HIGH-GLYCEMIC MEAL RESPONSES AFFECT FUNDAMENTAL COMPONENTS OF METABOLIC REGULATION IN GROWING QUARTER HORSE WEANLINGS

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by
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ABSTRACT

The primary objective of this study was to investigate relationships between fundamental components of the somatotropic axis and concentrate meal responses in young, growing horses. Twelve Quarter Horse weanlings were utilized in a crossover design consisting of two 21-day periods, where treatments consisted of a twice-daily feeding of a high-glycemic (HG) or low-glycemic (LG) concentrate meal. On the final day of each period, blood samples were drawn every 15 minutes for a 24-hour period and analyzed for plasma glucose, insulin, non-esterified fatty acids and growth hormone concentrations. Growth hormone secretory patterns were estimated utilizing deconvolution analysis by AutoDecon software. Glucose and insulin peak concentrations and AUC were greater in HG than LG ($P<0.05$) in response to AM and PM meals. Growth hormone secretion following meals was inhibited for a longer time in HG horses as compared to LG horses, with a greater amount of GH secreted following this inhibition in HG. Nocturnal growth hormone secretion was greater in HG horses than LG. Elevated insulin responses to the two daily meals may potentially alter growth hormone secretion from the pituitary. This study provides insight into the mechanisms by which growth hormone may be altered by feeding strategies in the growing horse. Ultimately, this may impact processes of growth and metabolic regulation.

Key words: deconvolution analysis, growth hormone, horse, meal feeding, nutrition
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CHAPTER 1

Review of the Literature

Introduction and Rationale

Nutritional management of the young horse has the potential to affect its value as a performance animal. More specifically, a strong foundation in terms of skeletal and muscular development may aid in improving performance and preventing lameness during the horse’s career. Additionally, the animal’s ability to sense and process nutrients is of growing importance in the equine industry, as nutrition-associated metabolic pathologies such as obesity and insulin resistance manifest.

The growing animal is actively partitioning nutrients to facilitate skeletal growth and it follows that nutritional management at this life stage could prove influential for the horse’s skeletal foundation and integrity throughout its life. Concordantly, this partitioning of nutrients incorporates a host of metabolic processes to digest nutrients from feed and allocate them to various parts of the body. The somatotropic axis is a chemical communication network that orchestrates metabolic and physiologic processes, providing a connection between nutritional management of the young horse and potential mechanisms for altering growth and metabolic regulation. Complex interplay between components of the somatotropic axis preclude any single constituent from governing the system; however, growth hormone’s actions throughout the body and its relationships
with nutrient processing are of key interest for investigating mechanisms between feeding practices and optimizing growth of the young horse.

The horse is a hindgut fermenter that evolved to survive by grazing throughout the day on a forage-based diet. In the foregut of the horse, prececal digestion facilitates the uptake of glucose from starches and sugars in the diet. The insoluble fiber (hemicelluloses, cellulose, lingo-cellulose), resistant starches, soluble fiber (gums, mucilages, pectins) components of the horse’s diet are fermented in the cecum and colon of the hindgut to provide volatile fatty acids as an energy source (Hoffman et al., 2001). To acquire sufficient energy on a diet composed primarily of forage, the feral horse spends approximately 14-18 hours per day eating grass. With the domestication of the horse for work and recreation, feeding practices have incorporated more energy-dense feedstuffs to address the increased energy needs of the working horse. In addition, the attrition of available grazing spaces for horses has required humans to feed horses in a manner that is often counter to a diet based solely on forage.

Common management of the domesticated horse often includes feeding of 2-3 meals of concentrated dietary energy in addition to providing hay and/or allowing access to pasture for grazing. Providing a concentrate energy source, or grain, is often a convenient method for equine caretakers to provide a consistent source of energy to the horse. This concentrate energy is typically composed of one or more cereal grains (corn, wheat, oats, barley, etc.) and is more energy dense than forage sources.

In discussing dietary energy in the horse, it is important to note the source of energy as well as how it is digested by the equine gastrointestinal tract. Chemical analysis of carbohydrates fractions according to equine physiology can be grouped into three main
groups: hydrolyzable carbohydrates, rapidly-fermentable carbohydrates, and slowly-fermentable carbohydrates (Hoffman et al., 2001). Hydrolyzable carbohydrates are digested in the foregut and consist of starches, some oligosaccharides, disaccharides, and hexoses, all of which provide energy in the form of rapidly-available glucose. Rapidly-fermentable carbohydrates are digested in the hindgut include starches that are resistant to foregut digestion, galacto-oligosaccharides, fructo-oligosaccharides, and soluble fiber, which provide energy through lactate and prioprionate. Slowly-fermentable carbohydrates are also digested in the hindgut and include hemicelluloses, cellulose, and lingo-cellulose, which provide energy through acetate and butyrate (Hoffman et al., 2001). Non-structural carbohydrates (NSC), as typically assessed in the ruminant, include hydrolyzable and rapidly-fermentable carbohydrates. However, due to the anatomy of the horse and the feedstuffs typically provided to the horse, this carbohydrate fraction may not be entirely appropriate. In cereal grains, NSC content is equivalent to hydrolyzable carbohydrate content, while hydrolyzable carbohydrate content in forages accounts for roughly 1/3 of the NSC content (Hoffman et al., 2001). In discussing NSC content in cereal grains, this may be equated as the sum of starch and water-soluble carbohydrates; while for forages, NSC content may be calculated by difference, incorporating water, protein, fat, ash, and neutral detergent fiber. In this review, NSC refers to the sum of water soluble carbohydrates and starch.

The NSC content of concentrate meals is important to consider as it elicits physiological meal responses in circulating hormones and metabolites and may impact the overall health of the horse’s gastrointestinal tract. In the event that the concentrate meal provides more NSC than the foregut can process, excess NSC may avoid digestion
in the foregut, causing them to enter the cecum, where they are rapidly fermented and may cause adverse changes in the bacterial population (Julliand et al., 2006). To avoid hindgut fermentation of NSC, concentrate meals consisting of less than 2 grams of starch per kilogram bodyweight are recommended (Julliand et al., 2006). In addition to the effects on the hindgut, greater amount of NSC in the diet and meal feeding are counter to the anatomical evolutionary adaptations of the horse’s gastrointestinal tract and may disrupt physiological processes (Clarke et al., 1990).

Diets based on concentrate meals high in non-structural carbohydrates have been associated with decreased insulin sensitivity in adult horses, which may contribute to the occurrence of other metabolic disturbances such as metabolic syndrome and laminitis (Hoffman et al., 2003). Decreased insulin sensitivity and the term “insulin resistance” are relative terms that indicate an impaired ability for insulin to facilitate glucose uptake into tissues. In addition, insulin resistance is a component of metabolic diseases such as equine metabolic syndrome and obesity. Insulin is a component of the somatotropic axis, and thus intertwines feeding with growth and metabolic regulation. The processes of nutrient partitioning, growth, and other metabolic actions are concordantly impacted by feeding management of the equid.

Feeding and managing the equid relies on the assumption that the horse can appropriately facilitate nutrient uptake and metabolic regulation. Alterations in nutrient processing (e.g. insulin resistance) may manifest in a variety of pathologies that affect the horse’s ability to work and perform. In the young animal, alterations in nutrient partitioning may alter growth. Increased availability of energy from non-structural carbohydrates may promote rapid skeletal growth; however, skeletal integrity may be
compromised. As a result, conditions such as developmental orthopedic disease may occur, compromising the horse’s ability to perform and work. It is the goal of equine caretakers to provide appropriate dietary energy and nutrients to promote ideal development of the growing horse. This goal includes development of a skeletal structure that is supportive of the athletic demands on the horse and a metabolism that adequately processes and partitions nutrients. By understanding the relationships between growth hormone and the meal feeding of concentrates, mechanisms of growth and metabolism may be elucidated and provide knowledge to optimize growth and avoid detrimental health conditions.

This review will discuss the known regulation of growth hormone secretion, with an emphasis on hormones and metabolites that intertwine with the meal response in the horse.

**Characteristics of growth hormone**

**General characteristics**

Growth hormone (GH) is a fundamental component of the somatotropic axis and is critical for postnatal growth and development in mammals. Growth hormone is secreted from the anterior pituitary in a pulsatile nature (Veldhuis et al., 1987). The regulation of growth hormone secretion is affected by a wide array of factors and a simplified schematic of this regulation is depicted in Figure 1-1. Additionally, factors such as age, sex, body composition, sleep, and nutritional status affect characteristics of
GH secretion (Jaffe et al., 1998; Takahashi et al., 1968; Veldhuis et al., 1991; Zadik et al., 1985).

After release from the anterior pituitary, GH acts directly on tissues and indirectly through its promotion of insulin-like growth factor I (IGF-I) secretion from the liver. The key function of GH and IGF-I is to promote bone growth. Insulin-like growth factor I acts to bolster the development of extracellular matrix proteins in cartilage and bone, while both GH and IGF-I promote osteoblast proliferation (Tauchmanova et al., 2007). Growth hormone influences the regulation of metabolic processes, such as protein, fat, carbohydrate, and bone metabolism. While processes of growth and metabolism are inherently complex, GH functions as a key regulator of these systems. Work regarding the regulation of growth hormone in the equid is limited compared to research in the human or in other animal models, yet there has been exploration into mechanisms that affect GH.

**Growth hormone in the horse**

Somatotrope cells in the anterior pituitary are the primary cells responsible for synthesis and release of growth hormone, though there is evidence that growth hormone may also be localized to mammosomatotropes (Rahmanian et al., 1997). Upon secretion from the somatotrope, the half-life of equine growth hormone has been estimated at approximately 18 minutes (de Graaf-Roelfsema, 2007). This is similar to the growth hormone half-life in the human, which is widely accepted to range from 12-20 minutes.
The pulsatile secretion of growth hormone and its relatively short half-life require frequent sampling to thoroughly assess its secretory patterns.

In the horse, peak detection analysis has been the most widely used analytical method for assessing characteristics of growth hormone. The general characteristics reported in the literature are included below. Subsequent sections of this review will address nutritional influences on GH in the horse.

Sex effects on growth hormone secretion have been discussed in the equine literature. Mares have been reported to have more frequent GH concentration peaks with smaller peak amplitude than gelding, though basal concentrations did not appear different (Christensen et al., 1997b). However, this study is potentially confounded with age, by comparing aged mares (20-26 years old) with geldings with a larger range in age (7-21 years old). Since growth hormone secretion is correlated with growth and age, the decrease in mares may be an artifact of older subjects. Younger growing animals have greater peaks in growth hormone concentration as compared to older animals, with maximal values reached in puberty. In hourly sampling, mares and stallions did not differ in GH characteristics; however, small sample size, peak detection analysis, and infrequent sampling reduce the ability to detect differences (Thompson et al., 1992). Quarter-hourly sampling over a 12-hour period did not elicit a sex difference in respect to average GH concentration and number of GH concentration peaks (DePew et al., 1994). Recent work in humans detected an increase in average GH concentration and amount of GH secreted in women as compared to men, though age, body-mass index, and IGF-I concentrations also impacted growth hormone (Veldhuis et al., 2011).
The effect of stimulation of the sympathetic nervous system and exercise on GH secretion has also been reported in the horse. Stallions exhibited immediate increases in GH concentrations during thirty minutes following acute physical exercise, sexual stimulation, twitching of the nose, and epinephrine injection (Thompson et al., 1992). Overtrained horses exhibit altered nocturnal growth hormone secretion, with smaller, more frequent peaks and a greater degree of disorder in secretion patterns as compared to horses in moderate training (de Graaf-Roelfsema et al., 2009).

The bulk of growth hormone literature in the horse lacks detail regarding the pulsatility of growth hormone secretion due to the software utilized in analysis. However, deconvolution analysis has been utilized in more recent equine growth hormone research (de Graaf-Roelfsema, 2007). By mathematically modeling growth hormone secretion utilizing deconvolution analysis, investigation of growth hormone in the horse may be explored more thoroughly and precisely. Advancing the analytical methods for growth hormone research allows for further understanding into factors that may alter growth hormone secretion in the horse.

**Neuroregulation of growth hormone secretion**

Growth hormone (GH) is secreted in a pulsatile manner as a “pulse” from the anterior pituitary (Veldhuis et al., 1987). The rapid rise (“peak”) and subsequent fall (“nadir”) in GH concentration is essential for the action and effect of growth hormone in the body (Urban et al., 1988). Classically, GH secretion is regulated by hypothalamic hormones, inhibited primarily by somatostatin (STT) and promoted by growth hormone
releasing hormone (GHRH). However, recent work supports acyl ghrelin (AG) as a promoter of GH synthesis and release from the somatotrope as well (Kojima et al., 1999). Somatostatin is released from the anterior periventricular nucleus, while GHRH is secreted from the arcuate nucleus in the pituitary. Acyl ghrelin originates predominantly from the stomach and the arcuate nucleus, to a lesser extent (Veldhuis and Bowers, 2010). These promoting and inhibiting factors act to facilitate the pulsatile patterns of growth hormone secretion from somatotropic cells. The regulation of GH secretion occurs concordantly through the actions of somatostatin, growth hormone releasing hormone, and acyl ghrelin at the hypothalamus and anterior pituitary. Actions upon these regulators and subsequent alterations in GH secretion may have later influences on metabolism and growth. This section will focus on the primary regulators: growth hormone releasing hormone, somatostatin, and acyl ghrelin, depicted in Figure 1-2.

**Growth hormone releasing hormone**

Growth hormone releasing hormone (GHRH) is released from the arcuate nucleus of the hypothalamus and stimulates growth hormone synthesis and release (Frohman and Kineman, 1999). The half-life of GHRH is relatively short and is estimated at approximately 7 minutes (Dieguez et al., 1988). After secretion from the hypothalamus GHRH travels through the hypothalamo-hypophyseal portal system to act at the anterior pituitary. The growth hormone releasing hormone receptor (GHRH-R) on the anterior pituitary is a G-protein coupled receptor and upon GHRH binding promotes the conversion of adenosine triphosphate (ATP) to 3’, 5’ cyclic adenosine monophosphate
(cAMP), which activates protein kinase A (PKA). Protein kinase A then translocates to the nucleus and phosphorylates cAMP-response element-binding protein (CREB), which promotes the transcription of GH, pituitary transcription factor 1, and GHRH-R genes (Frohman and Kineman, 1999). Pituitary transcription factor 1 further promotes the transcription of GH and GHRH-R genes. Following the translation of mRNA to form the growth hormone protein, GH is packaged into secretory vesicles. In addition to stimulating the synthesis of GH, GHRH also facilitates its secretion by a depolarization of the membrane via PKA, which phosphorylates Na+ and other cation channels, rendering them inactive, and by allowing the influx of calcium to depolarize the plasma membrane (Frohman and Kineman, 1999). Membrane depolarization stimulates growth hormone secretory vesicles to transiently bind to fusion pores (porosomes) on the plasma membrane and release of GH into the blood stream. Figure 1-2 depicts the actions of GHRH at the pituitary. Growth hormone negatively feeds back to GHRH at the arcuate nucleus, but the mechanisms behind this are relatively unknown (Frohman and Kineman, 1999).

**Somatostatin**

Somatostatin (STT) originates from a variety of tissues in the body, including the gut, pancreas, adrenal, placental, thyroid, and nervous system. Somatostatin originating from the periventricular nucleus in the hypothalamus acts to inhibit the secretion of growth hormone from the somatotrope. It also acts negatively on the synthesis of GHRH, though this mechanism has not been elucidated. At the anterior pituitary, somatostatin
reduces the ability of Ca\textsuperscript{2+} to enter somatotrope cells and increases K\textsuperscript{+} channels, preventing membrane depolarization and the subsequent release of GH from the somatotrope (Tannenbaum and Epelbaum, 1999).

Somatostatin originating from the hypothalamus is inhibited by glucose and stimulated by insulin (Patel, 1999). Insulin-like growth factor I may potentially promote STT release at the hypothalamic level, but the findings to this are equivocal (Tannenbaum and Epelbaum, 1999). mRNA for the somatostatin receptor has been demonstrated to decrease in metabolic states with low insulin concentration (starvation, type I diabetes mellitus) (Patel, 1999). Acyl ghrelin may negate the actions of STT (van der Lely et al., 2004; Veldhuis and Bowers, 2010).

**Acyl ghrelin**

Ghrelin is a peptide hormone that has been extensively investigated as a link between energy status and metabolic regulation, especially concerning the somatotropic axis (van der Lely et al., 2004). Biologically active ghrelin (n-octanoylated, acyl ghrelin, acylated ghrelin, AG) is an orexigenic, growth hormone secretagogue that is released primarily from endocrine X/A cells in the fundus of the stomach (Cummings et al., 2004; Date et al., 2000; Kojima et al., 1999). Desacyl ghrelin does not exhibit similar orexigenic effects or act as a GH secretagogue. In the equine stomach, ghrelin is abundant in the oxyntic glands, located in the corpus of the stomach (Hayashida et al., 2001). While ghrelin has been detected in other tissues, the stomach unequivocally is the primary source of circulating acyl ghrelin (van der Lely et al., 2004). Acylation of ghrelin
occurs in the stomach, pancreatic, and intestinal tissues with the addition of an octanoate group to the serine-3 of ghrelin by ghrelin O-acyltransferase (GOAT) (Gutierrez et al., 2008; Yang et al., 2008). The ghrelin receptor (GHS-R) has been identified in numerous tissues, but of primary interest for GH secretion is its presence at the hypothalamus and anterior pituitary (van der Lely et al., 2004).

The mechanism by which AG is able to cross the blood brain barrier and bind to receptors in the arcuate nucleus is unknown, though there are several theories that are beyond the scope of this review (Fry and Ferguson, 2010). In the hypothalamus, GHS-R is located on the arcuate nucleus and may stimulate GHRH neurons by increasing the rate but not pattern, of GHRH secretion (Osterstock et al., 2010). The pituitary also expresses GHS-R and the ghrelin binding at this location has been shown to release GH (Cruz and Smith, 2008; Kineman and Luque, 2007). In addition to amplifying GHRH-induced secretion, ghrelin may also have an antagonistic relationship with somatostatin (van der Lely et al., 2004; Veldhuis and Bowers, 2010). Though GHRH has long been thought of as the sole promoter of GH release from the pituitary, AG may induce a similar magnitude of GH secretion (Kineman and Luque, 2007). For its orexigenic effects, it is believed that GHS-R receptors on neuropeptide Y (NPY) and agouti-related peptide (ARGP) neurons (Fry and Ferguson, 2010).

Ghrelin also interacts with glucose and insulin metabolism, independent of its actions on GH (Sangiao-Alvarellos and Cordido, 2010). When infused, ghrelin often increases blood glucose and reduces insulin concentrations, potentially by suppressing insulin secretion from pancreatic beta cells (Sangiao-Alvarellos and Cordido, 2010). These observations fit well with ghrelin’s classification as an orexigenic hormone.
In humans, a rise in ghrelin concentrations prior to meal initiation is followed by a precipitous fall after eating (Cummings et al., 2004). In the horse, limited work has shown ghrelin concentrations continue to increase 20-minutes after meal initiation before decreasing (Gordon and McKeever, 2005). Ghrelin decays rapidly following blood sample collection and the assay utilized by Gordon and McKeever may have limited their ability to detect differences (Liu et al., 2008). In addition to meal feeding's regulation of AG, it has been reported in humans that postprandial ghrelin concentration is reflective of the meal’s macronutrient composition, with glucose and lipid doses causing significant decreases in postprandial ghrelin and protein causing no change (Greenman et al., 2004). In a similar study examining macronutrient composition on AG secretion, the greatest postprandial decrease was observed following a carbohydrate beverage (55% below pre-beverage baseline) as compared to a lipid or protein beverage (40% and 50% below baseline, respectively) (Foster-Schubert et al., 2008). Interestingly, the carbohydrate beverage also had the greatest postprandial increase following its nadir, rising above original baseline concentrations by greater than 30% while the lipid and protein beverages did not rise above baseline concentrations. The carbohydrate beverage elicited a peak glucose concentration of approximately 140 mg/dL and peak insulin concentration of 100 mIU/L, while the protein and lipid drinks did not display a postprandial rise in glucose. The insulin response of protein beverage was intermediate to glucose and lipid, reaching a peak of approximately 60 mIU/L, while the lipid drink reached 40 mIU/L. The carbohydrate drink reached a nadir in acyl ghrelin approximately 40 minutes after the beverage was consumed, returned to baseline approximately 200 minutes postprandial, reached a peak concentration at approximately 260 minutes, and returned to baseline at
320 minutes (Foster-Schubert et al., 2008). This “flux” in acyl ghrelin following a carbohydrate meal, in which AG rapidly drops and then rebounds, may have implications for postprandial GH secretion, though GH was not quantified in the described study.

In a study of meal feeding on ghrelin concentrations in pigs, pigs fed ad libitum had lower ghrelin concentrations than those adapted to once-daily meal feedings and failed to display any correlation between ghrelin and insulin concentrations. Meal-fed pigs had increased ghrelin concentrations prior to the meal that fell afterward and were negatively correlated to plasma insulin (Reynolds et al., 2010). It is important to note that Reynolds et al. (2010) does not report whether the ghrelin quantified was total ghrelin, desacyl ghrelin, or acyl ghrelin. Desacyl ghrelin is not able to act as a GH secretagogue and its role in the other physiological functions is debated.

The relationship between acyl ghrelin concentrations and patterns with growth hormone concentration patterns and concentrations is not clear in the literature. This may be due to differences in blood sampling frequency, assay sensitivity, or whether the experiment is measuring total ghrelin, acyl ghrelin, or desacyl ghrelin (Nass et al., 2011). In rats fed ad libitum, endogenous ghrelin secretion did not correlate to growth hormone secretion (Tolle et al., 2002). Synchronization between ghrelin secretion and increases in growth hormone concentrations were observed in meal-fed sheep (Sugino et al., 2002). In humans, there has been evidence for ghrelin-modulated growth hormone release in the fed state, with increases in AG correlating to rises in growth hormone concentration prior to a meal (Nass et al., 2008). Thus, modulation of acyl ghrelin concentrations through feeding practices may have further implications through its effects on growth hormone secretion.
The interplay between growth hormone releasing hormone, somatostatin, and acyl ghrelin at the level of the pituitary is complex. Figure 1-2 depicts the actions of these regulators at the somatotrope as well as their feedback and feedfoward mechanisms. The regulation at the pituitary is difficult to explore in vivo, given the difficulty of sampling blood from the hypothalamo-hypophyseal portal system. However, growth hormone pulses from the anterior pituitary can be estimated in vivo by quantifying growth hormone concentrations in circulation.

**Measurement and analysis of growth hormone secretion**

Frequent blood sampling intervals and advanced computer analysis are necessary to estimate GH regulatory mechanisms and address growth hormone’s pulsatile secretion, relatively short half-life, seemingly random concentration fluctuations, and high inter-individual variation in secretion. The concentration profile of GH in the plasma pool is a convolution interval, which illustrates secretory and clearance events simultaneously, as pictured in Figure 1-3 (Veldhuis et al., 1988). Investigation into secretory events requires understanding of characteristics and methods by which to separate secretion and elimination events. Computer-based algorithms objectively evaluate characteristics of secretory profiles to remove human bias through mathematically modeling secretory events.

Early work in quantification of GH profiles utilized pooled t-tests to identify discrete peaks and nadirs in growth hormone concentration. A cohort of these computer programs came to fruition in the late 1980s and early 1990s and included computer-based
algorithms, such as Pulsar, Cluster, Detect, and Ultra (Urban et al., 1988). These peak detection analyses define a “nadir” as a statistically significant concentration decrease in the series, followed by a statistically significant increase; by difference, “peaks” are the points between nadirs (Veldhuis and Johnson, 1986). Between programs, differences in the mathematical calculations and statistical evaluations contributed to discrepancies in identifying number of peaks in a given time series (Evans et al., 1992). In a simulated data set of pulsatile luteinizing hormone, Cluster (peak detection) analysis identified 15-25% fewer than AutoDecon (deconvolution) analysis, suggesting it is less accurate and precise in its detection abilities (Johnson et al., 2008). Peak detection only detects changes in concentration and does not offer insight into secretory episodes. Additionally, the peak detection methods are unable to separate secretion events from clearance, providing no insight to the characteristics of the secretory bursts from the anterior pituitary.

Deconvolution analysis solves the convolution interval, which has been defined by Veldhuis et al. as:

\[ C(t) = \int_0^t S(z)E(t-z)dz \]  

(Eq. 1)

Where \( C(t) \) is the concentration of the hormone in the serum at the given time, \( t \); \( S(z) \) is the impulse function or amount of the hormone secreted per unit distribution volume per unit time evaluated at time \( z \); and \( E(t-z) \) is the impulse-response function, the integral of which gives the amount of hormone elimination (clearance) that occurs at the time interval \( (t-z) \).

Secretion is defined as:
\[ S(z) = \sum_{i=0}^{n} A_i e^{-\frac{z}{\text{SD}}} \]

(Eq. 2)

Where \( A_i \) is the amplitude of the \( i \)th secretory burst (mass of hormone released per unit distribution volume per unit time); \( PPi \) is the \( i \)th secretory burst position in time; \( z \) is an instant in time; \( i \) is the peak number in the time series of \( n \) peaks; and \( \text{SD} \) is the standard deviation of the random (Gaussian) distribution of instantaneous molecular secretion rates comprising a pulse.

Clearance is defined as:

\[ E(t-z) = f e^{-\frac{0.693(t-z)}{HL}} \]

(Eq. 3)

For one half life component, or

\[ E(t-z) = f e^{-\frac{0.693(t-z)}{HL_1}} + (1 - f) e^{-\frac{0.693(t-z)}{HL_2}} \]

(Eq. 4)

For two half lives.

(Veldhuis et al., 1987; Veldhuis and Johnson, 1988)

Initial deconvolution approaches required a priori knowledge of number of secretory characteristics, such as temporal position, amplitude, and half-life, which incorporates a subjective factor in terms of the user-based input (Johnson et al., 2009a).

The AutoDecon program is fully automated and statistically evaluates secretory episodes utilizing three methods: “a parameter fitting module, an insertion module, and a triage module” (Johnson et al., 2008). The fitting module utilizes a weighted nonlinear least-squares parameter estimation to maximize the probability of the secretory function, Eq. 2, and the elimination function, Eq. 3, being correct. The insertion module inserts the next presumed secretion event to maximize the probable position index, \( PPi \):
models incorporate a weighting factor for the data point and a residual factor for the residual of the data point, which allows for heteroscedastic datasets (Johnson et al., 2009a). The triage module statistically evaluates the removal of a secretory event with two weight nonlinear least-squares parameter estimations, with and without the secretory event, at a user-defined probability level (Johnson et al., 2009a). Cycles of these three modules continue until further secretory events are not added and current model parameters are incorporated throughout the cycles (Johnson et al., 2009a).

The AutoDecon deconvolution program (Charlottesville, VA) offers a fully-automated method for estimating characteristics regarding secretory episodes. This program assumes the following: a secretion pattern composed of a series of Gaussian curves with an additive constant, positive secretory amplitudes, and equal standard deviation of the Gaussian secretion events, measurements of uncertainty that are variable for each data point (Johnson, 2011). AutoDecon does not make assumptions regarding the temporal position or amplitude of secretory events (Johnson, 2011). The ability of the AutoDecon deconvolution program to estimate secretory episodes accurately and precisely is impacted by the quality of the data set provided to the program. AutoDecon takes into account the precision of the hormone assay, in regard to minimal detectable concentration and the variation at this low concentration in its estimation of secretory pulses (Johnson et al., 2009a). Increased sampling frequency improves the ability of the
software to detect pulsatile events (Evans et al., 1987; Hartman et al., 1991). Sampling at a frequency of four samples per half-life is recommended for pulse detection (Johnson, 2011). Frequent sampling (as recommended) and a sensitive hormone assay are ideal for estimating pulsatile secretion; less frequent sampling and less sensitivity may reduce the number of pulses detected. The data set should reflect the goals of the experiment. For example, if large secretory pulses are of interest, less frequent sampling may be employed upon the understanding that small pulses may not be detected.

The utilization of deconvolution analysis to estimate secretory episodes allows for further prediction as to the mechanisms regulating secretion. Estimation of secretory GH pulses allows for further speculation into the regulatory actions at the pituitary. Deconvolution analysis of growth hormone has had limited use in the equid and employment of this modeling procedure may elucidate governing mechanisms that are relatively unexplored in the horse, especially concerning meal feeding.

**Physiologic responses to meal feeding**

The interplay between incoming energy and nutrients from feeding and metabolic processes under the regulation of the somatotropic axis is inherently complex. A host of hormones are constituents of the somatotropic axis, which are beyond the scope of this review. Key metabolites and hormones critical to our concerns in the horse fed concentrate meals include glucose, insulin, non-esterified fatty acids (NEFA), and growth hormone. While work in the horse integrating all of these constituents is somewhat limited, extensive studies in the human have been conducted. Compromised health states
in the human, such as type 1 and type 2 diabetes, acromegaly, growth hormone
deficiency, metabolic syndrome, and obesity, have been examined and compared to
healthy individuals in an attempt to understand the mechanisms and characteristics of
these relationships. In addition, work in vitro has elucidated potential mechanisms by
which meal feeding modulates GH secretion. The following section will investigate key
hormones and metabolites in the horse fed concentrate meals and how these meal
responses may impact the somatotropic axis.

**Glucose**

Glucose is a primary source of fuel in the body and is converted to other
carbohydrates, such as glycogen and ribose, for additional metabolic functions.
Regulation of plasma glucose concentration is primarily controlled by the pancreatic
hormones insulin and glucagon, though somatostatin, growth hormone, cortisol, and
catecholamines have also been demonstrated to affect plasma glucose (Rizza et al.,
1979). In the horse, acquisition of glucose occurs primarily through starch digestion in
the foregut (prececal). Elevated glucose concentrations cause the release of insulin from
pancreatic beta cells to facilitate glucose uptake into tissues for energy storage and
utilization.

The reference range for normal fasting blood glucose concentration in horses is
76-127 mg/dL (Colahan et al., 1999). This wide range in glucose concentrations may be
employed due to breed and age differences. In addition, in clinical veterinary practice, it
may be difficult to acquire a genuine “fasting” blood glucose concentration due to
variation in feeding practices and timing of blood sampling. Breed differences in fasting glucose have been reported between Dutch warmblood horses and Shetland ponies at 97.2 mg/dL and 68.4 mg/dL, respectively (Rijnen and van der Kolk, 2003). Variation between ponies and larger horses is consistent with previous data reporting higher fasting glucose concentrations in Thoroughbreds and Morgans than in Shetland ponies (78.7 and 79.8 mg/dL versus 55.5 mg/dL) (Robie et al., 1975). Considerably greater basal glucose concentrations in young horses provide evidence for an ontogenic decrease as the horse approaches maturity (George et al., 2009; Holdstock et al., 2004; Stull and Rodiek, 1988). A decrease in baseline glucose concentrations in foals from 5 days of age to 160 days of age occurred concordantly with a decrease in insulin sensitivity. At 5 days, baseline glucose concentrations averaged between 150-175 mg/dL, while they ranged between 135-140 mg/dL at 40 days, 120-135 mg/dL at 80 and 160 days in age (George et al., 2009). Within the first 10 days of life, Holdstock et al. reported glucose concentrations between 146-190 mg/dL (2004). In 2-year old horses, fasting glucose concentrations were reported at 96.4 mg/dL (Stull and Rodiek, 1988). These ontogenic changes in basal glucose concentrations may have relationships with growth hormone secretion, though this has not been explored in the horse.

Hypoglycemia, achieved through fasting or by insulin administration, has long been known as an impetus for growth hormone secretion in humans (Roth et al., 1963). In this early study, glucose infusion after fasting concomitantly increased glucose and insulin plasma concentrations and decreased growth hormone concentrations (Roth et al., 1963). Infusions of both glucose and insulin, particularly in earlier studies, tend to be superphysiological and may not reflect changes in glucose and insulin concentrations
following a meal. Low blood glucose concentrations (60-80 mg/dL) in individuals with anorexia nervosa coincide with elevated growth hormone concentrations compared to controls (Landon et al., 1966). The increases in pulsatile GH secretion in anorexic individuals include increased pulse mass, duration, and frequency (Misra and Klibanski, 2011). While GH increases, anorexics have decreased IGF-I concentrations, which is likely to impact bone metabolism (Misra and Klibanski, 2011). Decreased bone mineral density and increased bone resorption, higher fracture prevalence, and greater incidence of osteopenia and osteoporosis are observed in anorexic individuals compared to controls (Giustina et al., 2008). The connections between glucose concentrations, growth hormone secretion, and bone metabolism in anorexic individuals reinforced the importance and significance of investigating these relationships.

While hypoglycemia is a clear impetus for increasing GH secretion, the rate at which glucose concentrations reach hypoglycemia was explored as a potential causal mechanism for GH secretion. In humans, slow and rapid rates at which glucose fell to a hypoglycemic concentration (approximately 50 mg/dL) had similar responses in growth hormone secretion (Amiel et al., 1987). This precipitous fall in blood glucose, caused by withdrawing dextrose infusions during a euglycemic-hyperinsulinemic clamp test, may not be physiologically pertinent to normal eating patterns in either humans or horses. Regardless, growth hormone concentrations increased significantly in response to hypoglycemia, despite having elevated insulin concentrations of 108 mIU/L throughout the clamp (Amiel et al., 1987). While the actions of insulin will be discussed in the following section, this experiment provides support for hypoglycemia being one of the
strongest promoters of increased GH secretion, despite the potential antagonist relationship between insulin and GH (Amiel et al., 1987; Cryer, 1993).

Hyperglycemia and its effects on the somatotropic axis have been investigated in humans, primarily concerning diseases such as type 1 diabetes mellitus and acromegaly. Unregulated type 1 diabetes, where the body does not secrete endogenous insulin and thus is subject to hyperglycemia, has been associated with increase GH secretion. Prolonged hyperglycemia in type 1 diabetes individuals did not elicit a decrease in growth hormone concentrations and therefore, hyperglycemia may not be critical in reducing growth hormone (Fowelin et al., 1993). This highlights a potential relationship between insulin and growth hormone secretion, which will be further discussed in the following section. Acromegaly, a disorder where excess GH is secreted from the pituitary, has been associated with hyperglycemia. The inherent metabolic dysregulation in both type 1 diabetes and acromegaly makes it difficult to determine causal relationships between glucose, insulin, and growth hormone actions. However, knowledge garnered from these diseases may provide insight to potential regulatory mechanisms in the healthy animal.

**Insulin**

Insulin regulates increases in blood glucose by facilitating the uptake of glucose into tissues. Insulin is released from pancreatic beta cells in a pulsatile manner, with pulses estimated to occur every 6 minutes in humans (Song et al., 2002). Increases in insulin concentration in response to an increase in blood glucose are believed to result
from an increase in the amplitude of these secretory pulses (Song et al., 2002). The increase in blood sugar may result from the ingestion of non-structural carbohydrates (NSC) or, to a lesser extent, the carbon skeleton of amino acids. Non-structural carbohydrates in pasture and grass consist mainly of water-soluble carbohydrates, while concentrates have greater starch content. Thus, the NSC content of feed provided to horses impacts postprandial insulin concentrations in the horse.

Insulin receptors are located on insulin sensitive tissues, such as adipose tissue and skeletal and cardiac muscle. Circulating insulin binds to the receptor and promotes the translocation of the GLUT 4 glucose transporter to the plasma membrane where it is active. Insulin is also able to cross the blood brain barrier at physiological concentrations and has receptors on the anterior pituitary (Banks et al., 1997; Luque et al., 2006). Activity of insulin receptors in the pituitary, as assessed by phosphorylation of Akt, was comparable to that of insulin-sensitive adipose, muscle, and liver tissue (Luque et al., 2006).

In addition to its actions to facilitate glucose uptake in insulin-sensitive tissues, as a component of the somatotropic axis, insulin communicates with several body systems to relay information regarding energy and nutrient status. Insulin is a pleiotropic hormone and beyond its regulation of glucose, enhances the storage of fat by reducing lipolysis and increasing lipogenesis. The actions of hormone sensitive lipase and cAMP are decreased in the presence of insulin to reduce lipolysis and the action of acetyl-CoA carboxylase is promoted to facilitate energy storage. Thus, a rise in plasma insulin typically corresponds with a decrease in circulating non-esterified fatty acids.
Insulin secretion dynamics appear to undergo ontogenic changes in the horse. Between 5-160 days of age, no linear change in baseline insulin concentration occurred, though there was a decrease in insulin sensitivity. Concentrations reported during these ages ranged from 3.5-11 mIU/L (George et al., 2009). Basal insulin level in meal fed 2 year old horses was 4.7 mIU/L and was measured at 4.3 IU/L in similar horses fasted for 24 hours (Stull and Rodiek, 1988). In adult horses, fasting insulin concentrations less than 20 mIU/L are considered normal (Frank et al., 2009).

Insulin’s ability to circulate to insulin-sensitive tissues and pass through the blood brain barrier, combined with the expression insulin receptors (INSR) in somatotrope cells may allow for interactions between meal responses and regulation of the somatotropic axis. Insulin treatment in baboon pituitary cells decreased GH, growth hormone releasing hormone receptor (GHRH-R), and growth hormone secretagogue receptor (GHS-R) mRNA, with a decrease in GH release into culture as well (Luque et al., 2006). The mRNA expression for INSR in the pituitary was greater than INSR in fat, skeletal muscle, and liver, highlighting the pituitary as an insulin-sensitive tissue that may be regulated by nutritional status (Luque et al., 2006). Two models for obesity in mice (ob/ob and diet-induced obesity) displayed decreased pituitary mRNA expression of growth hormone and growth hormone secretagogue receptor (GHS-R) (Luque and Kineman, 2006). The ob/ob mouse model is leptin deficient and has an insatiable appetite that results in extreme obesity and has been heavily used as a model for type II diabetes mellitus in humans. The dietary-induced obesity mouse model utilizes a higher-calorie diet to generate an obese animal, which may be more reflective of obesity onset in the human and other animals (Luque and Kineman, 2006). This decrease in expression was
positively correlated with increases in glucose and insulin concentrations observed in both types of obese mice. While mRNA expression does not equate to protein expression, it does provide insight that may be physiologically relevant in investigating relationships between insulin concentration and GH secretion. However, it is important to recognize that the incubation period of 24-hours utilized by Luque et al. (2006) may not be reflective of postprandial actions. Instead it is more reflective of a chronic insulin status, such as seen in fasting or insulin resistance and may reflect and adaptation to this state instead of an immediate response.

Elevated insulin concentrations have been hypothesized to be the cause of reduced GH secretion in humans eating twice their normal dietary intake (Cornford et al., 2011). Over a period of 2 weeks, lean humans consumed a diet of approximately 4,000 calories per day, composed of 50% carbohydrate, 35% fat, and 15% protein, thus consuming about 75% more calories than necessary for weight maintenance. Average insulin concentration over a 24-hour period increased from approximately 15mIU/L before the increased caloric intake to nearly 30 mIU/L after only 3 days on the diet. This elevated average insulin concentration persisted through day 14 of the study. Reduction in average growth hormone concentration was observed after 3 and 14 days of overeating, beginning at 1.25 ng/mL before the study and dropping to 0.25 ng/mL at days 3 and 14. The decrease in average GH concentration was attributed to a reduction in average peak amplitude, going from 6.4 ng/mL at baseline to 2.5 and 1.5 ng/mL on days 3 and 14, respectively. Graphical representations of the data depict a consistent reduction in growth hormone throughout the 24-hour sampling period after 3 and 14 days on the increased energy diet. After 14 days of overeating, body mass, body mass index, body fat
percentage, and fat mass showed statistically significant increases, but did not place the subjects in the obese BMI category. The decreased GH concentrations typically observed in obese individuals may in fact be reflective of the actions of insulin as opposed to obesity, as the lean humans in this study reduced daily growth hormone secretion concordantly with an increase in plasma insulin concentrations prior to achieving obese body condition (Cornford et al., 2011).

In a contrasting study, a decreased insulin response in humans eating a high protein/low carbohydrate diet (60% fat, 5% carbohydrate, 35% protein) compared to a low protein/high carbohydrate diet composed of 60% carbohydrate, 30% fat, and 10% protein did not detect differences in mean growth hormone, pulse frequency, pulse amplitude, and basal concentration (Harber et al., 2005). Postprandial peak insulin concentrations in the high protein diet were less than 20mIU/L compared to 40mIU/L measured in the low protein diet. Both Harber et al. (2005) and Cornford et al. (2011) utilized frequent (20-minute) sampling with peak detection analysis software, which is not able to elucidate secretion events as accurately as deconvolution analysis.

It is important to note that the relationship between meal responses and growth hormone regulation is dynamic and all components of the system act in concert to regulate and counter regulate one another. Due to insulin’s lipogenic actions, insulin concentrations are inversely related to the concentration of non-esterified fatty acids in healthy individual. Separating elevated insulin concentrations from decreased NEFA concentrations is possible through pharmacologic means. In humans, decreasing circulating NEFA concentrations through the drug acipimox had an increased response to a GHRH bolus (GH peak 60 ng/mL) than acipimox with a low-dose insulin clamp.
(0.1 mU·kg⁻¹·min⁻¹; GH peak 45 ng/mL). Both were significantly greater than the response to GHRH in a high dose insulin clamp (0.4 mU·kg⁻¹·min⁻¹), which had a peak GH of approximately 15 ng/mL (Lanzi et al., 1997). While elevated insulin appears to inhibit GH secretion to a greater degree than decreased NEFA concentrations, non-esterified fatty acids are important components of the meal response and may contribute to GH secretion to some extent.

Non-esterified fatty acids

Non-esterified fatty acids (NEFA), or free fatty acids, are metabolically active components of the plasma lipid profile. Following digestion of dietary triglycerides, chylomicrons composed of triglycerides and cholesterol transport lipids throughout the body. Lipoprotein lipase (LPL) is constituent on the endothelial cells of adipose, heart, skeletal muscle and mammary tissue and breaks down triglycerides into glycerol and NEFA to facilitate uptake and storage. Insulin stimulates LPL, acting to promote the storage of energy. In times of energy deficit, hormone sensitive lipase releases fatty acids from adipose tissue and its relationship with insulin is opposite of that of LPL. Glucagon, cortisol, catecholamines, and growth hormone promote the actions of hormone sensitive lipase (HSL), which releases fatty acids by breaking down triglycerides in the extrahepatic tissues. Non-esterified fatty acids released into circulation are used as an energy source in the liver through beta oxidation. Non-esterified fatty acids are oxidized to provide ketone bodies for energy when glucose supply is limited, thus they typically rise during fasting and fall postprandially.
A wide range of NEFA concentrations has been reported in the equine literature. Fasting NEFA concentration in Shetland ponies (1,270 μEq/L) was greater in comparison to Morgans (784 μEq/L) and Thoroughbreds (536 μEq/L) (Robie et al., 1975). Adult horses fed hay ad libitum had NEFA concentrations averaging 46 μEq/L (Frank et al., 2002). Adult horses fed to obesity had NEFA concentrations of less than 100 μEq/L (Carter et al., 2009). In exercising horses fed thrice daily, mean NEFA concentrations ranged from 90-130 μEq/L over a 24-hour time course (Piccione et al., 2008). Elevated NEFA concentrations have been associated with obesity, insulin resistance, and metabolic syndrome in humans, though work in the horse has not been conclusive in this regard (Frank et al., 2010). In feed- deprived horses, NEFA concentrations rose above 800 μEq/L during fasting and fell to below 100 μEq/L upon refeeding (Christensen et al., 1997a; Sticker et al., 1995). In meal-fed horses, NEFA concentrations before feeding have been reported between 200-400 μEq/L and drop to concentrations below 100 μEq/L after eating (Powell et al., 2000).

Growth hormone has both lipogenic and lipolytic effects on adipose tissue and thus has a complicated relationship with plasma NEFA concentrations. Following a period of reduced GH, administration of GH has an insulin-like effect on adipose tissue, stimulating energy storage and reducing NEFA concentrations, for 1-2 hours (Casanueva, 1992). Growth hormone acts as a lipolytic agent when glucose availability is low by stimulating NEFA release from adipose tissue. Decreased NEFA concentrations elicit an increase in plasma GH, while elevated free fatty acids inhibit GH secretion (Casanueva, 1992; Quabbe et al., 1983). However, this is not absolute, as the use of NEFA as an energy source during hypoglycemia does not inhibit elevated growth hormone secretion.
Reduction in circulating NEFA in obese subject by pharmacological means enhances growth hormone response to a GHRH bolus (Quabbe et al., 1983). While concomitant reductions in NEFA and rises in plasma insulin are typical in normal physiological settings, through pharmaceutical means, these two mechanisms have been separated and independently affect GH secretion (Lanzi et al., 1997). The mechanisms by which NEFA may modulate GH secretion from the pituitary are unclear, though baboon pituitary cells exposed to NEFA had reduced GH, GHRH-R, and GHS-R mRNA as well as reduced GH release into culture (Luque et al., 2006).

Following the ingestion of a concentrate meal, non-esterified fatty acids, insulin, and glucose undergo dynamic changes. The intricate interactions between these hormones and metabolites present a variety of potential mechanisms by which growth hormone secretion may be regulated.

**Growth hormone and meal feeding in the horse**

The previously mentioned connections between blood glucose, insulin, and NEFA and growth hormone secretion naturally lead to an investigation on the effects of meal feeding on GH secretion. The bulk of the research on equine growth hormone does not use sampling frequencies or analytical methods that allow for elucidation of secretory episodes. In the equid, individual peaks in growth hormone concentration were observed during hours of darkness and also before both morning and afternoon concentrate feedings. Additionally, peaks were not detected for 2 hours after the morning meal or 5 hours after the evening meal in aged geldings with blood sampled every 20 minutes.
(Christensen et al., 1997b). In an 8 hour window, aged mares fed once daily exhibited a growth hormone peak during the first sample (0500 h) and GH peaks following a grain meal at 0800 h (Christensen et al., 1997b). Feed deprivation increased growth hormone concentrations from 1.02 ng/mL to 1.86 ng/mL after 24 hours and 1.25 ng/mL after 48 hours, though samples were taken every 12 hours, preventing a clear elucidation of GH secretion. However, upon refeeding, hourly samples measured an increase in GH to 3.22 ng/mL 3 hours postprandial (Christensen et al., 1997a). In a study of mares and stallions aged 5-15 years old, some horses appeared to have an increase in GH concentration prior to feeding at 1200 h; however the statistical analysis did not evaluate the relationship of times of feeding and times of GH peaks (DePew et al., 1994). Additionally, the figures reported in this study do not display an increase in growth hormone secretion until at least 2 hours after the meal (DePew et al., 1994). Concordant with these postprandial decreases in growth hormone secretion are rises in blood glucose and insulin concentrations and a decrease in non-esterified fatty acids (DePew et al., 1994), supporting the hypothesis that hyperinsulinemia may inhibit growth hormone release and contrasting the postprandial peak observed after 48 hours of feed deprivation (Christensen et al., 1997a).

In a comparison of Quarter horse weanlings (5-7.5 months old) fed a 34% NSC concentrate (CARB) or a 24% NSC diet supplemented with 10% fat (FAT) concentrate over a period of 60 days, mean growth hormone secretion appeared to differ by diet on day 30, but not on day 0 or 60 (Ropp et al., 2003). Day 30 growth hormone concentration had a peak in concentration at the time of feeding for the FAT weanlings, while the CARB weanlings showed a marked increase in GH concentration 2 hours after feeding.
Contrastingly, on day 60, FAT weanlings appeared to have concentration peaks 3 hours postprandially, with CARB animals exhibiting no significant increase in growth hormone. Day 30 glucose concentrations were significantly higher for weanlings on the CARB diet and had no difference in the insulinemic meal response. On day 60, glycemic responses did not differ, but insulinemic response was increased on the CARB diet 2-3 hours after the meal (Ropp et al., 2003). While insulin sensitivity was not measured directly in this study, the increased insulin response to the CARB diet over the 60-day period may be indicative of decreased insulin sensitivity. Young animals tend to be more insulin sensitive and the diets provided in this study may not have been sufficiently different in starch content to elicit a physiologically significant difference in insulin response before day 60. The increased insulin response on day 60 may indicate an adaptation to this diet though any effect on GH secretion is unclear. The mean growth hormone profiles presented in this study do not accurately represent individual growth hormone secretion, given the high variability in secretion and may not be appropriate for drawing conclusions regarding the impact of nutrition and meal feeding on the growth hormone. The potential adaptation to concentrate meals that elicit an elevated insulinemic response may have further implications beyond immediate effects on growth hormone secretion.

**Metabolic disorders in the horse**

In addition to examining the mechanisms connecting physiological meal responses to the regulation of growth hormone secretion, feeding practices in the young
horse may have implications for metabolic processes throughout the animal’s life. In the growing animal, skeletal growth and development may potentially be impacted through alterations in growth hormone secretion, resulting in various forms of developmental orthopedic disease. Routine feeding of concentrate meals that elicit an elevated insulin response and alterations in GH secretion may reduce insulin sensitivity and potentially manifest in health problems such as insulin resistance, obesity, equine metabolic syndrome or laminitis. Additionally, similar health issues in humans have been associated with altered growth hormone secretion, providing further impetus to investigate these problems in the young horse.

**Developmental orthopedic disease**

As defined by McIlwraith (2004), developmental orthopedic disease (DOD) encompasses the following disorders: “osteochondrosis dissecans (OCD), subchondral cystic lesions, angular limb deformities, physisitis, flexural deformities, cubodial bone abnormalities, and juvenile osteoarthritis”. This cluster of pathologies typically effects in the young animal, when skeletal development is occurring, but may also affect the animal later in its athletic career. Incidence of osteochondrosis has been reported in horses competing in a variety of disciplines and of a variety of breeds. Incidence of DOD has been reported as high as 61.7% in a population of yearling South German Coldblood horses (Wittwer et al., 2006). Osteochondrosis is a disease affecting growth cartilage, where the endochondral cartilage fails to develop properly and form bone, a process known as endochondral ossification (Ytrehus et al., 2007). Developmental orthopedic
disease is multifactorial, with evidence it is potentiated by factors such as nutrition, genetics, endocrine functions, metabolic regulation, and growth rate, which interfere with endochondral ossification (Thatcher, 1991). Growth hormone and IGF-I, which is regulated by growth hormone, facilitate longitudinal bone growth in young, growing animals and regulate bone mass in adult animals (Giustina et al., 2008). Growth hormone regulates bone remodeling by directly stimulating bone through a variety of mechanisms, including increased chondrocyte replication, endochondral bone formation, and proliferation of osteoblasts (Giustina et al., 2008). Abnormal regulation of growth hormone secretion, in both GH deficiency and excessive GH secretion has been associated with high rates of bone fractures (Giustina et al., 2008). In addition, insulin augments bone formation and lack of circulating insulin in humans with type I diabetes has been linked to osteopenia and osteoporosis (Fulzele and Clemens 2011). While the overall connection between nutrition, growth hormone secretion, and DOD in the horse is not clear, exploration between the relationships of growth hormone, a key regulator of bone metabolism, and nutrition may provide insight into these potential mechanisms.

**Insulin resistance**

As previously discussed, insulin’s role in facilitating glucose uptake and as a component in the somatotropic axis intertwines the feeding of the horse, potential health problems, and growth. Decreased insulin sensitivity, or insulin resistance (IR), is often associated with obesity, equine metabolic syndrome, and laminitis. Animals and humans with insulin resistance have a reduced ability to facilitate glucose uptake to insulin-
sensitive tissues such as muscle, liver, and adipose. Secretion of insulin from the pancreas is elevated to compensate for its lack of effectiveness, which may be manifested clinically with elevated glucose and insulin concentrations. Clinical diagnosis of IR in the horse may be made if the animal has a plasma insulin concentration greater than 20 mIU/L after an overnight fast (Frank et al., 2009).

In human and other animal models, there has research has demonstrated complex correlations between growth hormone secretion and insulin sensitivity. Exogenous administrations of GH and excessive endogenous GH production (e.g. acromegaly) have been associated with decreased insulin sensitivity through downregulation of insulin receptors (Domicini and Turyn 2002). Intracellular signaling pathways for growth hormone and insulin have been reported to converge and crosstalk through PI-3 kinase, though the extent of this activity is variable between tissues (Domicini and Turyn 2002). Through this crosstalk, growth hormone may act to facilitate insulin resistance (Domicini and Turyn 2002). Little work has successfully connected decreases in insulin sensitivity with alterations in the somatotropic axis in the horse. Weanlings adapted to a 49% non-structural carbohydrate (NSC) grain diet had greater insulin secretion during FSIGTT and had higher circulating IGF-I concentrations, potentially highlighting a connection between feeding practices and alterations in the somatotropic axis, which may play a role in the development of diseases such as metabolic syndrome or osteochondrosis (Treiber et al., 2005a). In equine and human literature, IR often occurs concurrently with other metabolic disturbances, such as obesity and metabolic syndrome. Focus on insulin resistance in horses has increased in recent years due to increasing prevalence of metabolic disturbances, such as obesity, and detrimental disease outcomes (Geor, 2008).
**Obesity**

Obesity, the excessive accumulation of body fat, and its associated metabolic disruptions and diseases result from increased dietary energy intake in human and animal models. Secretion of growth hormone is sensitive to nutrient status, and has been examined more thoroughly in the human and mouse models than in the equid. Awareness of the incidence of obesity in the horse is increasing and is similar to the obesity epidemic in humans, with similarities to human metabolic disorders such as insulin resistance, metabolic syndrome, hyperlipemia, and type 2 diabetes mellitus as well as those unique to the equine, such as laminitis and pituitary pars intermedia dysfunction (Durham et al., 2009; Johnson et al., 2009b). The management practices of the modern horse and incidence of obesity and its related disorders necessitates research into how the equine somatotropic axis may be affected. Feeding concentrate meals or diets high in non-structural carbohydrates is believed to contribute to the growing incidence of obesity and concurrent metabolic disturbances (Carter et al., 2009; Geor and Frank, 2009; Treiber et al., 2005b). In comparison to lean humans, obese children and adults tend to have a severely blunted growth hormone secretory pattern, with pulsatile episodes that are difficult to detect, decreased pulse amplitude, and an overall decrease in growth hormone secreted (Kreitschmann-Andermahr et al., 2010). Relationships between obesity and components of the somatotropic axis have been studied extensively in human subjects as compared to the horse and further exploration into networks may prove helpful in addressing obesity and obesity-related health disturbances.
Equine metabolic syndrome

Equine metabolic syndrome (EMS) has not been universally described, however, the recent consensus statement of the American College of Veterinary Internal Medicine describes the EMS phenotype as having increased regional or general adiposity, insulin resistance, and a predisposition toward laminitis “in the absence of recognized causes such as grain overload, colic, colitis, or retained placenta” (Frank et al., 2010). This rather broad collection of characteristics is similar to metabolic syndrome in humans (Geor and Frank, 2009; Kronfeld, 2003). The most obviously damaging manifestation of equine metabolic syndrome is laminitis, which is detrimental to the animal’s health and may result in euthanasia. Insulin resistance has been proposed as a key mechanism for connecting EMS and laminitis, by potentially reducing nutrient availability to hoof tissues due to impaired glucose uptake, compromising vasculature and favoring vasoconstriction, and increasing platelet accumulation (Frank, 2009). Growth hormone secretion has not been investigated in regard to EMS; however, the correlations between altered GH secretion, insulin resistance, and obesity imply that disturbances to the somatotropic axis are linked to EMS development.

Laminitis

Laminitis is a debilitating disease of the laminae in the equine foot, where vasculature to the distal phalange and surrounding tissues is compromised, separating the soft tissues from the hoof wall and resulting in severe pain, inflammation, and in extreme cases, prolapse of the pedal bone and euthanasia (1991). The exact or precise mechanism
causing laminitis is unknown; however, proposed mechanisms for destruction of the lamellae structure include altered blood flow dynamics, increased presence of inflammatory factors, and mechanical stress (Harris et al., 2006). Connections between the somatotropic axis and laminitis occurrence in the horse have not been explicitly investigated; however, previously described relationships between obesity, insulin resistance, and growth hormone secretion emphasize the multifactorial and complex nature of these health problems.

**Summary**

The connections between nutrition, growth, and potential pathological conditions in the horse necessitate investigation. In the young, growing animal, skeletal growth is of fundamental importance. During this time, the somatotropic axis actively participates in the skeletal growth and is sensitive to dietary energy and nutrient intake. Intake of concentrates eliciting an elevated glycemic and insulinemic response may also affect processes of growth. Growth hormone is a critical component of the somatotropic axis and its secretion has been affected by energy intake in other species. Due to the pulsatile secretion of growth hormone and the high degree of variation between individuals, deconvolution analysis is a method by which growth hormone secretory episodes may be modeled and characterized. The pattern of growth hormone secretion has not been investigated in response to meal feeding, yet there is evidence that nutrition regulates growth hormone secretion. The relationship between feeding practices and GH secretory
patterns has not been thoroughly investigated in the young growing horse using deconvolution as a tool to understand these mechanisms.

**Hypotheses and objectives**

In the young, growing horse, we hypothesize that feeding a high-glycemic concentrate meal will inhibit GH secretion postprandially for a period of 3-5 hours, with a subsequent greater amount of GH secreted in a pulse. Contrastingly, we hypothesize that horses fed a low-glycemic meal will not have this inhibition and subsequent elevated release of GH following a concentrate meal.

The objective of this study was to quantify concentrations of glucose, insulin, non-esterified fatty acids, and growth hormone over a 24-hour period in weanling Quarter horses fed a high-glycemic or low-glycemic concentrate meal twice daily. Furthermore, to assess growth hormone secretion, we will employ deconvolution analysis to estimate the mechanisms of the relationships between feeding concentrate meals and growth hormone secretory patterns.
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Figure 1-1. Neural and metabolic regulatory factors affecting growth hormone secretion.

Hormones and metabolites joined by a “+” indicate feedforward signal, those joined by a “—” indicate an antagonistic signal. Ach=acetylcholine, GHRH=growth hormone releasing hormone, STT=somatostatin, Arg=arginine, GH=growth hormone, NEFA=non-esterified fatty acids, IGF-I=insulin-like growth factor I. Adapted from Ghigo et al., 1999.

This simplified schematic of growth hormone regulation illustrates factors originating from the periphery and from the central nervous system. Regulation of GH is complex and the study at hand focuses on the components of this image that are associated with physiological meal responses (glucose, insulin, and NEFA). Somatostatin and growth hormone releasing hormone have bolded arrows as they are critical for the
regulation of GH secretion. Growth hormone is subsequently critical for IGF-I production and secretion.
Figure 1-2. Regulatory factors in growth hormone secretion from the anterior pituitary.

Lines ending in an arrow indicate a feed-forward mechanism. Those ending in a perpendicular line indicate an antagonistic relationship. Dotted lines represent potential models for acyl-ghrelin facilitated GH secretion that have not been thoroughly
investigated to this point. Adapted from Frohman and Kineman, 1999; Kineman and Luque, 2007.

Somatostatin (STT) from the periventricular nucleus (PVN) antagonizes growth hormone releasing hormone (GHRH) secretion from the arcuate nucleus (ARN). GHRH stimulates STT secretion. GHRH is secreted from ARC and binds to the G protein-coupled receptor, GHRH-R. GHRH-R undergoes a conformational change and the G,α subunit dissociates and stimulates adenosine cyclase (AC), which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). cAMP goes on to activate protein kinase A (PKA). The catalytic unit of PKA translocates to the nucleus, where it activates cAMP-response element-binding protein (CREB), which facilitates the transcription of genes for growth hormone (GH), growth hormone releasing hormone receptor (GHRH-R) and pituitary transcription factor-1 (PIT-1). Pit-1 further facilitates the transcription of ghrh-r and gh. PKA also phosphorylates Na+, K+, or other cation channels to prevent their influx. This depolarizes the cellular membrane and allows for the influx of Ca²⁺. Calcium influx in concert with intracellular calcium from the endoplasmic reticulum facilitates the secretion of GH vesicles out of the somatotrope. Acyl-ghrelin (AC) is secreted primarily from the stomach. It may bind at ARC and upregulate GHRH secretion. At the anterior pituitary, AC binds to the growth hormone secretagogue receptor (GHS-R). GHS-R is a G coupled-protein receptor and upon AG binding, G,α activates phospholipase C (PLC), which cleaves phosphatidylinositol 4, 5-bisphosphate (not pictured) to diacylglycerol (DAG) and inositol 1, 4, 5-triphosphate (IP3). DAG activates phosphokinase C (PKC), which potentially (dotted line) upregulates MAPK and PKA activity, facilitating GH secretion. STT binds to its receptor and blocks
calcium influx as well as cAMP production, thus reducing GH secretion and synthesis. Recent literature attributes the primary effect of STT to be on the secretion of GH, not negatively affecting synthesis. After being secreted from the anterior pituitary, GH feeds back negatively onto GHRH and positively to STT.
Figure 1-3. Convolution integral of plasma growth hormone concentration.

The concentration of growth hormone measured in circulation is a result of simultaneous events of secretion and elimination. Adapted from Veldhuis et al., 1987.
CHAPTER 2

High-glycemic meal responses and meal feeding affect fundamental components of the somatotropic axis in Quarter Horse weanlings

Abstract

Growth hormone is a key component of the somatotropic axis and is critical for the interplay between nutrition, regulation of metabolic functions, and subsequent processes of growth. The objective of this study was to investigate potential relationships between meal feeding concentrate diets differing in glycemic responses and growth hormone secretory patterns in young growing horses. Twelve Quarter Horse weanlings were used in a crossover design, consisting of two 21 d periods and two treatments, a high-glycemic (HG) or low-glycemic (LG) concentrate meal fed twice-daily. Horses were individually stabled and fed hay ad libitum. On the final day of each period, quarter-hourly blood samples were drawn for 24 continuous hours and analyzed for plasma glucose, insulin, non-esterified fatty acids, and growth hormone. Growth hormone secretory characteristics were estimated using AutoDeon deconvolution software. Following a meal, HG horses exhibited a longer inhibition of growth hormone secretion and increased amount of GH secreted in the initial postprandial secretory pulse as compared to LG horses. During nocturnal hours, HG horses secreted a greater amount of growth hormone than LG horses. These differences highlight relationships between glycemic responses and GH secretion, potentially through elevated insulin responses measured in HG horses. Dietary energy source and metabolic perturbations associated with meal feeding of high glycemic concentrates to young, growing horses has the
potential to alter growth hormone secretory patterns as compared to low glycemic concentrates.

Key words: deconvolution analysis, growth hormone, horse, meal feeding, nutrition

Introduction

The somatotropic axis is a chemical communication network that allows the body to respond to external stimuli, such as nutrition, by regulating metabolic processes that partition energy and nutrients. Several health problems, such as obesity, equine metabolic syndrome, laminitis, and developmental orthopedic disease have implicated components of the somatotropic axis as potential causal factors. Growth hormone (GH) is a key signal and regulator and integrates processes of nutrient partitioning with functions of growth and metabolism.

In the young growing animal, growth hormone and its associated hormones are particularly important for skeletal growth and development. In adult animals, GH acts primarily to regulate other systems, such as those used for nutrient and energy partitioning. A host of factors, particularly nutrient status, may influence growth hormone secretion. Obesity and overeating has been documented to blunt growth hormone secretion in adult humans (Cornford et al., 2011; Kreitschmann-Andermahr et al., 2010). Severe malnutrition, in the form of anorexia nervosa, typically results in elevated GH secretion, with increased pulse frequency, mass, and duration in women, concordant with GH resistance and lower bone density (Misra and Klibanski, 2011). Growth hormone secretion following a mixed meal in humans is inhibited, potentially due to glucose
promoting somatostatin, which inhibits GH release, and insulin inhibiting GH at the level of the anterior pituitary (Ghigo et al., 1999). However, details regarding the dynamics of physiological meal responses alongside growth hormone secretion have not been reported. Systemic alterations in circulating glucose and insulin observed in obesity, overeating, and anorexia nervosa have been strongly linked to modification of growth hormone secretion and bone density. Postprandial relationships between physiological meal responses and growth hormone secretion may also have implications for skeletal development and metabolic diseases.

Developmental orthopedic disease in the horse is multifactorial, with evidence it is potentiated by factors such as nutrition, genetics, endocrine functions, metabolic regulation, and growth rate, which interfere with endochondral ossification (Thatcher, 1991). The feeding of concentrate meals based on cereal grains impacts immediate physiological processes and may regulate an array of endocrine and metabolic processes that impact skeletal development. The non-structural carbohydrate (NSC) fraction of concentrates is equivalent to the hydrolyzable carbohydrate content and may be calculated as the sum of starch and water-soluble carbohydrates (Hoffman et al., 2001). Cereal-based concentrate meals elicited dose-dependent increases in glycemic and insulinemic responses as starch content of the feed increased (Vervuet et al., 2009). In response to a concentrate meal, young horses (<2 years old) with osteochondrosis dissecans (OCD) lesions displayed greater glycemic and insulinemic responses as compared to those with no radiological OCD lesions (Ralston, 1996). Additionally, abnormal regulation of growth hormone secretion, in both GH deficiency and excessive GH secretion has been associated with high rates of bone fractures (Giustina et al., 2008).
Associations between glycemic and insulinemic meal responses and altered GH secretion have not been investigated in healthy, growing horses. Alteration of the regulatory components of the somatotropic axis through meal feeding may affect skeletal growth and subsequent athletic ability. Limited work conducted previously in Quarter Horse weanlings showed inconclusive results regarding the impact of high glycemic feeds on growth hormone concentrations and did not explore variables of growth hormone secretion through deconvolution analysis (Ropp et al., 2003).

Circulating growth hormone concentrations encompass both secretory and clearance events, which may be mathematically modeled through deconvolution analysis (Veldhuis and Johnson, 1988). Modeling the secretory pulses allows for a precise characterization of the kinetics of growth hormone secretion and associations between nutrition and alterations in growth hormone secretion from the anterior pituitary. Deconvolution analysis has not been employed for analysis of growth hormone secretory patterns in the young, growing horse in response to perturbations in physiological meal responses. Utilizing this method for analysis provides information regarding the temporal timing and mass of GH secretion, among other secretory characteristics, to elucidate relationships between meal feeding and changes in growth hormone secretion.

Our hypothesis was that altering the metabolic profile that results from a meal would alter growth hormone secretory patterns—specifically, that horses fed a high-glycemic concentrate meal would exhibit an initial inhibition of GH secretion followed by an amplified GH release as compared to horses fed a low-glycemic concentrate meal. The objective of this study was to investigate glucose, insulin, non-esterified fatty acids,
and growth hormone secretory patterns in Quarter Horse weanlings fed a high-glycemic or low-glycemic concentrate over a 24-hour period.

Materials and Methods

Horses

Twelve Quarter Horse weanlings, five females and seven intact males, were utilized in a crossover design 42 d in length with two 21-d periods during the months of September and October. Horses were allotted to two treatment groups and balanced for sex, age, and body condition. At the beginning of the experiment, horses were 165 ± 13 d of age, weighed 228 ± 17 kg, and had body condition scores of 4.4 ± 0.3 (mean ± SD) (Henneke et al., 1983). Horses were individually housed and fed hay and concentrate meals in covered stables, with group exercise for 4 h per day (1600-2000 h) on a dry lot with ad libitum access to water. Body weight was measured weekly and 2 experienced individuals assessed body condition score. Animals were owned by the Pennsylvania State University and the experiment was approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC # 32764).

Treatments

Horses were fed either a high-glycemic (HG) or low-glycemic (LG) pelleted concentrate throughout the course of the study. Concentrate rations (Table 2-1, 2-2) were split into two daily meals (AM meal at 0700 h, PM meal at 1545 h) and animals were
gradually adapted to their respective diet over the course of 5 days (d 1-5) at the beginning of each period. Total concentrate fed daily to each horse was 0.75 kg on d 1; 1.5 kg on d 2 and 3, 2.25 kg on d 4 and 5 and 3 kg on d 6 through 21. Mixed mostly-grass hay (Table 2-2) was provided ad libitum, with fresh hay supplied at 0700 and 2000 h, following dry lot exercise. Upon visual assessment of hay wastage, we estimated horses consumed approximately 70% of the hay provided to them. Estimated hay intake is displayed in Table 2-3. Total nutrient intake is estimated in Table 2-4. Following 24 h of serial blood sampling on d 21, horses were transitioned to a new feed for the 0700 h feeding on d 1 of period 2. Period 2 consisted of the same feeding schematic as period 1 (Figure 2-1).

**Blood sampling**

Horses were fitted with jugular catheters between 1600-2000 h on day 20 of each period to facilitate blood sampling on d 21. Animals remained calm throughout blood sample collection and did not appear disturbed by the sampling personnel. On d 21 of each period, management practices were identical to previous days, except horses were not allowed group turn out and had red lights illuminating the stable during nocturnal hours (2345-0700 h) to reduce disturbances. Beginning at 0500 h on d 21, whole blood samples were drawn every 15 min for 24 consecutive hours. Each 12-mL blood sample was split equally into potassium ethylenediaminetetraacetic acid (EDTA) and sodium heparin vacutainer tubes, gently inverted 3-5 times, and then stored on ice. Within 45 min of collection, blood samples were centrifuged at 1400 x g as recommended by the
manufacturer at 4°C for 12 min (BD Vacutainer, Franklin Lakes, NJ). Plasma was then separated from erythrocytes and stored at -20°C until analysis. One horse on the HG treatment was removed from the study during period 1 due to difficulties with catheter patency.

Metabolite and hormone analysis

Growth hormone was measured every 15 min, while glucose, insulin, and non-esterified fatty acids (NEFA) were measured hourly, with more frequent sampling following meals (Figure 2-2). Hormone and metabolite responses to meals were grouped into AM meal (0700-1545 h), PM meal (1600-0045 h), and nocturnal (0100-0645 h). Plasma glucose was quantified by glucose oxidase methodology using an enzymatic colorimetric assay (Stanbio, Boerne, TX) with intra- and inter-assay CVs of 2.8% and 3.5%, respectively. Non-esterified fatty acids were quantified by enzymatic colorimetric assays (Wako Diagnostics, Richmond, VA) and had intra- and inter-assay CVs of 1.8% and 3.8%, respectively. Previously validated radioimmunoassays for insulin (Freestone et al., 1991) and growth hormone (Thompson et al., 1992) were used. Insulin intra- and inter-assay CVs were 23.3% and 6.3%, respectively. With the understanding that coefficient of variation values are considerably greater at lower concentrations, excluding plasma samples with an insulin concentration below 3mIU/L improved the intra-assay CV value to 11.6%. Intra- and inter-assay CVs for growth hormone were 9.0% and 6.9%, respectively. Assay procedures are outlined in Appendix A. Preliminary attempts at quantifying acyl ghrelin are described in Appendix D.
Basal glucose, insulin, and non-esterified fatty acid concentrations were based on four hourly samples during night hours (0100, 0200, 0300, and 0400 h) where the animals had access to hay but were not under the influence of concentrate meal feeding. Basal hormone and metabolite concentrations were assumed to be the homeostatically controlled set points for the animals. Meal baseline concentrations for glucose, insulin, and NEFA were based on the two consecutive samples immediately prior to each meal to establish baseline values for characterizing the AM and PM meal responses. Peak glucose and insulin were determined as the highest concentration in both the AM and PM time period. The incremental increases and decreases in glucose and insulin were determined by subtracting the meal baseline values from the peak and nadir, respectively. Nadir glucose, insulin, and NEFA were determined as the lowest point following each meal. Time to peak and nadir were measured in min after meals were fed. Area under the curve for glucose and insulin was considered as the area above baseline following each meal. Area under the curve for NEFA was calculated as the area beneath meal baseline following each meal. Hormone and metabolic AUC values were calculated using the STATA statistical package, version 10.1 (StataCorp, College Station, TX), which calculated the incremental area under the curve.

Growth hormone secretion for each horse on each treatment (n=23) was analyzed via deconvolution analysis with AutoDecon, a program within the Pulse XP software package (University of Virginia, Charlottesville, VA). A GH profile consisted of the growth hormone concentrations measured every 15 min throughout the 24-h period. AutoDecon is a fully automated program that calculates secretion events based on concentration values utilizing a weighted nonlinear least squares estimate to predict
probable secretory episodes and characteristics of secretion over the time course. Basal concentration, secretion standard deviation, and hormone half-life are variables that can be fixed in the algorithm to improve the ability to model the data. Three growth hormone profiles were not able to be fit by the automated program and reported physiologically impossible half-lives for growth hormone. Thus, these three profiles had the basal secretion fixed to provide the least residual variance of the model.

Variables of interest regarding 24 h profiles of growth hormone secretion included: GH half-life, number of secretory episodes (pulses), number of small pulses, number of large pulses, half-duration of secretory pulse, time between secretory pulse and concentration peak, time between secretory pulses, nocturnal GH secretion, total amount of GH secreted, total pulsatile GH secretion, and total basal GH secretion over the 24-h period. Since AutoDecon allows for the estimation of the time and amplitude of each secretory event, secretory pulses were classified as “large” and “small”. Large pulses (LP) were designated as secretion pulses with a mass constituting >7% of the daily pulsatile GH secretion. Small pulses (SP) were pulses that did not meet this criterion. Relationship of the timing and size of these pulses were of interest in the context of meal feeding, and thus the time to LP (TTLP) and time to SP (TTSP) after meal initiation were analyzed, as well as the amount of GH released in the first postprandial LP. The nocturnal hours not associated with meal feeding were analyzed for amount of pulsatile GH secreted, number of LP, and number of SP.
Statistical Analysis

Homoscedacity was assessed utilizing Levene’s test for all variables under consideration using STATA version 10.1 and transformations were made when data were determined to be heteroscedastic (StataCorp, College Station, TX). Natural log transformations were performed on meal baseline insulin, peak insulin, peak NEFA, and incremental increases in glucose, insulin and NEFA. A cubic transformation was performed on meal baseline glucose. Subsequent analyses were performed using a mixed model ANOVA and employed the PROC MIXED procedure in SAS version 9.2 (SAS Institute Inc., Cary, NC) and employed Tukey’s post-hoc test to assess differences for statistical significance.

A mixed model ANOVA with fixed effects diet and sex and random effect horse was utilized to analyze the effect of diet, period, sex, and diet by period interaction. Sex was removed from the model if it was not significant at a level of \( P<0.05 \). This analysis was employed for the following variables: basal glucose, basal insulin, basal NEFA, basal GH secreted, number of GH secretory pulses, number of large GH pulses, number of small GH pulses, half-duration of GH secretory pulse, secretory standard deviation of pulse, time from secretory pulse to concentration peak, pulsatile GH secreted, total GH secreted, nocturnal number of LP, nocturnal number of SP, and nocturnal pulsatile GH secretion.

To examine potential differences in AM and PM meal responses, a mixed model ANOVA with fixed effects of diet and sex, random effect horse, repeated measure meal, and subject of horse within diet was used to analyze the effects of diet, sex, meal, and diet
by meal interaction. Sex was removed from the model if it was not significant at a level of $P<0.05$. Meal effects were investigated for the following variables: baseline glucose, baseline insulin, baseline NEFA, peak insulin, peak glucose, nadir glucose, nadir insulin, nadir NEFA, incremental decrease in glucose, incremental decrease in NEFA, incremental increase in glucose, incremental increase in insulin, incremental increase in NEFA, glucose AUC, insulin AUC, NEFA AUC, time to glucose peak, time to insulin peak, time to NEFA nadir. Incremental increases were calculated by subtracting the meal baseline concentrations from meal peak concentration. Incremental decreases were calculated by subtracting the meal baseline from the meal nadir concentration.

For all statistical analyses, $P\leq 0.05$ was considered statistically significant and $P\leq 0.10$ was considered a trend. Data are reported as means ± standard deviation, without transformations, and reported $P$ values are from Type III tests of mixed effects. Due to the removal of a horse in period 1, the final sample size was 11 HG horses and 12 LG horses.

**Results**

**Intake & average daily gain**

Body condition score averaged 4.4-5.1 throughout the duration of the study. No significant differences in average daily gain were observed between diets ($P=0.95$). Average daily gain during decreased from period 1 to period 2 (Figure 2-3, Table 2-5). Estimated average daily hay intake was approximately $2.4 \pm 0.2\%$ of body weight and no
differences were detected between period \((P=0.98)\) or diet \((P=0.10, \text{Table 2-3})\). On day 21 of each period, all horses completely finished concentrate for each meal within 1 h, except for one horse on HG diet during period 2. This horse consumed 1.18 kg at the AM meal and 1.26 kg at the PM meal, 79% and 84% of the full amount, respectively. Time to eat the concentrate ration did not differ by diet \((P=0.74)\) or meal \((P=0.32)\) and averaged \(37 \pm 14\) min.

**Glucose, insulin, non-esterified fatty acids**

Concentration profiles over the 24-hour period for glucose, insulin, and non-esterified fatty acids are displayed in Figures 2-4, 2-5, and 2-6, respectively. Individual 24-h profiles of glucose, insulin, and NEFA concentrations are depicted in Appendix B. Basal concentrations were obtained from hourly samples taken at night, when animals had ad libitum access to hay. Basal glucose concentration averaged \(100.4 \pm 6.8\) mg/dL and did not differ by diet \((P=0.62)\). Basal NEFA was greater in LG horses \((33.7 \pm 8.3\) µEq/L) compared to HG horses \((25.3 \pm 5.3\) µEq/L, \(P=0.0136)\). Average basal NEFA concentration over both periods was \(30 \pm 8.1\) µEq/L. Basal insulin concentration did not differ between diets \((P=0.58)\), but averaged 1.6 mIU/L for period 1 and 1.0 mIU/L for period 2 \((P=0.0323)\). Sex was statistically significant \((P=0.0420)\) for basal insulin concentration, females averaged 1.6 ± 0.8 mIU/L and males averaged 1.0 ± 0.6 mIU/L. Average basal insulin concentration over both periods was \(1.3 \pm 0.8\) mIU/L.

Variables associated with the glycemic and insulinemic meal responses are listed in Table 2-5. A diet by meal interaction was detected for baseline glucose \((P=0.0080)\)
with diet also having an effect \((P=0.0300)\). At AM meal, baseline concentrations did not differ between HG and LG, however at PM meal baseline glucose was significantly lower for horses fed HG compared to LG \((HG=89.7 \pm 9.6, LG=97.1 \pm 4.9 \text{ mg/dL}, P=0.0057)\).

Insulin concentration at baseline was greater at PM meal compared to AM meal \((P<0.0001)\) and had no dietary differences \((P=0.1944)\). Peak insulin concentrations were greater for HG than LG \((P<0.0001)\) and for AM compared to PM \((P=0.0002)\). Postprandial changes in incremental increase in postprandial insulin concentrations were similar to those seen in peak insulin, HG was greater than LG \((P<0.0001)\) and AM was greater than PM \((P=0.0003)\). Peak glucose and incremental increase in glucose were significantly greater for HG than LG \((P<0.0001)\). AM meal had a tendency to have greater peak glucose than PM \((P=0.0550)\), while the incremental increase was significantly greater for AM than PM \((P=0.0083)\).

Time between feeding and peak glucose concentration had no effect of meal \((P=0.47)\) and a trend \((P=0.09)\) for being greater in HG \((129 \pm 46 \text{ min})\) compared to LG \((108 \pm 46 \text{ min})\). Time between feeding and peak insulin concentration for HG was \(209 \pm 148 \text{ min}\), while LG was \(70 \pm 60 \text{ min}\) \((P=0.0073)\) and did not differ by meal \((P=0.9131)\).

Nadir glucose concentrations were lower for HG compared to LG \((P<0.0001)\) and for AM meal compared to PM meal \((P=0.0041)\). Postprandial incremental decrease in glucose was greater (i.e. more negative) for HG than LG \((P=0.0178)\) with no meal effect \((P=0.1672)\). Glucose AUC was greater for HG than LG \((P<0.0001)\) and for AM than PM \((P=0.0314)\). Nadir insulin concentrations were not affected by diet \((P=0.9170)\) or meal \((P=0.1600)\). Insulin AUC was greater for HG compared to LG and AM compared to PM \((P<0.0001 \text{ for both comparisons})\) with a meal by diet interaction \((P=0.0133)\).
Baseline NEFA did not differ by diet ($P=0.9729$) or meal ($P=0.2733$) and averaged $33 \pm 17.5 \ \mu$Eq/L. Nadir NEFA was lower for HG than LG ($18.7 \pm 4.2$ vs. $24.4 \pm 6.2 \ \mu$Eq/L, $P=0.002$) and had was not affected by meal ($P=0.1486$). Postprandial incremental increase in NEFA was greater for LG than HG ($22.7 \pm 21.3$ vs. $13.7 \pm 12.6 \ \mu$Eq/L, $P=0.0484$) and tended to be greater for AM compared to PM ($23.7 \pm 20.0$ vs. $13.0 \pm 14.5 \ \mu$Eq/L, $P=0.0621$). Incremental decrease in NEFA did not differ by diet ($P=0.1919$) or meal ($P=0.4104$) and averaged $-11.3 \pm 17.0 \ \mu$Eq/L. Area under the curve for NEFA did not differ significantly between diet ($P=0.1615$) or meal ($P=0.4037$). Time to nadir NEFA did not differ by diet ($P=0.6936$) or meal ($P=0.3294$).

*Growth hormone*

Several growth hormone characteristics did not differ by diet and were pooled across treatments. Sex was statistically significant for growth hormone half-life, total pulsatile GH secretion, time from secretory pulse to concentration peak, and basal secretion at $P<0.05$. Characterizations of half-life, number of secretory pulses, number of large pulses, number of small pulses, secretory standard deviation of pulse, total amount of GH secreted, basal amount of GH secreted, and half-duration of secretory pulse are listed in Table 2-6, with sex differences represented where significant. Average growth hormone concentrations over the 24-hour period for all horses on both diets (n=23) are depicted in Figure 2-7. Individual horse GH secretion characteristics are listed in Appendix B.
Dietary differences were present in estimations of GH secretion over the 24-hour period, during nocturnal hours, and in postprandial periods (Table 2-8). Over the 24-hr period, pulsatile GH secretion in horses fed HG averaged 127.4 ± 42.6 ng compared to 95.7 ± 29.4 ng estimated in LG horses \((P=0.0497)\). Pulsatile secretion during nocturnal hours was 50.5 ± 16.0 ng in HG horses compared to 30.1 ± 13.7 ng in LG horses \((P=0.0020)\). Number of large pulses estimated during nocturnal hours was greater for HG \((2.4 ± 0.8 \text{ vs. } 1.7± 0.7, P=0.0421)\). Number of small peaks during the nocturnal hours did not differ by diet. During meal periods, large peaks averaged 2.0 ± 1.0 for AM meal and 1.6 ± 0.6 for PM \((P=0.0388)\). There was a trend \((P=0.0985)\) for LG \((5.7 ± 1.0)\) horses to have a greater number of large pulses compared to HG horses \((5.6 ± 1.1)\). Time from feeding to a large pulse was longer in HG horses, averaging 277 ± 131 min compared to 96 ± 29 min in LG horses \((P=0.0328)\). Mass secreted in the first postprandial LP was 17.7 ± 13.4 ng for HG horses and 11.0 ± 4.9 ng for LG horses \((P=0.0159)\). Time from feeding to a small peak tended to be longer for HG \((154 ± 124 \text{ min})\) than LG \((95 ± 64 \text{ min}, P=0.0782)\).

**Discussion**

The results of this study indicate that the source or amount of dietary energy has a significant impact on the growth hormone secretory pattern in growing horses. This finding has important implications concerning the ability of those feeding the horse to modulate important aspects of metabolic control through modifications to the macronutrient source of dietary energy. The goals of this study were to evaluate
differential glycemic and insulinemic meal responses on GH secretory patterns and to
that regard, our design successfully addressed the question at hand. The variation in
nutrient content between the two diets does not allow glycemic or insulinemic responses
to be isolated as the sole impetus for altered growth hormone secretion; however, recent
work indicates circulating insulin plays an important role in modulating growth hormone
secretion (Cornford et al., 2011; Lanzi et al., 1997; Luque and Kineman, 2006).

Horses fed a high glycemic meal displayed an elevated insulin concentration and
longer inhibition of GH secretion following a concentrate meal than horses fed a low
glycemic meal. Following this period of inhibition, the pulsatile release of GH was
greater in mass in HG horses as compared to LG horses. During nocturnal hours, HG
horses secreted a greater amount of GH and had a greater number of LP than LG horses.
These differences in growth hormone secretory patterns indicate that dietary energy
provided in concentrate meals plays an important role in the metabolic regulation of the
growing horse.

The dietary differences in glycemic and insulinemic responses were anticipated
due to HG diet having a nearly 5-fold greater NSC content as compared to LG. The bulk
of this difference is a result of increased starch content in HG compared to LG. Increased
starch content in a meal has been demonstrated to increase peak glucose, glucose AUC,
peak insulin, and insulin AUC, likely as a result of increased glucose availability in the
small intestine (Vervuet et al., 2009). This response is similar in humans and dogs, as
increased starch content in the diet increases glycemic and insulinemic meal responses
(Nguyen et al., 1998; Reaven, 1979). Increased incorporation of NSC into concentrate
meals consistently increases glycemic and insulinemic meal responses in the horse.
(Gordon et al., 2007; Williams et al., 2001). Horses on HG and LG concentrates received approximately 2.5 and 0.3 g starch/kg BW/meal, respectively. The HG concentrate is near the maximal amount of starch per meal suggested to avoid small intestinal starch overload, however a threshold for starch intake has not been established in the horse (Vervuet et al., 2009). However, both HG and LG diets reflect concentrates that are available in the equine industry and were designed to elicit differential glycemic responses.

Plasma NEFA concentrations in feed-deprived horses have been reported between 800-1000 μEq/L (Christensen et al., 1997a; Sticker et al., 1995). Refeeding of feed-deprived horses and routine concentrate meal feeding both elicit a precipitous drop in postprandial NEFA concentrations ranging from 87-180 μEq/L (Christensen et al., 1997a; Powell et al., 2000). Weanling horses in this study did not exhibit a typical postprandial NEFA response and had an average NEFA concentration less than 60 μEq/L throughout the sampling time course. Average NEFA concentrations of 46 μEq/L were reported in adult horses given ad libitum access to mixed grass hay (Frank et al., 2002). Adult horses fed to obesity had mean basal NEFA concentrations below 100 μEq/L (Carter et al., 2009). The weanlings in this study were in positive energy balance, gaining weight, and given access to hay ad libitum and these factors may have acted in concert to minimize circulating NEFA concentrations.

The pulsatile secretion of growth hormone from the anterior pituitary is essential for its actions (Urban et al., 1988). Deconvolution analysis of humans estimated an average of 12 secretory pulses over a 24 h period with an average of 85 min between secretory pulses (Hartman et al., 1991). Growth hormone secretion is dynamic and
impacted by a multitude of factors including age, sex, body composition, and sleep onset in humans (Jaffe et al., 1998; Takahashi et al., 1968; Veldhuis et al., 1991; Zadik et al., 1985). Sex differences have not been detected in total daily GH secretion; however, the pattern of GH secretion is sexually dimorphic (Jaffe et al., 1998). Male humans have larger nocturnal GH pulses and relatively smaller pulses during the day, while women exhibit more pulses of greater amplitude throughout the day in addition to a large nocturnal pulse (Jaffe et al., 1998). Obese men exhibit fewer secretory pulses and decreased growth hormone production compared to lean men (Veldhuis et al., 1991). Research examining the pulsatile secretion of GH in the equid is limited and 24-hour patterns of GH secretion have not been documented.

To date, the majority of literature regarding equine growth hormone utilizes peak detection analysis or presents average growth hormone concentrations (Christensen et al., 1997b; DePew et al., 1994; Ropp et al., 2003). Growth hormone concentrations measured in circulation represent a convolution integral that is inclusive of overlapping events of secretion and elimination (Veldhuis et al., 1987). Deconvolution analysis utilizes a mathematical model to deconstruct the concentration profile into variables describing secretion and elimination. Peak detection analysis statistically tests concentration profiles for significant increases and decreases in concentration and does not address the secretory mechanisms regulating growth hormone. The AutoDecon deconvolution software utilized in this study is able to detect secretory episodes with greater sensitivity than peak detection software and is a better method for assessing secretory characteristics of GH (Johnson et al., 2009). This study is the first of its kind to investigate relationships
between GH secretion and concentrate meal responses in horses utilizing deconvolution analysis.

Previous research in the horse utilizing deconvolution analysis of growth hormone has been limited to investigating nocturnal secretion in overtrained horses (de Graaf-Roelfsema et al., 2009). The half-life of endogenous equine growth hormone has been estimated through deconvolution analysis to range between 7.3-15.2 min for 2-year old horses subjected to exercise (de Graaf-Roelfsema et al., 2009). In response to an injection of GHRH, equine growth hormone half-life was estimated at 17.7 min (de Graaf-Roelfsema, 2007). Nocturnal sampling of growth hormone over an 8-h time course estimated 6.2-10.6 secretory pulses and extrapolation of this number to a 24-h time course would lead to greater pulses than detected in our study. Additionally, half-duration of the pulse ranged from 2.8-7.9 min with interval between pulses ranging from 38.9-70.4 min (de Graaf-Roelfsema et al., 2009). Sampling frequency in this study was every 5 min, in contrast to our sampling of every 15 min. Increased sampling frequency allows for a clearer depiction of growth hormone concentrations in the blood, which results in a greater number of detected concentration peaks and secretory pulses (Evans et al., 1987; Hartman et al., 1991). Given that secretion of growth hormone from the pituitary is pulsatile, the mass of growth hormone that may have been secreted by undetected pulses was likely accounted for by the basal secretion or coupled with larger pulses that were detected. Decreased sampling frequency may overestimate individual secretory episodes, as smaller pulses are unable to be discerned from those of larger magnitude (Hartman et al., 1991). Other factors, such as assay sensitivity, also play into the ability of AutoDecon to detect secretory episodes, as the lower limit of detection is a determining factor in the
model. The inability of AutoDecon to fit three profiles may also be due to the sampling frequency. Quarter-hourly sampling may reduce the precision in detecting nuances in growth hormone secretion and offer less information for modeling. Due to the design and goals of the study and physical limitations regarding blood sampling, deconvolution analysis appears to have appropriately predicted secretory episodes of major significance over a 24-h period. Deconvolution analysis has not characterized 24-h growth hormone secretory patterns in weanling horses prior to our study, so while differences between our work and that of de Graaf-Roelfsema and colleagues (2009) may be due to sampling protocol, it is also important to note that these may be indicative of ontogenous adaptations of the somatotropic axis or potentially due to breed or management differences.

Growth hormone is sensitive to energy balance and hypoglycemia, in the presence of elevated insulin concentrations, is an extremely robust impetus for increased growth hormone secretion in humans (Cryer, 1993; Roth et al., 1963). Hyperinsulinemia, at physiological concentrations in the absence of hypoglycemia, is a potential inhibitor of growth hormone secretion in humans (Cornford et al., 2011; Lanzi et al., 1999; Lanzi et al., 1997). The mechanism by which this occurs has not been elucidated. Insulin receptor mRNA has been detected in pituitaries of baboons and mice at levels comparable to that in liver, fat, and skeletal muscle (Luque et al., 2006; Luque and Kineman, 2006). In baboon pituitaries, physiological quantities of insulin reduced mRNA for GH, growth hormone releasing hormone receptor (GHRH-R), and growth hormone secretagogue receptor (GHS-R) and decreased GH secretion from the somatotrope (Luque et al., 2006). Two mice models of obesity (an ob/ob and diet-induced obesity) had elevated insulin
concentrations concomitant with reduced pituitary GH, GHRH-R, and GHS-R mRNA (Luque and Kineman, 2006). The ob/ob is a leptin-deficient mouse model has an insatiable appetite that results in extreme obesity and has been heavily used as a model for type 2 diabetes mellitus in humans. The dietary-induced obesity mouse model utilizes a higher-calorie diet to generate an obese animal, which may be more reflective of obesity onset in the human and other animals (Luque and Kineman, 2006). Lean humans consuming 75% more calories than needed for weight maintenance displayed elevated insulin concentrations and blunted growth hormone concentration profiles over a 24-hour period after 3 days of adaptation to the increased energy diet (Cornford et al., 2011). The elevated postprandial insulin response in HG horses did not alter total amount of GH secreted compared to LG horses. However, insulin may modulate postprandial GH secretion and therefore alter the pulsatile pattern of secretion.

The total amount of GH secreted in this study did not differ between HG and LG, rather the pattern of secretion was different, which lends evidence that the mechanisms of GH secretion, not GH production, so regulation at the pituitary or at the hypothalamus may be affected. Somatostatin is secreted from the hypothalamus and inhibits the secretion, but not synthesis of GH. Insulin has been reported to facilitate somatostatin release from the pituitary (Patel, 1999). Hyperinsulinemic mouse models did not exhibit increases in hypothalamic somatostatin mRNA (Luque and Kineman, 2006). Support for increased somatostatin as a causal mechanism for decreased GH secretion in obese humans is equivocal (Barnett, 2003). The actions of somatostatin may be regulating GH in response to glycemic and insulinemic response, but the design of our study prevents
the elucidation of the precise neuroregulatory mechanisms that alter GH secretion in HG horses.

Modulation of GH secretion is a dynamic process involving the communication and coordination of numerous hormones and metabolites. The promotion of GH secretion in the meal fed horse may also involve the orexigenic, GH secretagogue, acyl ghrelin. Acyl ghrelin has been demonstrated to rise prior to meal initiation and falls precipitously after eating in humans (Cummings et al., 2004). Growth hormone is modulated by acyl ghrelin in meal-fed humans and sheep (Nass et al., 2008; Sugino et al., 2002). This work has not been replicated in the horse (Gordon and McKeever, 2005).

The differences detected in growth hormone secretory patterns between horses fed a high-glycemic or low-glycemic concentrate meal demonstrate a role for nutrition to modulate actions of the somatotropic axis in the growing horse. Concentrates diets utilized in this study varied in nutrient composition and thus we cannot tease apart the effect of concentrate composition from the different glycemic and insulinemic responses to HG and LG. The objective in this study was to investigate the effect of contrasting glycemic meal responses on GH secretion and the diets utilized achieved this goal.

Conclusion

Meal feeding concentrate grains that elicit elevated glycemic and insulinemic responses alter patterns of GH secretion in Quarter Horse weanlings. Weanlings fed a high glycemic concentrate exhibited a longer inhibition of GH secretion than those fed a low glycemic concentrate. In addition, HG horses secreted a larger amount of growth hormone in their first postprandial secretory pulse than LG horses. Nocturnal GH
secretion in HG horses was greater than that of LG horses and had greater amounts of GH released in large pulses.

Alterations in the secretion of GH may have implications for processes of growth and metabolic regulation in the young horse. Further exploration into these regulatory mechanisms and potential long-term effects are necessary to understand the consequences of using nutrition to modulating the somatotropic axis in horses.
Literature Cited


Table 2-1. Composition of pelleted concentrates fed to weanling Quarter Horses\(^1\).

<table>
<thead>
<tr>
<th>Ingredient, %(^2)</th>
<th>HG</th>
<th>LG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground</td>
<td>57.7</td>
<td>–</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>21.0</td>
<td>20.9</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>0.3</td>
<td>37.5</td>
</tr>
<tr>
<td>Soybean, cooked</td>
<td>10.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>–</td>
<td>7.3</td>
</tr>
<tr>
<td>Oat byproduct</td>
<td>1.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Buck screenings</td>
<td>5.0</td>
<td>–</td>
</tr>
<tr>
<td>Alfalfa meal, 17% protein</td>
<td>–</td>
<td>2.5</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>–</td>
<td>2.2</td>
</tr>
<tr>
<td>Pleasure horse pelleted feed(^3)</td>
<td>0.2</td>
<td>–</td>
</tr>
<tr>
<td>Safe ‘n easy pelleted feed(^3)</td>
<td>–</td>
<td>0.1</td>
</tr>
<tr>
<td>Trace mineral premix</td>
<td>3.6</td>
<td>6.2</td>
</tr>
</tbody>
</table>

\(^1\)The information was provided by Buckeye Nutrition, a division of MARS horsecare (Dalton, OH).

\(^2\)Composition of the concentrates remained constant throughout the course of the study.

\(^3\)Available from Buckeye Nutrition.
Table 2-2. Nutrient composition\(^1\) of concentrates and hay.

<table>
<thead>
<tr>
<th>Item</th>
<th>HG (n=6)</th>
<th>LG (n=6)</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>89.4 ± 0.7</td>
<td>90.6 ± 0.3*</td>
<td>92.4 ± 0.7</td>
</tr>
<tr>
<td>CP, %</td>
<td>14.5 ± 0.4</td>
<td>14.4 ± 0.3</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>ADF(^2), %</td>
<td>7.0 ± 0.6</td>
<td>28.9 ± 1.3*</td>
<td>39.5 ± 1.4</td>
</tr>
<tr>
<td>NDF(^3), %</td>
<td>19.0 ± 1.0</td>
<td>48.6 ± 1.5*</td>
<td>68.2 ± 1.6</td>
</tr>
<tr>
<td>Starch(^4), %</td>
<td>45.9 ± 1.5</td>
<td>5.0 ± 0.5*</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>WSC(^5), %</td>
<td>6.1 ± 0.5</td>
<td>6.1 ± 0.4</td>
<td>12.3 ± 0.6</td>
</tr>
<tr>
<td>NSC(^6), %</td>
<td>52.0 ± 1.2</td>
<td>11.0 ± 0.7*</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>ESC(^7), %</td>
<td>3.3 ± 0.8</td>
<td>3.3 ± 0.6</td>
<td>5.8 ± 1.0</td>
</tr>
<tr>
<td>Crude Fat(^8), %</td>
<td>6.1 ± 0.5</td>
<td>10.2 ± 0.6*</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>NFC(^9), %</td>
<td>54.5 ± 0.9</td>
<td>17.2 ± 1.4*</td>
<td>12.9 ± 1.4</td>
</tr>
<tr>
<td>Ash, %</td>
<td>6.0 ± 0.4</td>
<td>9.7 ± 0.6*</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>Ca, %</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.0*</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>P, %</td>
<td>0.8 ± 0.1</td>
<td>1.6 ± 0.1*</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.3 ± 0.0</td>
<td>0.7 ± 0.0*</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>K, %</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.1*</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0*</td>
<td>–</td>
</tr>
<tr>
<td>Fe, ppm</td>
<td>299 ± 25</td>
<td>960 ± 44*</td>
<td>87 ± 32</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>193 ± 23</td>
<td>204 ± 19*</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Cu, ppm</td>
<td>75 ± 9</td>
<td>79 ± 8*</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Mn, ppm</td>
<td>136 ± 17</td>
<td>144 ± 2</td>
<td>75 ± 15</td>
</tr>
<tr>
<td>Mo, ppm</td>
<td>1.5 ± 0.2</td>
<td>1.9 ± 0.1*</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>DE(^10), Mcal/kg</td>
<td>3.4 ± 0.0</td>
<td>2.5 ± 0.0*</td>
<td>1.9 ± 0.0</td>
</tr>
</tbody>
</table>

Presented as means ± standard deviation, on a dry matter basis.

\(^\ast\) Indicates a significant difference between HG and LG at \(P<0.05\).

\(^1\) DM basis, as analyzed by Dairy One Forage Lab (Ithaca, NY).

\(^2\) Acid detergent fiber: cellulose and lignin component.

\(^3\) Neutral detergent fiber: hemicelluloses, cellulose, and lignin component.

\(^4\) Starch: polysaccharide component.

\(^5\) Water soluble carbohydrate: monosaccharides, disaccharides, and some polysaccharides (mainly fructan).

\(^6\) Non structural carbohydrate: starch + WSC.
7 Ethanol soluble carbohydrate: monosaccharides and disaccharides extracted in 80% ethanol.

8 Crude fat determined by ether extraction.

9 Non fibrous carbohydrate: estimate of non-cell wall carbohydrate. NFC = 100 - (%CP + %NDF + %Crude Fat + %Ash).

10 Digestible energy calculated as DE = 2.118 + 12.18(%CP) - 9.37(%ADF) - 3.83 (%NDF-%ADF) + 47.18 (%Crude Fat) + 20.35 (%NFC) - 26.3 (%Ash).
Table 2-3. Average individual hay daily intake\(^1\) on a dry matter\(^2\) and body weight basis\(^3\)

<table>
<thead>
<tr>
<th>Period</th>
<th>High Glycemic</th>
<th></th>
<th>Low Glycemic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/d</td>
<td>% BW/d</td>
<td>kg/d</td>
<td>% BW/d</td>
</tr>
<tr>
<td>1</td>
<td>5.2 ± 0.7</td>
<td>2.2 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>5.3 ± 0.4</td>
<td>2.2 ± 0.1</td>
<td>5.8 ± 0.5</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

Presented as mean ± standard deviation in kilograms and as a percentage of body weight during days 6-21 of each period. Overall, average hay intake was 5.3 ± 0.6 kg of DM per day, which was 2.2 ± 0.2 % of body weight.

\(^1\)Hay wasteage was estimated at 30% of hay fed.

\(^2\)Period effect, \(P=0.0001\), no effect of diet \((P=0.43)\) or period by diet interaction \((P=0.13)\).

\(^3\)Trend for period effect, \(P=0.051\), no effect of diet \((P=0.82)\) or period by diet interaction \((P=0.21)\).
Table 2-4. Estimate of total daily nutrient intake

<table>
<thead>
<tr>
<th>Item</th>
<th>HG&lt;sup&gt;1&lt;/sup&gt;</th>
<th>LG&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Hay&lt;sup&gt;2&lt;/sup&gt;</th>
<th>HG + Hay&lt;sup&gt;3&lt;/sup&gt;</th>
<th>LG + Hay&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, kg/d</td>
<td>2.7</td>
<td>2.7</td>
<td>5.3</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>CP, g/d</td>
<td>389</td>
<td>391</td>
<td>504</td>
<td>892</td>
<td>895</td>
</tr>
<tr>
<td>ADF, g/d</td>
<td>188</td>
<td>786</td>
<td>2094</td>
<td>2281</td>
<td>2879</td>
</tr>
<tr>
<td>NDF, g/d</td>
<td>510</td>
<td>1321</td>
<td>3615</td>
<td>4124</td>
<td>4936</td>
</tr>
<tr>
<td>Starch, g/d</td>
<td>1231</td>
<td>136</td>
<td>42</td>
<td>1273</td>
<td>178</td>
</tr>
<tr>
<td>WSC, g/d</td>
<td>164</td>
<td>166</td>
<td>652</td>
<td>816</td>
<td>818</td>
</tr>
<tr>
<td>NSC, g/d</td>
<td>1395</td>
<td>299</td>
<td>689</td>
<td>2084</td>
<td>988</td>
</tr>
<tr>
<td>ESC, g/d</td>
<td>89</td>
<td>90</td>
<td>307</td>
<td>396</td>
<td>397</td>
</tr>
<tr>
<td>Crude Fat, g/d</td>
<td>164</td>
<td>277</td>
<td>127</td>
<td>291</td>
<td>404</td>
</tr>
<tr>
<td>NFC, g/d</td>
<td>1462</td>
<td>467</td>
<td>684</td>
<td>2145</td>
<td>1151</td>
</tr>
<tr>
<td>Ash, g/d</td>
<td>161</td>
<td>264</td>
<td>376</td>
<td>537</td>
<td>640</td>
</tr>
<tr>
<td>Ca, g/d</td>
<td>25</td>
<td>35</td>
<td>22</td>
<td>48</td>
<td>57</td>
</tr>
<tr>
<td>P, g/d</td>
<td>22</td>
<td>44</td>
<td>15</td>
<td>37</td>
<td>59</td>
</tr>
<tr>
<td>Mg, g/d</td>
<td>8</td>
<td>18</td>
<td>11</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>K, g/d</td>
<td>24</td>
<td>36</td>
<td>105</td>
<td>129</td>
<td>141</td>
</tr>
<tr>
<td>Na, g/d</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Fe, ppm/d</td>
<td>8</td>
<td>26</td>
<td>5</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>Zn, ppm/d</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cu, ppm/d</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mn, ppm/d</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mo, ppm/d</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>DE, Mcal/d</td>
<td>9.1</td>
<td>6.8</td>
<td>10.3</td>
<td>19.4</td>
<td>17.1</td>
</tr>
</tbody>
</table>

<sup>1</sup>Calculations are based on the nutrient analysis in Table 2-1 and an intake of 3 kg concentrate per day.

<sup>2</sup>Calculations are based on nutrient analysis in Table 2-1 and average hay intake of 5.3 kg of DM per day calculated over all horses from Table 2-2.

<sup>3</sup>Calculations are based on the sum of nutrients from concentrate and hay.
Table 2-5. Average daily weight gain in kilograms/day

<table>
<thead>
<tr>
<th>Period</th>
<th>HG, kg/d</th>
<th>LG, kg/d</th>
<th>NRC Predicted&lt;sup&gt;1&lt;/sup&gt;, kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.9&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.8&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Average daily weight gain (ADG) during days 6-21 of each period. Presented as means ± standard errors. No significant differences were observed between diets ($P=0.95$) but there was a decrease in ADG in period 2 compared to period 1 ($P=0.0061$).

<sup>1</sup>Predicted from the equation ADG= Mature Weight * (6.97121 * e<sup>(-0.0772*Age))</sup>/(30.4*100). Mature weight is in kilograms, age is in months.

<sup>2</sup>Mature weight of 550 kg, age of 5 months.

<sup>3</sup>Mature weight of 550 kg, age of 6 months.
Table 2-6. Glycemic and insulinemic responses to AM and PM meals

<table>
<thead>
<tr>
<th>Variable</th>
<th>HG AM</th>
<th>PM</th>
<th>LG AM</th>
<th>PM</th>
<th>P-value</th>
<th>Diet¹</th>
<th>Meal²</th>
<th>Diet x Meal³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92.9 ± 6.9</td>
<td>89.7 ± 9.6</td>
<td>92.6 ± 5.4</td>
<td>97.1 ± 4.9</td>
<td>0.0300</td>
<td>0.4616</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>150.7 ± 17.8</td>
<td>141.4 ± 17.2</td>
<td>123.7 ± 13.1</td>
<td>119.5 ± 6.5</td>
<td>&lt;0.0001</td>
<td>0.0545</td>
<td>0.4612</td>
<td></td>
</tr>
<tr>
<td>Nadir</td>
<td>82.6 ± 10.8</td>
<td>87.0 ± 8.1</td>
<td>91.4 ± 4.6</td>
<td>93.5 ± 6.3</td>
<td>&lt;0.0001</td>
<td>0.0041</td>
<td>0.2859</td>
<td></td>
</tr>
<tr>
<td>Incremental increase</td>
<td>57.8 ± 20.1</td>
<td>51.7 ± 21.1</td>
<td>31.1 ± 13.0</td>
<td>22.4 ± 9.0</td>
<td>&lt;0.0001</td>
<td>0.0083</td>
<td>0.2629</td>
<td></td>
</tr>
<tr>
<td>Incremental decrease</td>
<td>-10.3 ± 7.2</td>
<td>-2.75 ± 9.3</td>
<td>-1.2 ± -3.9</td>
<td>-3.6 ± 5.8</td>
<td>0.0178</td>
<td>0.1672</td>
<td>0.0116</td>
<td></td>
</tr>
<tr>
<td>AUC (min<em>mg</em>dL^-1)</td>
<td>11,954 ± 4,071</td>
<td>11,297 ± 4,945</td>
<td>6704 ± 3763</td>
<td>3983 ± 2406</td>
<td>&lt;0.0001</td>
<td>0.0314</td>
<td>0.1784</td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.6 ± 0.5</td>
<td>2.5 ± 2.5</td>
<td>1.7 ± 2.2</td>
<td>2.0 ± 1.0</td>
<td>0.1944</td>
<td>&lt;0.0001</td>
<td>0.0594</td>
<td></td>
</tr>
<tr>
<td>Peak</td>
<td>18.5 ± 7.6</td>
<td>12.7 ± 6.2</td>
<td>8.8 ± 3.1</td>
<td>7.2 ± 3.4</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
<td>0.3828</td>
<td></td>
</tr>
<tr>
<td>Nadir</td>
<td>1.0 ± 1.1</td>
<td>1.6 ± 2.3</td>
<td>1.2 ± 1.1</td>
<td>1.3 ± 1.0</td>
<td>0.9170</td>
<td>0.1600</td>
<td>0.3931</td>
<td></td>
</tr>
<tr>
<td>Incremental increase</td>
<td>17.9 ± 7.4</td>
<td>10.2 ± 6.8</td>
<td>7.1 ± 3.2</td>
<td>5.1 ± 3.1</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
<td>0.3215</td>
<td></td>
</tr>
<tr>
<td>Incremental decrease</td>
<td>0.5 ± 0.9</td>
<td>-0.9 ± 0.7</td>
<td>-0.6 ± 1.6</td>
<td>-0.7 ± 0.6</td>
<td>0.2031</td>
<td>0.0214</td>
<td>0.0569</td>
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</tr>
<tr>
<td>AUC (min<em>mIU</em>L^-1)</td>
<td>4,181 ± 1,613</td>
<td>2,005 ± 1,523</td>
<td>1,560 ± 972</td>
<td>848 ± 514</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0133</td>
<td></td>
</tr>
</tbody>
</table>

Glycemic and insulinemic responses to two meals fed in a 24-h period. Meal 1 was fed at 0700 h and Meal 2 was fed at 1545 h.

¹High-glycemic meal compared to low-glycemic meal.

²AM meal compared to PM meal.

³Diet by meal interaction.
Table 2-7. Growth hormone secretory characteristics detected by deconvolution analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
<th>P-value for sex effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life (min)</td>
<td>23.1 ± 6.5</td>
<td>26 ± 5.4</td>
<td>19.5 ± 6.1</td>
<td>0.0326</td>
</tr>
<tr>
<td>Total GH secreted over 24-hr period (ng)</td>
<td>188.2 ± 69.4</td>
<td>159.8 ± 38</td>
<td>255.2 ± 84.7</td>
<td>0.0407</td>
</tr>
<tr>
<td>Basal GH secreted over 24-hr period (ng)</td>
<td>76.6 ± 59.2</td>
<td>53.5 ± 35.0</td>
<td>106.6 ± 71.9</td>
<td>0.0763</td>
</tr>
<tr>
<td>Time from secretory pulse to concentration peak (min)</td>
<td>14.2 ± 2.9</td>
<td>15.9 ± 2.4</td>
<td>12.0 ± 1.8</td>
<td>0.0031</td>
</tr>
<tr>
<td>24 hr Number of secretory pulses</td>
<td>13.5 ± 2.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24 hr Number of LP(^1)</td>
<td>5.6 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24 hr Number of SP(^2)</td>
<td>7.9 ± 3.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Secretory pulse SD (min)</td>
<td>12.8 ± 3.23</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time between secretory pulses (min)</td>
<td>96.6 ± 18.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Presented as means ± standard deviation. Sex effects are presented when sex was significant at a level of $P<0.05$.

\(^1\)Large pulse constituting >7% of daily pulsatile GH secretion.

\(^2\)Small pulse constituting <7% of daily pulsatile GH secretion.
Table 2-8. Growth hormone secretory characteristics affected by diet

<table>
<thead>
<tr>
<th>Variable</th>
<th>HG</th>
<th>LG</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr Pulsatile^1 (ng)</td>
<td>127.4 ± 42.6</td>
<td>95.7 ± 29.4</td>
<td>0.0497</td>
</tr>
<tr>
<td>Nocturnal Pulsatile^2 (ng)</td>
<td>50.5 ± 16.0</td>
<td>30.1 ± 13.7</td>
<td>0.0020</td>
</tr>
<tr>
<td>Nocturnal number of LP^3</td>
<td>2.4 ± 0.8</td>
<td>1.7 ± 0.7</td>
<td>0.0421</td>
</tr>
<tr>
<td>Meal number of LP^4</td>
<td>5.6 ± 1.1</td>
<td>5.7 ± 1.0</td>
<td>0.0985</td>
</tr>
<tr>
<td>Time from feeding to first LP^5 (min)</td>
<td>277 ± 131</td>
<td>96 ± 29</td>
<td>0.0124</td>
</tr>
<tr>
<td>Mass of first LP after feeding^6 (ng)</td>
<td>17.7 ± 13.4</td>
<td>11.0 ± 4.9</td>
<td>0.0159</td>
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<tr>
<td>Time from feeding to first SP^7 (min)</td>
<td>154 ± 124</td>
<td>95 ± 64</td>
<td>0.0715</td>
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</tbody>
</table>

Presented as means ± standard deviation

^1Amount GH secreted in pulses over the 24-hr period

^2Amount of GH secreted in pulses during nocturnal hours (0100-0700)

^3Number of large pulses occurring during nocturnal hours (0100-0700)

^4Number of large pulses occurring during postprandial times.

^5Time to first large pulse after concentrate feeding

^6Amount of GH secreted in first large postprandial pulse

^7Time to first small pulse after concentrate feeding
Figure 2-1. Visual representation of experimental design.

Twelve Quarter Horse weanlings were split into two groups and balanced by sex, weight, age, and body condition. Group 1, solid line, was on HG treatment for period 1 and LG for period 2. Group 2, dashed line, began on LG treatment before switching to HG. For the first 5 days of each period, horses were transitioned to the respective concentrate (indicated by the shaded boxes). Total concentrate was split into two meals per day (0700 h, 1545 h). Total concentrate fed on d 1 was 0.75 kg, d 2 and 3 were 1.5 kg, d 4 and 5 were 2.25 kg, with the full amount of 3 kg fed on d 6-21. The last day (d 21) of each period consisted of a 24-hour blood sample collection.
Figure 2-2. Schematic of hormone and metabolite analysis over 24-hour period.

Growth hormone was quantified in every sample. Glucose, insulin, and non-esterified fatty acids (NEFA) were measured hourly, with more frequent sampling following concentrate meal feeding. Meals were fed at 0700 h (AM) and 1545 h (PM).
Figure 2-3. Average daily weight gain by period.

Average daily weight gain by period, in kilograms/day, presented as means ± standard errors. Period 2 decreased compared to period 1 ($P=0.0061$).
**Figure 2-4.** Glucose concentrations over 24-h period in horses fed HG (high-glycemic) and LG (low-glycemic) concentrate meals at 0700 h and 1545 h.

Presented as means ± standard errors.
**Figure 2-5.** Insulin concentrations over 24-h period in horses fed HG (high-glycemic) and LG (low-glycemic) concentrate meals at 0700 h and 1545 h.

Presented as means ± standard errors.
Figure 2-6. Non-esterified fatty acid (NEFA) concentrations over 24-h period in horses fed HG (high-glycemic) and LG (low-glycemic) concentrate meals at 0700 h and 1545 h.
Figure 2-7. Growth hormone concentration over 24-h period in horses fed HG (high-glycemic) and LG (low-glycemic) concentrate meals at 0700 h and 1545 h.

Presented as means ± standard errors, average of all horses on both treatments (n=23).

Shaded areas represent hours when red lights were used for night time blood sampling.
CHAPTER 3
Further implications

This thesis has demonstrated that nutrition may be utilized to alter growth hormone secretion in young, growing Quarter Horses. Previous work in the horse has not thoroughly investigated growth hormone secretion utilizing deconvolution analysis and thus, most of the knowledge obtained from this work does not elucidate potential alterations in the regulation of GH secretion. It has been thoroughly documented that the pulsatile secretion of growth hormone, and other hormones, is critical for the downstream effects throughout the body (Desjardins, 1981, Urban et al., 1988, Veldhuis et al., 1987).

While few studies have investigated the effect of dietary energy sources on growth hormone secretion, there has been extensive work done in regard to GH’s role in lipid, protein, and carbohydrate metabolism, as well as in disease states such as insulin resistance, diabetes mellitus, obesity, and acromegaly. Based on the results of our study, it appears likely that by feeding the horse concentrate meals that elicit different physiological responses than would be expected in a grazing animal, it is likely that concentrate meal feeding is affecting growth hormone secretion in a manner that is counter to the animal’s evolutionary metabolism. Repeating this feeding practice daily throughout the life of the animal is likely to affect nutrient partitioning, growth, and potentially disease outcomes in the domesticated horse. Below I will highlight some of the areas that I think are most likely to be influenced by altered patterns of growth hormone secretion.
Skeletal development

In the growing horse, the goal is to raise an animal that has a sound skeletal foundation with strong bones to prevent lameness and a large size that may improve athletic ability. Growth hormone stimulates longitudinal bone growth through direct actions, by binding to growth hormone receptors on the chondrocyte, and through the stimulation of IGF-I production through endocrine, paracrine, and/or autocrine mechanisms (Isaksson et al., 1991). In a study of hypophysectomized rats, pulsatile infusions of GH lead to greater body weight gain in comparison to continuous GH administration, though the total amount of GH given did not vary (Clark et al., 1985). Bone growth of the proximal tibia was increased in animals given equivalent total amounts of GH split into 3 or 9 pulses per day compared to those given 1 pulse of GH per day (Clark et al., 1985). Pulsatile administration of GHRP-6, a synthetic ghrelin mimetic, increased skeletal growth, epiphyseal plate width, and IGF-I concentrations in growth retarded (Tgr) rats (Wells and Houston, 2001). While continuous GHRP-6 administration also increased these measurements of growth, the effects waned after 3 days of injections, while the rats receiving pulsatile GHRP-6 had a greater rate of weight gain and increased epiphyseal plate width after 7 days of injections (Wells and Houston, 2001).

There has been little research investigating alterations in pulsatility in wild-type mice or other animal models, so thus it is difficult to know whether the alterations in the growth hormone secretory patterns of the Quarter Horses in this study would elicit drastic changes in growth. However, the duration that horses are maintained on concentrate diets may be critical for investigating potential outcomes. In most husbandry circumstances,
horses are maintained on concentrate meals from weaning (4-6 months of age) through adulthood. During puberty, growth hormone concentrations are their highest as compared to other life stages in human and animal models. Corresponding increases in body mass and skeletal growth also occur at this time, and alterations in GH made through dietary energy source fed to the horse may impact skeletal growth. The HG horses in this study displayed greater GH pulse mass following a high-glycemic meal, potentially increasing rate of body weight gain and skeletal growth.

Histological examination of the growth plate cartilage of weanlings fed 130% of their digestible energy requirements had increased growth plate thickness alongside a more disorganized columnar structure than those fed 100% DE (Glade and Belling, 1984). While there is not great detail regarding the dietary energy of these horses, corn is listed as a primary nutrient source. Corn is known to cause a significant increase in glycemic and insulinemic response, particularly as a primary ingredient in the ration (Vervuert et al., 2004). Young horses (<2 years old) with osteochondrosis dissecans (OCD) lesions displayed greater glycemic and insulinemic responses to a meal lesions as compared to those with no radiological OCD lesions (Ralston, 1996). Thus, the glycemic and insulinemic responses to greater amounts of corn in the diet may further emphasize a link between dietary energy source, total amount of energy, growth hormone secretion, and impact on bone growth.

The relationship between glycemic and insulinemic responses to a concentrate meal and alterations in growth hormone secretion have been demonstrated in our study, providing a causal link between the reported associations of increased energy intake and glycemic and insulinemic responses with skeletal abnormalities in the horse. Work in the
rodent emphasizes the importance of GH pulsatility on its actions at skeletal tissue. Further work investigating skeletal integrity in horses with demonstrated alterations GH secretory patterns as well as monitoring animals for orthopedic disease outcomes would allow for further elucidation of these mechanisms.

**Metabolic perturbations and disease**

Altered growth hormone secretion has been linked extensively to conditions such as insulin resistance and obesity in human and animal models but has not been replicated to the same degree in the equine literature (Kreitschmann-Andermahr et al., 2010). Additionally, these metabolic disorders have been linked to diets with a greater non-structural carbohydrate fraction in the horse (Hoffman et al., 2003). The high-glycemic concentrate fed to horses in this study altered growth hormone secretion as compared to the low-glycemic concentrate, which may have further implications for insulin sensitivity and obesity. It is important to note that research investigating the pattern of growth hormone secretion, i.e. temporal occurrence of secretion and mass of secretory pulses, has not been conducted in healthy humans or animals in response to meal consumption. Findings are often reported in individuals with conditions such as acromegaly, growth hormone deficiency, insulin resistance, diabetes, or obesity. However, extrapolating this information to potential outcomes that may befall healthy young horses maintained on high-glycemic diets may be useful and points to areas in need of research.

The high-glycemic meals fed in this study elicited elevated insulin responses and increased growth hormone mass in postprandial secretory pulses. Independently, these
responses may perpetuate insulin resistance and their combined effect has not been
investigated in healthy human or animal models. Individuals receiving low-dose growth
hormone replacement therapy (9.6 μg/kg body weight per day) have decreased insulin
sensitivity following 6 months of daily injections (Bramnert et al., 2003). Overnight
infusions of physiological GH concentrations in growth hormone deficient individuals
also caused transient insulin resistance (Krusenstjerna-Hafstrom et al., 2011). Both
growth hormone and insulin are pleiotropic hormones and share signaling pathways and
cellular functions that promote cell proliferation and differentiation (Dominici and Turyn,
2002). This also may account for insulin-like functions of growth hormone, in which GH
facilitates glucose uptake into tissues in GH-deficient individuals. This “crosstalk”
between the signaling pathway of GH and insulin has been proposed as an explanation
for decreased insulin sensitivity in patients with excessive GH or receiving therapeutic
GH (Dominici and Turyn, 2002). The mechanisms by which excess GH decreases insulin
sensitivity have not been fully elucidated, though it has been proposed that GH
phosphorylates the insulin receptor by GH so that insulin is unable to bind (Dominici and
Turyn, 2002). While the high glycemic diet fed in this study may promote the
development of insulin resistance, it is important to remember that HG horses had an
inhibition of GH secretion while insulin concentrations were elevated and experienced
their first large pulse of GH approximately 70 min after peak insulin had been reached.
The suppression of GH during the time of elevated insulin concentration may not
immediately alter glucose and insulin metabolism. However, if diet alone is responsible
for the development of insulin resistance, then higher circulating insulin concentrations
may inhibit GH secretion, which would further support the hypothesis that insulin
inhibits GH secretion. Future work in this area is necessary, particularly in regard to the ontogenic alterations in insulin sensitivity and GH secretion in horses fed high glycemic meals.

Insulin resistance often occurs alongside a cluster of metabolic issues, including diabetes and obesity (Frank et al., 2010). Relationships between obesity and components of the somatotropic axis have been studied extensively in human subjects as compared to the horse. In comparison to lean individuals, obese children and adults tend to have a severely blunted growth hormone secretory pattern, with pulsatile episodes that are difficult to detect, decreased pulse amplitude, and an overall decrease in growth hormone secreted (Kreitschmann-Andermahr et al., 2010). However, investigation into alterations in the pattern of GH secretion have not been thoroughly documented, as the emphasis in the literature is on “blunting” of GH secretion observed in obese humans and animals. By administering GH to obese individuals in a pulsatile manner, 24-hour mean plasma GH concentrations increased to value comparable to those seen in lean controls and elicited a secretory profile with discernable pulses similar to those seen in controls (Surya et al., 2009). Continuous infusion of GH achieved 24-hour mean GH concentrations similar to that of lean controls, but secretion was still blunted as compared to pulsatile administration and lean controls (Surya et al., 2009). This emphasizes the importance not only of the amount of GH circulating, but that the pattern by which it is secreted is physiologically important. For the obese horse, integration of high-glycemic meals to augment GH pulses is likely not practical. High-glycemic concentrates may perpetuate concordant issues of insulin sensitivity and may be difficult to incorporate into a diet that should be focused on created an energy deficiency to promote weight loss.
Potential benefits for the adult horse

Modifying the diet of a horse is one of the easiest modifications available to the horse owner or professional. This study provides a more precise understanding of the relationship between nutrition and metabolism and indicates we may be able to more precisely regulate metabolism through nutrition. Growth hormone concentrations have been documented to decrease throughout adulthood in human and other animal models. Concordantly, aging is associated with sarcopenia, decreased muscle mass. Performance horses often compete at the highest levels well into adulthood, which is comparable to a 35-60 year old human athlete participating at the Olympic level. Exercise, which promotes GH secretion, is recommended in humans to prevent the occurrence of sarcopenia, in part due to GH’s protein-sparing effects. Aged horses may benefit from the larger mass of GH secreted following a high-glycemic concentrate meal observed in weanlings. Thus, modulating GH secretion through nutrition may be useful in addressing sarcopenia in aging populations.

Additionally, augmenting growth hormone secretion through nutrition may be useful in aging performance horses undergoing a layoff due to injury. Tendon and ligament injuries are common problems in the athletic horse and often require 3-6 months of limited exercise to facilitate healing, yet this undoubtedly reduces muscle mass. Promoting growth hormone secretion through larger secretory episodes as was reported in this study may assist these injured horses by utilizing growth hormone’s protein-sparing qualities to reduce muscle mass loss during the layoff or to decrease the time for the horse to return to its pre-injury level of performance.
Conclusions

This study demonstrates that growth hormone secretion may be modulated by nutrition, specifically in regard to meal feeding young horses. Given the broad actions of growth hormone in the body, these nutrition-induced alterations may have multiple outcomes, which may or may not be of benefit depending on the individual animal. Further research regarding equine growth hormone secretion is necessary to understand these mechanisms and it is critical that future work utilize sufficient methods of growth hormone analysis to address these questions.
Literature Cited


APPENDICES

Appendix A
Assay Procedures

Glucose Quantification

Stanbio Glucose Procedure No. 1075

The enzymatic glucose reagent provided was prepared as directed in the kit instructions (250 mL H₂O added to powdered reagent). A standard curve with six points was prepared by performing serial dilutions of a provided glucose standard (100 mg/dL). A laboratory control was utilized on all plates. Plasma samples collected in sodium heparin tubes were assayed in duplicate on a 96-well plate by pipetting 3 µL sample and 10 µL of triple-distilled H₂O in each well. Glucose reagent (300 µL) was added to each well for a 100:1 ratio as recommended in the kit protocol. Sample and reagent were mixed by placing plate on a microplate shaker at 650 rpm for 1 minute and allowed to incubate for 20 min. Samples were read using a Wallac2 plate reader at 490 nm. A linear regression was performed on the standard curve and unknown sample concentrations were interpolated from this equation.

Non-esterified Fatty Acid Quantification

Wako Diagnostics HR NEFA(2)
This colorimetric assay utilizes two enzymatic reactions to detect non-esterified fatty acids (NEFA) in plasma samples. A standard curve with six points was prepared by performing serial dilutions of a provided NEFA standard of oleic acid (1000 µEq/L). Controls were provided by the manufacturer and were prepared as directed. Color reagents A and B were prepared according to kit instructions. Plasma samples collected in EDTA tubes were assayed in duplicate on a 96-well plate by adding 5 µL of sample into each well. Color reagent A was added (200 µL/well) and mixed on a microplate shaker at 650 rpm for one min followed by a 5 min incubation at 37°C. Absorbance was measured at 550 nm on a Wallac2 plate reader, which served as the absorbance 2. Color reagent B was then added (100 µL/well), mixed on a microplate shaker at 650 rpm for one min and then incubated at 37°C for 5 min. Absorbance was measured at 550 nm on a Wallac2 plate reader, which served as absorbance 1. Final absorbance was determined by subtracting absorbance 1 from absorbance 2 for each well. A linear regression was performed on the standard curve values and unknown samples were interpolated from this equation.

**Insulin Quantification**

*Siemens Coat-A-Count Radioimmunoassay*

This competitive solid-phase radioimmunoassay utilizes ¹²⁵I-labeled insulin and antibody-coated tubes provide in the kit to quantify unknown insulin concentrations. Standards and controls were prepared by manufacturer’s direction, with additional standards created to elucidate unknown values below the original standard curve. Two
additional standards were created by diluting the lowest standard with a standard with no insulin, creating standards with a 1:2 and 1:4 dilution of the original lowest standard.

Standards were assayed in triplicate and samples were assayed in duplicate, with 200 µL of standards and sample added to an antibody-coated tube. Within 45 min, 1 mL of $^{125}\text{I}$-labeled insulin was added to each tube, vortexed for 30 seconds, and allowed to incubate overnight (18-24 hours) at room temperature. Following incubation, tubes were decanted to remove supernatant and isolate antibody-bound insulin. Radioactive counts for each tube were detected by counting each tube in a 1470 Wizard Automatic Gamma Counter for 60 seconds. Net counts for each standard and sample were calculated by subtracting the non-specific binding (NSB) tubes from the average counts per min (CPM) for the standard/sample. Then, percent binding was determined as net counts/net maximum binding counts. The standard curve was plotted after a log-logit transformation and sample values were determined.

**Growth hormone**

Growth hormone was quantified in the laboratory of Dr. Donald L. Thompson at Louisiana State University utilizing a double-antibody radioimmunoassay. Standards and samples were run in duplicate and laboratory controls were used. Radioiodinated porcine growth hormone (pGH) and an antiserum for pGH were utilized. Briefly, 300 µL antiserum containing antiserum against pGH in PBS buffer with 0.033 M EDTA, 0.112% normal rhesus monkey serum, and 33% nonreactive equine serum was added to each tube. Addition of 200 µL of unknown samples competed with radioiodinated pGH for
binding to the primary antibody. Antiserum against rhesus monkey IgG was used to precipitate the first antibody (200 µL/tube). The radioactive counts in the precipitate were quantified. The original validation reported a sensitivity of 0.1 ng.
Appendix B

Individual responses for glucose, insulin, non-esterfied acids, and growth hormone over 24 hours.

Horse 1 - Nic A Cee 2010
Horse 1- Nic A Cee 2010

- NEFA uEq/L

- Growth Hormone, ng/mL

Time of Day
Horse 2 - Verse 2010

![Graph showing glucose and insulin levels over time for high and low glycemic conditions.](image-url)
Horse 2- Verse 2010

[Graph showing NEFA (Ueq/L) levels over time with AM and PM meals indicated]

[Graph showing Growth Hormone (ng/mL) levels over time with AM and PM meals indicated]
Horse 3 - Te 2010
Horse 3- Te 2010

![Graph of NEFA uEq/L vs Time of Day with AM and PM Meal markers.]

![Graph of Growth Hormone ng/mL vs Time of Day with AM and PM Meal markers.]

- High Glycemic
- Low Glycemic
Horse 4- CJ 2010

**Glucose, mg/dL**

- AM Meal
- PM Meal

**Insulin, mU/L**

- AM Meal
- PM Meal

**Time of Day**
Horse 4- CJ 2010

Graph 1: NEFA uEq/L

Graph 2: Growth Hormone, ng/mL

Time of Day
Horse 5- Willy 2010

![Graph of Glucose Levels](image1)

![Graph of Insulin Levels](image2)
Horse 5- Willy 2010

**NEFA uEq/L**

**Time of Day**

**Growth Hormone, ng/mL**

**Time of Day**

- High Glycemic
- Low Glycemic
Horse 6- Blue 2010

![Graph of glucose levels over time with AM and PM meals indicated.]

![Graph of insulin levels over time with AM and PM meals indicated.]

- High Glycemic
- Low Glycemic
Horse 6 - Blue 2010
Horse 7- Tango 2010

Graph showing glucose levels over time with two meal times marked as AM and PM.

Graph showing insulin levels over time with two meal times marked as AM and PM.
Horse 7- Tango 2010

[Graphs showing NEFA and Growth Hormone levels over time with AM and PM meals indicated]
Horse 8- Patty 2010

Graph showing glucose levels and insulin levels over the course of a day with AM and PM meals indicated.
Horse 8- Patty 2010
Horse 9: Indy 2010

- Graphs showing glucose and insulin levels over time, with meal times indicated.
Horse 9- Indy 2010

Graph 1: NEFA uEq/L

Graph 2: Growth Hormone, ng/mL
Horse 10- Whisper 2010

The graphs show the glucose and insulin levels over the course of a day, with peaks during AM and PM meals. Glucose levels are depicted on the left graph, with ranges from 75 to 175 mg/dL, and insulin levels are shown on the right graph, with ranges from 0 to 20 mIU/L. The graphs illustrate the differences between high glycemic and low glycemic conditions.
Horse 10 - Whisper 2010

NEFA uEq/L

Time of Day

Growth Hormone, ng/mL

Time of Day
Horse 11 - Lady 2010

![Graph of Glucose levels over time showing AM and PM meals with high and low glycemic levels.](image)

![Graph of Insulin levels over time showing AM and PM meals with high and low glycemic levels.](image)
Horse 11- Lady 2010

[Graph showing NEFA uEq/L vs Time of Day with AM and PM Meals indicated, and data points for High Glycemic and Low Glycemic lines.

Graph showing Growth Hormone ng/ml vs Time of Day with AM and PM Meals indicated, and data points for High Glycemic and Low Glycemic lines.]
Horse 12- Cassie 2010

Graph showing glucose levels over time for AM and PM meals.

Graph showing insulin levels over time for AM and PM meals.
Horse 12- Cassie 2010

- NEFA uEq/L
- High Glycemic
- Low Glycemic

- AM Meal
- PM Meal

- Growth Hormone, ng/mL
- High Glycemic
- Low Glycemic

Time of Day
Appendix C

Growth hormone characteristics, by horse

<table>
<thead>
<tr>
<th>Horse</th>
<th>GH Half-Life (min)</th>
<th>Basal Secretion Rate (ng/mL)</th>
<th>Mean GH Concentration (ng/mL)</th>
<th>Number of Secretory Episodes</th>
<th>Growth Hormone AUC</th>
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Growth hormone secretory characteristics, by horse continued.

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<th>Horse</th>
<th>Total GH Secreted (ng/mL)</th>
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<th>% Pulsatile Secretion</th>
<th>Approximate Entropy Ratio</th>
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Appendix D

Ghrelin Quantification

Brief introduction

Acyl ghrelin is a growth-hormone secretagogue and may augment growth hormone secretion in the fed state. Additionally, dietary energy sources have been demonstrated to have differential effects in fluctuations of acylated ghrelin. Acylated-ghrelin is the “active” form of ghrelin, in that it promotes the release of growth hormone and is active orexigenically. Previous equine research examining ghrelin is limited and has utilized a radioimmunoassay for active ghrelin by Linco. However, a sandwich-type assay developed at the University of Virginia was demonstrated to be more sensitive in detecting fluctuations in acyl ghrelin, yet has not been utilized in the horse. The octanoyl group that defines ghrelin as acyl ghrelin is labile and is rapidly degraded by esterase. Immediate processing following blood sampling to accurately quantify active ghrelin.

Preparation of Samples

Plasma samples were prepared for ghrelin analysis using the protocol described in for the Linco Active Ghrelin RIA kit (GHRA-88HK). Blood samples were collected in EDTA tubes, kept on ice, and centrifuged at 1400 x g. A 1-mL aliquot of plasma was placed into a microcentrifuge tube containing 50 μL of 1 N HCl and 10 μL of
phenylmethylsulfonyl fluoride (PMSF). Phenylmethylsulfonyl fluoride was prepared in a solution of 100% methanol at a concentration of 10 mg/mL.

**Assay Selection**

Upon further investigation into the assays available for quantifying active ghrelin, the sandwich assay utilized by the University of Virginia was thought to be superior to the Linco RIA for addressing the questions in this study. A comparison of the two assays determined the UVA assay to be more sensitive (Prudom et al., 2010). However, the plasma preparation differed between assays and the protocol for the UVA assay is described in (Liu et al., 2008). The recommended plasma sample preparation for the UVA assay collected blood samples in to a chilled EDTA vacutainer loaded with 4-[2-aminoethyl benzene] sulfonyl fluoride (AEBSF) and kept on ice. Tubes were then centrifuged and plasma was acidified with 200 μL 1 N HCl per milliliter of plasma. The at increased HCl was fully effective at denaturing esterase, where addition 50 μL HCl/mL plasma the esterase only reduces activity to 80% of initial activity in human plasma (Gaylinn, personal communication).

**Preliminary Results**

Preliminary investigation into validating the UVA assay for use in the equine was conducted utilizing a subset of 10 samples. Acyl ghrelin flux, both above and below baseline, following a carbohydrate load (primarily consisting of glucose) was greater than
observed in protein or lipid loads (Foster-Schubert et al., 2008). Selected samples were taken from 5 horses fed the high-glycemic concentrate on d 2. Time points selected were 120 min (immediately prior to the morning meal) and 195 min based on typical peak and nadir acyl ghrelin peaks and nadirs observed in humans. Samples were shipped to the University of Virginia and acidified with HCl to a concentration of 200 μL 1 N HCl per milliliter of plasma. Samples were analyzed in duplicate for acyl and des-acyl ghrelin.

Previously documented active ghrelin in horses has been recorded between 20-60 pg/mL (Gordon and McKeever, 2005, 2006). The UVA assay did not appear to detect any greater concentration of acyl ghrelin than the Linco kit. The difference between pre- and post-prandial measurements did not appear to change significantly nor consistently in the subset. Further investigation into acyl ghrelin concentrations was discontinued based on these inconsistent results.

Table D-1. Preliminary ghrelin results

<table>
<thead>
<tr>
<th>Horse</th>
<th>Acyl-ghrelin (pg/mL) Before meal (120 min)</th>
<th>Acyl-ghrelin (pg/mL) After meal (195 min)</th>
<th>Des-acyl ghrelin (pg/mL) Before meal (120 min)</th>
<th>Des-acyl ghrelin (pg/mL) After meal (195 min)</th>
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</table>
Literature Cited


VITA

Sarah Marie Gray

Sarah Marie Gray graduated from Delton Kellogg High School in 2003 and received her B.S. in Animal Science from Michigan State University in 2007. She was an undergraduate research assistant under the tutelage of Dr. Hilary Clayton at the McPhail Equine Performance Center at Michigan State University’s College of Veterinary Medicine from 2005-2007. After receiving her B.S., Sarah worked internationally in the equine industry throughout Asia and Europe before enrolling as a graduate student at the Pennsylvania State University in 2009. Under the supervision of Dr. W. Burton Staniar, she investigated the effects of glycemic meal responses on growth hormone secretory patterns in horses utilizing deconvolution analysis. Her future plans are to amalgamate her love of show jumping, physiology, traveling, and teaching into a career.