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Leptin in horses: influences of body condition, gender, insulin insensitivity, feeding, and dexamethasone

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LEPTIN IN HORSES: INFLUENCES OF BODY CONDITION, GENDER, INSULIN
INSENSITIVITY, FEEDING, AND DEXAMETHASONE

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
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ABSTRACT

The experiments described herein were designed to answer questions that arose from initially attempting to determine whether treatment with dexamethasone increased concentrations of leptin in geldings. Dexamethasone treatment did in fact increase leptin concentrations in mares, geldings, and stallions. Additional experiments were designed to determine leptin's interaction, not only with the adrenal axis, but with the thyroid axis, the growth hormone/insulin-like growth factor-1 axis, as well as glucose and insulin metabolism in geldings, mares, and stallions. During the course of these experiments, differences in leptin concentrations in the horse were attributed to degree of body condition, gender, and feeding time. Additionally, it was found that horses with high body condition fell into two distinct groups based solely on circulating concentrations of leptin (high vs low). The obese, hyperleptinemic horses were found to be hyperglycemic and hyperinsulinemic and had elevated concentrations of triiodothyronine and decreased concentrations of growth hormone, a hormonal profile similar to that of a type II diabetic human. Indeed, this obesity-related hyperleptinemia was associated with a degree of insulin insensitivity evidenced by the increased insulin response to glucose in these horses. Thus, further experiments were conducted to determine the degree to which these horses were insensitive to insulin, as well as whether diet supplementation or feed restriction might alleviate this insulin insensitivity. Chromium propionate supplementation did not decrease plasma insulin or leptin; however, restricted nutrient intake (6 h of grazing per day) was successful in decreasing concentrations of leptin. It was concluded that leptin in the horse is affected by adrenal and thyroid hormones as well as by glucose/insulin metabolism, and that a syndrome of obesity-related

hyperleptinemia, hyperglycemia, and hyperinsulinemia exists in the horse that is similar to

type II diabetes in humans.

CHAPTER 1

INTRODUCTION

Leptin, a 16 kDa protein hormone secreted by adipocytes, is involved in regulating

feed intake and thermogenesis in rodents via receptors in the hypothalamus (Brunetti et al.,

1999) where it inhibits neuropeptide-Y function (Stephens et al., 1995). Infusion of leptin

into the third ventricle resulted in reduced feed intake in sheep (Henry et al., 1998; Morrison

et al., 2001) and pigs (Barb et al., 1998). Besides regulating feed intake, leptin is thought to

be the hormonal signal to the brain for the nutritional and energy status of the body

(Houseknecht et al., 1998). In fact, circulating concentrations of leptin are directly correlated

to body mass index and percentage of body fat in humans (Prolo et al., 1998) and ruminants

(Chilliard et al., 2000). Further, Gentry and co-workers (2001) showed that body condition

scores (BCS) based on the system described by Henneke and co-authors (1983), ultrasound

measurements of fat thickness, and circulating concentrations of leptin were all highly

correlated in mares.

Several other hormonal systems in the body have been shown to affect plasma leptin

concentrations both *in vivo* and *in vitro*. Dexamethasone (DEX) and insulin have been

reported to stimulate leptin secretion in humans and pigs (Larsson and Ahren, 1996; Miell et

al., 1996; Ramsay and White, 2000). In adipocytes cultured *in vitro*, DEX stimulated leptin

production and secretion (Hardie et al., 1996; Considine et al., 1997) as well as mRNA

content (Reul et al., 1997; Bradley and Cheatham, 1999). Hypothyroidism has been reported

to increase leptin secretion in humans (Leonhardt et al., 1998; Leonhardt et al., 1999; Pinkney

et al., 1998); however, others have reported that hypothyroidism is associated with decreased

leptin concentrations (Valcavi et al., 1997; Yoshida et al., 1998). Treatment with thyroxine

(T4) in humans (Pinkney et al., 2000) or T4 plus triiodothyronine (T3) in rats (Escobar-Morreale et al., 1997) decreased circulating concentrations of leptin. In horses, 24 h of feed restriction reduced leptin concentrations but had no effect on gonadotropin secretion (McManus and Fitzgerald, 2000).

Circulating concentrations of leptin are also influenced by gender. Buff and co-workers (2002) reported that leptin concentrations are greater in stallions and geldings compared to mares. However, after correcting for body fat, concentrations of leptin are generally greater in women than in men (Castracane et al., 1998; Pineiro et al., 1999). Concentrations of leptin were increased in ovariectomized rats treated with estradiol-17β (Tanaka et al., 2001). In contrast, androgen treatment decreased concentrations of leptin in healthy men (Simon et al., 2001). Therefore, gender differences in plasma leptin concentrations seem to vary with species.

It has been reported that feeding alters leptin concentrations as well. In humans (Dallongeville et al., 1998; Elimam and Marcus, 2002) and cats (Appleton et al., 2002), concentrations of leptin increase following a meal. After feeding both during the day and at night, Dallongeville et al. (1998) concluded that the increase in leptin concentrations in humans was in response to feeding and not the day/night cycle.

Leptin in the Horse

Concentrations of leptin decrease after foaling and remain lower for 4 weeks in lactating mares compared to non-lactating mares (Heidler et al., 2003). This decreased plasma leptin is thought to promote feed intake in lactating mares avoiding an energy deficit. Further, concentrations of leptin are greatest in whole and skimmed pre-suckled colostrums, decline sharply 6 h post-partum, and gradually decline thereafter (Salimei et al., 2002).

Mares in a non-pregnant state for successive years have increased percentage of body fat, and this condition may lead to continued reproductive activity during winter months (Fitzgerald et al., 2002). Fitzgerald and co-workers (2002) proposed that long-term regulation of seasonal reproduction in mares may involve recognition of body energy stores prior to changes in photoperiod or, in contrast, energy stores may need to reach a critical level before inhibitory daylength signals initiate seasonal anestrous.

Mares were monitored for an entire year during which changes in body weight, total body fat, and concentrations of leptin were followed (Fitzgerald and McManus, 2000). Young mares entered winter anestrous much earlier in the year compared to older, more mature mares. Treatment with melatonin did not induce mature mares to enter anestrous and only showed an inhibitory effect on concentrations of prolactin during the spring months. Mature mares had greater concentrations of leptin, body weights, and percentage of total body fat compared to young mares. Further, the authors reported that all three (leptin, body weight, and total body fat) were greatest during the summer than the winter in both young and mature mares. It was concluded that the reproductive response to both photoperiod and melatonin treatment is modified by energy availability, which may be signaled to the brain by changes in plasma leptin concentrations.

Treatment of mares with a beta(2)-adrenergic receptor agonist for 4 or 7 months decreased both leptin concentrations and body fat percentage except in the presence of melatonin and did not alter timing or proportion of mares exhibiting anestrous (McManus and Fitzgerald, 2003). Short-term feed restriction (1 d) successfully decreased plasma leptin concentrations in aged and young mares, however it did not alter concentrations of prolactin, follicle-stimulating hormone, or luteinizing hormone (McManus and Fitzgerald, 2000). In

that study, the authors concluded that the lack of change in glucose concentrations with an increase in free fatty acids indicated maintenance of metabolizable fuels which may have accounted for the absence of suppression rather than a failure of leptin to signal nutritional status to the reproductive axis in the mare. In contrast, Gentry and others (2002b) decreased plasma leptin and BCS in mares through long-term feed restriction (5 months). In that study, mares with low BCS (3.0 to 3.5) experienced a deep anovulatory period accompanied by decreased concentrations of leptin, IGF-1, and prolactin. In comparison, all but one mare in the high BCS group continued to cycle or had significant follicular activity on the ovaries throughout the winter. However, even though concentrations of leptin were lowest in low BCS mares and highest in high BCS mares, there tended to be a wide variation in leptin concentrations (< 5 ng/mL vs 7 to 20 ng/mL) in well-fed mares, possibly indicating other factor(s) may be influencing plasma leptin under these conditions of high BCS (Gentry et al., 2002b).

Subsequent observations on 18 of the mares used in the experiment of Gentry et al. (2002b) indicated that the high vs low leptin distinction was consistently observed up to two years later (*unpublished*; mares were had similar BCS), indicating that the underlying cause was relatively permanent and deserved further study.

Insulin Resistance and Diabetes Mellitus

As defined by Guyton and Hall (2000), diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. Type I diabetes (or insulin-dependant diabetes mellitus; IDDM) is caused by lack of insulin secretion (Guyton and Hall, 2000). Type II

diabetes (or non-insulin-dependant diabetes mellitus; NIDDM) is caused by insensitivity to insulin of target tissues, often called insulin resistance (Guyton and Hall, 2000).

The onset of IDDM may be initiated by injury or disease to the pancreas that impairs beta cell production of insulin. In the absence of injury or disease, beta cell degeneration may still occur indicating that IDDM may also be hereditary. Type I diabetes most commonly develops in children in their early teens and can develop rapidly over a few weeks or even days with principal symptoms being: 1) increased blood glucose, (2) increased utilization of fats for energy and for formation of cholesterol by the liver, and (3) depletion of body proteins (Guyton and Hall, 2000).

Insulin resistance is a primary symptom of NIDDM and is a risk factor for cardiovascular disease, hypertension, and cancer in humans (Reaven, 1988; Kim, 1998; Frayn, 2001). In horses, insulin resistance may be involved in colic (Hudson et al., 2001), exertional rhabdomyolysis (Valentine et al., 2001), and osteochondrosis (Ralston, 1996), and is associated with obesity and laminitis (Jeffcott et al., 1986; Pass et al., 1998). Type II diabetes is more common than IDDM and usually develops in adult humans over the age of 40 (Guyton and Hall, 2000). In contrast to IDDM, NIDDM is characterized by elevated concentrations of insulin. The increased levels of insulin are in response to elevated glucose, however hyperglycemia still occurs due to peripheral tissue resistance or insensitivity to insulin (Guyton and Hall, 2000).

The symptoms of NIDDM can be at least partially alleviated by specific dietary and lifestyle changes. Chromium supplementation improves insulin sensitivity in rats and humans (Sun et al., 2000; Ghosh et al., 2002; Keszthelyi et al., 2003). Ott and Kivipelto (1999) reported that yearling horses fed chromium at 420 µg/kg diet had increased measurements of

glucose clearance but no change in sensitivity to exogenous insulin. In addition, type II

diabetes in humans is managed by a healthy diet and exercise. In the horse, Powell and co-

workers (2002) reported greater insulin sensitivity in lean mares compared to obese mares and

that low intensity exercise increased insulin sensitivity in both groups of mares for 24 h.

Assessments of Glucose Metabolism

Glucose metabolism in the horse has been assessed by intravenous glucose tolerance

tests (IVGTT; Sticker et al., 1995ab), hyperinsulinemic-euglycemic clamp (Powell et al.,

2002; Rijnen and van der Kolk, 2003), and frequently sampled intravenous glucose tolerance

test (FSIGT; Hoffman et al., 2003) with minimal model analysis (Bergman and Lovejoy,

1997). The hyperinsulinemic euglycemic clamp method for measuring glucose clearance and

assessing insulin sensitivity is considered by many to be the "gold standard" in humans.

However, it is impractical and labor intensive for large studies. The general procedure

involves an overnight fast, a bolus injection of insulin to shut down endogenous

gluconeogenesis, and infusion of insulin at a constant rate and dose while varying the infusion

rate of glucose until euglycemia is obtained. Once euglycemia is reached, the glucose

infusion rate (GIR) is equal to the glucose degradation rate (GDR). Subjects with high GIR

are more sensitive to insulin than subjects with low GIR. A low GIR indicates some degree

of insulin insensitivity or resistance, possibly even NIDDM.

The general procedure for an IVGTT is a single i.v. injection of glucose with frequent

blood samples collected up to 180 min. The peak insulin response and the time it takes

plasma glucose to return to baseline are two important observations from the IVGTT.

Extremely high responses in insulin coupled with a slow rate of glucose clearance imply

insulin insensitivity. However, since the rate of glucose clearance is not linear, simply

calculating Kg (glucose clearance rate) is not accurate and led researchers to develop the minimal model analysis with FSIGT (Bergman and Lovejoy, 1997).

The FSIGT using minimal model analysis generally includes a bolus i.v. injection of glucose, an i.v. injection of insulin 20 min later, and frequent blood samples collected around injections for determination of concentrations of glucose and insulin. Through the use of minimal model analysis, it describes glucose metabolism as glucose effectiveness (Sg), the ability of glucose to mediate its own disposal, and insulin sensitivity (SI), the ability of insulin to promote glucose removal (Bergman et al., 1979; Ward et al., 1991, Bergman and Lovejoy, 1997).

The development of the minimal model and the components of glucose metabolism it is able to assess have been described in detail by Bergman and Lovejoy (1997). Besides Sg and Si, the model also assigns a value to acute insulin response to glucose (AIRg) and disposition index (DI). The plasma insulin response, above baseline, from 0 to 10 min after glucose injection is AIRg. The DI is a constant determined by the product of Si and AIRg and is a measure of beta-cell function.

Rationale for Present Research

Although reports in other species concerning plasma leptin and its effect on, or response to, various hormonal systems are numerous; few deal specifically with leptin secretion in the horse. The involvement of leptin in signaling the brain of the body's energy and nutritional status make it an important regulator of growth, reproduction, lactation, and pregnancy. Further, an understanding of how different hormonal systems affect concentrations of leptin in the horse may increase our ability to better manage the horse through different phases of production and use.

The experiments described in the subsequent chapters were designed to answer these questions about leptin and its interaction with other physiologic systems in the horse. We initially determined whether glucocorticoids and/or hypothyroidism altered concentrations of leptin in geldings, mares, and stallions. Apparent variations in the leptin response among genders led to further research on differences between geldings, mares, and stallions. During this time, it was also observed that a specific diurnal pattern in leptin secretion existed in stallions but was absent in mares and geldings. Further, it was discovered that there was a wide variation in resting concentrations of leptin in mares and geldings with high BCS. These high BCS horses also had elevated concentrations of glucose, insulin, and T3, as well as decreased concentrations of growth hormone. This elevation in T3 in high BCS horses along with the effects observed during hypothryroidism led to the hypothesis that elevated T4 might decrease concentrations of leptin. In addition, the obesity related hyperleptinemia, hyperinsulinemia, and hyperglycemia implied some degree of insulin insensitivity or resistance in these horses. Therefore, the final set of experiments were designed to determine if this syndrome could be alleviated and to what degree these horses were resistant to insulin.

CHAPTER 2

EFFECTS OF DEXAMETHASONE, GLUCOSE INFUSION, ADRENOCORTICOTROPIN, AND PROPYLTHIOURACIL ON PLASMA LEPTIN CONCENTRATIONS IN HORSES[1]

Introduction

Dexamethasone (DEX) and insulin have been reported to stimulate leptin secretion in various non-equine species (Larsson and Ahren, 1996; Miell et al., 1996; Ramsay and White, 2000). In adipocytes cultured *in vitro*, DEX stimulates leptin production and secretion (Hardie et al., 1996; Considine et al., 1997) as well as mRNA content (Reul et al., 1997; Bradley and Cheatham, 1999). Hypothyroidism has been reported to increase leptin secretion in humans (Leonhardt et al., 1998; Leonhardt et al., 1999; Pinkney et al., 1998); however, others have reported that hypothyroidism is associated with decreased leptin concentrations (Valcavi et al., 1997; Yoshida et al., 1998). Treatment with thyroxine (T4; Pinkney et al., 2000) or T4 plus triiodothyronine (T3; Escobar-Morreale et al., 1997) decreased circulating concentrations of leptin. In horses, 24 h of feed restriction reduced leptin concentrations but had no effect on gonadotropin secretion (McManus and Fitzgerald, 2000).

Due to the lack of information on the effect of glucocorticoids on leptin secretion in equine species and the controversial effects of thyroid hormones on leptin secretion in non-equine species we designed the following experiments. The series of experiments reported herein were designed to assess the effects of DEX, glucose infusion, adrenocorticotropin (ACTH), and 6-n-propyl-2-thiouracil (PTU; an inducer of hypothyroidism) on leptin secretion in horses.

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Materials and Methods

Experiment 1. Nine light horse geldings (462 to 577 kg BW) were used in three
replicates of a 3 x 3 Latin square with repeated measures. They were maintained on native
grass pasture and provided grass hay as needed to maintain good body condition (scores of 6
to 8; Henneke et al., 1983). The three geldings within each replicate were assigned randomly
to the three treatments: 1) i.m. injection of DEX in vegetable oil (125 µg/kg BW; Sigma
Chem. Co., St. Louis), 2) i.v. infusion of glucose (0.2 g/kg BW as a 50% solution in 0.155 M
saline), or 3) no injection (controls). The three replicates were performed simultaneously.
Treatments were administered in the morning at approximately 0800 for four consecutive
days (d 1 to 4). Samples of jugular blood were drawn via jugular venipuncture into
evacuated, heparinized tubes at 0 and 12 h relative to treatments on these days. Weekly blood
samples were subsequently collected on d 12, 19, 26, and 33.   The successive periods (2 and
3) of the Latin square were begun on d 34 after the initial treatment injection of the previous
period.

All blood samples were immediately centrifuged at 1,200 x g at 5°C and the plasma
was harvested and stored at -15°C.   Plasma concentrations of leptin were measured by RIA
using commercially available reagents (Multi-species leptin kit, Linco Research Inc., St.
Charles, MO). Similar to reported previously by McManus and Fitzgerald (2000), human
leptin standards from the kit, human recombinant leptin (0.16 to 10 ng/tube), and three horse
plasma pools (two mares and one gelding; 6.2 to 100 µL/tube) produced parallel inhibition
curves in the assay.   Moreover, five levels of human recombinant leptin (0.3 to 5 ng) added to
a pool of horse plasma were quantitatively recovered with an average of 111% and an R^2

value of 0.995. The intra- and interassay coefficients of variation and assay sensitivity were 4%, 8%, and 0.8 ng/mL.

Insulin (DePew et al., 1994), insulin-like growth factor-1 (IGF-1; Sticker et al., 1995b), and growth hormone (GH; Thompson et al., 1992) were determined by RIA previously validated for horse samples. Intra- and interassay coefficients of variation and assay sensitivities were 5%, 8%, and 0.1 ng/mL for insulin; 5%, 12%, and 8 ng/mL for IGF-1; and 8%, 11%, and 0.5 ng/mL for GH. Glucose concentrations were determined spectrophotometrically (Method No. 315; Sigma Chemical Co., St. Louis, MO, USA).

Experiment 2. Twelve light horse mares (465 to 560 kg BW; body condition scores of 6 to 8) were used. Six mares were assigned randomly to each of two groups, and then groups were selected randomly to receive either 1) DEX in oil (125 μg/kg BW i.m.) or 2) a similar volume of oil i.m. Injections were administered at approximately 0800 each morning for 4 d beginning on d 1. Blood samples were collected just prior to injections and 12 h later during the first 4 d. Additional samples were collected in the morning on d 5, 6, 7, 8, 10, 12, 19, 26, and 33. Samples were immediately centrifuged and plasma frozen for later determination of glucose, insulin, leptin, and IGF-1 concentrations as described for Exp. 1.

Experiment 3. Six light horse geldings (490 to 600 kg BW) not used in Exp. 1 were used in a completely randomized design with repeated measures. They were in good body condition (6 to 8) and were assigned randomly to receive either 1) 200 IU i.m. of porcine ACTH (Sigma; A6303) in saline containing 0.1 g/mL gelatin (n = 3) or 2) an equivalent volume of vehicle (n = 3) i.m. at 12-h intervals for four consecutive days (0, 12, 24, 36, 48, 60, 72, and 84 h). Blood samples were collected via jugular venipuncture at -1, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, 36, 42, 48, 54, 60, 66, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90,

92, 94, and 96 h. Additional blood samples were collected on d 12, 19, 26, and 33 relative to the first day of treatment. All blood samples were immediately centrifuged and the plasma harvested for later determination of cortisol (ICN Pharmaceuticals, Inc., Costa Mesa, CA; Kit # 07-221102), insulin, glucose, leptin, and IGF-1 concentrations.

Experiment 4. Plasma samples selected from a previous experiment (Pruett, 2000) were used for the measurement of leptin. In that experiment, eight adult light horse stallions (390 to 550 kg BW; body condition scores 5 to 7) were used. Stallions were on a diet of commercially available pelleted feed plus grass hay to maintain body condition. To induce hypothyroidism, four randomly selected stallions received PTU (Sigma; P3755) at 6 mg/kg BW administered in 250 g of a top dressing of a molasses-containing balanced grain ration; four control stallions were given the top dressing with no addition. Stallions were fed their rations daily at 0800 and were allowed to eat throughout the day; hay and water were available *ad libitum* throughout the experiment. Plasma samples drawn on d 1, 6, 13, 20, 27, 31, 36, 41, 45, and 52 relative to the start of treatment were selected for determination of leptin concentrations; in addition, samples from d 52 were assessed for T3 and T4 (ICN; Kit# 07-290102 and 07-292102, respectively) and TSH (Sticker et al., 2001) concentrations. Intra-assay coefficients of variation and assay sensitivities were 5% and 0.02 ng/mL for TSH; 5% and 5 ng/mL for T4; and 5% and 40 pg/mL for T3.

Statistical Analyses. Data were analyzed via the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data from repetitive sampling over time were analyzed in a split-plot ANOVA (Gill and Hafs, 1971) for a replicated Latin square (Exp. 1) or a completely randomized design (Exp. 2, 3, and 4; Steel and Torrie, 1980). In Exp. 1, the main effects of squares, treatments, horses, and days were tested by the square, treatment, horse, and day

interaction term; the time factor and its interaction with treatment were tested with residual error. In Exp. 2, 3, and 4, the main effect of treatment was tested with the horse within treatment term; the time factor and its interaction with treatment were tested with residual error. Differences between treatment groups in each time period in the treatment-day interactions were assessed by the LSD-test (Steel and Torrie, 1980) for Exp. 2, 3, and 4; differences among the three treatments in each time period in Exp. 1 were compared with Tukey's HSD test (Steel and Torrie, 1980). In all cases, the LSD or HSD calculated for $P < 0.01$ was used.

Results

  Experiment 1. Treatment with DEX increased ($P = 0.0001$) concentrations of plasma glucose and insulin (Figure 2.1a) within 24 h, and concentrations were at their highest on d 4. These elevated glucose and insulin concentrations persisted through d 12 ($P < 0.01$) but returned to concentrations similar ($P > 0.05$) to those in controls by d 19. Given the blood sampling schedule in this experiment, any effects of glucose infusion on the mornings of d 1 through 4 on glucose and insulin concentrations were not evident after 12 h (the next blood sample), and there was no effect ($P > 0.1$) in the ANOVA (Figure 2.1a and b).

  Treatment with DEX also increased ($P = 0.001$) plasma leptin concentrations (Figure 2.2a) within 24 h, and concentrations were at their highest on d 3. These elevated leptin concentrations persisted through d 12 ($P < 0.01$) but returned to concentrations similar to those in controls by d 19 ($P > 0.05$). There was no effect ($P > 0.1$) of glucose infusion on leptin concentrations. There was an increase ($P < 0.001$) in plasma IGF-1 concentrations in DEX-treated geldings (Figure 2.2b), however, this did not occur until treatment had ended (d
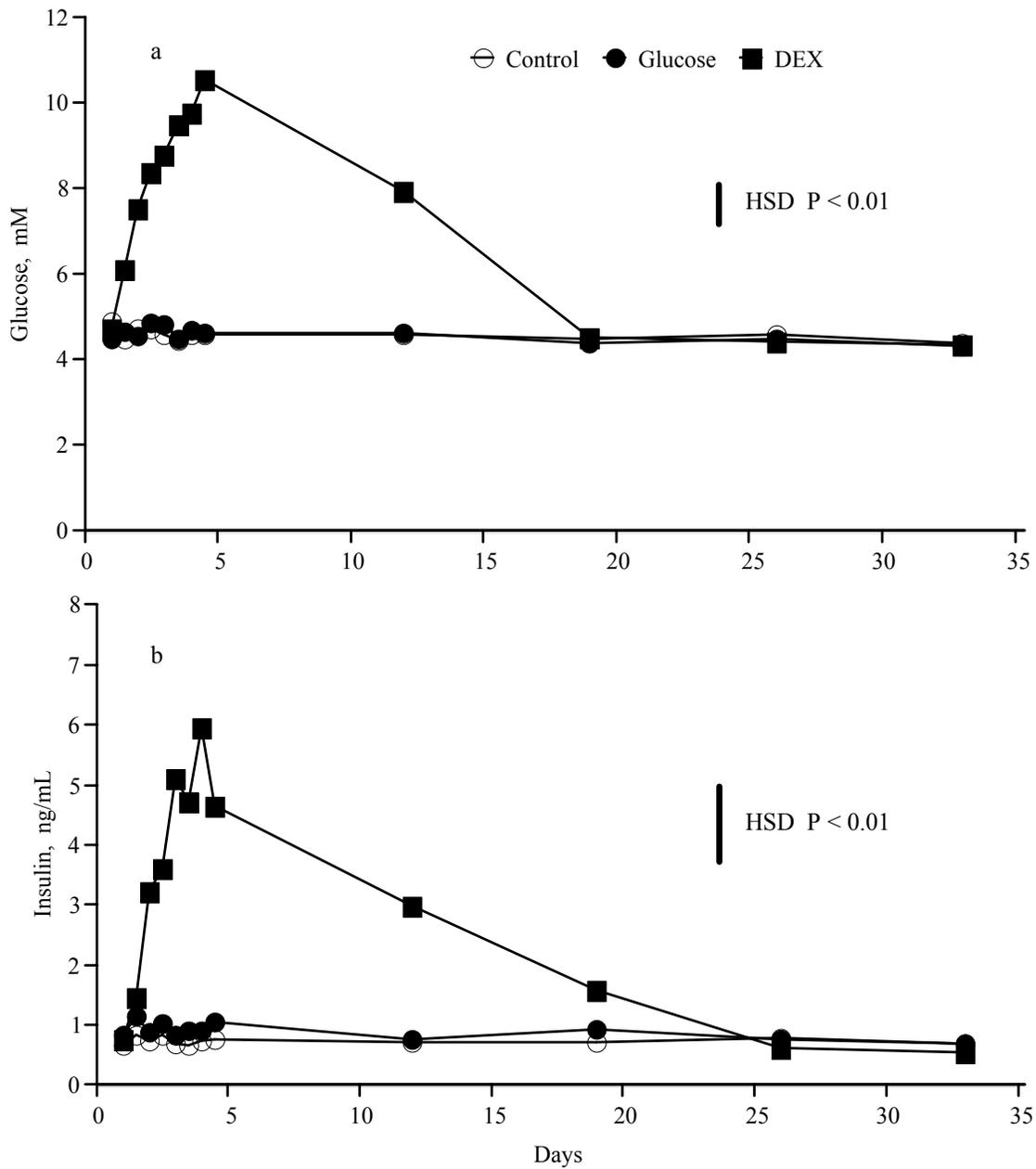
Figure 2.1. Concentrations of glucose (a) and insulin (b) in plasma of geldings receiving dexamethasone (DEX) injections, glucose infusion, or nothing at all (control) each morning on days 1 to 4 in Exp. 1. The pooled SEM were 0.25 mM for glucose and 0.32 ng/mL for insulin concentrations, respectively. Tukey's honest significant difference (HSD) value for comparison of means is indicated by the vertical bar in each graph.
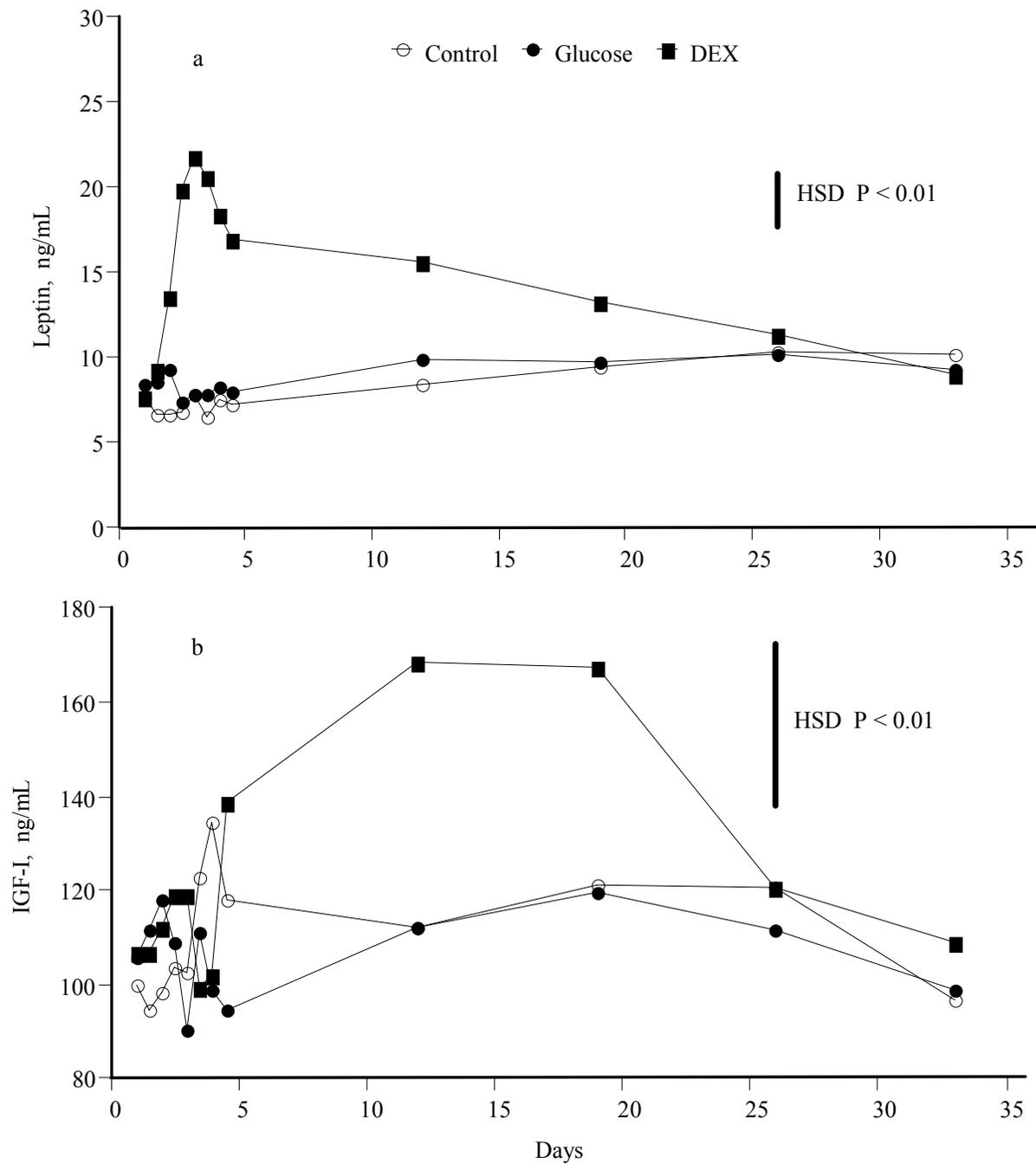
Figure 2.2. Concentrations of leptin (a) and insulin-like growth hormone (IGF-1; b) in plasma of geldings receiving dexamethasone (DEX) injections, glucose infusion, or nothing at all (control) each morning on days 1 to 4 in Exp. 1. The pooled SEM were 0.83 and 9.0 ng/mL for leptin and IGF-1 concentrations, respectively. Tukey's honest significant difference (HSD) value for comparison of means is indicated by the vertical bar in each graph.

12 and 19). Glucose treatment did not affect (P > 0.1) plasma IGF-1 concentrations. Neither DEX nor glucose treatment affected (P > 0.10) plasma GH concentrations (data not shown).

Experiment 2. Treatment with DEX increased (P < 0.01) concentrations of glucose and insulin (Figure 2.3a and b) in a manner similar to that in geldings in Exp. 1. Plasma glucose and insulin concentrations in DEX-treated mares were elevated by d 2, were highest around d 6, and remained elevated above that of controls until d 12. Plasma leptin concentrations (Figure 2.4a) following DEX administration were increased (P < 0.01) by d 2, were highest on d 3.5, and remained elevated relative to controls through d 12. Plasma IGF-1 concentrations (Figure 2.4b) following DEX treatment were also increased (P < 0.01), again only after the end of treatment, on d 10, 12, and 19.

Experiment 3. Treatment with ACTH increased (P < 0.01) plasma cortisol concentrations (Figure 2.5a) 4 to 6 h after injections on d 1, 2 and 4, but not d 3. In each case, cortisol concentrations in ACTH-treated geldings immediately before the next injection were not different (P > 0.10) from controls. Plasma glucose concentrations (Figure 2.5b) increased (P < 0.01) in both groups from d 1 to 5, and were higher (P<0.01) in geldings receiving ACTH than in controls on d 2. Similarly, there was a transient increase (P < 0.01) in insulin concentrations (Figure 2.5c) in ACTH-treated geldings on d 3.5. There was no effect (P > 0.10) of ACTH treatment on plasma concentrations of GH or IGF-1 (data not shown). Plasma leptin concentration differed between groups (P < 0.05) before onset of ACTH treatment (Figure 2.6a), thus the residual values relative to time 0 were calculated and analyzed separately (Figure 2.6b). For the residuals, treatment with ACTH increased (P < 0.01) leptin concentrations relative to controls from d 2.5 through 4. Unlike previous experiments, leptin
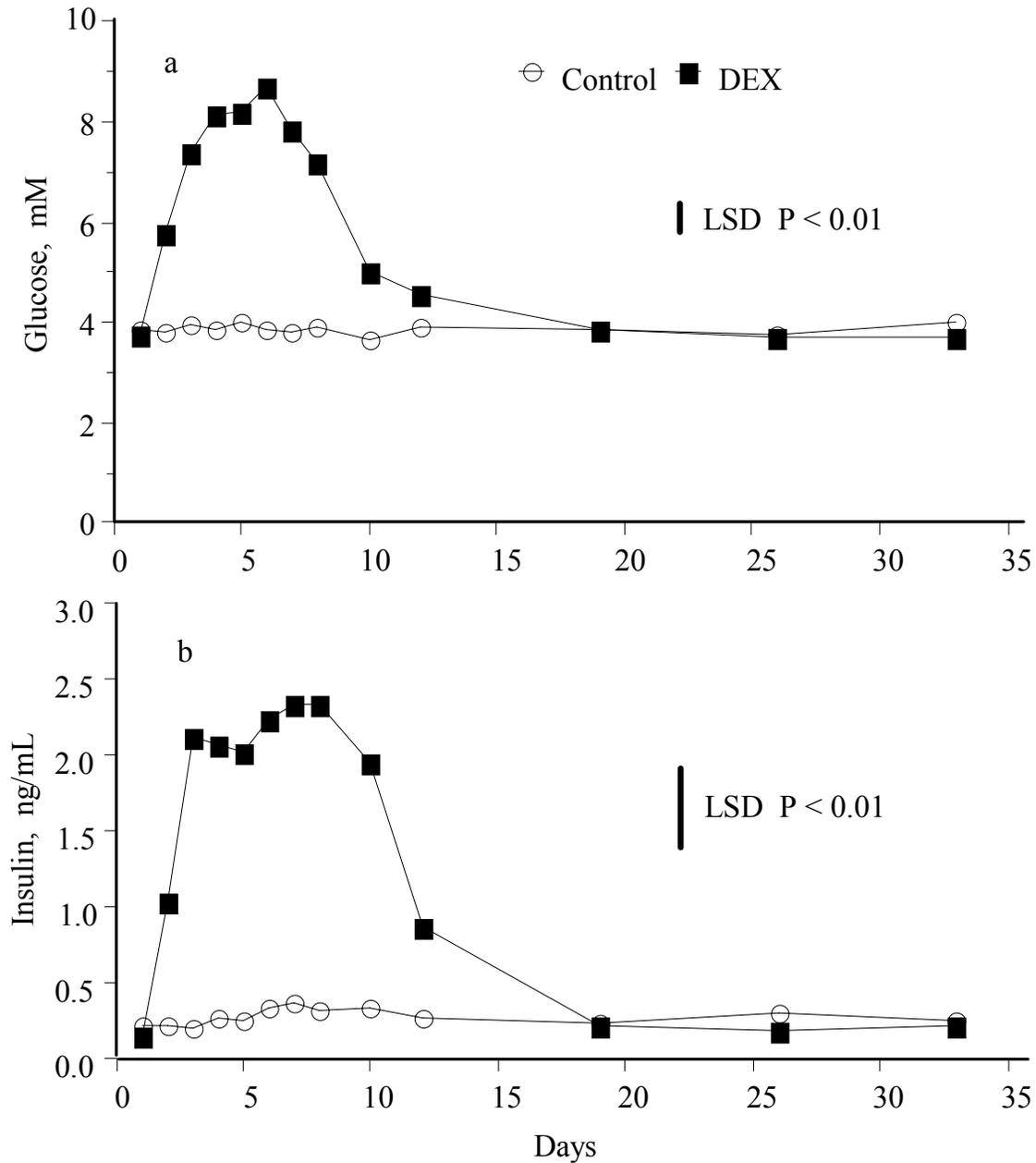
Figure 2.3. Concentrations of glucose (a) and insulin (b) in plasma of mares receiving dexamethasone (DEX) or vehicle (control) injections each morning on days 1 to 4 in Exp. 2. The pooled SEM were 0.17 mM for glucose and 0.73 ng/mL for insulin concentrations, respectively. The least significant difference (LSD) value for comparison of means is indicated by the vertical bar in each graph.
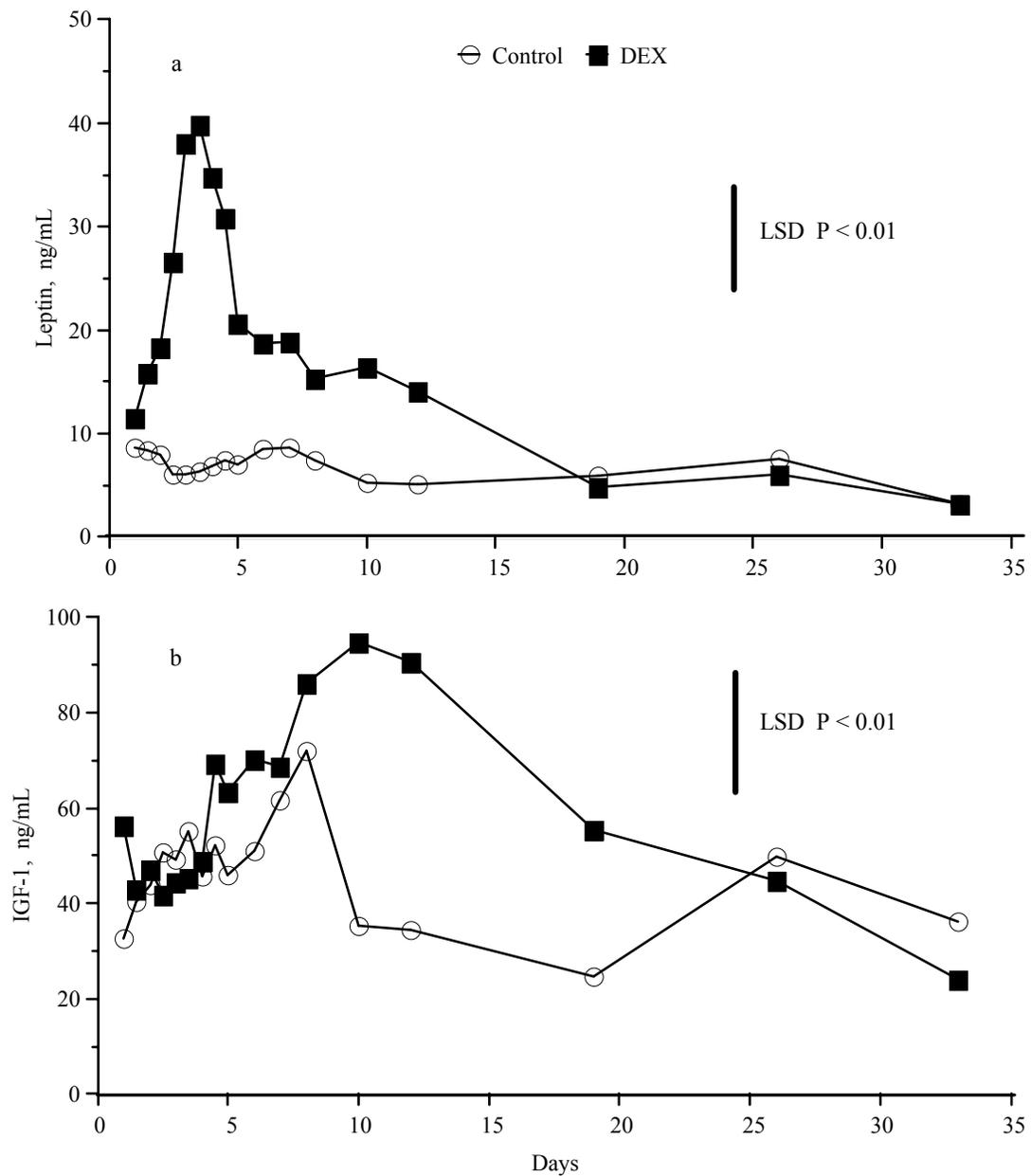
Figure 2.4. Concentrations of leptin (a) and insulin-like growth factor (IGF-1; b) in plasma of mares receiving dexamethasone (DEX) or vehicle (control) injections each morning on days 1 to 4 in Exp. 2. The pooled SEM were 3.2 and 11 ng/mL for leptin and IGF-1 concentrations, respectively. The least significant difference (LSD) value for comparison of means is indicated by the vertical bar in each graph.
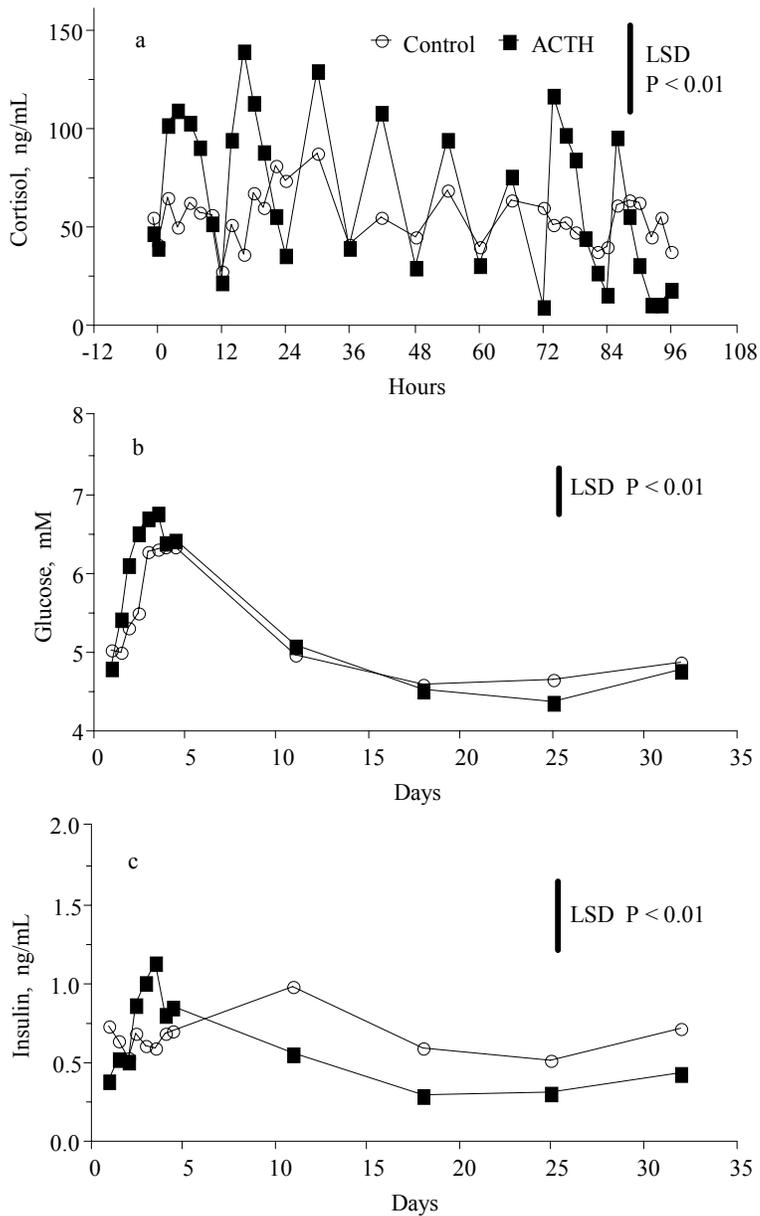
Figure 2.5. Concentrations of cortisol (a) glucose (b) and insulin (c) in plasma of geldings receiving adrenocorticotropin (ACTH) or vehicle (control) injections at 12-h intervals starting on the morning of day 1 and continuing through the evening of day 4 in Exp. 3. The pooled SEM were 12 ng/mL for cortisol, 0.17 mM for glucose, and 0.12 ng/mL for insulin concentrations, respectively. The least significant difference (LSD) value for comparison of means is indicated by the vertical bar in each graph.
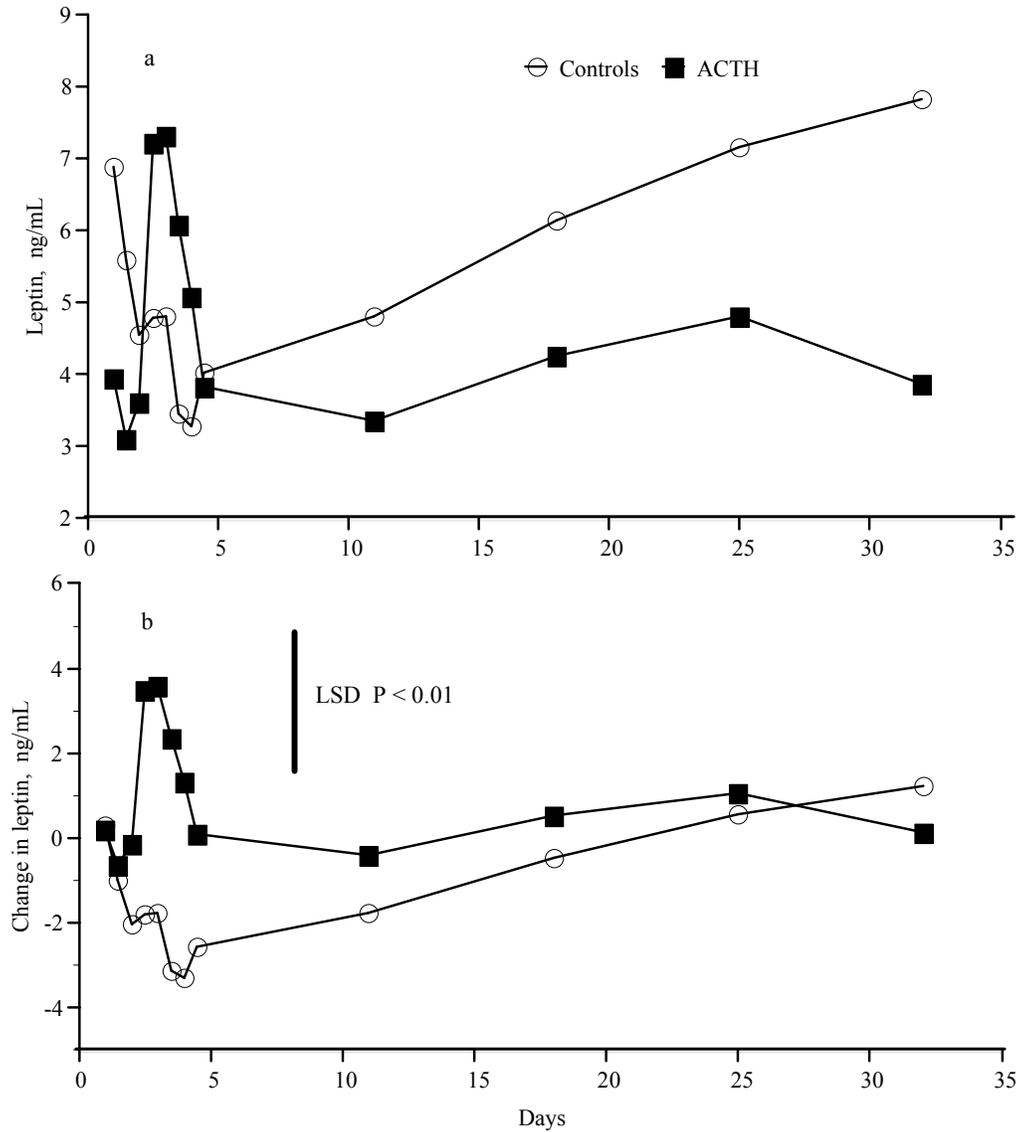
Figure 2.6. Concentrations of leptin (a) and the net change in leptin concentrations relative to pretreatment (b) in plasma of geldings receiving adrenocorticotropin (ACTH) or vehicle (control) injections at 12-h intervals starting on the morning of day 1 and continuing through the evening of day 4 in Exp. 3. The pooled SEM were 1.0 ng/mL and 1.0 ng/mL for leptin and net change in leptin concentrations, respectively. The least significant difference (LSD) value for comparison of means is indicated by the vertical bar in the bottom graph.

concentrations in control geldings tended to decrease from d 1 to 4, and gradually rebounded thereafter.

Experiment 4. On d 52 of PTU feeding, plasma concentrations of T4 were reduced ($P < 0.05$) from 21.3 to 12.9 ng/mL, concentrations of T3 were reduced ($P < 0.01$) from 329 to 95 pg/mL, and concentrations of TSH were elevated ($P < 0.01$) from 0.11 to 2.11 ng/mL in PTU-fed stallions relative to controls. Plasma concentrations of leptin (Figure 2.7) tended to decrease in control stallions after d 6 and increase in PTU-treated stallions after d 31, and were therefore different ($P < 0.01$) between groups from d 10 through 52.

Discussion

The hyperglycemia and hyperinsulinemia observed in the geldings in Exp. 1 and the mares in Exp. 2 are characteristic of excessive glucocorticoid exposure (Guyton and Hall, 1996) and confirm the biological potency of this dose of DEX in horses. The results from Exp. 1 and 2 also demonstrate that DEX at this dosage is a potent stimulator of plasma leptin concentrations in horses, which is assumed to be due to an increase in leptin secretion. This is in agreement with previous reports in humans (Larsson and Ahren, 1996; Miell et al., 1996; Janssen et al., 1998) and rats (Zakrzewska et al., 1999) in which DEX was shown, at varying doses and routes of administration, to stimulate leptin secretion.

In humans, circulating plasma leptin concentrations are higher in women than in men, even after correction for body fat (Castracane et al., 1998; Pineiro et al., 1999). Moreover, Blache et al. (2000) reported that plasma concentrations of leptin were higher in female sheep than in castrated or intact male sheep. Pretreatment leptin concentrations were similar for

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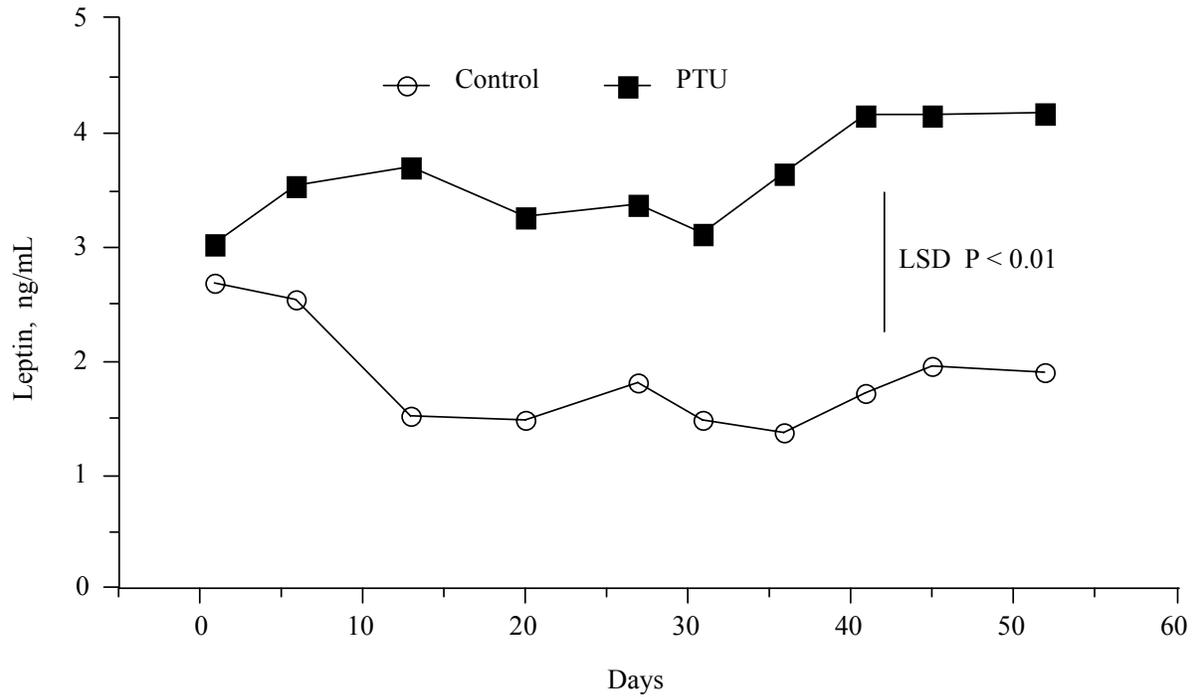
Figure 2.7. Concentrations of leptin in plasma of control stallions and those fed 6-n-propyl-2-thiouracil (PTU) to inhibit thyroid function for 60 d beginning on day 1. The pooled SEM was 0.30 ng/mL. The least significant difference (LSD) value for comparison of means is indicated by the vertical bar.

geldings in Exp. 1 and mares in Exp. 2, however the leptin response in mares was almost twice as large as that in the gelding, even though their body condition scores were similar. Thus, a sexual dichotomy may exist in horses for leptin responsiveness to DEX stimulation.

In both experiments, leptin concentrations peaked on d 3 (Exp. 1) or 3.5 (Exp. 2) and had begun a gradual decline even though treatment was continued through d 4. Thus, although DEX at this dose stimulates leptin secretion within 24 h, apparently the stimulatory effects are transient, or alternatively, continued exposure to DEX becomes inhibitory. The extra blood samples on d 5, 6, 7, 8, and 10 in Exp. 2 were added to better define this decline, which was fairly abrupt to d 5 and gradual thereafter. The fact that leptin concentrations in DEX-treated horses returned to, but did not go below, pretreatment levels may indicate that the decline was not due to an inhibitory effect.

Although the 12-h blood sampling regimen in Exp. 1 did not characterize the short-term (first few hours) glucose and insulin responses to the glucose infusions, we have previously reported such responses in geldings (Smith et al., 1999) and mares (Sticker et al., 1995a). In each case, insulin concentrations increased about 2 ng/mL above baseline within 10 min, and the elevated concentrations returned to pre-glucose levels within 3 h. We chose to use glucose infusion in Exp. 1 to elevate insulin concentrations rather than insulin injections per se due to the possible side effects of insulin injection. Given the temporary nature of the insulin elevations, the lack of effect of glucose infusion on leptin concentrations may have been due to insufficient insulin stimulation. *In vitro* studies with insulin and equine adipocytes are needed to directly test the hypothesis that insulin increases leptin production and secretion.

The involvement of insulin in the DEX-induced increase in leptin secretion cannot be ruled out *in vivo* due to the fact that glucose and insulin concentrations increased rapidly at the same time as leptin concentrations. Several previous reports indicated that DEX has direct effects on leptin production and secretion (Hardie et al., 1996; Considine et al., 1997) as well as mRNA content (Reul et al., 1997; Bradley and Cheatham, 1999) in adipocytes cultured *in vitro*. Thus, it is likely that DEX stimulated leptin secretion in these horses directly, although an interaction with the elevated insulin and/or glucose concentrations was possible.

The elevation in IGF-1 concentrations on d 12 and 19 in the DEX-treated geldings in Exp. 1 was unexpected, particularly given that GH concentrations were unaltered by treatment. The same increase was noted in the mares in Exp. 2, except that by d 19 concentrations had begun to decrease back to pretreatment levels. The increase occurred 6 to 8 d after the end of DEX injections, and it is possible that IGF-1 concentrations rose in response to the earlier elevation in insulin concentrations (Bereket et al., 1999; Thrailkill 2000). Another possibility is that increased concentrations of leptin resulted in the elevation in IGF-1. Houseknecht et al. (2000) observed a high correlation between IGF-1 mRNA and leptin mRNA after incubation of bovine adipose tissue with GH *in vitro*. In addition, it has been suggested that excess GH and IGF-1 reduces serum leptin concentrations (Miyakawa et al., 1998). Therefore, the possibility that an IGF-1/leptin feedback mechanism exists cannot be ruled out at this time.

In Exp. 3, ACTH was used to elevate plasma cortisol concentrations within physiologic limits for the horse. At this dose and frequency of injection, ACTH had a transient stimulatory effect on leptin concentrations. The magnitude of the leptin response in ACTH-treated geldings was considerably less than for the DEX-treated horses, which

probably reflects the difference in degree of glucocorticoid stimulation in the three experiments. Others have reported adrenal-leptin associations (Elimam et al., 1998). Spinedi and Gaillard (1998) observed decreased concentrations of leptin in adrenalectomized rats. Moreover, serum immunoreactive leptin levels are increased in human patients with Cushing's Syndrome (Leal Cerro et al., 1996), which some have attributed to the direct effect of glucocorticoids on adipocytes (Masuzaki et al., 1997) but others to the associated hyperinsulinemia and(or) impaired insulin sensitivity (Widjaja et al., 1998). The decrease in leptin concentrations observed in control geldings may be attributed to the abrupt change in feeding regime. Prior to the sampling times the geldings were kept on native pastures and grazed *ad libitum*. During the four days of sampling the geldings were kept in stalls and fed hay.

The elevated concentrations of TSH and the decreased concentrations of T3 and T4 in the stallions in Exp. 4 confirmed their status as hypothyroid relative to controls. The increase in leptin concentrations in those PTU-treated stallions, albeit small, is in agreement with reports in several species. Leonhardt et al. (1999) reported an elevation in concentrations of leptin following methimazole-induced hypothyroidism in rats, with the greatest concentrations noted after 28 d of treatment. Escobar-Morreale et al. (1997) reported that thyroidectomized rats had increased concentrations of leptin compared to controls and animals treated with T3 and T4. Similarly, in humans, hypothyroid subjects had elevated levels of leptin compared to controls and hyperthyroid subjects (Leonhardt et al., 1998; Pinkney et al., 1998; Pinkney et al., 2000). In contrast to these reports, some have reported lower leptin concentrations in human hypothyroid patients (Valcavi et al., 1997; Yoshida et al., 1998). The decrease in leptin concentrations in control stallions observed in this

experiment may have been due to the effect of season (samples were collected during September and October). A seasonal pattern in circulating leptin concentrations has been previously reported (Fitzgerald and McManus, 2000) in both young and mature mares, with leptin being greatest in the summer and lowest in the winter. Moreover, this seasonal trend was most evident in the older mares (Fitzgerald and McManus, 2000).

In conclusion, treatment of geldings or mares with DEX results in a consistent but apparently transient increase in plasma leptin concentrations in addition to the hyperglycemia and hyperinsulinemia associated with excess glucocorticoid stimulation. Stimulation with ACTH injections also increased leptin concentrations, but to a lesser extent than DEX treatment. Further, PTU-induced hypothyroidism in the horse is associated with increased concentrations of leptin.

CHAPTER 3

CHANGES IN LEPTIN CONCENTRATIONS IN HORSES FOLLOWING ADMINISTRATION OF DEXAMETHASONE OR TESTOSTERONE PROPIONATE

Introduction

We previously reported that dexamethasone (DEX) dramatically increases concentrations of leptin in mares and geldings (Gentry et al., 2002a; Cartmill et al., 2003a). Buff and co-workers (2002) reported that leptin concentrations are greater in stallions and geldings compared to mares. Concentrations of leptin were increased in ovariectomized rats treated with estradiol-17β (Tanaka et al., 2001). In contrast, androgen treatment decreased concentrations of leptin in healthy men (Simon et al., 2001). The objectives of the present experiments were to determine 1) whether multiple DEX injections increased leptin secretion in stallions as they do in mares and geldings, 2) whether concentrations of leptin differ in mares, geldings, and stallions following a single injection of DEX, 3) whether different doses of DEX would result in varying leptin responses in mares, and 4) whether testosterone propionate would decrease concentrations of leptin in mares.

Materials and Methods

Experiment 1. Nine adult, light horse stallions between 4 and 16 yr of age were maintained in paddocks and fed daily hay and grain rations for the duration of the experiment. Each stallion was randomly assigned to receive either dexamethasone (DEX; 125 μg/kg BW in vegetable oil; n = 5) or vehicle (n = 4) as an i.m. injection daily for 5 d beginning on d 1. Beginning immediately prior to the first injection, blood samples were collected via jugular venipuncture at 12 h intervals through d 5.5. Additional samples were collected on the mornings of d 8, 10, 14, 17, 21, 24, 28, and 31.

Experiment 2. Thirty light horses (10 stallions, 10 mares, and 10 geldings), ranging in age from 2 to 20 years, were randomly assigned to receive a single i.m. injection of DEX (125 μg/kg BW) once on d 0. Blood samples were collected via jugular venipuncture once on d -2, -1, and 0, then at 12 h intervals through d 4, and then once daily on d 5, 6, 8, and 10.

Prior to blood sampling, all horses were assigned a body condition score (BCS; Henneke et al., 1983), and an estimation of subcutaneous fat thickness (FT) was obtained via ultrasound at the withers, 10th rib, rump, and tailhead. Body condition scores and ultrasound measurements of fat thickness were collected again on d 14 relative to DEX injection.

Experiment 3. Sixteen light horse mares were assigned randomly to receive an i.m. injection of one of four different doses of DEX in vegetable oil (125, 62.5, 31.25, or 15.625 μg/kg BW). Blood samples were collected from each horse via jugular venipuncture at 0, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 h relative to injection.

Experiment 4. Twelve light horse mares were assigned to two groups of six based on plasma leptin concentrations (an equal number of high and low secretors in each group; Cartmill et al., 2003b). Groups were randomly assigned to receive either testosterone propionate (175 μg/kg BW i.m.) in oil or a similar volume of oil every other day for a total of five injections. Samples of jugular blood were collected from each mare daily beginning on d -2 relative to the first injection. On d 0, injections were started immediately after the daily blood samples were collected that morning. Injections each day followed blood sampling. Immediately following treatment injections on d 4, all mares received 50 μg/kg BW of DEX i.m. in vegetable oil. Blood samples were collected at 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 h relative to injection of DEX.

Handling and Analysis of Samples. Blood samples were immediately centrifuged at 1,200 x g for 20 min at 5°C upon collection. Plasma was harvested and stored at -15°C. Concentrations of insulin (DePew et al., 1994) and IGF-1 (Sticker et al., 1995b) were determined via RIA previously validated for horse samples. Concentrations of glucose were determined spectrophotometrically (method no. 315; Sigma Chem., St. Louis, MO). In Exp. 1 and 2, concentrations of leptin were determined using a commercial kit (Multi-species leptin kit, Linco Research Inc., St. Charles, MO) previously validated for horse samples (McManus and Fitzgerald, 2000; Cartmill, et al., 2003a). Subsequently, a newly developed RIA for measuring leptin (Cartmill et al., 2003b) was validated for horse samples, and this assay was used for samples in Exp. 3 and 4.

Statistical Analyses. For all experiments, data were analyzed by GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Single point variables were analyzed via a one-way ANOVA. Data from daily and frequent sampling periods were analyzed for effects of treatment, time, and treatment by time interactions via split-plot ANOVA (Gill and Hafs, 1971). The main effect of treatment was tested with the horse within treatment term as error. The effect of time and its interaction with treatment was tested with the residual error. The residual leptin concentrations in Exp. 1 were analyzed as well due to an apparent pre-DEX difference. Residual leptin concentrations were calculated by subtracting the concentrations of leptin prior to treatment from subsequent samples. In Exp. 2, simple correlations and stepwise regression analysis (Steel et al., 1997) were used to determine relationships of gender, estimates of body fatness, and age with pre-treatment concentrations of leptin and with the maximum leptin response following DEX.

Results

In Exp. 1, treatment with DEX increased ($P < 0.01$) concentrations of leptin, glucose, and insulin in stallions (Figure 3.1a, b, and c, respectively). Concentrations of IGF-1 in stallions receiving DEX decreased through d 5 then increased to become greater ($P < 0.01$) compared to controls on d 13 (Figure 3.1d).

In Exp. 2, there was a tendency ($P = 0.06$) for a main effect of gender in the ANOVA for leptin concentrations following a single injection of DEX, and there was a strong interaction ($P < 0.01$) between gender and time (Figure 3.2), with the leptin response being greater ($P < 0.01$) in mares and geldings compared to stallions. In addition, mares and geldings had greater ($P < 0.01$) weight, BCS, and ultrasound FT compared to stallions (Table 3.1). Simple correlation coefficients are shown in Table 3.2. Estimates of body fatness were highly correlated ($P < 0.01$) to concentrations of leptin prior to DEX treatment and to the maximum response to DEX. Based on stepwise regression analysis, BCS, FT over the withers, and gender best fit ($R^2 = 0.65$) the model for pre-DEX concentrations of leptin. For maximum leptin response to DEX, the best fitting model ($R^2 = 0.75$) included BCS, FT over the back, and gender.

In Exp. 3, there was an effect ($P < 0.01$) of time, indicating a response in leptin to treatment with DEX. However, there was no difference ($P > 0.10$) in concentrations of leptin (Figure 3.3) following any of the four doses of DEX.

In Exp. 4, there was no effect ($P > 0.10$) of treatment or interaction with time on leptin concentrations (Figure 3.4) in mares receiving testosterone propionate compared to mares
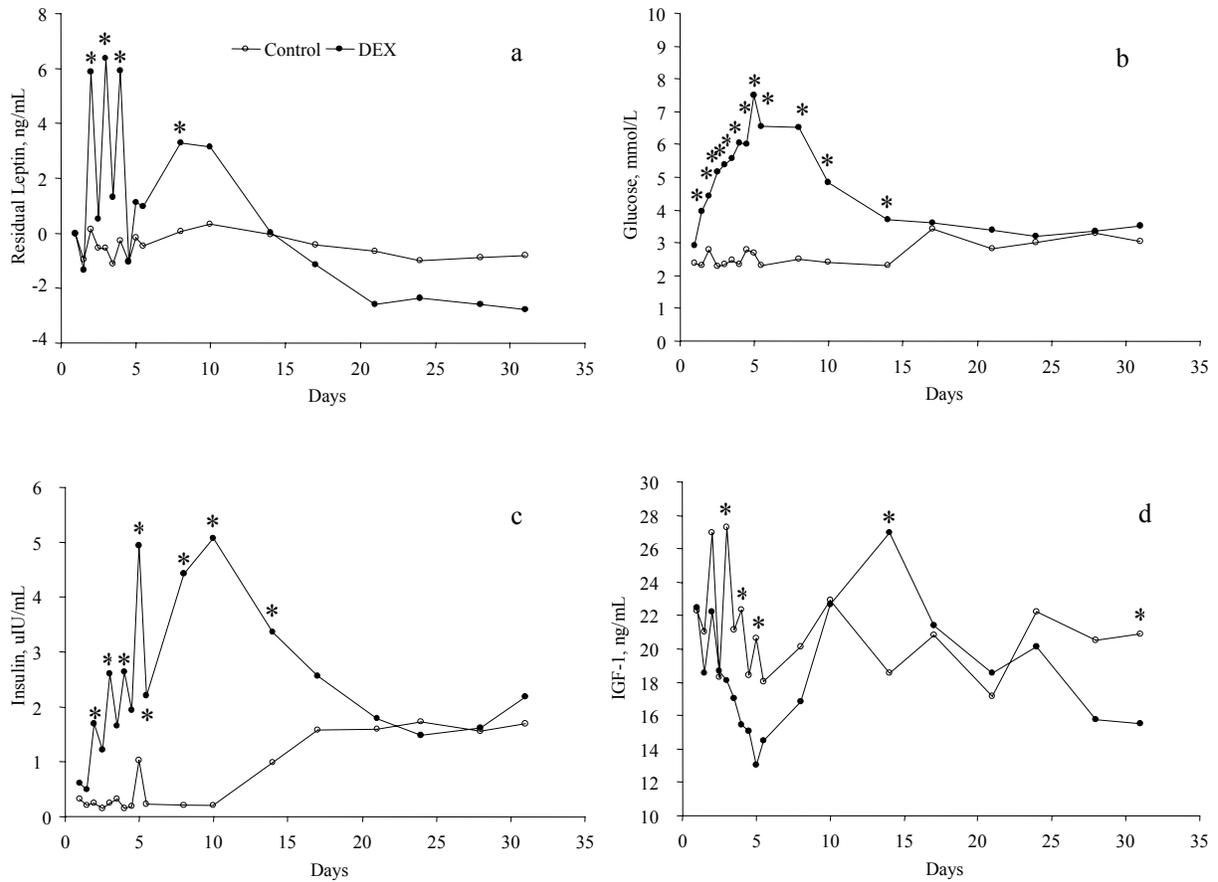
Figure 3.1. Residual leptin concentrations (a), mean concentrations of glucose (b), insulin (c), and IGF-1 (d) in stallions treated 5 consecutive days with dexamethasone (DEX; 125 µg/kg BW) or vehicle. Pooled SEM were 0.8 ng/mL, 0.2 mmol/L, 3.5 µIU/mL, and 12.4 ng/mL for residual leptin, glucose, insulin, and IGF-1 respectively. Asterisks indicate differences between groups (P < 0.05).
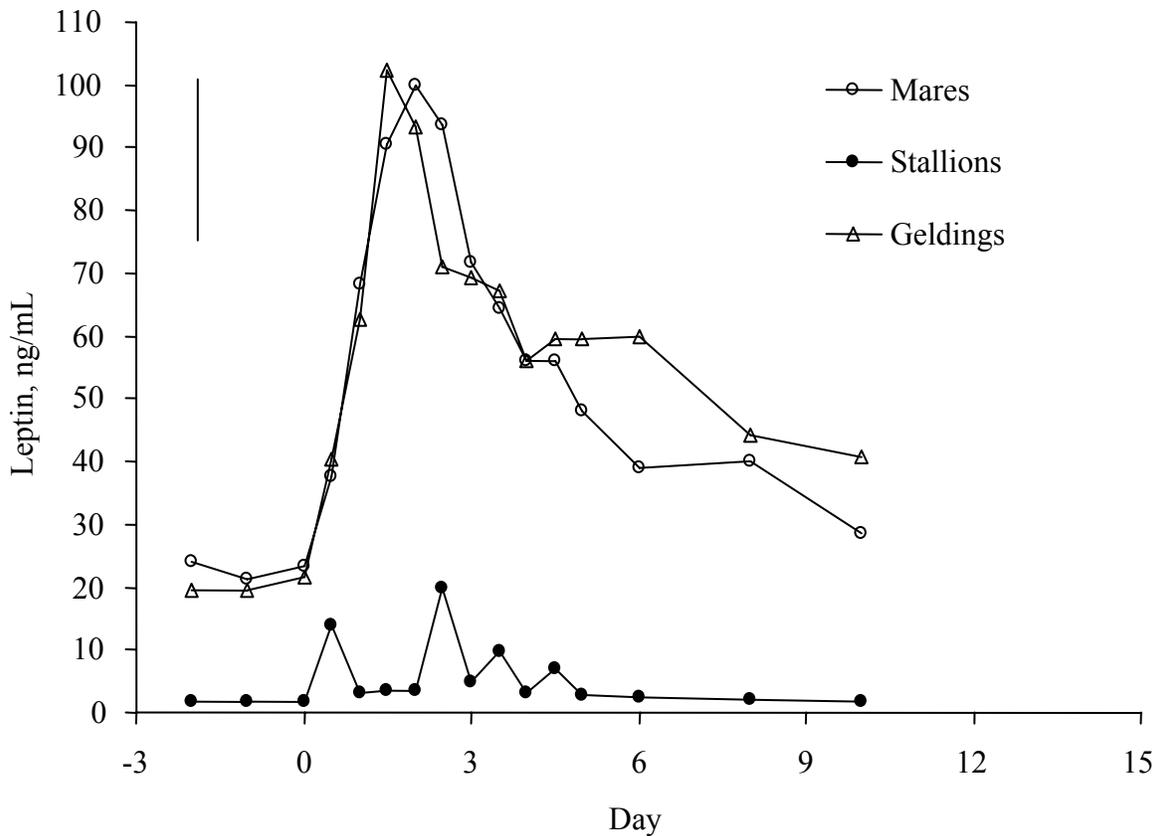
Figure 3.2. Concentrations of leptin in mares, stallions, and geldings following a single i.m. injection of dexamethasone (125 μg/kg BW) on d 0. Plasma leptin was greater ($P < 0.01$) in mares and geldings compared with stallions. Pooled SEM for leptin was 4.6 ng/mL. The vertical bar represents Tukey's HSD value = 26.7.

Table 3.1. Average age, weight, body condition scores (BCS), and ultrasound fat thicknesses at the tailhead, rump, back, and wither for geldings, mares, and stallions.

Gender	Age (yr)	Wt. (kg)	BCS	Tailhead (in)	Rump (in)	Back (in)	Wither (in)
Gelding	10.9 ± 5.2	515 ± 74.9[a]	6.6 ± 1.4[a]	0.67 ± 0.30[a]	0.17 ± 0.06[a]	0.39 ± 0.11[a]	0.48 ± 0.16[a]
Mare	11.4 ± 6.0	560 ± 36.8[a]	7.1 ± 1.3[a]	0.63 ± 0.23[a]	0.15 ± 0.05[a]	0.36 ± 0.13[a]	0.47 ± 0.19[a]
Stallion	13.8 ± 6.3	449 ± 55.1[b]	4.9 ± 1.0[b]	0.35 ± 0.07[b]	0.10 ± 0.02[b]	0.25 ± 0.05[b]	0.28 ± 0.05[b]

[ab]Values in a column with different superscripts differ ($P < 0.05$).

Table 3.2. Simple correlations of concentrations of leptin both before and after dexamethasone treatment in mares, geldings, and stallions with body condition score (BCS), age, gender, and ultrasound measurements of fat thickness.

Measurement	Pre-dexamethasone Leptin		Maximum Leptin Response	
	Pearson	Spearman	Pearson	Spearman
BCS	0.695 **	0.805 **	0.768 **	0.824 **
Fat thickness				
Withers	0.683 **	0.614 **	0.730 **	0.714 **
Rib	0.540 **	0.583 **	0.671 **	0.667 **
Back	0.670 **	0.649 **	0.730 **	0.707 **
Tailhead	0.620 **	0.610 **	0.719 **	0.699 **
Weight	0.380 *	0.510 **	0.379 *	0.437 *
Age	0.260	0.261	0.231	0.118
Gender	-0.237	-0.474 **	-0.300	-0.505 **

* Values indicated with single asterisks differ ($P < 0.05$).
** Values indicated with double asterisks differ ($P < 0.01$).
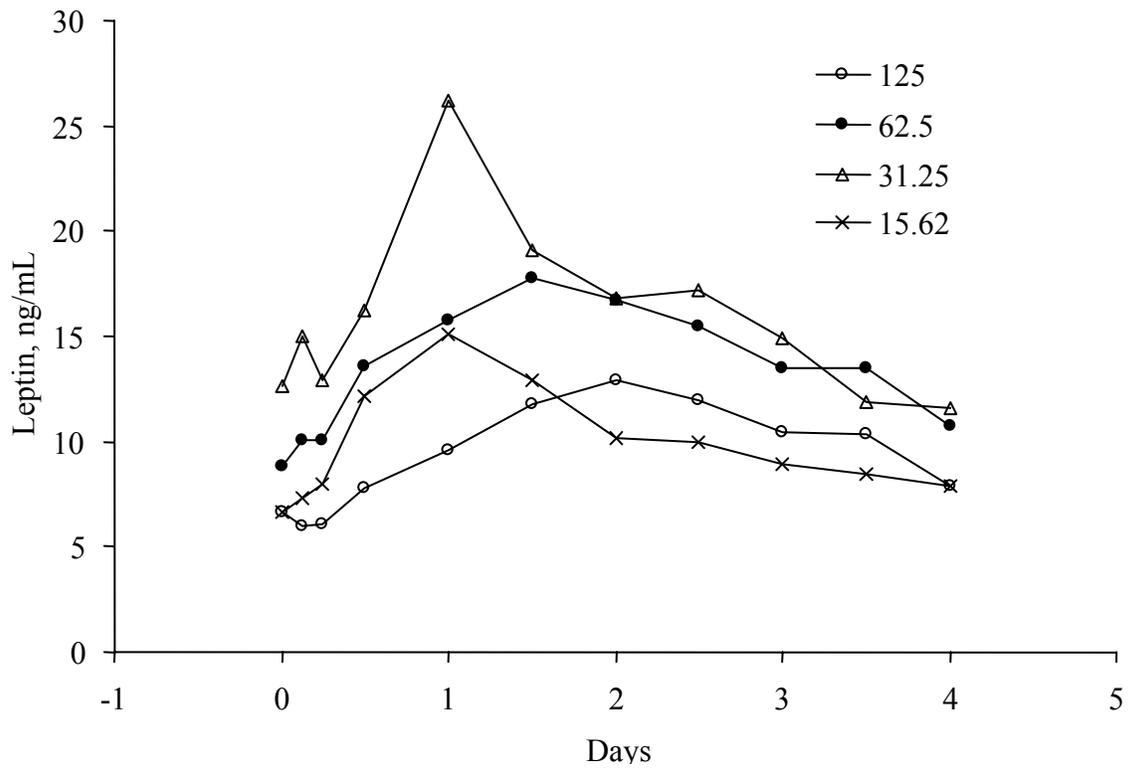
Figure 3.3. Concentrations of leptin in mares following a single i.m. injection of dexamethasone in oil (125 μg/kg BW, 62.5 μg/kg BW, 31.25 μg/kg BW, or 15.625 μg/kg BW). There was no difference between doses ($P > 0.10$). Pooled SEM for leptin was 0.71 ng/mL.
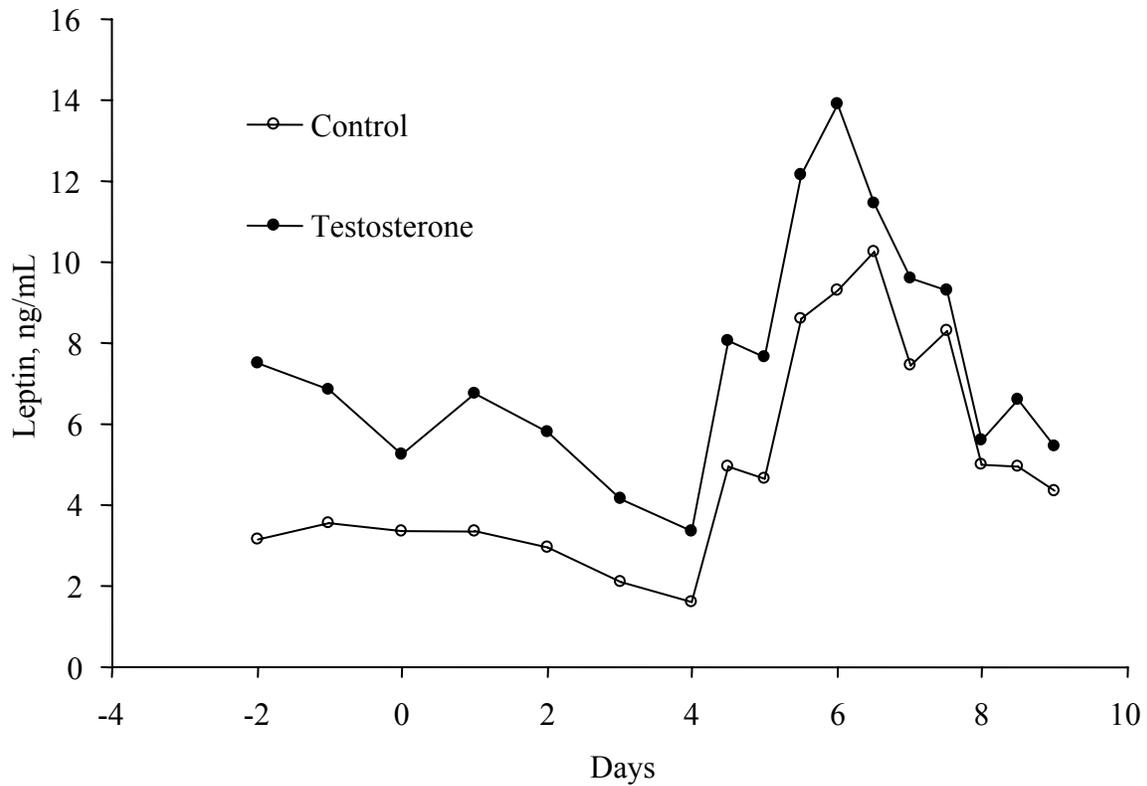
Figure 3.4. Concentrations of leptin in mares treated daily with testosterone proprionate (175 µg/kg BW) or vehicle for 5 d beginning on d0. On d 4, all mares received a single i.m. injection of dexamethasone (50 µg/kg BW). There was no difference between treatment groups (P > 0.10). Pooled SEM for leptin was 0.50 ng/mL.

receiving oil. However, as in Exp. 3, there was an effect of time (P < 0.01), indicating a

response in leptin to treatment with DEX.

Discussion

In Exp. 1, treatment of stallions with the potent glucocorticoid analog DEX resulted in

large increases in glucose, insulin, and leptin similar to those previously observed in mares

and geldings (Gentry et al., 2002a; Cartmill et al., 2003a). However, unlike mares and

geldings in high body condition, which displayed constantly high concentrations of leptin,

these stallions had a spike-like pattern of secretion in leptin and insulin, with peaks occurring

~12 h after each injection. In addition to the morning injections of DEX, these stallions were

meal-fed a concentrated grain mixture at 0700 everyday, which supplemented their *ad libitum*

hay and native grasses in their respective pens; mares and geldings in previous experiments

were maintained on pasture and likely grazed throughout the day. Thus, gender, lower BCS,

and feeding regimen are all possible factors in the difference between leptin patterns. Similar

to these stallions, Gentry and co-workers (2002a) reported that mares in low body condition

(BCS of 3.0 to 3.5) had leptin responses to DEX that peaked approximately 12 h after each

injection before returning to near pre-treatment concentrations by 24 h. Those low BCS

mares were only allowed to graze 2 h each morning, which likely mimicked the meal-feeding

situation of the stallions in the present experiment.

The increase in concentrations of IGF-1 in stallions receiving DEX was similar (i.e. a

delayed increase) to previous observations in both mares and geldings (Cartmill et al., 2003a).

However, it still remains unclear as to whether this increase in IGF-1 is due directly to

treatment with DEX or results from increases in leptin (Houseknecht et al., 2000) and/or

insulin (Bereket et al., 1999; Thrailkill 2000).

In Exp. 2, under the management conditions at the LSU Horse Farm, mares and geldings had much greater concentrations of leptin compared to stallions both before and after treatment with a single injection of DEX. In addition, the pattern of leptin secretion in mares and geldings in response to a single injection of DEX peaked on d 2 to 2.5. This response pattern is very similar to our previous observations (Cartmill et al., 2003a) in which mares and geldings received four daily injections of DEX and leptin concentrations peaked around d 3. The fact that leptin concentrations decreased after a peak at 2 to 3 d, regardless of whether horses get a single injection or four daily injections, indicates that the DEX stimulus on leptin is transient.

The lower concentrations of leptin in stallions (Exp. 2) are consistent with the results from Exp. 1. In human males, treatment with either testosterone or DHT decreased concentrations of leptin (Simon et al., 2001). Simon and co-workers (2001) reported that the decrease was due mainly to DHT treatment. In addition, treatment of men with anabolic-androgenic steroids decreased serum leptin, whereas suppression of endogenous testosterone increased serum leptin (Hislop et al., 1999). Therefore, testicular production of testosterone and/or DHT in stallions may contribute to lower concentrations of leptin compared to mares and geldings. In contrast to these results, Buff and co-workers (2002) reported that stallions and geldings had greater concentrations of leptin compared to mares, as well as a positive correlation of increasing concentrations of leptin with increasing age and body condition. It was not reported how gender and body condition were related or whether stallions were in greater body condition compared to the mares.

In the present experiment, results of the simple correlations showed that age was not highly correlated to concentrations of leptin either before or after administration of DEX.

Further, mares and geldings were of greater body condition than the stallions, and body condition is typically a major determinant in leptin secretion rates. In the multiple regression analysis, gender was highly correlated to circulating leptin even after accounting for BCS and fat thickness. Thus, the presence of testes, in addition to body condition, likely influences circulating concentrations of leptin in horses (given that geldings and mares had similar leptin characteristics).

In Exp. 3, the greatest dose of DEX used was the same used in our previous experiments (Gentry et al., 2002a; Cartmill et al., 2003a). The three lesser doses resulted in similar leptin responses as the highest dose. Therefore, it is likely that 125 µg/kg BW is on the saturation part of the dose-response curve. In future studies, a dose as low as 15.62 µg/kg BW should be adequate to elicit a leptin response.

The dose and frequency of testosterone propionate used in Exp. 4 was derived from the report of Thompson et al. (1980) and is known to produce testosterone concentrations in blood of geldings similar to those found naturally in breeding season stallions. Treatment of mares with testosterone propionate did not alter concentrations of leptin. This is in agreement with one report (Pineiro et al., 1999) in which no effect of testosterone on *in vitro* leptin secretion in adipocytes from either men or women was observed. However, Pineiro and co-workers (1999) reported that DHT, dehydorepiandrosterone sulphate, and stanozolol, a non-aromatizable androgen, decreased *in vitro* leptin secretion from female adipocytes but not adipocytes from men.

In contrast, others (Simon et al., 2001) reported that *in vivo* treatment with DHT and not testosterone decreased leptin in healthy human males with low plasma testosterone. Further, Jockenhovel and co-workers (1997) reported that *in vivo* treatment of obese

hypogonadal men with testosterone normalized elevated concentrations of leptin. In those studies where an effect of androgen treatment on *in vivo* leptin secretion was reported, human males and not females were used. Tanaka and co-workers (2001) observed an increase in leptin concentration in ovariectomized rats following administration of estradiol-17β. Therefore, one possibility why treatment of mares with testosterone propionate failed to reduce leptin concentrations is that the mares were at random unknown stages of the estrous cycle, and the presence of endogenous ovarian steroids may have also influenced leptin concentrations. Further studies of mares in known stages of the estrous cycle, as well as geldings treated with androgens, are needed to clarify the androgenic effect on leptin secretion in horses.

In summary, stallions exhibit a leptin response to multiple injections of DEX that is similar to the responses in mares and geldings, but differs in pattern. Feeding management likely influences the pattern of leptin response to DEX. Mares and geldings have greater resting concentrations of leptin as well as greater leptin responses to DEX compared to stallions, even after accounting for differences in fatness. In addition, a much lower dose of DEX than previously used seems sufficient in eliciting an increase in concentrations of leptin in horses. And finally, short-term testosterone propionate treatment does not decrease circulating leptin in mares.

CHAPTER 4

EFFECT OF DEXAMETHASONE OR FEEDING TIME ON PLASMA LEPTIN CONCENTRATIONS IN STALLIONS

Introduction

Concentrations of leptin, a protein hormone secreted by adipocytes, are increased in mares and geldings of high body condition following 4 d of daily injections of dexamethasone (DEX; Gentry et al., 2002a; Cartmill et al., 2003a; Chapter 3). A similar increase in plasma leptin concentrations was observed in stallions following 5 d of DEX administration (Chapter 3). In stallions, but not in mares or geldings, the leptin response to DEX varies diurnally, with peak concentrations occurring in the evening. This diurnal pattern in leptin was apparent in both treated and control stallions, but was greatly enhanced by DEX treatment. A similar diurnal pattern in leptin concentrations was reported by Gentry et al. (2002a) for mares in low body condition that were allowed to graze for only 2 h each morning. One common factor in these latter two experiments was the meal-fed nature of the horses' nutrient intake: the stallions received a portion of their diet as a concentrate, fed each morning, and the thin mares were limit grazed for 2 h. The mares and geldings of high body condition were maintained on pasture and grazed continuously *ad libitum*. In humans (Dallongeville et al., 1998; Elimam and Marcus, 2002) and cats (Appleton et al., 2002), concentrations of leptin increase following a meal. Moreover, Dallongeville et al. (1998) concluded that daily surges in leptin concentrations were in response to feeding and not the day-night cycle. The purpose of the present experiments was to characterize the effects of DEX and(or) feeding time on the pattern of leptin secretion in stallions.

Materials and Methods

Ten light horse stallions in average body condition (BCS 4 to 6; Henneke et al., 1983) were maintained, under normal management practices at the LSU horse farm, on minimal native grasses in individual pens, *ad libitum* hay and water, and a commercially prepared concentrate fed once daily at 0700. In Exp. 1, stallions were randomly assigned to receive either a single injection of DEX (125 μg/kg BW i.m.; n = 5) or vehicle (controls; n = 5) at 0700 on d -1. Beginning at 0700 the next day (0 h), blood samples were drawn every 2 h for 36 h via jugular venipuncture into heparinized tubes.

Experiment 2 was initiated 2 d after the conclusion of Exp. 1. In Exp. 2, the same 10 stallions were assigned to receive the concentrate portion of their diet every 24 h either at 0700 (AM; 0 h; n = 5) or 1900 (PM; 12 h; n = 5). Stallions were allotted to treatments so that a similar number of horses that received DEX in Exp. 1 were in both treatment groups. After a 14-d adjustment period to the feeding regimens, blood samples were collected via jugular venipuncture every 4 h for 48 h, then twice daily for an additional 5 d.

In both experiments, samples of blood were immediately centrifuged and plasma was harvested and stored at -15ºC. Concentrations of leptin (Multi-species Leptin Kit, Linco Research Inc., St. Charles, MO; McManus and Fitzgerald, 2000; Cartmill et al., 2003a), cortisol (Diagnostic Systems Laboratory, Webster, TX), and insulin (DSL) were determined using RIA. Concentrations of glucose were determined spectrophotometrically (Method no. 315; Sigma Chemical Co., St. Louis, MO). The assay sensitivities and intra- and interassay coefficients of variation were 0.8 ng/mL, 4%, and 8% for leptin; 0.11 μg/dL, 5%, and 8% for cortisol; and 2.0 mIU/mL, 5%, and 8% for insulin.

Data from both experiments were analyzed via the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a completely randomized design for Exp. 1 and a randomized block design for Exp. 2, both with repetitive sampling. The dependant variables were analyzed with horse, treatment, time, and appropriate interactions included in the model. Treatment was tested with horse within treatment as the error term. Time and its interaction with treatment was tested with residual error. Differences between treatments at individual time points were assessed via the LSD test. In Exp. 1, due to heterogeneity of variances in leptin concentrations after DEX, the log of the leptin data was used for analysis.

Results

In Exp. 1, concentrations of cortisol (Figure 4.1a) in control stallions varied ($P < 0.01$) with time, being greatest in the morning and lowest in the evening; treatment with DEX suppressed ($P < 0.01$) cortisol concentrations. Concentrations of insulin (Figure 4.1b) were greater ($P < 0.01$) in the DEX-treated group and increased ($P < 0.01$) in response to feeding in both DEX-treated and control stallions; the response in treated stallions was greater ($P < 0.01$) than that of controls. Concentrations of leptin (Figure 4.1c) in control stallions varied ($P < 0.01$) with respect to time in an apparent diurnal pattern, being highest in the evening. This pattern of leptin secretion was exaggerated ($P < 0.01$) by treatment with DEX (Figure 4.1d).

In Exp. 2, concentrations of cortisol varied diurnally ($P = 0.02$; Figure 4.2a) and were not altered ($P = 0.21$) by feeding time. Plasma concentrations of glucose, insulin, and leptin (Figure 4.2b, c, and d, respectively) all increased ($P < 0.01$) following feeding. However, the peaks in insulin and leptin concentrations were shifted 12 h by feeding at 1900 rather than 0700.
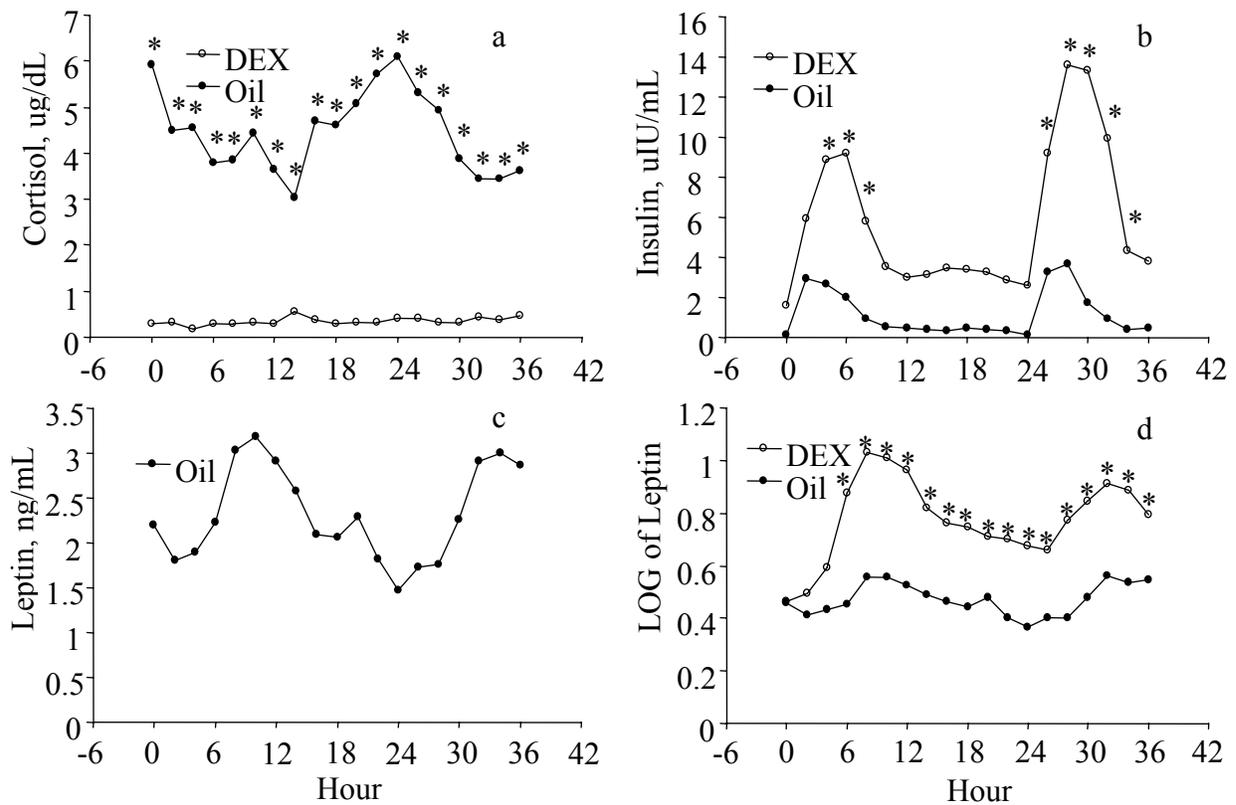
Figure 4.1. Concentrations of cortisol (a), insulin (b), leptin in control stallions only (c), and LOG of leptin concentrations (d) in stallions receiving dexamethasone (DEX) or oil. Points indicated with asterisks differ (P < 0.01). Pooled SEM = 0.31 μg/dL, 9.50 μIU/mL, 2.55 ng/mL, and 0.05 for cortisol, insulin, leptin, and log of leptin, respectively.
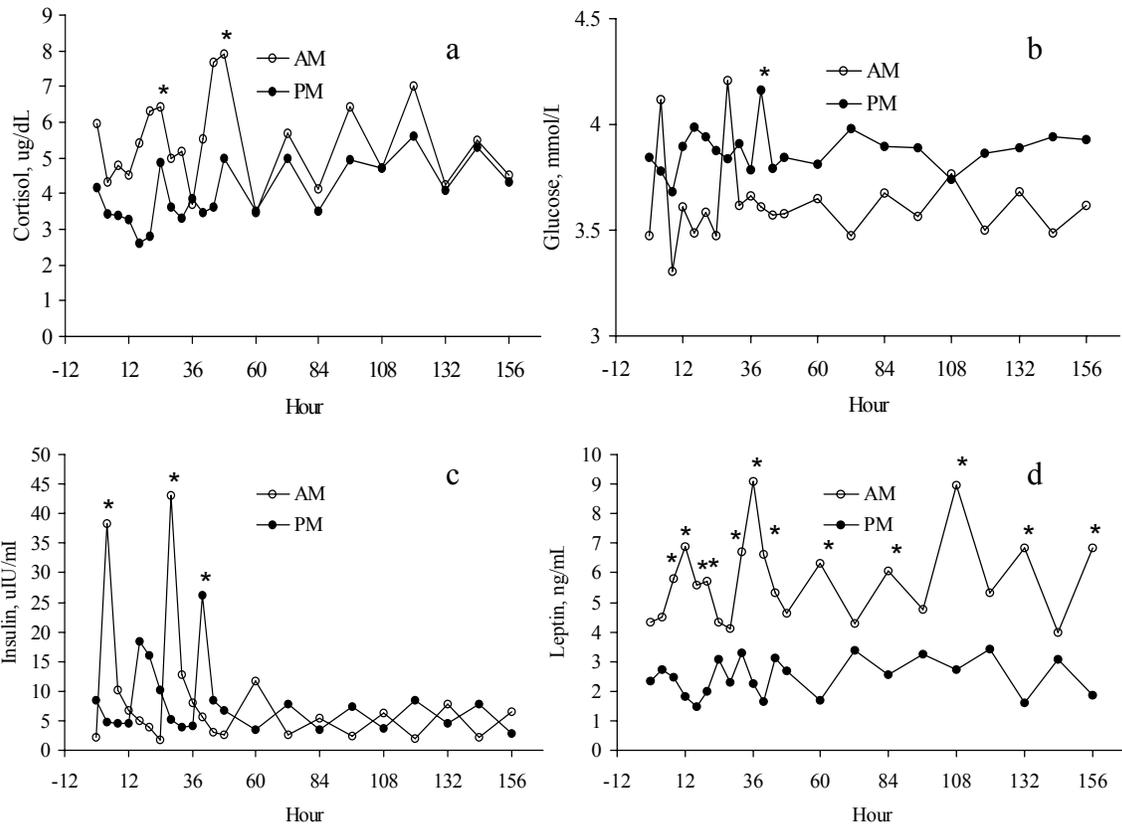
Figure 4.2. Concentrations of cortisol (a), glucose (b), insulin (c), and leptin (d) in stallions fed the concentrate portion of their diet at either 0700 (AM) or 1900 (PM). Points indicated with asterisks differ ($P < 0.01$). Pooled SEM = 0.61 μg/dL, 0.10 mmol/L, 3.79 μIU/mL, and 0.72 ng/mL for cortisol, glucose, insulin, and leptin, respectively.

Discussion

In Exp. 1, a diurnal pattern in concentrations of leptin was clearly seen in control stallions (Figure 4.1c). In these stallions, plasma leptin concentrations peaked ~10 h after feeding, which occurred at 0 and 24 h on the graph. This increase in leptin concentrations was preceded by an increase in insulin concentrations that peaked approximately 4 to 6 h after feeding. Administration of a single injection of DEX to stallions suppressed plasma cortisol concentrations and increased concentrations of insulin and leptin, which is similar to our previous observations in mares and geldings (Cartmill et al., 2003a). Treatment with DEX also enhanced the diurnal variation in leptin concentrations. This enhancement agrees with the pattern of leptin secretion in stallions receiving DEX daily for 5 d (Chapter 3). In that study, due to a less frequent sampling schedule, it appeared that leptin secretion peaked 12 h following administration of DEX and every 24 h thereafter for the remainder of the study. With the higher frequency blood sampling in the present experiment, it is clear that leptin secretion in stallions peaked at ~8 h after each feeding.

In Exp. 2, concentrations of insulin and leptin increased in response to feeding, which is consistent with the results of Exp 1. and with reports for other species (Dallongeville et al., 1998; Appleton et al., 2002; Elimam and Marcus, 2002). The patterns of concentrations of both insulin and leptin were shifted ~12 h by altering the feeding time. The peak in insulin concentrations occurred 4 h after feeding in both feeding groups, and was followed by a peak in leptin concentrations 8 h later (12 h after feeding). In cats, peak concentrations of leptin occurred 9 h after the insulin peak following a meal (Appleton et al., 2002). Because the diurnal patterns in cortisol concentrations were not shifted by altering the feeding schedule, it

is likely that the diurnal pattern in leptin concentrations in stallions is influenced more by

feeding time than by cortisol levels.

CHAPTER 5

ENDOCRINE RESPONSES IN MARES AND GELDINGS WITH HIGH BODY CONDITION SCORES GROUPED BY HIGH VERSUS LOW RESTING LEPTIN CONCENTRATIONS[2]

Introduction

Gentry et al. (2002a) reported that leptin concentrations in adult mares with high body condition scores (BCS) in September tended to vary widely, and mares tended to fit into two distinct groups based on leptin concentrations: low (< 5 ng/mL) and high (7 to 20 ng/mL). Subsequent observations on 18 of those same mares indicated that the high vs low distinction was consistently observed 2 years later (*unpublished*), indicating that the underlying cause was relatively permanent. Leptin secretion by adipocytes has been reported to be affected by various other hormones, including insulin (Sivitz et al., 1998; Ramsay and White, 2000), GH (Isozaki et al., 1999), glucocorticoids (Wang et al., 2002; Cartmill et al., 2003a), epinephrine (Cammisotto and Bukowiecki, 2002), prolactin (Mastronardi et al., 2000), and thyroid hormones (Ghizzoni et al., 2001; Nowak et al., 2002; Cartmill et al., 2003a). The purpose of the current experiment was to test the hypothesis that the high vs low leptin concentrations observed in horses of good body condition were due to some interaction of leptin secretion with other endocrine systems in the horse. Specific hormonal systems studied were insulin, GH/IGF-1, prolactin, adrenal glucocorticoids, and thyroid hormones.

Materials and Methods

Selection of Horses. All horses were of light horse breeds from the resident herd at the Louisiana Agricultural Experiment Station - Central Stations Horse Farm. They were routinely maintained on native grass pastures during the spring, summer, and fall and were

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ryegrass pasture in winter. On September 25, a total of 36 mares and 18 geldings were assigned a body condition score (BCS) as described by Henneke et al. (1983; 1 = extremely emaciated through 9 = extremely fat) by two independent, experienced technicians; the mean of the two estimates were used. Previous studies have documented that there are high correlations between direct estimates of empty body fat and backfat thickness determined by ultrasonography (Kane et al., 1987) and between BCS and backfat thickness determined by ultrasonography (Gentry et al., 2002b). Jugular blood samples were collected from each horse into heparinized tubes on the day of BCS estimation for determination of initial leptin concentration. Of the mares and geldings that had a mean BCS of at least 7.5, five each were selected that had the lowest (low leptin) and highest (high leptin) leptin concentrations. Preliminary analysis of variance indicated that BCS were approximately equivalent for the low leptin and high leptin groups, whereas geldings in the high leptin group had slightly higher BCS on the average (8.6 vs 7.8 to 7.9 ± 0.3 for the other groups; P = 0.052). Similar analysis of age indicated that horses selected for low leptin vs high leptin had similar mean ages (10.2 vs 12.2 yr ± 1.8 yr, respectively), whereas geldings were older (13.7 yr) than mares (8.7 yr; P = 0.013).

Daily Monitoring and Challenges. To establish baseline daily concentrations of the various hormones of interest, samples of jugular blood were collected at 12-h intervals beginning at 0700 on October 4 and continuing through 1900 on October 6. The horses were brought in from pasture on each occasion and were sampled within 1 h or less. These samples were drawn into evacuated, heparinized tubes and the resulting plasma was stored at -15º C.

For the subsequent challenges (manipulations) that were used to perturb the hormonal systems of interest, the general procedure was that horses were fitted with jugular catheters,

loosely tethered in a shed, and allowed to rest at least 1 h before blood sampling was initiated.

Frequent blood samples from the challenges were placed into tubes containing heparin and

were centrifuged within 15 min; plasma was stored at -15ºC. There was a minimum of 2 d

between consecutive challenges, administered in the order: i.v. glucose infusion, i.v.

thyrotropin releasing hormone (TRH) injection, exercise, and i.m. dexamethasone injection.

The doses of glucose (Sticker et al., 1995a), TRH (Thompson and Nett, 1984), and

dexamethasone (Cartmill et al., 2003a) were the same as those used in previous experiments;

all three were purchased from Sigma Chem. Co. (St. Louis, MO).

For the glucose infusion administered on October 7, all horses were deprived of feed

overnight and were administered glucose i.v. in the morning. Glucose (0.2 g/kg BW as a 0.5

g/mL solution in 0.155 M saline) was administered through the jugular catheter. Blood

samples drawn at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min relative to

onset of infusion were used to assess insulin response to glucose.

An i.v. TRH challenge (4 µg/kg BW) was administered to each horse at 1400 on

October 10. Blood samples collected at -10, 0, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min

relative to injection of TRH were used to assess the responses of prolactin, TSH, and thyroid

hormones.

A brief exercise bout on October 13 was used to assess the responses of GH, prolactin,

cortisol, and leptin. Beginning in the morning, each horse was lunged in a circular pen for 5

min, usually at a trot, but with occasional cantering. This regimen has been used previously

as a secretory stimulus for GH and prolactin in horses (Thompson et al., 1994). Blood

samples were collected at -10, 0, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min relative to

onset of exercise.

Lastly, on October 16 at 0700, each horse was administered dexamethasone at 125 μg/kg BW as an i.m. injection in corn oil. Blood samples were collected immediately before injection (time 0) and then at 12-h intervals through 0700 on October 20; daily samples were collected at 0700 on October 22, 24, and 26. Plasma from these samples was used for the measurement of leptin, insulin, glucose, IGF-1, and cortisol.

Sample Analyses. Concentrations of glucose were determined spectrophotometrically (Pointe Scientific, Inc., Lincoln Park, MI). Plasma concentrations of leptin (Cartmill et al., 2003b), insulin (DePew et al., 1994), IGF-1 (Sticker et al., 1995b), GH (Thompson et al., 1992), prolactin (Colborn et al., 1991), and TSH (Sticker et al., 2001) were assessed by RIA previously validated for horse samples. Plasma concentrations of cortisol (Diagnostic Systems Laboratories, Webster, TX), triiodothyronine (T3) and thyroxine (ICN Pharmaceuticals, Costa Mesa, CA; Kits 07-290102 and 07-292102, respectively) were assessed with commercially available RIA reagents. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 4%, and 0.2 ng/mL for leptin, 5%, 8%, and 0.5 μIU/mL for insulin; 5%, 12%, and 2 ng/mL for IGF-1; 8%, 11%, and 0.5 ng/mL for GH; 7%, 12%, and 0.2 ng/mL for prolactin; 5%, 8%, and 0.02 ng/mL for TSH; 6%, 8%, and 0.11 μg/dL for cortisol; 5%, 8%, and 3.7 ng/dL for T3; and 5%, 9%, and 1.2 μg/dL for thyroxine.

Statistical Analyses. Data were analyzed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a 2 x 2 factorial ANOVA (gender and group fixed; Steel and Torrie, 1980); repetitive sampling was taken into account when appropriate (split-plot; Gill and Hafs, 1971). Gender, group (low leptin or high leptin), and their interaction were tested with the horses within gender-group term; time and its interactions with gender and group were tested with residual error. Differences among the four groups of horses within each time

period were compared with the LSD test (Steel and Torrie, 1980) when a significant effect of group or an interaction involving group was detected; because four means were being compared within each time period, only differences at the $P < 0.01$ level were considered meaningful in these comparisons.

Results

 Daily Hormone Concentrations. Plasma leptin concentrations in horses selected for high leptin averaged 14.1 ng/mL over the 3-d period of daily sampling (Figure 5.1a), which was greater ($P < 0.0001$) than the 2.8 ng/mL in horses selected for low leptin. In addition, mares had greater ($P = 0.008$) concentrations of leptin than geldings (10.4 vs 6.6 ng/mL). Horses selected for high leptin had lower ($P = 0.027$) daily GH concentrations over the 3-d period than horses selected for low leptin (Figure 5.1b). In contrast, horses selected for high leptin had greater ($P = 0.0005$) daily insulin concentrations than horses selected for low leptin (Figure 5.1c).

Cortisol concentrations in daily samples were greater ($P = 0.0006$) in mares than in geldings, and there was an interaction ($P = 0.03$) between gender and group (Figure 5.2a); mares selected for high leptin had greater cortisol concentrations than mares selected for low leptin, whereas the opposite was true for geldings. There was also a time trend ($P = 0.084$) in cortisol concentrations, with highest concentrations observed in the mornings.

Horses selected for high leptin had greater ($P = 0.0005$) daily concentrations of T3 than horses selected for low leptin (Figure 5.2b). Moreover, there was an interaction ($P = 0.058$) of group with time, with T3 concentrations decreasing over the 3-d period more in the low leptin than the high leptin group. In contrast to T3 concentrations, there was no effect of group ($P = 0.6$) on concentrations of thyroxine. However, there was an interaction ($P =$
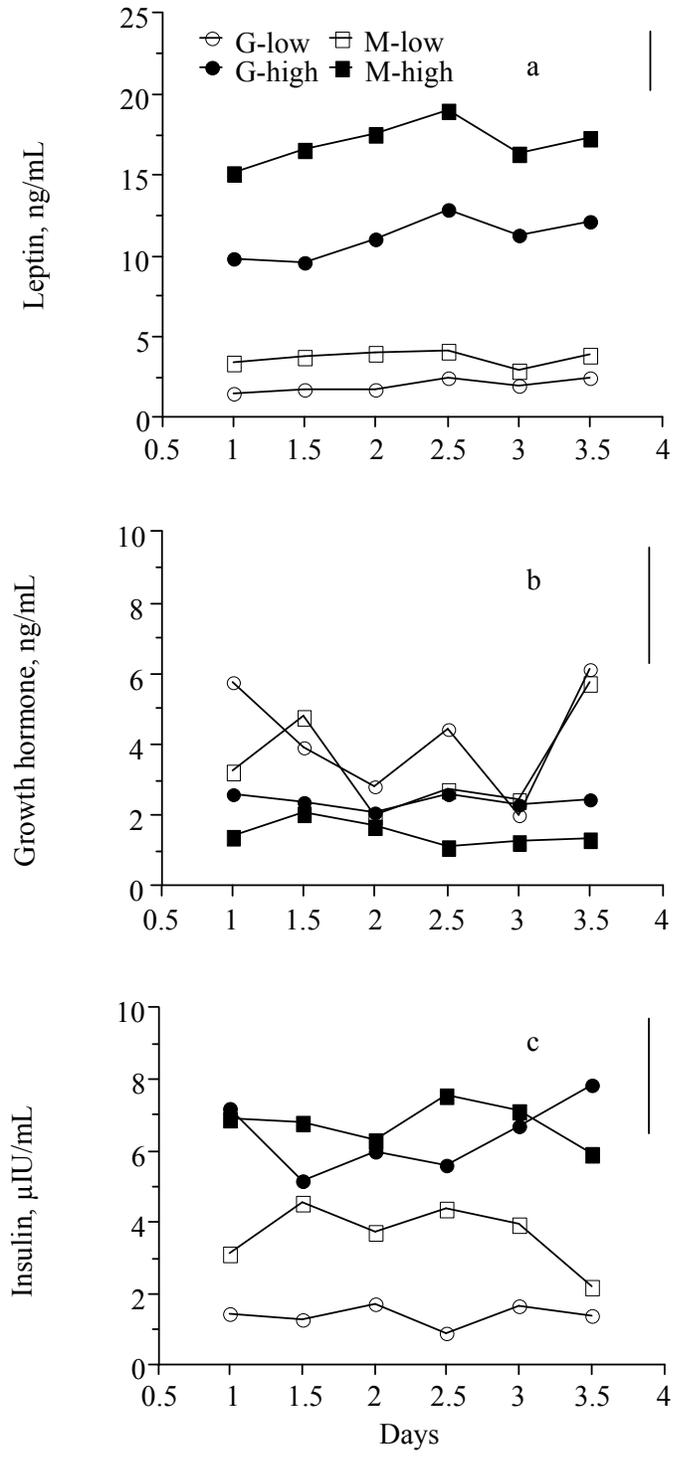
Figure 5.1. Resting concentrations of leptin (a), GH (b), and insulin (c) in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar in a panel indicates the least significant difference (LSD) value ($P < 0.01$) for comparison among groups within each time period. Pooled SEM were 0.95 ng/mL for leptin, 0.86 ng/mL for GH, and 0.83 µIU/mL for insulin.

0.015) of gender, group, and time for concentrations of thyroxine (Figure 5.2c). Mares

selected for low leptin tended to have greater thyroxine concentrations than those selected for

high leptin for most but not all time periods, whereas in geldings the opposite was true. There

was no effect ($P > 0.1$) of group or gender for daily TSH, IGF-1, or prolactin concentrations

(Figure 5.3).

     Responses to Challenges. Horses selected for high leptin had a greater ($P = 0.036$)

insulin response to glucose infusion than did horses selected for low leptin (Figure 5.4).

     Mares had a greater ($P = 0.0006$) TSH response to the i.v. injection of TRH than

geldings (Figure 5.5a), however there was no effect ($P > 0.1$) of group. In addition, mares

tended ($P = 0.088$) to have a greater prolactin response to TRH injection (Figure 5.5b).

Average concentrations of T3 during the TRH challenge were greater ($P = 0.047$) in horses

selected for high leptin compared to horses selected for low leptin (Figure 5.5c), and there

was an interaction between group and time ($P = 0.022$) for concentrations of thyroxine

(Figure 5.5d).

     Plasma concentrations of GH increased ($P = 0.0004$) in response to 5 min of exercise

(Figure 5.6a), as did cortisol concentrations (Figure 5.6b; $P < 0.0001$). However, there was

no effect ($P > 0.1$) of gender or group on the GH or cortisol responses. Leptin concentrations

in the four groups around the exercise bout (Figure 5.6c) were consistent with the daily

concentrations, but were not affected ($P > 0.1$) by exercise.

     A single injection of dexamethasone increased ($P < 0.0001$) plasma concentrations of

leptin in all groups (Figure 5.7a), and the response was greater ($P = 0.006$) in horses selected

for high leptin than in horses selected for low leptin. Similarly, the insulin response to

dexamethasone was also greater ($P < 0.0001$) in horses selected for high leptin compared to
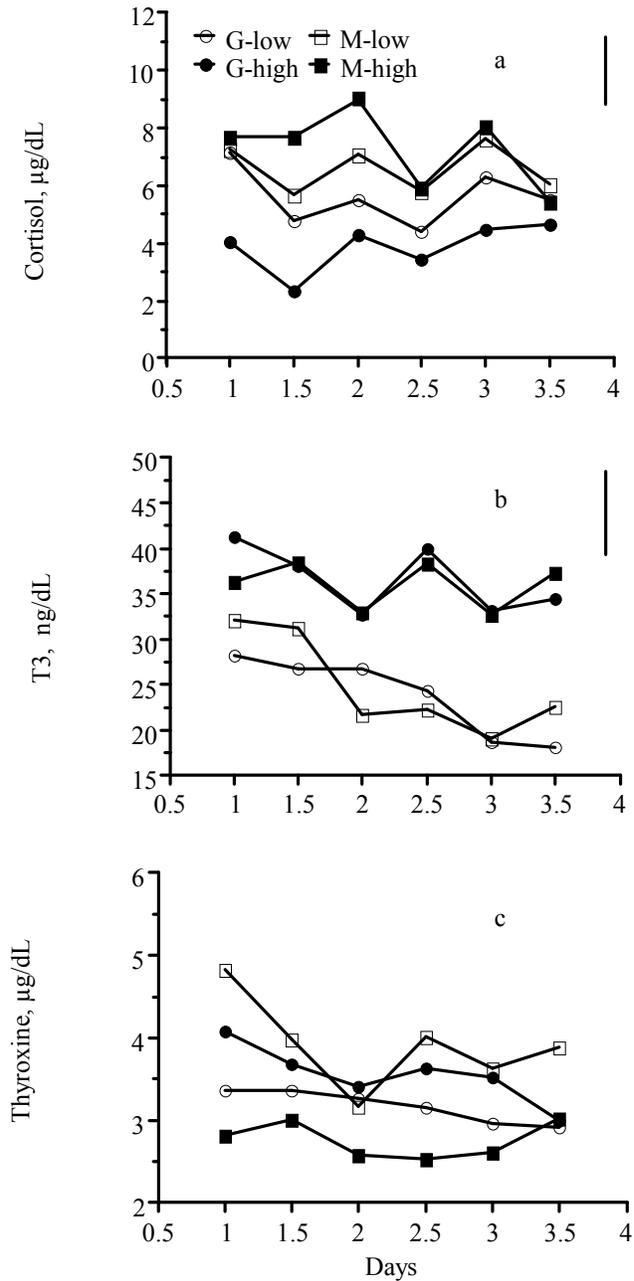
Figure 5.2. Resting concentrations of cortisol (a), triiodothyronine (T3; b), and thyroxine (c) in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar in a panel indicates the least significant difference (LSD) value ($P < 0.01$) for comparison among groups within each time period. Pooled SEM were 0.65 μg/dL for cortisol, 2.3 ng/dL for T_3, and 0.21 μg/dL for thyroxine. Thyroxine concentrations were not affected ($P = 0.6$) by group.
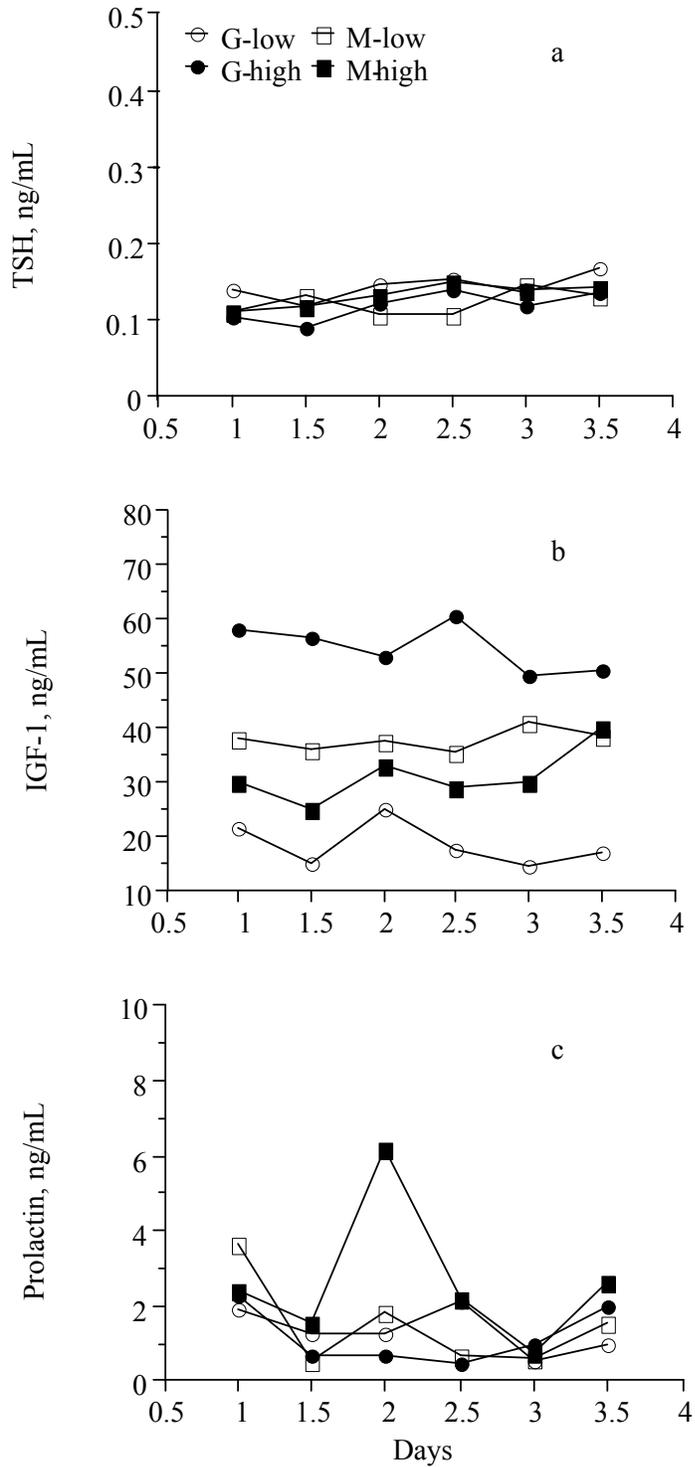
Figure 5.3. Resting concentrations of thyroid stimulating hormone (TSH; a), IGF-1 (b), and prolactin (c) in mares (M) and geldings (G) selected for high vs low leptin concentrations. Pooled SEM were 0.01 ng/mL for TSH, 3.7 ng/mL for IGF-1, and 0.87 ng/mL for prolactin. There was no effect (P > 0.1) of group for any hormone.
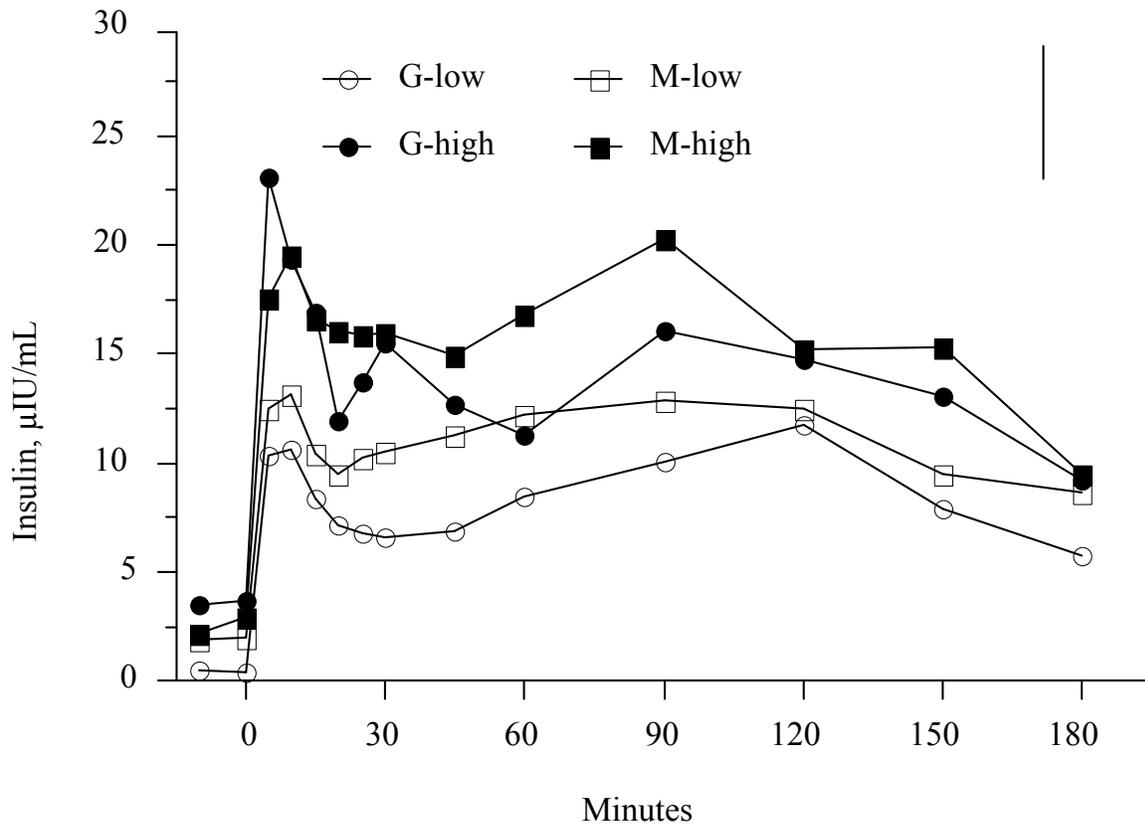
Figure 5.4. Concentrations of insulin following i.v. infusion of glucose in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar indicates the least significant difference (LSD) value (P < 0.01) for comparison among groups within each time period. Pooled SEM was 1.7 µIU/mL.
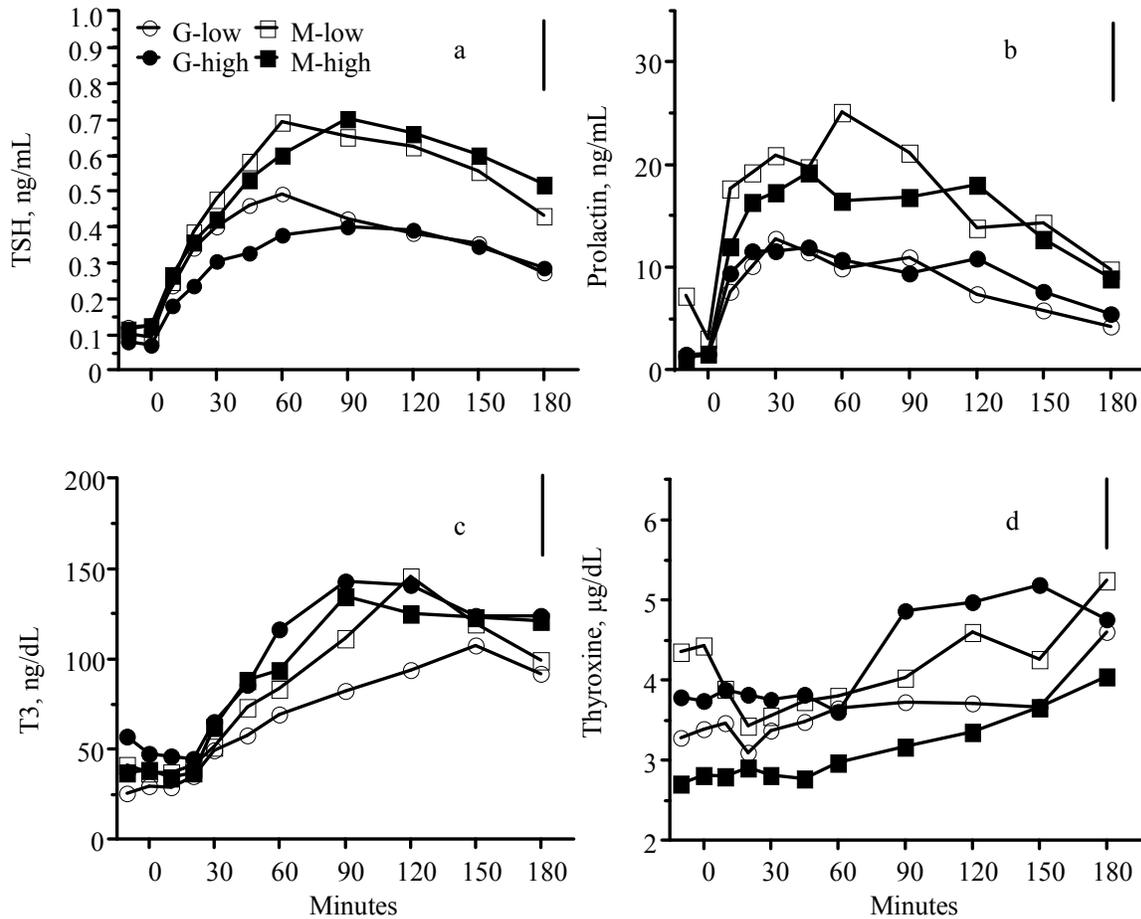
Figure 5.5. Concentrations of thyroid stimulating hormone (TSH; a), prolactin (b), triiodothyronine (T3; c), and thyroxine (d) following i.v. administration of thyrotropin releasing hormone (TRH) in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar in each panel indicates the least significant difference (LSD) value ($P < 0.01$) for comparison among groups within each time period. Pooled SEM were 0.05 ng/mL for TSH, 2.1 ng/mL for prolactin, 10.9 ng/dL for T3, and 0.23 μg/dL for thyroxine.
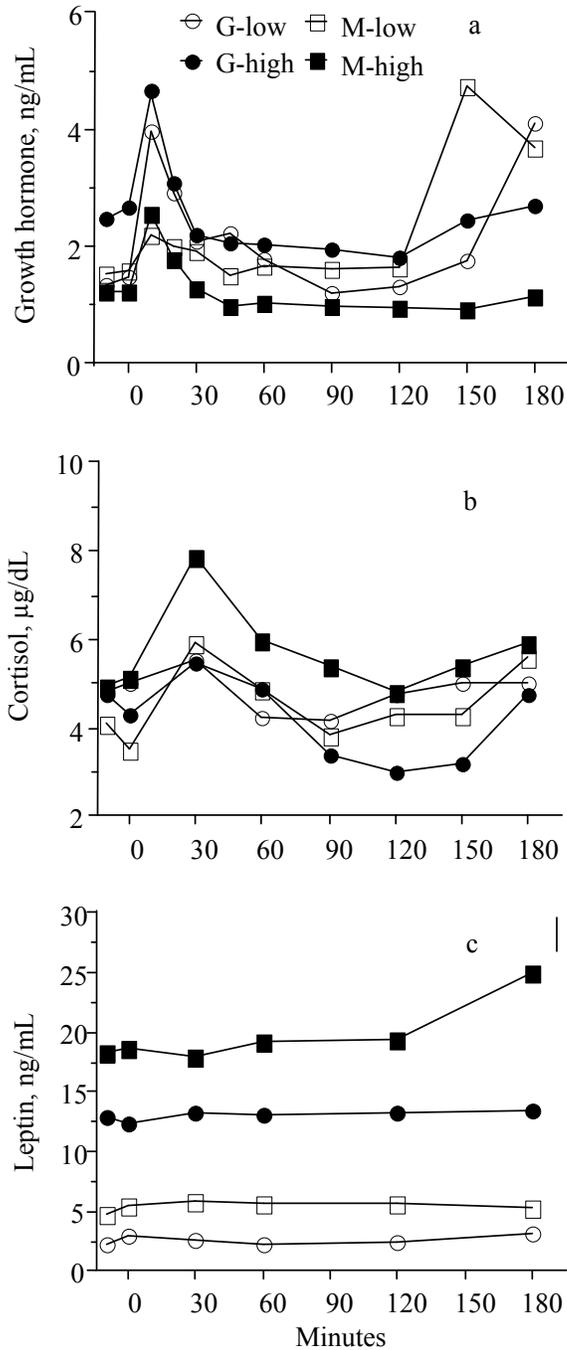
Figure 5.6. Concentrations of GH (a), cortisol (b), and leptin (c) following exercise in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar for leptin indicates the least significant difference (LSD) value ($P < 0.01$) for comparison among groups within each time period. Pooled SEM were 0.69 ng/mL for GH, 0.55 µg/dL for cortisol, and 0.81 ng/mL for leptin. Growth hormone and cortisol concentrations were affected by time ($P < 0.05$) but not by group ($P > 0.1$).

horses selected for low leptin (Figure 5.7b). Concentrations of glucose in response to

dexamethasone were greater (P = 0.0063) in horses selected for high leptin (Figure 5.7c). In

addition, the glucose response to dexamethasone was greater (P < 0.0001) in mares compared

with geldings.

There was no difference (P > 0.1) in IGF-1 concentrations due to group or gender

(Figure 5.8a), although there was an effect of time (P < 0.0001); IGF-1 concentrations

increased in all groups starting about 3 d after DEX injection. Cortisol concentrations were

equally suppressed (P = 0.029) by DEX in all groups (Figure 5.8b), and remained so through

6 d after injection, after which they gradually began to recover.

Discussion

In other species, leptin has been reported to be primarily derived from adipose tissue,

thus circulating concentrations are generally correlated to body fat mass (Prolo et al., 1998;

Chilliard et al., 2000). Similar results have been reported for the horse, in which body fat

mass was estimated by ultrasonography of backfat (Fitzgerald and McManus, 2000) or

visually by BCS (Buff et al., 2002; Gentry et al., 2002b). In the study of Gentry et al.

(2002b), 24 mares of BCS between 6.5 and 8.0 in September had widely variable plasma

leptin concentrations and tended to fit into two distinct groups of either high (7 to 20 ng/mL)

or low (<5 ng/mL) leptin concentrations. Subsequent nutrient restriction of half of those

mares resulted in a decrease in leptin concentrations, regardless of their initial starting point.

The question arose as to what factors might contribute to the large variability in plasma leptin

concentrations in a group of mares with similar body fat content. Approximately 2 years

later, we sampled 18 mares that were available from that experiment, all of which were still in
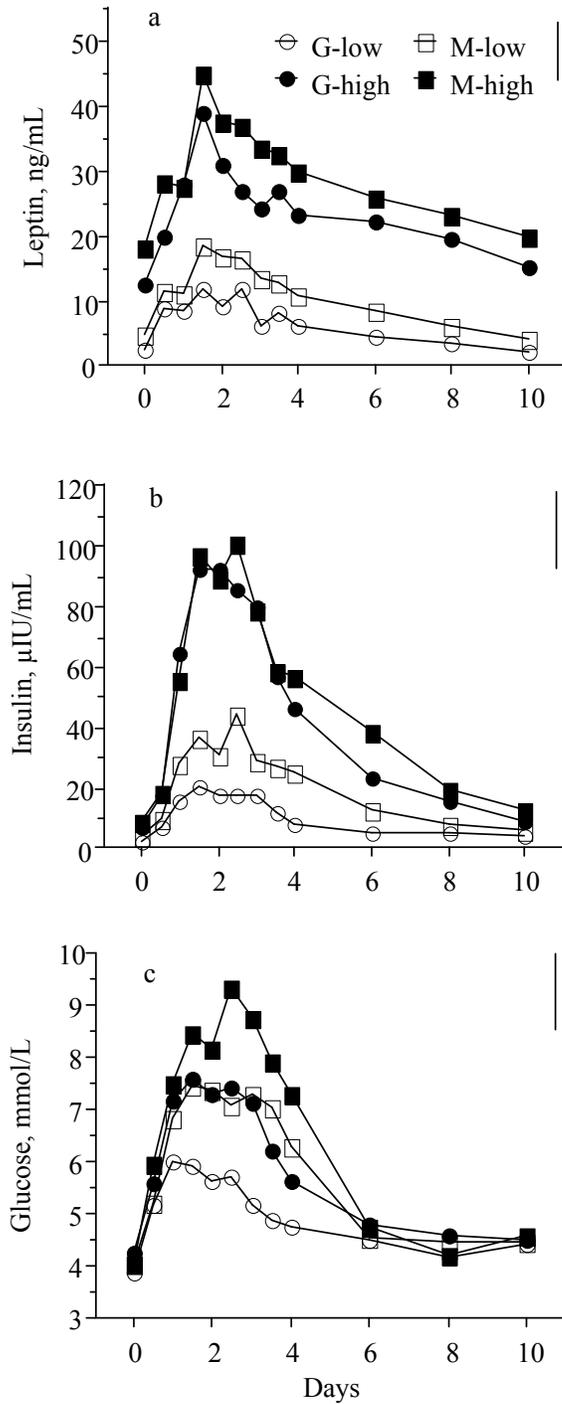
Figure 5.7. Concentrations of leptin (a), insulin (b), and glucose (c) following administration of dexamethasone in mares (M) and geldings (G) selected for high vs low leptin concentrations. The vertical bar in each panel indicates the least significant difference (LSD) value ($P < 0.01$) for comparison among groups within each time period. Pooled SEM were 2.4 ng/mL for leptin, 7.2 μIU/mL for insulin, and 0.40 mmol/L for glucose.
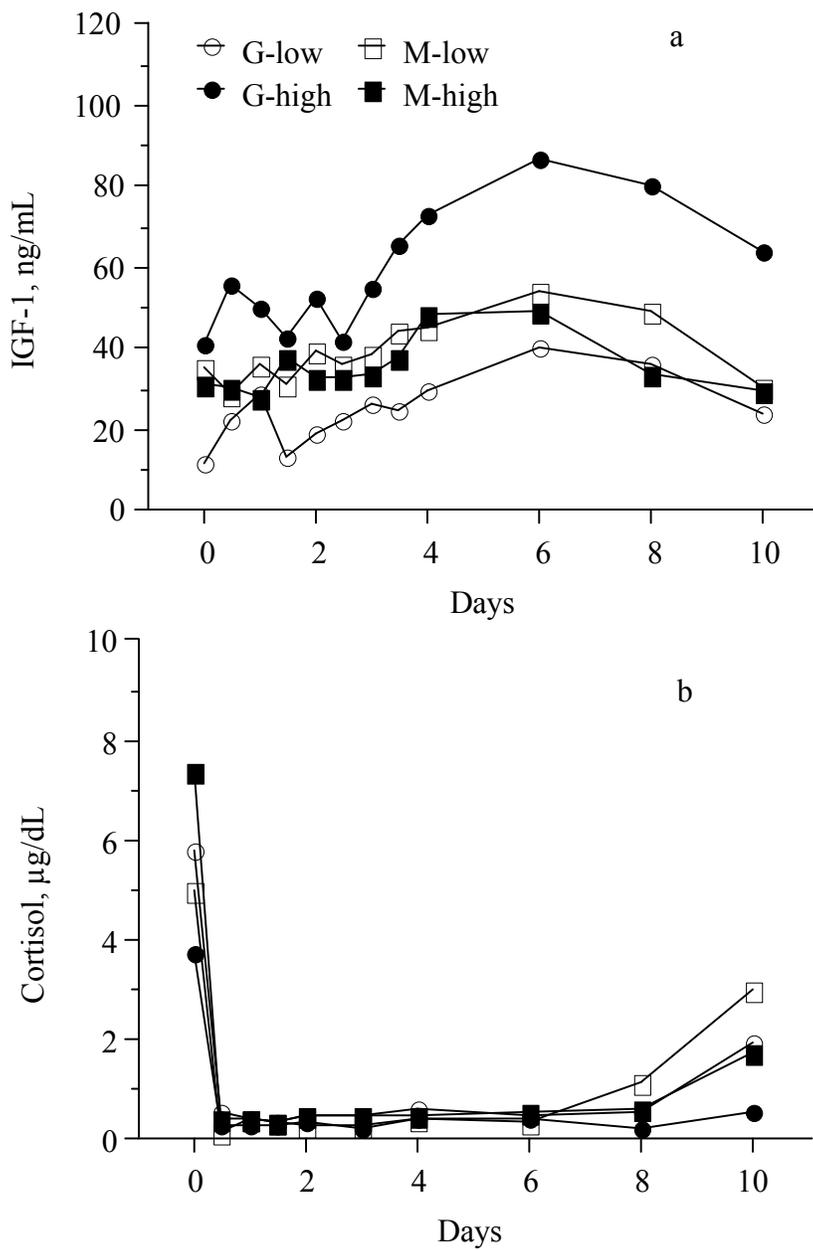
Figure 5.8. Concentrations of IGF-1 (a) and cortisol (b) following administration of dexamethasone on d 0 in mares (M) and geldings (G) selected for high vs low leptin concentrations. Pooled SEM were 6.4 ng/mL for IGF-1 and 0.45 μg/dL for cortisol. There was no effect ($P > 0.1$) of group on either hormone.

good body condition, and found that their original high vs low classification was repeatable (r = 0.91). This indicated that the underlying cause was relatively permanent, thus leading to the present experiment.

Similar to what was previously observed by Gentry and co-workers (2002a), the mares and geldings with BCS of 6.5 or greater originally sampled for the present experiment (n = 51 of the 54) had widely variable leptin concentrations in September; however, there was less of a dichotomy per se than that described by Gentry et al. (2002a). That is, there was more of a continuum of plasma leptin concentrations from 0.6 to 50 ng/mL, with 25 horses <5 ng/mL, 18 horses 10 ng/mL or greater, and 8 horses between those limits. The scattergram of leptin concentrations vs BCS was similar to that presented by Buff et al. (2002), with horses in the lower leptin range found up through BCS of 8, whereas no horses with leptin concentrations greater than 10 ng/mL were observed with BCS < 7. In contrast to the report of Buff et al. (2002), which indicated that serum leptin concentrations were greater in stallions and geldings compared to mares, mares in the present experiment had greater average leptin concentrations than geldings, albeit both genders had an equivalent number of animals fall into the high leptin and low leptin classifications. Moreover, the geldings in the present experiment had an average BCS slightly higher than the mares, indicating even a lesser leptin output per unit of fat mass.

Of the hormonal systems studied in these horses selected for high leptin vs low leptin, three seemed to be consistently different between the high leptin vs low leptin groups: GH, insulin, and T3. The greater daily concentrations of GH in horses with low leptin is consistent with reports in other species indicating a negative relationship between leptin and GH concentrations (Spurlock et al., 1998; Isozaki et al., 1999; Elimam et al., 2001), perhaps via a

direct inhibitory effect of GH on adipocytes. Long-term treatment of growing foals with GH (Capshaw et al., 2001) decreased plasma leptin concentrations by about 75% at 16 months of age (Gentry, 2001); however, that effect was likely due to decreased fat stores in the GH-treated horses (Kulinski et al., 2002).

Insulin is one hormone that consistently increases leptin secretion and(or) is associated with elevated leptin concentrations. Hyperinsulinemia has been shown to increase leptin concentrations within 3 to 5 h in both rats and humans (Cusin et al., 1995; Saladin et al., 1995), plasma leptin concentrations are markedly reduced under conditions of insulin deficiency and are rapidly increased by insulin treatment (Sivitz et al., 1998), and insulin directly stimulates leptin secretion from in vitro cultured adipocytes (Ramsay and White, 2000; Cammisotto and Bukowiecki, 2002). Horses selected for high leptin in the present experiment had greater resting concentrations of insulin as well as a greater insulin response to glucose infusion, all of which are characteristic of insulin insensitivity in other species (Boden et al., 1997; Haffner et al., 1997; Appleton et al., 2002). Therefore, it is possible that the elevated insulin observed in the horses selected for high leptin may have directly increased the adipocyte production and secretion of leptin. Alternatively, given that leptin administration enhances systemic insulin sensitivity and whole body glucose utilization in rats (Ogawa et al., 1999; Wang et al., 1999), the high insulin in horses selected for high leptin may be a result of them being leptin insensitive.

The lower daily concentrations of T3 in horses selected for low leptin in the present experiment did not seem consistent at first with our previous findings (Cartmill et al., 2003a) in which hypothyroidism (reduced T3 and thyroxine) induced by propylthiouracil feeding in stallions increased leptin concentrations. However, the results from both experiments are

consistent with the model proposed by Ghizzoni et al. (2001), which states that under baseline physiological conditions, the hypothalamic-pituitary-thyroid axis in humans has a prevailing inhibitory effect on leptin secretion, whereas leptin has a prevailing positive effect on the hypothalamic-pituitary-thyroid axis. Based on that model, the high leptin in the current experiment in horses selected for high leptin would be the causitive agent for the higher T3 concentrations. Similarly, in rats, prolonged treatment with leptin increased thyroid hormones while decreasing TSH (Nowak et al., 2002). Unlike T3, thyroxine and TSH concentrations in these horses did not vary in any significant manner.

In some species, IGF-1 and prolactin can directly affect concentrations of leptin (Gualillo et al., 1999; Mastronardi et al., 2000). In horses, IGF-1 concentrations are very responsive to alterations in feed intake (Sticker et al., 1995b), and prolactin concentrations rise consistently after a meal (DePew et al., 1994). However, no differences were observed for IGF-1 or prolactin concentrations between the horses selected for high leptin and low leptin in the current experiment, likely because their nutrient intakes, like their BCS, were similar.

Following administration of TRH, mares had greater concentrations of prolactin and TSH when compared to geldings. We previously reported (Thompson et al., 1994) that mares and stallions had greater resting prolactin concentrations than geldings in summer, although the exercise-induced rise in prolactin did not differ among genders. In rats, testosterone increased basal TSH and response to TRH, however estrogen had no effect (Christianson et al., 1981), whereas Donda et al. (1990) reported that androgens inhibited and estrogen stimulated TRH receptor content in the pituitary. This latter report is consistent with the possibility that mares may have greater TRH receptor numbers and therefore a greater TSH

response to TRH compared to geldings. Similar to the daily concentrations of T3, horses selected for high leptin had a greater T3 response to TRH administration.

The endocrine response to a single injection of dexamethasone in the current experiment was virtually identical to our previous observations (Gentry et al., 2002a; Cartmill et al., 2003a), even though horses in the previous experiments had been administered the same dose of dexamethasone (per injection) over four consecutive days. Concentrations of both leptin and insulin began increasing at 12 h after injection, peaked at 36 to 48 h, and returned to pre-dexamethasone values by d 10. Horses selected for high leptin had much greater leptin and insulin responses to dexamethasone compared to horses selected for low leptin. Gentry et al. (2002a) reported a similar difference between mares full fed and those feed-restricted to produce BCS of ~3. Full fed mares had an average pre-dexamethasone leptin concentration ~10 ng/mL, and increased to 40 to 50 ng/mL after dexamethasone, whereas mares with low BCS had an average pre-dexamethasone leptin concentration of 0.5 ng/mL and rose only to ~2.5 ng/mL after dexamethasone. A major difference between the present experiment and that of Gentry et al. (2002b) is that the horses in the present experiment all had high BCS, thus the differential effects on leptin and insulin response to dexamethasone were not due to large differences in body fat, but were likley due to the long-term differences in insulin sensitivity described herein.

The gradual rise and fall in plasma IGF-1 concentrations observed 2 to 10 d after dexamethasone administration in the present experiment has been consistently observed in previous experiments (Cartmill et al., 2003a) in which untreated (no dexamethasone) mares and geldings were included. Although the rise in IGF-1 concentrations does not coincide in time with the increases in leptin and insulin concentrations, both leptin (Miyakawa et al.,

1998; Houseknecht et al., 2000) and insulin (Bereket et al., 1999; Thrailkill et al., 2000) have been implicated in stimulating IGF-1 production and secretion in other species.

In conclusion, mares and geldings with similar high BCS selected for high vs low resting leptin concentrations had distinctly different insulin characteristics and daily GH and T3 concentrations. Based on reports in other species, the apparent insulin insensitivity/Type-II diabetic-like condition of the horses with high leptin likely contributes to their high resting leptin concentrations. The lower daily GH concentrations in horses selected for high leptin are consistent with models in other species in which a negative relationship between leptin and GH concentrations exists; however, the nature of the relationship (cause-and-effect) needs to be identified in future experiments. In a similar manner, there is evidence from other species that high leptin stimulates thyroid function, which may explain the higher T3 concentrations in horses selected for high leptin, but this too needs to be studied further in the horse.

CHAPTER 6

EFFECT OF THYROXINE ON PLASMA LEPTIN CONCENTRATIONS
IN MARES

Introduction

Thryoxine (T4) is considered a plasma reservoir for triiodothyronine (T3), which is the biologically active thyroid hormone (Guyton and Hall, 2000). Pharmacological elevation of T4 should therefore provide increased concentrations of T3 as well. We have previously shown that, in stallions, hypothyroidism induced by feeding of propylthiouracil (PTU) increased concentrations of leptin (Cartmill et al., 2003a; Chapter 2). Further, in mares and geldings selected for high vs low leptin concentrations (Cartmill et al., 2003b; Chapter 5), horses with hyperleptin/insulinemia also had elevated concentrations of T3 but not T4. These observations indicated a likely interaction between the production and secretion of leptin and thyroid hormones. Thus, the objective of the current experiment was to determine whether long-term elevation of plasma T4 altered leptin concentrations in mares.

Materials and Methods

Animals and Treatments. Fifteen healthy mares weighing 450 to 500 kg were used. Endpoints evaluated included injection site swelling scores, as well as plasma T4 and leptin concentrations. Blood samples were collected by jugular venipuncture 24 h prior to and immediately before treatment (d 0) and on d 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, and 30 after treatment. Three biodegradable lactide-glycolide microparticle formulations (blue, white, and green) containing 125 mg T4 were injected i.m. on d 0 with three horses per group. Control (n = 6) horses received an equivalent amount of vehicle.

Sample Analyses. Blood samples were immediately centrifuged and plasma harvested and frozen for later RIA. Concentrations of T4 were determined via a commercial kit (ICN Pharmaceuticals, Costa Mesa, CA). Concentrations of leptin were determined via RIA previously validated for horse samples (Cartmill et al., 2003b).   Intra- and interassay coefficients of variation and assay sensitivities were 6%, 4%, and 0.2 ng/mL for leptin, and 5%, 5%, and 5 ng/mL for T4.

Statistical Analyses. Concentrations of T4 and leptin were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with the effects of treatment, day, and their interaction included in the model. The main effect of treatment was tested with horse within treatment as the error term. The effects of day and its interaction with treatment were tested with residual error. When a significant F was detected ($P < 0.05$), differences between treatments were assessed by the LSD test.

Results

Concentrations of T4 were elevated ($P < 0.01$) in all 3 treatment groups compared to controls (Figure 6.1). Concentrations of T4 were greatest ($P < 0.02$) in the blue formulation which differed from all other groups. The red formulation was intermediate and different ($P < 0.02$) from controls but not the green formulation which did not differ ($P > 0.10$) from controls. However, there was no effect ($P > 0.10$) of T4 on concentrations of leptin (Figure 6.2). There was no noticeable injection site soreness or swelling in any of the mares.

Discussion

In a previous report (Cartmill et al., 2003a), hypothyroidism in stallions induced by propylthiouracil feeding resulted in a slight rise in leptin concentrations while leptin concentrations in control stallions gradually decreased, indicating an interaction between
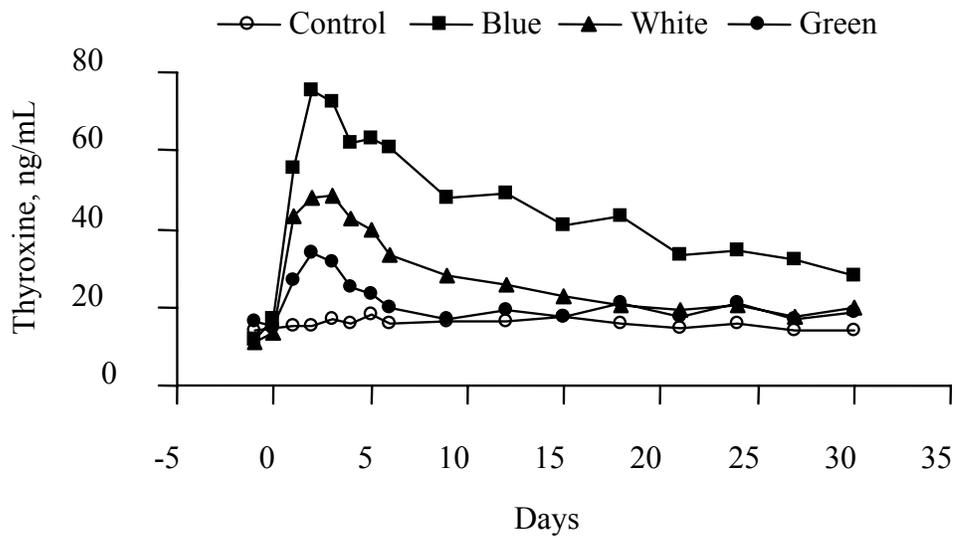
Figure 6.1. Concentrations of thyroxine in mares after receiving one of three biodegradable lactide-glycolide microparticles formulations (blue, white, and green) containing 125 mg of T4 or vehicle (control). All three formulations increased (P< 0.01) concentrations of thryoxine.
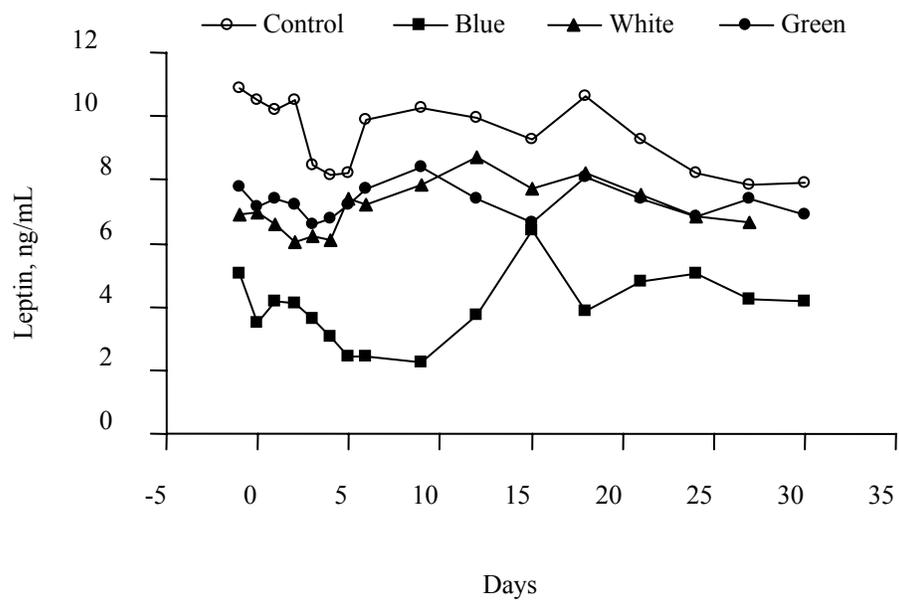
Figure 6.2. Concentrations of leptin in mares mares after receiving one of three biodegradable lactide-glycolide microparticles formulations (blue, white, and green) containing 125 mg of T4 or vehicle (control). There was no difference (P > 0.10) between treatments.

leptin and thyroid hormones. Further, mares and geldings with obesity-related

hyperleptin/insulinemia have elevated concentrations of T3 but not T4 (Cartmill et al.,

2003b). Others have reported decreases in concentrations of leptin following treatment with

T4 (Pinkney et al., 2000) or T4 plus T3 (Escobar-Morreale et al., 1997). However, in the

present experiment, dramatic increases in T4 failed to alter leptin concentrations.

Although T3 is the biologically active thyroid hormone, T4 is considered to be a

plasma reservoir for T3. Therefore, increasing circulating concentrations of T4 to the amount

achieved in this experiment would be expected to proportionally increase the availability of

T3 in plasma and cells. The lack of effect on plasma leptin concentrations in the present

experiment may indicate that thyroid hormones do not stimulate leptin production and

secretion, and that the elevated T3 observed in the hyperleptinemic horses of Cartmill et al.

(2003b) were stimulated by the increased levels of leptin, rather than vice versa. This latter

explanation is contrary to the fact that propylthiouracil-induced hypothyroidism was

associated with higher leptin secretion (Cartmill et al., 2003a), and it is apparent that more

research is needed to clarify the thyroid-leptin interactions observed to date.

CHAPTER 7

EFFECTS OF CHROMIUM SUPPLEMENTATION OR LIMIT GRAZING ON PLASMA INSULIN AND LEPTIN IN HORSES WITH ELEVATED CONCENTRATIONS OF LEPTIN

Introduction

Cartmill and others (2003b; Chapter 5) recently identified obese horses with hormone profiles similar to those in human type II diabetics. Chromium improves insulin sensitivity in some rats and humans (Sun et al., 2000; Ghosh et al., 2002; Keszthelyi et al., 2003). In addition, type II diabetes in humans is managed by reduced nutrient intake and increased exercise. Therefore, the objectives of these experiments were to determine whether 1) chromium supplementation or (2) reduction of BCS via limited grazing would alleviate the hyperleptinemia and hyperinsulinemia in horses exhibiting this syndrome.

Materials and Methods

Experiment 1. Twelve light horses (6 mares and 6 geldings), identified via blood sampling as having elevated concentrations of leptin and insulin (Cartmill et al., 2003b), were weighed and assessed for BCS (Henneke et al., 1983) by 2 independent evaluators (the average BCS was used in analysis). Beginning on April 13, all horses received ~400 g of a commercially available sweet feed once daily and were randomly assigned to receive either a top-dressing of limestone or 0.04% chromium propionate in a limestone carrier (~10 g/d total or 4 mg/d of chromium propionate) for 30 d beginning on d 0. Control horses were fed limestone so that groups would be receiving similar amounts of calcium in their diets. Blood samples were collected every 4 d via jugular venipuncture.

On d 15, an intravenous glucose tolerance test (IVGTT) was performed. During the IVGTT, blood samples were collected via an indwelling jugular catheter at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min relative to infusion of a 50% glucose solution (0.2 g/kg BW; Sigma Chem. Co., St. Louis, MO). On d 31, a frequently sampled intravenous glucose tolerance test (FSIGT) was performed. During the FSIGT, blood samples were collected via an indwelling jugular catheter at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 240, 300, and 360 min relative to glucose infusion (50% solution; 0.3 g/kg BW). Human insulin (30 mIU/kg BW; Sigma Chemical Co., St. Louis, MO) was infused at 20 min. Acute insulin response to glucose (AIRg), disposition index (DI), insulin sensitivity (SI), and glucose effectiveness (Sg) were calculated from FSIGT results using software (BeBos Assoc. 2001 version 5.7.7) at Pennington Biomedical Research Center, Baton Rouge, LA.

Experiment 2. The same twelve horses (6 mares and 6 geldings) previously identified as obese and having hormone profiles resembling type II diabetes (Cartmill et al., 2003b) used in Exp. 1 were again assigned a BCS (Henneke et al., 1983) by 2 independent evaluators (the average was used for analysis). On July 14, three mares and three geldings were assigned to grazing of native grasses for 6 h each day from 0700 to 1300 beginning on d 0 until a BCS of 5.0 to 5.5 was achieved or until d 45. The remaining six horses were allowed to continuously graze *ad libitum*. Assignments to treatments were such that each group in Exp. 2 contained three horses previously exposed to chromium propionate in Exp. 1.

Body condition scores and weight were assessed every 7 d. Blood samples were collected at 1300 beginning on d 0 and twice weekly thereafter. An FSIGT (as described in Exp. 1) was used to assess glucose dynamics and insulin sensitivity on d 0 and 45. On d 10,

20, and 30, blood samples were collected every 6 h for 36 h via jugular venipuncture to characterize concentrations of leptin and insulin.

Blood Samples. All samples were immediately centrifuged and the plasma harvested and frozen for later determination of plasma glucose, insulin, and leptin. Concentrations of leptin (Cartmill et al., 2003b), insulin (Depew et al., 1994), and glucose (Sigma Chemical Co., St. Louis, MO, USA) were determined from selected blood samples. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 4%, and 0.2 ng/mL for leptin, 5%, 8%, and 0.5 μIU/mL for insulin.

Statistical Analyses. Hormonal concentrations were analyzed by ANOVA using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with treatment, time, and the interaction included in the model. The main term of treatment was tested with horse within treatment as the error term, whereas time and its interaction with treatment were tested with residual error. When a significant F was detected ($P < 0.05$), differences between groups were assessed by the LSD test. The residual weights and BCS in Exp. 2 were analyzed due to apparent pre-treatment differences between groups. Residuals were calculated by subtracting the pre-treatment weights and BCS from subsequent days.

Results

In Exp. 1, mares had greater ($P < 0.01$) mean concentrations of leptin (Figure 7.1a) than geldings (12.9 vs 8.1 ng/mL, respectively). However, there was no effect ($P > 0.10$) of chromium supplementation on concentrations of leptin (Figure 7.1b) or insulin (data not shown). There was no difference ($P > 0.10$) in insulin response to glucose during the IVGTT on d 15 or the FSIGT on d 31. Chromium supplementation had no effect ($P > 0.10$) on AIRg,

DI, or Si (Table 7.1). However, there was a tendency (P = 0.055) for horses fed chromium to have a lower Sg compared to horses fed limestone.

In Exp. 2, grazing horses for just 6 h per day for 45 d resulted in lower mean body weight (P < 0.01) and tended (P = 0.054) to decrease mean BCS compared to horses allowed to graze *ad libitum*. There was an interaction (P < 0.01) of treatment and time for both BCS and weight in Exp. 2 (Figure 7.2). Limiting grazing time to 6 h/d for 45 d decreased both BCS and weight. There was no effect (P > 0.10) of gender for BCS or body weight.

In Exp. 2, limiting horses to 6 h of grazing per day decreased (P < 0.05) concentrations of leptin (Figure 7.3a). Geldings in the restriction group had greater (P = 0.018) mean concentrations of insulin compared to geldings allowed to graze continuously (15.6 vs 7.7 µIU/mL, respectively), whereas the opposite was true for mares (9.9 vs 20.6 µIU/mL for restricted and *ad libitum*, respectively). There was also an interaction (P = 0.011) of treatment and time for concentrations of insulin (Figure 7.3b). Horses that were limit-grazed had greater concentrations of insulin on d 4, where as horses allowed to graze continuously had greater plasma insulin on d 18; concentrations of insulin in both groups decreased (P < 0.01) over time.

Table 7.1. Acute insulin response to glucose (AIRg), disposition index (DI), insulin sensitivity (Si), and glucose effectiveness (Sg) in high leptin mares and geldings fed either chromium propionate or limestone.

Treatment	AIRg ($\mu IU \cdot mL^{-1} \cdot min$)	DI ($\times 10^{-2}$)	Si ($\times 10^{-4} \ mL \cdot uIU^{-1} \cdot min^{-1}$)	Sg * ($\times 10^{-2}$/min)
Chromium	361.4 ± 123.8	19.3 ± 23.3	5.84 ± 7.54	2.1 ± 0.8
Limestone	287.3 ± 68.6	14.2 ± 10.1	5.34 ± 4.14	3.2 ± 0.9

*Tendency for glucose effectiveness to differ between treatments (P = 0.055).
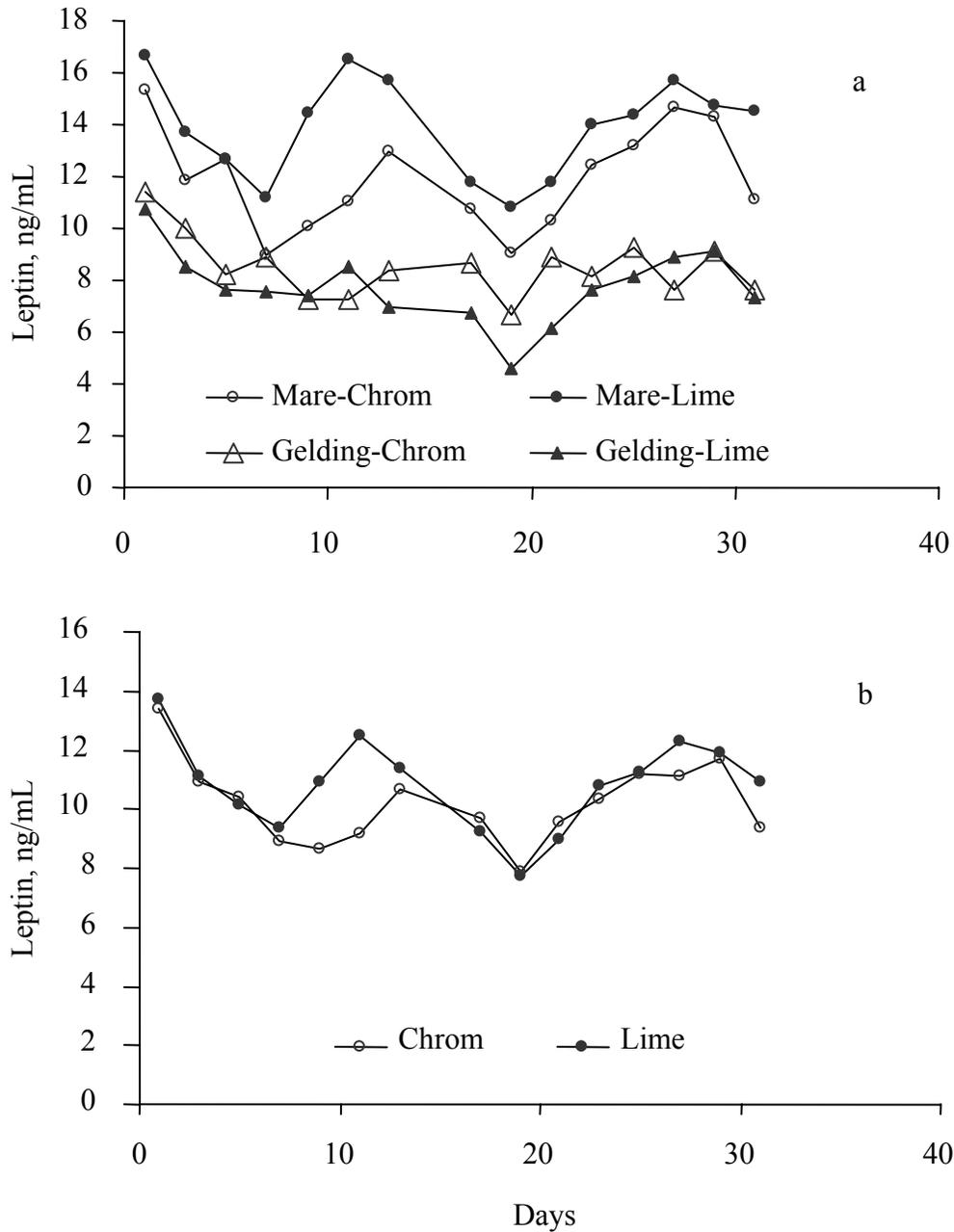
Figure 7.1. Concentrations of leptin in mares and geldings supplemented with either chromium propionate or limestone (a). Panel b shows the pooled means of both mares and geldings for each treatment group. Pooled SEM for leptin was 0.68 ng/mL.
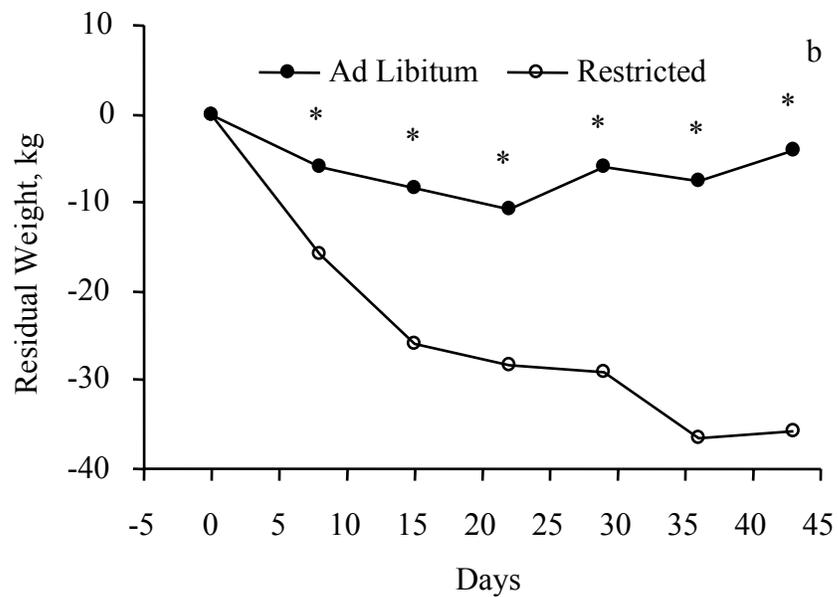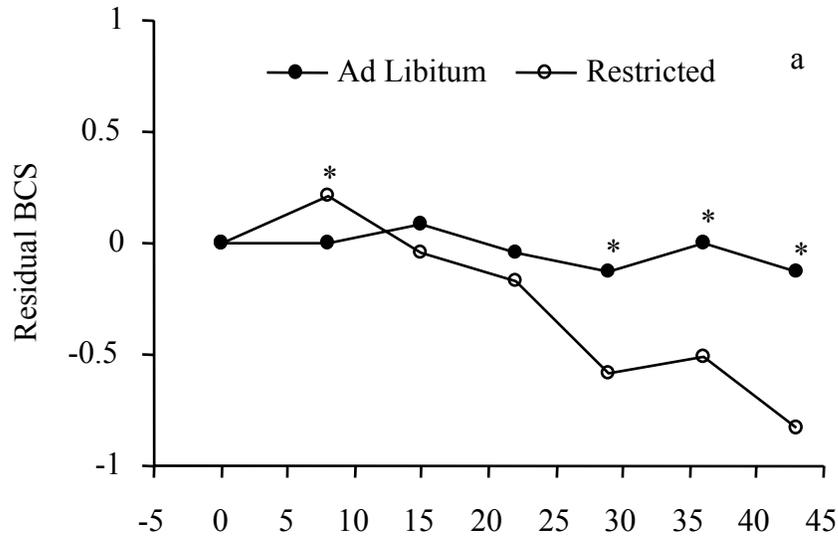
Figure 7.2. Residual body condition scores (BCS; a) and weights (b) for horses either restricted to 6 h of grazing per day for 45 d or allowed to graze *ad libitum*. Pooled SEM for BCS and weight were 0.044 and 1.3 kg, respectively. Asterisks indicate differences (P < 0.05) between groups for that day.
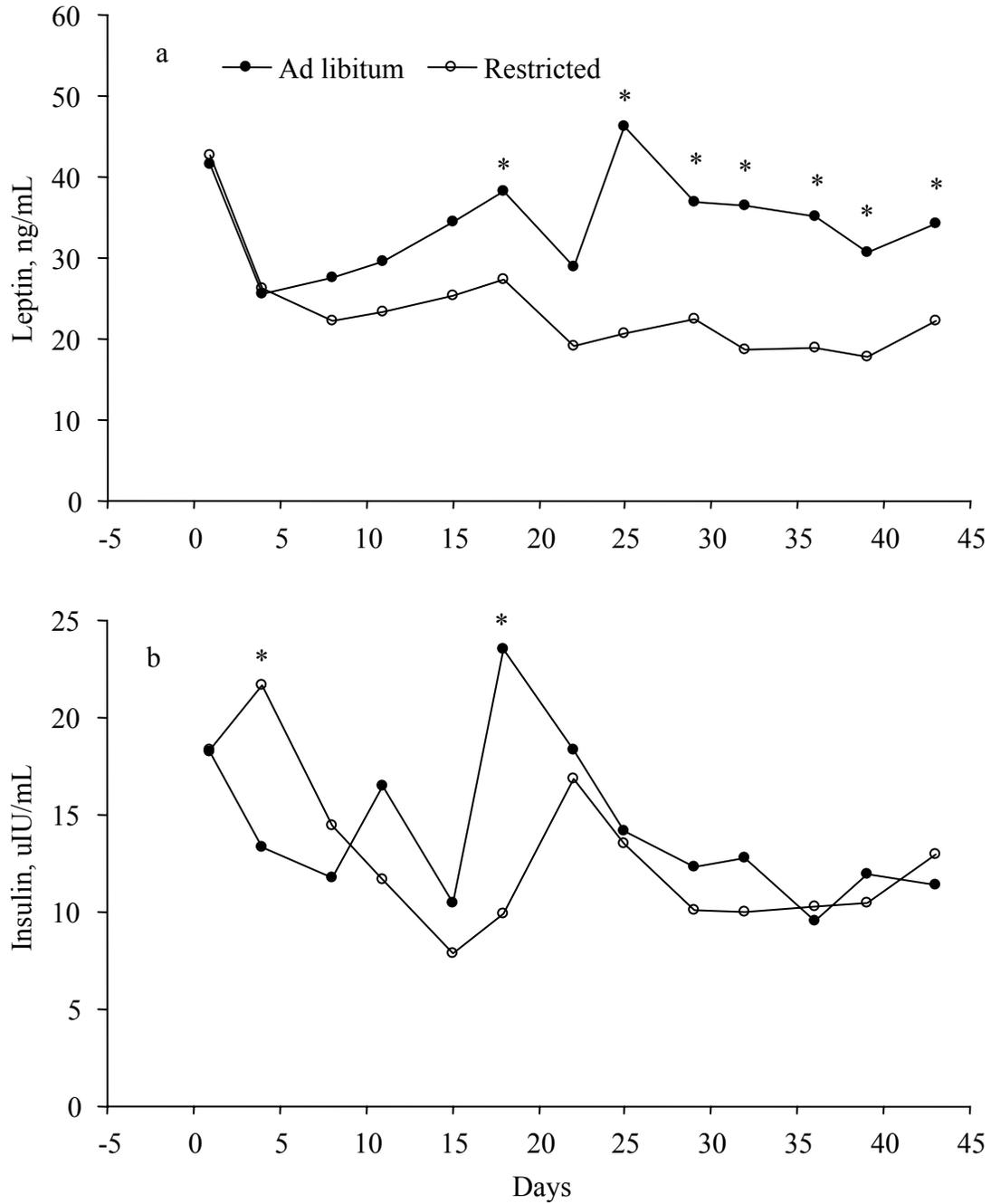
Figure 7.3. Twice weekly concenctrations of leptin (a) and insulin (b) in horses either allowed to graze *ad libitum* or restricted to 6 h per day. SEM for leptin and insulin were 2.7 ng/mL and 1.4 µIU/mL, respectively. Days indicated with an asterisks differ P < 0.05.

On d 10, 20, and 30, horses that were allowed to graze continuously had greater (P < 0.01) mean concentrations of leptin and insulin over a 36-h period compared to restricted horses (35.4 vs 21.0 ng/mL for leptin and 13.6 vs 7.1 µIU/mL for insulin, respectively). There were no differences in plasma leptin or insulin between d 10, 20, or 30, therefore the mean results for all three days are shown in Figure 7.4. There was a dramatic increase in concentrations of insulin in restricted horses during the 6 h they were allowed to graze. This increase in plasma insulin was followed approximately 6 h later by an increase in plasma leptin concentrations.

There was no difference (P > 0.10) in concentrations of glucose or insulin in horses either limit or continuously grazed during the the first FSIGT, the second FSIGT, or between FSIGTs. The results from minimal model analyses are shown in Table 7.2. No physiologically obvious effect or pattern resulting from treatments emerged. However, statistically, AIRg was different (P < 0.05) at the level of periods, and the interaction between group and treatment, whereas DI was not different (P > 0.05) at any level. In addition, there was an interaction (P < 0.05) of treatment and period for Si, and a three-way interaction (P < 0.01) of treatment, group, and period for Sg.

Discussion

Despite previous reports on the effects of chromium supplementation on concentrations of insulin in various species (Sun et al., 2000; Ghosh et al., 2002; Keszthelyi et al., 2003), there were no observable differences between groups in this experiment. It may be that chromium supplementation does not affect insulin sensitivity in horses. However, three other possibilities for the lack of effect reported herein may be: 1) the source of chromium fed was not available for absorption in the digestive tract, 2) amount of chromium
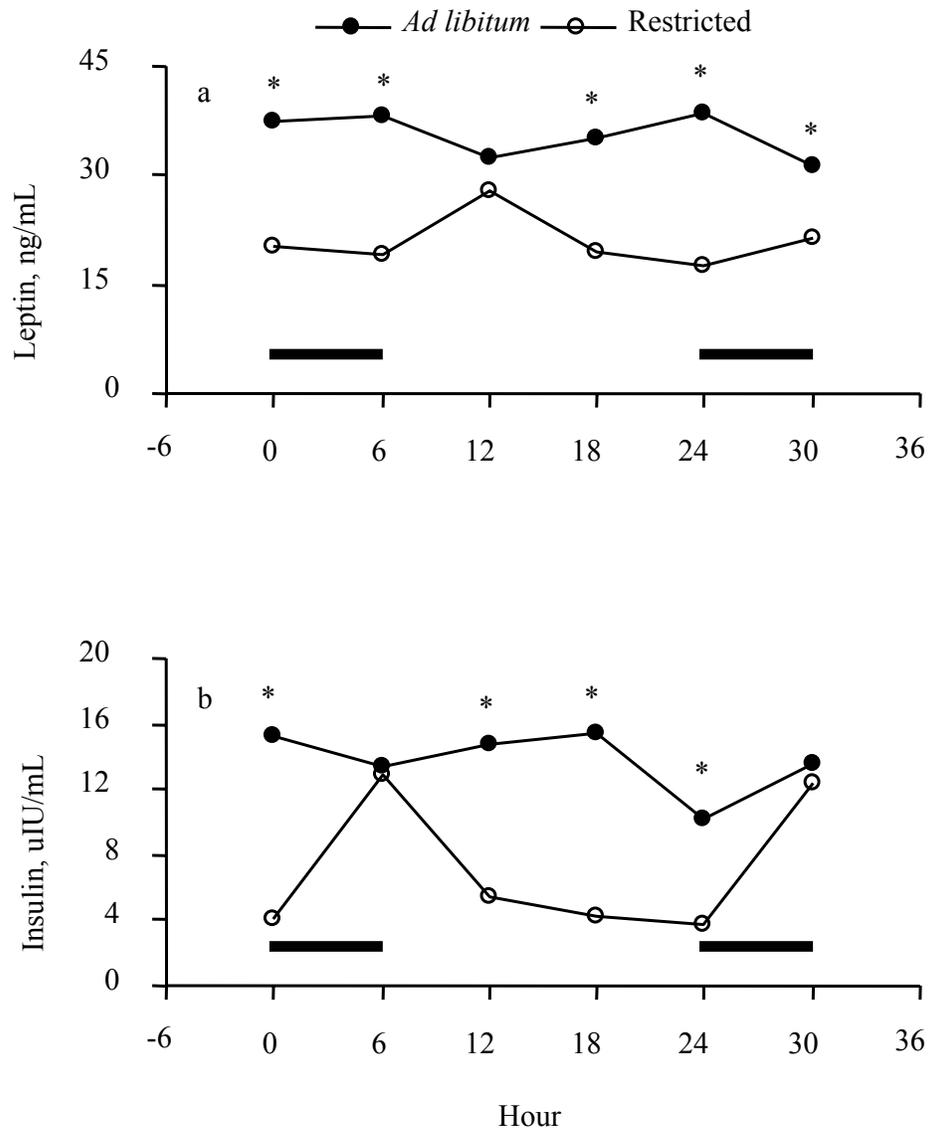
Figure 7.4. Pooled concentrations of leptin (a) and insulin (b) during a 36-h window on d 10, 20, and 30 in horses either allowed to graze *ad libitum* or restricted to 6 h per day. Horizontal bars indicate the 6-h period in which restricted horses were allowed to graze. Pooled SEM for leptin and insulin were 2.9 ng/mL and 1.5 μIU/mL, respectively. Times indicated with an asterisks differ $P < 0.05$.

Table 7.2. Acute insulin response to glucose (AIRg), dispostition (DI), insulin sensitivity (Si), and glucose effectiveness (Sg) for horses from either before or after 45 d of either limited (6 h per day) or *ad libitum* grazing[abcd] . (Mean ± SE).

	Restricted				Ad libitum			
	Gelding		Mare		Gelding		Mare	
	Before	After	Before	After	Before	After	Before	After
AIRg (μIU·mL^{-1}·min)	954.4 ± 214.2	563.8 ± 179.2	465.2 ± 249.7	415.2 ± 228.9	301.8 ± 75.6	217.5 ± 165.1	552.0 ± 250.1	560.4 ± 311.0
DI ($\times 10^{-2}$)	13.4 ± 8.4	19.7 ± 8.2	11.4 ± 8.7	13.8 ± 5.6	21.6 ± 21.4	10.6 ± 11.8	8.20 ± 2.3	11.7 ± 6.3
Si ($\times 10^{-4}$ mL·μIU^{-1}·min^{-1})	1.30 ± 0.67	3.49 ± 0.97	2.27 ± 0.62	3.55 ± 0.72	6.44 ± 5.39	3.81 ± 2.43	1.56 ± 0.30	2.18 ± 1.02
Sg ($\times 10^{-2}$/min)	1.59 ± 1.15	2.20 ± 0.92	2.37 ± 0.77	1.50 ± 0.41	1.63 ± 0.59	1.56 ± 0.46	1.30 ± 0.82	2.52 ± 0.55

[a]Effect of period for AIRg (P = 0.034).

[b]Interaction of treatment and gender for AIRg (P = 0.029).

[c]Interaction of treatment and period for Si (P = 0,024).

[d]Interaction of treatment, gender, and period for Sg (P < 0.01).

fed was not enough to improve insulin activity, or 3) the duration of supplementation may have been too short.

Glucose disappearance rate was increased and glucose half-life was decreased in pigs fed chromium picolinate (200 µg/kg of diet), however, insulin kinetics were not altered (Amoikon et al., 1995). Further, Matthews and co-workers (2001) reported that feeding barrows 200 ppb of chromium as either chromium picolinate or chromium propionate improved insulin sensitivity indicating that both chromium sources were bioavailable. Twelve week supplementation of chromium picolinate to type II diabetic humans improved glucose metabolism by improving insulin action rather than stimulating insulin secretion (Ghosh et al., 2002). Horses supplemented 5 mg of chromium, as yeast (an organic source of chromium), in a basal diet providing 12 mg/d of chromium, showed reduced insulin resistance, reduced stress to exercise, and improved energy metabolism (Frape, 1998). In contrast, chromium picolinate at a dose range of 20 to 60 µg/kg BW/d did not show any beneficial or harmful effects in insulin-treated diabetic dogs (Schachter et al., 2001). Further, healthy, aged, but non-obese humans receiving 1000 µg/d of chromium picolinate for 8 wk did not experience improved insulin sensitivity (Amato et al., 2000).

Our results show that supplementation of 4 mg/d of chromium propionate (a dose comparable to those used in other species; 90% that previously reported in horses) for 30 d does not improve insulin sensitivity in horses with obesity related hyperleptinemia, hyperglycemia, and hyperinsulinemia. In the current experiment, the duration of supplementation was shorter than reported for other species. Sun and co-workers (2000) reported an 8 wk supplementation time in rats fed a high fat diet with and without chromium lowered both concentrations of insulin and leptin.

Still yet another possibility exists, Clodfelder and co-workers (2004) reported greater urinary loss of chromium, greater movement of chromium from blood to tissues (i.e. skeletal muscle), and an alteration of chromium distribution in the blood in diabetic rats. Since all the horses in this experiment have hormone profiles similar to type II diabetes and were previously identified as having some degree of insulin insensitivity (Cartmill et al., 2003b), it may be possible that enough ingested chromium was lost in the urine to decrease the effectiveness of this dosage. Given these possibilities, further research into the effects of chromium supplementation on insulin concentrations and sensitivity in the horse is necessary to completely rule out its possible use in high leptin/type II diabetic-like horses.

In contrast, restricting horses to only a 6-h grazing period successfully lowered concentrations of leptin. Although there was a difference detected between treatments in both BCS and weight on d 1 prior to restriction, horses that were limited to a 6-h grazing period lost approximately 0.75 of a BCS and nearly 50 kg of BW in 45 d. In comparison, horses allowed to graze *ad libitum* maintained BCS, weight, and concentrations of leptin for the duration of this experiment.

Although a statistical difference was detected for plasma insulin, the physiological difference between groups was not great. The frequency of sampling may not have been adequate to detect changes in plasma insulin because restricted horses were sampled immediately prior to being penned and *ad libitum* horses may or may not have just finished eating prior to blood collection. In addition, there seemed to be a large variation in plasma insulin among horses in the same group. The length of time from when a horse last ate and when the sample was collected may account for this variation. The increasing plasma insulin in restricted horses observed over 36 h on d 10, 20, and 30 indicates that horses experience an

increase in concentrations of insulin while grazing. Therefore, since it is likely that *ad libitum* horses were grazing at random times a large variation in plasma insulin would be expected.

The increase in plasma insulin during the 36-h window in restricted horses was very similar to, although not as dramatic, as what we previously observed in meal-fed stallions allowed minimal grazing and hay *ad libitum*. In that study (Cartmill et al., 2003c; Chapter 4), insulin peaked approximately 6 to 8 h after the meal followed by leptin at 10 to 12 h regardless of feeding time. In this experiment, although the sampling regime was more limited, similar peaks in both insulin and leptin were observed indicating that 6 h of grazing has similar effects on insulin and leptin as a meal-fed concentrate.

Finally, the lack of consistent differences in the FSIGT-Minimal Model results may indicate no real change in insulin sensitivity, even though restricted horses lost body condition, weight, and experienced decreased levels of leptin. Hoffman et al. (2003) described horses with a BCS greater than 6.0 as either "moderately obese" or "obese" (7.0 or more) and reported differences in minimal model results only between the "obese" horses and horses with BCS less than 6.0. It may be possible that the reason no differences in glucose/insulin metabolism were detected after either chromium supplementation or restricted grazing is due to the fact that the horses in this study were all of the "moderately obese" to "obese" state.

In conclusion, chromium propionate supplementation at the dose and route of administration used herein was ineffective in reducing concentrations of insulin or leptin. In comparison, restricted grazing did reduce BCS, weight, and concentrations of leptin in mares and geldings. However, simply reducing BCS, weight, and plasma leptin was not sufficient to improve glucose/insulin metabolism in these horses. Further research is needed to determine

if a greater reduction would alleviate elevated concentrations of insulin and improve insulin

sensitivity in horses with high concentrations of leptin.

CHAPTER 8

COMPARISON OF HYPERINSULINEMIC-EUGLYCEMIC CLAMP, MINIMAL MODEL, AND IVGTT IN MARES WITH HIGH OR LOW CONCENTRATIONS OF LEPTIN AND IN STALLIONS

Introduction

Concentrations of leptin in mares with low and high body condition scores (BCS; Henneke et al., 1983) were monitored during spring transition (Gentry et al., 2002). In that study, mares with high BCS had a wide range in concentrations of leptin. Further, it was determined that mares and geldings with high BCS selected for high and low concentrations of leptin had different concentrations of insulin (Cartmill et al., 2003b). Horses with high leptin had a greater insulin response to glucose during the intravenous glucose tolerance test (IVGTT) compared to low leptin horses, indicating a possible insulin insensitivity in high leptin horses.

Characteristics of glucose metabolism in the horse have been assessed by IVGTT (Sticker et al., 1995ab; Cartmill et al., 2003b), hyperinsulinemic-euglycemic clamp (Powell et al., 2002; Rijnen et al., 2003), and frequently sampled intravenous glucose tolerance test (FSIGT; Hoffman et al., 2003). To our knowledge, a comparison of these three assessments of glucose metabolism in horses in the same experiment has not yet been performed. Thus, the objective of this experiment was to compare the results from the IVGTT, FSIGT, and hyperinsulinemic-euglycemic clamp in mares with either high or low concentrations of leptin and stallions.

Materials and Methods

   Horses.   Twelve horses (8 mares and 4 stallions) were selected from the research herd at Louisiana State University Agricultural Center Ben Hur Research Station. All horses were weighed and BCS was assessed (Henneke et al., 1983) by two independent evaluators prior to the experiment. The mean BCS was used in assigning mares to treatment groups. Mares were further grouped (n = 4) based on their resting leptin concentrations, determined by RIA (Cartmill et al., 2003b). Mares in the high-leptin and low-leptin groups had mean BCS and leptin concentrations of 7.625 and 3.23 ng/mL and 7.375 and 0.13 ng/mL, respectively. All horses were assigned three dates on which they would receive an FSIGT, an IVGTT, or a hyperinsulinemic-euglycemic clamp; for any given horse, there was at least 3 d but no more than 21 d between tests. Tests were assigned to individual horses in a group (i.e. stallions, low mares, and high mares) such that not all horses in that group received the tests in the same order.

   Description of Tests. Blood samples were collected via indwelling jugular catheters during all three tests. Blood samples were immediately centrifuged and plasma was harvested and frozen for later determination of concentrations of glucose (method no. 315: Sigma Chemical Co., St. Louis, MO, USA) and insulin (Depew et al., 1994). Horses were feed-deprived overnight for a minimum of 12 h prior to the IVGTT and hyperinsulinemic-euglycemic clamp procedures, but were not deprived prior to performing the FSIGT (Hoffman et al., 2003). During the FSIGT, samples were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 240, 300, and 360 min. All horses received glucose (0.3 g/kg BW in a 50% solution; Sigma Chemical Co., St. Louis, MO) i.v. at 0 min and insulin (30 mIU/kg BW; Sigma Chemical Co.,

St. Louis, MO) i.v. at 20 min. During the IVGTT, samples were collected at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min relative to an i.v. injection of glucose (0.2 g/kg BW in a 50% solution). During the hyperinsulinemic-euglycemic clamp, horses were fitted with a single indwelling jugular catheter on both sides of the neck. A Flo-Gard® 6300 dual channel volumetric infusion pump (I.V. Techonologies, Inc., Upperville, VA) was attached to one catheter and sampling was performed via the other. Blood glucose concentrations were monitored during infusions by a portable glucose monitor (Accu-chek®, Roche Diagnostics Corp., Indianapolis, IN) at -20, -10, and 0 min to establish basal concentrations.

After initiating the infusion, concentrations of glucose were monitored at 2.5-min intervals for the first 20 min and then at 5-min intervals for duration of the test. The pump rate for glucose was adjusted when needed to maintain blood glucose concentrations at pre-clamp levels. Once blood glucose levels were stabilized at their basal concentrations, additional blood samples were collected at 15 min intervals for 45 min. Other samples of blood were collected at -20, -10, 0 10, 20, 30, 60, 90, 120, 150, and 180 relative to initiating the pump for later assay of concentrations of insulin and glucose. Infusates (i.e., glucose and insulin) and bolus insulin injections were prepared as described by Powell and co-authors (2002). In the present experiment, the final volume of insulin infusate was 135 mL. The insulin pump rate was held constant at 30 mL/h and the concentration of insulin in the infusate was varied with individual BW (1.2 mIU/kg/min). In contrast, the concentration of glucose infusate was held constant and individual pump rates were determined based on individual BW (0.43 mg/kg/min).

Statistical Analyses. The main effect of group and its interaction with time for concentrations of insulin and glucose were analyzed using the GLM procedures of SAS (SAS

Institute Inc., Cary, NC). When a significant F (P < 0.05) was detected, differences between

groups were determined using Tukey's honest significant difference (HSD) test (Steel and

Torrie, 1980). Glucose infusion rates (mg/kg BW/min) were determined from the pump rate

obtained once euglycemia was achieved during the clamp procedure. Acute insulin response

to glucose (AIRg), disposition index (DI), insulin sensitivity (Si), and glucose effectiveness

(Sg) were calculated from FSIGT results using software (BeBos Assoc. 2001 version 5.7.7) at

Pennington Biomedical Research Center, Baton Rouge, LA. The GLM procedures of SAS

were also used to determine differences between groups in weight, BCS, glucose infusion

rates, AIRg, DI, Si, and Sg. When a significant F was detected (P > 0.05) the least significant

difference (LSD)-test (Steel and Torrie, 1980) was used to determine differences between

groups.

Results

Mares in the high leptin group had greater (P < 0.01) BCS than stallions, with mares

in the low leptin group being intermediate in BCS (Table 8.1). There were no differences in

age (P = 0.12) or weight (P = 0.77) among the three groups.

There were no differences (P = 0.35) in concentrations of glucose following the

IVGTT between high leptin mares, low leptin mares, or stallions (Figure 8.1a). However,

Table 8.1. Age, weight, and BCS for high leptin mares, low leptin mares, and stallions prior to IVGTT, FSIGT, and hyperinsulinemic-euglycemic clamp.

	Age, yr	Weight, kg	BCS
High Mares	10.5 ± 2.4*	513.75 ± 25.0	7.875 ± 0.25[a]
Low Mares	11.5 ± 2.6	513.75 ± 35.2	6.625 ± 0.48[b]
Stallions	16.5 ± 5.7	492.50 ± 68.5	5.750 ± 0.87[b]

*Means ± SE.
[ab]Body condition scores with different superscripts differ (P < 0.01).
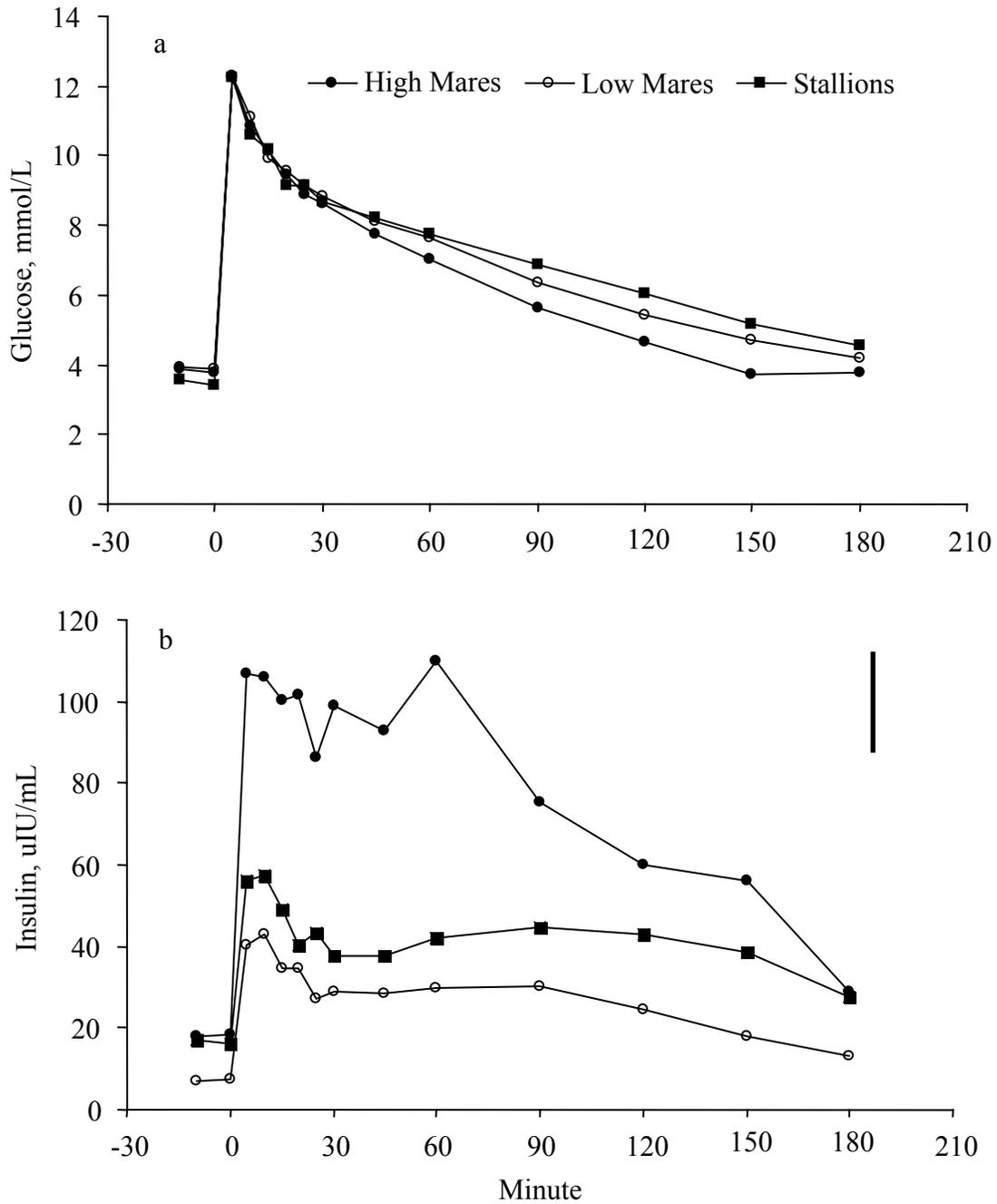
Figure 8.1. Concentrations of glucose (a) and insulin (b) in high leptin mares, low leptin mares, and stallions following administration of the intravenous glucose tolerance test. Pooled SEM were 0.18 mmol/L and 3.8 μIU/mL for glucose and insulin, respectively. The HSD value for comparison is indicated when a significant F was detected ($P < 0.05$).

high-leptin mares had greater (P < 0.01) concentrations of insulin in response to the bolus glucose infusion during the IVGTT (Figure 8.1b).

Similar to the IVGTT, there was no difference (P = 0.47) in concentrations of glucose during the FSIGT (Figure 8.2a). However, during the first 20 min of the FSIGT (Figure 8.3), mean concentrations of glucose tended (P = 0.055) to differ between groups (12.7 vs 12.2 vs 13.6 mmol/L for high mares, low mares, and stallions, respectively).

There was a main effect of group (P = 0.02) and interaction (P < 0.01) of group with time for concentrations of insulin during the FSIGT (Figure 8.2b). High leptin mares had greater mean concentrations of insulin during the FSIGT (143.8 µIU/mL) than stallions (118.9 µIU/mL) and low-leptin mares (84.1 µIU/mL). Further, during the first 20 min of the FSIGT, the endogenous insulin response to the glucose bolus was greater (P = 0.012) in high-leptin mares compared to low-leptin mares but was not different from stallions, which were intermediate (Figure 8.3). Mean concentrations of insulin only tended (P = 0.058) to be different between groups (114.2 vs 56.4 vs 89.7 µIU/mL for high mares, low mares, and stallions, respectively). Finally, due to large variation within groups, there was no difference (P > 0.10) in AIRg, DI, Si, or Sg among high mares, low mares, and stallions (Table 8.2).

Table 8.2. Acute insulin response to glucose (AIRg), disposition index (DI), insulin sensitivity (Si), and glucose effectiveness (Sg) in high leptin mares, low leptin mares, and stallions following FSIGT (Mean ± SE).

|	AIRg (uIU·mL^{-1}·min)	DI ($\times 10^{-2}$)	Si ($\times 10^{-4}$ mL·uIU^{-1}·min^{-1})	Sg ($\times 10^{-2}$/min)
High Mares	909.8 ± 450.6	36.8 ± 56.6	6.67 ± 7.98	2.55 ± 1.32
Low Mares	487.6 ± 111.0	5.13 ± 3.58	1.14 ± 0.81	2.03 ± 1.53
Stallions	574.2 ± 491.9	7.50 ± 8.03	1.46 ± 1.72	1.94 ± 1.02

Figure 8.2. Concentrations of glucose (a) and insulin (b) in high leptin mares, low leptin mares, and stallions following administration of the frequently sampled intravenous glucose tolerance test. Pooled SEM were 0.24 mmol/L and 10.8 μIU/mL for glucose and insulin, respectively. The HSD value for comparison is indicated when a significant F was detected ($P < 0.05$).
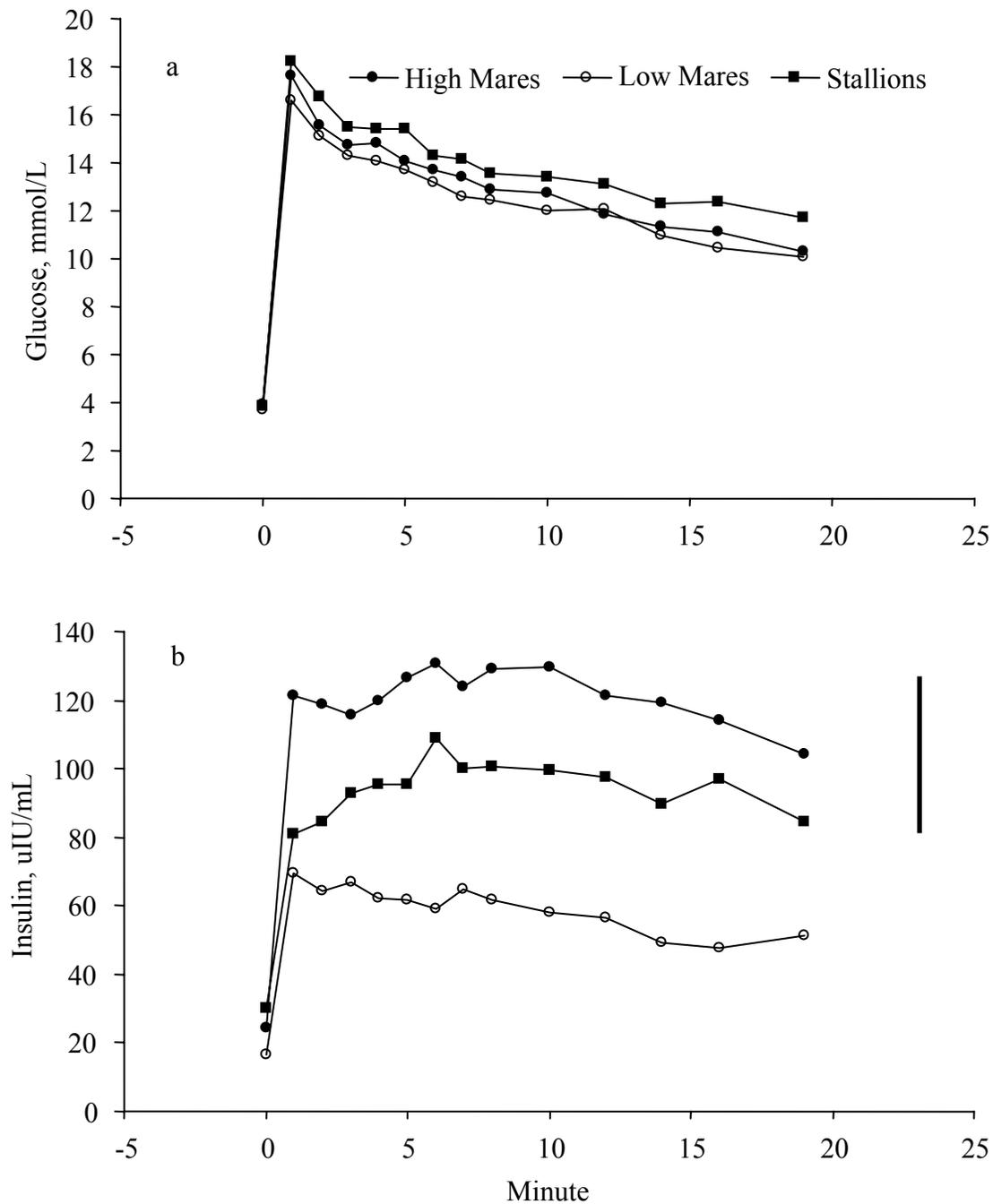
Figure 8.3. Concentrations of glucose (a) and insulin (b) in high leptin mares, low leptin mares, and stallions during the first 20 min of the frequently sampled intravenous glucose tolerance test. Pooled SEM were 0.18 mmol/L and 3.9 µIU/mL for glucose and insulin, respectively. The HSD value for comparison is indicated when a significant F was detected (P < 0.05).

The glucose infusion rate was 1.20, 2.00, and 1.88 mg/kg BW/min for high mares, low mares, and stallions, respectively, and was not different ($P = 0.128$) among groups. However, when the first 90 min of infusion only were analyzed, there was an interaction ($P < 0.01$) of group and time for concentrations of glucose (Figure 8.4), as well as a tendency ($P = 0.054$) for a main effect of group. Stallions had lower mean concentrations of glucose (3.39 mmol/L) compared to high mares (3.89 mmol/L) and low mares (3.94 mmol/L), which did not differ. Furthermore, stallions and low-leptin mares responded to infusion of insulin with more rapid declines in concentrations of glucose compared to high leptin mares. The concentration of insulin infusate was calculated based on individual BW and the infusion rate was held constant therefore we expected to find no difference in concentrations of insulin. In fact, there was only a tendency ($P = 0.065$) for a main effect of group and no interaction ($P = 0.21$) with time for concentrations of insulin during the first 90 min of the clamp procedure (Figure 8.4). Once euglycemia was achieved (between 90 and 150 min after initiating the clamp procedure), there was no difference ($P > 0.10$) between groups for concentrations of insulin or glucose.

Discussion

The difference in BCS, but not weight, between the stallions and high-leptin mares is most likely due to differences in degree of muscling. The stallions were more heavily muscled with less fat due to high levels of androgens in their blood compared to the mares. In addition, there were differences in diet between mares and stallions. Stallions were maintained in individual lots, fed a pelleted feed once per day, with access to native pasture as well as *ad libitum* hay and water. In comparison, mares were maintained on similar pastures
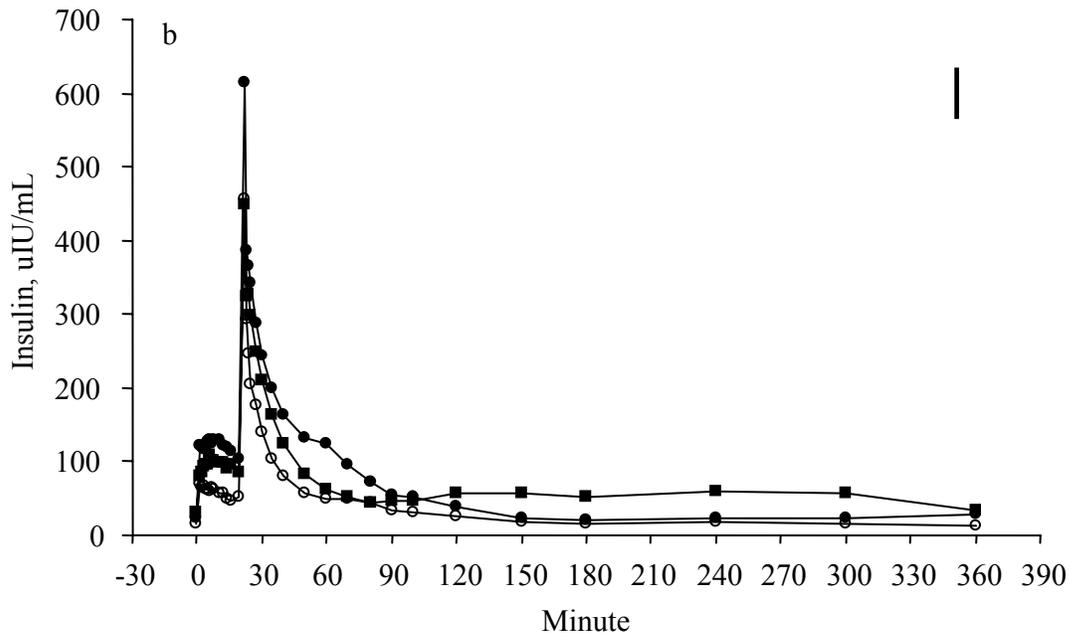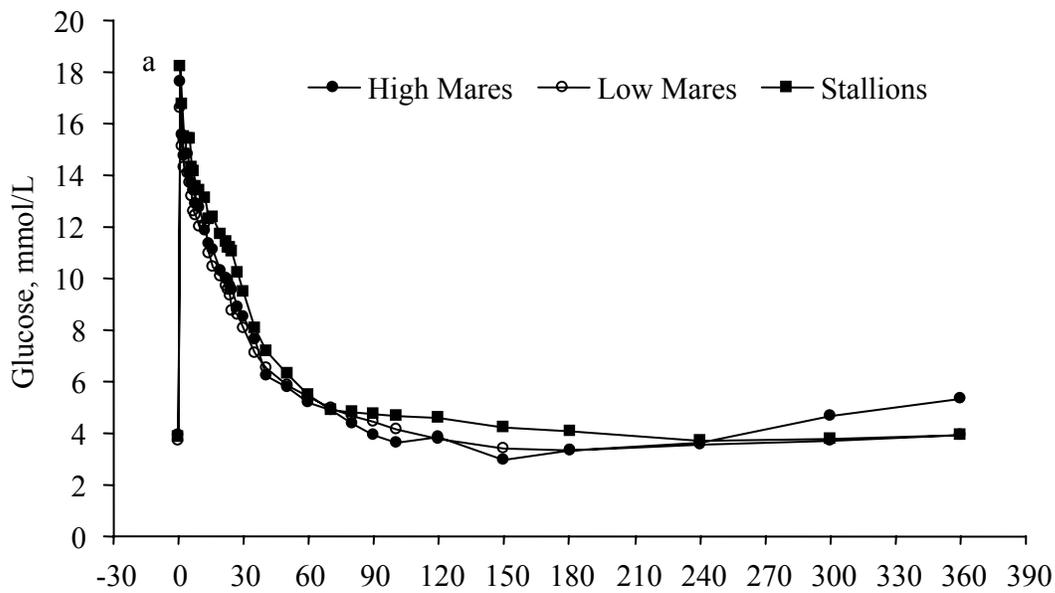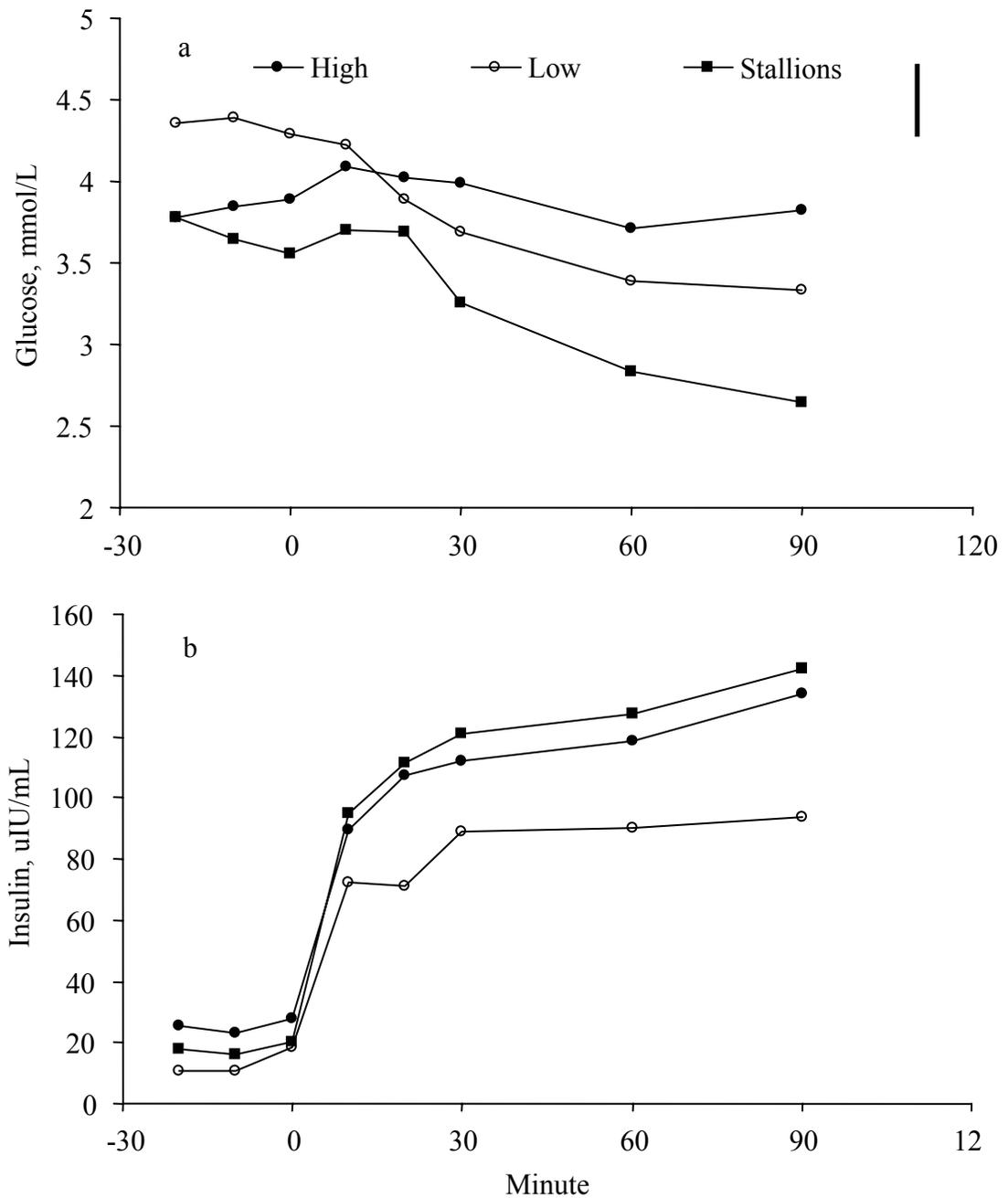
Figure 8.4. Concentrations of glucose (a) and insulin (b) in high leptin mares, low leptin mares, and stallions following administration of the hyperinsulinemic-euglycemic clamp. Pooled SEM were 0.072 mmol/L and 4.02 µIU/mL for glucose and insulin, respectively. The HSD value for comparison is indicated when a significant F was detected ($P < 0.05$).

with *ad libitum* hay and water, and were also allowed access to winter rye grass pasture for 4 to 6 h each day. Even though stallions were receiving additional energy in their diet in the form of a concentrate, they still put on less fat than the mares out on pasture further implicating the presence of androgens as the cause for the lower BCS observed in stallions.

Although mares were selected so that BCS was equal, they were different by the time the glucose metabolism assessments were performed (approximately 45 d later). This difference in BCS, but not weight, between high-leptin mares and low-leptin mares by the time testing was initiated is more difficult to explain. The BCS and leptin concentration assessments in these mares were done in late November, and the glucose assessments were started in early January. In November, the mean of both BCS and plasma leptin for high-leptin mares was 7.625 and 3.23 ng/mL, respectively. In comparison, the mean BCS and plasma leptin for low-leptin mares was 7.375 and 0.13 ng/mL, respectively. At the start of glucose/insulin testing, the BCS of high leptin-mares was 7.875 compared to 6.625 for low-leptin mares. Given that all mares were maintained on the same pastures during this time, the difference in BCS losses may indicate a difference between low- and high-leptin mares with regard to nutrient utilization and/or their ability to adjust intake to winter conditions. The lower concentrations of leptin during the winter observed herein compare favorably to previous observations during late summer and early fall (Cartmill et al., 2003b) that plasma leptin levels decline in horses from summer to winter (McManus and Fitzgerald, 2000; Gentry et al., 2002b).

Concentrations of glucose did not differ among high-leptin mares, low-leptin mares, and stallions. However, plasma insulin was greatly increased in high-leptin mares compared with low leptin mares and stallions. The similar concentrations of glucose between groups

indicate similar glucose clearance rates by endogenous insulin. The greater insulin response to glucose, but lack of increased glucose clearance in high-leptin mares, indicates insensitivity to insulin in these mares, to which they adjust by secreting more insulin. The results in Chapter 5 were similar following an IVGTT in high- vs low-leptin mares and geldings: high-leptin horses had greater concentrations of insulin in response to the glucose bolus compared to low-leptin horses. Further, in that experiment, there was no difference in concentrations of glucose regardless of the insulin response and it was concluded that elevated insulin in high-leptin horses may be indicative of insulin insensitivity and/or a type II diabetic-like state.

The concentrations of insulin and glucose obtained from the first 20 min of the FSIGT show a pattern of response similar to that in the IVGTT in high-leptin vs low-leptin horses. There was no detectable difference in glucose concentrations among groups of horses. It may be that even though stallions had lower BCS than mares, they were slightly older, and age may have contributed to their decreased glucose clearance (Elahi et al., 1993; Stout, 1994). However, in mares, geldings, and stallions receiving a single injection of dexamethasone, age was not highly correlated to concentrations of leptin both prior to treatment and in response to dexamethasone (Chapter 3). Both high- and low-leptin mares had virtually identical concentrations of glucose; however, as in the IVGTT, mares with elevated concentrations of leptin had a greater insulin response during the FSIGT. The elevated insulin response and similar glucose clearance in high-leptin mares may again indicate insulin insensitivity (as in the IVGTT).

Recently, Hoffman et al. (2003) studied FSIGT in horses and classified Thoroughbred geldings with BCS 5.0 to 5.9 as "nonobese", 6.0 to 6.9 as "moderately obese", and 7.0 to 9.0 as "obese" and found that Sg and AIRg were greater in obese geldings compared with non-

obese geldings. Further, they observed a decrease in Si but no change in DI between these groups of geldings. They concluded that lower Si and higher Sg found in obese vs non-obese geldings indicated that obese geldings rely primarily on glucose-mediated glucose disposal. In addition, they concluded the higher AIRg in conjunction with the elevated endogenous insulin during the first 20 min of the FSIGT indicated an increased ability of the equine to compensate for decreased glucose clearance and insulin ineffectiveness by secreting more insulin. They further suggested that the lack of difference in DI suggested this "compensatory" insulin secretion in response to glucose was adequate enough to make up for decreased Si. The results in the current experiment do not disagree nor do they agree with this previous report. In this experiment the extreme variation among horses within treatment groups contributed to the lack of detectable difference in any of these four variables (AIRg, Si, Sg, or DI). However, even with this large variation and lack of difference in AIRg, SI, Sg, and DI, it is still possible to conclude that high-leptin mares have some degree of insulin "insensitivity" that they compensate for by secreting more insulin in response to glucose while achieving the same glucose clearance (Figure 3).

The range in glucose infusion rates obtained in this study (1.20 to 2.00 mg/kg/min) were within the range previously reported (0.74 to 2.97 mg/kg/min; Powell et al., 2002). The lack of difference in final glucose infusion rate during the hyperinsulinemic-euglycemic clamp in this study was unexpected. The slightly greater age of the stallions in combination with the lower BCS of the low leptin mares compared with high leptin mares may have been enough to alter glucose metabolism and eliminate any detectable difference with this sample size. Type II diabetes most often manifests in humans over 40 yr old. Therefore, it may be possible that decreased insulin sensitivity may be more prevalent in older horses. However,

the increased decline in glucose concentrations to the same amount of insulin infused during the first 90 min in stallions and low leptin mares compared to high leptin mares indicates an increased insulin sensitivity in these low leptin horses.

This observation of insulin insensitivity in high leptin mares has been consistent in each of the three glucose/insulin tests in this experiment. High leptin mares have an increased insulin response to glucose (i.e., IVGTT and FSIGT) and decreased decline in glucose in response to insulin infusion (i.e., hyperinsulinemic-euglycemic clamp) compared to low leptin mares and stallions. Therefore, it may be possible to detect insulin insensitivity in horses without having to apply the minimal model or hyperinsulinemic-euglycemic clamp.

From a technical standpoint, three of the mares used in this study were also used in previous studies in which the FSIGT has been applied. They all have maintained similar body weights and BCS during this time, as well as being high-leptin mares. In retrospect, there appears to be a great deal of variation not only among horses within a group but also among multiple FSIGT within a given horse. This large variation and unpredictability in horses coupled with the intense bleeding schedule may make the IVGTT not only a more reliable but more easily applicable initial assessment of glucose/insulin metabolism in the horse.

CHAPTER 9

SUMMARY AND CONCLUSIONS

The goal of the initial experiments was to determine whether concentrations of leptin in horses were affected by adrenal function, thyroid function, and/or glucose and insulin metabolism. It has been reported, in species other than the horse, that dexamethasone, a highly potent synthetic glucocorticoid analog, and insulin increase concentration of leptin *in vivo* (Larsson and Ahren, 1996; Miell et al., 1996; Ramsay and White, 2000). Further, adipocytes cultured *in vitro* in the presence of dexamethasone secreted more leptin (Hardie et al., 1996; Considine et al., 1997) and had increased leptin mRNA content (Reul et al., 1997; Bradley and Cheatham, 1999). In addition, hyperinsulinemia has been shown to increase leptin concentrations within 3 to 5 h in both rats and humans (Cusin et al., 1995; Saladin et al., 1995), and plasma leptin concentrations are markedly reduced under conditions of insulin deficiency and are rapidly increased by insulin treatment (Sivitz et al., 1998). In contrast, hypothyroidism has been associated with elevated (Leonhardt et al., 1998; Leonhardt et al., 1999; Pinkney et al., 1998), as well as decreased (Valcavi et al., 1997; Yoshida et al., 1998) concentrations of leptin in humans. Treatment with thyroxine with and without triiodothyronine decreased concentrations of leptin (Pinkney et al., 2000; Escobar-Morreale et al., 1997).

The results reported here in show that concentrations of leptin in horses are affected by multiple systems in the body. Dexamethasone consistently increases concentrations of leptin in geldings, mares, and stallions. Further, a reduction in both triiodothryonine and thryoxine, but not an increase in thyroxine, resulted in alterations in leptin secretion in horses. Plasma leptin is also affected by glucose and insulin metabolism. Besides the affects

observed of these hormonal systems on plasma leptin in the horse, concentrations of leptin were also affected by degree of body condition, gender, and feeding time. Horses in greater body condition had greater concentrations of leptin compared with thinner horses. In addition, mares and geldings have greater concentrations of leptin compared with stallions. Further, meal feeding, whether as a concentrate or in a restricted grazing situation, increased both concentrations of leptin and insulin.

It was also found that horses with similar high body condition can be classified into two groups based on concentrations of leptin: hyperleptinemic and normal. The high leptin horses also have elevated concentrations of glucose, insulin, and triiodothyronine, as well as decreased concentrations of growth hormone. This obesity-related hyperleptinemia, hyperglycemia, and hyperinsulinemia syndrome is similar to type II diabetes in humans. Moreover, the horses with this high-leptin syndrome have some degree of insulin insensitivity based on increased insulin response to glucose injection while other horses with like BCS, but lower leptin, have an insulin response similar to thinner horses. Limited grazing to 6 h per day for 45 d, but not chromium supplementation for 30 d, decreased concentrations of leptin and BCS in high-leptin horses and neither improved insulin sensitivity. However, additional research is needed to determine the consistency of different glucose tolerance tests for assessing insulin sensitivity in the horse.

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APPENDIX A

LETTER OF PERMISSION

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APPENDIX B

LETTER OF PERMISSION

Sent by: Jean Rice <JEANR@assochq.org>
To: 'Joshua A Cartmill' <jcartm1@lsu.edu>
cc:
Subject: RE: permission to publish in dissertation

Joshua--

By this e-mail, I am granting you permission to use this material in your dissertation.

Best regards,

Jean Rice
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-----Original Message-----
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Endocrine responses in mares and geldings with high body condition scores grouped by high vs low resting leptin concentrations
J. Anim Sci. 2003 81: 2311-2321.

Thank you,

Joshua A. Cartmill

VITA

Joshua Allen Cartmill was born February 14, 1975, in Emporia, Kansas. Joshua began high school in Pleasant Hill, Missouri, before eventually graduating from Tigard High School in Tigard, Oregon, in 1993. He completed a Bachelor of Science degree in animal science-production management at Kansas State University in December, 1997. In January, 1997, he began work on his Master of Science degree in dairy cattle reproduction at Kansas State University with Dr. Jeff Stevenson. He received his Master of Science degree from Kansas State in December, 1999. During his time at Kansas State, he competed on the KSU Intercollegiate Rodeo Team and participated in the KSU Rodeo Club, serving as their President from 1997 to 1998. In January of 1999, he began work on his doctoral degree in animal science at Louisiana State University under the guidance of Dr. Don Thompson in the field of equine physiology. He is a candidate for his doctoral degree in May, 2004.