ENDOCRINOLOGY OF EQUINE METABOLIC PATHOPHYSIOLOGY

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by

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ENDOCRINOLOGY OF EQUINE METABOLIC PATHOPHYSIOLOGY

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DEDICATION

I would like to dedicate this dissertation to the memory of my granny Emily Larson, my grandma Alma Nyhus, and my auntie Wanda Larson. They were each, in their own way, a picture of strength, courage, and kindness and I miss them dearly.

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ENDOCRINOLOGY OF EQUINE METABOLIC PATHOPHYSIOLOGY

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ABSTRACT

Obesity in horses is an emerging field of study and while our understanding of obesity in horses is improving, much of our current understanding is based on data from human and rodent studies. In some aspects the human and rodent data is directly applicable to the horse, while in other aspects, the dramatic disparity that exists in the digestive system and feeding patterns of the horse limits extrapolation and application of the data to the horse. A unique concern with obese horses (that is not apparent in humans or rodents) is their predisposition to develop laminitis. Therefore, to understand the patho-physiology of obesity and related maladies in horses, a series of characterization and challenge studies were conducted.

Our initial studies focused on the earliest stages of growth and development of the horse. Data gleaned from rodent, human, and sheep studies provided evidence that nutrition of the dam and stress on the dam during gestation and lactation could manifest endocrine responses that would alter neonatal physiology of the offspring. Again, because of the limited data which exists in horses, we sought to identify metabolic hormones and growth factors present during the peri-parturient period that may affect neonatal physiology. Therefore, the objectives of the first study were to characterize the endocrine profiles of leptin, insulin-like growth factor-1 (IGF-1), and thyroid stimulating hormone (TSH) in the blood and milk of peri-parturient mares and in the blood of their

offspring. We found that in the milk, the highest concentration of all three hormones occurred in the milk sample taken within 2 h of parturition and before the foals first suckled. Not only did this work yield novel information on leptin concentrations in the neonatal foal, these data also complemented other studies on peri-parturient concentrations of serum IGF-1 in mares and foals.

In our second study, we examined the relationship between adipokines and adrenocorticoid hormones. It is well-documented that adiposity is positively correlated with peripheral leptin concentrations and it has been suggested that leptin plays a role in hypertension through activation of the sympathetic nervous system in obese individuals. Additionally, adipocytes have recently been found to secrete potent mineralocorticoid secretagogues providing evidence of a direct link between adiposity and hypertension. Therefore, in order to investigate the relationship between leptin, aldosterone, and cortisol, the mineralocorticoid receptor antagonist spironolactone was administered to pony mares for seven days. A single dose of spironolactone administered to ponies has previously been found to significantly increase sodium excretion (an action that is antagonistic to aldosterone) and peripheral concentrations of spironolactone have been found to peak at approximately three days in humans; therefore we felt seven days would be adequate to observe a difference in hormone profiles. Our hypothesis was that leptin concentrations would be altered in response to spironolactone treatment and that concentrations of aldosterone and cortisol would coincidently increase in the blood in response to spironolactone treatment. In contrast to our predicted outcome, we found no change in peripheral concentrations of cortisol or leptin in response to spironolactone, but as predicted, we observed a trend for a transient increase in aldosterone concentrations in

the blood of spironolactone treated ponies over time. The precise reason for the tendency of increasing aldosterone over time is not known. Based on reports in the literature, however, the antagonistic action of spironolactone on the mineralocorticoid receptors in the distal convoluted tubules of the kidneys likely prevented binding of aldosterone to those receptors. As a result of this disruption in the negative feedback loop, renin secretion from the kidney increased, ultimately elevating aldosterone concentrations in the blood (Garthwaite and McMahon, 2003). Because the receptors are bound by spironolactone, the body fails to recognize the elevated concentrations of aldosterone in the blood, thus the negative effects of hyperaldosteronism are abated. Further work is necessary to elucidate the relationship between adipokines and adrenocorticoids in equine.

In our third study, we investigated the effects of lipoic acid supplementation on blood glucose and insulin responses, as well as its effects on peripheral concentrations of leptin in pony mares. We hypothesized that lipoic acid treated ponies would have improved insulin responsiveness and glucose disposal, as well as altered blood concentrations of leptin. In contrast to our hypothesis, we found no difference in blood glucose or leptin concentrations due to lipoic acid treatment; however there was a trend for decreased insulin levels to occur during our modified i.v. glucose tolerance test. The decreased concentrations of insulin may be evidence that lipoic acid improved insulin effectiveness; since less insulin was required to dispose of the same amount of glucose from blood. Further investigation into the application of lipoic acid supplementation is warranted.

Investigation of the etiology and pathophysiology of obesity and related

conditions in equine is necessary to improve the understanding of these maladies. In addition, the development of sound management techniques based on scientific data is imperative to effectively control obesity as well as associated disorders in the horse. These studies provide the groundwork for further research in the area of equine obesity.

CHAPTER I

INTRODUCTION

Obesity is a growing concern in the horse industry that can be attributed to any number of genetic or environmental factors. Easy access to better quality of feedstuffs, combined with owners over-feeding their horses, are examples of contributing causes to the problem of equine obesity (Marcella, 2004). Additionally, the "thrifty genotype" and (or) "thrifty phenotype" hypotheses may help to explain the predisposition of some equine to becoming obese. Robert Eustace, Director of The Laminitis Clinic in Wiltshire, England has gone so far as to state that "*horse obesity* [should] *be declared a welfare concern*" (Jurga, 2003). It is obvious that owner education is necessary to resolve certain aspects of these issues relative to nutrition and feeding of the horse. Although this is being addressed by some individuals in the horse industry (The Horse, 2006), elucidation of the complex paradigm of appetite regulation, energy intake and expenditure, as well as the hormones and mechanisms involved in the metabolic physiology of equine is warranted.

The studies presented herein were designed to identify select metabolic hormones and growth factors present during the peri-parturient period in mares and foals and to investigate the relationship between adipokines, adrenocorticoids, and insulin sensitivity. In the first study, endocrine profiles of leptin, IGF-1, and TSH were characterized in mare and foal blood and in mare milk from 2 weeks prior to parturition until 2 months after parturition. The second study investigated the relationship between adipokines and

adrenocorticoids in equine by measuring peripheral concentrations of leptin, aldosterone, and cortisol after administration of a mineralocorticoid receptor antagonist for seven days. In the third and final study, leptin concentrations and glucose and insulin dynamics were evaluated after supplementation with an insulin sensitizing agent (lipoic acid) for 14 days. In short, our goals were to achieve a better understanding of equine metabolic physiology, as well as investigate possible strategies to ameliorate obesity and related maladies in equine.

CHAPTER II

LITERATURE REVIEW

Equine metabolism and energetics

Feeding Behavior

Feed intake in equine is dependent upon a number of factors, including state of energy balance, body condition score, stage of growth, quality and availability of feedstuffs, temperate environment, herd dynamics/housing, reproductive state, level of fitness, and health of the animal. The digestive tract of the horse is characterized by a relatively small stomach (8 % of GI tract) and comparatively large hindgut (40 – 50% of GI tract). The fact that the horses' stomach is small provides some indication that equine are not designed to consume one or two large meals each day. Rather, they are designed to consume small, frequent meals which coincide with their natural feeding pattern. Feral horses will graze for up to 15 hours per day (Duncan, 1979) with home ranges of up to 32 square kilometers (Feist and McCullough, 1976). It has been suggested that perhaps the reason feral horses spend nearly 65% of their day eating, is out of necessity (Dr. Gary Potter, personal communication). In the U.S., feral horses typically have access to low quality forage and therefore they *must* spend the majority of their time foraging in order to meet their daily energy and nutrient requirements. Similar to their feral counterparts, domesticated lactating mares with free access to adequate pasture reportedly graze up to 16 h per day (Crowell-Davis et al., 1985). In stark contrast, horses housed in stables have limited access to pasture (and thus grazing time) and, depending on management, spend

significantly less time eating throughout the day and more time standing idle (Fulmer, 1995). A reduction in foraging time and access to free exercise as well as increased intake of high-energy feeds has been implicated in the development and expression of stereotypical behavior in horses (Waters et al., 2002; McGreevy et al., 1995). In this context, a stereotype is defined as relatively unchanging, repeated patterns of behavior performed for no apparent purpose (Fraser and Broom, 1990), and expression of these behaviors may be an indication of reduced animal welfare (Goodwin et al., 2002; Waters et al., 2002; Fraser and Broom, 1990).

Appetite regulation

Regulation of appetite in mammals is a complex paradigm controlled by two general pathways that contribute to the body's awareness of current energy status. Briefly, the first pathway involves hormonal signals that are ultimately integrated in the hypothalamus. Peripheral hormone signals include leptin, ghrelin, insulin, cholecystokinin (CCK), and pancreatic polypeptide YY (PYY; Broberger, 2005); to mention a few of those involved. Two sets of neurons, having receptors for these peripheral endocrine signals, are located in the arcuate nucleus of the mediobasal hypothalamus. One set of neurons expresses anabolic/orexigenic (appetite stimulating) neuropeptide Y (NPY) and Agouti-related peptide (AgRP), and another set of neurons expresses catabolic/anorexigenic (causes loss of appetite) pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART; Cripps et al., 2005).

The second major pathway contributing to the body's awareness of current energy status involves the brainstem. The vagus nerve carries information from the

gastrointestinal tract to the brainstem nucleus tractus solitarii (nTS). This vagal input senses GI tract distension and the presence (or absence) of CCK and other appetite-regulating compounds (Broberger, 2005). These systems work together to stimulate or inhibit feeding behavior and are explained in greater detail hereafter.

NPY**.** Neuropeptide Y is considered a hunger signal and is secreted in response to increased energy demands and during the fasted state so as to increase feed intake (Cone, 2005; Erlanson-Albertson, and Zetterström, 2005). Few studies have investigated the role of NPY relative to appetite in the equine, however it has been demonstrated in sheep that intracerebroventricular (i.c.v.) injection of NPY increases feed intake and can overpower / suppress satiety factors such as artificial distension of the reticulorumen (Miner, 1992). Similar work in rodents revealed that NPY was a potent stimulator of appetite (Cripps et al., 2005).

POMC**.** Pro-opiomelanocortin is expressed in the skin, immune system, pituitary gland, the arcuate nucleus of the hypothalamus and the nTS (Pritchard et al., 2002). Pro-opiomelanocortin is a prohormone that is cleaved upon secretion into a number of peptides including beta endorphin, adrenocorticotrophic hormone (ACTH), and melanocyte stimulating hormones (MSH). The POMC pathway generally functions to induce satiety and increase fat metabolism. Donaldson (2004) reported that a correlation existed between α-MSH and body mass index (BMI) of horses. The investigators stated that further research would be required to determine whether this correlation was due to α-MSH plasma concentrations or a defect in melanocortin receptors.

Leptin. Leptin is an anorexigenic peptide synthesized and secreted primarily by white adipose tissue. Leptin receptors are found on NPY, AgRP, and POMC neurons in

the hypothalamus. During the fed state, leptin has an inhibitory effect on NPY and AgRP gene expression and a stimulatory effect on POMC gene expression; however during the fasted state, expression of AgRP and NPY mRNA are upregulated and expression of POMC mRNA are downregulated (Cone, 2005). Presumably leptin possess more of a permissive role in hunger signaling (i.e. via low leptin levels) rather than a role as a satiety signal (Erlanson-Albertson and Zetterström, 2005). The effect of leptin infusion in horses has not been investigated.

It is well documented that blood concentrations of leptin are positively correlated to BCS and fat mass in horses and other species (Buff et al., 2002; Kearns et al., 2005; Kershaw and Flier, 2004). However, feed restriction of up to 48-h decreased peripheral concentrations of leptin regardless of BCS in horses (Buff et al., 2005; McManus, 2000). Decreased levels of leptin in response to restricted nutrient intake makes physiologic sense due to leptin's anorexigenic effects, where decreased levels of leptin is permissive to facilitate satiation, albeit the anorexigenic effects of leptin has not been directly determined in horses. In a study by Gordon and McKeever (2006), plasma concentrations of leptin decreased due to a grain meal but increased as a result of i.v. dextrose administration. The decrease in plasma leptin after a grain meal was in contrast to that found in humans and other mammalian studies where either an increase or no change in plasma leptin concentrations were observed after a meal. The increase in plasma concentrations of leptin following an infusion of dextrose (as observed by Gordon and McKeever, 2006) does, however, agree with human and rodent studies. Modulation of peripheral concentrations of leptin by glucose and/or insulin has been suggested. Increased plasma leptin in horses have been demonstrated in response to a bolus injection

of insulin (Cartmill et al. 2005), as well as following an injection of dexamethasone into mares, geldings, and stallions (Cartmill et al., 2006). Gordon and McKeever (2005) found that peripheral concentrations of leptin were lower in fit vs. unfit horses and that fit horses also had lower BCS and % fat. The role of leptin as a signal of energy status deserves further investigation in the horse.

Insulin. Insulin is secreted from the beta cells of the pancreas in response to increased blood glucose and it functions to enhance glucose uptake by cells and stimulate lipogenesis. Insulin receptors are prominent in the arcuate nucleus of the hypothalamus and it has been reported that central (i.c.v.) administration of insulin decreases food intake, likely through inhibition of NPY (Cone, 2005; Cripps et al., 2005). Insulin anorexigenic effects are the natural physiological response to food intake (i.e. elevated blood glucose), however in the fasted state, insulin functions like leptin to modulate NPY and AgRP gene expression and decrease POMC gene expression (Cone, 2005).

Diet has been reported to influence glucose dynamics and insulin sensitivity in equine (Treiber et al., 2005). These investigators reported decreased insulin sensitivity and increased insulin secretion in seven month-old weanling horses fed a high-glycemic diet (high sugar and starch) twice daily since birth compared to those fed a low-glycemic diet (high fat and fiber) twice daily since birth. Similarly, Hoffman et al. (2003) reported lower insulin sensitivity in Thoroughbred geldings fed high- vs. low-glycemic diets over an eight week period. The authors noted that horses were of varying BCS and this may have confounded their results as obese horses are often insulin resistant. Aged mares adapted to a light exercise regimen for 7 days showed improved insulin sensitivity in response to a glucose challenge (Powell et al., 2002). The authors reported no changes in

BW, BCS, or % body fat in the mares, suggesting that improved insulin action was due to exercise and not fat loss. Diet and exercise appear to significantly affect insulin dynamics. Insulin resistance and hyperinsulinemia as related to obesity will be discussed in the following sections.

Ghrelin. Ghrelin is an orexigenic hormone secreted by the stomach tissue of horses, humans, rats, and other livestock species (Hayashida et al., 2001). Gordon and McKeever (2005) measured plasma ghrelin over 24-h and speculated that diurnal variations of ghrelin in horses may be attributed to feeding times. However, this does not appear to be exclusive, as ghrelin concentrations did not increase significantly in response to or in anticipation of a grain meal for horses in that study (Gordon and McKeever, 2005), nor in a follow-up study (Gordon and McKeever, 2006). This is contradictory to human and rat research that have determined that ghrelin concentrations typically increase in anticipation of a meal, stimulating feed intake and gastric emptying (Broberger, 2005). Gordon and McKeever (2005) concluded the reason there was no pre-prandial increase in ghrelin could be due to the fact that horses had hay in front of them throughout the sampling period and, as a result, constant hindgut activity may have prevented a pre-prandial rise in anticipation of a meal as observed in other species. Gordon et al. (2005) found ghrelin to be negatively correlated with body condition score in horses and reported greater fasting concentrations of active ghrelin in fit vs. unfit horses. This may be explained by the fact that a fit horse's brain predicts that it will require more intake of energy to meet the energy needs associated with training and increased physical activity.

In rats, exogenous and hypothalamic ghrelin treatments have been reported to

activate NPY and AgRP neurons and inhibit POMC neurons, thus increasing feed intake and weight gain. Treatments of NPY receptor antagonists block ghrelin-stimulated feed intake. Evidence for a vagal pathway for ghrelin signaling also exists because after vagotomy, stimulation of feed intake is blocked after peripheral (but not central; hypothalamic) administration of ghrelin (review by Cone, 2005). This indicates that by severing the vagal nerve, ghrelin secretion is not detected by the hypothalamic nuclei from the periphery.

CCK. Cholecystokinin is a well-characterized satiety signal secreted in response to the presence of fat or protein in the small intestine (Erlanson-Albertson and Zetterström, 2005). Receptors for CCK are present on the vagal nerve. Information regarding appetite is transmitted to the nTS via the vagus nerve and ultimately integrated in the brain. Functions of CCK include inhibition of gastric emptying (the stomach must be full for satiation), stimulation of intestinal motility and pancreatic enzyme secretion, and acute inhibition of feed intake (Cone, 2005). No information could be found relative to the role of CCK specifically in equine; however work by Lorenzo-Figueras et al. (2005) investigated the effects of a high-fat vs. high-carbohydrate diets on gastric emptying in horses. These investigators found no difference in gastric emptying times between the diets; suggesting that fat supplementation in horses may not have a remarkable effect on gastric motility, which is in contrast to most species. The reason for this disparity is perhaps because horses have evolved to eat a diet consisting primarily of carbohydrates and consume small, frequent meals throughout the day; thus a sense of satiety may be less developed in equine compared to humans. From a teological perspective, it would not make evolutionary sense to have a highly responsive satiety

system when the needs are to continually eat in order to consume adequate energy and nutrients for survival.

PYY. Pancreatic polypeptide YY is produced and secreted in the gastrointestinal tract of humans and rodents in response to lipids and carbohydrates (Cripps et al., 2005). Peripheral administration of PYY has been reported to decrease food intake and stimulate weight loss in rats and humans (Broberger, 2005). The role of PYY in the horse has not yet been investigated; however, because PYY secretion is stimulated by the presence of carbohydrates in the gut, it may be worth exploring in equine.

Adiponectin. Adiponectin is an adipocyte-derived hormone present in humans, horses, and a number of other species. There is evidence that adiponectin has insulin sensitizing properties in rodents (Haluzik et al., 2004), but this remains to be investigated in equine. Adiponectin has recently been reported to correlate negatively with fat mass in equine (Gordon et al., 2005; Kearns et al., 2005 and 2006), which is in agreement with human and rodent data, although the mechanisms behind this correlation are not fully understood. Plasma concentrations of adiponectin were found to be higher in fit vs. unfit standardbred horses (Gordon et al., 2005). These investigators hypothesized that increased adiponectin concentrations in fit horses could be related to a number of factors, but its inverse relationship to body mass (and therefore adiposity) was the most likely explanation. Further conclusions could not be drawn due to the non-descriptive nature of the study. Gordon and McKeever (2006) found no changes in plasma adiponectin concentrations in response to a grain meal or i.v. dextrose challenge, which is in agreement with studies of lean humans, and thus they concluded that acute glucose and insulin concentrations do not appear to regulate adiponectin secretion in horses. The role

of adiponectin in appetite regulation is yet to be elucidated.

Nutrient partitioning

The theory of "priority in partitioning of nutrients" proposed by Hammond (1950) maintains that tissues and organs compete for nutrients in proportion to their metabolic rate, thus tissues with higher metabolic rates take up nutrients more actively than those with lower metabolic rates (Figure 1). For example, the metabolic rate of the developing fetus is greater than that of the pregnant dam, so maternal resources are diverted first to the fetus and then to the dam. Bauman and Currie (1980) expanded on this notion and put forth the idea that the regulation of nutrient partitioning involved two types of control: homeostasis and homeorhesis. Homeostasis is defined here as "maintenance of physiological equilibrium or constant conditions in the internal environment" and homeorhesis is defined here as "orchestrated or coordinated changes in metabolism of body tissues necessary to support a physiological state." The authors use the lactating dairy cow as an example and reveal that during the first third of the lactation period, the nutrient demands of the mammary gland far outweigh that of the cow relative to total metabolism, consequently resulting in the cow being in an acute state of negative energy balance. It was recognized however that if a stressor of sufficient consequence was introduced (e.g. disease) that homestatic control for survival could essentially take over the homeorhetic control.

Donoghue et al. (1990) argue that minimization and prevention of tissue protein loss is perhaps the most important objective of animal nutrition. They contend that the cumulative loss of tissue protein via repeated mobilization of amino acids can be an

indicator of chronic stress in the animal and lead to immune system compromise, reduced fertility, and depressed activity. Moberg (2000) explains that when an animal has insufficient biological reserves to effectively deal with repeated stressors, those resources (e.g., tissue proteins) are shifted away from less essential functions (e.g., reproduction) leaving those biological functions are impaired. Ensuring minimal tissue protein loss may be achieved by providing adequate dietary protein and energy in a palatable form to the animal and in an environment conducive to feed consumption.

In contrast to protein and energy, adipose tissue mobilization is generally regarded as a beneficial physiologic mechanism (Donoghue et al., 1990) compared to protein mobilization. Catecholamines (dopamine, epinephrine, and norepinephrine) function to increase lipolysis through activation of β2-adrenergic receptors in subcutaneous adipocytes of horses (Carrington et al., 2003). Beta 2 adrenergic agonists, clenbuterol and ractopamine hydrochloride, enhance protein accretion (increase muscle mass) by minimizing protein degradation and increasing lipolysis by repartitioning nutrients away from fat deposition and towards lean tissue deposition.

In equine, clenbuterol is prescribed to alleviate symptoms of chronic obstructive pulmonary disease (COPD) because of its potent bronchodilation effects. However, it has been shown that chronic administration of therapeutic levels of clenbuterol to equine has a repartitioning action as well. Kearns et al. (2001) investigated the effects of exercise coupled with clenbuterol administration. They found significant decreases in body fat with clenbuterol treatment alone and with exercise at week 2 and with exercise alone at week 4 of the trial. These investigators speculated that one mode of action for the repartitioning effects of clenbuterol are due to its ability to stimulate lipase

production, which in turn increases adipocyte lipolysis. Kearns et al. (2006) investigated the effects of clenbuterol on adipokines and reported an increase in human equivalents of immunoreactive adiponectin (ir-adiponectin HE) and a decrease in human equivalents of immunoreactive leptin (ir-leptin HE) in horses treated with clenbuterol for 8 weeks.

Ractopamine hydrochloride is approved for use in swine and has been reported to improve ADG and feed efficiency, as well as decrease fat depth while increasing longissimus dorsi muscle area (Crome et al., 1996). Due to its effect on fat mass, the therapeutic use of ractopamine in obese pony mares was investigated by Buff et al. (2006a). These investigators reported a tendency for increased weight loss in ponies supplemented with ractopamine and demonstrated that a reduction of feed intake to 75% of *ad libitum* intake resulted in significant weight loss without exercise. The fact that weight loss occurred without exercise is important because it is not uncommon for obese ponies and horses to have foot pain associated with chronic laminitis, rendering them unable to exercise.

It should be recognized that both clenbuterol and ractopamine are banned substances under the purview of the United States Equestrian Federation, which is the governing body for all rated performance horse activities in the United States (U. S. Equestrian Federation, 2006). Penalties range from monetary fines to permanent suspension from the activity or sport. Detection of these substances in horses is accomplished by enzyme-linked immunosorbent assay (ELISA) of the urine (Lehner et al., 2004).

Physiology of equine obesity

Background

The accumulation of excess adipose tissue (obesity) is a growing problem in human and equine populations, giving rise to a multitude of detrimental conditions in both species. Obesity is a disorder of energy balance by which energy intake exceeds energy expenditure and excess energy is stored as fat (Margetic et al., 2002). In humans, obesity is often accompanied by hypertension (Pausova, 2006; Davy and Hall, 2004; Hall, 2000) and insulin resistance (Bastard et al., 2006; Kim et al., 2006), as well as subsequent development of Type II diabetes and cardiovascular disease (McGavock et al., 2006; Gogia and Agarwal 2006). In horses, obesity is associated with laminitis (Johnson, 2002), insulin resistance (Freestone et al., 1992; Jeffcott et al., 1986), and reduced reproductive performance (Sessions et al., 2004).

Thrifty genotype hypothesis

The thrifty genotype hypothesis was proposed by Neel in 1962 and maintains that a "thrifty genotype" is one that predisposes an individual to being highly efficient at food intake and utilization, as would have likely been necessary for the survival of our ancestors. Neel hypothesized that modern day diseases such as obesity, diabetes, and hypertension are the result of disharmony between the contemporary environment (sedentary, food-abundant Western lifestyle) and our genetics (that of hunter-gatherer peoples). Sharma (1998) expanded on this hypothesis suggesting unhealthy or "affected" individuals are in fact the normal and that healthy or "unaffected" individuals are those exhibiting recent mutations which lead to "loss of thriftiness." He provides evidence from current studies of the angiotensinogen gene in which a recently discovered mutation

reduced the risk of hypertension.

Similar observations have been made in equine populations. Researchers have speculated that horses and ponies, referred to as "easy keepers" have retained the metabolically efficient thrifty genotype (Johnson, 2002; Jeffcott et al., 1986), and that the "normal" metabolism of the modern equine could be the result of human intervention, breeding for traits "inconsistent with metabolic efficiency" (Buff et al., 2006). Support for this may exist in data from pony breeds that demonstrate an innate insulin resistance, and therefore a predisposition to obesity and laminitis (Jeffcott et al., 1986). Domesticated mustangs also often have a greater than average incidence of obesity and endocrinopathic laminitis than the average horse population (Johnson, 2002). Presumably the thrifty genotype of ponies and feral mustangs confers some survival benefits for grazing sparse, low quality forage but predisposes those same equine to becoming obese in modern management systems where they are often over-fed high quality forage and concentrate.

From a different perspective, consider that Holt and Byrne (2002) suggested that the intra-uterine environment was more important than genetic factors in determining growth rates in humans; estimating that 62% of infant birth weight variation was due to uterine environment and only 20% from maternal and 18% from paternal genes. Walton and Hammond (1938) uniquely illustrated this concept in horses with their classic studies involving crossing Shire horses with Shetland ponies. They and others (Allen et al., 2004; Tischner, 1985 and 1987; Tischner and Klimczak, 1989) demonstrated that maternal size, and therefore uterine size, has a marked influence on birth weight, as well as growth and size, that persists through maturity (Figure 2).

Thrifty phenotype hypothesis

The thrifty phenotype hypothesis is based on the idea of neonatal programming and proposes that poor nutrition (as opposed to adequate and/or excess nutrition) during gestation and lactation turns on different genes in the fetus, essentially preparing the offspring's endocrine and metabolic systems for an appropriate response to expected energy intake (Hales and Barker, 1992). The thrifty phenotype hypothesis stems from epidemiologic data where small birth size was related to the development of metabolic and/or cardiovascular disorders later in life (Cripps et al., 2005). Additionally, it seems that development of obesity in infants malnourished during gestation and lactation occurs predominately when their nutrition improves later in life. This was illustrated by the Dutch Famine Study whereby malnutrition during pregnancy resulted in offspring with increased incidence of overweight, obesity, and Type II diabetes as adults (Ravelli et al., 1976). Conversely, de Moura and Passos (2005) speculated that the lack of obesity in Russian children of women pregnant during the German siege of Leningrad in World War II was because food availability did not dramatically improve for those children in their lifetime. Thus metabolic programming for relative scarcity of resources most likely proved beneficial to those individuals.

Rodent models of nutrient restriction during pregnancy provide some support for these theories. Rat pups born to dams that were nutritionally restricted after 10 d of gestation until birth and subsequently allowed *ad libitum* access to rat chow had significantly greater percentage of body fat and weighed more than rat pups born to dams that were not nutritionally restricted during gestation (Desai et al., 2004). In another

study, pregnant rats were nutritionally restricted during the first 14 d of gestation then allowed ad libitum access to feed throughout lactation (Jones and Friedman, 1982). These investigators reported hyperphagia and overweight in male rat pups while no difference was observed between female pups and controls, supporting the argument that timing of nutrient restriction has important implications in the response of offspring to feed intake. Three year old sheep born to nutrient restricted ewes exhibited elevated blood pressure prior to feeding and responded with increased circulating leptin to injections of norepinephrine or infusions of angiotensin II (Gopalakrishnan et al., 2004). In another study, the effects of maternal nutritional restriction of ewes resulted in a significant decrease in the activity of an enzyme essential for fatty acid oxidation in their offspring (Zhu et al., 2006). This resulted in increased intramuscular triglyceride content in lambs which is associated with a disruption of the insulin signaling cascade, thus leading to insulin resistance.

Ironically, no reports could be found in the literature which described the endocrine profiles or physiology of foals during the immediate post-natal period, relative to maternal nutritional restriction during gestation or lactation. However, the effects of maternal size and uterine capacity on foal cardiovascular function (Giussani et al., 2003) and insulin dynamics (Forhead et al., 2004) has been reported. Giussani et al. (2003) transferred pony embryos into Thoroughbred mares (PinT) and Thoroughbred embryos into pony mares (TinP), as well as maintained two sets of control animals (Thoroughbred in Thoroughbred – TinT and pony in pony – PinP; five to eight animals per group). The authors reported that PinT foals had elevated basal arterial blood pressure, reduced baroflex sensitivity, and increased cortisol response to ACTH stimulation compared to all

other groups. In contrast, TinP foals had increased baroflex sensitivity, but arterial blood pressure and cortisol response were not different compared to all other groups. Because both growth-enhanced (PinT) and growth-restricted (TinP) foals had altered postnatal regulation of adrenal function and blood pressure, the authors suggested that deviations either above or below the normal growth trajectory may predispose individuals to cardiovascular disease later in life (Giussani et al., 2003).

Forhead et al. (2004) investigated the effects of the intrauterine environment on glucose and insulin dynamics in foals. Four groups of foals with seven or eight animals per group were produced using embryo transfer (TinT, PinP, PinT, and TinP). The authors reported that PinT foals had elevated basal insulin concentrations and a greater β pancreatic cell response to glucose administration during glucose tolerance tests compared to the other three groups of foals. No differences were seen between the TinP foals and control animals in glucose or insulin concentrations during the glucose tolerance tests. The authors concluded that overgrowth, as opposed to growth retardation *in utero,* was responsible for alteration of insulin response in the immediate postnatal period in equine (Forhead et al, 2004).

The thrifty phenotype hypothesis may also be a plausible explanation for the increased incidence of obesity and laminitis occurring in the pony and mustang populations described previously. Perhaps the metabolic programming occurring during gestation and lactation in those populations functions to prepare the offspring for relatively scarce and uncertain food resources, and not the abundant food supply often readily available in a domestic state. The latter circumstance thus predisposes those animals to experience a greater than average incidence of obesity related complications

than the general horse population. The thrifty genotype and thrifty phenotype hypotheses need not be mutually exclusive however, since gene expression for metabolic efficiency (thrifty genotype) may be up-regulated or down-regulated depending on maternal nutritional status during gestation and lactation (thrifty phenotype).

Obesity and leptin

The identification of leptin's role in obesity can be traced to experiments by Hervey (1959) and Coleman (1973 and 1978). The discovery of two genetic mutations in mice, described as obese (*ob*) and diabetic (*db*), served to contribute to the theory of a satiety factor. The phenotypes of these two mutations were similar and characterized by hyperphagia and obesity; however each responded differently in parabiosis studies. Parabiosis of the *ob/ob* mouse and normal mouse resulted in the *ob/ob* becoming hypophagic and losing weight while the normal mouse was unaffected. When the *db/db* and normal mouse were paired, the normal mouse decreased its food intake and lost weight while the *db/db* mouse was unaffected. Finally parabiosis of the *db/db* and *ob/ob* mice resulted in hypophagia and weight loss by *ob/ob* mouse. From these experiments, Coleman concluded that the *ob* mouse lacked the ability to produce a satiety factor, while the *db* mouse was unable to respond to the satiety factor. Identification of leptin by Zhang et al. (1994) as the satiety factor described by Coleman, coupled with additional experiments using the *ob/ob* (mutated leptin) and *db/db* (mutated leptin receptor) mouse models further reinforced the role of leptin as a regulator of food intake and energy expenditure (Houseknecht et al., 1998).

Obesity is associated with elevated plasma concentrations of leptin in humans and

horses (Hall, 2000; Buff et al., 2002), as well as selective leptin resistance (Rahmouni et al., 2006). Selective leptin resistance is characterized by rising concentrations of leptin simultaneously activating the sympathetic nervous system to increase blood pressure, yet failing to reduce food intake and weight gain. This has been attributed to the activation of different hypothalamic neurotransmitter systems by leptin in rodents and humans (Pausova, 2006). Johnson (2002) suggested that leptin resistance plays a role in equine obesity since similar hyperleptinemic profiles are exhibited by overweight horses.

Leptin normally functions to stimulate fatty acid oxidation and glucose uptake into tissues, as well as prevent lipid accumulation in non-adipose tissue such as muscle (Minokoshi et al., 2002). Slawik and Vidal-Puig (2006) describe lipotoxicity as a state resulting from accumulation of lipids in non-adipose tissue (muscle, heart, liver, pancreas, kidneys, or blood) due to "over-spill" from saturated adipose tissue. They further offer that peripheral leptin resistance can lead to lipid accretion in non-adipose tissue, potentiating the effects of lipotoxicity in those tissues.

Obesity and insulin

The primary biological function of insulin is to decrease plasma glucose concentrations. Its primary target tissues are the liver, skeletal muscle, and adipose tissue. In the normal fed state, insulin promotes hepatic glycogen synthesis and inhibits hepatic glucose release, enhances glucose transport into the muscle and fat via GLUT4 transporter mobilization, and inhibits lipase activity, which thereby reduces free fatty acid (FFA) plasma concentrations (Silverthorn, 2001). In the normal fasted state, insulin concentrations are low, permitting glycogenolysis and gluconeogenesis, fatty acid

oxidation, and lipolysis (Silverthorn, 2001).

The paradigm of insulin resistance (IR) is often associated with obesity in humans (Bastard et al., 2006; Kim et al., 2006) and horses (Jeffcott et al., 1986; Hoffman et al., 2003; Frank et al., 2006). Kahn (1978) defined IR as existing when "normal concentrations of insulin produces a less than normal biologic response." Treiber et al. (2006) state that, although the exact mechanisms are not wholly understood, insulin resistance often occurs as a result of insulin-receptor substrate-1 (IRS-1) not being phosphorylated, decreasing activation of phosphatidylinositol 3-kinase (PI 3-kinase), and ultimately reduced expression of glucose transporters. In turn, glucose concentrations remain elevated in the blood, consequently stimulating insulin secretion and thus creating a vicious self deprecating cycle. Chronic elevation of insulin may also contribute to lipotoxicity. Frank et al. (2006) cite evidence in humans that lipid accretion disrupts both β-cell function in the pancreas and the IRS-1 signaling cascade in skeletal muscle; thus creating a state of hyperglycemia and IR.

To further compound issues, obesity and insulin resistance are associated with laminitis in equine (Johnson, 2002); however the intricate details of this relationship have yet to be elucidated. Human studies demonstrate that obesity and insulin resistance are related to vascular and endothelial damage (Rahmouni et al., 2005) and inflammation (Greenberg and Obin, 2006), all of which are apparent in laminitis. It is important to note that the age range of horses affected with endocrinopathic laminitis (obesity-associated laminitis not necessarily brought about by an acute episode) is between 8 and 18 years of age. Slawik and Vidal Puig (2006) reports that the aging process causes physiological cellular degeneration that may be exacerbated by insults such as lipotoxicity, and vice

versa.

Frank et al., (2006) compared the neck circumferences of obese IR adult horses with non-obese adult horses of similar height and weight and found the neck circumference of the obese IR horses were significantly greater than the non-obese horses. The suggestion was made that as a result of localized fat deposition, neck circumference could be used as a screening tool for IR in obese horses and potentially thwart laminitic episodes.

It is documented in horses that weight loss, as a result of exercise (Powell et al., 2002) or in the absence of exercise (Freestone et al., 1992), improves insulin sensitivity in equine. Powell et al. (2002) speculated that improved insulin sensitivity of obese mares subjected to a 7 d regimen of light exercise was likely due to increased distribution of GLUT-4 transporters in the skeletal muscle as has been reported in rats. Additional support for this hypothesis is evident in a recent study by Jose-Cunilleras et al. (2005) who reported an increase in GLUT-4 expression in skeletal muscle during the post-exercise hours in horses. Improved insulin response can also be achieved by reducing the amount of soluble carbohydrates in equine diets (Hoffman et al., 2003; Treiber et al., 2005) and controlling feed intake of horses (Freestone et al., 1992; Buff et al., 2006a). This may be beneficial to equine because chronic hyperinsulinemia in young horses has been implicated as an associative factor in the development of osteochondritis dessecans (OCD) lesions (Ralston, 1996). Both insulin and IGF-1 play a role in chondrocyte maturation and exacerbations of these hormones by high carbohydrate diets may be manifested by developmental orthopedic diseases such as OCD (Henson et al., 1997). Insulin resistance has also been reported to increase the interovulatory interval in mares

(Sessions et al., 2004), although the exact mechanisms behind this effect remains unclear.

Obesity and glucocorticoids

Excess production or administration of glucocorticoids (also known as Cushing's syndrome) is characterized by hypercortisolism, hyperglycemia, insulin resistance, dyslipidemia, and omental (within the abdomen) obesity. Omental adipocytes have been reported to over-express 11 β-HSD1 (Johnson, 2002), which is the enzyme responsible for the conversion of cortisone (inactive form) to cortisol (active form). Accretion of omental vs. subcutaneous adipocytes has been reported to increase insulin resistance (Attallah et al., 2006). Additionally, increased secretion of leptin from adipocytes, stimulated by excess local glucocorticoid production may potentate the negative effects of hyperleptinemia in affected individuals (Johnson, 2002). Inhibition of 11βHSD1 may serve to protect against the maladaptive phenotype associated with omental obesity (Kershaw et al., 2005; Wake and Walker, 2006). Excess glucocorticoids are associated with laminitis as well, and an in-depth review on the subject has been written by Johnson et al. (2002). Briefly, exogenous administration of glucocorticoids to adult horses does not generally cause laminitis (a widely held view), although glucocorticoid-induced changes may predispose a horse to an episode of laminitis under certain conditions. Glucocorticoids have vasoconstrictive properties that may be involved in lamellar breakdown, as well as stimulatory effects on intestinal permeability which may result in the absorption of toxins which has been implicated as a causative factor in laminitis. Needless to say however, the role of glucocorticoids in laminitis and the associated mechanisms has yet to be elucidated.

Obesity induced hypertension

It is well-documented in the human population that increased weight gain is associated with increased blood pressure, although considerable individual variation exists (Davy and Hall, 2004). Activation of the sympathetic nervous system and the rennin-angiotensin-aldosterone system (RAAS) are likely the primary players in this condition (Rahmouni et al., 2005; Hall, 2000). Pausova (2006) suggested that activation of the sympathetic nervous system by leptin (which is elevated in obesity) may raise blood pressure via peripheral vasoconstriction and enhance re-absorption of renal sodium and water. Ehrhart-Bornstein et al. (2003) reported that human adipocytes secrete potent mineralocorticoid releasing factors, providing evidence of a direct connection between obesity, the RAAS, and hypertension in humans. Aldosterone is a steroid hormone synthesized by the zona glomerulosa cells of the adrenal cortex. Classically, it is secreted in response to increased extracellular potassium concentrations and to angiotensin II, while it is inhibited by an increase in extracellular osmolarity (Silverthorn, 2001). Aldosterone functions to promote sodium, and thus water reabsorption, which increases blood volume and subsequently increases blood pressure. Elevated aldosterone concentrations consequently lead to an increase in blood pressure. Pausova (2006) reported that all components of the RAAS are augmented in obese compared to lean individuals.

The most accurate assessment of blood pressure in horses requires arterial catheterization (invasive); however indirect (noninvasive) measures of blood pressure may be taken by using an occlusion cuff over the coccygeal artery and measuring

pressure by oscillometric methods (Magdesian, 2004). Evidence does exist for a hypertensive state in chronically laminitic (and often obese) ponies. However, it has been reported that hypertrophy of the left ventricle occurs in response to an exercise-induced increase in blood pressure and volume in horses (Buhl et al., 2005; Young et al., 2005). Rugh et al. (1987) compared the hearts of nine chronically laminitic ponies to nine apparently normal controls and found that the left ventricle was significantly thicker and heavier in the chronically laminitic ponies, providing evidence of hypertension. In a review of the literature, no published work apparently existed relevant to whether weight loss in obese equine parallels attenuation of blood pressure and left ventricular hypertrophy. It has been reported that weight loss in humans is accompanied by lowered blood pressure, and thus a decrease of its ill-effects (Davy and Hall, 2004; Rahmouni et al., 2005); therefore it seems an investigation of this phenomenon in equine would be productive.

Spironolactone

Structure and mechanism of action

Spironolactone is mineralocorticoid receptor (MR) antagonist (Figure 3). It competitively binds MR at sodium-potassium exchange sites in the distal convoluted tubule of the kidney. The consequence of its actions are increased water and sodium secretion while retaining potassium and magnesium ions; thus its actions are antagonistic to aldosterone (Figure 4). The antagonistic action of spironolactone at the MR both displaces and prevents binding of aldosterone at these sites and disrupts the negative feedback loop, stimulating renin secretion which ultimately leads to a transient increase

in aldosterone synthesis and release from the adrenal cortex (Garthwaite and McMahon, 2003). Elevated circulating concentrations of aldosterone, in response to spironolactone treatment, do not aggravate the negative consequences associated with elevated aldosterone levels due to pathology or obesity because the MR are occupied by spironolactone and are therefore unable to be activated by aldosterone (Garthwaite and McMahon, 2003).

Mineralocorticoid receptors have been reported to have a high affinity for glucocorticoids and become saturated by low concentrations of circulating glucocorticoids (Spencer et al., 1990; Spencer et al., 1993). Occupation of the MR by spironolactone not only prevents binding of aldosterone, but glucocorticoids as well. This antagonistic action on the negative feedback effects of glucocorticoids at the MR has been reported to activate the HPA axis, causing transient elevation of glucocorticoids in humans (Young et al., 1998) and rats (Bradbury et al., 1994).

Spironolactone also has mild anti-androgen effects; yet conflicting evidence exists for the mechanism of action by which spironolactone exerts these anti-androgen effects. Sert et al. (2003) suggested that spironolactone inhibits 5-α reductase activity and blocks dihydrotestosterone from binding its nuclear receptor, resulting in decreased testosterone production. Ménard (2004) provided evidence, citing a number of studies, that spironolactone displaces dihydrotestosterone but does not affect 5-α reductase activity. Therefore, alterations in testosterone-estrogen ratios and/or metabolic clearance of these hormones may be altered, causing anti-androgenic activity. Side effects of spironolactone treatment reportedly may include gynecomastia, menstrual irregularities, and decreased libido (Garthwaite and McMahon, 2004).

Use of Spironolactone

Spironolactone is primarily used as a diuretic to treat congestive heart failure and hypertension in humans (Rossing et al., 2005; Rogerson et al., 2003). *In vitro* work in rodents provides evidence that spironolactone may offer cardioprotective effects in addition to lowering blood pressure. Chai et al. (2005) treated rats with spironolactone and demonstrated a significant reduction in infarct size (% of heart at risk for damage) for rats' hearts that were perfused following ischemia. These investigators speculated that spironolactone may act on cardiac MR to counteract the negative effects of locally produced aldosterone which include increased free radical production and proarrhythmogenic (causing abnormal heartbeat) actions.

In other pathologies, spironolactone has been used to treat hirsuitism and polycystic ovary syndrome in women due to its anti-androgenic actions (Zulian et al., 2005; Archer and Chang, 2004).

In equine, spironolactone has been administered to affect electrolyte excretion in ponies (Alexander, 1982). A 1 mg/kg BW dose of spironolactone resulted in a significant increase in urinary sodium excretion which was augmented by an additional dose 24 h later. This response was expected due to the antagonistic effect of spironolactone on aldosterone action at the MR in the distal nephron. Aldosterone concentrations were not measured in that study.

Lipoic Acid

Structure and mechanism of action

Lipoic acid is a naturally occurring compound synthesized by humans and animals (Carreau et al, 1975). Although the complete pathway for its *de novo* synthesis is yet to be elucidated, it is known that lipoic acid is synthesized from octanoic acid to form an eight carbon structure with a disulfide bond in a 1,2-dithiolane ring with a chiral center at the C3 carbon to which a five carbon tail is attached. The disulfide bonds may be oxidized to yield lipoic acid or reduced to yield dihydrolipoic acid (Figure 5). It should be noted that the R-isomer of lipoic acid is the only one synthesized in the natural state and is more biologically active than the S-isomer (Biewenga et al., 1997). Finally, endogenously produced lipoic acid is transported peripherally bound to lysine by an amide linkage forming a lipoyl group, which is a component of the mitochondrial pyruvate dehydrogenase and the α-ketoglutarate dehydrogenase complexes (Biewenga et al., 1997).

Lipoic acid in glucose metabolism and insulin function

Lipoic acid has been reported to increase glucose transport into skeletal muscle and improve insulin sensitivity in both rodent (Henriksen, 2006; Lee et al., 2005; Saengsirisuwan et al., 2004) and in human studies (Konrad et al., 1999). Saengsirisuwan et al. (2004) administered 30 mg/kg BW of R-LA (the more biologically active isomer of lipoic acid) by intraperitoneal injection once daily for 15 days and reported a significant increase in insulin receptor substrate (IRS)-1 protein expression in the soleus muscle of treated rats compared to control animals. Lee et al. (2005) found improved insulin-stimulated whole body glucose uptake in diabetes-prone obese male rats fed racemic lipoic acid at 0.5% wt./wt. for three days compared to controls. These investigators

attributed the improved glucose uptake to activation of AMP-activated protein kinase (AMPK), which is an enzyme involved in cellular energy metabolism that stimulates glucose uptake into cells (Lee et al., 2005). Konrad et al. (1999) found that oral lipoic acid supplementation, delivered twice daily for 4 weeks, to lean and obese diabetic patients improved glucose-mediated glucose disposal (glucose effectiveness) as demonstrated by application of the minimal model to a modified glucose tolerance test performed before and after supplementation. The minimal model analysis of glucose and insulin dynamics estimates glucose effectiveness and insulin sensitivity (insulin-mediated glucose disposal) using data from glucose tolerance tests (Kronfeld et al., 2005).

Lipoic acid has also been shown to reduce feed intake and BW in rodents. Kim et al. (2004) fed lipoic acid at 0.25, 0.50, and 1.0 %, wt./wt. for 2 weeks and demonstrated a dose dependent reduction in feed intake and BW in rats. Additionally, intracerebroventricular (i.c.v.) administration of lipoic acid suppressed food intake in rats, suggesting that the anorexic effects of lipoic acid are mediated in the hypothalamic neurons (Kim et al., 2004).

Lipoic acid use in livestock

Lipoic acid has previously been fed experimentally to pigs (Maddock et al., 2003; Rentfrow et al., 2004), beef cattle (Schmidt et al.,2006), broiler chickens (Hamano, 2006), and horses (Williams et al., 2002). Schmidt et al. (2006) found that beef cattle supplemented with 32 mg/kg BW of lipoic acid had a more rapid recovery from infectious bovine rhinotracheitis virus challenge than did cattle supplemented with 16 mg/kg lipoic acid or with none at all. This positive effect on cattle health was likely a

result of lipoic acid's antioxidant capabilities and subsequent augmentation of the immune system. Hamano (2006) demonstrated enhanced whole body glucose uptake in broiler chickens supplemented with lipoic acid from 2 to 5 wk of age as demonstrated by the results of before and after hyperglycemic-euglycemic clamp tests. Supplementation of pasture managed horses with lipoic acid for two weeks reduced oxidative stress in treated animals as measured by glutathione and anti-oxidant enzymes present in the blood (Williams et al., 2002).

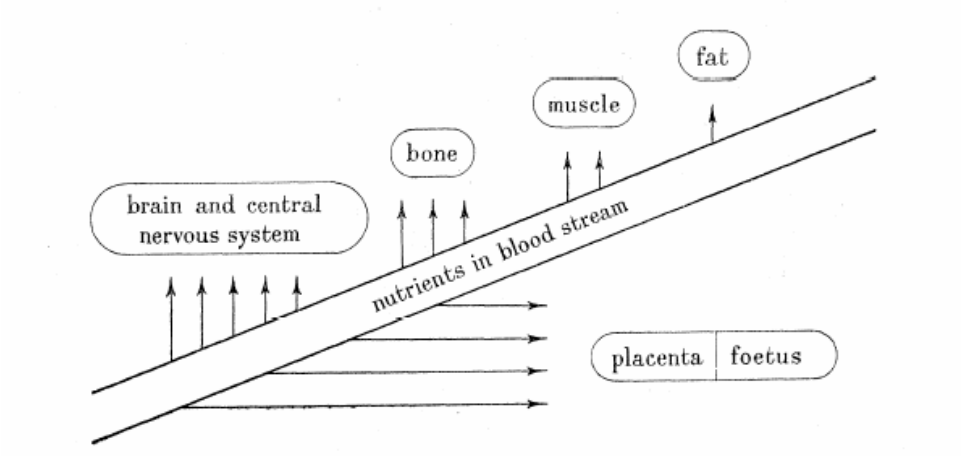


Figure 1. Hammond's (1950) theory of nutrient partitioning according to metabolic rate.

Figure 2. These photographs are from Allen et al. (2004) and demonstrate the dramatic effect of intra-uterine space on size and growth of offspring. Fourteen month old Thoroughbred (TB) colts are pictured in upper picture and the TB on the left was gestated in a pony (P) mare while the TB on the right was gestated in a TB mare. Fourteen month old P fillies are pictured in the lower picture and the P on the left was gestated in a TB mare while the P on the right was gestated in a P mare.

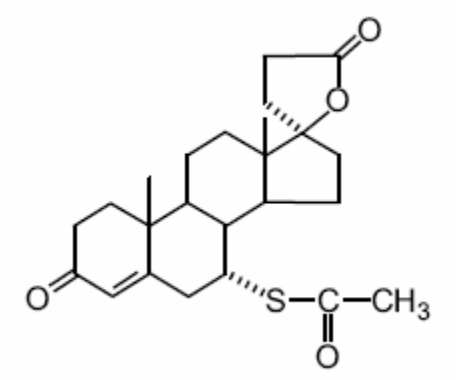


Figure 3. Molecular structure of spironolactone (Rogerson et al., 2003).

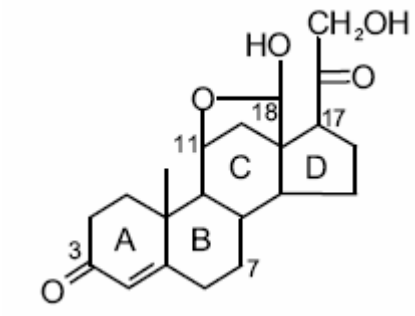


Figure 4. Molecular structure of aldosterone (Rogerson et al., 2003).

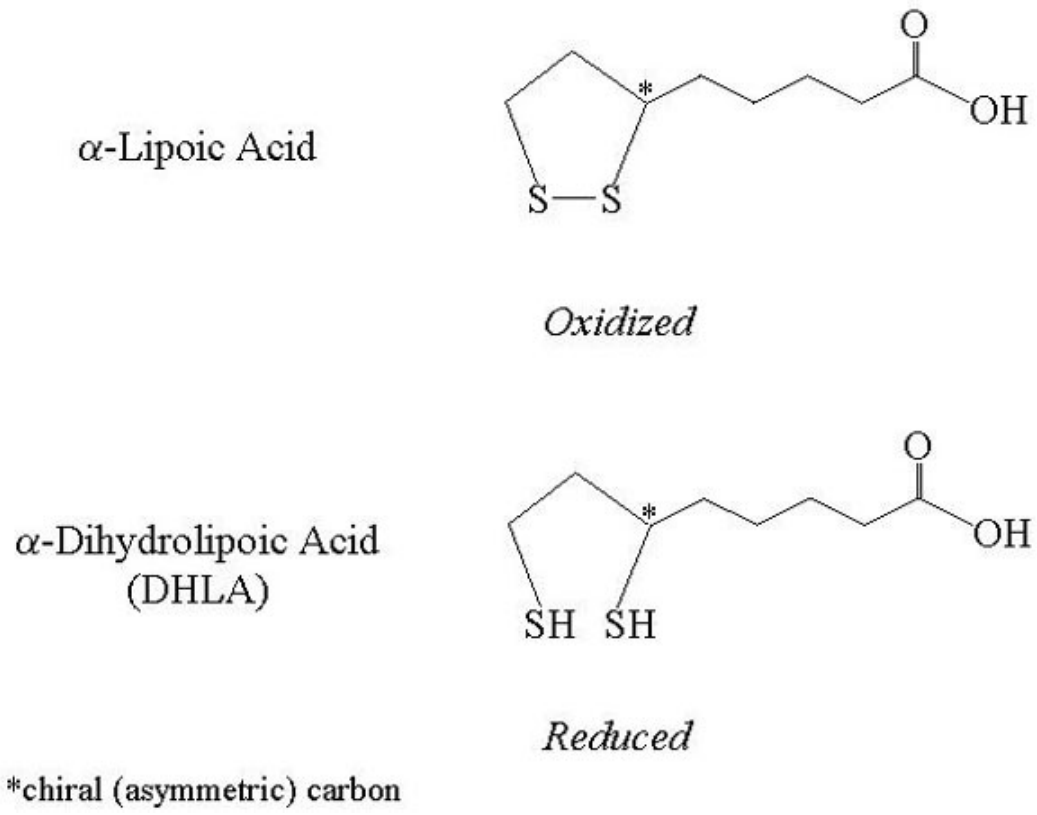


Figure 5. Molecular structures of lipoic acid and dihydrolipoic acid (http://lpi.oregonstate.edu/infocenter/othernuts/la/lastructure.jpg).

CHAPTER III

ENDOCRINE PROFILES OF PERI-PARTURIENT MARES AND THEIR FOALS

ABSTRACT: The aim of this study was to characterize concentrations of leptin, IGF-1, and TSH in the blood serum of mares pre- and post-partum, in the milk serum of mares post-partum, and in the blood serum of their foals. Nine pregnant Quarter Horse mares, aged 4 to 21 yr and their subsequent offspring were used in this study. Mares foaled between March 8 and May 15. Once weekly between 1000 and 1200 h for two weeks prior to their predicted parturition date, pregnant mares were weighed, assigned a BCS, and blood sampled via jugular venipuncture. Within 2 h of parturition and before foals nursed (d 0), blood samples were obtained from mares and foals, and a milk sample collected from the mares. Blood from foals and blood and milk from mares were collected again at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 4.5, 5, 12, 19, 26, 33, and 61 d post-partum. Mares and foals were weighed and assigned a BCS on d 0, 5, 12, 19, 26, 33, and 61 as well. Additionally, ultrasound images of fat depth and muscle area of the longissimus dorsi immediately cranial to and parallel with the last rib on the left side of foals were measured to characterize changes in fat depth and muscle area over time on d 5, 33, and 61.

Our observations were that there was no change in mare blood serum concentrations of IGF-1 ($P = 0.07$) or TSH ($P = 0.15$), nor were there any changes in foal blood serum concentrations of leptin ($P = 0.54$) or TSH ($P = 0.10$) during the trial period.

Mare blood serum concentrations of leptin were found to change over time ($P < 0.001$), initially decreasing then remaining relatively stable after d 5. Foal blood serum concentrations of IGF-1 increased initially, peaked at d 19 and stabilized thereafter ($P < 0.001$). Milk serum concentrations of leptin and TSH were greatest on day 0 and decreased over time ($P < 0.007$), reaching nadir concentrations at d 61. Milk serum concentrations of IGF-1 also changed over time ($P = 0.02$), being greatest on d 0 and undetectable by d 12. There was no difference in BCS (P = 0.94) in mares over time, but there was a difference between pre- and post-partum BW (P < 0.001) due to foaling. However, no differences were detected in pre- ($P = 0.70$) or post-partum BW (P = 0.76) of mares over time. Both mean ultrasonic fat depth and longissumus dorsi muscle area increased ($P < 0.04$) as foals aged, as did BCS and BW (P < 0.001). Recognizing changes in metabolic hormones surrounding the time of parturition in the mare and foal provides a basis for further determination of the role if any these hormones play in the milk, as well as in the neonate.

Key Words: Foal, Insulin-like Growth Factor-I, Leptin, Mare, Milk, Thyroid Stimulating Hormone

Introduction

Successful transition of the animal from the fetal to the neonatal status involves tremendous physiological adaptation on both the part of the neonate and the dam. The success or failure of this transition process equally dictates the survival of the offspring and subsequent recovery of the dam. Precocial species are initially dependent upon the

dam to provide adequate nutrition and immune protection. Typically, newborn foals do not ingest adequate amounts of solid feedstuffs for the first two to three months and therefore depend solely upon mare's milk to obtain the required nutrients during that time (Duncan et al., 1984). Additionally, because foals are born with a naïve immune system, ingestion of good quality maternal colostrum within the first 24 h of life is imperative (Robinson et al., 1993).

It has been suggested for a number of species that the concentrations of various hormones and growth factors present in colostrum and milk may serve to "program" the neonate's endocrine system in the acute post-partum period and therefore shape the body's response to feeding and stress later in life (see review by de Moura and Passos, 2005). This may be of relative importance to the current problem of obesity in horses, as identification and elucidation of the roles of various compounds in milk and subsequently the newborn, may provide initial insight into mechanisms involved in the development of obesity-related maladies in equine.

Hormones previously identified in mares' milk include insulin, IGF-1 (Hess-Dudan et al., 1994), leptin (Romagnoli et al., 2006; Salimei et al., 2002), progesterone (Laitinen, et al., 1981), and triiodothyronine (Slebodziniski et al., 1998). The possible roles of these hormones in milk have been described in a number of species and to some extent in the horse (Xu, 1996; Murray and Luba, 1993;Grosvenor et al., 1992). However, limited information is available on acute changes in metabolic hormones relative to parturition in mares and their foals. Therefore, our objective was to characterize a portion of the endocrine changes occurring in peri-parturient mares and their offspring. We quantified concentrations of leptin, IGF-1, and TSH in the blood serum of mares pre-

and post-partum, in the milk serum of lactating mares post-partum, and in the blood serum of their foals. Additionally, ultrasound images of fat depth and muscle area of the longissimus dorsi immediately cranial to and parallel with the last rib on the left side of foals were measured to estimate changes in fat depth and muscle area over time.

Materials and Methods

Management of Animals. Nine pregnant Quarter Horse mares, aged 4 to 21 yr and their subsequent offspring were used in this study. All mares, with the exception of one, were multiparous mares. The mares foaled March through May of 2004. Sixty days prior to expected parturition, mares were removed from their winter pasture of tall-fescue grass and maintained in a 4-acre dry paddock at the University of Missouri Horse Research and Teaching Farm. Mares had *ad libitum* access to orchardgrass / alfalfa hay, fresh water, and a plain salt block. Additionally, mares were fed a concentrate (Table 1) at 1% of their BW daily according to NRC requirements (1989). Two weeks prior to expected parturition date, mares were monitored daily between 1600 and 1800 h for any changes in physical characteristics related to parturition (udder distention, teat secretions, and tone and appearance of croup muscles). One mL of mammary secretions were obtained from mares at this time and mixed with 6 mL of deionized water to determine water hardness using Baker test strips (BVA Scientific, San Antonio, TX). Test strips for water hardness have been utilized as a predictor for impending parturition in the mare as demonstrated by Ley et al. (1989). Once there was a color change in at least 4 of the 5 zones on the test strip, indicating increased water hardness, mares were brought into 3.6 x 7.3 m stalls to be monitored for parturition throughout the night. If the mare did not foal,

she was turned back out into the 4-acre paddock and checked daily until parturition. If the mare foaled, the pair was maintained in the same 3.6 x 7.3 stall through d 5 post-partum with *ad libitum* access to fresh water, a plain salt block, orchardgrass / alfalfa hay and mares were fed 1 % of their BW in concentrate daily according to NRC requirements (1989). On d 6 post-partum mares and foals were turned out onto a 20-acre orchardgrass pasture with access to fresh water, a plain salt block, and fed 1 % of their BW in concentrate daily.

The mares were vaccinated against rhinopneumonitis Type 1 with a killed vaccine at 5, 7, and 9 months of gestation. Five weeks prior to expected parturition, mares were vaccinated against Eastern and Western equine encephalitis, West Nile virus, tetanus, and influenza. The mares were dewormed every 2 mo with alternating anthelmintic products (January and July with pyrantel pamoate, March and September with ivermectin, and May and November with moxidectin). Foals were dewormed with pyrantel pamoate at 1 mo of age, with ivermectin at 2 mo of age, and then adapted to the same de-worming program as the broodmares. The research protocol was approved prior to the study by the University of Missouri Animal Care and Use Committee.

Data Collection. A 10 mL blood sample was collected via jugular venipuncture from pregnant mares once weekly between 1000 and 1200 for two weeks prior to their predicted parturition date. Mares were also weighed and assigned a body condition score at these times. Body condition scoring was performed by the same individual throughout the trial period using a 1 to 9 scale according to Henneke et al. (1983). Within 2 h of parturition and before the foal nursed (d 0), a 10 mL blood sample was collected via jugular venipuncture from the foal, and a 10 mL blood sample and 5 mL milk sample

collected from the mare. Body weights were obtained for mares and foals within 24 h of parturition. Blood from foals and blood and milk from mares was collected again at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 4.5, 5, 12, 19, 26, 33, and 61 d post-partum. Additionally, on d 5, 12, 19, 26, 33, and 61 mares and foals were weighed and assigned a BCS. Ultrasound measurements of fat depth and of foals' longissimus dorsi muscle immediately cranial and parallel to the last rib on the left side were taken on d 5, 33, and 61.

Blood samples were collected into SST® gel and clot activator Vacutainer brand collection tubes (Benton Dickinson, Franklin Lakes, NJ), were allowed to clot for 20 minutes at room temperature and centrifuged for 25 minutes at 3,000 × *g*. Serum was harvested immediately and frozen at – 20°C for later analysis. Whole milk samples were collected into polyurethane tubes, immediately frozen at 0°C, and later centrifuged at 100,000 × *g* at 6°C for 1 h to obtain milk serum. The clear supernate was collected and stored at – 20°C for later analysis. Blood and milk samples were analyzed for leptin, IGF-1, and TSH by radioimmunoassay procedures described as follows.

Radioimmunoassays. Blood and milk serum concentrations of leptin were quantified using the double-antibody leptin radioimmunoassay procedures described by Delavaud et al. (2000) with one modification consisting of the substitution of the reported primary antiserum with rabbit anti-ovine leptin primary antiserum #7105. Briefly, standard concentrations of recombinant ovine leptin (Gertler et al., 1998; 0.1, 0.2, 0.3, 0.5, 0.8, 1.2, 2.0, 3.5, 5.0, and 7.5 ng in 300 μL/tube) and increasing volumes of serum (25, 40, 60, 100, 175, 250, and 300 μL) from a pool of serum collected from a fat mare were added to assay tubes in triplicate and the total volume balanced to 300 μL per tube with buffer consisting of 0.1% gelatin, 0.01 *M* EDTA, 0.9% NaCl, 0.01 *M* PO_4, 0.01%

sodium azide, 0.05%Tween-20, pH = 7.1 (**PABET**). Likewise, 200 µL of the serum samples to be quantified were added to assay tubes in triplicate and volume balanced to 300 µL per tube with PABET. Immediately thereafter, 100 µL of rabbit anti-ovine leptin primary antiserum (7105; final tube dilution of 1:15,000 in PABET) was added and samples and standards incubated at 4°C for 24 h. After the initial incubation, 100 µL of ^{125}I-ovine leptin (20,000 c.p.m.) were added to each tube and incubation continued for an additional 24 h at 4°C. The antigen-antibody complex was then precipitated following a 15 min, 22°C incubation with 100 µL of a precipitated sheep-anti-rabbit second antiserum by centrifugation at 3,000 × *g* for 30 min, and the supernatant removed by aspiration. Assay tubes containing the antigen-antibody pellet were counted for 1 min on a LKB1277 gamma counter (LKB Wallac, Turku, Finland).

Standards and pooled aliquots of serum from a single source of fat-mare serum were linear (log/logit transformation; $R^2 > 0.98$) and parallel over a mass of 0.1 to 7.5 ng/tube and a serum volume of 25 to 300 µL, respectively. Total specific binding was 42%, the minimum detectable concentration was 0.1 ng/tube, percentage recovery of mass was > 99% across the range of 25 to 300µL of sample and the inter- and intra-assay coefficients were < 10%.

Equine blood and milk serum concentrations of IGF-I were measured in triplicate after acidified extraction via a double antibody radioimmunoassay validated for use in our lab (Lamberson et al., 1995). Assay sensitivity was 8.6 ng/mL and the specific binding 41.8%. The intra- and inter-assay coefficients of variation were 6% and 9% respectively.

Equine blood and milk serum concentrations of TSH were performed in triplicate

with a double-antibody RIA using equine TSH antiserum (AFP-C33812) and equine TSH antigen (AFB-5144B) provided by A. F. Parlow (Harbor-UCLA Medical Center, Torrance, CA, USA). The intra- and inter-assay CV were < 10% and the sensitivity was 0.02 ng/mL.

Ultrasound Images. Ultrasonic images were captured using the AUSkey System Software (Animal Ultrasound Services: AUS, Ithaca, NY) using a 500V Aloka (Corometrics Medical Systems, Inc., Wallingford, CT, USA) ultrasound machine with a 3.5 MHz transducer fitted to a custom standoff (a gel fitted to contour the shape of the foal immediately cranial to the last rib). Longissimus dorsi muscle area and fat depth images were captured immediately cranial to and parallel with the last rib on the left side of each foal. Generous amounts of commercial vegetable oil were applied to the ultrasound site to reduce soundwave attenuation associated with hair coat. The same ultrasound technician performed the measurements throughout the study and final ultrasound images were approved by a AUS trained technician.

Statistical Analysis. Statistical analyses were performed to determine whether there was a change in leptin, IGF-1, or TSH concentrations in mare blood serum and milk serum, and foal blood serum over time, as well as fat depth and longissimus dorsi muscle area of foals over time. Data were analyzed as repeated measures using the general linear model procedure (PROC GLM) of SAS (SAS Inst., Inc., Cary, NC). Means separation procedures were performed using the LSMEANS statement. Results are expressed as means ± standard error of means (± SEM).

Results

Leptin. There was a significant day effect on mare blood and milk serum concentrations of leptin ($P < 0.001$), but not on foal blood serum concentrations of leptin ($P = 0.54$; Figure 6). Mean blood serum concentrations of leptin in mares were greatest at 14 d pre-partum (10.34 ± 1.38 ng/mL), declined until d 2, increased slightly and stabilized thereafter. Milk serum concentrations of leptin were 34.13 ± 1.45 ng/mL on d0 (pre-suckle), dropped to 7.36 ± 1.37 ng/mL by d 0.5, and declined to nadir concentrations by d 61. Although there was no statistical difference in foal blood serum concentrations of leptin throughout this trial, blood serum leptin concentrations were lowest at d 0 (0.38 ± 0.85 ng/mL), peaked at d 5 (3.13 ± 0.80 ng/mL) and remained relatively stable thereafter.

IGF-1. There was no significant effect of day on mare blood serum concentrations of IGF-1 ($P = 0.07$), but there was a significant effect of day on milk serum concentrations of IGF-1 ($P = 0.02$) and foal blood serum concentrations of IGF-1 ($P <0.001$; Figure 7). Milk serum concentrations of IGF-1 were greatest at d 0 (76.31 ± 13.63 ng/mL) and undetectable by d 12. Foal blood serum concentrations of IGF-1 increased initially, peaked at d 19 (257.70 ± 10.96 ng/mL) and stabilized thereafter ($P < 0.001$).

TSH. There was no significant day effect on mare ($P = 0.15$) or foal ($P = 0.10$) blood serum concentrations of TSH, but there was a significant day effect on milk serum concentrations of TSH ($P < 0.001$; Figure 8). Milk serum concentrations TSH were greatest on d 0 (18. 01 ± 0.67 ng/mL), decreased over time and reached nadir concentrations on d 61.

BW, BCS, and Ultrasound Measurements. As expected, pre- and post-partum BW's of mares differed ($P < 0.001$) due to foaling. Within the prepartum interval, no differences were detected in BW over time ($P = 0.70$) and similarly, within the post-partum interval BW did not differ over time ($P = 0.76$). Among foals, both mean ultrasonic fat depth (Figure 9) and longissumus dorsi muscle area (Figure 10) increased ($P = 0.03$ and $P < 0.001$, respectively) as foals aged, as did BW and BCS ($P < 0.001$).

Discussion

It has been suggested that the presence of hormones and growth factors in colostrum and milk may contribute to neonatal GI tract development, feed intake regulation, thermoregulation, as well as metabolic programming in the newborn (Romagnoli et al. 2006; de Moura and Passos, 2005; McFadin et al., 2002). We found peak concentrations of leptin, IGF-1, and TSH in the colostrum (d 0) when compared to milk samples taken 12 h post-partum and thereafter. This pattern is similar to that described in other studies of leptin and IGF-1 in mare's milk serum where pre-suckle concentrations of hormone were the greatest and gradually declined to nadir concentrations within days (Romagnoli et al. 2006; Salimei et al., 2002; Hess-Dudan et al., 1994). Similar endocrine profiles have been reported to exist in ewes (McFadin et al., 2002) and cows (Pinotti and Rosi, 2006; Taylor et al., 2004). Coincidentally, the equine neonatal gut is able to readily absorb whole proteins within the first 24 h of life (Jeffcott, 1975), corresponding with peak hormone concentrations in the milk. The elevated concentration of leptin and IGF-1 found in equine colostrum may be due to increased local production in the mammary gland, as expression of leptin mRNA in mammary

tissues has been reported in other species (Bonnet et al., 2002; Aoki et al., 1999; Smith-Kirwin et al., 1998) and IGF-1 mRNA (Berry et al., 2003; Forsyth et al., 1999). It has also been speculated that the reason for peak hormone concentrations in colostrum is due to a pooling effect of the proteins in the milk prior to suckling by the neonate (McFadin et al. 2002).

The absolute values of leptin in the pre-suckled mare milk serum in our study differed from values found by Romagnoli et al. (2006) and Salimei et al. (2002). They reported peak milk serum leptin concentrations of 11.7 ng/mL and 16.88 ng/mL, respectively, while we found peak concentrations of 34 ng/mL. This discrepancy may be due to variations in the assay procedures, sampling paradigms, nutritional status of mares, and (or) breed differences. The blood leptin concentration profiles for the mares in our study were similar to those reported by others (Romagnoli et al., 2006: Heidler et al., 2003). Despite unchanging BCS or BW in mares, serum leptin concentrations were significantly lower in the post-partum period and remained there throughout the duration of the study (61 d). It has been reported that peripheral concentrations of leptin are positively correlated to BCS in horses (Buff et al., 2002; Gentry et al., 2002). However, it has also been demonstrated in equine that feed restriction of up to 48-h decreases leptin secretion regardless of BCS (Buff et al., 2005; McManus and Fitzgerald, 2000), supporting leptin's role as an indicator of acute energy status in horses. During the fed state, leptin has an inhibitory effect on orexogenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) gene expression and a stimulatory effect on anorexogenic pro-opiomelanocortin (POMC) gene expression in the hypothalamus(Cone, 2005). During the fasted state, however, expression of AgRP and NPY mRNA are upregulated and

expression of POMC mRNA are downregulated (Cone, 2005). Although we saw no difference in BW or BCS in mares post-partum, the decrease in blood serum leptin may serve as a means to protect mares against negative energy balance by encouraging feed intake in mares during early lactation. Erlanson-Albertson and Zetterström (2005) argue that leptin has more of a permissive role in hunger signaling (low leptin levels = stimulus for feed intake) rather than a role as a satiety signal per se (high leptin levels = stimulus to decrease feed intake). It is logical that energy signaling during early lactation should be directed towards feed intake in order for mares to maintain good body condition and subsequently an adequate milk supply. Quarter horse mares in early lactation produce nearly 12 kg (approximately 3% of BW) of milk daily, with peak production at 30 d post-partum (Gibbs et al., 1982). The NRC (1989) suggests that protein and energy requirements for mares in early lactation are nearly double what horses at maintenance require.

Although we saw an increase in fat depth and BCS over time, albeit small, there was no correlated increase in leptin concentrations in foals. The fact that blood leptin fails to rise in rapidly growing foals may indicate that decreased leptin secretion is a signal to permit food intake in order for foals to ingest adequate energy for rapid early growth (similar to mares in early lactation). Furthermore, the low adipose tissue reserves a foal has when born could also account for decreased blood leptin concentrations compared to mares. Buff et al. (2002) examined serum leptin concentrations by age classification and found horses less than 2 years old had serum leptin concentrations lower than their older contemporaries.

To our knowledge, this study is the first to report blood leptin and TSH

concentrations in foals during the acute neonatal period. Interestingly, both leptin and TSH in the foal blood were at nadir concentrations before nursing at time 0 and concentrations had increased at least two-and four-fold respectively after nursing. Although we cannot delineate cause and effect relationships between maternal milk and autonomous fetal sources of hormone based on these data, in other species, exogenous hormone administration has a subsequent effect on neonatal hormone concentrations (Lins et al., 2005; Sanchez et al., 2005; Tenore et al., 1980). Sanchez et al. (2005) demonstrated that a significant quantity of leptin is readily absorbed in the GI tract of rat pups through d 4 post-partum. Additionally, rat pups supplemented with five times the amount of leptin ingested normally from maternal colostrum and milk, resulted in decreased production of leptin in the stomach and subcutaneous adipose tissue of rat pups, reduced food intake as demonstrated by decreased gastric content compared to controls, and finally reduced thermogenic capacity in brown adipose tissue. These latter data provide evidence that leptin may play a role in feed intake and thermoregulation in the neonatal rat.

Leptin has been reported to directly stimulate thyroid hormone secretion independent of TSH, as well as increase the transfer of iodine through milk in early lactation in rodents (Lins et al., 2005). This action of leptin on thyroid hormones may serve to augment thermogenesis in the neonate.

Thyroid hormones are known to play important roles in growth regulation, cellular function, and metabolism and are especially critical for post-partum neonatal thermogenesis (Silva, 2006; Irvine, 1984; Chen and Riley, 1981). Hypothyroid foals may be characterized by hypothermia, musculoskeletal weakness, lethargy, and a poor sucking

reflex (Irvine, 1984). Murray and Luba (1993) reported blood serum concentrations of T4 and T3 in foals to be greatest within one hour of parturition in samples obtained before foals nursed. Similarly, Irvine and Evans (1975) found post-natal blood serum concentrations of total thyroxine (T4) and triiodothyronine (T3) in foals to be fourteen and twelve times greater, respectively, than in the blood of the mature horse. It was not specified when blood samples were taken relative to nursing.

A study by Ślebodziński et al. (1998) reported concentrations of T3 in mare's milk peaking 4 d post-partum (0.74 ng/mL), then declining and stabilizing at d 7 through d 21 (0.46 ng/mL). No previous reports of TSH concentrations in mare's milk could be found, however TSH is present in the colostrum of humans and rodents (Tenore et al. 1980; 1981). It has been reported that oral administration of bovine TSH to suckling rats caused a subsequent rise in blood serum T4 and T3 concentrations in rat pups, providing evidence that TSH is absorbed as a whole, biologically active protein (Tenore et al., 1980). Thus, although elevated T4 and T3 blood serum concentrations have been reported in neonatal foals before having nursed, the presence of TSH in maternal milk may further stimulate the neonatal thyroid axis which is crucial for thermogenesis and normal development.

We also found no difference in mare blood serum concentrations of IGF-1 over time, although the pattern of IGF-1 secretion was similar to other reports in mares (Heidler et al., 2003; Hess-Dudan et al.,1994). Pre-partum IGF-1 concentrations increased during the week prior to parturition, decreasing gradually post-partum and stabilized thereafter. This is in contrast to the abrupt decline in IGF-1 seen post-partum in dairy cattle (Taylor et al., 2004).

The presence of IGF-I in colostrum and milk has been reported in a number of species, including horses (Hess-Dudan et al., 1994; Cymbaluk and Laarveld, 1996), and has been reported to play a role in neonatal gut development in some species (Xu, 1996; Grosvenor et al., 1992). Administration of porcine colostrum to piglets markedly enhanced intestinal development which was not apparent compared to water administration and oral administration of IGF-I to piglets tended to increase intestinal epithelial cell proliferation (Xu et al., 1996). Similar work in rats resulted in enhanced GI tract growth, increased brain and liver weights, and increased overall weight gain in rat pups supplemented with a rat milk substitute plus IGF-I versus rat pups supplemented with a rat milk substitute lacking IGF-I (Philips et al., 1997).

The endocrine profile and values of IGF-1 concentrations in foal blood exhibited during the two months post-partum is in agreement with other work conducted in horses (Hess-Dudan et al.,1994; Cymbaluk and Laarveld, 1996). It is likely that the rapid increase of IGF-1 concentrations seen during the foals' first two weeks of life, are associated with rapid growth occurring during that time as has been demonstrated in other species (Dunshea et al., 2002; Nosbush et al., 1996). Plasma concentrations of IGF-I have been reported to decrease gradually as foals age from two to ten months (Thomas et al., 1996) and upon stabilization have been used as an indicator for the end of puberty in Thoroughbred horses (Fortier et al., 2005).

Cymbaluk and Laarveld (1996) reported lower serum IGF-1 concentrations that persisted until 1 year of age in foals fed milk replacer compared to wholly mare-nursed foals, demonstrating that pre-weaning nutrition affected the metabolic programming of IGF-1 in foals. Foals fed milk replacer did receive maternal colostrum but were removed

from their dams by 24 h post-partum and fed milk replacer until weaning at approximately two months of age. Additionally, foals in the mare-nursed group gained more rapidly and had greater serum IGF-1 concentrations than milk-replacer foals in the first two weeks post-partum. The difference in gain seen between the two groups may be partially attributed to the stress of early weaning in milk-replacer foals as the weight difference was negligible by one year of age.

Implications

Recognizing changes in hormones surrounding the time of parturition in the mare and foal provides a basis for further determination of the role hormones may play in the milk, as well as in the neonate. It is apparent in a number of species, including equine, that gestation and lactation can profoundly affect the metabolic programming in neonates; therefore in order for us to elucidate the intricate endocrine patterns surrounding the peri-parturient period, we must continue to identify additional hormones present in equine colostrum and milk and work to reveal their function in the neonate. Improved understanding of endocrine profiles during the peri-parturient period in equine may assist in determining which horses are predisposed to metabolic disruptions later in life.

Table 1. Composition of concentrate fed to mares and foals at 1% BW daily

Item	% as-fed basis
Ingredient	
Cracked corn	37.40
Whole oats	40.90
Soybean meal	9.00
Wheat bran	5.00
Trace mineral salt	0.50
Dicalcium phosphate	0.70
Limestone	0.70
Vitamin A/D/E premix[a]	0.80
Molasses	5.00
Total	100.00
Analyzed composition	
Moisture, %	12.24
Fat, %	3.93
CP, %	13.44
Ca, %	0.61
P, %	0.56

[a]Vitamin premix provided per kilogram of grain concentrate: vitamin A, 5,000 IU; vitamin D_3, 408 IU; vitamin E, 132 IU.

Figure 6. Mean concentrations of leptin in mare blood serum, mare milk serum and foal blood serum over time. There was a significant day effect for leptin in mare blood ($P <$ 0.001) and milk ($P < 0.001$, but not foal blood ($P = 0.54$). The inset within the panel provides details of d -14 to 5 on an expanded scale.

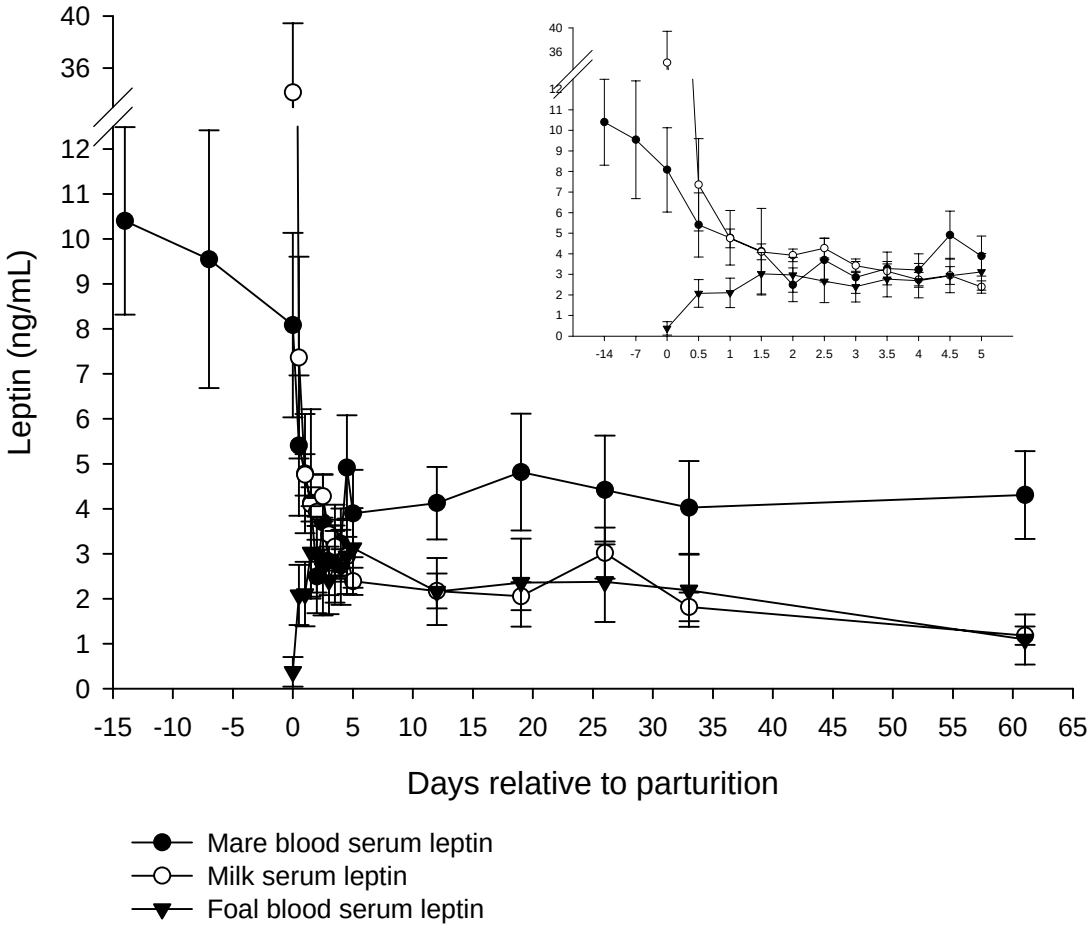


Figure 7. Mean concentrations of IGF-1 in mare blood serum and milk serum and foal blood serum over time. There was a significant day effect for IGF-1 in milk ($P = 0.02$) and foal blood ($P < 0.001$), but not mare blood ($P = 0.07$). The inset within the panel provides details of d -14 to 5 on an expanded scale.

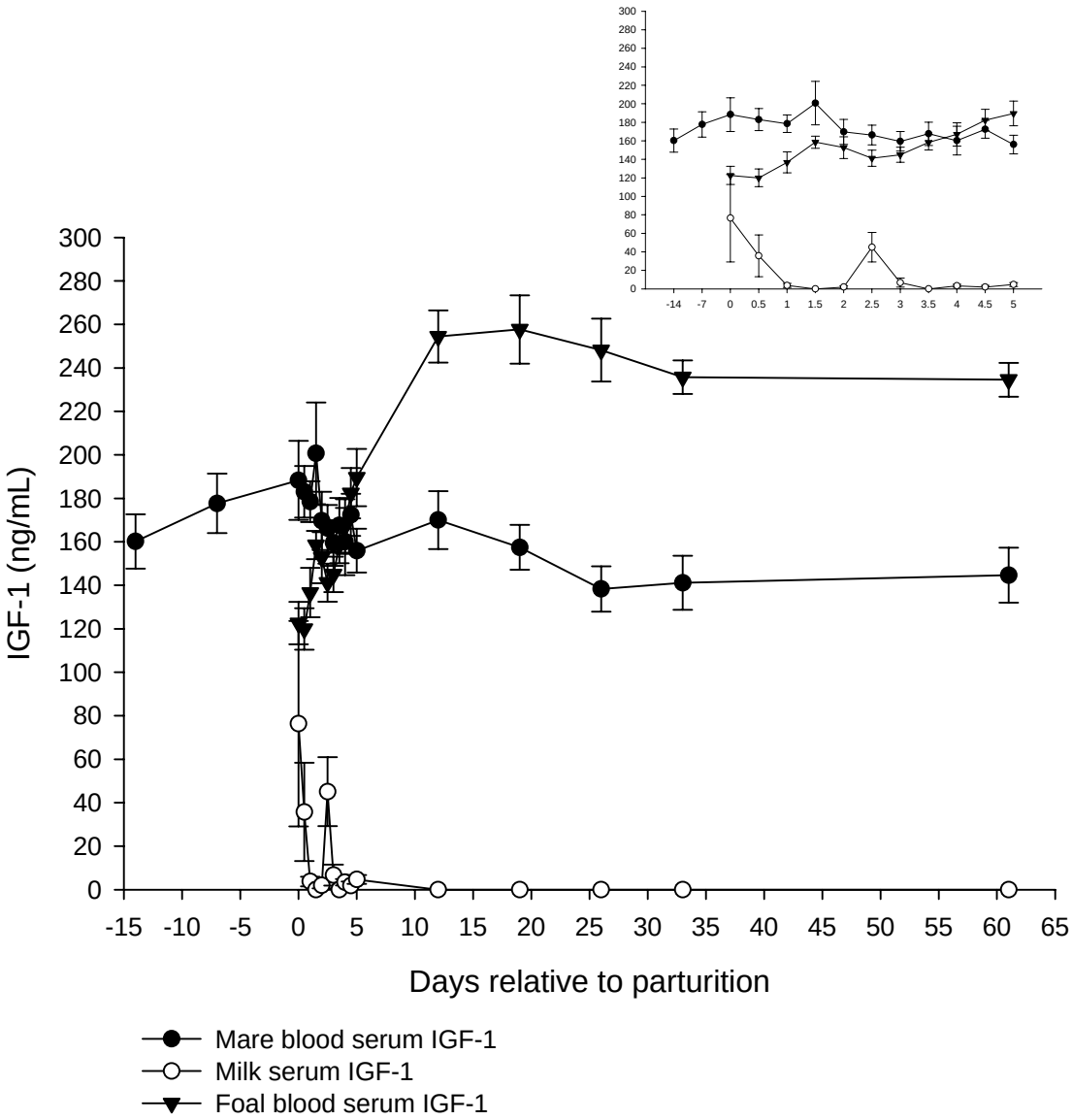


Figure 8. Mean concentrations of TSH in mare blood and milk serum and foal blood serum over time. There was a significant day effect for TSH in milk ($P = 0.02$), but not in mare blood ($P = 0.15$) or foal blood ($P = 0.10$). The inset within the panel provides details of d -14 to 5 on an expanded scale.

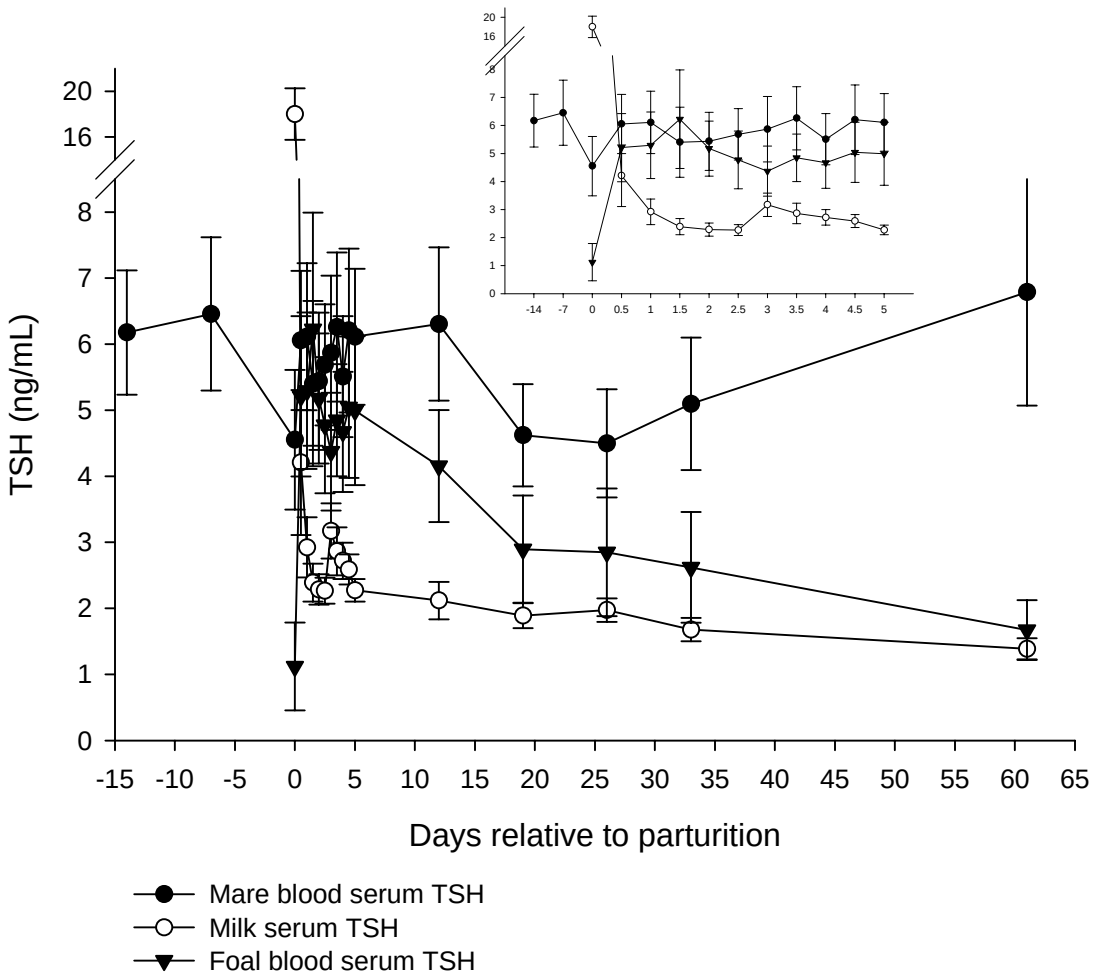


Figure 9. Estimate of mean ultrasonic fat depth (cm) immediately cranial to and parallel with the last rib on the left side of foals over time. Fat depth increased as the age of foals increased (P = 0.03)

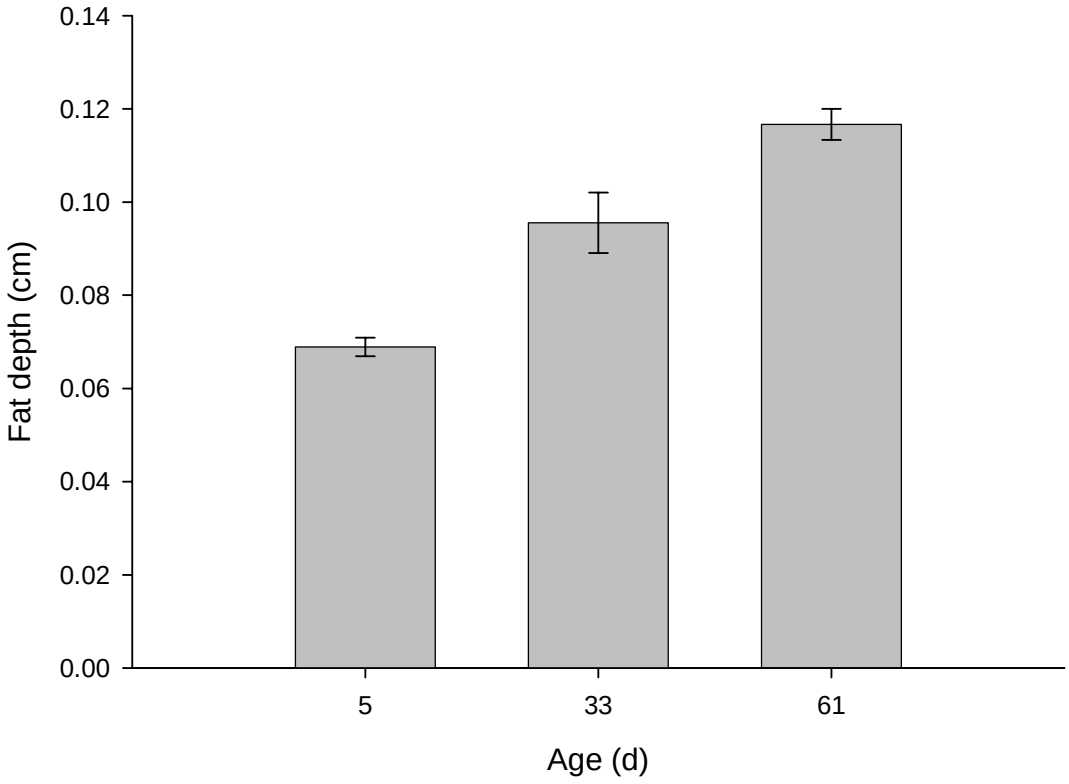
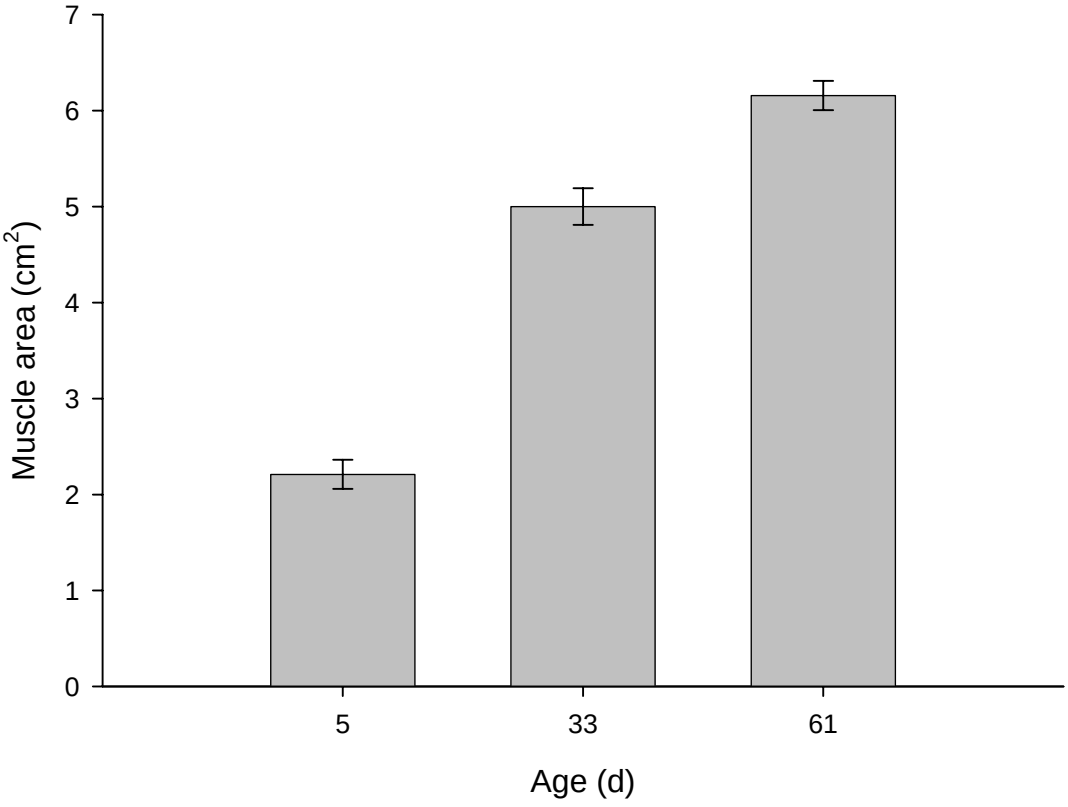


Figure 10. Estimate of mean ultrasonic muscle area (cm^2) of the longissimus dorsi muscle immediately cranial to and parallel with the last rib on the left side of foals over time. Muscle area increased as the age of foals increased ($P < 0.001$).



CHAPTER IV

EFFECTS OF SPIRONOLACTONE ON ALDOSTERONE, CORTISOL, AND LEPTIN IN FAT PONY MARES

ABSTRACT: Recent investigations have yielded evidence of a direct link between adipocytes and adrenocorticoid secretion in the pathology of obesity-related maladies. The objective of this study was to examine the effects of short-term administration of spironolactone (a mineralocorticoid receptor antagonist) on serum concentrations of aldosterone, cortisol, and leptin in overweight pony mares. Twelve pony mares ranging in age from 8 to 18 yr were blocked by BW and ovarian status [intact (**I**) or ovariectomized (**O**)], then randomly assigned to receive 1 mg/kg dose of spironolactone (**SPR;** Mutual Pharmaceutical Co., Inc; n = 6 [I = 4, O = 2]) or no spironolactone (**CON;** n = 6 [I = 3; O = 3]) daily for 7 d. Ponies were removed from their pasture two days prior the start of the study and maintained in 3.6 x 3.6 m stalls where ponies had visual contact with one another throughout the trial. Ponies had access to fresh water and TM salt blocks and were fed mixed grass hay at 2% of their BW daily that was split into two equal feedings at 0900 and 2000. Spironolactone tablets were crushed, mixed with 30 mL of syrup, and administered orally to each pony in the SPR group daily at 0800. The CON ponies each received 30 mL of syrup as a placebo daily at 0800. Orts were collected at 0830 daily. Blood was collected via jugular venipuncture three times daily (0700, 1300, and 1900) for later analysis of serum cortisol, aldosterone, and leptin. Data were analyzed with a repeated measures design using PROC MIXED of SAS. All ponies

lost BW ($P < 0.001$) regardless of treatment or ovarian status. There were no differences between treatment groups for feed intake ($P = 0.87$), leptin ($P = 0.47$), or cortisol ($P = 0.93$). Serum concentrations of cortisol changed over time ($P < 0.001$), while serum concentrations of leptin only tended to change over time ($P = 0.07$) for both SPR and CON ponies. Serum concentrations of aldosterone tended (time × treatment interaction; $P = 0.105$) to be greater in SPR than CON ponies over time. The relationship between leptin and adrenocorticoids in overweight equine is presently unclear. Further characterization and elucidation of the roles these hormones play in obesity and related maladies will improve our understanding and management of these disorders.

Key Words: Aldosterone, Cortisol, Leptin, Pony

Introduction

The condition of being overweight or obese is typically accompanied by hypertension in humans (Rossing et al., 2005; Saha et al., 2005), rats (Liu et al., 2000), and dogs (de Paula et al., 2004). Consequently, an association between adipocytes and adrenal activity has long been suspected in the pathology of obesity-related maladies. Recently, human adipocytes have been suspected of producing secretagogues that stimulate adrenal secretion of corticoids; and in particular, secretion of mineralocorticoids (Davy and Hall, 2004). Mineralocorticoids, specifically aldosterone, function to promote sodium and water resorption, resulting in increased blood volume and blood pressure. In 2003, Ehrhart-Bornstein et al., presented irrefutable evidence that adipocytes produced secretagogues that stimulated adrenal secretion of

mineralocorticoids. As such, mineralocorticoid receptor antagonists have also been reported to exhibit some attenuation of hypertension.

Increased adiposity is also associated with increased circulating concentrations of leptin in humans (Margetic, et al., 2002) and horses (Buff et al., 2002; Gentry et al., 2002). In addition to leptin's role as a regulator of appetite and energy balance, leptin has been reported to activate the sympathetic nervous system and more recently implicated in obesity-related hypertension in humans (Haynes, 2005; Tanida et al., 2006).

The condition of elevated blood pressure in obese and chronically laminitic ponies was reported by Johnson (2002). His indirect diagnosis of elevated blood pressure however was based on the pathology of left ventricular hypertrophy (**LVH**) of the heart muscle (Rugh et al., 1987). In reality, the condition and physiology associated with a hypertensive state in equine has yet to be identified.

Our objective was to characterize the endocrine association between obesity and adrenal function, and determine the short-term effects of spironolactone (a mineralocorticoid receptor antagonist) on serum concentrations of aldosterone, cortisol, and leptin in overweight pony mares.

Materials and Methods

Experimental Design and Treatments. Twelve pony mares ranging in age from 8 to 18 yr were blocked by BW and ovarian status [intact (**I**) or ovariectomized (**O**)], then randomly assigned to receive 1 mg/kg BW of spironolactone (**SPR;** Mutual Pharmaceutical Co., Inc; n = 6 [I = 4, O = 2]) or no spironolactone (**CON;** n = 6 [I = 3; O = 3]) daily for 7 d. The dose of spironolactone chosen was based on a previous study

in ponies (Alexander, 1982) that reported a significant increase in urinary sodium excretion with a single 1 mg/kg dose of spironolactone administered via nasogastric tube. Spironolactone pills were crushed, mixed with 30 mL of syrup, and administered orally to each pony in the SPR group daily at 0800. The CON ponies each received 30 mL of syrup as a placebo daily at 0800. Ponies readily consumed both the SPR and CON syrup. The research protocol was approved by the University of Missouri Animal Care and Use Committee prior to initiation of the study.

Management of Animals. Ponies were transported from their pasture of tall fescue and orchardgrass two days prior to the start of the study and maintained in 3.6 x 3.6 m stalls where ponies had visual and physical (able to touch noses) contact with one another for the duration of the 7 d trial. A two day acclimation interval was judged to be adequate because these ponies had been housed in the same facilities numerous times and other studies have provided evidence that peripheral concentrations of cortisol return to baseline within 1 d of transport (Stull and Rodiek, 2000). Ponies had access to fresh water and TM salt blocks throughout the study and were fed mixed grass hay at 2% of their BW daily that was split into two equal feedings at 0900 and 2000. Orts were collected and weighed daily at 0830. Ponies were weighed on d -1 and 8. Ponies were vaccinated against tetanus and Eastern and Western encephalitis, and dewormed with alternating anthelmintic products (February with febendazole, May with ivermectin, August with praziquantel, and November with moxidectin) according to the University of Missouri's standard operating procedures for the pony herd.

Measurements and Sample Collection. Blood was collected via jugular venipuncture three times daily (0700, 1300, and 1900) into Vaccutainer (Becton

Dickinson, Franklin Lakes, NJ) tubes with no additive. Samples were allowed to clot for 1 h at room temperature and were stored at 4°C for 4-12 h where they were later centrifuged at 3,000 × *g* for 20 minutes at 4°C for serum separation. Serum was collected and stored at – 20°C for later analysis of leptin, cortisol, and aldosterone.

Radioimmunoassay. Serum concentrations of leptin were measured in triplicate and were quantified using the double-antibody leptin radioimmunoassay procedures described by Delavaud et al. (2000) with one modification consisting of the substitution of the reported primary antiserum with a different rabbit anti-ovine leptin primary antiserum (number 7105). Briefly, standard concentrations of recombinant ovine leptin (Gertler et al., 1998; 0.1, 0.2, 0.3, 0.5, 0.8, 1.2, 2.0, 3.5, 5.0, and 7.5 ng in 300 µL/tube) and increasing volumes of serum (25, 40, 60, 100, 175, 250, and 300 µL) from a pool of serum collected from a fat mare were added to assay tubes in triplicate and the total volume balanced to 300 µL per tube with buffer consisting of 0.1% gelatin, 0.01 *M* EDTA, 0.9% NaCl, 0.01 *M* PO_4, 0.01% sodium azide, 0.05%Tween-20, pH = 7.1 (**PABET**). Likewise, 200 µL of the serum samples to be quantified were added to assay tubes in triplicate and volume balanced to 300 µL per tube with PABET. Immediately thereafter, 100 µL of rabbit anti-ovine leptin primary antiserum (7105; final tube dilution of 1:15,000 in PABET) was added and samples and standards incubated at 4°C for 24 h. After the initial incubation, 100 µL of ^{125}I-ovine leptin (20,000 c.p.m.) were added to each tube and incubation continued for an additional 24 h at 4°C. The antigen-antibody complex was then precipitated following a 15 min, 22°C incubation with 100 µL of a precipitated sheep-anti-rabbit second antiserum by centrifugation at 2,000 × *g* for 30 min, and the supernatant removed by aspiration. Assay tubes containing the pellets were

counted for 1 min on a LKB1277 gamma counter (LKB Wallac, Turku, Finland).

Standards and pooled aliquots of serum from a single source of fat-mare serum were linear (log/logit transformation; $R^2 > 0.96$) and parallel over a mass of 0.1 to 7.5 ng/tube and a serum volume of 25 to 300 µL, respectively. Total specific binding was 43 % , the minimum detectable concentration was 0.1 ng/tube, percentage recovery of mass was > 98% across the range of 25 to 300µL of sample and the inter- and intra-assay CV were < 10%.

Serum concentrations of cortisol were measured in duplicate using a commercial RIA kit previously validated for use on horse serum in our laboratory (Coated Tube RIA, Diagnostic Systems Laboratories, Webster, TX). Standards and pooled aliquots of serum from a single source of fat-mare serum were linear (log/logit transformation; $R^2 > 0.98$) and parallel over a mass of 5 to 600 ng/tube and a serum volume of 25 to 125 µL, respectively. Sensitivity of the cortisol assay was 5 ng/mL and the intra- and inter-assay CV was < 10%. No cross reactivity with aldosterone was reported by the manufacturers of this assay.

Serum aldosterone concentrations were measured in duplicate using a commercial RIA kit previously validated for use on horse serum in our laboratory (Coated Tube RIA, Diagnostic Systems Laboratories, Webster, TX). Standards and pooled aliquots of serum from a single source of fat-mare serum were linear (log/logit transformation; $R^2 > 0.92$) and parallel over a mass 25 to 1600 pg/tube and a serum volume of 50 to 400 µL, respectively. Sensitivity of the aldosterone assay was 25 pg/mL and the intra- and inter-assay CV was < 10%. No detectable cross-reactivity was reported with cortisol or spironolactone by the manufacturers of this assay.

Statistical Analysis. Data were analyzed with a repeated measures design using PROC MIXED of SAS (SAS Institute, Cary, NC). The model included the fixed effects of ovarian status, time, and treatment and all interactions of the previous variables on feed intake and serum concentrations of leptin, aldosterone, cortisol, and BW. Initial individual BW was used as a covariate because differences existed between I and O mares before treatment began. The variable of time was used in the repeated statement, and the error term used was animal within treatment × ovarian status. Least squares means were generated for ovarian status, time, treatment, and all interactions. No differences between I and O ponies were found in feed intake, leptin, cortisol, or aldosterone concentrations; therefore these data were pooled and analyzed using PROC MIXED of SAS. The model included time, treatment, and time × treatment. Least square means were generated for time, treatment, and time × treatment. All data are presented as means ± SEM.

Results

All ponies lost BW ($P < 0.001$) regardless of treatment or ovarian status. No differences were found between I and O ponies in feed intake ($P = 0.32$), serum leptin ($P = 0.23$), cortisol ($P = 0.43$), or aldosterone ($P = 0.63$), therefore these data were pooled and analyzed as described in Materials and Methods. When the data were pooled, there were no differences between treatment groups for feed intake ($P = 0.87$), leptin ($P = 0.47$) or cortisol ($P = 0.93$). Serum concentrations of cortisol changed over time (Figure 11; $P < 0.001$), and serum concentrations of leptin tended to change over time (Figure 12; $P = 0.07$). There was a trend among serum concentrations of aldosterone for a time ×

treatment interaction (Figure 13; $P = 0.105$) such that serum aldosterone was greater in SPR than CON ponies over time.

Discussion

This study was designed to investigate the relationship between leptin and adrenocorticoids (cortisol and aldosterone) in equine and in response to administration of the mineralocorticoid receptor antagonist spironolactone. Spironolactone competitively binds and displaces aldosterone at the MR at the sodium-potassium exchange sites in the distal convoluted tubule of the kidneys, causing increased water and sodium secretion while retaining potassium and magnesium ions. Chronically elevated aldosterone concentrations in the blood are often associated with obesity, and are thought to play a major role in obesity-induced hypertension (Pausova, 2006; de Paula et al., 2004). An *in vitro* study by Ehrhart-Bornstein et al. (2003) demonstrated that human adipocytes secrete potent mineralocorticoid releasing factors, suggesting a direct link between obesity and hypertension. Pausova (2006) suggested that activation of the sympathetic nervous system by leptin (which is elevated in obesity) may augment the effects of aldosterone on peripheral vasoconstriction and enhance reabsorption of renal sodium and water, thus elevating blood pressure. A hypertensive state in equine as measured by LVH has previously been reported in horses affected with chronic laminitis (Rugh et al., 1987) who are often obese (Johnson, 2002); however endocrine profiles associated with hypertension in equine have yet to be identified.

Chronic hyperleptinemia associated with obesity and a high fat diet has been shown to elevate blood pressure in rodents (Tanida et al., 2006) likely due to peripheral

vasoconstriction and increased sodium and water retention (Pausova, 2006). It has been reported that blood concentrations of leptin are positively correlated with BCS (Buff et al., 2002) and fat mass in horses (Kearns et al., 2005), as well as BMI in humans (Hall et al., 2000). Notwithstanding these associations, we found no difference between CON and SPR ponies in serum concentrations of leptin in the present study. Haluzik et al. (2002) compared serum concentrations of leptin and insulin sensitivity in eleven patients with primary hyperaldosteronism (PA) to eleven healthy, age-, gender-, and body mass index (BMI)-matched individuals. They reported impaired insulin sensitivity in PA individuals before surgical or pharmacological (spironolactone) treatment, and no differences in leptin concentrations between healthy and PA individuals. However, after at least six mo of treatment, insulin sensitivity improved significantly and leptin concentrations increased in individuals treated for PA with no significant change in BMI, which was not expected. Since hyperleptinemia often accompanies insulin resistance, not improved insulin sensitivity, no explanation could be offered for this rise in leptin based on the Haluzik et al. (2002) study. The authors speculated though that chronic elevation of aldosterone and/or decreased concentration of renin may somehow affect leptin synthesis. Additionally, it was suggested that reduced concentrations of leptin among patients with elevated aldosterone concentrations may have been a compensatory mechanism to regulate hypertension (Haluzik et al., 2002).

We saw no changes in serum concentrations of cortisol between CON and SPR mares which was unexpected based on human reports. Young et al. (1998) reported that one single 400 mg dose of spironolactone administered to healthy adult men caused a transient rise in blood cortisol concentrations compared to placebo treatment.

Glucocorticoids have a high affinity for the MR, which become saturated by low concentrations of glucocorticoids (Spencer et al., 1990; Spencer et al., 1993; Young et al., 1998). The transient rise in cortisol reported in human studies may be explained by spironolactone's occupation of the MR, preventing binding of glucocorticoids to those receptors, causing a temporary increase in circulating glucocorticoid concentrations. Our inability to resolve a difference in serum concentrations of cortisol may have been due to an inadequate sampling frequency, compared to the human study by Young et al. (1998) where samples were collected every 30 min for 6 h. Additionally, there may be species differences in the glucocorticoid response to spironolactone.

The cortisol response may also have been altered as a result of the jugular venipuncture three times per day. We observed that while some of the ponies were less reactive to venipuncture with time, other ponies were more responsive to venipuncture. Thus our sampling method may have unexpectedly skewed or obscured the cortisol response in some ponies.

The tendency of aldosterone to be increased over time in SPR compared to CON groups is in agreement with the literature (Ménard, 2004; Garthwaite and McMahon, 2003). The antagonistic action of spironolactone at the MR in the distal convoluted tubules of the kidneys both displaces and prevents binding of aldosterone at these sites, disrupting the negative feedback loop. Because of occupation by spironolactone, the MR do not respond to the circulating aldosterone, which stimulates renin secretion, ultimately leading to increased aldosterone synthesis and release from the adrenal cortex (Garthwaite and McMahon, 2003). Chronic elevated circulating aldosterone concentrations in response to spironolactone treatment are unlikely to cause the negative

consequences associated with elevated aldosterone concentrations due to pathology or obesity because the MR are occupied by spironolactone and are therefore unable to be activated by aldosterone (Garthwaite and McMahon, 2003). Alexander (1982) administered spironolactone to equine to examine its effects on electrolyte excretion in ponies. A 1 mg/kg BW dose of spironolactone resulted in a significant increase in urinary sodium excretion which was augmented by an additional dose 24 h later, presumably as a result of spironolactone's antagonistic effect at the MR on aldosterone action. The authors did not measure peripheral concentrations of aldosterone in their study.

Clarke et al. (1988) investigated the effects of meal frequency and size on the rennin-angiotensin-aldosterone system (RAAS) in horses. These investigators found that a single large meal caused significant changes in the RAAS of horses that were not exhibited when horses were fed six equal meals at 4-h intervals throughout the day. Since horses are designed to eat small, frequent meals throughout the day, not one or two large meals as is the current industry practice, the consequences of this management strategy may warrant further investigation (Clarke et al., 1988).

It is well-documented in the human population that increased weight gain is associated with increased blood pressure, although great individual variation exists (Davy and Hall, 2004). Activation of the sympathetic nervous system and the rennin-angiotensin-aldosterone system (RAAS) are likely the primary influences in this condition (Rahmouni et al., 2005; Hall, 2000) as it has been reported that all components of the RAAS are augmented in obese compared to lean individuals (Pausova, 2006). Although we did not measure blood pressure in our study, indirect measures of blood

pressure may be taken in equine by using an occlusion cuff over the coccygeal artery and measuring pressure by oscillometric methods (Magdesian, 2004). No published work could be found investigating whether weight loss in obese equine parallel attenuation of blood pressure. It has been reported that weight loss in humans is accompanied by lowered blood pressure, and thus a decrease of its ill-effects (Davy and Hall, 2004; Rahmouni et al., 2005); therefore it seems an investigation of this phenomenon in equine would be worth pursing.

Implications

The relationship between leptin and adrenocorticoids in overweight equine is presently unclear. In addition, the implication of a hypertensive state in equine remains unknown and warrants further investigation. Further characterization and elucidation of the roles that adipokines and adrenocorticoids play in equine hypertension, as it relates to obesity, insulin resistance, and laminitis in the horse deserve additional investigations.

Figure 11. Serum concentrations of cortisol in control (CON; ●,n = 6) vs. spironolactone (SPR; ○,n = 6) treated pony mares over time. The numbers on the x-axis indicate the sample day and the letters a, b, and c represent sampling times of 0700, 1300, and 1900 within each day, respectively. Serum concentrations of cortisol changed over time ($P < 0.001$); however no differences ($P = 0.93$) were seen between treatments.

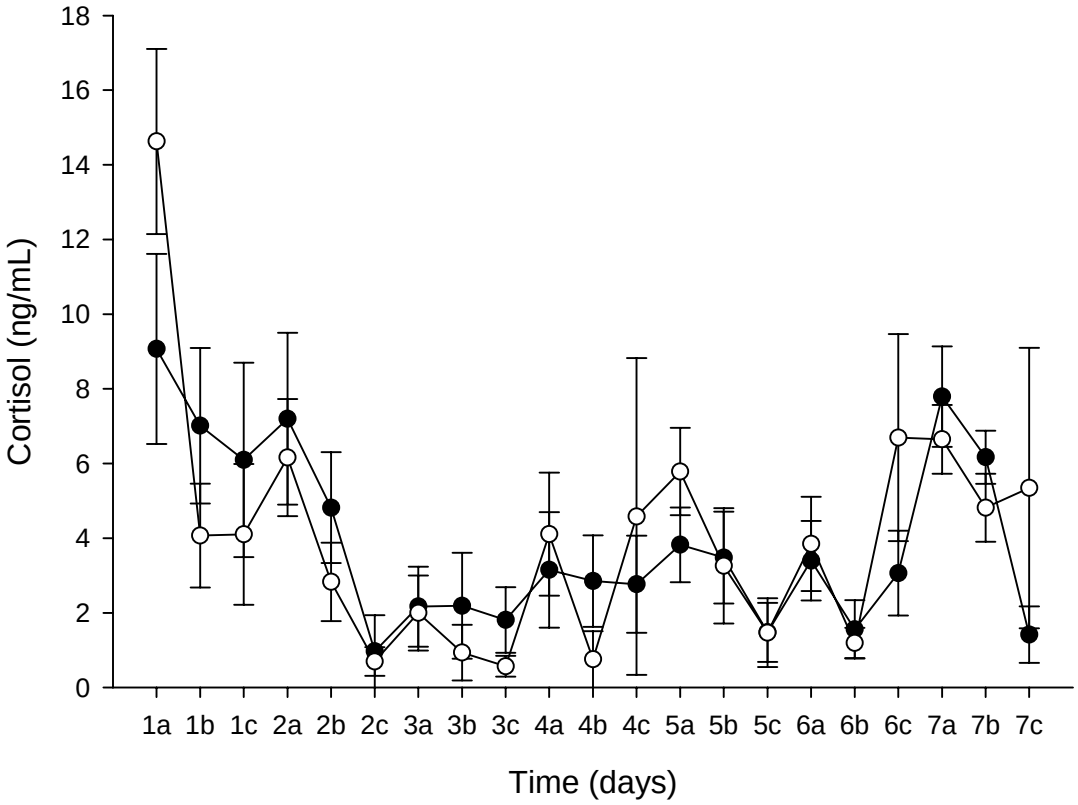


Figure 12. Serum concentrations of leptin in control (CON;▲, n = 6) vs. spironolactone (SPR; Δ, n = 6) treated pony mares over time. The numbers on the x-axis indicate the sample day and the letters a, b, and c represent sampling times of 0700, 1300, and 1900 within each day, respectively. Serum concentrations of leptin tended to change over time ($P = 0.07$); but did not differ ($P = 0.47$) with respect to treatment.

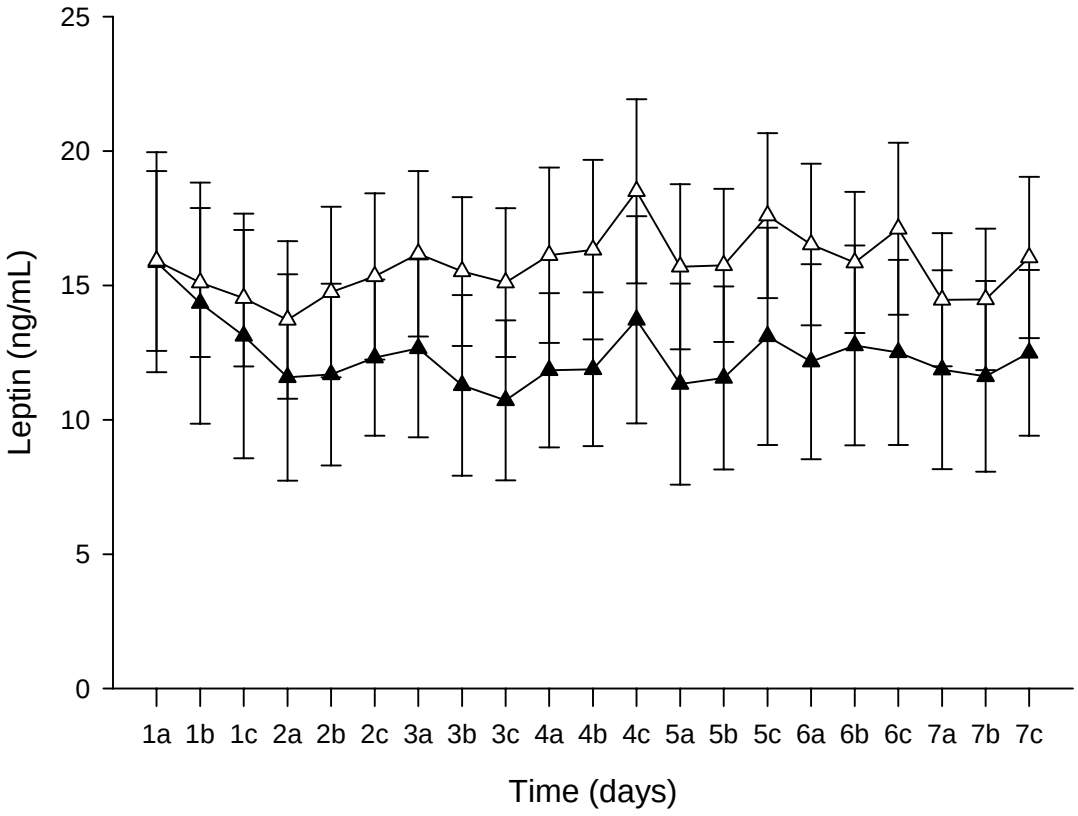
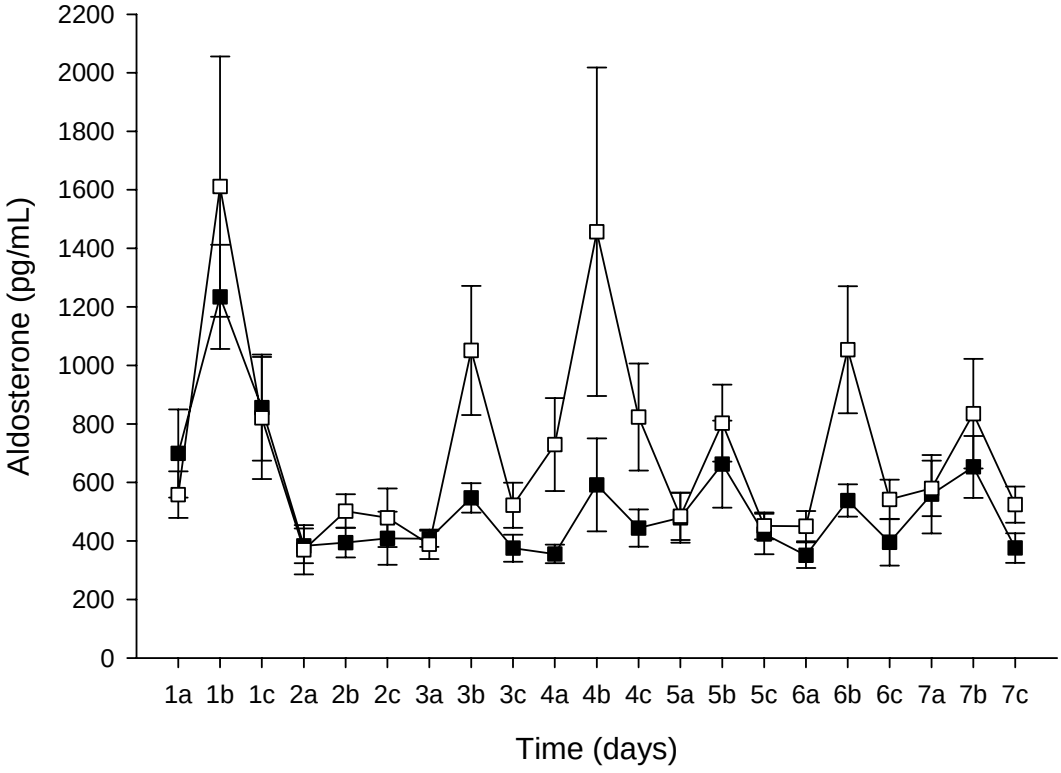


Figure 13. Serum concentrations of aldosterone in control (CON; ■, n = 6) vs. spironolactone (SPR;□, n = 6) treated pony mares over time. The numbers on the x-axis indicate the sample day and the letters a, b, and c represent sampling times of 0700, 1300, and 1900 within each day, respectively. Serum concentrations of aldosterone tended to differ according to a time × treatment interaction ($P = 0.105$), and serum concentrations of aldosterone were greater in SPR than CON ponies over time.



CHAPTER V

BIOAVAILABILITY OF LIPOIC ACID IN EQUINE

ABSTRACT: Lipoic acid is a small molecule that is rapidly absorbed by the gastrointestinal tract and metabolized by a variety of tissues. Lipoic acid is also a cofactor in a number of multi-enzyme complexes and supplementation with lipoic acid has been shown to improve insulin effectiveness and glucose disposal. No evidence could be found in the literature to establish the absorption rate of lipoic acid into equine blood after oral dosing. Therefore the objective of this trial was to determine whether lipoic acid was absorbed into equine blood after oral administration and if so, the associated pharmacokinetics. Three pony mares were fitted with indwelling jugular catheters 3 d prior to initiation of the lipoic acid bioavailability trial. A baseline sample was taken at time 0 h, and then a 10 mg/kg BW dose of lipoic acid was mixed with 30 mL of syrup and administered orally to each animal. Subsequent blood samples were taken at 0.02, 0.08, 0.50, 1, 2, 4, 6, 8, 10, 12, and 24 h after LA administration. Serum was harvested and stored at – 80°C for later HPLC analysis. Our observations were that oral dosing of lipoic acid rapidly resulted in elevated serum concentrations of lipoic acid, peaking at 30 min post-dosing and returning to baseline concentrations by 6 h post-dosing. We conclude that oral administration of lipoic acid to equine is an effective method of lipoic acid supplementation.

Introduction

It has been reported that oral dosing of rats with lipoic acid resulted in rapid absorption by the gastrointestinal tract, metabolism by various tissues including the liver, skeletal muscle, and heart, and then excretion (Bustamante et al., 1998). The small molecular size of lipoic acid (approximately 43 kD; Yasuno and Wada, 1998) likely facilitates its rapid absorption into biological membranes.

No evidence could be found in the literature to establish the bioavailability of orally administered lipoic acid to equine. Therefore, the purpose of this trial was to determine whether orally administered lipoic acid would be absorbed by the equine gut and the pharmacodynamics of lipoic acid in the blood.

Materials and Methods

Three pony mares were fitted with indwelling jugular catheters (Extended Use MILACATH®, Florence, KY) 3 d prior to study. A baseline sample was taken at time 0 h, and then a 10 mg/kg BW dose of lipoic acid was mixed with 30 mL of syrup and administered orally to each animal. The mixture was readily consumed by all ponies. Subsequent blood samples were taken at 0.02, 0.08, 0.50, 1, 2, 4, 6, 8, 10, 12, and 24 h after lipoic acid dosing. Blood samples were collected into Monoject tubes with no additive (Tyco Healthcare Group, Mansfield, MA) and allowed to clot for 1 h at room temperature. Blood samples were then centrifuged at 3,000 × *g* for 20 minutes at 4°C. Serum was harvested and stored at – 80°C for later high-performance liquid chromatography (HPLC) analysis.

Samples were briefly thawed and 250 µl of serum was placed into 1.5 ml microtube. A 0 h reference sample was created to establish recovery rates, by adding 100

µl of 1 ppm lipoic acid standard to 500 µl of diluted alcalase buffer. After vortexing, samples were incubated in a 37°C water bath for 30 min. After incubation, 1 ml of distilled water and 1 ml of trichloroacetic acid were added and tubes and vortexed. The samples were then slowly loaded on a pre-conditioned Baker SPE phenyl column and washed with 1 to 2 mL of distilled water. Lipoic acid was eluted with 4 mL of methanol washed through the column. The samples were then reconstituted in 1 mL HPLC grade methanol:water (80:20) mixture, vortexed, and rested for 30 minutes until analysis. Cloudy samples were transferred to microtubes and centrifuged at 13,000 × *g* for 5 min and then transferred to HPLC vials for analysis.

Results

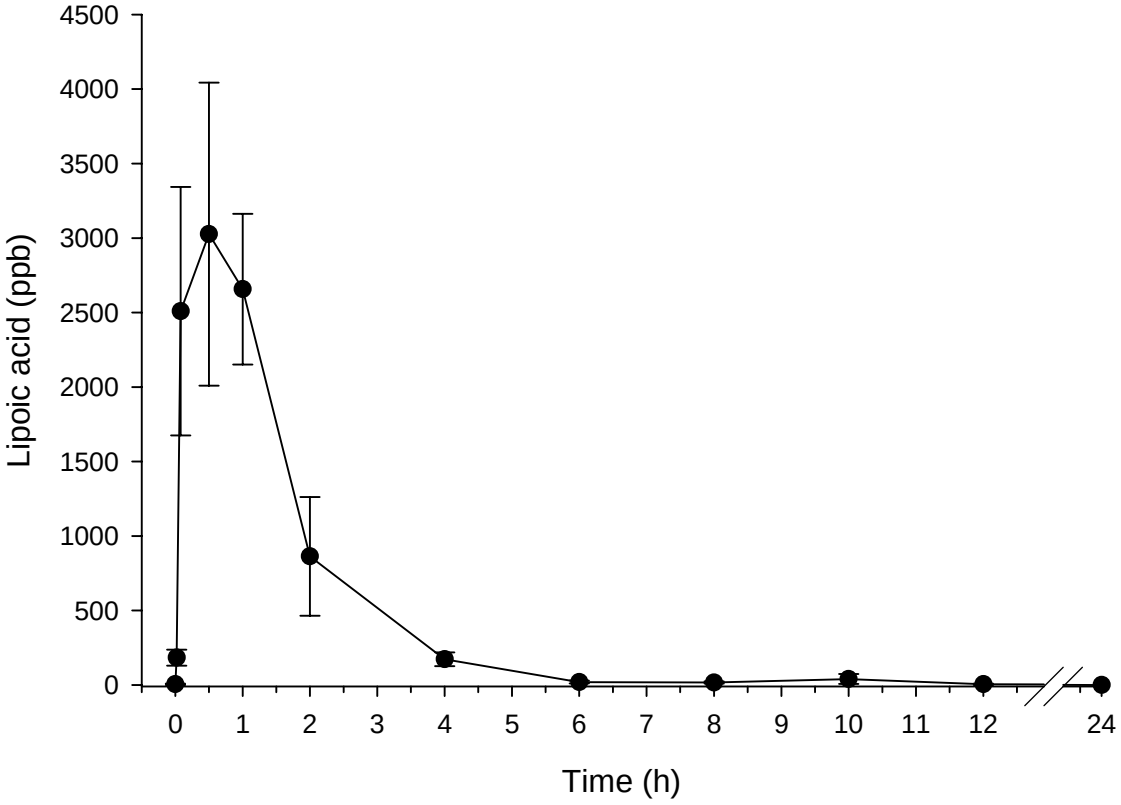
Concentrations of lipoic acid increased 3,000 times baseline concentrations by 30 min post-dosing and rapidly decreased as well, declining to 800 times baseline concentrations by 2 h post-dosing, and returning to baseline concentrations by 6 h post-dosing (Figure 2).

Discussion

This trial establishes that lipoic acid is rapidly absorbed into equine blood after oral dosing, similar to findings in other species. Bustamante et al. (1998) reported maximum urinary excretion of lipoic acid 3 to 6 h after administration to rats, supporting the idea that lipoic acid is rapidly absorbed and metabolized by biological membranes. Schmidt (2004) reported a 6,000-fold increase in blood serum concentrations of lipoic acid 3 h after oral dosing of lipoic acid in sheep, compared to only an 80-fold increase

when lipoic acid was placed directly into the rumen of cannulated steers. Schmidt and coworkers (2004) speculated that due to lipoic acid's small size, oral dosing resulted in a much more rapid increase in blood levels of lipoic acid due to absorption of lipoic acid in the mouth and esophagus of orally dosed animals, as opposed to rumen absorption. We concluded that oral dosing equine with lipoic acid is an effective method of lipoic acid supplementation.

Figure 14. Serum concentrations of lipoic acid in the blood of pony mares following oral administration of 10 mg/kg lipoic acid at the time 0 blood sample.



CHAPTER VI

EFFECTS OF LIPOIC ACID SUPPLEMENTATION ON GLUCOSE, INSULIN, AND LEPTIN CONCENTRATIONS IN PONY MARES

ABSTRACT: Equine obesity is a growing industry concern often associated with insulin resistance. Dietary supplements containing lipoic acid have been found to improve the effectiveness of insulin and glucose disposal for a number of species. The primary objective of this study was to determine whether daily dietary supplementation of 10 mg/kg lipoic acid for 14 d to fat pony mares would improve insulin effectiveness and glucose disposal as measured by an i.v. glucose tolerance test (IVGTT). In Experiment 1, eleven pony mares were blocked by BCS and ovarian status (ovary intact = I or ovariectomized = O) and randomly assigned to receive saline (SAL) or insulin (INS) during a IVGTT on d 0. In Experiment 2, the same pony mares were again blocked by BCS and ovarian status and randomly assigned to receive 10 mg/kg lipoic acid daily (LA; n = 6 [I = 4, O = 2]) or control (CON; n = 5 [I = 3, O = 2]) treatment for 14 d. An IVGTT was performed on d 14 of the trial period. The IVGTT consisted of baseline blood samples collected one hour (-1) preceding initiation of the IVGTT on d 0 and 14 and immediately prior to administration of the glucose bolus (d 0 and d 14; 50% dextrose solution, Fort Dodge Animal Health, Fort Dodge, IA) administered at the rate of 0.3 g/kg of BW. In Experiment 1, an insulin bolus of 0.030 U/kg was injected through the jugular catheter 20 min after glucose dosing of INS ponies while an equal dose of saline was administered to SAL ponies. In Experiment 2, an insulin bolus of 0.030 U/kg

was injected through the jugular catheter 20 min after glucose dosing to the both CON and LA ponies. Blood samples were collected at 3, 6, 9, 12, 15, 18, 22, 25, 28, 31, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 150, and 180 min following glucose administration. Additional blood samples were obtained twice daily from LA and CON ponies on d 1 through 14 for leptin analysis. In Experiment 1, there was a trend (P = 0.099) for mean concentrations of glucose to be lower in the INS than in the SAL group, however no differences were found in mean serum concentrations of insulin (P = 0.16) or leptin (P = 0.65) during the d 0 IVGTT. In Experiment 2, there was a trend (P = 0.06) for mean serum concentrations of insulin to be lower in the LA ponies compared to CON; however no differences were found in mean serum concentrations of glucose (P = 0.61) or leptin (P = 0.81) between treatments during the d 14 IVGTT**.** No differences (P = 0.80) were found between treatment groups for mean serum concentrations of leptin over the 14 d sampling period. All ponies lost BW (P = 0.002) and BCS (P = 0.02) regardless of treatment or ovarian status. These data provide evidence that lipoic acid supplementation to ponies for 14 d may improve insulin effectiveness, suggesting that further work to investigate the potential of lipoic acid as an insulin sensitizing agent in equine is worth pursuing.

Key Words: Lipoic acid, Equine, Glucose, Insulin, Leptin

Introduction

Both insulin and leptin are recognized as biological regulators of energy balance (Schwartz et al., 2000). The primary role of insulin is glucose homeostasis and it is an

acute indicator of energy status (Benoit et al., 2006). Leptin is an adipocyte-derived protein hormone that plays an important role in appetite regulation (Schwartz et al., 2000) and is a relatively stable indicator of body fat in animals (Benoit et al., 2006). It has been demonstrated that obese equine often have elevated concentrations of both leptin (Buff et al., 2002; Gentry et al. 2002; Kearns et al., 2005) and insulin (Jeffcott et al., 1986), however the exact relationship between the two hormones is yet to be defined.

Lipoic acid is a naturally occurring eight carbon compound synthesized by animals and plants (Carreau et al, 1975), although the complete pathway for its *de novo* synthesis is yet to be elucidated. Endogenously produced lipoic acid travels bound to lysine by an amide linkage forming a lipoyl group that is a component of the mitochondrial pyruvate dehydrogenase and the α-ketoglutarate dehydrogenase complexes which are involved in energy metabolism (Biewenga et al., 1997). Although two isomers (R-LA and S-LA) of lipoic acid may be synthetically produced and supplemented, only R-LA is synthesized in the natural state and is more biologically active than S-LA (Biewenga et al., 1997).

Lipoic acid supplementation has been found to improve glucose utilization and insulin action in humans, rodents, and chickens. Saengsirisuwan et al. (2001) found that supplementation of R-LA to obese Zucker rats increased glucose transport into isolated skeletal muscle as assessed by *in vitro* rates of 2-deoxyglucose uptake into muscle. Hamano (2006) reported improved whole body glucose disposal in broiler chickens supplemented daily with 200 mg/kg lipoic acid as estimated by hyperglycemic-euglycemic clamp technique. In addition, lipoic acid supplementation has been reported to improve insulin effectiveness as evidenced by increased expression of insulin receptor

substrate-1 (IRS-1) signaling in skeletal muscle (Saengsirisuwan et al., 2004; Henriksen, 2006), a primary site for glucose uptake and storage.

The objectives of this study were to determine whether lipoic acid supplementation to pony mares for 14 d: 1) improved glucose uptake and insulin effectiveness (as measured by a modified frequent sampled i.v. glucose tolerance test) and (or) 2) altered serum concentrations of leptin.

Materials and Methods

Animal Management and Blood Collection for Experiments 1 and 2. Eleven pony mares between 8 and 18 years of age with an initial average BW of 252.5 ± 18.3 kg were used for this trial. Two days prior to the initiation of Experiment 1, ponies were removed from a dry lot and placed in 3.6 x 3.6 m^2 stalls. While on test, ponies continued to be fed mixed grass hay (Table 2) from the same source and load as had been fed while in the dry lot. Ponies were maintained in the 3.6 x 3.6 m^2 stalls for the duration of both studies and fed mixed grass hay at 2% of BW daily split into two equal feedings. The morning hay was fed at 0830 and evening hay at 2030. Ponies had *ad libitum* access to fresh water and TM salt blocks throughout the study. All ponies were vaccinated against tetanus and Eastern and Western encephalitis and dewormed with alternating anthelmintic products (February with febendazole, May with ivermectin, August with praziquantel/ivermectin combination, and November with moxidectin) according to University of Missouri's standard operating procedures.

Experiment 1. Ponies were weighed and assigned a BCS prior to the initiation of Experiment 1 (d 0). Body condition scoring was performed by the same individual

throughout the trial period using a 1 to 9 scale according to Henneke et al. (1983). Ponies were blocked by BCS and ovarian status [intact (**I**) or ovariectomized (**O**)], and randomly received a 0.030 U/kg (**INS;** n = 6 [I = 4, O = 2]) insulin bolus or an equivalent volume of saline (**SAL**; n = 5 [I = 3, O = 2]) during a modified frequent sampled i.v. glucose tolerance test (**IVGTT**) on d 0.

Experiment 2. Ponies were again blocked by BCS and ovarian status [intact (**I**) or ovariectomized (**O**)], then randomly assigned to receive either 10 mg/kg BW of racemic (50:50 mixture of R- and S-isomer) lipoic acid (**LA;** MTC Industries, New York, NY; n = 6 [I = 4, O = 2]) on d 1 through 14 or no lipoic acid (**CON**; n = 5 [I = 3, O = 2]). The dose of lipoic acid chosen was based on a previous study (Williams et al., 2002) that reported reduced oxidative stress in horses orally supplemented with 10 mg/kg lipoic acid for 14 d. The lipoic acid was mixed with 30 mL of generic brand maple syrup and administered orally to each pony in the LA group at 0800 while each CON pony received 30 mL of syrup daily as a placebo at 0800. Both CON and LA ponies readily consumed the treatment dose. Blood samples were collected daily at 0700 and 1900 on d 1 through 13 and once at 0700 on d 14 for subsequent leptin analysis. Ponies were weighed and assigned a BCS prior to administration of a IVGTT on d 14.

Modified IVGTT for Experiments 1 and 2. Modified IVGTT were administered on d 0 and 14. Ponies were fitted with extended use indwelling jugular catheters (Extended Use MILACATH®, Florence, KY) the morning of the IVGTT on d 0. Each pony had a minimum of two hours from the time of catheterization to initiation of the IVGTT at 1430. Ponies were allowed access to hay until the initiation of the IVGTT (Eiler et al., 2005; Frank et al., 2006) as fasting has been shown to disrupt the insulin

response (Forhead and Dobson, 1997). Baseline blood samples were collected one hour (-1 h) preceding initiation of the IVGTT and immediately prior to administration of the glucose bolus (0 h; dextrose solution 50%, Fort Dodge Animal Health, Fort Dodge, IA) dosed at 0.3g/kg BW. The glucose bolus was injected through the catheter in less than 2.5 min. In Experiment 1, an insulin bolus (Humulin R, Eli Lilly and Co., Indianapolis, IN) of 0.030 U/kg was injected through the catheter 20 min after glucose administration to INS ponies while an equal dose of saline was administered to SAL ponies. In Experiment 2, an insulin bolus of 0.030 U/kg was injected through the catheter 20 min after glucose administration to the both CON and LA ponies. The doses of glucose and insulin administered have previously been used in horses by Hoffman et al. (2003). Blood samples were collected at 3, 6, 9, 12, 15, 18, 22, 25, 28, 31, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 150, and 180 min following glucose administration. Jugular venous blood was collected for both plasma and serum yield. The blood for plasma was immediately placed into sodium heparinized Venoject tubes (Terumo Medical Corporation, Elkton, MD) and kept on ice until plasma was harvested within 60 minutes of collection. The blood for serum was placed into Monoject tubes with no additive (Tyco Healthcare Group, Mansfield, MA) and allowed to clot for 1 h at room temperature. Samples for plasma and serum were centrifuged at 3,000 × *g* for 20 minutes at 4°C. After harvest, plasma and serum were stored at –20°C for later analysis.

Glucose and Hormone Analysis from Blood. Plasma concentrations of glucose were analyzed by enzymatic assay (Infinity Glucose Hexokinase, Thermo DMA, Pittsburgh, PA). Plasma concentrations of insulin were determined in duplicate using a commercial RIA kit (Coat-A-Count Insulin, Diagnostic Products Corp., Los Angeles,

CA) which was previously validated for equine insulin determinations (Freestone et al., 1991). The intra- and interassay CV for glucose was 5% and <10% for insulin. Serum concentrations of leptin were measured in triplicate and were quantified using the double-antibody leptin radioimmunoassay procedures described by Delavaud et al. (2000) with one modification consisting of the substitution of the reported primary antiserum with a different rabbit anti-ovine leptin primary antiserum (number 7105). Standards and pooled aliquots of serum from a single source of fat-mare serum were linear (log/logit transformation; $R^2 > 0.97$) and parallel over a mass of 0.1 to 7.5 ng/tube and a serum volume of 25 to 300 µL, respectively. Total specific binding was 43%, the minimum detectable concentration was 0.1 ng/tube, percentage recovery of mass was > 98% across the range of 25 to 300µL of sample and the inter- and intra-assay CV were < 10%.

Statistical Analysis. In Experiment 1, analyses were conducted to determine treatment differences over time for glucose, insulin, and leptin during the IVGTT on d 0 as a result of the insulin dose. Data were analyzed with a repeated measures design using the mixed procedures of SAS evaluating the effects of treatment (SAL vs. INS) and ovarian status (I vs. O) for differences in least squares means of glucose, insulin, and leptin with treatment as the repeated measure and animal within ovarian status as the error term. No differences were found between I and O animals, therefore these data were pooled and analyzed using the mixed procedure of SAS evaluating the main effect of treatment (SAL vs. INS) for differences in least squares means of glucose, insulin, and leptin with treatment as the repeated measure and animal as the error term. Least square means ± SEM were generated for treatment**.**

In Experiment 2, analyses were conducted to determine whether differences

existed in leptin, BW, and BCS due to lipoic acid treatment for 14 d. Leptin, BW, and BCS data were analyzed with a repeated measures design using the mixed procedure of SAS (SAS Institute, Cary, NC). Effects used in the model were treatment (CON vs. LA), time, ovarian status (I vs. O), and all interactions of the previous variables on leptin, BW, and BCS. The variable of time was used in the repeated statement, and the error term used was animal within treatment × ovarian status. Least squares means were generated for treatment, time, ovarian status, and all interactions. No differences ($P > 0.05$) were found between I and O ponies, therefore these data were pooled and analyzed using mixed procedures of SAS and the model used including time, treatment, and time × treatment. Least square means were generated for time, treatment, and time × treatment**.**

Analyses were also performed to determine whether differences existed between glucose, insulin, and leptin during the d 14 IVGTT as a result of lipoic acid treatment. Data were analyzed with a repeated measures design using the mixed procedures of SAS evaluating the effects of treatment (CON vs. LA) and ovarian status (I vs. O) for differences in least squares means of glucose, insulin, and leptin concentrations with treatment as the repeated measure and animal within ovarian status as the error term. No differences ($P > 0.05$) were found between I and O ponies, therefore data were pooled and analyzed using the mixed procedure of SAS, evaluating the main effect of treatment (CON vs. LA) for differences in least squares means of glucose, insulin, and leptin concentrations with treatment as the repeated measure and animal as the error term. Least square means ± SEM were generated for treatment**.**

Results

Experiment 1. Mean concentrations of glucose across all time points associated with the IVGTT tended ($P = 0.099$) to be lower in INS (154.03 ± 9.23 mg/dL) compared to SAL (179.18 ± 10.14 mg/dL) treated ponies. No differences were found in mean insulin (113.65 ± 36.22 and 188.91 ± 33.16 μU/mL for SAL and INS, respectively; $P = 0.16$) or leptin (6.87 ± 2.21 and 5.48 ± 2.02 ng/mL for SAL and INS, respectively; $P = 0.65$) concentrations across all time points of the IVGTT between treatments. There was a difference in mean concentrations of insulin (120.63 ± 52.31 and 296.13 ± 47.75 μU/mL for SAL and INS, respectively; $P = 0.035$) and glucose (191.55 ± 10.51 and 159.10 ± 9.59 mg/dL for SAL and INS, respectively; $P = 0.049$) between the time points of 22 min (2 min post-dosing) and 55 min of the IVGTT. Response variables are presented in Figures 15 through 17 for glucose, insulin, and leptin, respectively.

Experiment 2. All ponies lost BW (252.5 ± 18.3 and 246.2 ± 18.3 kg for d 0 and d 14, respectively; $P = 0.002$) and BCS (6.82 ± 0.32 and 6.44 ± 0.32 for d 0 and d 14, respectively; $P = 0.02$) regardless of treatment (CON or LA) or ovarian status (I or O). No differences ($P = 0.80$) were found between treatment groups for serum concentrations of leptin over time (Figure 18). During the d 14 IVGTT, concentrations of insulin tended ($P = 0.06$) to be higher among CON (304.10 ± 45.90 μU/mL) than LA (167.86 ± 41.95 μU/mL) treated ponies. Interestingly, no differences ($P = 0.61$) were found in mean concentrations of glucose between CON (162.06 ± 10.65 mg/dL) and LA (169.61 ± 9.74 mg/dL). Likewise, concentrations of leptin for CON (5.46 ± 1.75 ng/mL) and LA (4.87 ± 1.60 mg/dL) did not differ ($P = 0.81$). Response variables during the IVGTT are presented in Figures 19 through 21 for glucose, insulin, and leptin, respectively.

Discussion

Experiment 1. The primary biological function of insulin is to decrease blood concentrations of glucose. Although we saw no difference in mean concentrations of insulin across all time points of the IVGTT, we did observe a significant increase in concentrations of insulin during the period from 22 to 55 min of the IVGTT. This was accompanied by reduced concentrations of glucose during that same interval. The 22 to 55 min interval was chosen and analyzed discretely, as we anticipated this intervalwould yield the greatest difference in insulin and glucose, if one existed, between INS and SAL ponies in the period following exogenous insulin administration at 20 min. The end point of 55 min was chosen because the half-life of insulin is approximately 2 to 3 minutes (Benoit et al., 2004), therefore we expected the exogenous insulin to have been cleared from the blood in approximately 30 min (time 55). A trend for reduced concentrations of glucose in the INS ponies was maintained across all time points.

We did not observe an increase in concentrations of leptin during the 180 min IVGTT in either SAL or INS treated ponies. Cartmill et al. (2005) reported that leptin had not increased by 3 h after stallions had been administered exogenous insulin or a high carbohydrate grain meal. However, by approximately 8 hr post-treatment, significant peaks in serum concentrations of leptin were evident while no increase was observed in stallions that were not fed and did not receive exogenous insulin. Similarly, Gordon and McKeever (2005) reported no increase in plasma leptin within 3 hr of a grain meal, but did see an increase in concentrations of leptin approximately 7 h after increased plasma concentrations of insulin as the result of a morning grain meal. In the same study, this

did not hold true for the afternoon meal as a subsequent rise in leptin was not observed 7 to 9 h post-feeding. Had we continued to sample ponies for a longer period of time, we speculate that we may have observed an increase in leptin response due to the insulin dose. Buff et al. (2006b) recently reported a decrease in leptin concentrations 6 h after feed removal in obese pony mares. Similar work in equine supports the idea that feed deprivation suppresses leptin secretion (McManus and Fitzgerald, 2000; Cartmill et al., 2005).

Experiment 2. Insulin resistance is described by Kahn (1978) as existing "whenever normal concentrations of hormone produce a less than normal biologic response." In other words, if more insulin than normal is required to maintain homeostatic concentrations of blood glucose, as is often the case with obese individuals, the condition is described as insulin resistance. Obese Zucker (*fa/fa*) rats are an animal model for severe insulin resistance in skeletal muscle. These rats have a defect in the insulin signaling pathway which leads to decreased insulin-stimulated GLUT4 expression, resulting in reduced glucose uptake by skeletal muscle (Henriksen, 2006). Saengsirisuwan et al. (2004) orally supplemented R-LA at 30 mg/kg BW daily for 2 weeks to obese Zucker rats and reported a significant improvement in insulin action on glucose transport activity in skeletal muscle. This improvement was attributed to increased expression of key factors in the insulin-signaling pathway, including IRS-1 and PI-3 kinase. Lee et al. (2005) demonstrated that oral supplementation of a racemic lipoic acid mixture at 0.5% (wt/wt) for 3 d to obese male Otsuka Long Evans Tokushima Fatty rats improved insulin-stimulated glucose disposal in whole body and in skeletal muscle as determined by hyperglycemic-euglycemic clamp studies. We observed a trend for

reduced mean concentrations of insulin in LA ponies after 14 d of supplementation and, although we saw no difference in concentrations of glucose, these data may indicate that lipoic acid indeed improved insulin effectiveness since less insulin was required to clear the same amount of glucose. Another possible explanation for the apparent improvement in insulin effectiveness with no difference in glucose concentrations may be due to the fact that the majority of glucose was disposed of via non-insulin dependent mechanisms as has been demonstrated in humans (Gottesman et al., 1983; Huang et al., 1980). So although lipoic acid supplementation did affect insulin secretion and perhaps improved insulin effectiveness, lipoic acid does not appear to have aided in glucose disposal in this study because no differences in glucose concentrations were observed.

Our glucose results are in contrast to a number of studies in humans and rodents that reported differences between lipoic acid supplemented and control animals. Konrad et al. (1999) reported that oral lipoic acid supplementation to humans twice daily for four weeks increased glucose transport via non-insulin-dependent mechanisms, as determined by application of the minimal model to i.v. glucose tolerance data. The minimal model of glucose and insulin dynamics is a model which predicts the disappearance rate of glucose from blood and separates glucose-mediated glucose disposal (non-insulin-dependent) from insulin-mediated glucose disposal (Bergman, 2005). Hamano (2006) also demonstrated enhanced whole body glucose uptake in broiler chickens supplemented with lipoic acid from 2 to 5 wk of age as demonstrated by the results of before and after hyperglycemic-euglycemic clamp tests. Differences in glucose response between other studies and the present study may be attributed to the isomer of lipoic acid supplemented (pure R-LA as opposed to racemic lipoic acid), the lipoic acid dose and dosing frequency,

or species differences. Another possibility may be the timing of lipoic acid supplementation in relation to the glucose tolerance tests. Lipoic acid is rapidly absorbed into equine blood after oral supplementation with peak concentrations in serum occurring 30 min post-dosing and returning to basal concentrations by 6 h post-dosing (our unpublished observations). The optimal time to measure the maximum effects of lipoic acid supplementation on glucose disposal may be at the peak appearance of lipoic acid in the blood. In addition, it could be that twice daily supplementation would improve the effect of lipoic acid on glucose disposal. More studies are necessary to determine the optimal time for lipoic acid dosing in relation to an expected rise in blood glucose concentrations.

The effects of lipoic acid may also be more dramatic in those individuals with impaired insulin action. Saengsirisuwan et al. (2002) investigated the effects of lipoic acid and exercise on glucose and insulin response during an oral glucose tolerance test in lean (insulin-sensitive) Zucker rats and found no evidence that lipoic acid treatment alone enhanced glucose uptake in skeletal muscle. Additionally, Lee et al. (2005) found no improvement in insulin effectiveness in normal (insulin-sensitive) Long-Evans Tokushiuma Otsuka rats orally supplemented with 0.5% (wt/wt) racemic lipoic acid for 3 d. The ponies used in this study were fat (mean d 14 BCS of 6.44 ± 0.32). Insulin resistance is often associated with excess adiposity in humans (Bastard et al., 2006; Kim et al., 2006) and horses (Jeffcott et al., 1986; Hoffman et al., 2003; Frank et al., 2006). Admittedly, we did not assess insulin resistance per se, but by assuming that insulin resistance accompanies obesity, is possible that the ponies used in this study were either not fat or insulin resistant "enough" for lipoic acid supplementation to affect glucose

uptake from the blood; thus the reason we saw no differences.

We also found no differences in BW between LA and CON ponies, which is in contrast to studies in rodents. Cremer et al. (2006) conducted a 2 year trial for toxicity testing of lipoic acid and found a significant reduction in BW in lipoic acid supplemented rats with no signs of toxicity. Kim et al. (2004) fed LA at 0.25, 0.50, and 1.0 % (wt/wt) for 2 weeks and demonstrated a dose dependent reduction in feed intake and BW in rats. Additionally, intracerebroventricular (i.c.v.) administration of lipoic acid suppressed food intake in rats, suggesting that the anorexic effects of lipoic acid are mediated via the hypothalamic neurons (Kim et al., 2004). In partial agreement with our work, body mass index did not change in humans supplemented twice daily with 600 mg lipoic acid for 4 weeks (Konrad et al., 1999). In order to elicit weight loss in equine, a greater dose may be necessary to sufficiently reduce feeding behavior. Additionally, it may be that lipoic acid must be supplemented for a longer period of time in equine for weight loss to be realized.

Lipoic acid treatment had no effect on serum concentrations of leptin during the IVGTT or throughout the 14 d trial period. This is in agreement with work done by Schmidt et al. (2006) who reported no differences in concentrations of leptin in cattle fed lipoic acid (16 mg/kg or 32 mg/kg BW) for 42 d and challenged with infectious bovine rhinotracheitis virus at 21 d. No other reports of leptin concentrations being measured in lipoic acid trials could be found; however Lee et al. (2006) report that lipoic acid mimics the anorexogenic effect of leptin on hypothalamic nuclei. The fact that neither we, nor Schmidt et al. (2006) detected any differences in concentrations of leptin may be a result of the infrequent sampling paradigm in both studies (once and twice daily, respectively).

A diurnal pattern of concentrations of leptin has been reported in both feed-restricted and *ad libitum* fed ponies (Buff et al., 2006b). Additionally, lipoic acid may have no direct affect on leptin and therefore we did not observe a difference.

Implications

Insulin resistance in horses is associated with a number of maladies including laminitis, disruption of the estrous cycle, and osteochondrosis dessecans. Delivery of lipoic acid to pony mares appeared to improve insulin effectiveness, suggesting that further work on the potential of lipoic acid as an insulin sensitizing agent in equine is worth pursuing.

Table 2. Chemical analysis (DM basis) of hay fed to pony mares at 2% BW daily throughout the 14 day trial period [a]

Item	**%**
CP	9.7
ADF	47.1
NDF	66.4
NFC	12.9
Fat	1.7
Ash	9.3
Ca	0.4
P	0.2

[a] Analyses were performed at Custom Laboratory Inc., Golden City, MO.

[b] Non-fiber carbohydrate, NFC = 100 – (CP + ash + fat + NDF).

Figure 15. Serum concentrations of glucose over time in pony mares during the d 0 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and either saline (SAL, ●) or 0.030 U/kg (INS, ○) insulin administered i.v. at 20 min.

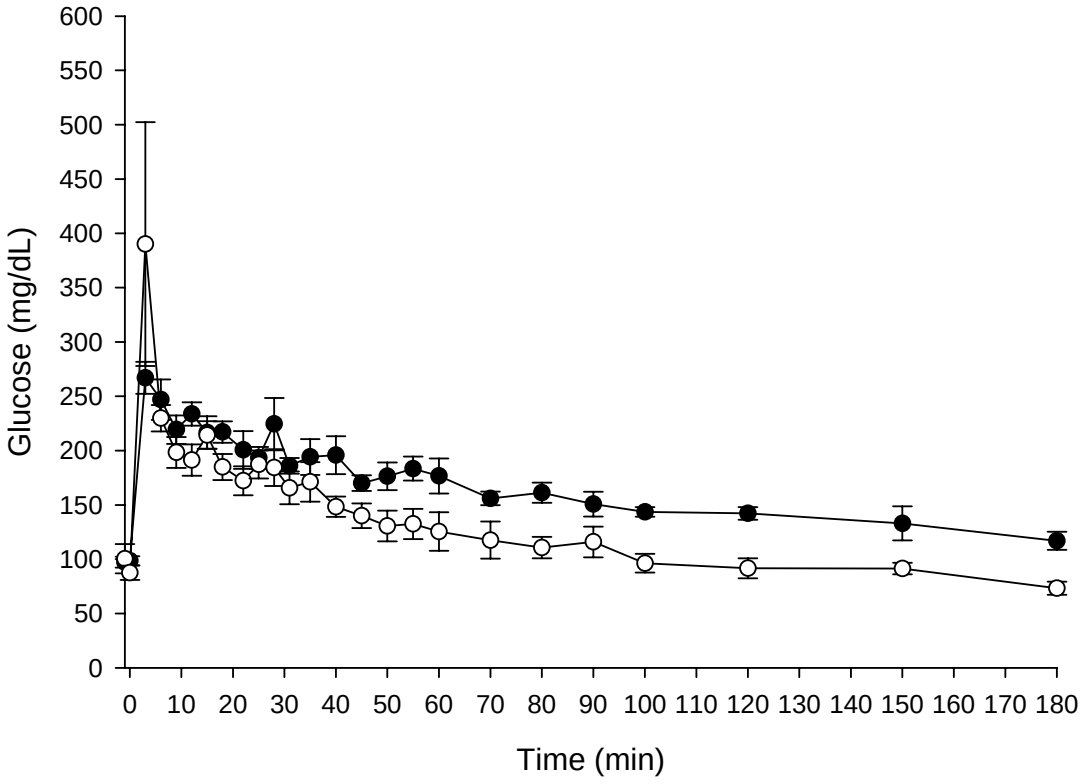


Figure 16. Serum concentrations of insulin over time in pony mares during the d 0 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and saline (SAL, ■) or 0.030 U/kg (INS, □) insulin administered i.v. at 20 min.

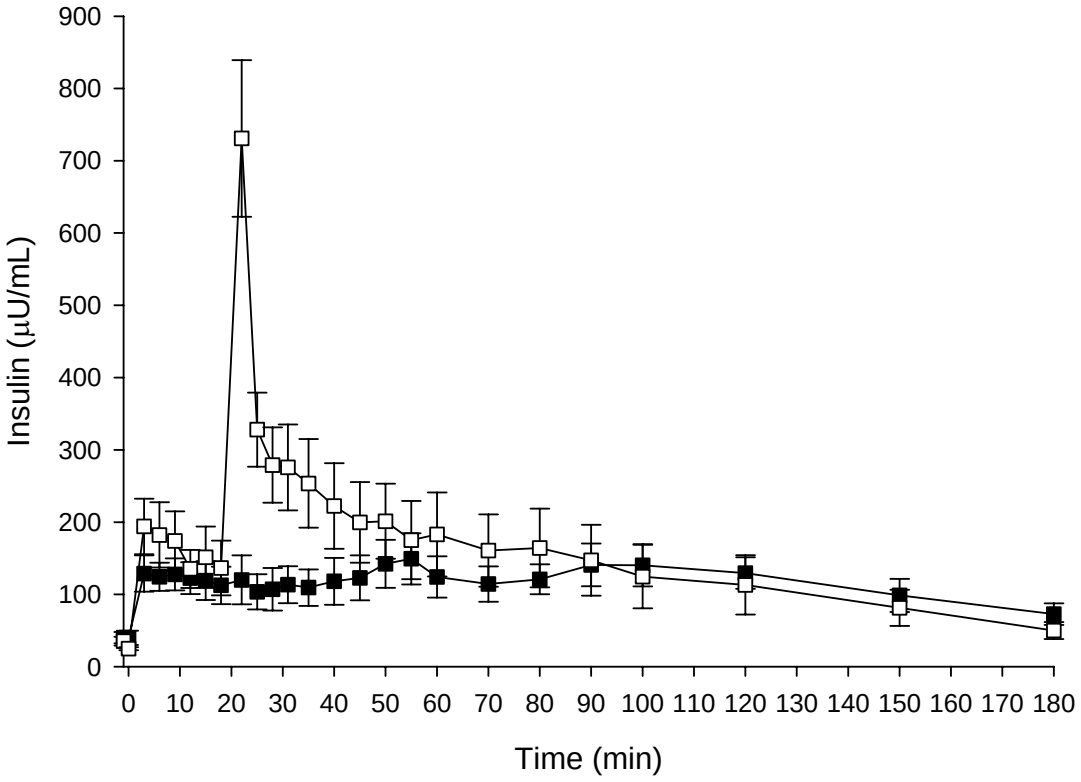


Figure 17. Serum concentrations of leptin over time in pony mares during the d 0 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and either saline (SAL, ▲) or 0.030 U/kg (INS, Δ) insulin administered i.v. at 20 min.

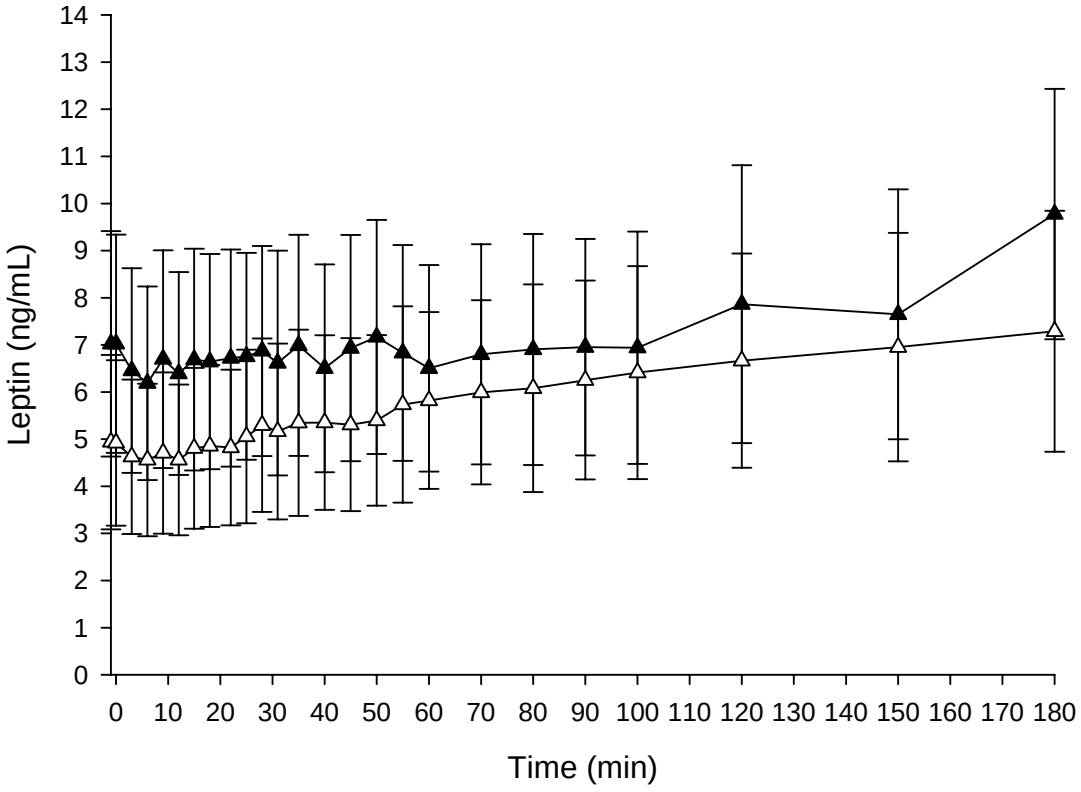


Figure 18. Serum concentrations of leptin over time in pony mares supplemented with 10 mg/kg lipoic acid daily (LA, Δ) or with sham treatment (CON, ▲) for 14 d. The numbers on the x-axis indicate the sample day and the letters "a" and "b" represent sampling times of 0700 and 1900, respectively, within each day.

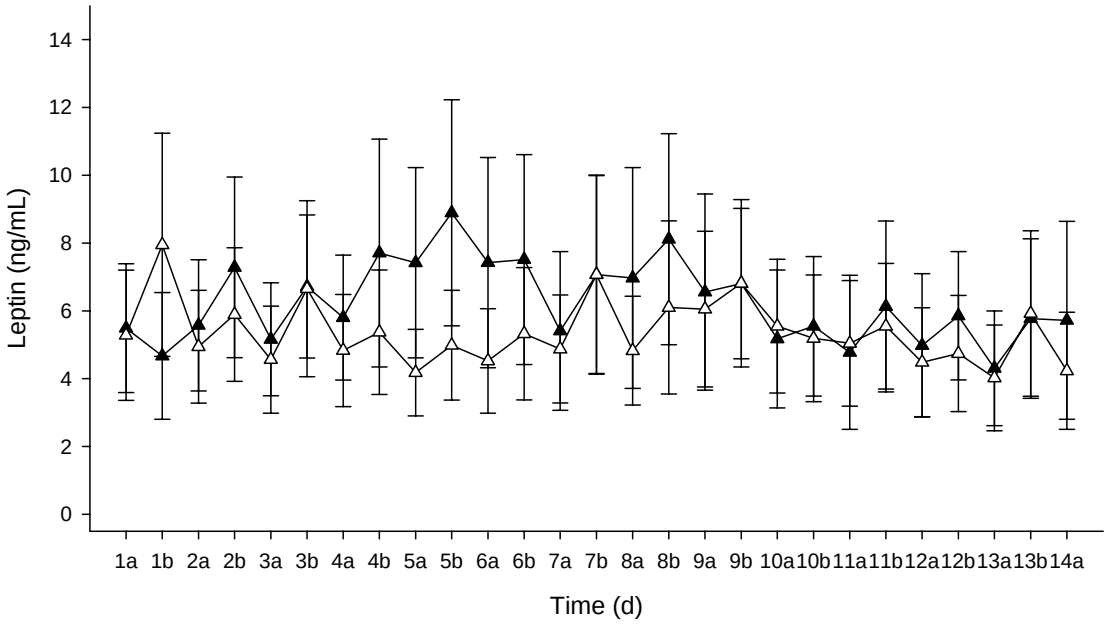


Figure 19. Plasma concentrations of glucose over time in pony mares during the d 14 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and 0.030U/kg insulin administered i.v. at 20 min. Ponies were supplemented with 10 mg/kg of lipoic acid (LA, ○) daily for 14 d or control treatment (CON, ●).

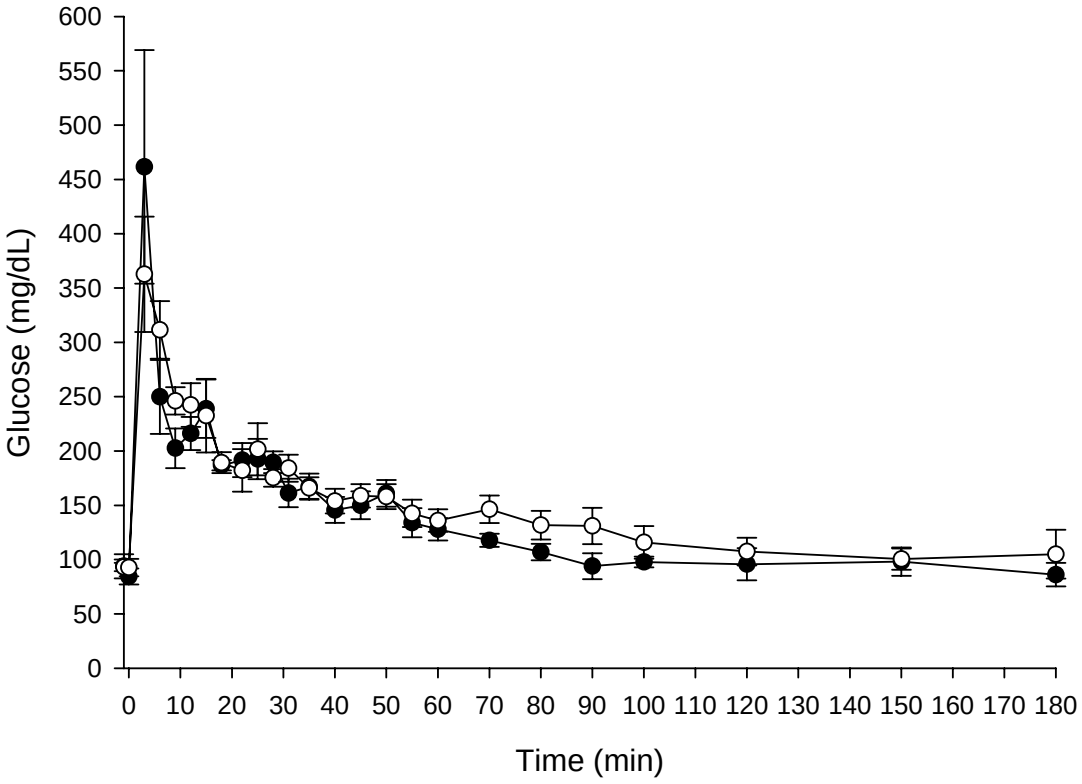


Figure 20. Plasma concentrations of insulin over time in pony mares during the d 14 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and 0.030U/kg insulin administered i.v. at 20 min. Ponies were supplemented with 10 mg/kg of lipoic acid (LA, □) daily for 14 d or control treatment (CON, ■).

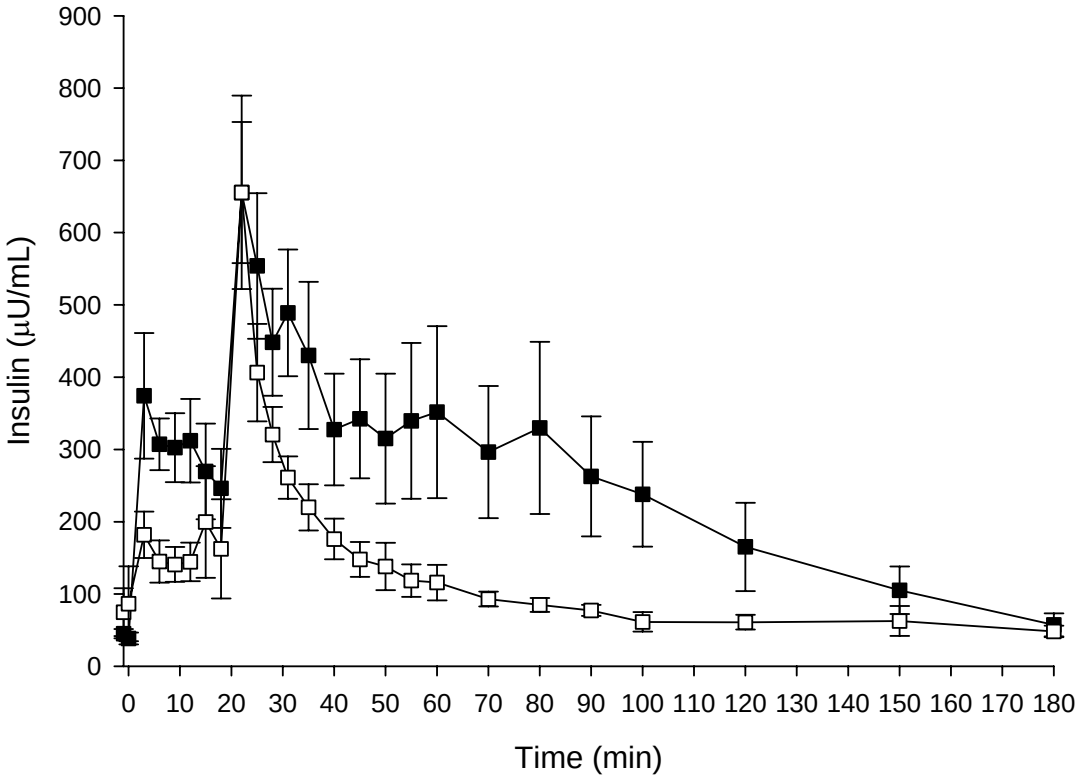
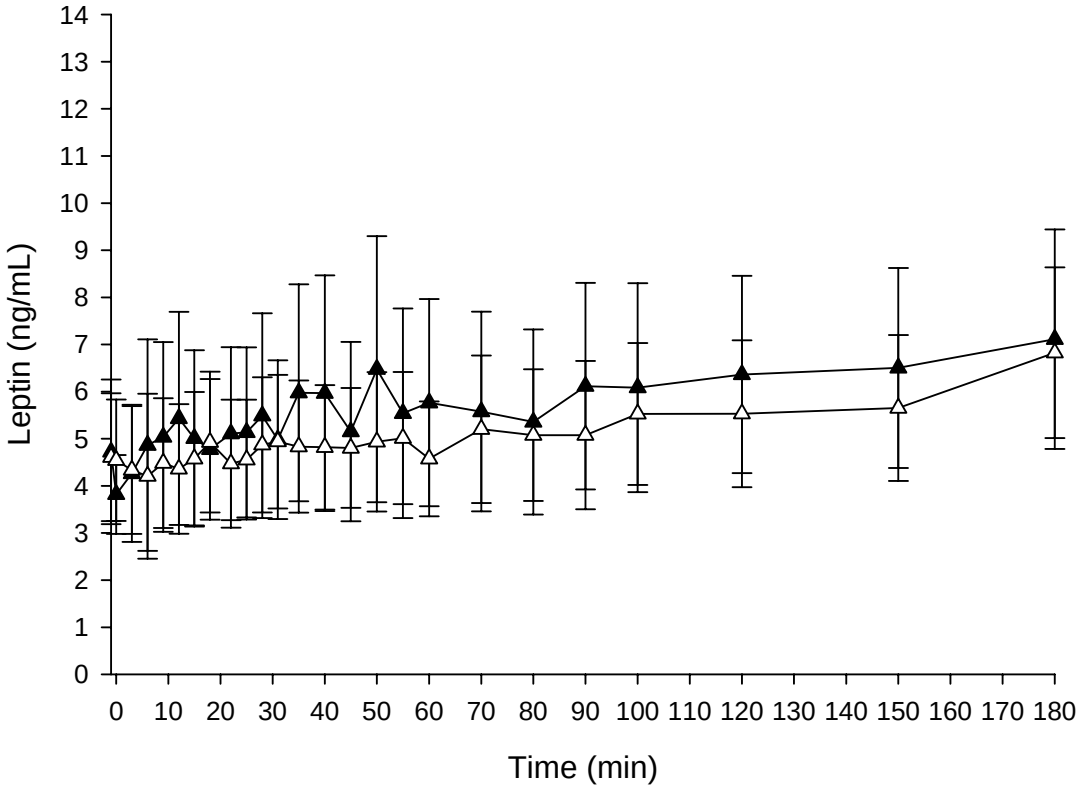


Figure 21. Serum concentrations of leptin over time in pony mares during the d 14 modified frequent sampled i.v. glucose tolerance test with 0.3 g/kg glucose administered i.v. at 0 min and 0.030U/kg insulin administered i.v. at 20 min. Ponies were supplemented with 10 mg/kg of lipoic acid (LA, Δ) daily for 14 d or control treatment (CON, ▲).



LITERATURE CITED

Alexander, F. 1982. The effect of ethacrynic acid, bumetanide, fursemide, spironolactone and ADH on electrolyte excretion in ponies. J. Vet. Pharmacol. Therap. 5:153-160.

Allen, W. R., S. Wilsher, C. Tiplady, and R. M. Butterfield. 2004. The influence of maternal size on pre- and post-natal growth in the horse: III postnatal growth. Reprod. 127:67-77.

Aoki, N., M. Kawamura, and T. Matsuda. 1999. Lactation-dependent down regulation of leptin production in mouse mammary gland. Biochem. Biophys. Acta. 1427:298-306.

Archer, J. S. and R. J. Chang. 2004. Hirsuitism and acne in polycystic ovary syndrome. Best Prac. Res. Clin. Obst. Gynecol. 18:737-754.

Attallah, H., A. L. Friedlander, and A. R. Hoffman. 2006. Visceral obesity, impaired glucose tolerance, metabolic syndrome, and growth hormone therapy. Growth Horm. IGF Res. 16 Suppl:62-67.

Aoki, N., M. Kawamura, and T. Matsuda. 1999. Lactation-dependent down regulation of leptin production in mouse mammary gland. Biochem. Biophys. Acta. 1427:298-306.

Bastard, J. P., M. Maachi, C. Lagathu, M. J. Kim, M. Caron, H. Vidal, J. Capeau, and B. Feve. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur. Cytokine Netw. 17:4-12.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and Homeorhesis. J. Dairy Sci. 63:1514-1529.

Benoit, S. C., D. J. Clegg, R. J. Seeley, and S. C. Woods. 2004. Insulin and leptin as adiposity signals. Recent Prog. Horm. Res. 59:267-285.

Bergman, R. N. 2005. Minimal model: perspective from 2005. Horm. Res. 64(Suppl):8-15.

Berry, S. D., R. D. Howard, P. M. Jobst, H. Jiang, R. M. Akers. 2003. Interactions between the ovary and the local IGF-1 axis modulate mammary development in prepubertal heifers. 177:295-304. J. Endocrinol.

Biddinger, S. B. and C. R. Kahn. 2006. From mice to men: insights into the insulin resistance syndromes. Annu. Rev. Physiol. 68:123-158.

Biewanga, G. P., G. R. M. M. Haenen, and A. Bast. 1997. An overview of lipoate chemistry. In: J. Fuchs, L. Packer, and G. Zimmer (eds.). Lipoic acid in health and disease, p. 1-32. Dekker, New York, New York.

Bonnet, M., I. Gourgou, C. Leroux, Y. Chilliard, J. Dijane. 2002. Leptin expression in the ovine mammary gland: putative sequential involvement of adipose, epithelial, and myoepithelial cells during pregnancy and lactation. J. Anim. Sci. 80:723-728.

Bradbury, M. J., S. F. Akana, M. F. Dallmah. 1994. Roles of type I and II corticosteroid receptors in regulation of basal activity in the hypothalamo-pituitary-adrenal axis during the diurnal trough and the peak: evidence for a nonadditive effect of combined receptor occupation. Endocrinol. 134:1286-1296.

Broberger, C. 2005. Brain regulation of food intake and appetite: Molecules and networks. J. Intern. Med. 258:301-327.

Buff, P. R., P. J. Johnson, C. E. Wiedmeyer, V. K. Ganjam, N. T. Messer IV, D. H. Keisler. 2006a. Modulation of leptin, insulin and growth hormone in obese pony mares under chronic nutritional restriction and supplementation with ractopamine hydrochloride. Vet. Ther. 7:64-72.

Buff, P. R., B. R. Spader, C. D. Morrison, and D. H. Keisler. 2006b. Endocrine responses in mares undergoing abrupt changes in nutritional management. J. Anim. Sci. 84:2700-2707.

Buff, P. R., C. D. Morrison, V. K. Ganjam, and D. H. Keisler. 2005. Effects of short-term feed deprivation and melatonin implants on circadian patterns of leptin in the horse. J. Anim. Sci. 83:1023-1032.

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

Buhl, R., A. K. Ersboll, L. Eriksen, and J. Koch. 2005. Changes over time in echocardiographic measurements in young Standardbred racehorses undergoing training and racing and association with racing performance. J. Am. Vet. Med. Assoc. 226:1881-1887.

Bustamante, J., J. K. Lodge, L. Marcocci, H. J. Tritschler, L. Packer, and B. H. Rihn. 1998. Alpha-lipoic acid in liver metabolism and disease. Free Radic. Biol. Med. 24:1023-1039.

Carreau, J. P., D. Lapous, and J. Raulin. 1975. A possible essential metabolite of linoleic acid: lipoic acid, the universal coenzyme of alpha-keto acid oxidation. J. Physiol. 71:327A-335A.

Carrington, E. F., M. Desautels, and J. M. Naylor. 2003. beta-Adrenergic stimulated lipolysis in pony adipocytes is exclusively via a beta2-subtype and is not affected by

lactation. Comp. Biochem. Physiol. Mol. Integr. Physiol. 136:311-320.

Cartmill, J. A., D. L. Thompson, Jr., R. P. Del Vecchio, W. A. Storer, and J. C. Crowley. 2006. Leptin secretion in horses: effects of dexamethasone, gender, and testosterone. Domest. Anim. Endocrinol. 31:197-210.

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

Chai, W., I. M. Garrelds, U. Arulmani, R. G. Schoemaker, J. M. J. Lamers, and A. H. Jan Danser. 20005. Genomic and nongenomic effects of aldosterone in the rat heart: why is spironolactone cardioprotective? Brit. J. Pharmacol. 145:664-671.

Chen, C. L. and A. M. Riley. 1981. Serum thyroxine and triiodothyronine concentrations in neonatal foals and mature horses. Am. J. Vet. Res. 42:1415-1417.

Clarke, L. L., V. K. Ganjam, B. Fichtenbaum. D. Hatfield., and H. E. Garner. 1988. Effect of feeding on rennin-angiotensin-aldosterone system of the horse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 254:524-530.

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

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

Cone, R. D. 2005. Anatomy and regulation of the central melanocortin system. Nature Neurosci. 8:571-578.

Cremer, D. R., R. Rabeler, A. Roberts, and B. Lynch. 2006. Long-term safety of alpha-lipoic acid consumption: a 2-year study. Regul. Toxicol. Pharmacol. Online. Available: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6WPT-4KKNNFS-2-1&_cdi=6999&_user=3419478&_orig=search&_coverDate=08%2F08%2F2006&_qd=1&_sk=999999999&view=c&_alid=454933173&_rdoc=1&wchp=dGLbVzb-zSkzS&md5=b771a68cccc999c946f639529c0491c8&ie=/sdarticle.pdf. Accessed July 12, 2006.

Cripps, R. L., M. S. Martin-Gronert, and S. E. Ozanne. 2005. Fetal and perinatal programming of appetite. Clin. Sci. 109:1 – 11.

Crome, P. K., F. K. McKeith, T. R. Carr, D. J. Jones, D. H. Mowrey, and J. E. Cannon. 1996. Effect of ractopamine on growth performance, carcass composition, and cutting yields of pigs slaughtered at 107 and 125 kilograms. J. Anim. Sci. 74:709-716.

Crowell-Davis, S. L., K. A. Houpt, and J. Carnevale. 1985. Feeding and drinking

behavior of mares and foals with free access to pasture and water. J. Anim. Sci. 60:883-889.

Cymbaluk, N. F and B. Laarveld. 1996. The ontogeny of serum insulin-like growth factor-I concentration in foals: effects of dam parity, diet, and age at weaning. Domest. Anim. Endocrinol. 13:197-209.

Davy, K. P. and J. E. Hall. 2004. Obesity and hypertension: two epidemics or one? Am. J. Physiol. Reful. Integr. Comp. Physiol. 286:R803-R813.

Delavaud , C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: effect of nutrition status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519-526.

de Moura, E. G. and M. C. F. Passos. 2005. Neonatal programming of body weight regulation and energetic metabolism. Bioscience Reports. 25:251-269.

de Paula, R. B., A. A. da Silva, and J. E. Hall. 2004. Aldosterone antagonism attenuates obesity-induced hypertension and glomerular hyperfiltration. Hypertension. 43:41-47.

Desai, M., D. Gayle, J. Babu, and M. G. Ross. 2004. Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. Am. J. Physiol. Integr. Comp. Physiol. 288:R91-R96.

Donaldson, M. T., D. McFarlane, A. J. R. Jorgensen, and J. Beech. 2004. Correlation between plasma α-melanocyte-stimulating hormone concentration and body mass index in healthy horses. Am. J. Vet. Res. 65:1469-1473.

Donoghue, S., T. N. Meacham, and D. S. Kronfeld. 1990. A conceptual approach to optimal nutrition of brood mares. Vet. Clin. North America Equine Prac. 6:373-391.

Duncan, P. 1979. Time budgets of Camarugue horses. Behaviour. 72:26-49.

Duncan, P., P. H. Harvey, and S. M. Wells. 1984. On lactation and associated behaviour in a natural herd of horses. Anim. Behav. 32:255-263

Dunshea, F. R., C. S. Chung, P. C. Owens, J. F. Ballard, and P. E. Walton. 2002. Insulin-like growth factor-I and analogues increase growth in artificially-reared neonatal pigs. Br. J. Nutr. 87:587-593.

Ehrhart-Bornstein, M., V. Lamounier-Zepter, A. Schraven, J. Langenback, H. S. Willendberg, A. Barthel, H. Hauner, S. M. McCann, W. A. Scherbaum, and S. R. Bornstein. 2003. Human adipocytes secrete mineralocorticoid-releasing factors. PNAS. 24:14211-14216.

Eiler, H., N. Frank, F. M. Andrews, J. W. Oliver, and K. A. Fecteau. 2005. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. Am. J. Vet. Res. 66:1598-1604.

Erlanson- Albertson, C. and R. Zetterström. 2005. The global obesity epidemic: Snacking and obesity may start with free meals during infant feeding. Acta Pediac. 94:1523-1531.

Feist, J. D. and D. R. McCullough. 1976. Behavior patterns and communication in feral horses. Z. Tierpsychol. 41:337-371.

Forhead, A. J., J. C. Ousey, W. R. Allen, and A. L. Fowden. 2004. Postnatal insulin secretion and sensitivity after manipulation of fetal growth by embryo transfer in the horse. J. Endocrinol. 181:459-467.

Forhead, A. J. and H. Dobson. 1997. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. Res. Vet. Sci. 62:265-269.

Fortier, L. A., M. A. Kornatowski, H. O. Mohammed, M. T. Jordan, L. C. O'Cain, W. B. Stevens. 2005. Age-related changes in serum insulin-like growth factor-I, insulin-like growth factor-I binding protein-3 and articular cartilage structure in Thoroughbred horses. Equine Vet. J. 37:37-42.

Frank, N., S. B. Elliott, L. E. Brandt, D. H. Keisler. 2006. Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance. J. Am. Vet. Med. Assoc. 228:1383-1390.

Fraser, A. F. and D. M. Broom. 1990. Farm animal behaviour and welfare. 3rd ed. Bailliere Tindall W. B. Saunders London England.

Freestone, J. F., K. Shoemaker, R. Bessin, and K. J. Wolfsheimer. 1992. Insulin and glucose response following oral glucose administration in well-conditioned ponies. Equine Vet. J. Suppl. 11:13-17.

Fulmer, B. K. 1995. A 24-hour stall ethogram on 23 horses in a commercial stable during two seasons. M.S. Thesis, Purdue Univ., West Lafayette, IN.

Garthwaite, S. M. and E. G. McMahon. 2003. The evolution of aldosterone antagonists. Mol. Cell. Endocrinol. 217:27-31.

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

Gertler, A., J. Simmons, and D. H. Keisler. 1998. Large-scale preparation of

biologically active recombinant ovine obese protein (leptin). FEBS Letters. 422:137-140.

Gibbs, P. G., G. D. Potter, R. W. Blake and W. C. McMullan. 1982. Milk production of Quarter horse mares during 150 days lactation. J. Anim. Sci. 54:496-499.

Giussani, D. A., A. J. Forhead, D. S. Gardner, A. J. W. Fletcher, W. R. Allen, and A. L. Fowden. 2003. Postnatal cardiovascular function after manipulation of fetal growth by embryo transfer in the horse. J. Physiol. 547:67-77.

Gogia, A. and P. K. Agarwal. 2006. Metabolic Syndrome. Indian J. Med. Sci. 60:72-81.

Goodwin, D., H. P. Davidson, and P. Harris. 2002. Foraging enrichment for stabled horses: effects of behaviour and selection. Equine Vet. J. 34:686-691.

Gopalakrishnan, G. S., D. S. Gardner, S. M. Rhind, M. T. Rae, D. E. Kyle, A. N. Brooks, R. M. Waler, M. M. Ramsay, D. H. Keisler, T. Stephenson, and M. E. Symonds. 2004. Programming of adult cardiovascular function after early maternal undernutrition in sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287:R12-R20.

Gordon, M. E. and K. H. McKeever. 2006. Oral and intravenous carbohydrate challenges decrease active ghrelin concentrations and alter hormones related to control of energy metabolism in horses. J. Anim. Sci. 84:1682-1690.

Gordon, M. E. and K. H. McKeever. 2005. Diurnal variation of ghrelin, leptin , and adiponectin in Standardbred mares. J. Anim. Sci. 83:2365-2471.

Greenberg, A. S. and M. S. Obin. 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am. J. Clin. Nutr. 83:461S-465S.

Grosvenor, C. E., M. F. Picciano, and C. R. Baumrucker. 1992. Hormones and growth factors in milk. Endo. Rev. 14:710-728.

Gordon, M. E., K. H. McKeever, C. L. Betros, and H. C. Manso Filho. 2005. Plasma leptin, ghrelin, and adiponectin concentrations in young fit racehorses versus mature unfit standardbreds. Vet. J. Available: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6WXN-4HWXB0X-2-8&_cdi=7163&_user=3419478&_orig=search&_coverDate=12%2F27%2F2005&_sk=999999999&view=c&_alid=459869849&_rdoc=2&wchp=dGLbVlz-zSkzk&md5=38f9a770e30c4bc8d67cd123793a94f2&ie=/sdarticle.pdf. Accessed May 23, 2006.

Gottesman, I., L. J. Mandarino, and J. Gerich. 1983. Estimation and kinetic analysis of insulin independent glucose uptake in human subjects. Am. J. Physiol. 6:E6322-E6325.

Hales, C. N. and D. J. P. Barker. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 35:595-601.

Hall, J. E. 2000. Pathophysiology of obesity hypertension. Curr. Hypertension Rep. 2:139-147.

Haluzik, M., J. Parizkova, and M. M. Haluzik. 2004. Adiponectin and its role in the obesity-induced insulin resistance and related complications. Physiol. Res. 53:123-129.

Halizuk, M., G. Sindelka, J. Widimsky, Ju. M. Prazny, T. Zalinka, and J. Skrha. 2002. Serum leptin levels in patients with primary hyperaldosteronism before and after treatment: relationships to insulin sensitivity. J. Human Hyperten. 16:41-45.

Hamano, Y. 2006. Effects of dietary lipoic acid on plasma lipid, in vivo insulin sensitivity, metabolic response to corticosterone and in vitro lipolysis in broiler chickens. Br. J. Nutri. 95:1094-1101.

Hammond, J. 1950. Measuring growth in farm animals. Proc. Soc. London. 137:452-456.

Hayashida, T., K. Murakami, K. Mogi, M. Nishihara, M. Nakazato, M. S. Mondal, Y. Horii, M. Kojima, K. Kangawa, and N. Murakami. 2001. Ghrelin in domestic animals: distribution in stomach and its possible role. Domes. Anim. Endocrinol. 21:17-24.

Haynes, W. G. 2005. Role of leptin in obesity-related hypertension. Exp. Physiol. 90:683-688.

Heidler, B., N. Parvizi, H. Sauerwein, R. M. Bruckmaier, U. Heintges, J. E. Aurich, C. Aurich. 2003. Effects of lactation on metabolic and reproductive hormones in Lipizzaner mares. Domest. Anim. Endocrinol. 25:47-59.

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

Henrikson, E. J. 2006. Exercise training and the antioxidant α-lipoic acid in the treatment of insulin resistance and type 2 diabetes. Free Radical Biol. Med. 40:3-12.

Henson, F. M., C. Davenport, L. Butler, I. Moran, W. D. Shingleton, L. B. Jeffcott, and P. N. Schofield. 1997. Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and neonatal chondrocytes. Equine Vet. J. 29:441-447.

Hervey, G. R. 1959. The effects of lesions in the hypothalamus in parabiotic rats. J. Physiol. 145:336-352.

Hess-Dudan, F., P. Y. Vacher, R. M. Bruckmaier, M. A. Weishaupt, D. Burger, J. W.

Blum. 1994. Immunoreactive insulin-like growth factor I and insulin in blood plasma and milk of mares and in blood plasma of foals. Equine Vet. J. 26:134-139.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Holt, R. I., and C. D. Byrne. 2002. Intrauterine growth, the vascular system and the metabolic syndrome. Semin. Vasc. Med. 2:33-43.

The Horse. 2006. Center offers new hope for unwanted horses. Available: http://www.thehorse.com/ViewArticle.aspx?ID=6926&kw=unwanted%20horse. Accessed May 20, 2006.

Houseknecht, K. L., C. A. Baile, R. L. Matteri, and M. E. Spurlock. 1998. The biology of leptin: a review. J. Anim. Sci. 76:1405-1420.

Huang, S. C., M. E. Phelps, E. J. Hoffman, K. Sideris, C. J. Selin, and D. E. Kuhl. 1980. Noninvasive determination of local cerebral metabolic rate of glucose in man. Am. J. Physiol. 238:E69-E82.

Irvine, C. H. G. 1984. Hypothyroidism in the foal. Equine Vet. J. 16:302-306.

Irvine, C. H. G. and M. J. Evans. 1975. Postnatal changes in total and free thyroxine and triiodothyronine in foal serum. J. Repro. Fert. Suppl. 23:709-715.

Jeffcott, L. B. 1975. The transfer of passive immunity to the foal and its relation to immune status after birth. J. Reprod. Fertil. Suppl. 23:727-733.

Jeffcott, L. B., J. R. Field., J. G. McLean, and K. O'dea. 1986. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Vet. J. 18:97-101.

Johnson, P. J. 2002. The equine metabolic syndrome peripheral Cushing's syndrome. Vet. Clin. Equine. 18:271-293.

Johnson, P. J., S. H. Slight, V. K. Ganjam and J. M. Kreeger. 2002. Glucocorticoids and laminitis in the horse. Vet. Clin. Equine. 18:219-236.

Jones, A. P. and M. I. Friedman. 1982. Obesity and adipocyte abnormalities in offspring of rats undernourished during pregnancy. Science. 215:1518-1519.

Jose-Cunilleras, E., K. A. Hayes, R. E. Toribio, L. E. Mathes, K. W. Hinchcliff. 2005. Expression of equine glucose transporter type 4 in skeletal muscle after glycogen-depleting exercise. Am. J. Vet. Res. 66:379-385.

Jurga, F. 2003. Obesity is dangerous, warns UK researcher.

http://www.thehorse.com/printarticle.aspx?ID=4368. Accessed October 16, 2005.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metabolism 27(Suppl.2):1893:1902.

Kearns, C. F., K. H. McKeever, and K. Malinowski. 2006. Changes in adiponectin, leptin, and fat mass after clenbuterol treatment in horses. Med. Sci. Sports Exer. 38:262-267.

Kearns, C. F., K. H. McKeever, V. Roegner, S. M. Brady, and K. Malinowski. 2005. Adiponectin and leptin are related to fat mass in horses. Vet. J. Available: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6WXN-4GHBPS4-1-6&_cdi=7163&_user=3419478&_orig=search&_coverDate=06%2F29%2F2005&_sk=999999999&view=c&_alid=459869849&_rdoc=3&wchp=dGLbVlz-zSkzk&md5=2cd01c0165ad25b8d606266905e5c09e&ie=/sdarticle.pdf. Accessed May 23, 2006.

Kearns, C. F., K. H. McKeever, K. Maliniowski, M. B. Struck, and T. Abe. 2001. Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent. J. Appl. Physiol. 91:2064-2070.

Kershaw, E. E. and J. S. Flier. 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metabol. 89:2548-2556.

Kershaw, E. E., N. M. Morton, H. Dhillon, L. Ramage, J. R. Seckl, and J. S. Flier. 2005. Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. Diabetes. 54:1023-1031.

Kim, C., J. Park, J. Park, E. Kang, C. Ahn, B. Cha, S. Lim, K. Kim, and H. Lee. 2006. Comparison of body fat composition and serum adiponectin levels in diabetic obesity and non-diabetic obesity. Obesity. 14:1164-1171.

Kim, M., J. Park, J., C. Namkoong, P. Jang, J. Ryu, H. Song, J. Yun, I. Namgoong, J. Ha, I. Park, I. Lee, B. Viollet, J. Y. Youn, H. Lee, and K. Lee. 2004. Anti-obesity effects of α-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. Nature Med. 10:727-733.

Konrad, T., P. Vicini, K. Kusterer, A. Hoflich, A. Assadkhani, H. J. Bohles, A. Sewell, H. J. Tritschler, C. Cobelli, and K. H. Usadel. 1999. α-lipoic acid treatment decreases serum lactate and pyruvate concentrations and improves glucose effectiveness in lean and obese patients with Type 2 diabetes. Diabetes Care. 22:280-287.

Kronfeld, D. S., K. H. Treiber, T. M. Hess, and R. C. Boston. 2005. Insulin resistance in the horse: Definition, detection and dietetics. J. Anim. Sci. 83(E. Suppl.):E22-E31.

Laitinen, J., E. Remes, O. Hanninen, M. Alanko, and V. Simanainen. 1981. Oestrus and

pregnancy diagnosis by milk progesterone assay in the mare. Br. Vet. J. 137:478-484.

Lamberson, W.R., T. J. Safranski, R. O. Bates, D. H. Keisler, and R. L. Matteri. 1995. Relationships of serum IGF-I concentrations to growth, composition and reproductive traits of swine. J Animal Sci 73:3241-3245.

Lee, W., K. Song, E. H. Koh, J.C. Won, H. S. Kim, H. Park, M. Kim, S. Kim, K. Lee. And J. Park. 2005. α-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle. Biochem. Biophys. Res. Comm. 332:885-891.

Lehner, A. F., C. G. Hughes, J. D. Harkins, C. Nickerson, B. Mollett, L. Dirikolu, J. Bosken, F. Camargo, J. Boyles, A. Troppmann, W. W. Karpiesiuk, W. E. Woods, and T. Tobin. 2004. Detection and confirmation of ractopamine and its metabolites in horse urine after Paylean administration. J. Anal. Toxicol. 28:226-238.

Ley, W. B., J. L. Hoffman, T. M. Meacham, T. L. Sullivan, R. L. Kiracofe, and M. L. Wilson. 1989. Daytime management of the mare. 1: Pre-foaling mammary secretions testing. Equine Vet. Sci. 9:88-94.

Lins, M. C., M. C. F. Passos, P. C. Lisboa, I. T. Bonomo, E. G. de Moura. 2005. Leptin treatment during lactation increases transfer of iodine through the milk. J. Nutri. Biochem. 16:138-143.

Liu, Y., Y. Nakagawa, K. Toya, Y. Wang, H. Saegusa, T. Nakanishi, and T. Ohzeki. 2000. Effects of spironolactone on systolic blood pressure in experimental diabetic rats. Kidney International. 57:2064.

Lorenzo-Figueras, M., T. Preston, E. A. Ott, A. M. Merritt. 2005. Meal-induced gastric relaxation and emptying in horses after ingestion of high-fat versus high-carbohydrate diets. Am. J. Vet. Res. 66:897-906.

Maddock, K. R. 2001. Lipoic acid on health and performance of weanling pigs. M.S. Thesis. Univ. of Missouri, Columbia, MO.

Magdesian, K. G. 2004. Monitoring the critically ill equine patient. Vet. Clin. N. Am. Equine Prac. 20:11-29.

Marcella, K. 2004. Equine Obesity. DVM Newsmagazine. May 1.

Margetic, S., C. Gazzola, G. G. Peff, and R. A. Hill. 2002. Leptin: a review of its peripheral actions and interactions. International J. Obesity. 26:1407-1433.

McFadin, E. L., C. D. Morrison, P. R. Buff, N. C. Whitley, and D. H. Keisler. 2002. Leptin concentrations in peri-parturient ewes and their subsequent offspring. J. Anim. Sci. 80:738-743.

McGavock, J. M., R. G. Victor, R. H. Unger, and L. S. Szczepaniak. 2006. Adiposity of the heart, revisited. Ann. Intern. Med. 144:517-524.

McGreevy, P. D., N. P. French, and C. J. Nicol. 1995. The prevalence of abnormal behaviours in dressage, eventing and endurance horses in relation to stabling. Vet. Rec. 137:36-37.

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

Ménard, J. 2004. The 45-year story of the development of an anti-aldosterone more specific than spironolactone. Mol. Cell. Endocrinol. 217:45-52.

Miner, J. L. 1992. Recent advances in the central control of intake in ruminants. J. Anim. Sci. 70:1283-1289.

Minokoshi, Y., Y. B. Kim, O. D. Peroni, L. G. D. Fryer, C. Müller, D. Carling, and B. B. Kahn. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature. 415:339-343.

Moberg, G. P. 2000. Biological response to stress: Implications for animal welfare. The Biology of Animal Stress. CAB International Eds. G. P. Moberg and J. A. Mench.

Murray, M. J. and N. K. Luba. 1993. Plasma gastrin and somatostatin, and serum thyroxine, triiodothyronine, reverse triiodothyronine and cortisol concentrations in foals from birth to 28 days of age. Equine Vet. J. 25:237-239.

Neel, J. V. 1962. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? Am. J. Human Genetics. 14:353-362.

Nosbush, B. B., J. G. Linn, W. A. Eisenbeisz, J. E. Wheaton, and M. E. White. 1996. Effect of concentrate source and amount in diets on plasma hormone concentrations of prepubertal heifers. J. Dairy Sci. 79:1400-1409.

NRC. 1989. Nutrient requirements of horses.

Pausova, Z. 2006. From big fat cells to high blood pressure: A pathway to obesity-associated hypertension. Curr. Opin. Nephrol. Hypertens. 15:173-178.

Philipps, A. G., G. G. Anderson, B. Dvorak, C. S. Williams, M. Lake, A. V. Lebouton, O. Koldovsky. 1997. Growth of artificially fed infant rats: effect of supplementation with insulin-like growth factor I. 272:R1532-R1539.

Pinotti and Rosi. 2006. Leptin in bovine colostrum and milk. Horm. Metab. Res. 38:89-93.

Pritchard, L. E., A. V. Turnbull, and A. White. 2002. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signaling and obesity. J. Endocrinol. 172:411-421.

Rahmouni, K., M. L. G. Correia, W. G. Haynes, and A. L. Mark. 2005. Obesity-associated hypertension: new insights into mechanisms. Hypertension. 45:9-14.

Ralston, S. L. 1996. Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesions. Pferdeheilkunde. 12:320-322.

Ravelli, G. P., Z. Stein, and M. Susser. 1976. Obesity in young men after famine exposure in utero and early infancy. N. Engl. J. Med. 295:349-353.

Rentfrow, G., M. L. Linville, C. A. Stahl, K. C. Olson, and E. P. Berg. 2004. The effects of the antioxidant lipoic acid on beef longissimus bloom time. J. Anim. Sci. 82:3034-3037.

Robinson, J. A., G. K. Allen, E. M. Green, W. H. Fales, W. E. Loch, and C. G. Wilkerson. 1993. A prospective study of septicaemia in colostrum-deprived foals. Equine Vet J. 25:214-219.

Rogerson, F. M., Y.Z. Yao, G. J. Smith, N. Dimopoulos, and P. J. Fuller. 2003. Determinants of spironolactone binding specificity in the mineralocorticoid receptor. J. Mol. Endocrinol. 31:573-582.

Romagnoli, U., E. Macchi, G. Romano, M. Motta, P. Accornero, and M. Baratta. 2006. Leptin concentration in plasma and in milk during the interpartum period in the mare. Anim. Reprod. Sci. Online. Available: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T43-4JF8HCP-2-9&_cdi=4963&_user=3419478&_orig=search&_coverDate=03%2F09%2F2006&_qd=1&_sk=999999999&view=c&_alid=454933758&_rdoc=1&wchp=dGLbVlz-zSkWz&md5=483fef1f2281a0fe695adb8df7cdc61c&ie=/sdarticle.pdf. Accessed March 22, 2006.

Rossing, K., K. J. Schjoedt, U. M. Smidt, F. Boomsma, and H. H. Parving. 2005. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy. Diab. Care 28:2106-2112.

Rugh, K. S., H. E. Garner, R. F. Sprouse, and D. G. Hatfield. 1987. Left ventricular hypertrophy in chronically hypertensive ponies. Lab. Anim. Sci. 137:335-338.

Saengsirisuwan, V. F. R. Perez, J. A. Sloniger, T. Maier, and E. J. Henriksen. 2004. Interactions of exercise training and α-lipoic acid on insulin signaling in skeletal muscle of obese Zucker rats. Am. J. Physiol. Endocrinol. Metab. 287:E529-E536.

Saengsirisuwan, V., F. R. Perez, T. R. Kinnick, and E. J. Henriksen. 2002. Effects of exercise training and antioxidant R-ALA on glucose transport in insulin-sensitive rat skeletal muscle. J. Appl. Physiol. 92:50-58.

Saengsirisuwan, V., T. R. Kinnick, M. B. Schmidt, and E. J. Henriksen. 2001. Interactions of exercise training and lipoic acid on skeletal muscle glucose transport in obese Zucker rats. J. Appl. Physiol. 91:145-153.

Saha, C. G. J. Eckert, W. T. Ambrosius, T. Chun, M. A. Wagner, Q. Zhao, and J. H. Pratt. 2005. Improvement in blood pressure with inhibition of the epithelial sodium channel in blacks with hypertension. Hypertension. 46:481-487.

Salimei, E., G. Varisco, and F. Rosi. 2002. Major constituents, leptin, and non-protein nitrogen compounds in mares' colostrums and milk. Reprod. Nutr. Dev. 42:65-72.

Sànchez, J., P. Oliver, O. Miralles, E. Ceresi, C. Picó, and A. Palou. 2005. Leptin orally supplied to neonate rats is directly uptaken by the immature stomach and may regulate short-term feeding. Endocrinol. 146:2575-2582.

Schmidt, T. B. 2004. Supplementation of lipoic acid to finishing steers. Ph.D. dissertation. Univ. of Missouri, Columbia, Missouri.

Schmidt, T. B., M. S. Brown, R. L. Larson, S. B. Kleiboeker, K. C. Olson, D. H. Keisler, J. A. Carroll, and E. P. Berg. 2006. Effect of dietary lipoic acid on metabolic hormones and acute-phase proteins during challenge with infectious bovine rhinotracheitis virus in cattle. Am. J. Vet. Res. 67:1192-1198.

Schwartz, M. W., S. C. Woods, D. Porte, Jr., R. J. Seeley, and D. G. Baskin. 2000. Central nervous system control of food intake. Nature. 404:661-671.

Sert, M., T. Tetiker, and S. Kirim. 2003. Comparison of the efficiency of anti-androgenic regimens consisting of spironolactone, diane 35, and cyproterone acetate in hirsuitism. Act. Med. Okayama. 57:73-76.

Sessions, D. R., S. E. Reedy, M. M. Vick, B. A. Murphy, and B. P. Fitzgerald. 2004. Development of a model for inducing transient insulin resistance in the mare: Preliminary implications regarding the estrous cycle. 82:2321-2328.

Sharma, A. M. 1998. The thrifty-genotype hypothesis and its implications for the study of complex genetic disorders in man. J. Mol. Med. 76:568-571.

Shirakami, A., T. Toyonaga, K. Tsuruzoe, T. Shirotani, K. Matsumoto, K. Yoshizato, J. Kawashima, Y. Hirashima, N. Miyamura, C. R. Kahn, and E. Araki. 2002. Heterozygous knockout of the IRS-1 gene in mice enhances obesity-linked insulin resistance: a possible model for the development of type 2 diabetes. J. Endocrinol.

174:309-319.

Silva, J. E. 2006. Thermogenic mechanisms and their hormonal regulation. Physiol. Rev. 86:435-464.

Silverthorn, D. U. 2001. Human physiology: an integrated approach. 2nd ed. Prentice Hall, New Jersey.

Slawik, M., A. J. Vidal-Puig. 2006. Lipotoxicity, overnutrition and energy metabolism and aging. Ageing Res. Rev. 5:144-164.

Ślebodziński, A. B., E. Brzezińska- Ślebodziński, J. Nowak, and K. Kowalska. 1998. Triiodiothyronine, insulin, and characteristics of 5'-monodeiodinase in mare's milk from parturition to 21 days post-partum. Reprod. Nutri. Dev. 38:235-244.

Smith-Kirwin, S. M., D. M. O'Connor, J. de Johnston, E. D. Lancy, S. G. Hassink, and V. L. Fuanage. 1998. Leptin expression in human mammary epithelial cells and breast milk. J. Clin. Endcrinol. Metab. 5:1810-1813.

Spencer, R. L., A. H. Miller, H. Moday, M. Stein, B. S. McEwen. 1993. Diurnal differences in basal and acute stress levels of type I and type II adrenal steroid receptor activation in neural and immune tissues. Endocrinol. 133:1941-1950.

Spencer, R. L., E. A. Young, P. H., Choo, and B. S. McEwen. 1990. Adrenal steroid type I and type II receptor binding; estimates of in-vivo receptor number, occupancy, and activation with varying level of steroid. Brain Res. 514:37-48.

Stull, C. L. and A. V. Rodiek. 2000. Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions. J. Anim. Sci. 78:1458-1466.

Tanida, M., S. Iwashita, N. Terui, Y. Ootsuka, M. Shu, D. Kang, and M. Suzuki. 2006. Effect of peripheral administration of leptin on the renal sympathetic nerve activity in high-fat diet-related hypertensive rats. Life Sci. 78:1149-1154.

Taylor, V. J., Z. Cheng, P. G. A. Pushpakumara, D. E. Beever, and D. C. Wathes. 2004. Relationships between the plasma concentrations of insulin-like growth factor-1 in dairy cows and their fertility and milk yield. Vet. Rec. 155:583-588.

Tenore, A., J. S. Parks, M. Gasparo, and O. Koldovsky. 1980. Thyroidal response to peroral TSH in suckling and weaned rats. Am. Physiol. Soc. 238:E428-E430.

Tenore, A., L. V. Oberkotter, O. Koldovsky, J. S. Parks, and C. M. Vandenberg. 1981. Thyrotropin in human breast milk. Horm. Res. 14:193-200.

Thomas, M.G., K. Bennett-Wimbush, D.H. Keisler and W.E. Loch. 1996.
Tonic secretion of growth hormone differs in prepuberty Quarter Horses and ponies with

respect to age but not breed. J. An. Sci. 74:156 (Abstr).

Treiber, K. H., R. C. Boston, D. S. Kronfeld, W. B. Staniar, and P. A. Harris. 2005. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic diets. J. Anim. Sci. 83:2357-2364.

Trischner, M. 1987. Development of Polish-pony foals born after embryo transfer to large mares. J. Reprod. Fert. Suppl. 35:705-709.

Trischner, M. 1985. Embryo recovery from Polish-pony mares and preliminary observations on foal size after transfer of embryos to large mares. Equine Vet. J. Suppl. 3:96-98.

Trischner, M. and M Klimczak. 1989. The development of Polish ponies born after embryo transfer to large recipients. Equine Vet. J. Suppl. 8:62-63.

U. S. Equestrian Federation. 2006. Equine Drugs Medications Pamphlet. Available: http://www.usef.org/documents/drugsMeds/USEF_EquineDrugsMedicationsPamphlet.pdf . Accessed May 16, 2006.

Wake, D. J. and B. R. Walker. 2006. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 in obesity. Endocrine. 29:101-108.

Walton, A. and J. Hammond. 1938. The maternal effects of growth and conformation in Shire horse – Shetland pony crosses. Proc. Soc. London. 125:311-335.

Waters, A. J., C. J. Nicol, and N. P. French. 2002. Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. Equine. Vet. J. 34:572-579.

Williams, C. A., R. M. Hoffman, D. S. Kronfeld, T. M. Hess, K. E. Saker, and P. A. Harris. 2001. Lipoic acid as an antioxidant in mature thoroughbred geldings: a preliminary study. J. Nutri. 132:1628-1632.

Xu, R. J. 1996. Development of the newborn GI tract and its relation to colostrum/milk intake: a review. Reprod. Fertil. Devel. 8:35-48.

Xu, R. J., D. J. Mellor, M. J. Birtles, G. W. Reynolds, H. V. Simpson, B. H. Breier, and P. D. Gluckman. 1996. Morphological changes in the oesophagus of newborn pigs: effects of age, diet, and oral insulin-like growth factor I (IGF-I) or IGF-II. Reprod. Fertil. Dev. 8:903:909.

Yasuno, R. and H. Wada. 1998. Biosynthesis of lipoic acid in Arabidopsis: cloning and characterization of the cDNA for lipoic acid synthase. Plant Physiol. 118:935-943.

Young, L. E., K. Rogers, and J. L. N. Wood. 2006. Left ventricular size and systolic

function in Thoroughbred racehorses and their relationship to race performance. 2005. J. Appl. Physiol. 99:1278-1285.

Young, E. A., J. F. Lopez, V. Murphy-Weinberg, St. J. Watson, and H. Akil. 1998. The role of mineralocorticoid receptors in hypothalamic-pituitary-adrenal axis regulation in humans. J. Clin. Endocrinol. Metab. 83:3339-3345.

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

Zhu, M. J., S. P. Ford. W. J. Means, B. W. Hess, P. W. Nathanielsz, and M. Du. 2006. Maternal nutrient restriction affects properties of skeletal muscle in offspring. J. Physiol. E-Pub June 8, 2006.

Zulian, E., P. Sartorato, S. Benedini., G. Baro, D. Armanini, F. Mantero, and C. Scaroni. 2005. Spironolactone in the treatment of polycystic ovary syndrome: effects on clinical features, insulin sensitivity, and lipid profile. J. Endocrinol. Invest. 28:49-53.

VITA

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