IMPACT OF BODY CONDITION ON PLASMA LEPTIN AND INSULIN-LIKE GROWTH FACTOR-I CONCENTRATIONS IN STALLIONS AND GELDINGS

A Thesis

by

TOMMY NEAL CHANCELLOR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Animal Science

IMPACT OF BODY CONDITION ON PLASMA LEPTIN AND INSULIN-LIKE GROWTH FACTOR-I CONCENTRATIONS IN STALLIONS AND GELDINGS

A Thesis

by

TOMMY NEAL CHANCELLOR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Martha M. Vogelsang
Committee Members,	David D. Forrest
	Duane Kraemer
	Brett D. Scott
Head of Department,	Gary R. Acuff

December 2006

Major Subject: Animal Science

ABSTRACT

Impact of Body Condition on Plasma Leptin and Insulin-like Growth Factor-I Concentrations in Stallions and Geldings. (December 2006)

Tommy Neal Chancellor, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Martha M. Vogelsang

The objective of this study was to more clearly define the relationship between body condition, plasma leptin and insulin-like growth factor-I (IGF-I) in stallions and geldings in moderate (5.0-5.5) versus fleshy (7.0-7.5) body condition. Data analyses of physical measurements showed that there was a difference for BCS ($P<0.001$) even though the fat group only achieved a mean BCS of 6.3 ± 0.2 as compared with a mean BCS of 5.3 ± 0.1 for the moderate group. Differences also existed for rump fat ($P<0.05$) and percent body fat ($P<0.05$) between BCS groups. Analysis of physical measurements revealed that there was no sex effect as geldings and stallions within each group were not significantly different. Analysis of plasma leptin concurred with previous reports as a difference ($P<0.001$) existed between the BCS groups. Mean leptin concentrations were 2.13 ± 0.1 ng/ml for the fat group and 1.44 ± 0.1 ng/ml for the moderate group. After normalization of the data, changes in leptin concentrations still revealed a significant difference ($P<0.05$) between BCS groups, yet no difference in leptin concentrations between stallions and geldings was seen. Dexamethasone (DEX) treatment on d 0 caused a subsequent 24 h rise in plasma leptin in both groups. Analysis of plasma IGF-I revealed no difference in IGF-I concentrations between BCS groups. Mean plasma IGF-I was 347.2 ± 11.4 ng/ml for the fat group and 344.3 ± 10.0 ng/ml for the moderate group.

There was however a difference ($P<0.05$) between geldings and stallions. Geldings exhibited an overall mean plasma IGF-I concentration of 360.6 $\pm$ 9.1 ng/ml with stallions exhibiting a mean IGF-I concentration of 329.1 $\pm$ 12.1 ng/ml. The post DEX challenge rise seen with leptin was not evident when analyzing the change in plasma IGF-I concentrations. In conclusion, the data presented herein have provided a more accurate profile of circulating concentrations of leptin and IGF-I in stallions and geldings of moderate and fleshy body condition.

ACKNOWLEDGEMENTS

I would like to first and foremost thank my committee chair, Dr. Martha Vogelsang, for giving me the opportunity to pursue a master's degree. Thank you for having enough belief in me so as to give me the opportunity to achieve something that took much consideration and prayer. To my committee members (alphabetically), Dr. David Forrest, Dr. Duane Kraemer, and Dr. Brett Scott, I thank you for your guidance, your time and your patience during this journey. I feel privileged that each of you agreed to be a part of this project. Your experience and guidance were invaluable.

Secondly, I want to thank the friends who were more than willing to help me along. To Trinette Ross, Denise Manhart, and Chris Mortensen, I thank you for your help collecting samples in those early morning hours. To Rusty Kriewald, I thank you for your willingness to help with the care and details of my research animals. To Elena Eller, you have my gratitude for your guidance in the lab and statistical input. And to Clay Cavinder, thank you for your support, counsel, and just generally listening to me whine when I couldn't get things figured out.

Last, but definitely not least, I would like to thank my family. To my wife Dana, I thank you for your unwavering character in standing by me through my struggles and triumphs. You had the courage to walk this journey with me, listen to me when times were tough, admonish me when I needed it, but above all else, there was your support and love. I admire your fortitude and love you for the rock that you are. To my sons, Layton and Aaron, you are both a part of the reason for pursuing this degree. It is my hope that the sacrifice of my time in being with you will eventually benefit you both. I

pray that I can provide you both with tools and skills that will enable you to be better than I am. Whatever endeavors may present themselves to you, I pray that I will have the strength to provide you the support that has been shown to me. Daddy loves you. To my parents Linda and Tommy, you have always been there to encourage my dreams and aspirations. You were my biggest proponents when the idea of graduate school came to me. The belief in me and aid in helping me finish this task were a comfort and gift that most people dream of. Words and deeds cannot express my appreciation, nor will I ever be able to fully repay you for being there for me in this endeavor. All my love to you both. Lastly, I want to give thanks to God. Though him all things are possible.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

In the twentieth century, research in endocrinology has expanded the understanding of hormones from hypotheses and educated conclusions to solid answers based upon experimental data. This process continues on into the twenty-first century with the knowledge base growing ever larger. With the myriad of hormones that operate within endocrine systems research interests are diverse and broad. Two hormones of particular interest that this paper seeks to address are the adipocyte hormone leptin and insulin-like growth factor-I (IGF-I).

Leptin is a polypeptide hormone secreted mainly by white adipocytes. Its primary function is to relay the body's nutritional status to the brain and to regulate energy homeostasis and body weight. Circulating concentrations of leptin have been shown to be sensitive to changes in energy intake as well as being positively correlated to the body's degree of fatness. Insulin-like growth factor-I on the other hand is a growth promoting polypeptide that primarily mediates the effects of growth hormone (GH) on the skeletal system. Like leptin, IGF-I can exhibit changes in circulating concentrations with changes in body fat. Insulin-like growth factor-I has also been found to decrease along with decreases in feed intake and body condition. Besides changes in nutritional intake, other factors can influence the circulating concentrations of these two hormones. Factors that regulate IGF-I production include thyroid hormones, sex steroids and insulin. Leptin secretion can be inhibited by factors such as GH and β-agonists and stimulated by insulin, glucose, neuropeptide-Y (NPY) and glucocorticoids.

This thesis follows the style and format of the Journal of Animal Science.

The effect of nutritional intake and BCS and their relation to leptin and IGF-I have been previously reported for mares, stallions and geldings. In addition, the effect of the synthetic glucocorticoid dexamethasone (DEX) on leptin secretion in the horse has also been determined. First, both leptin and IGF-I concentrations have been proven to correlate positively with BCS in horses and to fluctuate with changes in energy intake. Secondly, DEX has been shown to affect an acute increase in leptin secretion with reports indicating a possible positive response in IGF-I levels. Lastly, a sexual dichotomy has also been established with geldings and stallions exhibiting greater circulating leptin concentrations than mares.

Though circulating leptin concentrations and the effect of DEX in stallions and geldings have been compared, variations in feeding regimens, body condition and sampling schedules have led to conflicting results. Therefore, the objective of the present study was to more clearly define the relationships among body condition, leptin and IGF-I in stallions and geldings in moderate (5.0-5.5) versus fat (7.0-7.5) body condition with the addition of a DEX challenge. To ensure accurate profiles, horses were fed under controlled conditions and analyzed for BCS, rump fat thickness and percent body fat so that target BCS could be reached and maintained.

CHAPTER II

REVIEW OF LITERATURE

Body Condition

The evaluation of an animal's fatness level or body condition scoring (BCS) is a subjective method that incorporates visual appraisal usually combined with manual palpation of fat reserves. Methods for condition scoring have been developed for beef cattle (Herd and Sprott, 1985), dairy cattle (Wildman et al., 1982) and horses (Henneke et al., 1983a). Body condition scoring, when performed correctly, can be an excellent tool in visually determining an animal's nutritional status. This information can be used to make decisions in relation to production, reproduction, exercise and health. Though the principals for condition scoring are similar, the criteria and the numerical score assigned may differ for each species. Dairy cows are scored on a scale of 1 to 5 with 1 indicating poor condition and 5 representing above normal condition with only the rump and back being considered for appraisal (Wildman et al., 1982). In contrast, a larger numerical system is utilized when evaluating beef cows and horses. For these species, a scale of 1 through 9 is used with 1 representing a poor, or thin condition, and 9 designating obesity (Henneke et al., 1983a; Herd and Sprott, 1985). With regard to evaluation criteria, beef cattle are appraised at the shoulder, ribs, loin, and rump (Herd and Sprott, 1985). Horses, on the other hand, are evaluated at the neck, withers, ribs, girth, back, rump and tailhead (Henneke et al., 1983a). In determining the final condition score, all points of interest are considered, with no one point being valued above the others (Wildman et al., 1982; Henneke et al., 1983a; Herd and Sprott, 1985). Weight and body size do not correlate to

BCS and are not considered when making a final appraisal (Wildman et al., 1982; Henneke et al., 1983b; Herd and Sprott, 1985).

In addition to visual and tactile appraisal, ultrasonic measurement has become a valuable resource in determining an animal's fat composition . Ultrasonography has been used to determine fatness in cattle (Faulkner et al., 1990; Perkins et al., 1992; Brethour, 2000) and in horses (Westervelt et al., 1976; Kane et al., 1987). Westervelt et al. (1976) developed a method to correlate ultrasonic measurement to fat deposition in order to calculate percent body fat. It was determined that ultrasonic rump fat measurements (taken 5 cm from the midline at the center of the pelvic bone) in horses were positively correlated ($r = .93$) to actual rump fat measured after slaughter (Westervelt et al., 1976). In addition, Westervelt et al. (1976) developed an equation ($Y + 8.64 + 4.70 X$) to convert ultrasonic rump fat measurements (X = cm) to actual fat percentage (Y + %). Kane et al. (1987) also found that true and ultrasonic rump fat measurements were highly correlated in 4 out of 5 measurement sites at the rump.

Ultrasonography has also been utilized in correlating BCS to body fat in dairy cattle (Domecq et al., 1995) and in horses (Henneke et al., 1983b; Gentry et al., 2004). Henneke et al. (1983b) found that percent body fat and body condition correlated positively ($r = 0.65$, $P<0.001$) after taking ultrasonic rump fat measurements from 32 mares at different body conditions. Calculations for percent body fat for this study followed the guidelines and calculations set forth by Westervelt et al. (1976). It has also been determined that no correlation exists for weight, height or heart girth in relation to percent body fat (Henneke et al., 1983b). Gentry et al. (2004) also conducted a study to correlate BCS to body fat using ultrasonic measurements scanned at the thirteenth rib,

withers, tailhead and rump of mares. It was discovered that correlation coefficients for the rump, thirteenth rib, and withers were 0.84, 0.82 and 0.86 with the tailhead correlating highest at 0.87 (Gentry et al., 2004).

Leptin

Leptin is a 14-16 kDa peptide made up of 167 amino acids secreted mainly by white adipose tissue. Through positional cloning, leptin was first described by Zhang et al. (1994) as a protein product of the obese (ob) gene. Early work conducted with the ob/ob mouse would conclude that leptin's primary role is to relay the body's nutritional status to the hypothalamus and to aid in controlling energy homeostasis and body weight (Zhang et al., 1994; Halaas et al., 1995; Pellymounter et al., 1995).

The majority of work originally done to clarify leptin's actions was conducted using obese (ob/ob) mice. Through parabiosis experiments with ob/ob mice and diabetic (db/db) mice, Coleman et al. (1973) concluded that ob/ob mice were unable to synthesize a circulating "satiety" factor. Due to an inability to produce this factor, ob/ob mice exhibited obesity and a host of conditions such as hyperphagia, decreased immunity, poor thermoregulation, hyperinsulinemia, hypogonadism and infertility (Coleman et al., 1978). This illusive satiety factor was later discovered to be leptin. Zhang et al. (1994) identified a nonsense mutation in the genetic background of C57BL/6J ob/ob mice. It was found that these mice were unable to produce ob RNA (leptin) (Zhang et al., 1994). To confirm these findings, studies have been conducted in which ob/ob mice were treated with exogenous leptin. Ob/ob mice treated with leptin exhibit a reduction in food intake accompanied with reduced body weight, increased thermogenesis, increased metabolism,

and reduced insulinemia (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995)

Through expression cloning, the leptin receptor (Ob-R) was initially discovered to be located in the mouse choroid plexus (Tartaglia et al., 1995). Within the same research study conducted by Tartaglia et al.(1995), Ob-R was also found to have homology with interleukin-6 (IL-6) receptors and thus was identified as a class I cytokine (Tartaglia et al., 1995). The hypothalamus has been identified in the past as the control center regulating body weight and homeostasis, yet the factor linking the brain and the body was unknown (Hetherington and Ranson, 1940). Six splice variants of the Ob-R gene have also been identified with the Ob-Rb (long) form being the primary signaling form (Lee et al., 1996). The use of in situ hybridization found the greatest congregation of Ob-Rb to be within hypothalamic arcuate, ventromedial, paraventricular and premammillary nuclei (Mercer et al., 1996). Due to its high expression in the hypothalamus, Ob-Rb has been determined to be the signaling form that links leptin's action in the body to the brain (Lee et al., 1996; Mercer et al., 1996). McCann et al. (2003) also concluded that ultimately, leptin acts directly on the hypothalamic-pituitary axis by enacting nitric oxide (NO) release.

Though the brain has been the primary focus for leptin receptors, leptin receptors have also been discovered in peripheral tissues such as heart, lung, liver, kidney, skeletal muscle, adipose tissue, testes and pancreatic β-islets (Tartaglia et al., 1995; Lee et al., 1996; Emillson et al., 1997). It has also been determined that leptin in the human is 84% identical to murine leptin, 84% identical to bovine leptin and 85% identical to porcine

leptin (Zhang et al., 1994; Doyon et al., 2001). Therefore, results of studies in leptin can possibly be applied across species.

With the discovery of leptin in mice, other studies followed in order to identify leptin and its receptors in sheep, cattle, and pigs (Neunschwander et al., 1996; Pfister-Genskow et al., 1996; Dyer et al., 1997b). In the equine, Buff et al. (2002) utilized reverse transcriptase polymerase chain reaction (RT-PCR) to identify leptin receptor mRNA in liver, lung, testis, ovary, choroid plexus, hypothalamus, and subcutaneous adipose tissue. As well as being an "adipostatic" hormone that regulates food intake, leptin has been identified as a modulator of other metabolic processes such as hematopoesis, bone formation, immunity, angiogenesis, diabetes and fertility (Henson et al., 2003). As early as the 1950s, ob/ob female mice were found to be sterile and unable to reproduce (Ingalls et al., 1950; Lane and Dickie, 1954). Leptin was found to be the key as the treatment of female ob/ob mice with recombinant leptin rescued gonadotropin secretion, increased secondary sex organ weight and function, and restored fertility (Barash et al., 1996; Chehab et al., 1996). Similar evidence has been obtained in ob/ob male mice. Although some male ob/ob mice can reproduce when fed a restricted diet, the majority of male ob/ob mice demonstrate the same sterility condition observed in female ob/ob mice (Lane and Dickie, 1954). Male ob/ob mice exhibit conditions such as low testicular weight and low testosterone concentration (Mounzih et al., 1997). In addition, these mice exhibit a combination of few mature sperm cells within the seminiferous tubules along with severely atrophied Leydig cells (Mounzih et al., 1997). Once treatment with recombinant leptin was initiated in these mice, testicular weights returned

to normal followed by normal Leydig cell function and viable sperm cell production (Mounzih et al., 1997).

Through work with ob/ob mice it has been shown that basic physiological similarities exist for individuals of opposing sexes experiencing disturbances in leptin secretion. Despite this fact there is a sexual dimorphism that exists between the male and female genders. In humans, women have been reported to have a fasting serum leptin concentration of 15.2 ng/ml compared to 6.9 ng/ml in men (Hickey et al., 1996). Similar results have been found in sheep in which ewes have higher circulating leptin concentrations than wethers or rams (Blache et al., 2000). Blache et al. (2000) also denoted that adjusting for back-fat thickness could account for only 30% of the variation in leptin concentrations seen between genders. Similar results have been reported for leptin differences in humans after adjustment for body mass (Rosenbaum and Leibel, 1999). Though leptin concentrations have been found to be higher in human and ovine females, the opposite holds true for horses. In a study to better define peripheral leptin concentrations in the horse, Buff et al. (2002) found that leptin concentrations were higher in stallions and geldings when compared to mares ($P = 0.0002$).

Due to persistent differences in leptin concentrations between genders even after adjustment for fat levels, investigators have conducted studies to examine the possibility of testosterone acting as the limiting factor. Numerous studies have suggested the likelihood that testosterone can depress circulating leptin concentrations (Blum et al., 1997; Garcia-Mayor et al., 1997; Mantzoros et al., 1997; Steiner et al., 2003). Others have reported data to refute testosterone's negative effect on leptin. Piniero et al. (1999) denoted no change in leptin secretion from human omental adipose tissue cultured in

vitro with testosterone. Blache et al. (2000) suggested that testosterone might not play as large a role in regulating leptin as suspected due to little difference seen in leptin concentrations between castrated and intact male sheep. Taking into account this information, the exact interaction of leptin and testosterone is still unclear.

Some hormones and factors play an agonistic role to enhance leptin secretion while other factors play an antagonistic role thus inhibiting leptin's influence. Leptin secretion can be increased by insulin, glucose, neruopeptide-Y (NPY) and glucocorticoids (Barr et al., 1997; Dyer et al., 1997a; Elimam et al., 1998; Mueller et al., 1998). Factors that serve to down-regulate leptin synthesis include fasting, decreased adiposity, β-agonists and growth hormone (Ahren et al., 1997; Considine and Caro, 1997; Houseknecht et al., 2000). A stimulant of leptin secretion that has gained great attention is the synthetic glucocorticoid dexamethasone (DEX). Considine et al. (1997) found that cultured human adipocytes had an 83 $\pm$ 30% increase in ob mRNA followed by leptin secretion when co-incubated with DEX. In a study conducted by Houseknecht et al. (2000), DEX increased leptin mRNA above basal levels when incubated with tissue explants from steers. Similarly, DEX treatment has also been found to increase circulating leptin concentrations in mares, stallions and geldings after treatment with DEX (Cartmill et al., 2003; Cartmill, 2004; Cartmill et al., 2005).

In the area of nutrition, lower feed consumption and a body mass reduction have been shown to produce a negative result on leptin synthesis. Short and long-term systems exist within the body to regulate energy homeostasis and meal intake (Friedman and Halaas, 1998). The short-term system appears to regulate meal intake while the long-term system governs equilibrium between nutrient accumulation and usage, thus

providing energy homeostasis (Friedman and Halaas, 1998). Leptin has been found to operate mainly within this long-term system (Friedman and Halaas, 1998). In addition, it has been revealed that a single meal does not significantly change leptin concentration, nor does leptin alone result in limiting food intake (Maffei et al., 1995; Considine et al., 1996; Dickson et al., 2002). This calls into question the widely held belief that leptin acts as a classic satiety factor (Dickson et al., 2002).

With the knowledge that nutrient intake can effect leptin secretion, trials have been conducted to determine the effects of feed restriction and increased nutrient intake on leptin concentrations. Ahima et al. (1996) discovered that reductions in leptin and insulin levels were observed following a 48 h fast in mice. Decreases in circulating leptin produce a 23% increase in arcuate neuropeptide –Y (NPY) production signaling to the brain the body's requirement for increased food intake and energy homeostasis restoration via hypothalamic pathways (Hahn et al., 1998). During feed restriction, cortisol concentrations increase in response to the stress of reduced feed intake and declining leptin levels (Bornstein et al., 1997). The subsequent release of cortisol signals to the brain a requirement for increased nutrient intake (Chilliard et al., 2001). If nutrient uptake is initiated, a subsequent stimulation of insulin secretion follows, which under high cortisol levels, elicits secretion of leptin (Chilliard et al., 2001). The rise of leptin levels in turn can reduce cortisol release (Bornstein et al., 1997). In contrast to reduced energy stores, increases in adiposity following positive energy intake increases leptin levels, signaling the body to reduce feed intake (Dickson et al., 2002).

Many studies have been conducted in which animals of varying species were placed under feed restriction to determine if any effects on leptin secretion existed.

Short-term feed restriction in young heifers has been found to cause a decrease in insulin, IGF-I, leptin and LH pulses (Amstalden et al., 2000; Williams et al., 2002). Also, McManus and Fitzgerald (2000) conducted a study on aged and young mares to determine serum changes in leptin, gonadotropins, prolactin and metabolites. With both aged and young mares in good body condition, short term feed deprivation caused a decrease in leptin levels, yet serum levels of LH and FSH were not affected (McManus and Fitzgerald, 2000). An explanation for this observation is that species with a large body mass have greater available stores of oxidizable energy to counteract the negative response of the hypothalamic-pituitary-gonadal axis to feed reduction (McManus and Fitzgerald, 2000). Though much of the effect of nutrition on leptin has focused on nutritional restriction, the effect of nutritional increases on leptin concentrations have also been investigated. Zhang et al. (2004) noted an increase of circulating leptin within 7 h of feeding a high energy diet to mature Merino rams.

Adipocyte size also plays a role in regulating leptin. Hamilton et al. (1995) found that genetic expression of the ob gene was lower in smaller adipocytes when compared with larger adipocytes acquired from a single portion of adipose tissue. In agreement with this observation, Lonqvist et al. (1997) discovered that fat cell volume and lipid content accounted for 70-80% of variation seen in circulating leptin between obese and non-obese women. Fasting can reduce adipose tissue volume resulting in a decrease in leptin as opposed to an increase in leptin concentrations seen with increases in adipose tissue mass (Maffei et al., 1995; Considine and Caro, 1997; Friedman and Halaas, 1998).

The overall fatness of an animal, or body condition, has been found to be a good indicator of circulating leptin concentrations. Body condition has been found to be

positively correlated with leptin levels in cattle (Delevaud et al., 2000) and sheep (Yildiz et al., 2003). Comparison of individuals within the same species confirms the difference in leptin between individuals of high and low BCS. In an experiment conducted by Buff et al. (2002), leptin levels were positively correlated to body condition score (BCS) based upon the condition scoring system set forth by Henneke et al. (1983a). Serum leptin was found to be higher in horses of fleshier body condition when compared to their thinner counterparts (Buff et al., 2002). These results were confirmed by Gentry et al. (2002a) who found that mares with a high BCS (7.5-8.5) had higher circulating levels of leptin, IGF-I, and luteinizing hormone (LH) compared to mares in low body condition (3.0-3.5). Thus leptin concentrations can fall into two categories; (1) lower levels with lower numbers of adipocytes and (2) higher levels following an increase in adipocyte stores (Considine, 2003).

Insulin-like Growth Factor-I

Insulin-like growth factors are "growth promoting" polypeptides weighing 7.5 kDa that are secreted mainly by the liver and are controlled by the body's nutritional state and by other hormones (Phillips et al., 1990; Thissen et al., 2006). Rinderknecht and Humbel (1978) found the sequence of IGF-I to have 48% similarity with human proinsulin and thus coined the term "insulin-like". The principal role of IGF-I is to mediate growth hormone's (GH) effect on the skeletal system (Phillips and Vassilopoulou-Sellin, 1980a,b) and growth hormone has been found to elicit a response in IGF-I secretion from the liver (Jones and Clemmons, 1995). The majority of IGF-I in circulation (80%) is bound to IGF-binding protein-3 (IGFBP-3) with the remainder of

IGF-I in the "free/dissociable" state (Jones and Clemmons, 1995; Bereket et al., 1996). Other factors including thyroid hormones, insulin, sex steroids and nutrition can regulate IGF-I production (Philips et al., 1990; Buonomo and Baile, 1991).

Since GH can stimulate IGF-I secretion, it would logically be assumed that an increase in GH would be paralleled by a subsequent rise in IGF-I levels. In swine and sheep, feed restriction has been found to increase the amount of GH in plasma (Thomas et al., 1990; Buonomo and Baile, 1991; Yambayamba et al., 1996). Despite an increase in GH concentration during feed restriction, GH receptors and IGF-I concentration in plasma are decreased (Breier, 1999). Philips et al. (1990) found that IGF-I concentrations fell in fasted rats with a restoration of IGF-I concentrations after refeeding. Other studies have also shown that IGF-I levels consequently decrease with reductions in energy availability in humans (Musey et al., 1993), swine (Buonomo and Baile, 1991), sheep (Thomas et al., 1990) and cattle (Richards et al., 1991; Leon et al., 2004; Lents et al., 2005). The relationship between nutrient intake and IGF-I concentrations has also been investigated in the horse. Sticker et al. (1995) reported that mares fed a diet with energy reduced to 50% below maintenance levels had lower levels of IGF-I compared to mares on a control diet (100% of energy required for maintenance). Gentry et al. (2002a,b) found that mares in high BCS had greater levels of plasma IGF-I than mares in low BCS.

Reports also indicate the possibility that DEX treatment can positively stimulate IGF-I production. Ajuwon et al. (2003) discovered that porcine hepatocytes cultured in vitro with DEX caused a significant increase in IGF-I concentrations. Cartmill et al. (2003) noted a similar in vivo effect of DEX on IGF-I in horses. In this study, rises in

IGF-I levels after DEX injection were detected on d 12 and 19 relative to treatment in geldings, and on d 10, 12 and 19 in mares (Cartmill et al., 2003).

Interaction of Leptin and IGF-I

In many studies reporting the effect of nutrient intake on leptin, circulating leptin and IGF-I concentrations have mirrored one another. Both hormones exhibit lower concentrations subsequent with low BCS and feed restriction as opposed to higher concentrations in both hormones during positive energy balance and body fat levels. The parallel changes detected in leptin and IGF-I have led to the hypothesis that the two hormones may somehow directly influence one another.

To test this hypothesis, experiments have been conducted in which adipocytes have been cultured in vitro with IGF-I. Hardie et al. (1996) harvested mature adipocytes from epidydimal fat pads in male Zucker rats and cultured the adipocytes in vitro with IGF-I and GH. Neither IGF-I nor GH stimulated any response in leptin secretion from the cultured adipocytes (Hardie et al., 1996). Similar results have been obtained from in vivo studies using ”fatty” Zucker rats. Zucker rats infused with IGF-I (200 μg/kg/day) exhibited no change in body fat percentage or the amount of leptin mRNA (Isozaki et al., 1999).

Similarly, studies have also been conducted to determine if leptin is responsible for the increases seen in circulating IGF-I. In 1998, Barb et al. conducted an experiment to test the effects of recombinant porcine leptin on GH and IGF-I in gilts. No effect on either GH or IGF-I was detected after 10, 50, and 100 μg doses were administered (Barb

et al., 1998). Human studies have also revealed a lack of relationship between leptin and IGF-I concentrations (Janssen et al., 1998; Gomez et al., 2003).

Contrary to these findings, Ajuwon et al. (2003) found that at low doses, leptin reduced serum IGF-I concentrations dose-dependently and blocked hepatic IGF-I production. Also, Houseknecht et al. (2000) reported that leptin concentrations were positively correlated to the amount of IGF-I mRNA detected in adipose tissue from cattle. Taken together, the information presented reveals that a relationship between IGF-I and leptin, either direct or indirect, is complex and still unclear.

CHAPTER III

MATERIALS AND METHODS

Management of Horses

Five mature stallions and five geldings, of Stock Horse and Thoroughbred breeding, were used for the purposes of this study (Appendix Table 7A). All horses used in this study were property of the Texas A&M University Department of Animal Science and were maintained at the Texas A&M University Horse Center and N.W. Freeman Arena. Maintenance of the horses for this study conformed with guidelines set forth by the Institutional Agricultural Animal Care and Use Committee (AUP# 2005-252).

Horses were housed in 10' X 10' stalls and paddocks with access to regulated exercise to maintain the required BCS for each animal. Diets were adjusted accordingly to obtain and subsequently maintain the desired body condition for each horse. Meals for each horse were administered in accordance with the current ration utilized for each horse by the Texas A&M University Horse Center. This ration included a commercially produced concentrate (Producer's Cooperative Association, Bryan, TX) containing 13% crude protein. Horses were also fed coastal bermudagrass hay and were provided ad libitum access to water. All feedings were conducted at 0630 and 1730 daily.

Physical Measurements

All horses were initially evaluated for BCS utilizing the consensus of at least two objective observers beginning October 10, 2005. At this time, each horse was grouped into the appropriate BCS group to which it most closely fit. Concordantly, each horse

was weighed and rump fat measurements were taken using ultrasonic scanning equipment with a 5MHz transducer (Medison SonoVet 600®, Universal, Bedford Hills, NY.). Rump fat measurements were taken from an area measuring 10.00 cm from the point of the hip and 5.00 cm from the midline. The purpose of this measurement was to correlate fat deposition, or removal, to BCS. Following initial observations, each horse's diet was adjusted to ensure that the proper BCS group for each horse was obtained.

Once target BCS was met, week one of the initial trial began on November 7, 2005. Weight and rump fat measurements were taken on a weekly basis throughout both trial periods and every other week during the transition period. Weights and rump fat measurements for each horse were taken on the same day. Body condition score evaluations were conducted every two weeks throughout the entire project with the aid of at least two objective observers.

Experimental Design

A switchback design was utilized to maximize observations for all groups (gelding vs. stallion, BCS 5.0 vs. BCS 7.0). Each horse from one of the two male categories was initially evaluated and placed in an appropriate treatment group. The trial began initially with five horses (two stallions, three geldings) in BCS of 7.0 and five horses (three stallions, two geldings) in BCS of 5.0. Following the first 29 day blood collection period (beginning Nov. 7 and ending on Dec. 5, 2005), each horse's diet was adjusted to achieve the alternate body condition. Once the desired BCS was obtained, the second blood collection period began on March 7, 2006 with five horses (three stallions, two geldings) in BCS of 7.0 and five horses (two stallions, three geldings) in BCS of 5.0.

The adjustment period required approximately 12 weeks beginning on December 6, 2005 and ending on March 6, 2006.

Plasma Sampling

Blood was collected from each horse using jugular venipuncture on d -2, -1, 0, 1, 2, 3, 4, 5, 12, 19, and 26 for laboratory analysis. Blood samples were obtained at 0600 before feeding to minimize fluctuation in circulating hormones. On d 0, a single intravenous injection of DEX was administered to each horse after blood samples were collected. Each DEX dosage was concordant with the test subject weight (.045 mg/kg BW). Upon collection, all blood samples were stored on ice in preparation for centrifugation. All samples were centrifuged within 1 h of collection in a refrigerated centrifuge. Centrifuge temperature was set for 5°C and samples were spun at 2500 x G for 30 min. Once centrifuged, plasma was stored in microcentrifuge tubes at -20°C for later analysis.

Radioimmunoassay (RIA) Procedures

Plasma concentrations of leptin (McManus and Fitzgerald, 2000) and IGF-I (Sticker et al., 1995) were analyzed utilizing RIA as previously documented for horse samples. Leptin and IGF-I RIA kits were provided with all reagents required for completing each assay. Leptin and IGF-I assays were analyzed using a Packard Cobra II® gamma counter. All samples from each horse were analyzed in a single assay for each hormone. All assay samples for each horse were run in duplicate with standards and reagents being run in triplicate.

Plasma leptin concentrations were measured using a multispecies leptin RIA kit (Linco Research, Inc., St. Charles, MO). A sample volume of 100 µl was used with a sensitivity of 1.0 ng/ml. Plasma leptin intra-assay coefficient of variation (CV) was 6.2% and inter-assay CV was 16.6% (n = 7 assays).

Plasma IGF-I concentrations were measured using an RIA kit (Diagnostic Systems Laboratories, Inc., Webster, TX). A sample volume of 50 µl was used with a sensitivity of 0.8 ng/µl. Plasma IGF-I intra-assay CV was 7.8% and inter-assay CV was 6.0% (n = 7 assays).

Statistical Analyses

Data from BCS, rump fat thickness and body fat percentage were analyzed by analysis of variance (ANOVA) using STATA 8 statistical analysis software (Stata Corp., College Station, TX). Each parameter was also analyzed for group effects (BCS 5 vs. BCS 7 and stallions vs. geldings), and period effect.

Plasma leptin and IGF-I were analyzed using piecewise regression. A structural time break was modeled in order to assess the effect of the DEX challenge on hormone concentrations. Where necessary, data for each horse were normalized to baseline (d 0) values in order to better characterize changes from baseline. Data from leptin and IGF-I concentrations were also analyzed for day effect, period effect, and group effects by ANOVA.

CHAPTER IV

RESULTS

Body Condition

Body condition evaluations were performed semi-monthly on both stallions and geldings insuring that both castrate and intact males were correctly grouped by the body condition to which they were designated. Body condition evaluations were also performed to maintain a significant difference between the fat and moderate groups. It should be noted that due to unforeseen circumstances, one stallion was removed from the study during the second trial period. Horses in the fat group achieved a mean BCS of 6.3 $\pm$ 0.2 whereas horses in the moderate group achieved a mean BCS of 5.3 $\pm$ 0.1 (Table 1). Though the horses for the fat group did not achieve a mean body condition level of 7.0, analysis of body condition evaluations revealed a difference between both fat (BCS 7.0) and moderate (BCS 5.0) groups ($P<0.001$). Weekly rump fat measurements were also taken and converted to percent body fat to correlate BCS to body fat percentage. Weekly rump fat measurements and percent body fat analyses also showed a difference ($P<0.05$) between groups. Mean rump fat for the moderate group was 1.1 $\pm$ 1.5 cm versus 1.6 $\pm$ 1.2 cm for the fat group (Table 1). A greater rump fat measurement translated into a higher body fat percentage. This was illustrated by mean body fat percentage for the fat group of 16.2 $\pm$ 0.6% compared to a mean body fat percentage of 14.0 $\pm$ 0.7% for the moderate group (Table 1). The difference in means for rump fat and percent body fat between the fat and moderate groups further demonstrated that BCS groups were significantly different. No period effect was evident as mean body condition scores,

rump fat thickness and body fat percentage were not significantly different within each BCS group between the first and second periods.

Differences between stallions and geldings were also investigated. No sex effect was detected for BCS, rump fat thickness or percent body fat between geldings and stallions within each BCS group. Geldings in the fat group had a mean BCS of 6.4 ± 0.3 with stallions revealing a mean BCS of 6.1 ± 0.3 (Table 2). In comparison, geldings in the moderate group had a mean BCS of 5.4 ± 0.1 while stallions held a mean BCS of 5.3 ± 0.1 (Table 2). Though the percent body fat and rump fat thickness tended to be higher for geldings, there was no significant difference between either measurement for both groups.

Table 1. Mean (± SE) body condition, rump fat thickness, and percent body fat of fat- vs. moderate-conditioned groups for periods 1 and 2.

Group	n	BCS	Rump Fat (cm)	% Body Fat
Fat				
Period 1:	5	6.2^{a} ± 0.3	1.6^{c} ± 1.2	16.0^{c} ± 0.6
Period 2:	4	6.4^{a} ± 0.2	1.7^{c} ± 2.5	16.5^{c} ± 1.2
Overall Total:		6.3^{a} ± 0.2	1.6^{c} ± 1.2	16.2^{c} ± 0.6
Moderate				
Period 1:	5	5.2^{b} ± 0.2	0.8^{d} ± 2.0	12.6^{d} ± 1.0
Period 2:	5	5.5^{b} ± 0.1	1.4^{d} ± 1.6	15.3^{d} ± 0.7
Overall Total:		5.3^{b} ± 0.1	1.1^{d} ± 1.5	14.0^{d} ± 0.7

[a,b] Means within column lacking common superscripts differ (P<0.001)
[c,d] Means within column lacking common superscripts differ (P<0.05)

Table 2. Mean (± SE) body condition, rump fat thickness and percent body fat of geldings vs. stallions in fat and moderate BCS groups.

Group	n	BCS	Rump Fat (cm)	% Body Fat
Fat				
Geldings:	5	$6.4^{a} \pm 0.3$	$1.8^{c} \pm 1.1$	$17.8^{c} \pm 1.1$
Stallions:	4	$6.1^{a} \pm 0.3$	$1.4^{c} \pm 2.0$	$14.0^{c} \pm 2.0$
Moderate				
Geldings:	5	$5.4^{b} \pm 0.1$	$1.2^{d} \pm 0.7$	$12.2^{d} \pm 0.7$
Stallions:	5	$5.3^{b} \pm 0.1$	$1.0^{d} \pm 3.1$	$10.4^{d} \pm 3.1$

[a,b] Means within column lacking common superscripts differ ($P<0.001$)
[c,d] Means within column lacking common superscripts differ ($P<0.05$)

Hormone Analyses: Plasma Leptin

Mean plasma leptin concentration over the entire trial indicated that the fat group was 2.13 ± 0.1 ng/ml which was different ($P<0.001$) from a mean plasma leptin concentration of 1.44 ± 0.1 ng/ml for the moderate group (Table 3). It should be noted that analysis of leptin was initially conducted investigating only the difference between BCS groups. As there was a difference for leptin concentrations between the BCS groups, the difference between castrate and intact males was analyzed within BCS groups rather than between BCS groups. With regards to castrate versus intact males there was no significant difference between stallions and geldings as mean plasma leptin was 1.84 ± 0.1 ng/ml for stallions and 1.70 ± 0.1 ng/ml for geldings (Table 4). These values are slightly lower than the 3.37 ± 0.38 ng/ml for geldings and 3.50 ± 0.45 ng/ml for stallions reported by Buff et al. (2002).

Table 3. Mean (± SE) plasma leptin concentrations (ng/ml) for horses in moderate vs. fat BCS groups by day.

Day	BCS 5	BCS 7
-2	1.46 ± 0.3	2.06 ± 0.3
-1	1.32 ± 0.3	1.92 ± 0.4
0	1.36 ± 0.2	2.18 ± 0.4
1	1.66 ± 0.2	3.66 ± 0.8
2	1.62 ± 0.2	2.80 ± 0.5
3	1.37 ± 0.1	1.67 ± 0.2
4	1.38 ± 0.1	1.76 ± 0.4
5	1.75 ± 0.3	1.91 ± 0.3
12	1.33 ± 0.2	1.82 ± 0.3
19	1.40 ± 0.2	2.03 ± 0.3
26	1.22 ± 0.2	1.60 ± 0.2
Overall Total	1.44^{a} ± 0.1	2.13^{b} ± 0.1

[a,b] Means between columns lacking common superscripts differ (P<0.001)

Table 4. Mean (± SE) plasma leptin concentrations (ng/ml) for stallions vs. geldings by day.

Day	Stallions	Geldings
-2	1.98 ± 0.3	1.53 + 0.3
-1	1.73 ± 0.3	1.49 + 0.4
0	2.00 ± 0.4	1.52 + 0.3
1	2.44 ± 0.5	2.76 + 0.7
2	2.10 ± 0.3	2.25 + 0.5
3	1.53 ± 0.2	1.50 + 0.2
4	1.50 ± 0.2	1.61 + 0.3
5	2.02 ± 0.4	1.64 + 0.2
12	1.63 ± 0.3	1.50 + 0.2
19	1.70 ± 0.3	1.69 + 0.3
26	1.66 ± 0.2	1.17 + 0.2
Overall Total	1.84 ± 0.1	1.70 + 0.1

Due to initial variability in circulating concentrations of leptin between the two BCS groups, the data were normalized by subtracting leptin concentrations for each horse from baseline values. Regression analysis indicated that leptin concentration in response to the DEX challenge was different (P<0.05) between the fat and moderate groups with the fat group being higher (Figure 1). There was no effect of period, however, there was

an effect of day (P<0.001). DEX treatment on d 0 resulted in a rise in plasma leptin concentration within 24 h for both groups (Figure 1). Though leptin concentrations began to decrease following d 1, leptin concentrations remained different (P<0.05) between the two groups with leptin concentrations for both groups returning to baseline levels by d 5 (Figure 1).

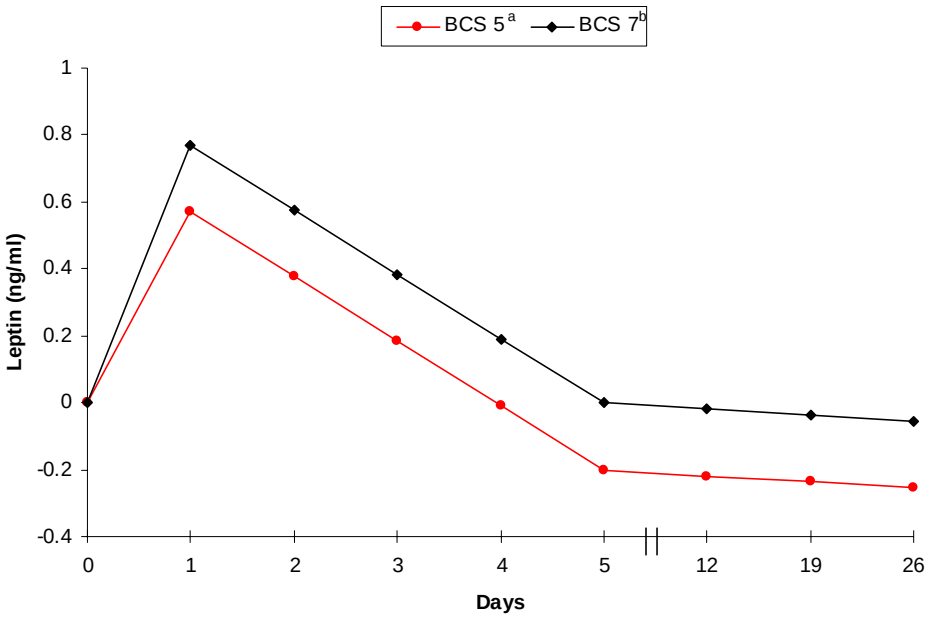

Figure 1. Regression analysis of change in plasma leptin concentrations between horses in moderate vs. fat BCS groups after DEX treatment. [a,b] Groups not sharing common superscripts differ (P<0.05)

In relation to stallions versus geldings, no significant difference was detected when comparing the change in plasma leptin concentrations. An increase in plasma leptin concentration was detected for both stallions and geldings within 24 h of DEX

treatment on d 0 (Figure 2). Leptin concentrations began to decrease after d 1 returning to baseline levels on d 5 (Figure 2).

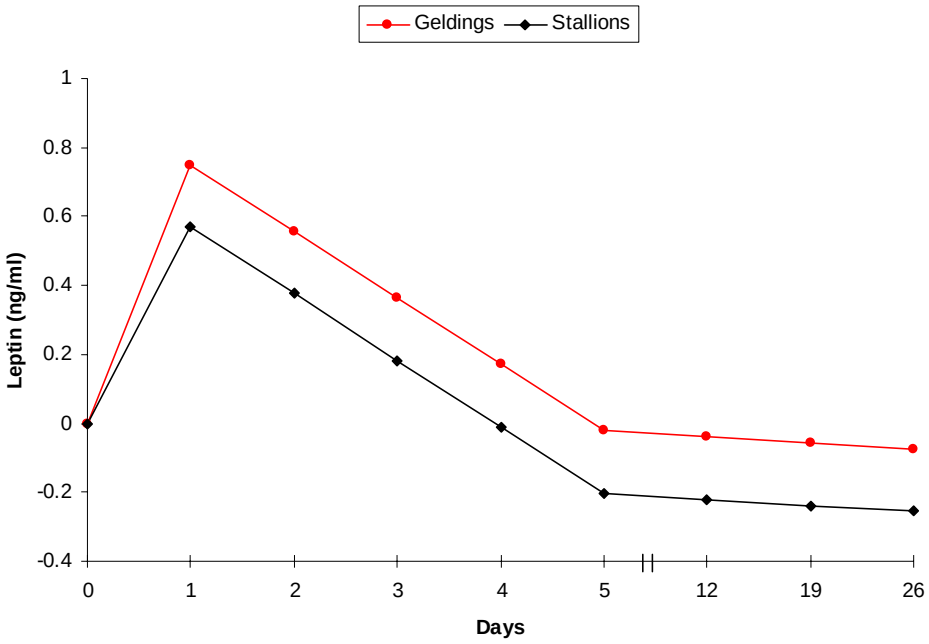

Figure 2. Regression analysis of change in plasma leptin concentrations between stallions vs. geldings after DEX treatment.

Hormone Analyses: Plasma IGF-I

Regression analysis of IGF-I revealed that IGF-I did not respond to the DEX challenge nor did it change by day. Therefore, plasma IGF-I data were analyzed by ANOVA. Mean plasma IGF-I concentration over the entire trial indicated no significant difference between BCS groups as plasma IGF-I concentration for the fat group was 347.2 $\pm$ 11.4 ng/ml versus a mean plasma leptin concentration of 344.3 $\pm$ 10.0 ng/ml for the moderate group (Table 5). Insulin-like growth factor-I concentrations were different ($P<0.05$) between geldings and stallions with the geldings having higher plasma IGF-I

concentrations. Mean plasma IGF-I was 329.1 ± 12.1 ng/ml for stallions while the geldings achieved a mean plasma IGF-I concentration of 360.5 ± 9.1 ng/ml (Table 6). No effect of period was detected.

Table 5. Mean (± SE) plasma IGF-I concentrations (ng/ml) for horses in moderate vs. fat BCS groups by day.

Day	BCS 5	BCS 7
-2	314.0 ± 34.6	353.0 ± 41.3
-1	322.8 ± 30.5	334.6 ± 33.0
0	322.7 ± 42.0	339.3 ± 45.5
1	368.7 ± 31.8	353.4 ± 40.5
2	383.8 ± 37.4	366.6 ± 42.9
3	339.7 ± 34.1	366.4 ± 37.3
4	378.7 ± 36.9	352.1 ± 36.0
5	329.0 ± 34.7	354.0 ± 45.4
12	330.9 ± 31.2	327.5 ± 32.8
19	355.1 ± 31.3	354.2 ± 39.5
26	340.2 ± 28.2	320.0 ± 40.4
Overall Total	344.3 ± 10.0	347.2 ± 11.4

Table 6. Mean (± SE) plasma IGF-I concentrations (ng/ml) for stallions vs. geldings by day.

Day	Stallions	Geldings
-2	312.5 ± 41.9	350.5 ± 34.1
-1	306.5 ± 36.0	348.1 ± 26.0
0	306.0 ± 47.0	352.6 ± 39.4
1	354.0 ± 39.7	368.2 ± 32.7
2	356.4 ± 44.9	392.0 ± 35.3
3	330.0 ± 42.5	372.4 ± 27.9
4	344.5 ± 47.0	385.5 ± 24.1
5	345.0 ± 42.8	338.1 ± 38.2
12	304.7 ± 33.7	351.4 ± 28.5
19	343.8 ± 41.5	364.4 ± 28.7
26	319.5 ± 42.8	340.6 ± 25.2
Overall Total	329.1^{a} ± 12.1	360.6^{b} ± 9.1

[a,b] Means between columns lacking common superscipts differ ($P<0.05$)

CHAPTER V

DISCUSSION

Body Condition

Both geldings and stallions were fed under controlled conditions and monitored to ensure that the proper BCS group to which each horse was assigned could possibly be achieved. Steps were also taken in designing the experiment to reduce the possibility of a period effect by grouping stallions and geldings together within a respective BCS group in a switchback design.

Body condition was evaluated by at least two objective individuals in accordance with BCS guidelines set forth by Henneke et al. (1983a). Statistical analysis of BCS revealed that horses designated for the fat (BCS 7.0) group had a mean BCS of 6.3 ± 0.2. Despite efforts to control conditions and feed intake, the horses in the fat group came in under the target BCS. This could be due to the fact that some individual horses may have required longer than the allotted 12 wk transition period to fully attain the appropriate BCS. As well, some of the horses used in this study may never have (due to physiology, breed type, etc.), been able to fully attain a BCS of 7.0 despite all efforts. Obviously, these individuals would ultimately lower the mean BCS for the fat group. It should be noted that all horses used in this study were healthy and experienced no insults to physical condition that would have made depositing fat a more arduous process. In contrast, horses assigned to the moderate (BCS 5.0) group performed well as the mean BCS for the moderate group was 5.3 ± 0.1. Despite the fact that a mean BCS of 7.0 was not achieved by the fat group, body condition analysis indicated a difference ($P<0.001$) between the two BCS groups.

In addition to BCS evaluations, weekly rump fat scans were performed with the use of ultrasonography and later converted to percent body fat using the conversion equation set forth by Westervelt et al. (1976). Studies have shown that ultrasonic rump fat measurements correlate positively to percent body fat in horses (Westervelt et al., 1976; Kane et al., 1987). Ultrasonography has also been used to positively correlate percent body fat to body condition (Henneke et al., 1983b; Gentry et al., 2004). The results of this study concur with the previous findings. Analysis of rump fat and percent body fat data revealed a difference ($P<0.05$) between BCS groups. Mean rump fat and percent body fat for the fat group were 1.6 ± 1.2 cm and $16.2 \pm 0.6\%$ respectively. The same analysis for the moderate group revealed a mean rump fat of 1.1 ± 1.5 cm and mean percent body fat of $14.0 \pm 0.7\%$. Greater rump fat measurements translate into greater percent body fat and ultimately a higher BCS. Therefore, the data served to confirm the difference seen between BCS groups.

In addition to analysis of overall BCS groups, the difference between stallions and geldings was also investigated. No sex effect was evident as there was no significant difference detected between stallions and geldings. Geldings within the fat group achieved a mean BCS of 6.4 ± 0.3 with the stallions achieving a mean BCS of 6.1 ± 0.3. For the moderate group, geldings held a mean BCS of 5.4 ± 0.1 with the stallions holding a mean BCS of 5.3 ± 0.1. Lastly, data were analyzed to determine if any period effect existed. The results showed that there was no period effect as there was no significant difference for rump fat or body fat percentage within each BCS group between the first and second periods.

Leptin

Leptin is an adipocyte hormone that serves to maintain energy homeostasis and body weight by relaying the body's nutritional state to the hypothalamus (Zhang et al., 1994; Halaas et al., 1995; Pellymounter et al., 1995). As a hormone secreted mainly by adipose tissue, it is logical to conclude that changes in nutrition and body fat can influence the levels of leptin secreted. Studies have shown that increases or reductions in feed consumption can have a positive or negative effect on leptin concentrations (Ahima et al., 1996; Dickson et al., 2002). Consequently, the overall size and volume of individual adipocytes, which indicate the body's degree of fatness, can also influence circulating leptin concentrations (Lonqvist et al., 1997). Taken together, changes in energy intake can influence body condition thereby influencing leptin secretion (Mafei et al., 1995; Considine and Caro, 1997; Friedman and Halaas, 1998).

Plasma leptin has been positively correlated to BCS in sheep (Yildiz et al., 2003) and cattle (Delevaud et al., 2000). A positive relation between body condition and leptin has also been shown to exist in the horse (Buff et al., 2002). Furthermore, a sexual dichotomy exists within the horse as circulating leptin concentrations are typically higher in stallions and geldings when compared to mares in similar BCS (Buff et al., 2002; Cartmill, 2004). In agreement with previous studies, the present study also shows a positive correlation between BCS and plasma leptin concentrations. Mean plasma leptin concentrations overall were different as mean plasma leptin was 2.13 ± 0.1 ng/ml for the fat group versus 1.44 ± 0.1 ng/ml for the moderate group ($P<0.001$). After normalizing the data to account for initial variability, the change in leptin concentrations seen in response to the DEX challenge again revealed a significant ($P<0.05$) difference between

BCS groups. Dexamethasone (DEX) has been proven to increase circulating leptin concentrations in humans (Considine et al., 1997), cattle (Houseknecht et al., 2000) and horses (Cartmill et al., 2003; Cartmill, 2004; Cartmill et al., 2005). The current data confirm these findings as a day effect was detected for circulating leptin ($P<0.001$). Both BCS groups experienced a 24 h rise in circulating leptin after treatment with DEX with horses in the fat BCS group experiencing a greater increase in circulating leptin concentrations compared to the moderate BCS group. The day effect was a direct result of the concomitant rise in leptin concentrations seen within 24 h of DEX treatment. This same rise was also seen in analysis of stallions and geldings with both groups exhibiting the same 24 h increase in circulating leptin concentrations seen with BCS group analysis.

Comparison of circulating leptin concentrations between stallions and geldings has previously been reported with geldings exhibiting higher leptin concentrations (Cartmill, 2004). Differences for leptin concentrations between geldings and stallions were also investigated within the present study with no significant difference in leptin concentrations seen between stallions and geldings within the same BCS group. These results differ from results observed by Cartmill (2004) and are in agreement with published data by Buff et al. (2002) who also saw no difference in leptin concentrations between stallions and geldings. The results for stallions and geldings noted by Buff et al. (2002) were slightly higher than the values for stallions and geldings reported in the present study. This could possibly be a result of the difference in RIA kits used between the studies. It should also be noted that in the study conducted by Cartmill (2004), stallions and geldings were not fed alike nor were they assigned to similar BCS groups. Geldings had a greater BCS of 6.6 ± 1.4 with stallions in an average BCS of 4.9 ± 1.0

(Cartmill, 2004). This would explain the contradiction between reports for stallions and geldings as the horses used in the present study were fed and maintained under controlled conditions as well as being assigned to similar BCS groups. It has also been postulated that testosterone may not play quite the antagonistic role on leptin previously thought (Blache et al., 2000). With no difference seen in leptin concentrations between stallions and geldings of similar BCS, the same conclusion could also be drawn from the current data.

IGF-I

Insulin-like growth factor-I (IGF-I) is a 7.5 kDa polypeptide that is secreted primarily from the liver and is regulated by growth hormone (GH) and nutritional state as well as other factors and hormones (Phillips et al., 1990). As with leptin, publications addressing IGF-I have reported reductions in IGF-I concentrations during feed restriction (Breir, 1999) and with subsequent increases in IGF-I after nutrient intake (Phillips et al., 1990). Insulin-like growth factor-I concentrations have also been correlated to BCS as differences in circulating IGF-I were noted between mares in high versus low BCS (Gentry et al., 2002a,b).

In the present study, no significant difference for IGF-I concentration was noted between BCS groups. Overall mean plasma IGF-I concentrations were 347.2 $\pm$ 11.4 ng/ml for the fat group versus 344.3 $\pm$ 10.0 ng/ml for the moderate group. This result could possibly be explained by the fact that even though BCS groups were deemed significantly different in the physical data, the difference may not have been large enough to effect significant differences in IGF-I concentrations.

Though no significant difference was detected for IGF-I concentrations between groups, there was a significant difference ($P<0.05$) detected for IGF-I concentrations between stallions and geldings. Geldings were found to have higher circulating IGF-I with an overall mean plasma concentration of 360.5 $\pm$ 9.1 ng/ml compared to 329.1 $\pm$ 12.1 ng/ml in stallions. These results differ from prior research conducted to investigate the difference in circulating IGF-I between stallions and geldings. Champion et al. (2002) found that stallions exhibited higher concentrations of IGF-I (307.4 $\pm$ 26.9 ng/ml) than did geldings (249.2 $\pm$ 5.3 ng/ml). The hypothesis was that a stallion's higher testosterone profile is somehow connected to the difference observed in IGF-I concentrations. With IGF-I concentrations in the current study being higher in geldings, it is possible that testosterone may not be responsible for stimulating IGF-I secretion. Since no other hormonal profiles were performed in the present study, further research is warranted to determine a specific factor responsible for the difference in IGF-I concentrations between stallions and geldings.

Unlike the results for leptin, DEX treatment did not produce any acute effects on circulating IGF-I in any of the groups, as there was no significant difference between days. Insulin-like growth factor-I has been reported to positively correlate to rising leptin levels in cattle (Houseknecht et al., 2000). Since there was no significant difference in IGF-I during the trial as was noted in leptin, it is possible that there is not a consistent relationship between circulating leptin and IGF-I.

CHAPTER VI

SUMMARY

In the present study, the analysis of stallions and geldings in either a fat (BCS 7.0) or moderate (BCS 5.0) condition suggests that plasma leptin is possibly more sensitive to smaller changes in BCS than is circulating IGF-I. Physical measurements (BCS, rump fat thickness, percent body fat) that are proven indicators of body fatness and effectors of the hormones assayed were collected and analyzed. This was done to ensure a clear understanding of the relation of hormonal profiles to the groups studied.

Leptin is an adipocyte hormone that has been firmly established as a modulator of the body's fat stores via the hypothalamic-pituitary-axis. Insulin-like growth factor-I has also been proven to relate closely with nutrition and body fatness as circulating IGF-I concentrations can be positively or negatively affected by changes in diet and body condition.

Leptin levels were shown in the present trial to correlate positively with body condition, yet no sex effect was evident as leptin levels varied little between castrate and intact males. These data serve to confirm previously published data that leptin is closely associated with BCS and changes in adiposity. Earlier studies have shown a difference in leptin profiles between castrate and intact male horses with geldings being relatively higher. Due to little variation noted between stallions and geldings in the present study, the role of testosterone as a leptin antagonist can be called into question.

Insulin-like growth factor-I profiles indicated that BCS within the range studied had little influence on circulating IGF-I. This can possibly be explained by the fact that despite a significant difference observed between BCS groups, all horses were in good

body condition (BCS $\geq$ 5). In previous studies, animals exhibiting differences in IGF-I profiles were primarily in poor versus good body condition. Had the horses in the present study been separated more widely by BCS, a difference in circulating IGF-I may have been seen. In relation to the difference between castrate and intact males, there was a significant difference detected with geldings having higher circulating IGF-I. These finding conflict with previously published data, and since no other hormonal profiles beyond leptin and IGF-I were taken, the reason for this difference cannot be concluded.

Dexamethasone has been shown to stimulate leptin secretion, and has been found in some reports to also positively effect IGF-I secretion. The present trial was found to be in agreement with previous findings as regression analysis of leptin indicated a day effect indicatory of the rise in leptin seen after DEX treatment. A similar rise was not seen in the IGF-I profile, therefore it could not be concluded from these data that DEX has any effect of circulating IGF-I. Due also to the differences in day effects seen between the two hormones presently studied a direct effect between leptin and IGF-I could not be concluded.

The data analyzed and presented in this experiment have provided a more accurate profile of leptin and IGF-I between geldings and stallions in moderate and fleshy body condition. Also, the analysis and results from this trial can be used to increase the knowledge base of equine endocrinology above that which was previously available.

LITERATURE CITED

Ahima, R. S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell, E. Maratos-Flier, and J. Flier. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382:250-252.

Ahren, B., S. Mansson, R. L. Gingerich, and P. J. Havel. 1997. Regulation of plasma leptin in mice: Influence of age, high-fat diet, and fasting. Am. J. Physiol. 273:R113-R120.

Ajuwon, K. M., J. L. Kuske, D. Ragland, O. Adeola, D. L. Hancock, D. B. Anderson, and M. E. Spurlock. 2003. The regulation of IGF-1 by leptin in the pig is tissue specific and independent of changes in growth hormone. J. Nutr. Biochem. 14:522-530.

Amstalden, M., M. R. Garcia, S. W. Williams, R. L. Stanko, S. E. Nizielski, C. D. Morrison, D. H. Keisler, and G. L. Willims. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: Relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63:127-133.

Barash, I. A., C. C. Cheung, D. S. Weigle, H. Ren, E. B. Kabigting, J. L. Kuijper, D. K. Clifton, and R. A. Steiner. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144-3147.

Barb, C. R., X. Yan, M. J. Azain, R. R. Kraeling, G. B. Rampacek, and T. G. Ramsay. 1998. Recombinant porcine leptin reduces feed intake and stimulates growth hormone secretion in swine. Domest. Anim. Endocrinol. 15(1):77-86.

Barr, V. A., D. Malide, M. J. Zarnowski, S. I. Taylor, and S. W. Cushman. 1997. Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinology 138(10):4463-4472.

Bereket, A., T. A. Wilson, S. L. Blethen, J. Fan, R. A. Frost, M. C. Gelato, and C. H. Lang. 1996. Effect of short-term fasting on free/dissociable insulin-like growth factor I concentrations in normal human serum. J. Clin. Endocrinol. Metab. 81:4379-4384.

Blache, D., R. L. Tellam, L. M. Chagas, M. A. Blackberry, P. E. Vercoe, and G. B. Martin. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. J. Endocrinol. 165:625-637.

Blum, W. F., P. Englaro, S. Hanitsch, A. Juul, N. T. Hertel, J. Muller, N. E. Skakkebaek, M. L. Birkett, A. M. Attanasio, W. Kiess, and W. Rascher. 1997. Plasma leptin levels in healthy children and adolescents: Dependence in body mass index, body fat mass, gender, pubertal stage and testosterone. J. Clin. Endocrinol. Metab. 82:2904-2910.

Bornstein, S. R., K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, and W. A. Scherbaum. 1997. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland. Diabetes 46:1235-1238.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol. 17:209-218,

Brethour, J. R. 2000. Using serial ultrasound measures to generate models of marbling and backfat thickness changes in feedlot cattle. J. Anim. Sci. 78:2055-2061.

Buff, P. R., A. C. Dodds, C. D. Morrison, N. C. Whitley, E. L. McFadin, J. A. Daniel, J. Djiane, and D. H. Keisler. 2002. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. J. Anim. Sci. 80:2942-2948.

Buonomo, F. C., and C. A. Baile. 1990. Influence of nutritional deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. J. Anim. Sci. 69:755-760.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos, and P. Burn. 1995. Recombinant mouse ob protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549.

Cartmill, J. A. 2004. Leptin in horses: Influences of body condition, gender, insulin insensitivity, feeding, and dexamethasone. Ph.D. Diss., Louisiana State Univ., Baton Rouge.

Cartmill, J. A., D. L. Thompson, Jr., L. R. Gentry, H. E. Pruett, and C. A. Johnson. 2003. Effects of dexamethasone, glucose infusion, adrenocorticotropin, and propylthiouracil on plasma leptin concentrations in horses. Domest. Anim. Endocrinol. 24:1-14.

Cartmill, J. A., D. L. Thompson, W. A. Storer, J. C. Crowley, N. K. Huff, and C. A. Waller. 2005. Effect of dexamethasone, feeding time, and insulin infusion on leptin concentrations in stallions. J. Anim. Sci. 83:1875-1881.

Champion, Z. J., B. H. Breier, W. E. Ewen, T. T. Tobin, and P. J. Casey. 2002. Blood plasma concentrations of insulin-like growth factor-I (IGF-I) in resting Standardbred horses. Vet. J. 163:45-50.

Chehab, F. F., M. E. Lim, and E. Lu. 1996. Corrrection of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Genet. 12:318-320.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Lerouz, J. Djiane, and F. Bocquier. 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest. Anim. Endocrinol. 21:271-295.

Coleman, D. L. 1973. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294-298.

Coleman, D. L. 1978. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14:141-148.

Considine, R. V. 2003. Endocrine regulation of leptin production. Pages 39-47 in Leptin and Reproduction. M. Henson, and D. Castracane, ed. Kluwer Academic/Plenum Publishers, New York, NY.

Considine, R. V., and J. F. Caro. 1997. Leptin and the regulation of body weight. Int. J. Biochem. Cell. Biol. 29:1255-1272.

Considine, R. V., M. R. Nyce, J. W. Kolaczynski, P. L. Zhang, J. P. Ohannesian, J. H. Moore, Jr., J. W. Fox, and J. F. Caro. 1997. Dexamethasone stimulates leptin release from human adipocytes: Unexpected inhibition by insulin. J. Cell. Biochem. 65:254-258.

Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohannesian, C. C. Marco, L. J. McKee, T. L. Bauer, and J. F. Caro. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334:292-295.

Delevaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165(2):516-526.

Dickson, S. L., L. Y. C. Tung, S. Lall, J.-O. Jansson, and A. K. Hewson. 2002. Interactions of growth hormone secretagogues with leptin-sensitive brain networks. Pages 1-14. in Brain somatic Cross-Talk and the Central Control of Metabolism. C. Kordon, I. Robinson, J. Hanoune, R. Dantzer,and Y. Christen, ed. Springer Verlag, Heidelberg, Germany.

Domecq, J. J., A. L. Skidmore, J. W. Lloyd, and J. B. Kaneene. 1995. Validation of body condition scores with ultrasound measurements of subcutaneous fat of dairy cows. J. Dairy Sci. 78:2308-2313.

Doyon, C., G. Drouin, V. L. Trudeau, and T. W. Moon. 2001. Molecular evolution of leptin. Gen. Comp. Endocrinol. 124:188-198.

Dyer, C. J., J. M. Simmons, R. L. Matteri, and D. H. Keisler. 1997a. cDNA cloning and tissue-specific gene expression of ovine leptin, NPY-Y1 receptor and NPY-Y2 receptor. Domest. Anim. Endocrinol. 14:295-303.

Dyer, C. J., J. M. Simmons, R. L. Matteri, and D. H. Keisler. 1997b. Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed restricted ewes. Domest. Anim. Endocrinol. 14:119-128.

Elimam, A., U. Knutsson, M. Bronnegard, P. Stierna, K. Albertsson-Wikland, and C. Marcus. 1998. Variations in glucocorticoid levels within the physiological range affect plasma leptin levels. Eur. J. Endocrinol. 139:615-620.

Emilsson, V., Y-L. Liu, M. A. Cawthorne, N. M. Morton, and M. Davenport. 1997. Expression of the functional leptin receptor mRNA n pancreatic islets and direct inhibitory action of leptin on insulin secretion. Diabetes 46:313-316.

Faulkner, D. B., D. F. Parrett, F. K. McKeith, and L. L. Berger. 1990. Prediction of fat cover and carcass composition from live and carcass measurements. J. Anim. Sci. 68:604-610.

Friedman, J. M., and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763-770.

Garcia-Mayor, R. V., M. A. Andrade, M. Rios, M. Lage, C. Dieguez, and F. F. Casanueva. 1997. Serum leptin levels in normal children: Relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J. Clin. Endocrinol. Metab. 82:2849-2855.

Gentry, L. R., D. L. Thompson, Jr., G. T. Gentry, Jr., K. A. Davis, and R. A. Godke. 2002a. High versus low body condition in mares: Interaction with responses to somatotropin, GnRH analog, and dexamethasone. J. Anim. Sci. 80:3277-3285.

Gentry, L. R., D. L. Thompson, Jr., G. T. Gentry, Jr., K. A. Davis, R. A. Godke, and J. A. Catmill. 2002b. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. J. Anim. Sci. 80:2695-2703.

Gentry, L. R., D. L. Thompson, G. T. Gentry, Jr., R. P. Del Vecchio, K. A. Davis, and P. M. Del Vecchio. 2004. The Relationship between body condition score and ultrasonic fat measurements in mares of high versus low body condition. J. Equine Vet. Sci. 24(5):198-203.

Gomez, J. M., F. J. Maravall, N. Gomez, M. A. Navarro, R. Casamitjana, and J. Soler. 2003. Interactions between serum leptin, the insulin-like growth factor system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin. Endocrinol. 58:213-219.

Hahn, T. M., J. F. Breininger, D. G. Baskin, and M. W. Schwartz. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Neurosci. 1:271-272.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lalone, S. K. Burley, and J. M. Friedman. 1995. Weight reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546.

Hamilton, B. S., D. Paglia, A. Y. M. Kwan, and M. Deitel. 1995. Increased obese mRNA expression in omental fat cells from massively obese humans. Nat. Med. 1:953-956.

Hardie, L. J., N. Guilhot, and P. Trayhurn. 1996. Regulation of leptin production in cultured mature white adipocytes. Horm. Metab. Res. 28:685-689.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983a. A scoring system for comparing body condition in horses. Equine Vet. J. 16:371.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983b. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15(4):371-372.

Henson, M. C., V. D. Castracane, and D. E. Edwards. 2003. Leptin: From satiety signal to reproductive regulator. Pages 3-11 in Leptin and Reproduction. M. Henson, and D. Castracane, ed. Kluwer Academic/Plenum Publishers, New York, NY.

Herd, D. B., and L. R. Sprott. 1985. Body condition, nutrition and reproduction of beef cows. Texas Agric. Ext. Bull. No. 1526, College Station, TX.

Hetherington, A. W., and S. W. Ranson. 1940. Hypothalamic lesions and adiposity in the rat. Anat. Res. 78:149-172.

Hickey, M. S., R. G. Israel, S. N. Gardiner, R. V. Considine, M. R. McCammon, G. L. Tyndall, J. A. Houmard, R. H. Marks, and J. F. Caro. 1996. Gender differences in serum leptin levels in humans. Biochem. Mol. Med. 59(1):1-6.

Houseknecht, K. L., C. P. Portocarrero, S. Ji, R. Lemanger, and M. E. Spurlock. 2000. Growth hormone regulates leptin gene expression in bovine adipose tissue: Correlation with adipose IGF-I expression. J. Endocrinol. 164:51-57.

Ingalls, A. M., M. M. Dickie, and G. D. Snell. 1950. Obese, a new mutation in the house mouse. J. Hered. 41:317-318.

Isozaki, O., T. Tsushima, M. Miyakawa, H. Demura, and H. Deki. 1999. Interaction between leptin and growth hormone (GH)/IGF-I axis. Endocrine J. 46(Suppl):S17-S24.

Janssen, J. M., N. A. Huizenga, R. P. Stolk, D. E. Grobbee, H. A. Pols, F. H. deJong, A. M. Attanasio, W. F. Blum, and S. W. Lamberts. 1998. The acute effect of dexamethasone on plasma leptin concentrations and the relationships between fasting leptin, the IGF-I/IGFBP system, dehydroepiandrosterone, androstenedione and testosterone in an elderly population. Clin. Endocrinol. 48(5):621-626.

Jones, J. I., and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: Biological actions. Endocrine Rev. 16:1-34.

Kane, R. A., M. Fischer, D. Parrett, and L. M. Lawrence. 1987. Estimating fatness in horses. Page 127 in Proc. 10th Equine Nutr. Phys. Symp., Ft. Collins, CO.

Lane, P. W., and M. M. Dickie. 1954. Fertile obese male mice. Relative sterility in obese males corrected by dietary restriction. J. Hered. 45:56-58.

Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee, and J. M. Friedman. 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632-635.

Lents, C. A., R. P. Wettemann, F. J. White, I. Rubio, N. H. Ciccioli, L. J. Spicer, D. H. Keisler, and M. E. Payton. 2005. Influence of nutrient intake and body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. J. Anim. Sci. 83:586-596.

Leon, H. V., J. Hernandez-Ceron, D. H. Keisler, and C. G. Gutierrez. 2004. Plasma concentrations of leptin, insulin-like growth factor-I, and insulin in relation to changes in body condition score in heifers. J. Anim. Sci. 82:445-451.

Lonqvist, F., L. Nordfors, M. Jansson, A. Thorne, M. Schalling, and P. Arner. 1997. Leptin secretion from adipose tissue of women. Relationship to plasma levels and gene expression. J. Clin. Invest. 99:2398-2404.

Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P. A. Kern, and J. M. Friedman. 1995. Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1:1155-1161.

Mantzoros, C. S., J. S. Flier, and A. D. Rogol. 1997. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J. Clin. Endocrinol. Metab. 82(4):1066-1070.

McCann, S. M., S. Karanath, W. H. Yu, and C. A. Mastonardi. 2003. Neuroendocrine regulation of leptin secretion and its role in nitric-oxide secretion. Pages 15-37 in Leptin and Reproduction. M. Henson, and D. Castracane, ed. Kluwer Academic/Plenum Publishers, New York, NY.

McManus, C. J., and B. P. Fitzgerald. 2000. Effects of a single day of feed restriction on changes in serum leptin, gonadotropins, prolactin, and metabolites in aged and young mares. Dom. Anim. Endocrinol. 19:1-13.

Mercer, J. G., N. Hoggard, L. M. Williams, C. B. Lawrence, L. T. Hannah, and P. Trayhurn. 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in muse hypothalamus and adjacent brain regions by in situ hybridization. F. E. B. S. Lett. 387(2-3):113-116.

Mounzih, K., R. Lu, and F. F. Chehab. 1997. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 138(3):1190-1193.

Mueller, W. M., F. M. Gregoire, K. L. Stanhope, C. V. Mobbs, T. M. Mizuno, C. H. Warden, J. S. Stern, and P. J. Havel. 1998. Evidence that glucose metabolism regulated leptin secretion from cultured rat adipocytes. Endocrinology 139(2):551-558.

Musey, V. C., S. Goldstein, P. K. Farmer, P. B. Moore, and L. S. Phillips. 1993. Differential regulation of IGF-1 and IGF-binding protein-1 by dietary composition in humans. Am. J. Med. Sci. 305:131-138.

Neuenschwander, S., G. Rettenberger, E. Meijerink, H. Jorg, and G. Stranzinger. 1996. Partial characterization of porcine obesity gene (OBS) and its localization to chromosome 18 by somatic cell hybrids. Anim. Genet. 27:275-278.

Nutrition Research Council. 1989. Nutrient Requirements of Horses. 5th rev. ed. National Academy Press, Washington, DC.

Pellymounter, M. A., M. J. Cullen, M. B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-543.

Perkins, T. L., R. D. Green, and K. E. Hamlin. 1992. Evaluation of ultrasonic estimates of carcass fat thickness and longissimus muscle area in beef cattle. J. Anim. Sci. 70:1002-1010.

Pfister-Genskow, M. A., H. Hayes, A. Eggen, and M. D. Bishop. 1996. Chromosomal localization of the bovine obese (OB) gene. Mamm. Genome 7:398-399.

Phillips, L. S., and R. Vassilopoulou-Sellin. 1980a. Somatomedins . N. Engl. J. Med. 302:371-380.

Phillips, L. S., and R. Vassilopoulou-Sellin. 1980b. Somatomedins . N. Engl. J. Med. 302:438-446.

Phillips, L. S., J. B. Harp, S. Goldstein, J. Klein, and C. I. Pao. 1990. Regulation and action of insulin-like growth factors at the cellular level. Proc. Nutr. Soc. 49:451-458.

Piniero, V., X. Casabiell, R. Peino, M. Lage, J. P. Camina, C. Menendez, J. Baltar, C. Dieguez, and F. F. Casnueva. 1999. Dihydrotestosterone, stanozolol, androstenedione, and dehydroepiandrosterone sulphate inhibit leptin secretion in female but not in male samples of omental adipose tissue in vitro: Lack of effect of testosterone. J. Endocrinol. 140:468-473.

Richards, M. W., R. P. Wettemann, L. J. Spicer, and G. L. Morgan. 1991. Nutritional anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biol. Reprod. 44:961-966.

Rinderknecht, E., and R. E. Humbel. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J. Biol. Chem. 253:2769-2776.

Rosenbaum, M., and R. L. Leibel. 1999. Role of gonadal steroids in the sexual dimorphisms in body composition and circulating concentrations of leptin. J. Clin. Endocrinol. Metab. 84:1784-1789.

Steiner, J., N. LaPaglia, L. Kirsteins, M. Emanuele, and N. Emanuele. 2003. The response of the hypothalami-pituitary-gonadal axis to fasting is modulated by leptin. Endocrine Res. 29(2):107-117.

Sticker, L. S., D. L. Thompson, Jr., J. M. Fernandez, L. D. Bunting, and C. L. Depew. 1995. Dietary protein and (or) energy restriction in mares: Plasma growth hormone, IGF-I, prolactin, cortisol, and thyroid hormone responses to feeding, glucose, and epinephrine. J. Anim. Sci. 73:1424-1432.

Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, and J. Deeds. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263-1271.

Thissen, J. P., J. M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocrinol. Rev. 15:80-101.

Thomas, G. B., J. E. Mercer, T. Kaaralis, A. Rao, J. T. Cummins, and L. J. Clarke. 1990. Effect of restricted feeding on the concentrations of growth hormone (GH), gonadotropins, and prolactin (PRL) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotropin subunits, and PRL in the pituitary glands of adult ovariectomized ewes. Endocrinology 126:1361-1367.

Westervelt, R. G., J. R. Stouffer, H. F. Hintz, and H. F. Schryver. 1976. Estimating fatness in horses and ponies. J. Anim. Sci. 43(4):781-785.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Williams, G. L., M. Amstalden, M. R. Garcia, R. L. Stanko, S. E. Nizialski, C. D. Morrison, and D. H. Keisler. 2002. Leptin and its role in the central regulation of reproduction in cattle. Domest. Anim. Endocrinol. 23:339-349.

Yambayamba, E. S., M. A. Price, and G. R. Foxcraft, 1996. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. J. Anim. Sci. 74:57-69.

Yildiz, S., D. Blache, F. Celebi, I. Kaya, M. Cenesiz, and B. Guven. 2003. Effects of short term high carbohydrate or fat intakes on leptin, growth hormone, and luteinizing hormone secretions in prepubertal fat-tailed Tuj lambs. Reprod. Dom. Anim. 38:182-186.

Zhang, S., D. Blache, M. A. Blackberry, and G. B. Martin. 2004. Dynamics of the response in secretion of luteinising hormone, leptin and insulin following an acute increase in nutrition in mature male sheep. Reprod. Fertil. Dev. 16:823-829.

Zhang, Y., R. Proenca, M. Maffei, M. Barrone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432.

APPENDIX

Appendix Table 1A. ANOVA table for body condition score.

Source	DF	Partial SS	Mean Square	F Value	Pr>F
Model	3	4.49147493	1.49715831	6.31	0.0055
Period	1	.238235446	.238235446	1.00	0.3322
Group	1	4.2024719	4.2024719	17.72	0.0008
Sex	1	.062235175	.062235175	0.26	0.6160
Residual	15	3.55799928	.237199952		
Total	18	8.04947421	.447193012		

Appendix Table 2A. ANOVA table for rump fat thickness.

Source	DF	Partial SS	Mean Square	F Value	Pr>F
Model	3	201.544079	67.1813596	4.40	0.0207
Period	1	56.4183824	56.4183824	3.69	0.0738
Group	1	111.265441	111.265441	7.29	0.0165
Sex	1	30.3007353	30.3007353	1.98	0.1794
Residual	15	229.0875	15.2725		
Total	18	430.631579	23.9239766		

Appendix Table 3A. ANOVA table for body fat percentage.

Source	DF	Partial SS	Mean Square	F Value	Pr>F
Model	3	44.2539734	14.7513245	4.36	0.0214
Period	1	12.2550076	12.2550076	3.62	0.0765
Group	1	24.3291303	24.3291303	7.18	0.0171
Sex	1	6.84724332	6.84724332	2.02	0.1755
Residual	15	50.8028782	3.38685854		
Total	18	95.0568515	5.2809362		

Appendix Table 4A. Normalized regression table for plasma leptin between groups.

Source	DF	Partial SS	Mean Square
Model	6	20.2664512	3.37774187
Residual	165	97.3474012	.58998425
Total	171	117.613852	.687800307

Leptin	Coef.	Std. Error	t	P> [t]
Day	-.4192323	.1460869	-2.87	0.005
Period	-.0947913	.1077513	-0.88	0.380
BCS	.1080006	.039687	2.72	0.007
Sex	-.1598771	.1077513	-1.48	0.140

Appendix Table 5A. Regression table for plasma IGF-I.

Source	DF	Partial SS	Mean Square
Model	4	68036.8558	17009.2319
Residual	202	2359110.7	11678.7658
Total	206	2427147.55	11782.2697

Leptin	Coef.	Std. Error	t	P> [t]
Day	.421547	2.366767	0.18	0.859
Period	-17.97574	15.10427	-1.19	0.235
BCS	-.1487325	7.552216	-0.02	0.984
Sex	-32.51352	15.10443	-2.15	0.033

Appendix Table 6A. ANOVA table for plasma IGF-I.

Source	DF	Partial SS	Mean Square	F Value	Pr>F
Model	13	120845.186	9295.78353	0.78	0.6829
Day	10	53178.8212	5317.88212	0.45	0.9225
Period	1	15987.1448	15987.1448	1.34	0.2488
BCS	1	.395669018	.395669018	0.00	0.9954
Sex	1	52807.5162	52807.5162	4.42	0.0368
Residual	193	2306302.37	11949.7532		
Total	206	2427147.55	11782.2697		

Appendix Table 7A. Description of project horses.

Horse	Age	Sex	Breed Type
Ed	21	Stallion	Quarter Horse
Special	14	Stallion	Quarter Horse
Peppy	6	Stallion	Quarter Horse
Menace	6	Stallion	Thoroughbred
Dundee	10	Stallion	Quarter Horse
Ben	10	Gelding	Quarter Horse
Jerry	14	Gelding	Paint Horse
Oliver	15	Gelding	Thoroughbred
Roman	10	Gelding	Appaloosa
Zippy	10	Gelding	Quarter Horse

VITA

Tommy Neal Chancellor was born February 1974 in Chickasha, OK and would eventually leave in 1983 and follow his family throughout the southern part of Texas, graduating from Kingwood High School in May of 1992. He would go on to attend Texas A&M University at College Station, Texas in January of 1994 after briefly attending junior college. He was awarded a Bachelor of Science in animal science in August of 1997.

After college he married Dana Lynn Stephens of Comanche, TX in June of 1998 and was employed as stable manager at B-7 Stables & Polo in March of 1999. In the spring of 2003 he eventually decided to re-enter college at the graduate level under the tutelage of Dr. Martha Vogelsang.

Although not hired as a research assistant, he would assist with various projects and workshops within the equine section. He also maintained full-time employment and helped his wife in raising their two sons while pursuing his graduate degree. Following completion of his Master of Science degree in animal science (December 2006), Tommy will begin pursuing a career either in equine assisted reproductive technologies or pharmaceutical sales with animal health divisions.

Tommy Neal Chancellor and his family currently reside at 1108 Chesapeake Ln., College Station, TX. 77845.