyronine, thyroxine, and cortisol concentrations did not differ between treatments. These results suggest that pulsatile injections of GHRF over long experimental periods may induce an anabolic response in sheep.
INTRODUCTION

Availability of growth hormone-releasing factor (GHRF) offers an alternative method to study growth hormone (GH) physiology because exogenous GHRF can be used to change the endogenous GH secretion pattern. Clark and Robinson (1) mimicked GH secretory patterns of male rats in female rats by pulse injections of GHRF every 3 hr for 12 days. The superimposed male pattern promoted an increase of 30% in body weight gain over control female rats. Administration of monoclonal antibodies against rat hypothalamic GHRF to male rats abolished GH surges and decreased growth rate (2-3). Simultaneous administration of GHRF antibodies and antisomatostatin serum to male rats did not alter the inhibitory effect of GHRF antibodies on GH release, suggesting that hypothalamic GHRF release was required for pulsatile GH secretion (2). Although cattle and sheep secrete GH episodically, the pattern is not as well defined as in rats (4-9). The objectives of the present experiments were to determine if pulsatile injection of GHRF could consistently release GH over time and to determine if GHRF injections affected nitrogen metabolism in growing sheep.
MATERIALS AND METHODS

Experiments were conducted to establish the dose of GHRF required to release endogenous GH in sheep within the physiological range (Exp. 1), to determine if sheep respond to multiple injections of GHRF with a persistent increase in GH (Exp. 2), and to evaluate the anabolic response to repeated injections of GHRF in growing sheep (Exp. 3).

For all experiments, crossbred wethers were adapted to metabolism crates and housed in an environmentally controlled room at 18°C with 12-hr photoperiods. The sheep were fed the diet described in Table 1 at 12 hr intervals at a level to allow for orls. Animals were weighed, and an indwelling catheter (vinyl, 1.02 mm i.d., Becton-Dickson) was inserted in a jugular vein, 1 day before experimentation. The catheters were filled with sterile saline containing 100 U heparin/ml between injections and sterile saline with 20 U heparin/ml when blood samples were being taken. Animals received injections of human pancreatic (hp) (1-40)-OH GHRF (10). The GHRF was dissolved in 0.1% acetic acid (1 ug/ul) and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818% NaCl) containing 0.01 M NaH$_2$PO$_4$·H$_2$O, pH 7.0; 0.01% ascorbic acid and 0.13 ml sheep plasma/ml. Solutions for injection were prepared on alternate days and stored at 4°C until used.

Blood samples were treated with heparin (40 U/10 ml) and stored at -15°C until analyzed. All plasma samples were assayed for GH (11). The GH assay had intra- and inter-assay coefficients of variation of 3% and 17%, respectively. Insulin concentrations were measured in
Exp. 2 and 3 (12). Intra- and inter-assay coefficients of variation of the insulin assays were 2% and 3%, respectively. Plasma samples from individual animals for each day in Exp. 3 were pooled and assayed for somatomedin-C (Sm-C), triiodothyronine (Diagnostics Product Corp., Los Angeles, CA), thyroxine (Diagnostics Product Corp., Los Angeles, CA), cortisol (Diagnostics Product Corp., Los Angeles, CA), and plasma urea nitrogen (Micro-urea, Technicon N-10a, Autoanalyzer). All radioimmunoassay kits were tested for parallelism by using ovine plasma at a minimum of three volumes. All the samples were assayed within one assay for each hormone. The intraassay coefficients of variation ranged from 1.4% to 1.8%.

Sm-C concentrations were determined by using a double antibody disequilibrium radioimmunoassay procedure purchased from Immuno Nuclear Corp. (Stillwater, MN). The procedure was modified by using acidification to remove the binding protein as previously described for ovine plasma (13), rather than using the octadecasilyl (ODS)-silica extraction column included in the kit. Plasma samples were acidified by adding an equal volume of pH 3.2, 0.1 M glycine-HCl and incubating at 37°C for 24 hr. After incubation, samples were neutralized with 0.1 M NaOH. Purified Sm-C isolated from human serum served as standard. Samples of extracted plasma were incubated with rabbit anti-serum against the synthetic 53-70 amino acid fragment of Sm-C for 2 hr at 4°C, followed by a second incubation with ¹²⁵I 53-70 fragment for 20 hr at 4°C. Normal rabbit serum, pre-precipitated with goat anti-rabbit serum and polyethylene glycol diluted in a bovine serum-albumin borate buffer
with merthiolate, was then added in a single step, and after 2 hr at 4°C, the tubes were centrifuged, and the supernatant was decanted.

Table 1. Composition of diet (dry-matter basis)

<table>
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<tr>
<th>Component</th>
<th>Percent</th>
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<tr>
<td>Corn, cracked</td>
<td>44.7</td>
</tr>
<tr>
<td>Cobs, ground</td>
<td>35.2</td>
</tr>
<tr>
<td>Molasses, cane</td>
<td>11.3</td>
</tr>
<tr>
<td>Soybean meal, solvent-extracted</td>
<td>7.2</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.5</td>
</tr>
<tr>
<td>Salt, iodized</td>
<td>0.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.3</td>
</tr>
<tr>
<td>Trace mineral mixture</td>
<td>0.1</td>
</tr>
<tr>
<td>Sulfur, elemental</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin A (5.2 million IU/kg)</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>150 IU/kg</td>
</tr>
</tbody>
</table>

The results were expressed as nMol/liter, which can be converted to ng/ml by multiplying by 7.7. The six standards provided in the kit ranged from 2.19 to 70.0 nMol/liter. Two quality-control plasma samples were included in each assay with mean concentrations of 15.2 +/- 1.7 and 22.6 +/- 5.1 nMol/liter.

To verify removal of the binding protein from Sm-C, dilution curves using normal ovine plasma, acid-treated ovine and bovine plasma, and ODS-silica column-extracted bovine plasma were tested. Normal ovine
plasma assayed at 12.5, 25 and 50 ul was parallel with the standard curve and had a mean concentration of 6.05 +/- 0.79 nMol SmC/liter. Acidified ovine plasma assayed at 12.5, 25 and 50 ul was parallel to the standard and had a mean concentration of 8.22 +/- 0.17 nMol/liter. Acidified bovine plasma samples from a heifer and bull, assayed at 25, 50 and 100 ul, were parallel with the standard curve and had concentrations of 20.5 +/- 4.0 and 36.0 +/- 4.5 nMol/liter, respectively. The bovine samples extracted with the ODS column were also parallel with the standard curve and had concentrations of 18.4 +/- 0.80 nMol/liter for the heifer and 48.0 +/- 8.5 nMol/liter for the bull. Addition of 8.75 nMol/liter of the standard to ovine plasma resulted in a recovery of 102%. The intra- and inter-assay coefficients of variation were 16% and 15%, respectively.

In Exp. 1, four wethers (43 kg) were randomly assigned to a Latin square design with dose of GHRF as treatment. GHRF was administered at 0, 0.05, 0.01, and 0.15 ug hpGHRF/kg body weight (bwt). Blood was collected 20, 10 and 1 min before and 5, 10, 15, 20, 40, 60, 80, and 100 min after GHRF injection. In Exp. 2, four wethers (28 kg) were injected with 0.1 ug hpGHRF/kg bwt every 4 hr for 24 hr. Blood was sampled 20, 10 and 1 min before and 5, 10, 15, 20, 40, and 60 min after each GHRF injection. In Exp. 3, eight wethers (42 kg) were randomly assigned to a crossover design, with the animals receiving the buffer solution with or without 0.067 ug hpGHRF/kg bwt for 5 days. Animals were rested for 5 days, and then treatments were reversed. Animals were injected every 4 hr during the treatment periods. The first injection of GHRF was 12 hr
before the initiation of the collection period. Blood was sampled daily 20, 10 and 1 min before and 5, 10, 15, 20, 40, and 60 min after the afternoon injection 5 hr after feeding.

Urine, feces, orts, and feed were collected for 5 days in Exp. 3. Samples of feed, orts, feces, and urine were collected daily, composited and stored at -15°C until analysis. Feed, orts and feces were chopped and then ground frozen in liquid nitrogen by using a heavy-duty blender (Waring, Model 32BL75, Waring Commercial, New Hartford, CT). Urine samples were thawed, mixed, and an aliquot taken for chemical analysis. Nitrogen was determined by the Kjeldahl procedure using a Kjeltec System II (Tecator, Hoganas, Sweden) (14). Dry-matter contents of feed, ort and feces were determined by drying at 60°C (14).

Analysis of variance was conducted using the Statistical Analysis System (15) for all experiments. Data from Exp. 1 were analyzed as a Latin square comparing GHRF treatments. The experimental model in Exp. 3 was a crossover design tested with animals nested within treatment. Growth hormone data were averaged into preGHRF (20, 10, and 1 min before GHRF injection), GHRF (5, 10, 15, and 20 min postinjection) and postGHRF (40, 60, 80, and 100 min after injection in Exp. 1 and 40 and 60 min after injection in Exp. 2 and 3) preceding final statistical analysis.
RESULTS

Mean plasma GH responses to 0, 0.05, 0.1, and 0.15 ug hpGHRF/kg bwt are listed in Table 2. The pattern of response to GHRF in all experiments was a rapid rise in plasma GH peaking within 5 to 15 min postinjection, then declining to concentrations similar to preinjection within 40 min. The preGHRF and postGHRF GH concentrations were not different between treatments. During the GHRF period, only 50% of the sheep responded to the 0.05-ug dose, whereas all sheep responded to the 0.1-ug and 0.15-ug doses. The 0.1-ug dose increased mean plasma GH from 1.2 to 46.8 ng/ml as compared with no increase in the animals injected with buffer alone (P<0.1). The 0.15-ug GHRF dose elevated GH to 31.9 ng/ml (P<0.13), which was not different from the response to 0.1 ug and suggesting that the peak of the dose titration curve was near 0.1 ug hpGHRF/kg bwt.

In the second experiment, all sheep responded to each injection of 0.1 ug hpGHRF/kg bwt given every 4 hr for 24 hr. The mean response is shown in Fig. 1. Insulin concentrations for the sheep ranged from 0.05 to 1.02 ng/ml, with a mean concentration of 0.44 ng/ml and a SE of 0.09. In these animals, 0.1 ug of GHRF/kg caused an increase in GH, which was considered to be above the normal physiological range for sheep. A dose of GHRF between 0.1 and 0.05 ug/kg bwt would consistently obtain a GH response in all sheep of less than 40 ng/ml. The dose of 0.067 ug GHRF/kg bwt, which had previously been used for studies in cattle (16-17), was selected for the anabolic studies.
Table 2. Effects of increasing growth hormone-releasing factor on plasma growth hormone in sheep (Exp. 1)

<table>
<thead>
<tr>
<th>Dose ug GHRF/kg body weight</th>
<th>Period</th>
<th>PreGHRF&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GHRF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PostGHRF&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0.8</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>0.05</td>
<td></td>
<td>1.2</td>
<td>0.6</td>
<td>4.6</td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td>1.2</td>
<td>0.8</td>
<td>46.8</td>
</tr>
<tr>
<td>0.15</td>
<td></td>
<td>1.4</td>
<td>0.6</td>
<td>31.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of 20, 10 and 1 min before GHRF injection.

<sup>b</sup>Mean of 5, 10, 15 and 20 min after GHRF injection.

<sup>c</sup>Mean of 40, 60, 80 and 100 min after GHRF injection.

Pulse injections of 0.067 ug GHRF/kg every 4 hr caused increases in plasma GH to concentrations ranging between 5 and 40 ng/ml. Plasma GH concentrations were elevated by GHRF in 36 of 40 (90%) of the injection periods sampled. Average GH responses are given in Table 3. There were no differences in preinjection GH concentrations between control and treated sheep. Mean response to GHRF at the 1500 hr injection was 16.4 ng/ml compared with 2.3 ng/ml in control animals (P<0.01).
Fig. 1. Mean concentrations of plasma GH in response to intravenous injections of 0.1 ug (1-40)-OH GHRF/kg bwt in sheep every 4 hr (n=4). The SE for the samples at 10 min before and after injection of GHRF are indicated by the bars. The times of feeding and GHRF administration are indicated by the arrows.
Table 3. Effect of growth hormone-releasing factor on plasma hormone concentrations (Exp. 3)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GHRF</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH, (preGHRF) (ng/ml)(^a)</td>
<td>2.7</td>
<td>2.3</td>
<td>0.4</td>
</tr>
<tr>
<td>GH, (GHRF) (ng/ml)(^b)</td>
<td>2.3</td>
<td>16.4</td>
<td>3.9*</td>
</tr>
<tr>
<td>Somatomedin-C (nMol/liter)</td>
<td>14.0</td>
<td>15.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>2.9</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Triiodothyronine (ng/dl)</td>
<td>74.2</td>
<td>72.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Thyroxine (ug/dl)</td>
<td>18.1</td>
<td>18.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Cortisol (ug/dl)</td>
<td>1.7</td>
<td>1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^a\)Mean of 20, 10 and 1 min before injection.

\(^b\)Mean of 5, 10, 15, and 20 min after injection.

\(^*P<0.01.\)

Urinary nitrogen decreased 2.9 g during the 5-day period (\(P<0.03\)) with injection of GHRF (Table 4). Fecal nitrogen (14 g, GHRF versus 13 g, control), intake nitrogen (86 g, GHRF versus 83 g, control), and dry-matter intake (5634 g, GHRF versus 5461 g, control) did not change with treatment. Even though nitrogen retention and weight gain increased 17% and 29%, respectively, in wethers given GHRF, these increases were not significantly different. Apparent digestibility of nitrogen and dry matter were not changed by GHRF. Apparent biological value of dietary protein and net protein utilization both increased 19%
with GHRF (P<0.2). Plasma urea nitrogen was 8% lower in animals given GHRF but was not statistically different from control values.

Table 4. Effect of intravenous pulse injections of growth hormone-releasing factor on nitrogen metabolism, weight gain and feed intake in sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GHRF</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary nitrogen, g/5 d</td>
<td>42.1</td>
<td>39.2</td>
<td>0.7*</td>
</tr>
<tr>
<td>Nitrogen retention, g/5 d</td>
<td>27.5</td>
<td>32.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Plasma urea nitrogen, mg/dl</td>
<td>8.7</td>
<td>8.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Weight gain, g/5 d</td>
<td>482</td>
<td>624</td>
<td>310</td>
</tr>
<tr>
<td>Dry-matter intake, g/5 d</td>
<td>5461</td>
<td>5634</td>
<td>195</td>
</tr>
<tr>
<td>Apparent dry-matter digestibility, %</td>
<td>72.6</td>
<td>76.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Apparent nitrogen digestibility, %</td>
<td>84.0</td>
<td>83.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Apparent biological value, %</td>
<td>37.0</td>
<td>44.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Net protein utilization, %</td>
<td>30.8</td>
<td>36.7</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*P<0.03.

The concentrations of other metabolic hormones are listed in Table 3. Although Sm-C concentrations increased 12% over the 5-day period with GHRF, the elevation was not significant. Insulin, triiodothyronine, thyroxine, and cortisol were not affected by GHRF treatment.
DISCUSSION

Growth hormone-releasing factor induced a plasma GH response in all our experimental animals. In the first experiment, GH was released in a dose-dependent manner. A similar dose-dependent relationship between GHRF administration and plasma GH response was obtained previously with sheep and cattle (16-20). In all experiments, plasma GH peaked within 5 to 15 min after injection of GHRF. The peak was followed by a return of GH to preinjection concentrations within 15 to 45 min.

The ability of our experimental animals to respond to multiple injections of GHRF, by releasing GH into the plasma, was demonstrated in Exp. 2 and 3. In the second experiment, in which GHRF was administered every 4 hr for 24 hr, all animals responded to every GHRF injection. In an extended study, in which GHRF was administered every 4 hr over a 5-day period (Exp. 3), the sheep maintained their overall ability to release GH in response to exogenous GHRF. Every sheep, however, did not always respond to each GHRF administration with elevated plasma GH. Of the measured GH responses, the sheep responded to 85% of the injections. The capability to respond to exogenous GHRF with repeated administration over time has also been observed in ruminants by Hart et al. (20), Moseley et al. (21-22), and Della-Fera et al. (23). Our results are in agreement with the findings of Hart et al. (20), who showed consistent plasma GH responses over 4 days of injecting GHRF every 2
hr in sheep. In contrast to these results, Della-Fera et al. (23) reported that, after the initial injection of GHRF to sheep, the amount of GH released decreased with subsequent GHRF administration. They suspected that the declining GH response was due to a refractoriness occurring at the pituitary level. Some of the injections that were administered were not always effective in stimulating a GH response, but this has been observed in similar experiments with cattle (19, 24), pigs (25), rats, rabbits, and humans (26-27). In an attempt to explain the variation in effectiveness of GHRF to release GH, Jansson et al. (26) suggested that the inconsistency was due to physiologically competing events, such as feedback, from endogenous GHRF, GH, and Sm-C, or elevated levels of somatostatin, which is a noncompetitive inhibitor of GHRF. It has been reported that, when GHRF is administered during an exogenous pulse of GH, a higher plasma GH peak is observed than when GHRF is administered between GH peaks (28-29). Furthermore, Shibasaki et al. (30) demonstrated that, when the interval between GHRF pulses was 2 hr, the second injection was ineffective. Nevertheless, this study's results, overall, showed that GHRF induced a significantly elevated GH response in sheep when administered at a dose of 0.067 ug/kg bwt every 4 hr.

In the present study, it was found that GHRF administered intermittently over a 5-day period (Exp. 3) altered nitrogen utilization in sheep. Decreased urinary nitrogen excretion (P<0.03) occurred with GHRF treatment without affecting intake or fecal nitrogen. This resulted in 17% greater nitrogen retention as compared with control
animals. Similar results were obtained by Moseley et al. (22) with constant infusion of GHRF in calves for 20 days. They noted that, during days 9 to 14 of treatment, urinary nitrogen decreased 14% ($P < 0.02$), whereas intake and fecal nitrogen were not affected by treatment. The 18% greater nitrogen retention in the GHRF treated calves, however, was significant ($P < 0.04$). The plasma urea nitrogen of our sheep declined 8% with GHRF treatment. This is similar to the observations of Hart et al. (20), who administered GHRF to ewes every 2 hr for 4 days and observed a 3.5% reduction in plasma urea nitrogen.

Elevated levels of plasma Sm-C have been associated with enhanced growth rates in ruminants (31), suggesting a positive relationship between growth and Sm-C. Although the 12% increase in Sm-C in GHRF-treated sheep was not significant, the rise in Sm-C along with a significant decline in urinary nitrogen and enhanced nitrogen retention, biological value, and weight gain, suggested that growth was occurring at an accelerated rate with GHRF treatment.

Growth responses, such as enhanced nitrogen retention or increased tibia length, have been known to depend on frequency of GH administration (32-33). The most effective pattern of GH that promotes growth of rats seems to be an intermittent plasma GH pulse with low intervening GH levels (26, 34-35). Similarly, in experiments with GHRF, Clark and Robinson (1) found that pulse injections administered every 3 hr for 12 days, accelerated body growth in female rats and male rats with GHRF deficiency, whereas continuous infusion had no effect on growth. The superimposed male GH pattern created by exogenous GHRF in
female rats promoted an increase of 30\% in body weight gain over control rats. In addition, pituitary GH content was increased by pulsatile but not continuous infusion of GHRF. Frohman and Jansson (27) postulated that intermittent exposure to a small dose of GHRF caused a more favorable GH synthesis-release ratio than constant GHRF exposure, resulting in larger readily releasable pools of GH in pituitaries. In our experiment, we found that GHRF administered as a pulse every 4 hr for 5 days decreased the urinary nitrogen losses from the body, suggesting that GHRF was stimulating growth responses. Multiple injections of GHRF did not alter the baseline of plasma GH in either Exp. 2 or 3, which is consistent with a GH pattern known to stimulate rapid growth in rats (34-35) and ruminants (84-9).

The present study demonstrated that intermittent intravenous injections of GHRF were able to release GH in sheep within the normal range of their plasma GH over extended periods. Although only conservation of urinary nitrogen was significant, all the other measurements suggested that there was an anabolic response to GHRF in sheep. These experiments suggested that pulsatile administration of GHRF could be used to increase growth rates in domestic sheep.
LITERATURE CITED


SECTION II. GROWTH HORMONE-RELEASING FACTOR AND SOMATOSTATIN ON GROWTH HORMONE SECRETION IN PREPUBERTAL CALVES
Growth hormone-releasing factor and somatostatin on growth hormone secretion in prepubertal calves$^{1,2,3}$

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$^{1}$Presented in part at the 75th Annual Meeting of the American Society of Animal Science, Pullman, WA (Abstract 173) and the 19th Meeting of the Midwestern Section of the American Society of Animal Science, Des Moines, IA (Abstract 106).

$^{2}$Journal Paper No. J-12789 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Projects 2444, 2528, 2644, and 2273, the last a contributing project to the North Central Regional Research Project NC-113). These studies were supported in part by the U.S. Department of Agriculture, ARS, CSRS Special Grant 59-2191-1-2-033-0.

$^{3}$Acknowledgments: We thank Douglas L. Hard and Jose R. Molina for their expertise, and Joan Rettig, Mahlon E. Shell, Craig R. Bohnker, Rod Berryman, and Carl Johnson for technical assistance.
The effects of intravenous (iv) administration of growth hormone-releasing factor (GHRF) and somatostatin (SRIH) on growth hormone (GH) secretion and GHRF on nitrogen metabolism were measured in prepubertal calves. Crossbred beef heifers (111 kg) were used in a Latin square design to test the effects of 0, 0.01, 0.033, 0.067, and 0.1 ug (1-40)-OH human pancreatic (hp) GHRF/kg body weight (bwt) on plasma GH concentrations. When they were given doses of 0.067 and 0.1 ug hpGHRF/kg bwt, plasma GH increased (P<0.05) within 5-15 min, compared with injections of control buffer, and then returned to preinjection levels. The response to 0.067 ug (1-40)-OH hpGHRF/kg bwt injected every 3 hr for 42 hr was studied in five heifers (137 kg bwt). The animals responded to 20 of 40 hpGHRF injections during the sampling periods. They only responded to one of the two GHRF injections with an increase in plasma GH during every 6-hr period measured. In two heifers (183 kg bwt) given 0.067 ug (1-40)-OH hp GHRF/kg bwt during infusions of 0.033 and 0.067 ug SRIH(SS)-14/(kg bwt*min) for 70 min, SRIH suppressed the GH response to the GHRF. Nitrogen retention and weight gain were measured in five bull calves (90 kg bwt) administered 0 or 0.067 ug [Nle^{27}] (1-29)-NH_{2} rat hypothalamic (rh) GHRF/kg bwt every 4 hr for 10 days. Although increases of 16% in nitrogen retention and 36% in weight gain with pulsatile GHRF treatment were variable and statistically similar to those of controls, these and other metabolic parameters were interpreted to indicate an anabolic response to GHRF. These results indicate that
GHRF induces peak GH secretion within 15 min in prepubertal calves, that the secretion of GH is suppressed by iv infusion of SRIH, and that GHRF may be anabolic in prepubertal calves under different regimens of peptide administration.
INTRODUCTION

Hypothalamic regulation of growth hormone (GH) secretion is mediated by a stimulatory factor, growth hormone-releasing factor (GHRF), and an inhibitory factor, growth hormone-releasing-inhibiting hormone, somatostatin (SRIH). Administration of GHRF results in a pulse release of GH into the plasma, creating a spike similar to that of endogenous GH secretion. It has been suggested that this episodic pattern of GH secretion is important in the regulation of growth (1, 2). There are also age-related changes in GH secretion in cattle, in which the secretion of plasma GH declines as the animals age (3). To understand the role of GH regulation in controlling growth, investigations are needed to determine the response of young ruminant animals to exogenous releasing and inhibiting GH peptides.

The objective of this study was to determine the role of GHRF on the secretion of GH in prepubertal calves by studying the effects of GHRF on GH secretion, the changes in plasma GH with multiple injections of GHRF, the interaction of exogenous GH-release-inhibiting hormone, somatostatin (SRIH), and GHRF on GH secretion, and the effect of pulsatile GHRF administration on nitrogen metabolism in calves.
MATERIALS AND METHODS

Animals

Crossbred beef heifer calves individually penned with straw bedding in an environmentally controlled room at 18°C with 12-hr photoperiods were used in Exp. 1, 2 and 3. In Exp. 4, five Holstein bull calves were maintained under similar conditions, although they were adjusted to metabolism crates for 3 wks before the experiment. Calves were fed the diets described in Table 1 at 12-hr intervals unless otherwise indicated. An indwelling catheter (Tygon microbore tubing, 1.27 mm i.d., Fisher Scientific, Pittsburgh, PA) was inserted in a jugular vein at least 1 day before experimentation. The catheters were filled with sterile saline containing 100 U heparin/ml between experiments and with sterile saline with 40 U heparin/ml when blood samples were being taken. Blood samples were treated with heparin (4 U/ml), and plasma was stored at -15°C until analyzed. All samples were analyzed for GH (4). The GH assay had intra- and inter-assay coefficients of variation of 1.7% and 9.0%, respectively.
Table 1. Composition of diets (dry-matter basis)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1,2,3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, cracked</td>
<td>49.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Alfalfa, dehydrated</td>
<td>39.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Soybean meal, solvent</td>
<td>5.6</td>
<td>9.5</td>
</tr>
<tr>
<td>extracted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molasses, cane</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Salt, iodized</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Trace mineral</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin A (5.2 million IU/kg)</td>
<td>0.1</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Peptides

Animals received bolus intravenous (iv) injections of human pancreatic (hp) (1-40)-OH GHRF in Exp. 1-3 (5) and [Nle$^{27}$] (1-29)-NH$_2$ rat hypothalamic (rh) GHRF in Exp. 4 (6). Somatostatin (SS)-14 (SRIH) was administered iv by continuous-infusion pumps (Harvard, Model 1201, Harvard Apparatus, Millis, MA). The GHRF and SRIH were dissolved in 0.1% acetic acid (1 ug/ul) and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818% NaCl) containing 0.01 M NaH$_2$PO$_4$·H$_2$O, pH 7.0, 0.01% ascorbic acid, and 1% bovine serum albumin. Solutions for injection and infusion were prepared the day of experimentation.
Dose Titration (Exp. 1)

Five 4-mo-old heifers (111 kg bwt) were randomly assigned to a Latin square design to test the plasma GH response to 0, 0.01, 0.033, 0.067, and 0.1 ug GHRF/kg bwt. Blood samples were collected 20, 10 and 1 min before injection and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after injection. Animals were fed the evening before the experiment and were allowed several hours to eat before the feed was removed.

Multiple Injections (Exp. 2)

Five 5-mo-old heifers (137 kg bwt) were randomly assigned to a crossover design testing 0 or 0.067 ug hpGHRF/kg bwt administered at 3-hr intervals for 42 hr. The treatments were reversed, and the experiment repeated after 1 wk. In order to monitor acute GH changes in response to GHRF after multiple injections, blood was sampled during four periods of 5 or 6 hr during the 42 hr to monitor plasma GH concentrations. During the 8-hr intervals that were not monitored for plasma GH, the calves received GHRF at 3-hr intervals. Two injections of GHRF were administered during each of the four sampling periods. Within each sampling period, the animals were fed between the GHRF injections. Blood was collected at 20-min intervals except immediately after a GHRF injection, when they were taken at 5-min intervals for 20 min.

SRIH and GHRF (Exp. 3)

Two heifers (183 kg bwt) at 6 mo of age were infused with 0.033 or 0.067 ug SRIH/(kg bwt*min) for 70 min in a crossover design. After 30
min of SRIH infusion, 0.067 ug GHRF/kg bwt was administered iv as a bolus injection. Plasma was sampled 30, 15 and 1 min before SRIH infusion; 15, 29, 40, 45, 50, and 70 min during SRIH infusion and GHRF injection; and 90, 110, 130, and 150 min after SRIH infusion. The animals were fed in the morning, and the experiments conducted in the afternoon.

Nitrogen Balance (Exp. 4)

Five Holstein bull calves at 3.5 mo of age (90 kg bwt) were assigned randomly to a crossover design to test the effects of administration of GHRF or control buffer every 4 hr for two consecutive 5-day periods on nitrogen metabolism. They received 0 or 0.067 ug [Nle²⁷] (1-29)-NH₂ rhGHRF/kg bwt via a jugular catheter at 4-hr intervals. After the 10-day period, the animals were rested for 7 days; then treatments were reversed. Blood was sampled once daily for 80 min surrounding the afternoon GHRF injection, as well as 20, 10 and 1 min before injection and 5, 10, 20, 40, and 60 min after injection.

In addition to GH, all plasma samples from Exp. 4 were analyzed for insulin (7), plasma urea nitrogen (Micro-urea, Technicon-N-10a, Autoanalyzer), and plasma glucose (Worthington Flozyme Glucose, Worthington, Diagnostic Systems, Inc., Freehold, NJ). Intra- and inter-assay coefficients of variation for the insulin assays were 1.1% and 1.3%, respectively. Coefficients of variation for plasma urea nitrogen were 5.8%, intraassay, and 7.3%, interassay. Plasma glucose intraassay coefficient of variation was 3.1%, whereas interassay coefficient of variation was 7.1%. The plasma samples taken before GHRF
injection were pooled for each day for analysis of somatomedin-C (Immuno Nuclear Corp., Stillwater, MN, as modified by Plouzek and Trenkle (8), triiodothyronine (Amerlex T-3 RIA kit, Amersham Corp., Arlington Heights, IL), thyroxine (Amerlex T-4 RIA kit, Amersham Corp., Arlington Heights, IL), and cortisol (Cortisol Double Antibody, Diagnostics Product Corp., Los Angeles, CA). The pooled samples were analyzed in one assay. The resulting intraassay coefficients of variation were 1.9% for somatomedin-C, 1.5% for thyroxine, 1.8% for triiodothyronine, and 1.4% for cortisol.

Samples of feed, feces, urine, and orts were collected daily, composited over 5 days and stored at -15°C until analysis. Feed, feces and orts were chopped and then ground frozen in liquid nitrogen by using a heavy duty blender (Waring, Model 32BL75, Waring Commercial, New Hartford, CT). Urine samples were thawed and mixed, and an aliquot was removed for chemical analysis. Nitrogen was determined by the Kjeldahl procedure (9) using a Kjeltec System II (Tecator, Hoganas, Sweden). Dry-matter contents of feed, orts and feces were determined by drying at 60°C (9).

Statistical Analysis

GH concentrations before GHRF injection (20, 10 and 1 min) were averaged, as well as 5, 10, 15, and 20 min after GHRF injection and 40, 60, 80, 100, and 120 min after GHRF injection for statistical analysis in Exp. 1. The means were analyzed as a Latin square design with dose, period and animal as main effects (10-11). The GH data in Exp. 2 were
analyzed as a crossover design with treatment (0 or 0.067 ug GHRF/kg bwt) and animal as main effects ($10^{11}$).

The experimental units in this study were the individual calves. Daily concentrations of somatomedin-C, triiodothyronine, thyroxine, cortisol, plasma glucose, and plasma urea nitrogen in Exp. 4 were averaged before statistical analysis. The model used to test the crossover design in Exp. 4 was animal nested within treatment as the error term to test treatment and period effects.
RESULTS

Dose Titration

Plasma GH increased in a dose-dependent manner, with a peak occurring between 5-15 min after injection of hpGHRF, and then it declined rapidly to basal levels within 60 min (Fig. 1). One animal did not respond to any dosage of GHRF. Overall, the animals responded to 66% of the GHRF injections. Before injection of GHRF, GH averaged 5.5 +/- 1.2 ng/ml (+/- SE). During the first 20 min after injection of GHRF, GH averaged 7 +/- 1.9, 19 +/- 11, 20 +/- 8, 43 +/- 17, and 54 +/- 17 ng/ml for 0, 0.01, 0.033, 0.067, and 0.1 ug GHRF/kg bwt, respectively. GH levels during the 20-min period after injection of 0.067 (P<0.05) and 0.1 ug GHRF/kg bwt (P<0.025) were greater than those of controls. Mean GH for all treatments at 40-120 min after GHRF injection was 6 +/- 1.9 ng/ml.

Multiple Injections

The pattern of GH release in response to injections of 0.067 ug hpGHRF/kg bwt every 3 hr for 42 hr, as shown in Fig. 2, was similar to that observed in Exp. 1. Of the 20 responses, 12 occurred after the first GHRF injection, and 8 responses were observed after the second. Each animal responded to only one of the two injections in a 5-hr sampling period. Three animals responded to the first injection of GHRF, but they did not respond to GHRF after feeding. The injection to which a given animal responded remained consistent.
Fig. 1. Mean concentration of plasma GH in crossbred beef calves in response to intravenous injections of 0, 0.01, 0.033, 0.067, and 0.1 ug (1-40)-OH hpGHRF/kg bwt (n=5). The dose of GHRF is indicated above the arrow at the time of administration. Representative SE for the samples at 10 min before and after injection of GHRF are indicated by the bars.
Fig. 2. The average plasma GH concentrations of calves receiving 0 or 0.067 ug (1-40)-OH hpGHRF/kg bw at 3-hr intervals for 42 hr. The thick line illustrates the response of animals receiving GHRF (n=5), and the thin line illustrates the plasma response of animals receiving buffer without GHRF (n=5). Standard errors, indicated by the bars, are shown for samples 40 min before GHRF injection and 10 min after injection. Times of feeding and GHRF administration are indicated by the arrows.
throughout the experiment with two exceptions: 1) One animal responded to both injections of GHRF in one period, and 2) one animal did not respond to GHRF during one sampling period. The average GH response within the four sampling periods to the first GHRF injection was 37 +/- 20 ng/ml and 22 +/- 16 ng/ml to the second GHRF injection. The response to GHRF during sampling periods was the same. The overall GH mean was 3 +/- 2.0 ng/ml for control animals.

**SRIH and GHRF**

After a preliminary study to determine effective doses of SRIH infusion to suppress GH secretion in calves, 0.033 and 0.067 ug SRIH/(kg bwt*min) were infused for 70 min and 0.067 ug hpGHRF/kg bwt was injected iv as a bolus 30 min after these infusions began (Fig. 3). GH averaged 9 +/- 1.2 and 13 +/- 2.0 ng/ml before infusion of 0.033 and 0.067 ug SRIH, respectively. During SRIH infusion, basal GH declined to 8 +/- 0.3 and 9 +/- 0.7 ng/ml for the 0.033 and 0.067 ug SRIH doses. When GHRF was injected during the 0.033 ug SRIH infusion, GH increased to 16 +/- 5.4 ng/ml; concomitantly, the GH response to GHRF during infusion of 0.067 ug SRIH was 14 +/- 1.6 ng/ml. When the SRIH infusions were stopped, GH was released as a spike, with mean concentrations of 15 and 12 ng/ml for the 0.033 and 0.067 ug SRIH doses, respectively (Fig. 3).
Fig. 3. Mean concentrations of plasma GH in calves being infused with 0.033 or 0.067 ug somatostatin (SS)-14 (SRIH)/(kg bwt*min) and injected with 0.067 ug (1-40)-OH hpGHRF/kg bwt (n=2). Arrows illustrate the time of GHRF injection, and the hatched area indicates the period of SRIH infusion.
Nitrogen Balance

Mean concentrations of plasma hormones in animals injected with buffer or GHRF for 10 days are given in Table 2. Since the 5-day periods did not differ from each other, the 10-day means are presented. The mean GH response in animals given GHRF was 25 ng/ml (P<0.05), compared with 7 ng/ml in control calves. The calves responded to 46 (77%) of the 60 GHRF injections in which sequential blood samples were obtained. The amplitude of the GH response to GHRF was similar to the beginning and end of the 10-day period. GH averaged 6.5 and 8.7 ng/ml before injections of GHRF and buffer. Although concentrations of somatomedin-C were 23% greater in calves given GHRF, these differences were not statistically significant. Insulin, triiodothyronine, thyroxine, and cortisol were not altered with administration of GHRF.

Although nitrogen retention increased 16% in calves given GHRF, it was not different (P>0.05) from that of controls. Urinary, fecal and intake nitrogen did not significantly change with GHRF treatment (Table 3). However, nitrogen intake increased 12% and urinary nitrogen increased 4%, which, by calculation, caused a 3% decrease in the percentage of intake nitrogen excreted in the urine with GHRF treatment. Apparent nitrogen digestibility and biological value were not affected by GHRF treatment. Plasma urea nitrogen, which declined 6% with GHRF treatment, and glucose were not significantly different between treatments. Although body weight gain increased 36% with GHRF administration, the increase was not significant. Dry-matter intake also increased 14%, but it was not statistically different from control
values. Fecal dry matter and dry-matter digestibility were not altered by GHRF treatment.

Table 2. Effect of multiple injections of GHRF on plasma hormone means over 10 days (Exp. 4)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GHRF</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH, ng/ml\textsuperscript{a}</td>
<td>8.7</td>
<td>6.5</td>
<td>1.5</td>
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<tr>
<td>GH, ng/ml\textsuperscript{b}</td>
<td>7.0</td>
<td>25.1\textsuperscript{c}</td>
<td>8.0</td>
</tr>
<tr>
<td>GH, ng/ml\textsuperscript{d}</td>
<td>7.8</td>
<td>9.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Somatomedin-C, nMol/liter</td>
<td>6.6</td>
<td>8.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.51</td>
<td>0.54</td>
<td>0.12</td>
</tr>
<tr>
<td>Triiodothyronine, ng/dl</td>
<td>1.34</td>
<td>1.31</td>
<td>0.22</td>
</tr>
<tr>
<td>Thyroxine, ug/dl</td>
<td>7.4</td>
<td>7.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Cortisol, ug/dl</td>
<td>1.8</td>
<td>2.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Mean of 20, 10 and 1 min before GHRF injection.

\textsuperscript{b}Mean of 5, 10, 15, and 20 min after GHRF injection.

\textsuperscript{c}Mean significantly different (P<0.05) from corresponding control mean.

\textsuperscript{d}Mean of 40 and 60 min after GHRF injection.
Table 3. Effect of multiple injections of GHRF on nitrogen metabolism, weight gain and feed intake (Exp. 4)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GHRF</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen intake, g/10 day</td>
<td>274</td>
<td>306</td>
<td>53</td>
</tr>
<tr>
<td>Urinary nitrogen, g/10 day</td>
<td>93.8</td>
<td>97.5</td>
<td>10.5</td>
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<tr>
<td>Apparent nitrogen digestibility, %</td>
<td>90.0</td>
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<tr>
<td>Nitrogen retention, g/10 day</td>
<td>156</td>
<td>181</td>
<td>41</td>
</tr>
<tr>
<td>Apparent biological value, %</td>
<td>60.4</td>
<td>63.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Intake nitrogen excreted in urine, %</td>
<td>35.9</td>
<td>33.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Plasma urea nitrogen, mg/dl</td>
<td>6.9</td>
<td>6.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>90.3</td>
<td>94.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Weight gain, kg/10 day</td>
<td>1.95</td>
<td>2.66</td>
<td>0.79</td>
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<tr>
<td>Dry-matter intake, kg/10 day</td>
<td>11.0</td>
<td>12.5</td>
<td>2.2</td>
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<tr>
<td>Apparent dry-matter digestibility, %</td>
<td>69.1</td>
<td>69.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>
DISCUSSION

Prepubertal calves responded to GHRF in a dose-dependent manner and to multiple injections of GHRF without a decline in peak amplitude of GH. These results are similar to studies with cattle and sheep (8, 12-15). In contrast, prepubertal lambs given multiple injections of GHRF declined in their GH response over 3 days (16).

Five-mo-old calves receiving GHRF every 3 hr for 42 hr responded to 50% of the GHRF injections measured (Exp. 2), whereas a greater degree of responses (77%) was observed in 3.5-mo-old calves given GHRF every 4 hr for 10 days (Exp. 4). A similar lack of response to some GHRF injections has been observed in studies with cattle and sheep (8, 13, 17), pigs (18, 19), rats (20), rabbits, and humans (21, 22). The response to GHRF may be related to physiological events occurring during GHRF administration, such as secretion of hypothalamic SRIH.

Prepubertal calves were sensitive to SRIH administration. The GH response to GHRF was blunted when SRIH was continuously infused. When the calves were given 0.067 μg hpGHRF/kg bwt without SRIH, mean GH increased to 28 ng/ml. This response declined to 16 ng/ml (41% suppression) when 0.033 μg SRIH/kg bwt was infused. This is similar to results with rat somatotrophs cultured with GHRF and SRIH simultaneously, in which GH secretion was reduced in comparison with GHRF in the absence of SRIH. Furthermore, increasing doses of SRIH resulted in a concomitant decrease in GH release stimulated by GHRF (23-24). In this study, after SRIH infusion in the calves, GH rebounded to levels
comparable to the GHRF-induced release of GH. A similar increase in GH release after withdrawal of SRIH has been observed with cultured somatotrophs (25-27). Kraicer et al. (27) demonstrated that the amplitude of the GH rebound after withdrawal of SRIH increased if GHRF was administered with SRIH.

Administration of 0.067 ug hpGHRF/kg bwt every 4 hr for 10 days to 90-kg calves increased nitrogen retention 16%, but this difference was not statistically significant. Although the endocrine and metabolic data were not significantly altered by GHRF treatment, the changes in the values indicated that a minor anabolic effect may be occurring in the calves. These results may be compared with the observations of Moseley et al. (12) where heavier calves, continuously infused with 2.5 ug (1-44)-NH₂ hpGHRF/min for 20 days, had an 18% increase in nitrogen retention and a 14% decline in urinary nitrogen on days 9-14. Similar to our study, they observed no significant changes in feed intake or protein digestion (12). The results of the present study may be contrasted with those in adult sheep, in which they were given the same dose of 0.067 ug GHRF/kg bwt at 4-hr intervals as the young calves in this study (8). The sheep given GHRF had a significant decrease in urinary nitrogen, which was not observed in these calves. The dissimilar effect of GHRF when administered in the same manner in these two studies may result from differences between the two species, fewer experimental units in the calf trial or differences due to age of the animals in as much as younger animals secrete more GH than do adults (28). The difference in the pattern of secretion of GH by the younger,
rapidly growing animals may have caused their limited ability to achieve additional growth by pulsatile administration of physiological dosages of GHRF.

In conclusion, prepubertal calves were sensitive to exogenous GHRF and responded by repetitive release of GH similar to that of adults. These calves responded to exogenous SRIH by suppression of GH secretion and a blunted GH response to GHRF. Although the nitrogen retention in calves increased 16% and weight gain was enhanced 36% in response to GHRF pulsed at 4^hr intervals for 10 days, the variation between animals prevented these increases from acquiring significance.


SECTION III. EFFECTS OF GROWTH HORMONE-RELEASING FACTOR AND SOMATOSTATIN ON GROWTH HORMONE SECRETION IN HYPOPHYSIAL STALK-TRANSECTED BEEF CALVES
Effects of Growth Hormone-Releasing Factor and Somatostatin in Growth Hormone Secretion in Hypophysial Stalk-Transected Beef Calves


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1Presented in part at the 76th Annual Meeting of the American Society of Animal Science, Columbia, MO (Abstract 173). These studies were supported in part by the U.S. Department of Agriculture, ARS, CSRS Special Grant 59-2191-1-2-033-0. Journal Paper J-12829 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Projects 2443, 2444, 2528, 2644, and 2273, the last a contributing project to the North Central Regional Research Project NC-113).

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ABSTRACT

The effects of growth hormone-releasing factor (GHRF) on growth hormone (GH) secretion were studied in beef calves after hypophysial stalk transection (HST). Peripheral GH concentration during surgery was elevated for 60 min after the initiation of anesthesia (to 15 ng/ml), which was greater than plasma levels after HST and during the recovery period (0-30 hr mean, 3 ng/ml; P<0.05). Episodic GH secretion normally seen in sham-operated controls (SOC) was abolished after HST. HST calves responded to every challenge of GHRF with an increase in plasma GH. A dose of 0.067 ug human pancreatic (hp) (1-40)-OH GHRF/kg body weight (bwt) 3 days after HST increased plasma GH to 55 ng/ml from a control period mean of 5 ng/ml (P<0.04). On day 8, HST calves received two injections of 0.067 ug hpGHRF/kg bwt at 3-hr intervals, with feeding 70 min after the first injection. During the preinjection control periods, basal GH averaged < 4 ng/ml and increased to 17 (P<0.02) and 9 (P<0.04) ng/ml immediately after the first and second injections of hpGHRF. On days 19 and 20, HST calves were infused iv with 0.033 and 0.067 ug somatostatin(SS)-14 (SRIH)/(kg bwt*min), during which a pulse injection of 0.067 ug hpGHRF/kg bwt was administered. GH increased to 9 and 5 ng/ml during the 0.033- and 0.067-ug SRIH infusions after GHRF; no somatotropic rebound was observed after the SRIH was discontinued. Five and six months after HST, the responses to two analogs of rat hypothalamic GHRF were similar to those in SOC calves. These results indicate that HST calves responded to exogenous stimuli of GHRF with an
abrupt increase in plasma GH, but GH response to GHRF during SRIH infusion was severely inhibited.
INTRODUCTION

The hypothalamus regulates episodic growth hormone (GH) secretion from the pituitary by release of growth hormone-releasing factor (GHRF) and growth hormone-releasing-inhibiting hormone, somatostatin (SRIH). The neurohypophysial link between the hypothalamus and pituitary for these releasing and inhibiting hormones is the hypophysial stalk. After surgical hypophysial-stalk transection (HST), normal episodic secretion of GH is abolished in cattle and pigs (1, 2) and these animals have depressed growth rates (3). Unlike hypophysectomized animals, HST animals can serve as a model to study the isolated effects of brain releasing and inhibiting hormones on pituitary hormone secretions. It is known that GHRF can stimulate an endogenous GH release in man, rats, pigs, sheep, and cattle (4-6). The effects of GHRF on GH release in HST animals, however, have not been reported. The purpose of this study was to investigate the effects of GHRF on GH release as a single- or multiple-pulse dose, to verify that the pituitary would respond to other exogenous stimuli, and to examine the interactions of GHRF and SRIH in prepubertal HST calves.
MATERIALS AND METHODS

Animals

Five crossbred beef heifers were individually penned with straw bedding in an environmentally controlled room at 18°C with 12-hr photoperiods. They were fed at 12-hr intervals a diet consisting of 49% cracked corn, 39% dehydrated alfalfa, 5.6% solvent-extracted soybean meal, 5.6% cane molasses, 0.5% dicalcium phosphate, 0.2% iodized salt, 0.02% trace mineral, and 0.1% vitamin A (5.2 million IU/kg). Animals were fed in the morning, and experiments conducted in the afternoon. An indwelling catheter (Tygon microbore tubing, 1.27 mm i.d., Fisher Scientific, Pittsburgh, PA) was inserted in a jugular vein before surgery and maintained for all experiments. The catheters were filled with sterile saline containing 100 U heparin/ml between experiments and with sterile saline with 40 U heparin/ml when blood samples were being obtained. Blood samples were treated with heparin (4 U/ml), and the plasma was stored at -15°C until radioimmunoassay for GH (7). The GH assay had intra- and inter-assay coefficients of variation of 1.7% and 9.0%, respectively.

Peptides

Animals received bolus intravenous (iv) injections of human pancreatic (hp) (1-40)-OH GHRF (8), (1-32)-OH rat hypothalamic (rh) GHRF, [Nle²⁷] (1-29)-NH₂rhGHRF (9), and thyrotropin-releasing hormone (TRH, Sigma Chemical Co., St. Louis, MO). Somatostatin(SS)-14 (SRIH)
was administered iv by continuous-infusion pumps (Harvard, Model 1201, Harvard Apparatus, Millis, MA). The GHRF, TRH and SRIH were dissolved in 0.1% acetic acid (1 µg/ul) and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818% NaCl) containing 0.01 M NaH₂PO₄·H₂O, pH 7.0; 0.01% ascorbic acid; and 1% bovine serum albumin. Solutions for injection and infusion were prepared the day of experimentation.

Surgery

Commencing 3 hr before anesthesia and surgery and continuing throughout surgical intervention, blood was sampled at 15-min intervals. Subsequently, blood was sampled hourly for 5 hr, then at 6-hr intervals for 30 hr. HST was performed on five calves by a supraorbital approach described previously (10-11). Two sham-operated controls (SOC) were subjected to the same surgical procedures, with the exception that the hypophysial stalk was not severed. Anesthesia was induced by iv injection of thiamylal sodium (0.5-1.0 g, Surital, Parke-Davis, Morris Plains, NJ) and maintained by a closed-circuit system of halothane (1-4%, Ayerst Laboratories, New York, NY) and O₂ (400-800 ml/min). After the hypophysial stalk was severed, a nylon disc (8.0 mm diameter and 0.45 mm thickness) was inserted between the severed ends of the tubular stalk to prevent regeneration between the hypothalamus and pituitary gland. Water and food intake returned to normal 6-24 hr after surgery. Postmortem examination of each heifer confirmed the completeness of stalk transection, the nylon disc was in the proper location and had prevented vascular regeneration of the stalk in each
calf. The pituitary gland was cut transversely and fixed in Susa's solution for histological evaluation. The glands were sectioned at 6 μm and stained with performic acid-Alcian blue-periodic acid-Schiff-orange G by the method of Heath (12), whereas other sections were stained with hematoxylin and eosin.

hpGHRF Dose Response (Exp. 1)

Three and four days after surgery, the HST calves (170 kg) received 0.067 and 0.133 μg hpGHRF/kg bwt, respectively, to test the GH secretory response. Plasma samples were collected 20, 10 and 1 min before the saline control period; 5, 10, 15, 20, 40, and 60 min during the control period; and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after GHRF injection.

TRH Response (Exp. 2)

Five days after surgery, the HST calves were injected iv with 100 μg TRH. Plasma sampling was similar to that in Exp. 1.

Multiple Injections of GHRF (Exp. 3)

Eight days after surgery, the HST calves received two iv injections of 0.067 μg hpGHRF/kg bwt at 3-hr intervals, with feeding 70 min after the first GHRF injection. Plasma was sampled at 20-min intervals before the first GHRF injection and at 5-min intervals for the first 20 min after GHRF injection and then sampling at 20-min intervals thereafter.
SRIH and GHRF on GH Secretion (Exp. 4)

Nineteen days after surgery, two HST calves (183 kg) were infused with 0.033 or 0.067 ug SRIH/(kg bwt*min) for 75 min in a crossover design. After 30 min of SRIH infusion, 0.067 ug hpGHRF/kg bwt was administered iv as a bolus injection. Plasma was sampled 30, 15 and 1 min before SRIH infusion; 15, 29, 35, 40, 45, 50, and 70 min during SRIH infusion and GHRF injection; and 90, 110, 130, and 150 min after SRIH infusion.

rhGHRF Dose Response (Exp 5)

Five mo after surgery, the GH response to [Nle^{27}] (1-29)-NH_2 rhGHRF was compared in three HST calves (245 kg bwt) and two SOC calves (304 kg bwt). The doses tested were 0, 0.0083, 0.0165, 0.033, 0.067, and 0.133 ug rhGHRF/kg bwt. Plasma was collected 20, 10 and 1 min before injection and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after injection. The experiment was repeated at 6-mo after surgery using the same dosages of another analog, rhGHRF(1-32)-OH.

Statistical Analysis

The experimental units in this study were the individual calves. Data were analyzed by using the Student's "t" test for comparisons among treatment groups (13-14).
RESULTS

GH secretion in these animals is presented: 1) during presurgery and anesthesia, and the effects of HST and SOC during the first 30 hr after surgery as seen in Fig. 1; 2) comparisons of pre- and post-GHRF or TRH treatments in HST calves as seen Figs. 3-5; and 3) comparisons of GHRF treatment during SRIH infusions before and after HST as seen in Fig. 6.

Surgery

In the seven calves subjected to either HST or SOC, the plasma GH levels during surgery and immediately in the postoperative recovery period are depicted in Fig. 1. GH secretion during the preanesthesia period, 9 +/- 3.9 ng/ml (+/- SE), (mean values from -345 to -195 min) was variable and similar to that during surgery and postoperative recovery period. The first 60 min after the initiation of anesthesia (-180 to -135 min), peripheral GH increased to a mean of 15 +/- 4.6 ng/ml, which was greater than in SOC calves during the first 90 min after time zero (3 +/- 0.7, P<0.05) as well as all the periods (P<0.05) following time zero in the HST calves (0-90 min, 4 +/- 0.8 ng/ml; 2-5 hr, 2.5 +/- 0.8 ng/ml; 6-30 hr, 3 +/- 0.8 ng/ml). A postanesthesia GH surge occurred at 3-4 hr in the SOC calves, which was greater than that in the HST calves (10 +/- 1.8 vs 3 +/- 1.0 ng/ml; P<0.05).
Fig. 1. Plasma GH concentrations in beef calves before and after surgery are illustrated. The GH response indicated by the open circles (o) illustrates the period before surgical intervention, which is indicated by the open arrow (n=7). Plasma GH after hypothalamic-stalk transection (HST) is illustrated by closed triangles (▲) (n=5), and GH after sham-operated controls (SOC) is indicated by squares (□), (n=2). Values are the mean +/- SE. SE bars for samples taken 2 and 3 hr after surgery are within the symbol.
Histology

Histological examination of pituitary glands from HST calves indicated the persistence of at least some secretory cells in the same areas of the adenohypophysis as in SOC calves (Fig 2). In sections from HST and SOC calves stained with performic acid-Alcian blue-periodic acid-Schiff-orange G, acidophils, basophils, and chromophobes were present. Pituitary gland weight was 35% less (P<0.01) in HST (0.91 +/- 0.05 g; 0.2 +/- 0.01 g/100 kg bwt) compared with SOC (2.59 +/- 0.20 g; 0.6 +/- 0.03 g/100 kg bwt) calves.

hpGHFR Dose Response (Exp. 1)

The HST calves responded to 0.067 and 0.133 ug hpGHFR/kg bwt with a rapid increase in plasma GH, which peaked within 10-20 min and then declined to preinjection concentrations within 60 min (Fig. 3). After HST, all animals responded to 100% of the GHRF injections during the control period, mean GH (5-20 min after injection of saline) was 5 +/- 0.3 ng/ml, which was less than 55 +/- 16 ng/ml (P<0.04) during the 0.067-ug GHRF period (5-20 min after GHRF). The mean of the control period during the 0.133-ug GHRF trial was 4 +/- 0.4 ng/ml, which contrasts with the GHRF period mean of 33 +/- 11 ng/ml (P<0.06). The GH response between the two GHRF doses was not statistically different.
Fig. 2. Photomicrographs of adenohypophysis of four hypothalamic-stalk transected (HST) (a, b, c, and d) and two sham-operated controls (SOC) (e and f) calves. Histological cross sections are from the middle one third of the anteromedial part of the adenohypophysis. Acidophils with cytoplasm were dispersed in anteromedial regions of the adenohypophysis in both groups of HST and SOC calves. Acidophils are associated with somatotrophs, lactotrophs and adrenocorticotrophs. Chromophobes were evident throughout the adenohypophysis in HST and SOC calves. Histological sections indicate survival of adenohypophysial cells in HST calves (x 360).
TRH Response (Exp. 2)

GH secretion in response to TRH was variable in HST calves. Only two of the five HST calves responded to TRH injections by an increase in GH similar to that evoked by hpGHRF which accounts for the increased variability in the GH peak as seen in Fig. 4. Although only 40% of them responded to a TRH challenge, all HST calves released GH in subsequent treatments with hpGHRF and rhGHRF.

Multiple Injections of GHRF (Exp. 3)

Eight days after surgery, all HST calves responded to the two injections of hpGHRF (Fig. 5). Plasma concentrations of GH were the same before each injection of hpGHRF (3.7 and 3.6 ng/ml) and significantly lower than after hpGHRF (P<0.05). The second injection of hpGHRF, which occurred after feeding, did not increase plasma GH to the same extent as the first injection (9 vs 17 ng/ml, P<0.09).

SRIH and GHRF on GH Secretion (Exp. 4)

Infusion of 0.033 and 0.067 ug SRIH/kg bwt*min depressed GH release when a bolus of 0.067 ug hpGHRF/kg bwt was administered (Fig. 6). GH levels 20 min after GHRF were 9 +/- 1.6 ng/ml during infusion of 0.033 ug SRIH and 5 +/- 0.4 ng/ml during infusion of 0.067 ug SRIH. The changes in plasma GH during the infusion of either dose of SRIH were not significantly different in any part of the experiment or between doses of SRIH. After the infusion of SRIH, GH concentrations remained stable.
Fig. 3. Plasma GH concentrations in hypothalamic-stalk (HST) calves in response to 0, 0.067 and 0.133 ug (1-40)-OH hpGHRF/kg bwt are illustrated by the closed triangles (▲), (n=5). For comparison, the response to 0.067 and 0.10 ug (1-40)-OH hpGHRF/kg bwt before surgery in these animals is indicated by the open circles (○) in the left and right panels, respectively. The GH response to 0.067 ug hpGHRF is shown in the left panel, and the right panel indicates the effect of the more concentrated dose of hpGHRF. Arrows indicate the time of vehicle or GHRF administration. Values are the mean +/- SE
Fig. 4. The GH response of hypothalamic-stalk transected (HST) calves receiving 0 and 100 μg TRH are illustrated (n=5). The time of vehicle and TRH injection is indicated by the arrows. Values are the mean +/- SE.
Plasma GH was stimulated in a dose-dependent manner to two analogs of rhGHRF in HST and SOC calves (Tables 1 and 2). All animals responded to either analog of rhGHRF when the dosage was 0.0165 ug rhGHRF/kg bwt or greater. No differences in plasma GH response between HST and SOC calves were observed.
Fig. 5. Plasma GH in hypothalamic-stalk transected (HST) calves receiving 0.067 µg hpGHRF/kg bwt at 3-hr intervals for 6 hr are indicated by the closed triangles (▲), (n=5). The response to the same treatment before HST is shown by the open circles (○). The time of feeding and hpGHRF injections are illustrated by arrows. Values are the mean +/- SE.
Fig. 6. Mean concentration of plasma GH in hypothalamic-stalk transected (HST) calves during iv infusion of 0.033 and 0.067 ug somatostatin(SS)-14 (SRIH)/(kg bwt*min) and injected with 0.067 ug (1-40)-OH hpGHRF/kg bwt are illustrated by closed triangles (A), (n=2). The same animals' response to these hormone treatments before surgical intervention is shown by the open circles (o). Arrows indicate the time of GHRF injections, and the hatched areas illustrate the period of SRIH infusion.
Table 1. Effects of rat hypothalamic GHRF, [Nle^{27}] rhGHRF(1-29)-NH_2, on plasma GH levels in HST and SOC calves 5 mo after surgery

<table>
<thead>
<tr>
<th>Dose of [Nle^{27}]rhGHRF(1-29)NH_2 given iv (ug/kg bwt)</th>
<th>Plasma GH concentration(^a) ng/ml</th>
<th>HST calves</th>
<th>SOC calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000(^b)</td>
<td>3.0 +/- 0.6</td>
<td>5.6 +/- 1.2</td>
<td></td>
</tr>
<tr>
<td>0.0083</td>
<td>6.7 +/- 2.1</td>
<td>9.4 +/- 0.9</td>
<td></td>
</tr>
<tr>
<td>0.0165</td>
<td>12.2 +/- 3.7</td>
<td>10.9 +/- 3.4</td>
<td></td>
</tr>
<tr>
<td>0.033</td>
<td>12.8 +/- 5.4</td>
<td>14.7 +/- 2.0</td>
<td></td>
</tr>
<tr>
<td>0.067</td>
<td>16.5 +/- 5.8</td>
<td>14.4 +/- 5.6</td>
<td></td>
</tr>
<tr>
<td>0.133</td>
<td>24.6 +/- 7.4</td>
<td>8.5 +/- 1.8</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Values are means +/- SE.

\(^b\)Vehicle consisting of 0.01 M NaH_2PO_4\*H_2O, 0.01% ascorbic acid and 1% bovine serum albumin.
Table 2. Effects of rat hypothalamic GHRF, rhGHRF(1-32)-OH, on plasma GH levels in HST and SOC calves 6 mo after surgery

<table>
<thead>
<tr>
<th>Dose of rhGHRF(1-32)-OH given iv (µg/kg bwt)</th>
<th>Plasma GH concentration(^a) ng/ml</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HST calves</td>
<td>SOC calves</td>
<td></td>
</tr>
<tr>
<td>0.0000(^b)</td>
<td>1.7 +/- 0.2</td>
<td>2.1 +/- 0.2</td>
<td></td>
</tr>
<tr>
<td>0.0083</td>
<td>3.1 +/- 0.8</td>
<td>3.2 +/- 0.8</td>
<td></td>
</tr>
<tr>
<td>0.0165</td>
<td>3.4 +/- 0.5</td>
<td>4.2 +/- 0.6</td>
<td></td>
</tr>
<tr>
<td>0.033</td>
<td>5.9 +/- 1.9</td>
<td>7.3 +/- 1.5</td>
<td></td>
</tr>
<tr>
<td>0.067</td>
<td>7.4 +/- 2.9</td>
<td>19.0 +/- 6.5</td>
<td></td>
</tr>
<tr>
<td>0.133</td>
<td>17.4 +/- 9.9</td>
<td>26.0 +/- 3.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Values are means +/- SE.

\(^b\)Vehicle consisting of 0.01 M NaH\(_2\)PO\(_4\)*H\(_2\)O, 0.01% ascorbic acid and 1% bovine serum albumin.
DISCUSSION

Plasma GH concentrations were acutely altered by the surgical procedures of HST and SOC in prepubertal calves. When the anesthesia was administered, GH increased abruptly for 60 min. After the anesthesia was discontinued, GH remained elevated in the SOC calves, whereas it dropped to basal levels in HST calves. The return of episodic GH secretion in SOC calves and its absence as well as depressed GH release in HST calves are similar to results reported by Anderson et al. (1, 15). In contrast, basal GH secretion in HST pigs remains greater than that in SOC animals (2, 16). Although plasma prolactin levels were not altered by anesthesia in these calves, HST caused consistently greater prolactin secretion compared with that in SOC animals (17-18).

The pattern of abrupt GH release in response to GHRF in HST calves was similar to that seen before surgery as well as in SOC animals. The high amplitude of the GH response to the first injection of hpGHRF at 3 days after surgery could not be replicated by later challenges. There was a dose dependency to rhGHRF in HST calves several months after surgery, which is similar to the GH response to hpGHRF or rhGHRF seen in HST pigs (16). Furthermore, both analogs of rat hypothalamic GHRF were effective in causing GH release in HST as well as SOC calves.

The pituitary in HST calves was capable of releasing GH in response to TRH, but only 40% of the animals responded to the TRH injections, whereas 100% of them responded to hpGHRF and rhGHRF. This variable
response to exogenous TRH has also been observed in intact cattle (19). Szabo (20) demonstrated that GHRF and TRH act through different mechanisms in the pituitary to release GH. The release of GH by GHRF is cAMP- and Ca\(^{2+}\)-mediated, whereas TRH mediates GH release by a cAMP-independent, Ca\(^{2+}\)-dependent process. The different mechanisms for mediation of GH release by the peptides may account for the variation in the ability to respond to the stimuli.

When HST calves were subjected to GHRF at 3-hr intervals, they responded to the first hormone injection with greater GH release than to the second GHRF injection, which occurred after feeding. The depression in GH response to the same dose of GHRF after 3 hr may be related to effects of feeding, number of GH-release challenges since surgery, or down regulation of the pituitary. These results are similar to the animals' response before surgical manipulation, however, the magnitude of the GH response to 0.067 ug GHRF before and after feeding was enhanced by surgery.

During SRIH infusion, GHRF did not significantly increase GH release in HST calves. After SRIH infusion, plasma GH was not altered. This contrasts with intact calves in which a somatotropic rebound was observed after SRIH withdrawal (21-24). Because the pituitary was no longer under exogenous SRIH and GHRF regulation after HST, it may not need to compensate for the GH-release suppression during SRIH infusion periods in these calves. For example, when these same treatment regimens of GHRF and SRIH were conducted before surgical manipulation, the variation of GH secretion was much greater than after HST. The mean
of all experimental coefficients of variation before surgery was 61%, whereas after HST, it was reduced to 17% (P<0.001). The reduced variation of GH secretion after HST may be explained by the lack of episodic secretion of GH that has been observed previously in HST animals (1, 2).

In conclusion, this is the first demonstration of a GH response to hpGHRF and rhGHRF injections in HST calves. Without the hypophysial stalk connection, the calves always responded to GHRF challenges. After HST, the pituitary remains capable of responding to other exogenous substances such as TRH, as well as of secreting greater amounts of prolactin than found in SOC calves. Finally, the somatotropic rebound observed in intact calves after SRIH withdrawal is not observed after HST. After HST, the pituitary was able to respond to a variety of stimuli; however, desensitization of the pituitary may occur during the period immediately after surgery, but the calves remain responsive to GHRF challenges several months later.
LITERATURE CITED


SECTION IV. GROWTH HORMONE (GH) PROFILES, METABOLIC CLEARANCE RATES, SECRETION RATES, HALF-LIFE, AND GH RESPONSE TO GH-RELEASING FACTOR AT FOUR AGES IN INTACT AND CASTRATE MALE AND FEMALE CATTLE
Growth hormone (GH) profiles, metabolic clearance rates, secretion rates, half-life, and GH response to GH-releasing factor at four ages in intact and castrate male and female cattle$^1,2,3$

Cathie A. Plouzek and Allen Trenkle

$^1$Presented in part at the 77th Annual Meeting of the American Society of Animal Science, Athens, GA (Abstract 109) and the 67th Annual Meeting of the Endocrine Society, Baltimore, MD (Abstract 476).

$^2$Journal Paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project 2644).

$^3$Acknowledgments: We thank Drs. Wylie Vale and Jean Rivier of The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, San Diego, CA for their generous gift of growth hormone-releasing factor; Carol Pochart Johnson for the SAS hormone secretory spike program; and Joan Rettig, John Lawrence, Rod Berryman, and Carl Johnson for their technical assistance.
ABSTRACT

Growth hormone (GH) parameters were examined in intact and castrated male and female cattle (4 of each), at 5, 8, 12, and 15 mo (+/- 2 wks) of age. Plasma GH profiles, secretion rates (SR), metabolic clearance rates (MCR), half-life of intravenously infused GH, and GH response to 0.0165, 0.067 and 0.267 ug of growth hormone-releasing factor (GHRF)/kg body weight (bwt) were studied. As cattle aged, overall GH levels in the plasma decreased, GH baseline declined, the frequency and amplitude of GH spikes and secretory periods diminished (P<0.05), SR decreased (P<0.001), half-life of infused GH decreased (P<0.01), and the GH response to intravenous (iv) injections of GHRF declined (P<0.05). Bulls had an elevated overall GH mean and GH baseline concentration, greater amplitude of GH spikes and a greater number of secretory periods (P<0.01), faster SR (P<0.05), and a greater GH response to 0.267 ug GHRF/kg bwt than steers, heifers, or ovariectomized heifers. Males had more frequent GH spikes and secretory periods of higher amplitude, greater SR, and greater GH responses to 0.0165 ug GHRF/kg bwt (P<0.05) than females. Heifers had a lower MCR than ovariectomized heifers (P<0.05). Intact and castrated cattle did not differ in GH parameters. These results suggested that testosterone and its metabolites enhanced GH secretion, and decreasing GH concentrations were associated with increasing age.
INTRODUCTION

There are many factors known to affect growth hormone (GH) concentrations in ruminants, such as nutrition, breed, age, sex, and castration (1-7). GH in ruminants is secreted over time as peaks occurring at random (2, 8-9). Due to this random secretion, GH has been difficult to define without serial sampling in ruminants. Characterization of GH profiles in ruminants has been attempted by defining the overall concentration of GH, GH baseline, and number and amplitude of GH secretory periods. However, these studies have only examined intact and castrate males or ovariectomized ewes (2, 8-9). In the rat, the pattern of GH secretion has been considered to be related to the differences in growth rates between males and females (10). In order to determine if differences in growth rates between intact and castrate male and female cattle are related to differences in GH secretory patterns, a characterization of their GH profiles is required.

The concentration of GH in blood is generally the result of the balance of secretion rate (SR) and metabolic clearance rate (MCR). Although SR and MCR comparisons have been conducted in males and females at different ages, they have not been studied in conjunction with GH profiles (11).

The purpose of this study, therefore, was to evaluate the GH status in growing male and female cattle, both intact and castrated, over a range of 10 months. This was accomplished by measuring GH concentration in the plasma at 20-min intervals over a 12-hr period and then
characterizing the profiles, determining MCR, SR and half-life of GH, and examining the GH response to 3 doses of exogenous GH-releasing factor (GHRF).
MATERIALS AND METHODS

Animals

Sixteen crossbred cattle (8 male and 8 female) were studied at 5, 8, 12, and 15 mo of age. Four male and 4 female animals were castrated at 6 months. The calves remained with their dams on pasture until 6 mo of age. The animals were then placed in a sheltered feedlot and fed a diet of 49% cracked corn, 30% ground cob, 10% molasses, 10% solvent extracted soybean meal, and 1% salt, minerals, and vitamin A. At 10 mos, the cattle were individually fed the diet using electronic broadbent headgates (American Calan Inc., Northwood, NH) to allow unrestricted feed intake. Two indwelling catheters (Tygon microbore tubing, 1.27 mm i.d., Fisher Scientific, Pittsburgh, PA) were inserted into each jugular vein at least 1 day before experiments began. Patency of catheters was maintained overnight with sterile physiological saline containing 100 U heparin/ml, and sterile saline containing 20 U heparin/ml between sampling. One catheter was used for infusion, and another for blood samples. Blood samples were treated with heparin (4 U/ml) and plasma stored at -15°C until analyzed for GH (12). The GH assay had intra- and inter-assay coefficients of variation of 1.8% and 13.8%, respectively.

Peptides

GH was purified using the methods of Dellacha and Sonnenberg (13), Wallis and Dixon (14) and Lorenson and Ellis (15) as modified by J. L. Bobbitt (Eli Lilly Inc., Indianapolis, IN; personal communication). The
purified GH paralleled the GH standard in the GH radioimmunoassay and was 4% less reactive than the GH standard. Purified GH was dissolved in a sodium bicarbonate buffer, pH 11.0 (4.24 g Na_2CO_3; 7.45 g KCl; 3.0 ml 1M HCl; to 500 ml with H_2O) for infusion. [Nle^27] (1-29)-NH_2 rat hypothalamic (rh) GHRF (16) was dissolved in 0.1% acetic acid (1 ug/ul) before making to volume in sterile buffer, pH 7.0. The buffer was physiological saline (0.818% NaCl) containing 0.01 M NaH_2PO_4·H_2O, 0.01% ascorbic acid and 1% bovine serum albumin. Peptide solutions were prepared the day of experimentation.

Experiments

On the first day of experimentation, 10-ml blood samples were drawn at 20-min intervals for 12 hr to determine the pattern of GH release. Immediately following the 12-hr profile, a dose of 0.0165 or 0.067 ug rhGHRF/kg bwt was administered to the animals. Blood samples were taken 1, 10 and 20 min before the bolus injection of GHRF and 5, 10, 15, 20, 40, and 60 min after injection. On day 2, administration of 0.0165 or 0.067 ug GHRF/kg bwt was administered to the cattle and blood sampled as described for day 1. One to two hr later, a calculated concentration of GH was infused to attain a steady concentration of 40 ng/ml (12), and averaged 47.4 ng/ml when the plasma was verified later by radioimmunoassay. GH was infused at a rate of 0.37 ml/min using peristaltic pumps (Harvard, Model 1505, Harvard Apparatus, Millis, NJ) to determine MCR, SR and half-life of GH. Samples were taken 1 min before infusion, 10, 20, 30, 70, 80, and 90 min during infusion, and 5, 10, 15, 20, 25, 30, 40, and 50 min after the infusion. Eight of the
cattle received 0.2667 ug rhGHRF/kg bwt after a 5-hr rest period. The blood sampling was similar to the GHRF administration described previously, with the exception that blood was sampled an additional 60 min at 20-min intervals. At 8, 12 and 15 mo of age, animals were fed 5 hr after the start of the experiment on both days.

The calculations to determine the MCR, SR and half-life of GH were similar to those described by Trenkle (11) with the SR calculated from the MCR and the average baseline GH concentrations during the control infusion period. The MCR and SR were adjusted to kg bwt basis for statistical analysis to compare the animals at different ages.

Statistics

The 12-hr GH profile analysis was based on Christian et al. (17) skewness coefficients as adapted to SAS procedures (18) with several additional modifications (Appendix). Spearman correlation coefficients as described by SAS (18) were used to determine if the GH concentrations were changing with time the animals were sorted by linear, quadratic or no correlation based on the Spearman correlation coefficients of the GH profiles. After adjusting for the trends, autocorrelations with a lag of one on the residuals were run to determine spike locations. The 15 maximum points were tested as outliers using the skewness test and the univariate procedure. If the autoregression residual value was equal or greater than 1.0, then the corresponding GH value was considered a spike. If the GH concentration returned to baseline or below for 1 or more samples, the spikes surrounded by the baseline samples were considered to be a period of secretion. The hormone concentration at an
identified spike was the amplitude for the hormone spike. The average of the GH concentration of the identified spikes within a secretory period was considered to be the amplitude of a period of secretion. The overall mean was the average of all GH samples taken in the profile. The baseline mean was determined by removing the samples identified as spikes from the average.

After different segments of the GH profile were identified, they were included in a SAS analysis with the other GH data in the experiments for the split-plot analysis (19) using the general linear model procedure (18).
RESULTS

Overall mean GH, baseline, number of spikes, spike amplitude, and secretory period amplitude significantly declined as the cattle aged (Figs. 1-4, Table 1). Baseline GH declined between 5 and 15 mo (P<0.009). An average decrease of two GH spikes in a 12-hr period occurred between 5 and 15 mo in the cattle (P<0.004). There were fewer spikes of GH at 15 mo as well as 27% less amplitude of the spikes than at 5 mo (P<0.04). Although the number of periods of GH secretion was not changed as the animal aged, the average amplitude of the secretory periods declined between 5 and 15 mo (P<0.04).

Bulls had different plasma GH profiles than steers, heifers or ovariectomized heifers (Figs. 1-4; Table 2). Considerable variation between animals was observed (Figs. 1-4). The overall mean of GH in the bulls was greater than the other animals (P<0.02). Even by the removal of the spikes of GH secretion from the overall mean, the baseline concentration of GH in bulls was significantly greater than steers, heifers and ovariectomized heifers. Males had more spikes of GH as well as more periods of GH secretion than the females (P<0.02). Bulls had a greater mean amplitude of the secretory periods and GH spikes (P<0.01) compared with steers, heifers or ovariectomized heifers.

A statistical interaction between males and females at different ages occurred with the number of spikes in a 12-hr period (P<0.05). In females, the number of GH spikes decreased with age (5.5, 3.5, 3.3, and
2.5 at 5, 8, 12, and 15 mo, respectively); while the number of GH spikes in males was 5.5, 4.4, 5.1, and 4.5, respectively, for the same periods.

The MCR and SR of GH are summarized in Table 3 for the four groups. MCR was significantly less in intact heifers than other animals (P<0.02). Bulls, steers and ovariectomized heifers had similar MCR. Bulls had a greater SR of GH than the other animals (P<0.03). Males had higher SR than females (P<0.0001). The half-life of GH was not different between intact and castrated male and female cattle.

MCR and SR according to age of cattle are summarized in Table 4). MCR significantly increased at 8 mo (P<0.02) but declined to levels similar to 5 mo at 12 and 15 mo. Secretion of GH was greater at 5 and 8 mo than 12 and 15 mo (P<0.0006). The half-life of GH declined (P<0.0001) between 5 and 8 mo and remained unchanged thereafter.
Fig. 1. Profiles of plasma GH over 12 hr in bulls at 5, 8, 12, and 15 mo of age. Each panel illustrates GH concentrations for each animal. Individuals have the same symbol in each panel.
Fig. 2. Profiles of plasma GH over 12 hr in steers at 5, 8, 12, and 15 mo of age. Each panel illustrates GH concentrations for each animal. Individuals have the same symbol in each panel.
Fig. 3. Profiles of plasma GH over 12 hr in heifers at 5, 8, 12, and 15 mo of age. Each panel illustrates GH concentrations for each animal. Individuals have the same symbol in each panel.
Fig. 4. Profiles of plasma GH over 12 hr in ovariectomized heifers at 5, 8, 12, and 15 mo of age. Each panel illustrates GH concentrations for each animal. Individuals have the same symbol in each panel.
Table 1. Plasma growth hormone profiles in cattle at different ages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5 mo</th>
<th>8 mo</th>
<th>12 mo</th>
<th>15 mo</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall GH concentration, ng/ml(^b)</td>
<td>5.1</td>
<td>4.4</td>
<td>3.7</td>
<td>3.1</td>
<td>0.3</td>
</tr>
<tr>
<td>GH baseline, ng/ml(^c)</td>
<td>3.9</td>
<td>3.3</td>
<td>2.8</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of GH secretory periods</td>
<td>2.7</td>
<td>2.9</td>
<td>3.0</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Mean amplitude of GH secretory periods, ng/ml(^d)</td>
<td>9.2</td>
<td>9.9</td>
<td>6.8</td>
<td>6.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Number of GH spikes(^e)</td>
<td>5.5</td>
<td>3.9</td>
<td>4.2</td>
<td>3.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean amplitude of GH spikes, ng/ml(^f)</td>
<td>8.1</td>
<td>9.1</td>
<td>6.4</td>
<td>5.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\(^a\) Average of all animals (bulls, steers, heifers, and ovariectomized heifers) over 12 hr.

\(^b\) Overall GH concentrations at 5 mo is different from 12 and 15 mo (P<0.006) and 8 mo is different from 15 mo (P<0.009).

\(^c\) Baseline GH at 5 mo is different from 12 and 15 mo (P<0.009) and 8 mo is different from 15 mo (P<0.03).

\(^d\) Mean amplitude of GH secretory periods at 8 mo is different from 12 and 15 mo (P<0.02) and 5 mo is different from 15 mo (P<0.04).

\(^e\) Number of GH spikes at 5 mo is greater than at 8, 12 or 15 mo (P<0.004).

\(^f\) Mean amplitude of GH spikes at 5 and 8 mo are greater than at 15 mo (P<0.04) and 8 mo is greater than 12 mo (P<0.02).
Table 2. Plasma growth hormone profiles in intact and castrated males and females

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomy Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall GH concentration, ng/ml^b</td>
<td>5.3</td>
<td>4.2</td>
<td>3.2</td>
<td>3.4</td>
<td>0.3</td>
</tr>
<tr>
<td>GH baseline, ng/ml^c</td>
<td>3.9</td>
<td>3.1</td>
<td>2.5</td>
<td>2.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of GH secretory periods^d</td>
<td>3.2</td>
<td>3.0</td>
<td>2.1</td>
<td>2.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean amplitude of GH secretory periods, ng/ml^e</td>
<td>11.4</td>
<td>8.0</td>
<td>6.8</td>
<td>6.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Number of GH spikes^f</td>
<td>4.9</td>
<td>4.9</td>
<td>3.5</td>
<td>3.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean amplitude of GH spikes, ng/ml^g</td>
<td>10.1</td>
<td>7.4</td>
<td>6.3</td>
<td>5.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

^a Average of 12-hr periods at 5, 8, 12, and 15 mo of age.

^b Overall GH concentration of bulls is different from all others at (P<0.02) and steers are different from heifers (P<0.05).

^c GH baseline in bulls is different from others (P<0.03).

^d Heifers have fewer secretory periods than bulls and steers (P<0.02).

^e Mean amplitude of GH secretory periods of bulls is different from all others (P<0.01).

^f Number of GH spikes of males is greater than females (P<0.02).

^g Mean amplitude of GH spikes of bulls is greater than others (P<0.01).
Table 3. Metabolic clearance rate, secretion rate and half-life of growth hormone in intact and castrated males and females

<table>
<thead>
<tr>
<th>Item</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic clearance rate, liter/(hr*kg) (^{a})</td>
<td>0.143</td>
<td>0.140</td>
<td>0.132</td>
<td>0.149</td>
<td>0.005</td>
</tr>
<tr>
<td>Secretion rate, ug/(hr*kg) (^{b})</td>
<td>0.791</td>
<td>0.640</td>
<td>0.450</td>
<td>0.513</td>
<td>0.046</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>15.0</td>
<td>15.8</td>
<td>15.5</td>
<td>15.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Average body weight, kg (^{c})</td>
<td>319</td>
<td>278</td>
<td>303</td>
<td>268</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^{a}\) Heifers are different from ovariectomized heifers (P<0.02).

\(^{b}\) Bulls are different from all other animals (P<0.03). Heifers are different from steers (P<0.01).

\(^{c}\) All groups are different from each other (P<0.02).
Table 4. Metabolic clearance rate, secretion rate and half-life of growth hormone in cattle at different ages

<table>
<thead>
<tr>
<th>Item</th>
<th>5 mo</th>
<th>8 mo</th>
<th>12 mo</th>
<th>15 mo</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic clearance rate, liter/(hr*kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.130</td>
<td>0.159</td>
<td>0.134</td>
<td>0.141</td>
<td>0.005</td>
</tr>
<tr>
<td>Secretion rate, ug/(hr*kg)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.695</td>
<td>0.737</td>
<td>0.506</td>
<td>0.456</td>
<td>0.046</td>
</tr>
<tr>
<td>Half-life, min&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.7</td>
<td>14.8</td>
<td>14.5</td>
<td>14.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Body weight, kg&lt;sup&gt;d&lt;/sup&gt;</td>
<td>139</td>
<td>222</td>
<td>367</td>
<td>440</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Metabolic clearance rate at 8 mo is greater than 5, 12 and 15 mo (P<0.02).

<sup>b</sup>Secretion rate at 5 and 8 mo is greater than 12 and 15 mo (P<0.0006).

<sup>c</sup>Half-life at 5 mo is faster than 8, 12 and 15 mo (P<0.008).

<sup>d</sup>All ages are different from each other (P<0.0001).
Response to injections of rhGHRF as the animals aged is listed in Table 5. The mean concentrations of GH before injections (20, 10 and 1 min; preGHRF) of 0.0165, 0.067 and 0.267 ug rhGHRF/kg bwt declined from concentrations above 5.0 ng/ml at 5 mo to concentrations between 2.0-3.0 ng/ml at 15 mo (P<0.02). The response (calculated from the average of 5, 10, 15, and 20 min samples after injection) to 0.0165 ug GHRF/kg bwt was greater at 5 mo than at 8 mo (P<0.01). However, the response at 5 mo was not different from the responses at 12 and 15 mo. At 5 mo, the GH response to 0.067 ug GHRF/kg bwt was greater than the responses at 8, 12 and 15 mo (P<0.01). A similar level of response was observed when 0.267 ug GHRF/kg bwt was administered at 5 mo. This level was also greater than the responses at 8, 12 or 15 mo (P<0.04).

GH response to rhGHRF in castrated and intact male and female cattle is summarized in Table 6. The bulls had a greater preGHRF concentration at the 0.0165-ug GHRF dose than females and a greater response to the dose than females (P<0.02). However, when these values were converted to a percentage change basis from the preGHRF period to the GHRF response period, there was no difference among bulls; the percentage increase among steers, 24%; heifers 52%; and ovariectomized heifers, 16%. The percentage change in GH concentration from the preGHRF to the 0.067-ug GHRF response was 142% in bulls, 78% in steers, 163% in heifers, and 139% in ovariectomized heifers. Bulls had a greater response to 0.267 ug GHRF/kg bwt than intact or ovariectomized heifers (P<0.02). The percent increase from 0.267 preGHRF to 0.267 GHRF
response in bulls, however, was 198%, and was not different from intact (146%) and ovariectomized (204%) heifers.

Over both sexes, castration did not have an effect on GH profiles, metabolic parameters or GH response to GHRF. When comparing intacts and castrates of the same sex, castrate males had lower GH baseline, GH amplitude of secretory periods and spikes, and GH SR/kg bwt than intact males, and ovariectomized heifers had higher MCR/kg bwt than intact heifers.
Table 5. Response to growth hormone-releasing factor in cattle of different ages

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>Age</th>
<th>5 mo</th>
<th>8 mo</th>
<th>12 mo</th>
<th>15 mo</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0165</td>
<td>PreGHRF&lt;sub&gt;a,b&lt;/sub&gt;</td>
<td>5.8</td>
<td>3.6</td>
<td>4.5</td>
<td>2.9</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GHRF&lt;sub&gt;c,d&lt;/sub&gt;</td>
<td>6.1</td>
<td>3.9</td>
<td>5.1</td>
<td>4.7</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>0.067</td>
<td>PreGHRF&lt;sub&gt;a,e&lt;/sub&gt;</td>
<td>5.1</td>
<td>4.4</td>
<td>4.5</td>
<td>2.7</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GHRF&lt;sub&gt;c,f&lt;/sub&gt;</td>
<td>14.3</td>
<td>6.5</td>
<td>9.2</td>
<td>8.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>0.267</td>
<td>PreGHRF&lt;sub&gt;a,e&lt;/sub&gt;</td>
<td>5.2</td>
<td>3.6</td>
<td>3.0</td>
<td>2.3</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GHRF&lt;sub&gt;c,g&lt;/sub&gt;</td>
<td>14.2</td>
<td>9.7</td>
<td>7.8</td>
<td>5.8</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean of 20, 10 and 1 min before GHRF injection.

<sup>b</sup>GH at 5 mo is different from 8 and 15 mo (P<0.02).

<sup>c</sup>Mean of 5, 10, 15, and 20 min after GHRF injection.

<sup>d</sup>Plasma GH response at 5 mo is different from 8 mo (P<0.01).

<sup>e</sup>Plasma GH at 5 mo is different from 15 mo (P<0.02).

<sup>f</sup>Plasma GH response at 5 mo is different from 8, 12 and 15 mo (P<0.01).

<sup>g</sup>Plasma GH response at 5 mo is different from 8, 12 and 15 mo (P<0.04).
Table 6. Response to growth hormone-releasing factor in castrated and intact male and female cattle

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0165</td>
<td>PreGHRFa,b</td>
<td>5.9</td>
<td>4.2</td>
<td>2.9</td>
<td>3.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>GHRFc,d</td>
<td>5.9</td>
<td>5.2</td>
<td>4.4</td>
<td>4.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.067</td>
<td>PreGHRFa,e</td>
<td>4.3</td>
<td>5.4</td>
<td>4.0</td>
<td>3.1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>GHRFc</td>
<td>10.4</td>
<td>9.6</td>
<td>10.5</td>
<td>7.4</td>
<td>1.4</td>
</tr>
<tr>
<td>0.267</td>
<td>PreGHRFa,f</td>
<td>4.5</td>
<td>4.2</td>
<td>2.8</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>GHRDb,c,f</td>
<td>13.4</td>
<td>9.5</td>
<td>6.9</td>
<td>7.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

aMean of 20, 10 and 1 min before GHRF injection.

bBulls are different from heifers and ovariectomized heifers (P<0.02).

cMean of 5, 10, 15, and 20 min after GHRF injection.

dFemales are different from males (P<0.03).

eSteers and ovariectomized heifers are different (P<0.04).

fOnly two animals per group.
DISCUSSION

The data presented here are part of a more extensive study of growing male and female cattle, both intact and castrated, in which the GH status as well as steroid and metabolic hormones and changes in body composition were examined as the cattle aged. The overall purpose of these studies was to define endocrine relationships related to growth as influenced by sex, castration and age.

Plasma levels of GH were quite low in the present study which may have been related to the nutritional regime. Breier et al. (5) reported that at high levels of feeding, GH peaks were of low amplitude and short duration in cattle, whereas, at medium and low planes of nutrition, GH peaks were high with multiple-phasic release and slow decay. Thus, the low amplitude of the few GH secretory periods in this study may have resulted from feeding cattle the high energy diet at a high level of intake.

The different diet that the animals received at 5 mo and the rest of the experiment may have caused differences in the MCR and half-life of GH which occurred during those periods. At 5 mo, the calves were nursing their dams. When being sampled at 5 mo, the calves did not have access to their dams and may be considered to have been fasting. At 8, 12 and 15 mo, the cattle had access to their diet during the experiment and were, therefore, in a fed state. Trenkle reported that fasting ruminants had longer GH half-life and lower MCR (12). Therefore, differences in MCR and half-life of GH between 5 mo and 8, 12
and 15 mo may not have been age related, but may have been due to nutritional intake differences.

With aging, overall GH concentration in plasma significantly declined in cattle. This was a result of a decline in baseline GH, number of spikes of GH, amplitude of GH spikes, and decreased SR on a kg bwt basis. The decrease of GH with age has been observed previously in cattle (20-21). The decline in SR/kg bwt has also been observed in cattle (21) and pigs (22), and may be the result of less pituitary tissue per unit of body weight with increase in body size.

The declining number and amplitude of GH spikes and secretory periods with increasing age may have been caused by decreased pituitary responsiveness to GHRF or less pituitary per unit of body weight. The GH response to all doses of GHRF was greater at 5 mo than 8, 12 or 15 mo. This was similar to the results of another study with sheep in which basal GH and GH response to GHRF decreased with age (23). In humans, the GH response to GHRF was shown to peak between 20-30 years and then decline after 40 years of age (24). On the other hand, the differences in GH response to GHRF between 5 mo and 8, 12 and 15 mo, may have been related to nutrition. Because the calves at 5 mo could be considered fasting, they may have responded in a similar manner to sheep on a restricted plane of nutrition injected with GHRF in which the GH pulses were more persistent than sheep with free access to food (25).

Whereas steers and intact and ovariectomized heifers gradually lost their episodic secretion of GH during aging, bulls maintained secretory periods of high amplitude at 15 mo. Intact male cattle have been
reported to maintain periodicity of GH secretion at 2, 3 and 5 years (1). This may be due to high androgen concentrations in bulls, because androgens have been related to increased amplitude of GH (26), increased overall GH secretion (2, 27), enhanced response of pituitary to other stimuli (27), and increased GH content of the pituitary (28). Bulls had a higher concentration of total estrogens in their plasma than the other groups (29) which may have been caused by aromatization of testosterone in bulls (30). High concentrations of endogenous estrogens may have stimulated GH secretion similar to exogenous estrogen administration. Exogenous estrogens in ruminants have been shown to enhance GH secretion by an increased frequency of GH secretory periods (4), and Trenkle (31) concluded that in cattle, estrogen increased pituitary size, GH content of the pituitary per unit bwt and GH in the plasma.

The difference in GH secretory patterns between bulls and steers was in agreement with other studies (2, 9). Males had more periods of secretion and spikes of GH than females. Bulls had a faster SR without an increase in MCR while intact heifers had a faster MCR with a decreased SR. The faster MCR and lower levels of GH of heifers were similar to results reported by Trenkle (11). Trenkle (32) also noted that changes in plasma hormone concentration were affected more by SR than MCR.

The response of GH to GHRF is unclear, some studies detected no differences in the GH response in humans between males and females, whereas others have shown that high doses of GHRF caused males to have a greater GH response than females (33-34). The sex differences observed
in this experiment were dependent upon the dose of GHRF administered: with the low dose, males responded with more GH release to GHRF administration than females; with the medium dose, no difference in the GH response was observed; and with the high dose, bulls secreted more GH than the other animals. Greater GH response to GHRF has been seen in 60-day-old male rats compared with female rats (35), but this sex difference in GH response was absent in 30-day-old rats. When testosterone treatment was given to intact and gonadectomized 60-day-old male rats, the GH response to GHRF again increased (35). Evans et al. (36) demonstrated in vitro that testosterone but not estradiol, enhanced the GHRF-mediated GH release by perifused male rat pituitary cells. Webb et al. (37), noted that estrogens did not affect basal GH or GHRF response but increased net synthesis of GH in the pituitary without affecting GH release. It has been suggested that estrogens play a minor role in the modulation of GH response to GHRF, whereas testosterone may increase pituitary cell content and enhance the GH response to GHRF in males (35-36). Hoeffler and Frawley (38) observed that the sex differences of GH release was due to differences in the secretory capacity of individual somatotropes rather than differences in the number of GH cells in the pituitary of rats.
LITERATURE CITED


SECTION V. METABOLIC HORMONES IN THE PLASMA OF GROWING MALE AND FEMALE, INTACT AND CASTRATED, CATTLE
Metabolic hormones in the plasma of growing male and female, intact and castrated, cattle\(^1,2\)

Cathie A. Plouzek and Allen Trenkle

\(^1\)Journal Paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project 2644).

\(^2\)Acknowledgments: We thank Joan Rettig, John Lawrence, Rod Berryman, and Carl Johnson for technical assistance.
ABSTRACT

The influence of age, sex and castration on plasma concentrations of metabolic hormones related to growth were examined in beef cattle. Plasma was sampled from four bulls, four steers, four heifers, and four ovariectomized heifers at 20-min intervals for 12 hr at 5, 8, 12, and 15 mo of age. Plasma was composited for analysis of somatomedin-C (Sm-C), testosterone, total estrogens, thyroxine, triiodothyronine, insulin, and glucose. Sm-C increased in the plasma from 5 to 12 mo of age (P<0.0001). Bulls had greater levels of Sm-C than steers, heifers or ovariectomized heifers (P<0.0001). In bulls, testosterone increased from 0.2 ng/ml at 5 mo to 5.6 ng/ml at 15 mo (P<0.0002). Total estrogens were highest in bulls (P<0.002), and were elevated in all animals during the summer months when they were 5 and 15 mo of age (P<0.0005). Triiodothyronine concentration was greater in ovariectomized heifers than bulls (P<0.004) or steers (P<0.04). Triiodothyronine was also increased at 8 and 12 mo (P<0.0001), which coincided with winter and early spring. Females had higher concentrations of thyroxine than males (P<0.002). At 5 mo, the thyroxine level was less than at any other age (P<0.001). Insulin and glucose levels were not influenced by sex, castration or time of feeding. Insulin increased with age (P<0.001), whereas plasma glucose was elevated at 8 and 12 mo of age (P<0.004). Age or sexual maturation influenced the levels of metabolic hormones. High concentrations of
testosterone and its metabolites were associated with greater concentrations of Sm-C and total estrogens.
INTRODUCTION

Endocrine regulation of growth is a balance of complex interactions of several hormones and their receptors. Growth hormone (GH) and somatomedin (Sm-C) are often considered the principal hormones regulating growth. However, GH and Sm-C have limited influence on growth without interaction with thyroid hormones, insulin, estrogens, androgens, and glucocorticoids. Some characterization of these endocrine relationships as cattle and sheep mature has been reported (1-8). However, these studies were limited to either one sex or did not compare intact and castrated animals (1-8). Furthermore, most of the studies examined only a few of the growth-related hormones or sampled blood infrequently, so that the overall endocrine pattern associated with growth was lost (1-5).

The purpose of this study was to assess the influence of age, sex and castration on plasma concentrations of Sm-C, testosterone, estrogen, thyroxine, triiodothyronine, and insulin in cattle.
MATERIALS AND METHODS

The animals used in the study have been previously described (9). Briefly, bulls, steers, heifers, and ovariectomized heifers, four of each, were sampled for plasma hormone concentrations at 5, 8, 12, and 15 mo of age. Blood samples were drawn via an indwelling jugular catheter every 20 min over 12 hr. Aliquots of the plasma were composited at 1-, 4- or 12-hr intervals. Sm-C was measured in the 12-hr composite using a double antibody radioimmunoassay (Immuno Nuclear Corp., Stillwater, MN) as modified by Plouzek and Trenkle (10). All samples were assayed at one time and had an intraassay coefficient of variation (COV) of 0.8%. Total estrogens were assayed using a double antibody radioimmunoassay (Radioassay Systems Laboratories, Inc., Carson, CA) on the 12-hr composite. The intraassay COV was 2.1%. Testosterone was measured in the 4-hr composites using a solid-phase radioimmunoassay that used antibodies covalently bound to the inner surface of plastic tubes (Immuno Nuclear Corp., Stillwater, MN) with an intraassay COV of 3.3%. Because the 3 composited samples did not differ, the results of the testosterone assay were averaged for statistical analysis. The 4-hr composite of plasma was used for triiodothyronine and thyroxine analysis by solid-phase radioimmunoassay in which the antibodies were covalently bound to polymer particles (Amersham Corp., Arlington Heights, IL) and had intraassay COV of 3.7% and 4.1%, respectively. In all hormone assays, bovine plasma paralleled the assay standards. Plasma insulin (11) and glucose (Worthington Diagnostics, Inc., Freehold, NJ) were
analyzed using the hourly composites. Insulin had an interassay COV of 5.7% and an intraassay COV of 5.3%. The interassay and intraassay COVs for plasma glucose were 0.9% and 1.1%, respectively. The results from the hourly analyses of insulin and glucose were averaged over 4-hr periods to evaluate levels before and after feeding. Animals were fed during the 5th hr of the 12-hr sampling periods. A split-plot model (12) of the general linear model procedure of SAS (13) was used to analyze the endocrine data.
RESULTS

Plasma Sm-C concentrations were significantly affected by sex and age of the animal (Table 1). Bulls had significantly higher concentrations of Sm-C than other animals ($P<0.0001$). The increase in Sm-C between 5 and 8 mo was greater in bulls than in other animals. This difference in bulls was maintained at 12 and 15 mo. Sm-C levels increased in all animals from 5 to 8 mo ($P<0.0001$) and from 8 to 12 mo ($P<0.0001$), but did not change between 12 and 15 mo. Due to higher Sm-C concentrations in bulls, males had greater Sm-C concentrations than females ($P<0.0001$), and intact animals had higher Sm-C concentrations than castrated cattle ($P<0.0001$).

Bulls had measurable testosterone levels at 5 mo (Table 2), which increased at 8 mo ($P<0.001$) and 12 mo ($P<0.0002$). Testosterone concentrations in bulls leveled off between 12 and 15 mo. Steers, heifers and ovariectomized heifers only had trace concentrations of testosterone.

Sex and castration affected plasma concentrations of total estrogens, triiodothyronine and thyroxine (Table 3). Over all ages, bulls had higher mean concentrations of total estrogens ($P<0.02$). Total estrogen concentrations were 9.6 and 7.9 pg/ml in males and females, respectively ($P<0.0001$), and 9.4 and 6.5 pg/ml in intact and castrated animals, respectively ($P<0.001$).
Table 1. Somatomedin-C concentrations at different ages in intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Age</th>
<th>Bulls a</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariec-tonized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 b</td>
<td>6.4</td>
<td>5.3</td>
<td>7.2</td>
<td>8.2</td>
<td>1.5</td>
</tr>
<tr>
<td>8 b</td>
<td>21.8</td>
<td>11.0</td>
<td>14.6</td>
<td>13.0</td>
<td>1.5</td>
</tr>
<tr>
<td>12 c</td>
<td>31.1</td>
<td>20.2</td>
<td>16.8</td>
<td>14.5</td>
<td>1.5</td>
</tr>
<tr>
<td>15 c</td>
<td>28.0</td>
<td>19.5</td>
<td>13.6</td>
<td>14.4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a Mean somatomedin-C concentration over the four ages of bulls (21.8 nMol/liter) is greater than mean somatomedin-C concentrations of steers, heifers and ovariectomized heifers (14.0, 13.0, and 12.5 nMol/liter, respectively, SE=0.8) (P<0.0001).

b Mean somatomedin-C concentrations over the four groups at 5 mo (6.8 nMol/liter) are less than 8 mo (15.1 nMol/liter) (P<0.0001).

c Mean somatomedin-C concentrations over the four groups at 12 and 15 mo (20.6 and 18.9 nMol/liter, respectively) are similar to each other but different from 5 mo (6.8 nMol/liter), (P<0.0001), and 8 mo (15.1 nMol/liter), (P<0.0001).

Triiodothyronine concentrations were highest in ovariectomized heifers (P<0.04). Castrated animals had greater triiodothyronine concentrations than intacts (P<0.04); and females had higher concentrations than males (P<0.001). Thyroxine concentrations were not affected by castration but were significantly higher in females than males (P<0.002).
Table 2. Testosterone concentrations at different ages in intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Bulls&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.15</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>8</td>
<td>3.72</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.29</td>
</tr>
<tr>
<td>12</td>
<td>5.45</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>15</td>
<td>5.64</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.29</td>
</tr>
</tbody>
</table>

<sup>a</sup>Testosterone concentrations of bulls at 8, 12, and 15 mo are different than all other animals at all ages (P<0.001). Testosterone level at 8 mo in bulls differs from 12 and 15 mo (P<0.0002).

The age of the animals or the season affected concentrations of total estrogens, triiodothyronine and thyroxine (Table 4). Total estrogens were higher at 5 and 15 mo which coincided with summer than at 8 and 12 mo, the winter and spring months (P<0.0005). Triiodothyronine concentrations were higher at 8 and 12 mo and lower at 5 and 15 mo (P<0.0001). At 5 mo, thyroxine was 7.0 ug/dl, which was increased at 8, 12 or 15 mo (P<0.0001).
Table 3. Total estrogens, triiodothyronine and thyroxine concentrations intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total estrogens, pg/ml</td>
<td>11.9</td>
<td>7.2</td>
<td>8.5</td>
<td>7.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Triiodothyronine, ng/ml</td>
<td>1.5</td>
<td>1.7</td>
<td>1.7</td>
<td>2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Thyroxine, ug/dl</td>
<td>7.9</td>
<td>8.6</td>
<td>11.0</td>
<td>11.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a Concentrations of total estrogens of bulls are greater than other animals (P<0.002).
b Triiodothyronine concentrations of ovariectomized heifers are greater than bulls (P<0.004) or steers (P<0.04).
c Thyroxine concentrations of females are greater than males (P<0.002).

Age, but not the sex of the animal, affected insulin and glucose concentrations (Table 5). Significantly lower levels of insulin were found at 5 mo than at 8, 12 or 15 mo (P<0.009). The difference at 5 mo may have been due to differences in diet, since the animals were still nursing at 5 mo of age. The insulin levels before and after feeding were not different. Plasma glucose concentrations were similar at 8 and 12 mo, but higher than concentrations at 5 or 15 mo (P<0.04). Glucose concentrations dropped 0-4 hr after feeding and then returned at 4-8 hr after feeding to levels similar to those before feeding. The glucose:insulin ratio steadily declined from 3.39 at 5 mo to 1.05 at 8 mo, 1.14 at 12 mo, and 0.76 at 15 mo.
Table 4. Total estrogens, triiodothyronine and thyroxine concentrations in cattle at different ages

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Age</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mo</td>
<td>8 mo</td>
<td>12 mo</td>
<td>15 mo</td>
<td>SE</td>
</tr>
<tr>
<td>Total estrogens, pg/ml</td>
<td>11.1</td>
<td>7.3</td>
<td>5.9</td>
<td>10.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Triiodothyronine, ng/ml</td>
<td>1.4</td>
<td>2.1</td>
<td>2.1</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Thyroxine, ug/dl</td>
<td>7.0</td>
<td>10.5</td>
<td>11.3</td>
<td>10.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^a\)Concentrations of total estrogens at 5 and 15 mo are greater than those at 8 and 12 mo (P<0.0005).

\(^b\)Triiodothyronine concentrations at 5 and 15 mo are less than those at 8 and 12 mo (P<0.0001).

\(^c\)Thyroxine concentration at 5 mo is less than the following ages (P<0.001).
Table 5. Insulin and glucose concentrations before and after feeding in cattle at different ages<sup>a</sup>

<table>
<thead>
<tr>
<th>Period</th>
<th>5 mo</th>
<th>8 mo</th>
<th>12 mo</th>
<th>15 mo</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, ng/ml&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32</td>
<td>0.76</td>
<td>0.80</td>
<td>1.28</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose, mg/dl&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.4</td>
<td>93.3</td>
<td>90.8</td>
<td>85.8</td>
<td>1.7</td>
</tr>
<tr>
<td>0-4 hr Postfeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, ng/ml&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.82</td>
<td>0.77</td>
<td>1.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose, mg/dl&lt;sup&gt;e&lt;/sup&gt;</td>
<td>73.7</td>
<td>86.9</td>
<td>87.6</td>
<td>80.1</td>
<td>1.4</td>
</tr>
<tr>
<td>4-8 hr Postfeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, ng/ml&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.19</td>
<td>1.06</td>
<td>0.79</td>
<td>0.99</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose, mg/dl&lt;sup&gt;g&lt;/sup&gt;</td>
<td>74.0</td>
<td>90.8</td>
<td>90.9</td>
<td>83.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Intact and castrated male and female values were not different.

<sup>b</sup>Insulin at 5 mo is less than any other age (P<0.0001), while at 15 mo is greater than any other age (P<0.0001).

<sup>c</sup>Glucose at 8 and 12 mo differs from 5 mo (P<0.001) and 15 mo (P<0.04).

<sup>d</sup>Insulin at 5 mo is less than any other age (P<0.001), while at 15 mo is greater than any other age (P<0.02).

<sup>e</sup>Glucose at 8 and 12 mo is similar, but all other values differ from each other (P<0.0002).

<sup>f</sup>Insulin at 8 and 15 is the same, however, all other values differ from each other (P<0.009).

<sup>g</sup>Glucose at 8 and 12 mo is similar, but all other values differ from each other (P<0.004).
DISCUSSION

The data presented here are part of an extensive study of growing intact and castrated male and female cattle in which GH secretion patterns, GH secretion rates, GH metabolic clearance rates, GH half-life, GH response to GH-releasing factor, Smíc, growth-related hormones, and changes in body composition were examined as the cattle aged.

Intact males had higher levels of sex steroids than steers, heifers or ovariectomized heifers. Bulls had the only measurable concentrations of testosterone, which increased from 5 mo to 12 mo and then leveled off. Others have observed an elevation in testosterone beginning at 6 to 8 mo (14). In that study, a rise in testosterone coincided with spermatogenesis which commenced around 5 mo (14). In our study, bulls also had elevated concentrations of total estrogens, which may have been due to the aromatization of testosterone (15). Both testosterone and estrogens have been shown to increase GH secretion (16-21). In the previous paper (9), it was shown that as these bulls aged they maintained a greater baseline GH concentration in the plasma with more secretion periods of greater amplitude than steers, heifers or ovariectomized heifers. The high frequency and amplitude of GH secretory periods and spikes at 12 and 15 mo of age observed only in bulls may have been due to higher testosterone concentrations in the plasma or due to the combined effects of testosterone and estrogen.
Many of the effects of GH on growth have been considered to be mediated through Sm-C (16, 22-24). In the present study, we observed that bulls had higher concentrations of Sm-C, which increased dramatically from 5 to 8 mo and continued to increase up to 12 mo of age. Sm-C concentrations increased in all animals up to 12 mo while plasma concentrations of GH declined. In humans, Sm-C also increased with age while GH declined (16, 24). In bulls, the episodic pattern of GH secretion that was sustained during aging may have been responsible for the elevated Sm-C concentrations. Several authors have suggested that the greater Sm-C levels were more related to sexual maturity than to the aging process (24-25). The changes in Sm-C concentrations in the plasma of bulls were similar to changes in testosterone. In human, Sm-C levels have been observed to increase rapidly in prepubertal boys that were able to secrete GH (26-27). In ruminants, elevated concentrations of Sm-C have been associated with faster growing breeds of cattle and sheep (23) and adequate nutrition (28).

The sex steroids have also been associated with alterations in thyroid hormone status of animals (15, 20-21). Thyroxine levels were higher in females than male cattle and triiodothyronine concentrations were elevated in ovariectomized heifers. It has been reported that testosterone and estrogens decreased concentrations of free thyroid hormones in the plasma which, in turn, decreased the energy requirement because of decreased protein turnover (21). Elevation of triiodothyronine in ovariectomized heifers may have been caused by low concentrations of sex steroids in plasma.
Insulin and plasma glucose concentrations in plasma were not altered by castration, or between sexes. Similar insulin concentrations have been observed between steers and heifers, as well as between bulls and steers (29). Although insulin has been associated with increased amino acid uptake, protein synthesis and Sm-C secretion, and decreased proteolysis to enhance growth (16, 29-30), the authors of this study did not observe differences in insulin between the sexes in the study to account for differences in growth.

Insulin levels increased with age in the cattle, while plasma glucose concentrations were elevated at 8 and 12 mo of age. The insulin increase with age in the present study was similar to other studies in ruminants in which insulin increased with increasing body weight (7-8, 16, 31-32). The glucose:insulin ratio decreased as the animals aged in this study, indicating that more insulin was required to metabolize the same quantity of glucose. It has been suggested that increased insulin during aging may be the result of decreased metabolic clearance rate, decreased extracellular fluid volume, decreased insulin receptor binding, or reduced potency of insulin degradation in cells as body weight increases (31).

Plasma concentrations of insulin were not altered by feeding in this experiment. The animals normally had free access to their feed. Ruminants that had free access to feed had fewer changes in plasma insulin and glucose levels as well as decreased GH oscillations (33-34). In contrast, when ruminants are meal fed once or twice daily, insulin
increased 2 hr after feeding and peaked within 4 hr and glucose increased 4 to 6 hr after feeding (31, 35-38).

Several hormone concentrations may have been confounded by age and season. The warm months of summer and fall occurred when the cattle were 5 and 15 mo of age, whereas cattle 8 and 12 mo in age were tested in winter and spring. At 8 and 12 mo, total estrogen levels were low, while triiodothyronine and glucose were high in the cattle. Anderson et al. (39) also observed increased triiodothyronine in growing cattle in winter than in summer. However, Verde and Trenkle (8) did not observe seasonal cycles of thyroid hormones in cattle.
LITERATURE CITED


SECTION VI. EFFECT OF AGE, SEX AND CASTRATION ON BODY COMPOSITION DURING AGING: INFLUENCE OF ENDOCRINE CHANGES ON BODY COMPOSITION
Effect of age, sex and castration on body composition during aging: Influence of endocrine changes on body composition

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2Acknowledgments: We thank Joan Rettig, Ralph Arnold, John Lawrence, Rod Berryman, and Carl Johnson for technical assistance.
ABSTRACT

The influence of age, sex, and castration on body composition in beef cattle was studied in bulls, steers, heifers, and ovariectomized heifers at 8, 12 and 15 mo of age. Body composition was determined by use of dilution of deuterium oxide. Between 8 and 12 mo of age, the rate of daily protein and fat deposition (kg/d) was: heifers, 0.12, 0.52; steers, 0.16, 0.42; ovariectomized heifers, 0.15, 0.40; and bulls, 0.21, 0.31. Bulls had different amounts of both protein and fat gain than the other animals. Differences in percent body protein, water, fat, and ash between 8 and 12 mo were associated with a decrease in growth hormone (GH) secretion rate/kg body weight (SR/kg bwt). Between 12 and 15 mo of age, the rate of daily protein and fat deposition was: ovariectomized heifers, 0.05, 0.65 kg; steers, 0.13, 0.54 kg; bulls, 0.15, 0.50 kg; and heifers, 0.08, 0.46 kg. Daily protein deposition was higher in bulls and steers than intact or ovariectomized heifers. An increase in plasma insulin was associated with an increased rate of fat gain between 12 and 15 mo of age. Overall GH concentrations over time were negatively related to percent empty body fat and positively related to percent empty body protein as the cattle aged. GH SR/kg bwt was positively related to percent body protein and negatively related to percent empty body fat during aging, and kidney, heart and pelvic fat, subcutaneous fat over the ribs and yield grade of the carcasses at 15 mo. The GH:insulin ratio was positively associated with percent empty body protein and negatively associated with percent empty body fat and
carcass kidney, heart and pelvic fat. GH and insulin had the strongest relationships with rates of protein and fat deposition.
INTRODUCTION

Growth of animals is characterized by an increase in tissue and organ mass. Because bone, muscle and fat grow at different rates (1), body composition varies according to the stage of development. Utilization of nutrients for synthesis of body tissues can be altered by factors such as nutrient intake, breed, sex, and age. Because the administration of growth hormone (GH) to domestic animals increased lean carcass mass (2-3), some studies have examined the association between endogenous GH concentration and body composition (4-6). In cattle, GH secretion rate (SR) has been positively correlated with growth of lean tissue, while negatively correlated with carcass adipose (4). However, this study was only conducted with steers and a study with male and female cattle was inconclusive (5). Differences in GH concentrations existed between intact and castrate male ruminants (7) as well as between males and females (8). Also, the body composition of bulls, steers, heifers, and ovariectomized heifers was shown to be different at slaughter (9). Therefore, the relationship between body composition and GH needs to be compared in intact and castrated male and female cattle. In some cases, positive relationships between insulin concentrations and adipose deposition have been observed in cattle (4-5), whereas other studies have failed to substantiate this (10). Somatomedin-C (Sm-C), thyroid hormones and sex steroids are required for stimulation of muscle growth but their relationships with body composition in cattle have not been established.
In order to resolve some of the endocrine correlations with body protein and fat deposition during growth, bulls, steers, heifers, and ovariectomized heifers at three stages of development served as models to study body composition changes as well as the relationships existing between empty body composition and measurements of growth-related hormones.
MATERIALS AND METHODS

The animals and diet used in this study have been described elsewhere (11). Individual feed intake was recorded daily and animals were weighed every 14 days from 10 to 15 mo of age. Bulls, heifers, steers, and ovariectomized heifers, four of each, were injected via an indwelling jugular catheter with 0.15 g deuterium oxide (D2O)/kg bwt followed by 5 ml of sterile saline. Injections were given at 8, 12 and 15 mo of age. A 2-ml blood sample was withdrawn to clear the catheters before taking 10-ml samples at 4, 7, 10, 14, 24, 30, 36, 48, 72, and 120 hr after D2O injection. Blood samples were placed in dry, screw-top culture tubes containing 20 U of heparin and stored at 4°C for analysis. Water from the samples was isolated by vacuum sublimation and assayed for D2O by infrared spectrophotometry as described by Arnold et al. (12). A single-compartment model (13) was used to estimate empty body composition of cattle from concentrations of D2O in blood water.

Animals were slaughtered at 15 mo of age in a commercial abattoir 3 weeks after the last D2O injection. Quality grades and percent kidney, heart and pelvic (KHP) fat of each carcass were determined by an official of the United States Department of Agriculture. All measurements except carcass weight were taken after chilling for 24 hr. Yield grade was calculated using an equation described by Rust (14):

yield grade = 2.5 + 2.5(Ribfat, inches) + 0.2(KHP fat) + 0.0038 (hot carcass weight, pounds) - 0.32(Ribeye area, inches).
Data were analyzed using a split-plot model (15) of the general linear models procedures of SAS (16). Linear regressions were calculated over time to determine the relationship between hormone concentrations and body composition in the cattle. The corresponding hormone concentrations and percent empty body fat, protein, water, and ash at each age for all groups of cattle were regressed.
RESULTS

Both sex and castration influenced daily gain and feed intake (Table 1). Between 10 and 12 mo of age, heifers gained less than bulls, steers or ovariectomized heifers (P<0.04) despite the fact that feed intake for heifers was similar to bulls and greater than steers (P<0.03). Heifers had the greatest feed requirement per kg of gain (P<0.0003). Between 12 and 15 mo of age, heifers and ovariectomized heifers gained less than steers (P<0.04) and bulls (P<0.01). Heifers (P<0.008) and ovariectomized heifers (P<0.02) consumed less than bulls during this period. The feed:gain ratio was not altered by sex or castration over the 12 to 15 mo period.

Changes in empty body composition between 8 and 12 mo were affected by sex and castration (Table 2). At 8 mo and a body weight of 239 kg, bulls were composed of 21.7% fat, 17.4% protein and 3.9% ash on an empty body basis. Steers and ovariectomized heifers were not different from bulls at 8 mo, being 22.3% and 23.7% fat, 17.3% and 17.1% protein, and 3.9% and 3.8% ash at body weights of 200 and 203 kg, respectively. At 8 mo, heifers (247 kg) differed from steers (P<0.009) and bulls (P<0.003) in percent fat (26.0%), protein (16.7%) and ash (3.8%). Bulls had a greater rate of gain between 8 and 12 mo than heifers (P<0.03) and ovariectomized heifers (P<0.05). Fat gain was the greatest for heifers (52% empty body weight gain) followed by steers and ovariectomized heifers (38% empty body weight gain), and then bulls (25% empty body weight gain). The high fat gain in heifers was offset by lower gains of
water, protein and ash. Water gain was the greatest in bulls (P<0.02), and steers gained more water than heifers (P<0.03). Protein and ash gain were highest in bulls (P<0.03). Steers had greater daily gains of protein and ash than heifers (P<0.05).

Sex and castration influenced the gain of water, protein, fat, and ash between 12 and 15 mo (Table 3). Bulls and steers had higher average daily gains than heifers (P<0.01 and P<0.03, respectively) and ovariectomized heifers (P<0.02 and P<0.04, respectively). Daily water gain was the lowest in ovariectomized heifers and was lower than bulls (P<0.0008) and steers (P<0.004). Heifers also had less water gain than bulls (P<0.01). Daily gain of fat was not different between bulls, steers, heifers, or ovariectomized heifers, however, the percent of the daily gain deposited as fat was 44% in bulls, 50% in steers, 61% in heifers, and 83% in ovariectomized heifers. Protein gains were lower in females than bulls (P<0.006) or steers (P<0.03). Ash gains were also lower in females than bulls (P<0.0007) or steers (P<0.004).

Carcass measurements were affected by sex and castration (Table 4). Live weight of ovariectomized heifers was less than males (P<0.02). Hot carcass weight was less for ovariectomized heifers than bulls (P<0.004) and heifers (P<0.04). Bulls had larger ribeye areas than steers and ovariectomized heifers (P<0.03). Bulls had less ribfat (P<0.02) and KHP fat (P<0.01) than steers, heifers or ovariectomized heifers. Heifers had the greatest amount of subcutaneous fat over the ribs (P<0.04). They also had greater amounts of KHP fat than steers (P<0.01). Bulls had lower quality (P<0.005) and yield grades (P<0.02) than steers,
heifers or ovariectomized heifers. Dressing percentage was not different between sexes or between intact and castrated animals.

Table 1. Daily feed intake, body weight gain and feed:gain ratio in intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Period</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-12 mo</td>
<td>1.49</td>
<td>1.48</td>
<td>1.10</td>
<td>1.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Average gain, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed intake, kg/day</td>
<td>9.3</td>
<td>8.1</td>
<td>9.6</td>
<td>8.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Feed/gain</td>
<td>6.3</td>
<td>5.5</td>
<td>8.3</td>
<td>6.4</td>
<td>0.3</td>
</tr>
<tr>
<td>12-15 mo</td>
<td>1.17</td>
<td>1.10</td>
<td>0.76</td>
<td>0.81</td>
<td>0.1</td>
</tr>
<tr>
<td>Average gain, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed intake, kg/day</td>
<td>11.4</td>
<td>10.0</td>
<td>9.0</td>
<td>9.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Feed/gain</td>
<td>9.8</td>
<td>9.6</td>
<td>11.7</td>
<td>12.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

aHeifers gained less than any other group (P<0.04).
bSteers ate less than heifers (P<0.03) and bulls (P<0.05).
cHeifers had the highest feed:gain of all animals (P<0.0003).
dHeifers and ovariectomized heifers had less gain than steers (P<0.04) and bulls (P<0.01).

eBulls ate more than heifers (P<0.008) and ovariectomized heifers (P<0.02).
Table 2. Changes in empty body gain between 8 and 12 mo in intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Item</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily gain, kg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24</td>
<td>1.11</td>
<td>1.00</td>
<td>1.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Total weight gain, kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>164</td>
<td>147</td>
<td>133</td>
<td>137</td>
<td>8</td>
</tr>
<tr>
<td>Water gain, kg/day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.68</td>
<td>0.50</td>
<td>0.34</td>
<td>0.46</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat gain, kg/day&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.31</td>
<td>0.42</td>
<td>0.52</td>
<td>0.40</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein gain, kg/day&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.16</td>
<td>0.12</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Ash gain, kg/day&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.047</td>
<td>0.036</td>
<td>0.027</td>
<td>0.034</td>
<td>0.003</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bulls differ from heifers (P<0.03) and ovariectomized heifers (P<0.05).

<sup>b</sup>Empty body basis.

<sup>c</sup>Bulls differ from all other animals (P<0.02). Steers differ from heifers (P<0.03).

<sup>d</sup>Bulls differ from steers (P<0.04) and heifers (P<0.0009). Heifers differ from ovariectomized heifers (P<0.03).

<sup>e</sup>Bulls differ from all other animals (P<0.03). Steers differ from heifers (P<0.05).
Table 3. Changes in empty body gain between 12 and 15 mo in intact and castrated, male and female, cattle

<table>
<thead>
<tr>
<th>Item</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total weight gain, kg(^a)</td>
<td>89</td>
<td>84</td>
<td>59</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Water gain, kg/day(^b,c)</td>
<td>0.46</td>
<td>0.38</td>
<td>0.20</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Fat gain, kg/day(^b)</td>
<td>0.50</td>
<td>0.54</td>
<td>0.46</td>
<td>0.65</td>
<td>0.09</td>
</tr>
<tr>
<td>Protein gain, kg/day(^b,d)</td>
<td>0.15</td>
<td>0.13</td>
<td>0.08</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Ash gain, kg/day(^b,d)</td>
<td>0.034</td>
<td>0.030</td>
<td>0.017</td>
<td>0.012</td>
<td>0.004</td>
</tr>
</tbody>
</table>

\(^a\) Ovariectomized heifers differ from bulls (P<0.02) and steers (P<0.04). Heifers differ from bulls (P<0.01) and steers (P<0.03).

\(^b\) Empty body basis.

\(^c\) Ovariectomized heifers differ from bulls (P<0.0008) and steers (P<0.004). Heifers differ from bulls (P<0.01).

\(^d\) Ovariectomized heifers differ from bulls (P<0.0007) and steers (P<0.004). Heifers differ from bulls (P<0.006) and steers (P<0.03).
Table 4. Carcass measurements in intact and castrated, male and female, cattle at slaughter

<table>
<thead>
<tr>
<th>Item</th>
<th>Bulls</th>
<th>Steers</th>
<th>Heifers</th>
<th>Ovariectomized Heifers</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liveweight, kg(^a)</td>
<td>504</td>
<td>469</td>
<td>458</td>
<td>423</td>
<td>15</td>
</tr>
<tr>
<td>Carcass weight, kg(^b)</td>
<td>299</td>
<td>272</td>
<td>282</td>
<td>247</td>
<td>10</td>
</tr>
<tr>
<td>Ribeye area, cm(^2),(^c)</td>
<td>81</td>
<td>72</td>
<td>75</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>Ribfat, cm(^d)</td>
<td>0.7</td>
<td>1.2</td>
<td>1.7</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Kidney, heart and pelvic fat, %(^e)</td>
<td>1.4</td>
<td>2.5</td>
<td>3.6</td>
<td>3.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Quality grade(^f),(^g)</td>
<td>4.3</td>
<td>8.0</td>
<td>7.3</td>
<td>7.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Yield grade(^h)</td>
<td>1.9</td>
<td>2.9</td>
<td>3.5</td>
<td>2.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Dressing %</td>
<td>59</td>
<td>58</td>
<td>62</td>
<td>58</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^a\)Ovariectomized heifers weigh less than males (P<0.02).
\(^b\)Ovariectomized heifers weigh less than bulls (P<0.004) and heifers (P<0.04).
\(^c\)Bulls have a larger ribeye area than steers and ovariectomized heifers (P<0.03).
\(^d\)Bulls have less ribfat than all other animals (P<0.02), while heifers have more ribfat than any others (P<0.04).
\(^e\)Bulls have less kidney, heart and pelvic fat than other groups (P<0.01), while heifers have more than steers (P<0.01).
\(^f\)Choice+=9, choice=8, choice-=7, good+=6, good=5, and good-=4.
\(^g\)Bulls have a lower quality grade than any other group (P<0.005).
\(^h\)Bulls have a lower yield grade than any other group (P<0.02).
DISCUSSION

In this study, the rate of protein and fat deposition differed between bulls, steers, heifers, and ovariectomized heifers as they matured. These data corresponded with other studies, which showed that females fattened at lighter body weight than castrate or intact males (9, 17). This was most likely the result of higher testosterone in bulls, which has been reported to accelerate muscle growth by hypertrophy and possibly potentiating the effects of GH on muscular growth (18).

Between 8 and 12 mo, a significant decrease in GH SR occurred, whereas, between 12 and 15 mo, the concentration of plasma insulin significantly increased in all the animals. Meanwhile, composition of the gain changed from less protein and water to more fat from the 8 to 12 mo period to the 12 to 15 mo period. These endocrine changes as related to specific periods of aging and body composition changes have not previously been reported. The decline in GH SR between 8 and 12 mo of age may have been a signal to shift the type of tissue deposition from protein to fat, and the increase in insulin between 12 and 15 mo of age may have enhanced the rate of adipose deposition.

During the 8 to 15 mo period, several relationships were observed between the endocrine system and body composition in cattle. Overall GH concentrations and GH SR/kg body weight were negatively related to body fat (r=-0.82 and -0.90, respectively). GH SR/kg body weight at 15 mo also was negatively related to KHP fat, subcutaneous fat over the ribs
and yield grades \( r = -0.96, -0.99 \) and \( -0.98 \), respectively). These results were similar to earlier studies in which negative relationships between GH and GH SR/kg body weight with carcass adipose and fat thickness over the ribs were observed \( (4-5) \). Percent body protein was positively related to overall GH concentrations, GH metabolic clearance rate/kg body weight and GH SR/kg body weight \( (r = +0.82, +0.53 \) and \( +0.90 \), respectively), again similar to previous results \( (4) \). The relationships between GH and protein and fat deposition were in agreement with the accepted role of GH in stimulating protein synthesis and decreasing the quantity of adipose tissue.

An association between insulin and percent body protein \( (r = -0.41) \) and fat \( (r = +0.42) \) was observed in this study. It is known that insulin increases the cell size of skeletal muscle cells and GH is important for increasing the number of nuclei in muscle cells, both hormones enhancing amino acid uptake into the cells \( (19-20) \). The effect of GH:insulin ratio on growth has not been established, but a high ratio is usually associated with muscle deposition and a low ratio with fat deposition \( (21-22) \). Large breeds of cattle have more GH and less insulin, which is associated with prolonged growth of skeletal muscle and a delay in adipose tissue deposition \( (21) \). In the present study, the GH:insulin ratio was positively related to body protein \( (+0.82) \) and negatively related to percent body fat \( (+0.83) \) and KHP fat in the carcass \( (-0.99) \). Increased adipose deposition, which coincided with increased plasma insulin and decreased GH per unit of body tissue, was associated with increasing age, time and body weight.
Sm-C, reported to be positively associated with growth (23), had low correlations with changes in body composition between 8 and 15 mo of age in the present study. It was negatively related to percent body fat \( (r=-0.31) \) and positively related to protein \( (r=+0.31) \). It has been shown that GH actions on adipose were not mediated by Sm-C (24). Therefore, the low correlations between changes in body composition and plasma concentrations of Sm-C may have been due to adipose being the most rapidly growing tissue during the 8 to 15 mo period.

The different rates of fat and protein deposition between 8 to 12, and 12 to 15 mo of age may have influenced reductions in GH secretion. It is possible that the tissue being deposited at the highest rate may have had some feedback on endocrine glands. Several researchers have noted that GH release by GH-releasing factor was impaired during periods of obesity in humans (25-28). In rats, an elevation of free fatty acids stimulates somatostatin secretion which in turn suppressed secretion of GH by GH-releasing factor (28).


GENERAL DISCUSSION

Administration of growth hormone-releasing factor (GHRF) induced growth hormone (GH) secretion in ruminants (Sections I-IV). The animals responded to both single and multiple injections of GHRF with a release of GH, however, animals with an intact hypothalamic stalk did not always respond (Section II). In the hypothalamic stalk-transected (HST) calves, GHRF always induced GH secretion (Section III). Continuous infusion of somatostatin (SRIH) severely inhibited the GH response to GHRF after HST. Both SRIH and GHRF induced GH responses similar to those observed in the animals before surgery. Therefore, it was concluded that the pituitary was still capable of responding to stimuli. The major difference in response to SRIH infusion between the intact and HST calves, was the lack of somatotrophic rebound after SRIH infusion was discontinued in the HST calves. The differences in GH secretion in response to GHRF before and after HST may be that in HST animals, endogenous GHRF and SRIH were not acting on the pituitary prior to treatment with exogenous GHRF or SRIH. In the absence of endogenous GHRF, the HST pituitary did not have stored GH. Thus, after SRIH infusion, a somatotrophic rebound was not observed. Similarly, endogenous SRIH did not prevent the HST pituitary from releasing GH after an injection of GHRF. Without SRIH acting on the pituitary in HST animals, GH was released after every injection of GHRF. Therefore, intact animals are more likely to have variable responses to exogenous GHRF unless endogenous SRIH release is suppressed.
When GHRF was injected every 4 hr for 24 hr in sheep (Section I) or in cattle at 3-hr intervals for 42 hr (Section II), the amplitude of the GH response varied. The GH response to GHRF decreased after feeding and slowly increased with each injection of GHRF until the next feeding. This difference in amplitude of GH release induced by a similar dose of GHRF may be related to gut SRIH release. Exogenous SRIH suppressed the GH response to GHRF in calves (Sections II-III). In an additional study, the GH response to GHRF in sheep that were fed or had the cranial sac of the rumen artificially distended with a water-filled balloon to simulate gut fill caused by feeding, was suppressed compared to animals that were fasting (1). Based on these findings, it was concluded that SRIH or neural transmission from tension receptors in the cranial sac of the rumen transmitted via the vagus nerve (2) inhibited the ability of GHRF to release GH.

Higher concentrations of GH and GH secretion rates (SR) were associated with higher amounts of protein and less adipose deposition (Sections IV and VI). The GH profiles, rates of secretion and response to GHRF declined with increasing age. Intact males had higher GH SR and GH baseline with more periods of episodic secretion than the castrate males or females, or intact females. Bulls did not lose their episodic secretion of GH as they aged, whereas the other groups did. The decline in GH SR, GH profiles and GH response to GHRF as the animals aged may be related to the differences in nitrogen retention observed between older sheep and prepubertal calves when they were administered similar doses of GHRF at 4-hr intervals (Sections I-II). After 5 days of GHRF
treatment, the older sheep had a significant reduction of urinary nitrogen, indicating an increased postabsorptive metabolic effect on nitrogen. After 5 or 10 days of GHRF treatment, prepubertal bull calves did not have significant improvements in any of the nitrogen measurements. Because the older sheep had a low baseline of GH, probably with few periods of secretion, these animals may not have needed much GH stimulation to induce a positive metabolic change in their nitrogen utilization. The calves had higher GH baseline, probably with many periods of GH secretion of high amplitude. The additional GHRF administered to calves seemingly was not adequate to induce significant improvements in nitrogen measurements.

Because the endocrine and nitrogen measurements in the calves indicated a general improvement in nitrogen efficiency, GHRF treatment may be effective in stimulating nitrogen retention in young animals. When GHRF was administered as a continuous infusion to steers, the amplitude of endogenous GH pulses was increased (3), while in bull calves, episodic secretion of GH was stimulated (4). Moseley et al. (5) demonstrated that continuous infusion of GHRF increased nitrogen retention in young bull calves. Based on the evidence presented, young animals may need either greater quantities of GHRF administered as a pulse injection or require continuous administration of GHRF to cause a significant increase in nitrogen retention.

An additional possibility for stimulating GH secretion may be the simultaneous administration of testosterone and GHRF. Whereas endogenous testosterone helped to sustain GH secretory profiles during
aging in bulls, the simultaneous administration of low concentrations of testosterone with GHRF may potentiate or prolong the GH response in ruminants.

Even though GH concentrations may be increased with GHRF administration, the resulting growth responses may be limited by inadequate nutrition as well as an imbalance of endogenous concentrations of thyroid hormones, insulin or other growth factors. The optimum response to an anabolic agent may be dependent upon the overall balance of the endocrine system. Nevertheless, our results showed that with high levels of nutrition, GHRF could be used to enhance the utilization of absorbed nitrogen in older sheep. Although statistically significant increases in nitrogen retention with GHRF treatment were not observed in young calves, the trends toward increased somatomedin-C concentrations, GH concentrations, nitrogen retention, weight gains, and feed intake indicated the potential for significant enhancement of nitrogen retention with GHRF under different administration.
ADDITIONAL LITERATURE CITED


ACKNOWLEDGMENTS

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APPENDIX. STATISTICAL PROCEDURES FOR GROWTH HORMONE PROFILE ANALYSIS

The statistical method for detection of hormone secretory spikes consisted of partitioning the growth hormone (GH) concentrations from sequential plasma samples for each experimental unit (animal) into two subgroups by the skewness coefficient test. The first subgroup, inliers, was those observations which constituted the normal, random variation expected among samples. It was assumed that inliers did not deviate significantly from a normal distribution. This subgroup was considered as the baseline population and was used to determine mean baseline GH concentrations. The second subgroup, outliers, contained GH concentrations which were abnormally high but occurred in a random manner. Outliers were obtained by dropping points from the right side of the frequency distribution until the normal distribution of inliers was obtained. Thus, outliers constituted the skewness and, therefore, constituted the secretory spikes. It was assumed that both subgroups did not deviate from a normal distribution, but if the outlier population, in particular, were found to conform to some other distribution, nonparametric tests rather than the parametric tests were used.

After entering the GH concentrations and corresponding time data for each experimental unit, the existence of linear, quadratic or no trends were determined by calculating Spearman correlation coefficients. Linear trends indicated that the samples varied about a straight line,
quadratic trends indicated a curved baseline and no trends had no patterns. The baseline was adjusted for linear and quadratic trends before time series analysis to exclude long-term changes in the baseline from affecting the frequency and amplitude of secretory spikes. Because secretory periods of equal amplitude raised observed levels to different maximum values when the baseline changes, removing trends was essential for peak identification. If Spearman correlation coefficients were significant, the observations were regressed on time and the adjusted regressed values used in a time series analysis. If Spearman correlation coefficients were not significant, the data were entered directly into the time series analysis. The time series analysis was used to remove positive serial correlation normally observed between adjacent observations of sequentially collected data. Since an assumption of independence of observations was required in the statistical tests, such as skewness coefficient test, a moving average from the time series analysis of the first order was fit to remove bias.

The time series analysis was performed to adjust either the original data or the adjusted regressed values for positive serial correlation. The data as adjusted by time series and autocorrelations were calculated with a lag of one on the residuals to determine spike locations. The resulting 15 maximum points of the time series residuals were tested as outliers using the skewness coefficient test within the univariate procedure of SAS (1). This procedure eliminates in each succeeding case the maximum value, so that the total number of observations reduces by one each time the next maximum value was
eliminated. If the skewness value calculated was larger than the table skewness value at a significance of 5%, it was considered a GH spike or part of a spike. If the calculated skewness value was equal or less than the table value, the value was considered part of the baseline and regressions stopped. The GH baseline value was calculated by averaging the remaining GH values after the outliers or spikes were eliminated. Frequency of GH spikes was the number of separate spikes located over the time period. Amplitude of GH spikes was obtained from the corresponding GH concentration for those skewness values. Averaging the hormone values for each spike gave the spike amplitude. If the GH concentration returned to baseline or below for one or more samples, the spikes surrounded by the baseline samples were considered to be a period of secretion. The amplitude of the period of secretion was the mean of all GH samples with a residual greater than the skewness table value within the period. The overall mean was the average of all GH samples for each experimental unit.
LITERATURE CITED