ABSTRACT

OWENS, KELLY MARIE. The Effect of Changes in Body Condition on Insulin Sensitivity, Leptin, and Adiponectin in Horses fed Forage-Only Diets. (Under the direction of Shannon Pratt-Phillips.)

An association between insensitivity to insulin and obesity has been reported in horses. Adipocytokines leptin and adiponectin have been identified as regulators of energy intake and an insulin-sensitizing hormone, respectively, where a positive correlation exists between adiposity and leptin, while the opposite is true for adiponectin. The nature of these relationships to obesity in horses is not fully understood, nor is there a recommended ideal level of adiposity. Therefore, this study was designed to determine how differences in body composition, achieved through differences in forage-only dietary energy intake, affect insulin sensitivity (IS), leptin and adiponectin in the horse.

Seventeen mature, light-breed gelding horses, 8.0 ± 4.6 yr, were used in this two-phase study. Prior to day 0 horses were started on grass-alfalfa mix hay cubes and fed to achieve a moderate body condition score (BCS) of 5. Horses were randomly assigned to one of three treatment groups: gain to lose (GL), control (C), and lose to gain (LG). Three types of cubes were fed for weight gain or loss (High Energy and Low Energy cubes, respectively), or maintenance of condition (Bale-in-a-Bag cubes) during Phase 1 (P1). Diets were reversed following day 130 when approximate target changes in BCS were observed in GL (BCS = 7) and LG (BCS = 3) treatment groups, and horses were fed to return to a BCS of 5 in Phase 2 (P2). Body weight was assessed weekly. Rump fat depth (RFD) and abdominal fat depth (AFD), BCS, IS assessed via the euglycemic-
hyperinsulinemic clamp (EHC), and serum for leptin and adiponectin were collected and assessed at days 0, 65, 130, 195, and 260, and analyzed using a switch-back, repeated measures ANOVA. Parameters for each treatment group (12 horses, GL n = 3, C n = 5, LG n = 4) were analyzed using PROC MIXED of SAS. Pearson correlations were also assessed at day 130.

Mean BCS for treatment groups at day 0 was 4.8 ± 0.1 and 5.1 ± 0.1 at day 260. Significant changes in BCS were observed at day 130 compared to days 0 and 260 in the GL and LG groups, where BCS at day 130 was 6.5 ± 0.3 and 3.6 ± 0.4, respectively. At day 130 significantly smaller RFD was observed in the LG group versus day 0 (1.2 ± 0.8 cm), while mean RFD in the GL group tended to be larger (8.3 ± 0.8 cm), however no differences were seen in either group when day 130 and day 260 were compared. No significant changes in IS or leptin were observed as a result of body condition gain or loss, nor were there any correlations with measures of adiposity. However, leptin was positively correlated with IS (r = 0.83, P = 0.01). A significant time effect (P < 0.01) on adiponectin was observed in control horses, such that adiponectin was higher in summer and lower in winter/early spring.

Our results indicate the differences in adiposity achieved were not enough to elicit the expected alterations in IS seen in other studies. However, it does support the notion that moderate changes in adiposity in horses fed forage-only diets should not increase their risk of developing problems associated with metabolic disturbance. Additionally, adiponectin dynamics are different from previous findings, indicating potential seasonal influences.
Further research is needed to identify mechanisms behind the regulation of insulin sensitivity, leptin, and adiponectin and their application to individual animal populations.
The Effect of Changes in Body Condition on Insulin Sensitivity, Leptin, and Adiponectin in Horses fed Forage-Only Diets

by
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DEDICATION

“Peace. It does not mean to be in a place where there is no noise, trouble or hard work. It means to be in the midst of those things and still be calm in your heart.” – Unknown

To mom and dad for calming my heart.

And to Grampa for giving me something to aspire to.
BIOGRAPHY

Kelly Marie Owens was born and raised in Orange County, California. She graduated with honors from Irvine High School in 2001 to pursue her undergraduate degree in Animal Science at the California Polytechnic State University at San Luis Obispo. Her experience offered insight into all aspects of the equine industry and planted the graduate-school seed. After earning her BS and graduating Cum Laude from Cal Poly in 2005, she accepted a position as an Intensive Care Unit veterinary technician at the renowned Alamo Pintado Equine Medical Center in Santa Ynez, CA, which ultimately helped her pinpoint her research interests. In 2007 she began her graduate career at North Carolina State University under the direction of Dr. Shannon Pratt-Phillips and in summer of 2009 earned her Master of Science degree in Equine Nutrition. In her spare time she enjoys the outdoors, boating and water sports, listening to music, painting and drawing, and, of course, horseback riding.
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“Pom pom-pom

Pom pom-pom (ping)

Pom pom-pom

Pom pom-pom (ping)

Friends sing together

La La La La

Friends do things together

La La La La

Friends laugh together

Ha Ha Ha Ha

Friends make graphs together

La La La La …..”
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CHAPTER 1
LITERATURE REVIEW

Research Problem

Insulin sensitivity is the ability of insulin to exert its physiological effect of facilitating glucose uptake into target tissues. Insulin resistance then is the opposite of insulin sensitivity. Several equine conditions have been linked to decreases in insulin sensitivity, or insulin resistance, such as laminitis (Kronfeld, Treiber et al. 2006; Treiber, Kronfeld et al. 2006), hyperlipemia (Jeffcott and Field 1985), Cushing’s disease (Pituitary Pars Intermedia Dysfunction, (Reeves, Lees et al. 2001; Johnson, Messer et al. 2004)), and Equine Metabolic Syndrome (Johnson, Messer et al. 2004). Both dietary energy source and exercise have been shown to affect insulin sensitivity, such that diets rich in starch and sugar (> 30% NSC, (Pratt, Geor et al. 2006)) decrease insulin sensitivity (Hoffman, Boston et al. 2003; Pratt, Geor et al. 2006), while increases in physical activity, or exercise conditioning, increase insulin sensitivity (Stewart - Hunt, Geor et al. 2006). Obesity, defined in horses as a body condition score (BCS) >7 (Henneke, Potter et al. 1983), and insensitivity to insulin has also been reported in horses (Hoffman, Boston et al. 2003; Vick, Adams et al. 2007), though the dynamics of this association are not fully understood. Additionally, there is no ideal recommended level of adiposity for optimized insulin sensitivity. With obesity in horses recently identified as a growing problem (Pleasant,
Thatcher et al. 2006; Wyse, McNie et al. 2008), it is of interest to investigate this relationship further.

Adipocytokines, leptin and adiponectin, synthesized and secreted by adipose tissue, are believed to have functions associated with regulation of energy intake (Friedman and Halaas 1998; Radin, Sharkey et al. 2009) and insulin sensitivity (Kadowaki and Yamauchi 2005; Radin, Sharkey et al. 2009), respectively. Previous research in horses has shown a positive correlation between leptin and body fat, such that horses with greater levels of adiposity (rump fat depth = 3.30 cm) have higher circulating leptin concentrations than horses with a lesser degree of adiposity (rump fat depth = 0.69 cm), with the opposite holding true for adiponectin (Kearns, McKeever et al. 2006).

Therefore, the purpose of this research was to determine how changes in body condition (adiposity), achieved through the modification of forage-only dietary energy intake (to avoid confounding factors such as DE source (Hoffman, Boston et al. 2003; Pratt, Geor et al. 2006)), influence insulin sensitivity, as well as adipose-tissue derived hormones leptin and adiponectin, and ultimately offer insight for horse owners to improve equine health through proper nutritional management.

**Insulin and Insulin Sensitivity**

Insulin is one of the key energy metabolism-regulating hormones in the body. The most notable function of this hormone is maintenance of blood glucose homeostasis via
stimulation of glucose uptake into insulin-sensitive tissues such as skeletal muscle, adipose, and liver. Insulin acts by binding to an insulin receptor at the target tissue and activating a series of secondary messengers, which ultimately results in the translocation of the insulin-sensitive glucose transporter (GLUT4) vesicles to the cell surface membrane and allows glucose uptake into the cell (Figure 1).

Insulin sensitivity (IS) is the ability of insulin to exert its physiological effect of facilitating glucose uptake into target tissue cells, while insulin resistance (IR) is the inability of insulin to exert its physiological effects at these tissues. Kahn identified an insulin resistant state as a less than normal physiological response to normal concentrations of a hormone that could occur before, at, or after the cell receptor in a signal transduction pathway (Kahn 1978; Kronfeld, Treiber et al. 2005).
Figure 1.1. Schematic of insulin binding and GLUT4 translocation.

Assessing Insulin Sensitivity

Many techniques are available to assess insulin and glucose dynamics, however, the euglycemic-hyperinsulinemic clamp (EHC) method has been identified as the “gold standard” for IS evaluation in human medicine (Bergman, Ider et al. 1979; DeFronzo, Tobin et al. 1979). The EHC is often used in assessment of equine IS (Rijnen, Johannes et al. 2003; Pratt, Geor et al. 2005; Vick, Adams et al. 2007) and has been shown to correlate well with Minimal Model (MinMod) analysis of frequently sampled intravenous glucose
tolerance test (FSIGT) data (Pratt, Geor et al. 2005). Both the EHC and FSIGT methods quantify insulin action on glucose disposal, however, the EHC approach has proved more repeatable (Pratt, Geor et al. 2005). Techniques such as oral and intravenous glucose and insulin tolerance tests, and single, point-in-time basal concentrations of insulin and glucose are also available to assess this relationship, however, they do not specifically quantify the effect of insulin on glucose. Additionally, the oral glucose tolerance test method is complicated by individual animal variations in intestinal absorption and gastric emptying; and insulin tolerance tests by endogenous insulin and other hypoglycemia-reversing hormones, making them less precise in assessing IS (Kronfeld, Treiber et al. 2005).

The EHC technique functions by imposing supra-maximal steady state insulin concentrations concurrently with glucose infused at a rate necessary to maintain euglycemia (Firshman and Valberg 2007), typically 5 mmol/L or 90 mg/dL in horses (Kronfeld, Treiber et al. 2005). A value of insulin sensitivity of muscle and adipose tissue is derived from the amount of glucose utilized in order to maintain euglycemia (M) divided by blood insulin concentration (I) during steady-state euglycemia (DeFronzo, Tobin et al. 1979; Radziuk 2000; Pacini and Mari 2003). Horses with a greater sensitivity to insulin would require higher glucose infusion rates to maintain euglycemia, while horses with reduced insulin sensitivity would require lower rates.

While the EHC method remains the gold standard in assessing IS, the technique is labor intense and requires significant technical skill. The EHC is also a time-consuming
test because it takes between 90 to 180 minutes to achieve steady-state conditions from which glucose disposal rate is calculated using the last 30 to 60 minutes of data (Kronfeld, Treiber et al. 2005). Another potential problem with the EHC is that due to the supra-physiological insulin concentrations achieved, interpretation of data does not offer insight into glucose utilization under normal insulin conditions (Kronfeld, Treiber et al. 2005). Despite these concerns, the EHC remains the optimal method for directly quantifying insulin effects on glucose disposal.

Previous studies in horses have used the EHC method to successfully detect higher insulin sensitivity values in animals with polysaccharide storage myopathy (Annandale, Valberg et al. 2004; Firshman, Valberg et al. 2008), as well as to determine effects of obesity (Vick, Adams et al. 2007) and exercise (Powell, Reedy et al. 2002) on insulin sensitivity, and to establish reference ranges in ponies and horses (Rijnen, Johannes et al. 2003). In each of the aforementioned studies, specifics of the EHC method varied in that insulin was administered and infused at different priming doses and rates leading to diverse ranges in glucose disposal. Rijnen and van der Kolk initially used a priming dose of 646 μmol/kg BW insulin followed by the infusion dose of 43μmol/kg/min BW (Rijnen, Johannes et al. 2003). However, researchers found that horses became hypoglycemic and they were unable to increase glucose concentrations due to the limited capacity of syringe pumps being used, thus the priming dose was adjusted to 323 μmol/kg BW insulin (Rijnen, Johannes et al. 2003). In their investigation of the effects of exercise on lean and obese
mares, Powell and colleagues infused insulin at a rate of 1.2 mU/kg BW/min for 120 min after first administering a priming dose of 0.4 mU/kg BW insulin (Powell, Reedy et al. 2003). Similarly, Vick and coworkers used methods established by Powell in their research on obesity and IS (Vick, Adams et al. 2007). Pratt et al evaluated the repeatability of the EHC, infusing insulin at a rate of 21.3 pmol/min/kg BW (3mU/min/kg BW, (Pratt, Geor et al. 2005)). Mean coefficient of variation (CV) for the repeatability of the EHC method of assessing of IS has been reported as 12.1% (Pratt, Geor et al. 2005).

Effect of Obesity and Weight Change on Insulin Sensitivity

Obesity in horses has been defined as a BCS > 7 (Henneke, Potter et al. 1983), while human obesity is defined as a body mass index (BMI) > 30% (Silha, Krsek et al. 2003). The link between obesity and insulin resistance (IR) is well established in human medicine (Silha, Krsek et al. 2003), and obesity-related decreases in insulin sensitivity (IS) are indicated in the pathogenesis of conditions such as cardiovascular disease and type II diabetes mellitus. The relationship between obesity and IR has also been established in other species (Hotamisligil, Shargill et al. 1993; Hoenig, Thomaseth et al. 2007), including horses (Hoffman, Boston et al. 2003). Silha and colleagues measured IR in lean and obese human subjects using the homeostasis model assessment ratio (HOMA-R) formula:

\[
\text{HOMA} = \frac{\text{fasting plasma glucose} \times \text{fasting plasma insulin}}{22.5}
\]
These authors found insulin concentrations and IR were significantly greater in obese subjects compared to lean control subjects (Silha, Krsek et al. 2003). Similarly, Hoening and colleagues used the EHC method to determine that obesity in mature, neutered cats led to a marked decrease in glucose effectiveness and IS, such that an approximate 30% decrease in IS was observed for each kilogram increase in body fat weight (Hoenig, Thomaseth et al. 2007). Note that while IS is a measure of the physiological ability of insulin to facilitate glucose uptake into target tissues, glucose effectiveness is the ability of glucose to suppress endogenous glucose production and stimulate glucose uptake (Treiber, Kronfeld et al. 2005). Another study in adult, neutered cats observed IS and glucose tolerance in response to induced obesity (approximately 30% increase in body weight achieved via ad libitum feeding) and the subsequent reversal of obese body condition to original body weights (Biourge, Nelson et al. 1997). Biourge and coworkers found post-weight-gain IS decreased such that obese animals developed mild insulin resistance. Interestingly, the correction of obesity and return to body weights observed prior to commencing the study resulted in a normalization of glucose disposal and complete reversal of IR (Biourge, Nelson et al. 1997). Previous studies in human subjects show similar increases in insulin sensitivity following weight reduction via exercise or restricted dietary intake (Bogardus, Ravussin et al. 1984), or bariatric surgery (Bikman, Zheng et al. 2008).
As with other species, in horses, the mechanisms involved in the relationship between obesity and IR are complex and not fully understood. Hoffman and coworkers used a modified FSIGT procedure to investigate IS in non-laminitic Thoroughbred geldings with varying degrees of adiposity (Hoffman, Boston et al. 2003). Horses in this study were body condition scored (Henneke, Potter et al. 1983) and categorized as nonobese (BCS 5 to 5.9), moderately obese (BCS 6 to 6.9), and obese (BCS 7 to 9). Results showed IS was approximately 80% lower in obese versus nonobese horses at \(0.37 \times 10^{-4} \text{L/mU}^{-1}/\text{min}^{-1}\) versus \(1.94 \times 10^{-4} \text{L/mU}^{-1}/\text{min}^{-1}\), respectively (Hoffman, Boston et al. 2003). One horse with a BCS of 7.3 had an undetectable response to insulin and was deemed severely insulin resistant (Hoffman, Boston et al. 2003). Results from another study in non-laminitic horses using the EHC method to quantify IS as it relates to obesity and inflammatory markers, such as TNFα, further supports previous research in horses and other species (Vick, Adams et al. 2007). Vick and colleagues identified strong negative correlations between measures of adiposity (BCS and percent body fat assessed via ultrasound) and IS, as well as between TNFα and IS, such that IS decreased with increasing adiposity and TNFα (Vick, Adams et al. 2007). Similar to findings in rodents (Hotamisligil, Shargill et al. 1993) and humans (Hotamisligil, Arner et al. 1995), this study identified a possible mechanism behind the obesity-IR relationship in the horse, where accumulation of adipose tissue associated with obesity could be responsible for both local and systemic inflammation and decreases in IS, though the need for subsequent research was indicated.
While the equine studies previously mentioned established relationships between obesity and decreases in insulin sensitivity, they did not look at the effects of weight change. Van Weyenberg and coworkers investigated the effect of severe weight loss through caloric restriction on glucose tolerance in ponies. Ponies were fed 70% of maintenance energy requirement, decreasing to 50% and 35% over the duration of the trial (Van Weyenberg, Hesta et al. 2008). The 18 weeks of extreme caloric restriction resulted in weight loss at a rate of 1% per week with an approximate 4-unit change in BCS from 8 or 9 to 4 or 5, changes not previously researched in horses. The results of the OGTT showed significant decreases in baseline insulin concentrations, such that basal insulin concentrations at week 17 were approximately 50% of those observed prior to initiating the study at 10.1 mU/L and 19.7 mU/L, respectively. Weight loss also resulted in lower area under the curve (AUC) for glucose and insulin, suggesting improved glucose tolerance (Van Weyenberg, Hesta et al. 2008). It was noted, however, that due to the limitations previously discussed with the OGTT, the improved glucose tolerance could have been the result of both glucose-mediated glucose uptake or increased insulin sensitivity. The present study aimed to determine if changes in body weight would affect insulin sensitivity by using the EHC method previously described.
**Effect of Innate Factors on Insulin Sensitivity**

In addition to the influence of obesity on insulin sensitivity, other factors, both innate and environmental, also influence insulin and glucose dynamics. Innate factors include factors such as age, breed, sex and reproductive status (ie. pregnant or lactating), seasonal fluctuations and adipocytokine influences, while major environmental factors are diet (energy source) and physical activity.

Previous research in humans suggests IS decreases with age, though it is believed the effects of age on IS are more likely in combination with the influence of diet, physical exercise, genetic and other factors (Barbieri, Rizzo et al. 2001). Murphy and colleagues found a similar relationship between age and IS in ponies, such that foals aged 6 to 9 months were more sensitive to insulin than older ponies aged 6 to 13 years (Murphy, Reid et al. 1997). Another study investigating IS in foals from mares fed high- or low-starch diets found IS was greatest at 5 days of age and gradually declined at 40, 80, and 160 days of age (George, Staniar et al. 2009). Conversely, Ralston suggested that mature horses were more sensitive to insulin than weanlings or foals (Ralston 1996). These conflicting results suggest the need for more research on the effect of age on IS to elucidate the mechanisms behind this relationship.

In horses, few studies have investigated the relationship between IS and breed. Potential breed differences suggest a possible genetic link with IS. It is a common belief in the equine community that certain breeds considered to be “easy keepers”, such as Paso
Finos, Morgans, and Quarter Horses, are more prone to decreased IS. Previous research suggests that ponies may be more prone to IR than horses (Jeffcott, Field et al. 1986; Rijnen, Johannes et al. 2003). Jeffcott and Field found ponies were less sensitive to insulin than Standardbred horses (Jeffcott, Field et al. 1986). Similarly, Rijnen and van der Kolk used the EHC to determine ponies had lower sensitivity to insulin compared to Dutch Warmblood horses (Rijnen, Johannes et al. 2003). Interestingly, one study examining glucose tolerance in horses, ponies, and donkeys found that donkeys appeared to have lower IS than both ponies and horses (June, Soderholm et al. 1992). In this case, insulin concentrations at 2 and 6 hours during the OGTT were not different between ponies and horses, though glucose concentrations were lower in ponies (June, Soderholm et al. 1992). In contrast, McLean and colleagues used a FSIGT to assess IS in donkeys of three body condition categories (McLean, Nielson et al. 2009). Results suggest that IS values for donkeys may fall within ranges found in mature horses with similar degrees of adiposity (McLean, Nielson et al. 2009). Recently, another survey study identified differences in basal insulin concentrations between breeds, such that Warmbloods and ponies had higher insulin concentrations than Quarter Horse, Arabian, Paint, and Morgan horse breeds (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION). Though these basal concentrations did not assess true IS, results indicate the possibility of breed effects on IS and the need for confirmation of these effects through quantitative methods.
Previous research in humans and mice suggests differences in IS due to sex (Foley, Kashiwagi et al. 1984; Macotela, Boucher et al. 2009). One study evaluating the IS and glucose metabolism of both abdominal and subcutaneous adipose tissue from normal, castrated, or steroid-implanted mice found female mice had increased insulin signaling and IS compared to male mice, while the opposite was true following castration, indicating an insulin sensitizing role of estrogen in females (Macotela, Boucher et al. 2009). Generally, females have a higher percentage of body fat and a greater accumulation of subcutaneous fat than males, whereas males have a lower percentage of body fat and more visceral, or abdominal, fat. In humans and rodents, location of fat deposition plays a role in adipose tissue biological activity, with subcutaneous tissue responsible for the majority of adipocytokine production (Lafontan and Berlan 2003; Radin, Sharkey et al. 2009). It appears then that female humans and rodents are more sensitive to insulin than males, possibly due to a combination of sex steroids and fat depot location and activity.

There has been little work done on the effect of sex on IS in horses. In a survey study of 366 horses, Pratt-Phillips and colleagues found significant effects of sex on basal insulin concentrations, such that geldings had higher insulin concentrations than mares (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION). Though not a quantitative test of IS, these resting concentrations suggest mares may be more sensitive to insulin than geldings given the association between high resting insulin concentrations and insulin resistance. This is contrary to another study in horses in which geldings had greater
sensitivity to insulin than mares as determined using the EHC method (Pratt, Geor et al. 2005). In humans, there is evidence that menstrual cycle phase influences IS in females, such that IS is lower in the luteal phase and higher in the follicular phase (Escalante Pulido and Alpizar Salazar 1999). Similar influences of the estrous cycle on IS have been identified in the mare (Cubitt, George et al. 2007). It is important to note that phase of estrous cycle was not indicated in the survey study (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION), and in both this and Pratt, Goer et al 2005, body condition score was not different between sexes. It is unknown if body condition is a confounding factor in determining sex effects on IS, therefore more research is needed to distinguish sex effects on IS in horses and if there are similarities to potential mechanisms described in human and rodent research.

There are conflicting data regarding seasonal differences in IS in humans (Gravholt, Holck et al. 2000; Bunout, Barrera et al. 2003). In a 15-month study of healthy men ages 21 to 27, no changes in IS due to season were observed when assessed using the FSIGT (Gravholt, Holck et al. 2000). Conversely, using the homeostasis model assessment (HOMA) as an index of IS, Bunout and colleagues found seasonal effects on IS in healthy elderly people (≥ 70 years), such that IS was higher in the colder months and lower in the warmer months (Bunout, Barrera et al. 2003). No significant changes in body weight or composition were observed thus changes in IS due to accumulation of adipose tissue was dismissed. Contrary to studies indicating seasonal variations in other hormones such as
cortisol (Donaldson, McDonnell et al. 2005), to our knowledge, circannual effects on IS have not been demonstrated in the horse.

**Effect of Diet and Exercise on Insulin Sensitivity**

Major environmental factors that contribute to insulin and glucose dynamics are dietary energy source and physical activity. Investigations into the effect of dietary energy source on IS in horses have focused primarily on starch and fat content of feeds. Previous research in horses suggests diets rich in starch and sugar negatively affect IS, such that grain-based meals decrease sensitivity to insulin compared to high fat and fiber feeds (Williams, Kronfeld et al. 2001; Hoffman, Boston et al. 2003; Treiber, Boston et al. 2005; Pratt, Geor et al. 2006). Early inquires into hay versus hay and grain supplemented diets in healthy horses found horses supplemented with grain showed increases in both glucose and insulin responses to an IVGTT (Garcia and Beech 1986). However, results from this study did not identify significant differences in plasma insulin-glucose ratios between treatment diets. Hoffman and colleagues investigated the effects of diet in Thoroughbred (TB) geldings fed concentrates, with either a majority of the calories derived from starch and sugar or from fat and fiber, while being maintained on mixed grass/legume pasture (8 week feeding periods for each supplement). Results of a modified FSIGT showed decreases in IS in horses supplemented with the high starch/sugar feed compared to both the fat/fiber supplemented horses, and control horses maintained on pasture and hay alone (Hoffman,
Boston et al. 2003). It is important to note non-structural carbohydrate (NSC) content of the starch/sugar supplement was three times greater than the fat/fiber supplement at 46% and 14%, respectively. Similarly, over a 15 week period, Williams and colleagues saw significant decreases in peak glucose and insulin concentrations, as well as smaller glucose and insulin AUC, in fat/fiber (NSC = 24.7%) supplemented TB mares compared to starch/sugar (NSC = 64.5%) supplemented mares (Williams, Kronfeld et al. 2001). Another study found a 37% decrease in IS in weanling TB horses adapted to high starch/sugar (NSC = 49%) meals versus fat/fiber (NSC = 12%) meals, over an approximately 6-month period, as determined through Minimal Model analysis of a FSIGT (Treiber, Boston et al. 2005). Euglycemic-hyperinsulinemic clamp analysis of IS also confirmed previous findings in that a 30% decrease in IS was observed in horses fed a high starch/sugar (NSC = 55%) supplement over 6 weeks compared to horses fed a high fat/fiber (NSC = 9.2%) supplement with hay cubes (Pratt, Geor et al. 2006). As previous research demonstrates the negative effect of diets high in starch and sugar on IS, current research in this field is directed toward establishing a threshold level of NSC consumption. Recently, Hoffman and colleagues evaluated glycemic response in 8 horses fed oat/beet pulp meals in differing ratios to achieve NSC doses ranging from 0.6 to 2.0 g/kg BW. Glucose response (AUC for glucose) was used to evaluate glycemic response threshold, and regression analysis showed that NSC consumption above 0.296 g/kg BW altered glucose response (Hoffman, Haffner et al. 2009).
Positive effects of physical conditioning on insulin sensitivity has been demonstrated in human and rodent studies (Rodgers, Yamamoto et al. 1988; Hughes, Fiatarone et al. 1993). Though relatively few studies on the effects of exercise on glucose and insulin dynamics have been done in horses, previous research indicates improved glucose tolerance as a result of increased physical activity (Freestone, Beadle et al. 1992; Powell, Reedy et al. 2002). Freestone and coworkers observed an overall reduction in insulin response to an OGTT in hyperinsulinemic ponies after 6 weeks of submaximal exercise (heart rate < 140 beats/min), with the greatest increase in glucose tolerance at 2 weeks of training (Freestone, Beadle et al. 1992). Glucose response to the OGTT in this study remained unchanged post-exercise compared to pre-exercise values, suggesting the need for a true assessment of IS. Studies of short-term exercise on IS in obese and lean mares (Powell, Reedy et al. 2002) and mature Standardbred horses (Stewart-Hunt, Geor et al. 2006) found decreases in IS following 7 days of training as determined by the EHC method. Interestingly, post-training effects on IS were conflicting in that Powell et al observed a return to pre-exercise IS values in both lean and obese exercised groups 9 days following training (Powell, Reedy et al. 2002), while Stewart-Hunt et al found positive effects of physical activity on IS still evident 5 days post-training, though the degree of intensity of exercise in the Stewart-Hunt study was significantly greater than in the Powell study (Stewart-Hunt, Geor et al. 2006). Another study by Pratt and coworkers examined IS in horses following a single bout of moderate-intensity exercise and found no
differences at 30 min, and 4 and 24 hr following physical activity compared to control horses (Pratt, Geor et al. 2007). Rate or extent of GLUT4 translocation to cell membranes was suggested as a possible explanation of the lack of change in IS following exercise (Pratt, Geor et al. 2007). In studies investigating the combined effects of diet and exercise training on IS, Treiber and colleagues found moderate-intensity exercise increased IS in horses fed a high starch/sugar supplement and in those horses fed a high fat/fiber supplement (Treiber, T.M. et al. 2006). Pratt and coworkers found IS decreased in horses fed a high starch/sugar concentrate and observed a reversal of these effects after 7 weeks of moderately intense physical conditioning, further confirming the positive effect of exercise training on IS (Pratt, Geor et al. 2006)

**Adipocytokines**

Adipose tissue is classified as either white adipose tissue (WAT) or brown adipose tissue (BAT), with the latter identified by its multilocular fat droplets and its role in thermoregulation, and the former identified by a single large lipid droplet (Radin, Sharkey et al. 2009). White adipose tissue is comprised of mature adipocytes, pre-adipocytes (those not yet infused with fat for storage), endothelial cells, fibroblasts, and macrophages (Tilg and Moschen 2007). Once thought to function solely as a depot for triglyceride (TG) storage, it is now recognized that adipose tissue is an important secretory organ (Tilg and Moschen 2007; Radin, Sharkey et al. 2009). Adipocytokines are biologically active
proteins synthesized and secreted by adipocytes that act in a paracrine, autocrine, or endocrine manner to regulate local tissue and whole organism physiology (Tilg and Moschen 2007; Radin, Sharkey et al. 2009). Two such adipocytokines involved in energy utilization and metabolism are leptin and adiponectin.

**Mechanism of Action and Function of Leptin**

Leptin, encoded by the *ob* gene, is a highly conserved 167 amino acid protein hormone (Radin, Sharkey et al. 2009). It is considered pro-inflammatory because of the structural similarity to other pro-inflammatory cytokines such as interleukin-6 (IL-6, (Tilg and Moschen 2007)). Leptin is produced almost exclusively by differentiated, or mature, adipocytes, and in humans, is expressed in higher concentrations in subcutaneous fat versus visceral fat (Lafontan and Berlan 2003). Leptin mRNA has also been identified, though in smaller concentrations, in placenta, liver, mammary gland, skeletal muscle, and stomach tissue (Tilg and Moschen 2007). The leptin receptor (Ob-R) is expressed in the highest concentrations in the satiety centers of the brain, though variable-length isoforms exist and can be found in different tissues throughout the body. It is believed that the longest receptor (Ob-Rb) is responsible for mediating most of the physiological functions of leptin (Radin, Sharkey et al. 2009)

Leptin is a regulator of energy intake and whole body energy homeostasis. Leptin acts by binding to the Ob-Rb receptor located in the satiety center of the hypothalamus,
resulting in appetite suppression and increased thermogenesis. Appetite suppression is achieved through stimulation of neurotransmitters such as α-melanocyte stimulating hormone (α-MSH) and neuropeptide-Y, which stimulate anorexigenic and suppress orexigenic neurons, respectively (Radin, Sharkey et al. 2009). Concurrently, increases in thermogenesis occur via activation of brown adipose tissue. It has been suggested that leptin may increase insulin sensitivity by promoting fat oxidation (Dyck, Heigenhauser et al. 2006), however a state of partial leptin resistance or hyperleptinemia is associated with obesity (Radin, Sharkey et al. 2009). In this case, regulation of energy homeostasis is hindered by either saturated leptin receptors or downstream malfunctions in the signal transduction pathway leading to decreases in insulin sensitivity.

Mechanism of Action and Function of Adiponectin

Adiponectin is a 244 amino acid protein characterized by its N-terminal collagen-like domain and C-terminal globular domain (Kadowaki and Yamauchi 2005). Adiponectin can exist in several forms which influence the biological activity of the adipocytokine. The full-length protein exists as a trimer (low molecular weight, LMW), a hexamer (middle molecular weight, MMW), or a 12-18 multimer (high molecular weight, HMW) (Kadowaki and Yamauchi 2005). It can also exist as the globular domain fragment independent of the rest of the molecule (Kadowaki and Yamauchi 2005). It is currently believed that the HMW form is the most biologically active form and is correlated with
insulin sensitivity (Radin, Sharkey et al. 2009). Adiponectin is secreted almost exclusively by mature adipocytes, however, it has also been identified in skeletal and cardiac myocytes and endothelial cells (Tilg and Moschen 2007). In contrast to leptin, adiponectin is found in greater concentrations in visceral fat compared to subcutaneous fat (Lafontan and Berlan 2003).

Along with anti-atherosclerotic (vasoprotective) and anti-inflammatory functions, adiponectin also functions as an insulin sensitizer (Radin, Sharkey et al. 2009). Insulin sensitizing effects occur via two mechanisms: the activation of AMP-activated protein kinase (AMPK) and decreased mRNA expression of the enzymes phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G-6-Pase) (Figure 2, (Kadowaki and Yamauchi 2005)). Binding of adiponectin to receptors (AdipoR1) in skeletal muscle results in the activation of AMPK, ultimately resulting in the regulation of intracellular malonyl CoA through the inhibition of acetyl CoA carboxylase. The decrease in malonyl CoA causes a subsequent decrease in lipogenesis and increase in mitochondrial fatty acid β-oxidation. The increase in AMPK activity also increases GLUT4 translocation to the cell membrane and thus glucose uptake into the cell (Kadowaki and Yamauchi 2005).

Concurrently, binding of adiponectin to the AdipoR2 receptor in liver tissue results in a decrease in mRNA expression of key gluconeogenic enzymes, PEPCK and G-6-Pase, with a subsequent decrease in blood glucose (Kadowaki and Yamauchi 2005). It is believed that the AdipoR1 receptor, found predominantly in skeletal muscle tissue, has a high affinity
for the globular and LMW forms of adiponectin, while the AdipoR2 receptor, predominantly in the liver, has a high affinity for the MMW and HMW forms of adiponectin (Kadowaki and Yamauchi 2005).

**Figure 1.2.** Schematic of mechanisms of action for the insulin sensitizing effects of adiponectin. AMP, adenosine monophosphate; AMPK, 5’AMP-activated protein kinase; G6Pase, glucose 6-phosphatase; HMW, high molecular weight; IS, insulin sensitivity; LMW, low molecular weight; MMW, middle molecular weight; PEPCK, phosphoenol pyruvate carboxykinase. Adapted from Kadowaki and Yamauchi 2005.
**Quantifying Leptin and Adiponectin**

Leptin and adiponectin are typically assessed using radioimmunoassays (RIA), and either serum or plasma can be used to detect the adipocytokines. Previous research quantifying leptin concentrations in horses has used commercially available multispecies RIA kits (Linco Research/Millipore, St. Charles, MO (Gordon, Betros et al. 2004; Pratt, Geor et al. 2005; Kearns, McKeever et al. 2006; Van Weyenberg, Hesta et al. 2008; Van Weyenberg, Hesta et al. 2008)). These commercial kits utilize $^{125}$I-labeled recombinant human leptin, guinea pig multispecies leptin primary antibody, and goat anti-guinea pig IgG serum for the precipitating reagent (Kearns, McKeever et al. 2006). Samples are run in duplicate to ensure reliability of results. Previous within assay coefficients of variation (CV) for leptin in horses were between 5.2% and 13.6%.

Adiponectin has also been quantified in horses using a commercially available kit (Linco Research/Millipore, St. Charles, MO), though these studies are few in number (Gordon, Betros et al. 2004; Pratt, Geor et al. 2005; Kearns, McKeever et al. 2006). The kit utilizes $^{125}$I-labeled murine adiponectin, multispecies adiponectin rabbit antiserum, and goat anti-rabbit IgG serum in the precipitating reagent (Kearns, McKeever et al. 2006). Validation for use in horses was accomplished by comparing purified human recombinant adiponectin standards with standards spiked with equine plasma, as well as serial dilutions of horse plasma and the control. Results from samples run in duplicate demonstrated linear parallelism over a range of concentrations, with an intra-assay CV of 9.1% (Kearns,
McKeever et al. 2006). Subsequent studies investigating equine adiponectin utilized these methods with reported intra-assay CVs of <10% (Gordon, Betros et al. 2004) and 11.6% (Pratt, Geor et al. 2005).

**Effect of Obesity and Weight Change on Leptin and Adiponectin**

Obesity is associated with lack of leptin production in mutant ob/ob mice as well as failure to produce functional leptin receptors as seen in mutant db/db mice (Radin, Sharkey et al. 2009). These mutations are not established in companion animal species and occur minimally in humans (Radin, Sharkey et al. 2009). It is understood that obesity then is more likely the result of excessive caloric intake in conjunction with minimal physical exercise than specific leptin gene mutations, which results in some degree of leptin resistance (Frank, Elliott et al. 2006; Wyse, McNie et al. 2008).

Previous research across all species suggest a positive correlation between adiposity and circulating leptin (Considine 1996; Kearns, McKeever et al. 2006; Hoenig, Thomaseth et al. 2007). For example, one study in dogs revealed experimental increases in body condition or body fat mass resulted in increased leptin concentrations (Ishioka, Soliman et al. 2002). The same correlation was observed in a clinical setting in pet dogs with higher BCS (Ishioka, Hosoya et al. 2007). Conversely, studies in both dogs and cats show loss of body condition (via caloric restriction) results in lower plasma leptin concentrations similar to those seen in lean animals (Jeusette, Detilleux et al. 2005; Hoenig, Thomaseth et al. 2007).
Several studies evaluating the relationship between peripheral concentrations of leptin and body condition and fat mass in horses found leptin concentrations increased with adiposity, though effects of changes in adiposity are conflicting (Buff, Dodds et al. 2002; Kearns, McKeever et al. 2006). Buff et al found leptin concentrations did not change when fat ponies (BCS ≥ 6) lost body weight and condition, nor when thin ponies (BCS ≤ 5) gained weight and condition (Buff, Dodds et al. 2002). This is in opposition to Van Weyenberg et al who found plasma leptin significantly decreased following extreme body weight and condition loss from caloric restriction (Van Weyenberg, Hesta et al. 2008). This discrepancy may have been due to the degree of weight loss in that the former observed adiposity changes of no more than 2 units in BCS (Buff, Dodds et al. 2002), while the later observed a total body weight loss of approximately 18% of initial body weight, corresponding to an approximate 4 unit change in BCS (BCS = 8 or 9 to BCS = 4 or 5, (Van Weyenberg, Hesta et al. 2008)).

While little is known about the dynamics of the relationship between adiponectin and obesity, it has been well established that there is a negative correlation between adiponectin and body fat mass (Yang, Lee et al. 2001; Ishioka, Omachi et al. 2006; Kearns, McKeever et al. 2006; Hoenig, Thomaseth et al. 2007). This negative correlation has been demonstrated in human and rodent research, such that increases in body condition and adiposity result in decreased circulating adiponectin, while weight and condition loss result in increased circulating adiponectin (Yang, Lee et al. 2001).
Studies involving companion animals offer the same conclusions. One study in dogs investigated changes in plasma adiponectin following an approximate 30% weight gain. Dogs were fed a high-energy diet for 14 weeks, after which adiponectin was found to have decreased significantly versus prior to weight gain (Ishioka, Omachi et al. 2006). The same study used qualitative visual measures of adiposity via body condition scoring (1-5 scale, 3 = optimal, 5 = obese) on 71 dogs in a clinical setting and found that obese animals (BCS = 5) had significantly lower circulating adiponectin than those dogs with an optimal BCS of 3 (Ishioka, Omachi et al. 2006). Interestingly, even dogs not considered truly obese but rather overweight (BCS = 4) still showed decreases in circulating adiponectin, suggesting circulating adiponectin is sensitive to small increases in body condition.

A similar study in cats evaluated the effect of obesity and weight loss on adiponectin. Again, adiponectin was negatively correlated with quantitative measures (magnetic resonance imaging of abdominal fat) of adiposity, with obese cats having lower concentrations than lean cats (Hoenig, Thomaseth et al. 2007). Body weight of obese cats was decreased at a rate of 1.5% per week to reach lean weights, resulting in significant increases in circulating adiponectin not different from concentrations seen in cats maintained at lean weights (Hoenig, Thomaseth et al. 2007).

In horses, studies evaluating the relationship between adiponectin and body weight, as well as both qualitative visual (BCS) and quantitative (percent body fat) measures of adiposity found the same negative correlations (Gordon, Betros et al. 2004; Kearns,
McKeever et al. 2006). Percent body fat in each case was evaluated using B-mode ultrasonography at a location of maximal fat thickness (over the rump) described by Westervelt et al (Westervelt, Stouffer et al. 1976). The descriptive nature of these studies calls for the investigation of the dynamics between adiponectin and both positive and negative changes in body condition in the horse. Our research aimed to elucidate these dynamics during active changes in body condition.

**Effect of Innate Factors on Leptin and Adiponectin**

As with insulin sensitivity, other factors, both innate (age, sex, breed, and seasonal changes) and environmental (diet and exercise), have been identified to have effects on leptin and adiponectin production and action, though the body of evidence behind these relationships is relatively small in comparison to work on IS. Previous research in dogs and horses investigating the effect of age on leptin suggests circulating leptin concentrations increase with age (Buff, Dodds et al. 2002; Ishioka, Hosoya et al. 2007). Ishioka and colleagues grouped 151 dogs by BCS (BCS = 3, 4, or 5 on 1-5 scale) and evaluated age effects on plasma leptin, finding no differences in leptin concentrations (Ishioka, Hosoya et al. 2007). However, upon separate analysis, 4 puppies in one of the groups (BCS = 3) that were less than 1 year of age tended to have lower leptin concentrations (Ishioka, Hosoya et al. 2007). Given the positive correlation between leptin and adipose tissue, where circulating leptin increases with adiposity, researchers proposed the lack of body fat
accumulation and high energy requirements for growing animals as cause for these results. Relatively few studies in horses have looked at age effects on leptin (Fitzgerald and McManus 2000; Buff, Dodds et al. 2002; Kearns, McKeever et al. 2006). Kearns and coworkers observed lower leptin concentrations in weanlings compared to mature mares (Kearns, McKeever et al. 2006). Similarly, in a study of 71 Quarter Horses aged 8 days to 24 years, leptin concentrations tended to increase with age (Buff, Dodds et al. 2002). In contrast, a recent study by Pratt-Phillips and colleagues found no correlation between age and leptin concentration in 366 horses (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION).

There are little data available on the effect of age on adiponectin. Previous research in rodents suggests adiponectin concentration remains unchanged with advancing age in rats fed either ad libitum or restricted diets (Escriva, Gavete et al. 2007). Escriva and coworkers postulated that though no change in total adiponectin was observed in rats, the development of age-associated insulin resistance may be the result of potential differences in the proportion of LMW or MMW isomers of adiponectin compared to the HMW form (believed to have the greatest insulin sensitizing activity (Radin, Sharkey et al. 2009). One study in horses found weanlings had a significantly higher ratio of adiponectin to leptin, as well as greater circulating adiponectin compared to mares (Kearns, McKeever et al. 2006). As in the study by Ishioka et al (Ishioka, Hosoya et al. 2007), it was speculated that these findings were the result of relatively low body fat accumulation in young, growing horses.
(Kearns, McKeever et al. 2006). Conflicting results from the aforementioned studies suggest the difficulty of discriminating effects of age itself on leptin and adiponectin versus confounding factors (i.e. changes in body composition that occur with aging), and suggest the need for further research.

Breed effects on circulating leptin concentration have not been well established in horses. Though effects were minimal, one study in canines that found differences in circulating leptin between Miniature Dachshunds and Shetland Sheepdog at BCS of 5 (1-5 scale, (Ishioka, Hosoya et al. 2007)) demonstrated the possibility for a genetic component to leptin dynamics in horses. A study comparing methods for assessing body condition in horses and ponies found median (25-75%) leptin concentration was greater in ponies than in horses, at 5.8 and 3.8 ng/mL, respectively (Carter, Geor et al. 2009). Recently, Pratt-Phillips and colleagues found ponies and gaited horses had higher circulating leptin concentrations than both Quarter Horses and Thoroughbreds (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION). It should be noted that some of these results may be due to confounding factors such as BCS given ponies had significantly greater BCS than several other breeds. Currently, there are no available data on the effect of breed on adiponectin in horses. This, combined with limited information on the relationship between leptin and breed, suggests the need for future research (under controlled conditions) to identify and confirm potential breed influence on adipocytokine production and activity.
Sex differences in adipocytokine production and activity are well established in humans (Considine 1996; Hickey, Houmard et al. 1997; Kennedy, Gettys et al. 1997; Plaisance, Grandjean et al. 2009) and rodents (Watanobe and Suda 1999; Xu, Chan et al. 2005), such that circulating leptin and adiponectin are significantly higher in females compared to males. Recently, Plaisance and colleagues found that serum adiponectin and leptin concentrations were higher (26% and 30%, respectively) in women than in men of similar age, body mass index, and waist circumference (Plaisance, Grandjean et al. 2009). Upon further evaluation of lean and obese men and women, adiponectin concentrations did not differ between lean men, and both lean and obese women, though lower concentrations were observed in obese men (Plaisance, Grandjean et al. 2009). Previous studies in castrated male rats suggest that testosterone may be the primary factor accounting for differences in adipocytokine secretion and activity, given plasma leptin concentration increased following orchidectomy and decreased when physiological doses of replacement testosterone were administered (Watanobe and Suda 1999). Similar effects of testosterone on adiponectin have been observed (Xu, Chan et al. 2005). Interestingly, in the study by Plaisance and coworkers, though circulating adiponectin was higher in lean versus obese men, testosterone concentrations were similar, suggesting other factors besides sex steroids may be responsible for sex differences in adipocytokines. Similarly, in horses, Buff and colleagues found serum leptin concentrations were greater in geldings and stallions than in mares (Buff, Dodds et al. 2002). Here it was suggested that sex effects on leptin might be
species specific. Conversely, Gordon and colleagues identified differences in circulating leptin concentrations between fit and unfit mares and geldings, such that mares had higher plasma leptin than geldings (Gordon, McKeever et al. 2007). This is in agreement with previous research in humans and rodents (Watanobe and Suda 1999; Plaisance, Grandjean et al. 2009). It was noted by Gordon, however, that when horses in the fit group were evaluated independently of the unfit group there were no apparent sex effects on adipocytokines (Gordon, McKeever et al. 2007). Additionally, two studies in horses failed to identify differences in circulating leptin or adiponectin between mares and geldings (Pratt, Geor et al. 2005), and between mares, geldings, and stallions (Pratt-Phillips, Owens et al. ACCEPTED FOR PUBLICATION). Given the conflicting results from previous studies, more research is needed in horses to determine the effect of sex on adipocytokine secretion and to better understand sex-steroid regulatory mechanisms.

Currently, there is limited data on seasonal variations in circulating adipocytokines in the horse. Previous research in hibernating mammals suggests possible circannual changes in these adipocytokines, such that leptin begins to increase in autumn/winter months as animals gain weight and decreases during summer months, while adiponectin appears to be higher in the summer to maintain hepatic insulin sensitivity while animals are eating (Florant, Porst et al. 2004). As seen in other species (Kearns, McKeever et al. 2006; Hoenig, Thomaseth et al. 2007), leptin and adiponectin were highly correlated with body fat in the study by Florant and coworkers. Interestingly, major decreases in leptin
concentration occurred prior to any significant decrease in fat mass (Florant, Porst et al. 2004). To our knowledge, while there is no data on the effect of seasonal changes on adiponectin in the horse, previous research on seasonal influences on leptin has focused primarily on leptin as it relates to onset and duration of seasonal ovarian activity in mares (Fitzgerald and McManus 2000; Gentry, Thomspson et al. 2002). Fitzgerald and McManus found circulating leptin decreased during winter months (December to January) in both young and mature mares, and this decrease was correlated with a loss of body weight and percent body fat (Fitzgerald and McManus 2000). However, leptin concentrations in the two groups were similar between January and April while body fat was not, suggesting changes in body fat mass may not fully account for the changes in leptin concentrations (Fitzgerald and McManus 2000). It is important to note that though an apparent seasonal effect on leptin was observed, experimental design did not permit direct evaluation of whether the decline in leptin was directly related to a decrease in day length. A recent study in Lippizeran fillies and mares (aged 1 to 4 years) observed lower leptin concentrations between January and June, and higher leptin concentrations in summer months (peaking in August to September, (Cebulj-Kadunc, Kosec et al. 2009)). In this study body condition remained stable throughout the year and seasonal changes in leptin were attributed to potential changes in quality of feed or environmental influences, specifically reduced ambient temperature and photoperiod as demonstrated in sheep (Bocquier, Bonnet et al. 1998). Though possible seasonal changes in leptin concentrations
have been indicated in mares (Fitzgerald and McManus 2000; Cebulj-Kadunc, Kosec et al. 2009), further research is needed to determine if there is a direct effect of photoperiod or ambient temperature on adipocytokine production and activity in the horse.

**Effect of Diet and Exercise on Leptin and Adiponectin**

In horses, the effects of diet composition on leptin and adiponectin concentration are not currently known. Previous research has focused primarily on effects of fed versus fasted states and post-prandial changes on adipocytokines rather than dietary energy source itself (Piccione, Bertolucci et al. 2004; Buff, Morrison et al. 2005; Gordon and McKeever 2005). Findings suggest leptin exhibits a circadian pattern in which circulating concentrations are highest at night and lowest during the day (Piccione, Bertolucci et al. 2004; Buff, Morrison et al. 2005). However, Gordon and coworkers found peak leptin concentration occurred midday at 1550 hrs, while the lowest leptin concentrations were observed at 0650 (Gordon and McKeever 2005). It was hypothesized this may have been the result of feeding schedule, where horses in this study received large amounts of grass hay (essentially allowing *ad libitum* feed) and small quantities of grain as opposed to large bolus meals (Gordon and McKeever 2005). It has also been established that feeding and subsequent increases in insulin cause an increase in leptin concentrations (Cartmill, Thompson et al. 2005). It may be possible then that dietary energy source may effect circulating leptin concentrations given two factors: 1) increased leptin is associated with
post-feeding rises in insulin, and 2) previous research suggests greater insulin response in horses fed diets high in NSC (Pratt, Geor et al. 2006). It is important to note, however, that this has not been demonstrated in horses. In contrast to leptin, previous research in horses suggests there are no fluctuations in circulating adiponectin concentrations in response to feeding (Gordon and McKeever 2005) and the effect of dietary energy source has not been reported.

Inquiries into the effect of physical activity on leptin and adiponectin have been performed in humans (Hickey, Houmard et al. 1997; Landt, Lawson et al. 1997; Kraemer, Chu et al. 2002; Simpson and Fiatarone Singh 2008). Generally, long-term exercise training appears to result in minimal increases in circulating adiponectin, while effects of short-term, acute exercise appear to be influenced by intensity of exercise and individual fitness levels (Simpson and Fiatarone Singh 2008), suggesting a more complicated mechanism behind these dynamics. Gordon and colleagues studied the effects of physical exercise in horses and found no significant changes in adiponectin in response to short-term, high-intensity exercise (Gordon, McKeever et al. 2007).

Similar variability exists in previous research investigating the influence of exercise on circulating leptin. Results from human studies suggest that, in general, bouts of acute short-term (AST, < 60 min) and acute long-term (ALT, > 60 min) exercise do not affect leptin concentrations (Kraemer, Chu et al. 2002). However, acute bouts of extreme exercise (i.e. marathon running) show decreases in circulating leptin, hypothesized to most
likely result from energy balance disruption (Landt, Lawson et al. 1997). Generally, exercise training of short-term (ETST, < 12 wk) and long-term (ETLT, > 12 wk) periods does not result in decreased circulating leptin (Kraemer, Chu et al. 2002), with the exception of type II diabetic individuals (Ishii, Yamakita et al. 2001) and those decreases in leptin resulting from significant loss of body fat (Kohrt, Landt et al. 1996). However, some ETLT studies have identified decreases in leptin despite the lack of change in body fat. For example, Hickey and colleagues found serum leptin decreased by 17.5% in females versus males following 12 weeks of aerobic exercise training, though no alterations in fat mass were observed in either group (Hickey, Houmard et al. 1997). This suggests a more complicated underlying mechanism behind the effect of exercise on circulating leptin.

Few studies in horses have investigated the influence of physical activity on leptin. Piccione and colleagues did not observe changes in serum leptin as a result of physical exercise in horses that underwent 60 days of moderately-intense training (Piccione, Bertolucci et al. 2004). Another study found plasma leptin concentrations were unchanged during short-term, high-intensity exercise, but decreased 24 hr post-exercise (Gordon, McKeever et al. 2007). Gordon and coworkers hypothesized there may be an energy expenditure threshold that must be exceeded before changes in leptin concentrations can be detected (Gordon, McKeever et al. 2007). The relatively small number of studies in horses investigating the effects of physical activity on leptin and adiponectin secretion and activity necessitates further research in this area, including underlying mechanisms of action.
Current Research Objectives

Several equine conditions have been linked to decreases in insulin sensitivity, or insulin resistance, such as laminitis (Kronfeld, Treiber et al. 2006; Treiber, Kronfeld et al. 2006), hyperlipemia (Jeffcott and Field 1985), and Cushing’s Disease (Reeves, Lees et al. 2001; Johnson, Messer et al. 2004). Insulin resistance has been identified as a multifactorial problem with both innate and environmental influences. Additionally, adipose tissue hormones, leptin and adiponectin, are suspected to play an important role in the pathogenesis of insulin resistant disease states due to functions associated with energy homeostasis and insulin sensitivity, respectively (Radin, Sharkey et al. 2009). In the horse, obesity has been linked to decreases in insulin sensitivity (Hoffman, Boston et al. 2003; Vick, Adams et al. 2007) and adiponectin concentrations, and increases in circulating leptin concentrations (Kearns, McKeever et al. 2006). With obesity in horses recently identified as a growing problem (Pleasant, Thatcher et al. 2006; Wyse, McNie et al. 2008) and no clear understanding of the dynamics behind the obesity-insulin sensitivity-adipocytokine association, it is of interest to investigate this relationship further. The objectives of the current research were to determine how changes in body condition (adiposity), achieved through the modification of forage-only dietary energy intake (to avoid confounding factors such as DE source (Hoffman, Boston et al. 2003; Pratt, Geor et al. 2006)), influence insulin sensitivity, as well as adipocytokines leptin and adiponectin, and ultimately offer insight on potential “ideal” levels of adiposity to horse owners and industry professionals.
References


CHAPTER TWO
THE EFFECT OF CHANGES IN BODY CONDITION ON INSULIN SENSITIVITY, LEPTIN, AND ADIPONECTIN IN HORSES FED FORAGE-ONLY DIETS

Introduction

Insensitivity to insulin (insulin resistance) has been linked to several equine conditions including laminitis (Kronfeld, Treiber et al. 2006; Treiber, Kronfeld et al. 2006) and hyperlipemia (Jeffcott and Field 1985). Previous research has shown that dietary energy source, specifically high glycemic-index feeds, negatively affects insulin sensitivity (Hoffman, Boston et al. 2003; Pratt, Geor et al. 2006). In contrast, exercise conditioning appears to increase tissue sensitivity to insulin (Stewart-Hunt, Geor et al. 2006). An association between obesity and insulin resistance has been reported in horses (Hoffman, Boston et al. 2003; Vick, Adams et al. 2007), though the nature of this relationship is not fully understood. Currently there is no recommended ideal level of adiposity for insulin sensitivity in horses therefore it is of interest to investigate this further. Leptin and adiponectin, secreted by adipose tissue, are believed to function as a regulator of energy intake (Friedman and Halaas 1998; Radin, Sharkey et al. 2009) and an insulin sensitizing agent (Kadowaki and Yamauchi 2005; Radin, Sharkey et al. 2009), respectively. Previous research in horses has shown a negative correlation between adiponectin and body fat, with the opposite holding true for leptin (Kearns, McKeever et al. 2006). Therefore the
objective of this study was to determine how changes in body condition (adiposity), achieved through the modification of forage-only dietary energy intake, influences insulin sensitivity, leptin, and adiponectin. It was hypothesized that an increase in body condition, regardless of starting point (i.e. BCS of 3 to 5 or BCS of 5 to 7), would result in decreases in both insulin sensitivity and serum adiponectin, as well as increased serum leptin, with the opposite holding true for a decrease in body condition. Additionally, it was hypothesized that regardless of any changes in IS, leptin, or adiponectin that occurred with weight gain or loss, these changes would return to baseline values when body condition returned to baseline.

**Materials and Methods**

**Animals**

All procedures were approved by North Carolina State University’s Institutional Animal Care and Use Committee. Seventeen mature gelding horses, aged 3-14 years (8.0 ± 1.1), were used in this study that was performed from August 2008 to April 2009. Breeds consisted mostly of Quarter Horses (QH, n = 4), Quarter Horse crosses (QHx, n = 2), and Quarter Horse-type (QHt, n = 3), either Paint or Appaloosa, horses. Other breeds included Arabian crosses (Arabx, n = 3), Thoroughbred or Thoroughbred crosses (TB and TBx respectively, n = 4), and Warmbloods (WB, n = 1). Horses were maintained on tall
fescue/orchard grass mix pasture and supplemented with free choice, round-bale timothy-orchard grass mix hay for approximately 25 weeks prior to commencing the study. Horses were moved from pasture to approximately 4 m x 11 m dry-lot stalls 6 weeks prior to day 0 collections and started on grass-alfalfa mix hay cubes (Bale-in-a-Bag, Idle Acres Farms, Cokato, MN). Hay cubes were fed between 1.5 and 2.5% of BW at day 0 to achieve a body condition score (BCS) of 5 (Henneke, Potter et al. 1983) prior to start of the study.

**Experimental Design**

This study was a repeated measures, switch-back design with three experimental treatment groups and two phases (P1 and P2, respectively). Horses were blocked by age (young 3-4 yr, mid 5-9 yr, and old 10-14 yr) and randomly assigned to one of three treatment groups: weight gain to loss (GL, n = 6), weight loss to gain (LG, n = 6), and maintenance, or control, (C, n = 5). Horses in the GL and LG treatment groups were fed to gain (fleshy, BCS = 7) or lose (thin, BCS = 3) weight and body condition, respectively, for the first 130 days, after which treatment diets for the groups were reversed and horses were fed to return to a BCS of 5 in the second 130-day period. Control horses were fed the same ration throughout the study to maintain body weight and condition.

Body weight changes were assessed weekly to monitor treatment integrity. Rump and abdominal fat depth assessed via ultrasound, insulin sensitivity (IS) using the euglycemic-hyperinsulinemic clamp (EHC) method, and blood serum samples for
quantification of adipocytokines, leptin and adiponectin, were collected and assessed at
days 0, 65 and 130, 195, and 260 (Figure 2.1). Concurrently, muscle and adipose tissue
biopsies were collected according to Bergstrom procedure, at a maximum tissue depth of
approximately 5 cm, under sterile conditions following desensitization of the mid-gluteal
muscle. Biopsy sites were alternated at each collection time, and samples were flash-frozen
in liquid nitrogen and stored at –80 °C until analysis (to be presented elsewhere).

Diets

Following day 0 collections to establish baseline parameters, BW and body
condition changes were achieved by feeding rations consisting of one of three types of
grass-alfalfa mix hay cube products (Idle Acres, Cokato, MN). Details of feedstuff energy
and protein content can be seen in Table 2.1. Horses gaining and losing weight were
adapted to High Energy cubes or Low Energy cubes, respectively, over a 1-week period,
while control horses were continued on Bale-in-a-Bag cubes. Cubes were fed as a
percentage of required digestible energy for maintenance (DEm) as specified by the
National Research Council (National Research Council 2007), where target consumption
for horses gaining weight was approximately 130% of DEm requirements, and target
consumption for horses losing weight was 70% of DEm requirements. Target intake for
control horses was 100% of DEm requirements.
Horses were fed pre-weighed cubes three times per day (0700-0800 hr, 1100-1200 hr, and 1600-1700 hr) for approximately 10 weeks from day 0 to day 65, after which flaked novel-endophyte fescue hay (MaxQ®, Pennington Seed, Madison, GA) was added to the ration in an amount supplying 20% of calculated digestible energy per day. Horses were then fed three times per day with cubes fed at morning and midday meals and hay fed at the evening meal only. Daily hay and cube feed refusals (orts) were collected separately and weighed. Horses had free access to fresh water and a vitamin/mineral supplement (Nature’s Essentials Free Balance 12:12, Purina Mills, Gray Summit, MO). Two horses in the GL treatment were supplemented with corn oil two times per day by dose syringe, in the amount of 3.51 MCal total per day, during P1 due to lack of weight gain on forage alone.

**Body Condition Score**

Body condition score (BCS) was assessed by one of two trained persons, blind to treatment, at days 0, 65, 130, 195, and 260 and is reported based on the Henneke 1-9 scale (Henneke, Potter et al. 1983). If both persons assessed BCS, the two scores were averaged.

**Body Weight**

Body weight (BW) was assessed on a weekly basis to monitor changes and track progress. Horses were weighed at 0900 hours in P1 and at 0700 hours, prior to feeding, in
P2 using a digital livestock-grade scale (Smart Scale 200, Gallagher Group Ltd., Hamilton, New Zealand).

**Rump and Abdominal Fat Depth**

Rump and abdominal fat depth (cm) were measured bilaterally by a trained technician at days 0, 65, 130, 195, and 260 via ultrasound. The same individual performed all evaluations. Rump fat depth (RFD) was measured in a 13 x 13 cm square centrally located between the top of the croup, point of the hip, and point of the buttock, opposite the current biopsy site (Kearns, McKeever et al. 2006). Abdominal fat depth (AFD) location was determined by counting five ribs spaces caudal to cranial, from the last rib, at a height between the ventral midline and stifle (Figure 2.2).

**Euglycemic-Hyperinsulinemic Clamp**

Feed was withheld from horses for a period of at least 12 hours. The morning of the clamp, horses were weighed and two indwelling 14 GA, 13 cm Angiocath catheters (BD, Franklin Lakes, NJ) were placed in each jugular vein using aseptic technique, following subcutaneous local anesthesia with 2% mepivacaine hydrochloride (Carbocaine®-V, Pharmacia and Upjohn Co., Pfizer, New York, NY) and sterile prep.

Insulin infusate was prepared by mixing 2 mL of each horse’s serum (to prevent binding or absorption of insulin to plastic surfaces) with 5 mL human recombinant DNA
insulin (Humulin®R, 100 U/mL, Hospira Inc., Lake Forest, IL) and 493 mL saline for injection (0.9% Sodium Chloride Injection, USP, Hospira Inc., Lake Forest, IL). After collection of baseline blood samples (time 0) and pre-clamp muscle and adipose biopsies, a priming insulin bolus in the amount of 18 mU/kg BW was administered and infusion of insulin via controlled-rate infusion (CRI) pump (Vet-Pro VIP 2000, Caesarea Medical Electronics Ltd., Lichtenstein, Germany) at a rate of 3 mU/min/kg BW (21.3 pmol/min/kg BW, (Pratt, Geor et al. 2005)) was started and maintained through the duration of the clamp. Concurrently, a variable rate infusion of glucose solution (50% w/v Dextrose Injection, Hospira Inc., Lake Forest, IL) using a CRI pump was started for maintenance of euglycemia, defined as blood glucose concentration of 90 mg/dL ± 10%. Blood samples (3 mL) were collected from one of the indwelling jugular catheters at 5-minute intervals throughout the clamp for determination of blood glucose concentration by precision glucometer (One Touch Ultra Mini, Life Scan Inc., Milpitas, CA). If blood glucose deviated from euglycemia by more than 15%, the glucose infusion rate was adjusted. Additional blood samples (17 mL) were collected every 15 minutes. Additive-free 10 mL Vacutainer tubes (BD, Franklin Lakes, NJ) were allowed 20 minutes to clot, and, along with 7 mL K2EDTA (10.8 mg) Vacutainer tubes (BD, Franklin Lakes, NJ), were centrifuged for 30 minutes at 905 x g, and serum and plasma collected and stored at -20 °C until further analysis.
After completing the EHC, insulin infusion was stopped while glucose infusion continued, at the same rate as that established to maintain euglycemia, for approximately 20-30 minutes. Horses were immediately offered feed and one of the two catheters was removed. Both catheter insertion sites were dressed with triple antibiotic ointment and dry gauze, and wrapped with Elastikon (Johnson and Johnson, Langhorne, PA). Horses were returned to stalls and blood glucose was monitored every 30 minutes for an additional 2 hours, after which the remaining catheter was pulled.

**Analysis of Serum Insulin and Quantification of Insulin Sensitivity**

Frozen serum samples were thawed to room temperature before analysis. Insulin concentration was assessed with serum samples using commercially available radioactive immunoassay (RIA) kits validated for use in horses (Coat-a-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, (Reimers, Cowan et al. 1982)). A ratio of mean steady state, defined as a period of euglycemia where glucose infusion rate (GIR) varies by no more than 25 mL/hr, insulin concentrations (I, µU/mL) and GIR (M, µg/min/kg BW) were used to calculate IS (Pratt, Geor et al. 2005), where:

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\text{IS} = \frac{M}{I}
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Analysis of Serum Leptin and Adiponectin

Serum samples were thawed to room temperature before analysis. Leptin and adiponectin were assessed using commercially available RIA kits validated for use in horses (Millipore, Billerica, MA, (Pratt 2005; Kearns, McKeever et al. 2006)). Serum for quantification of adiponectin was diluted at a ratio of 1:1000 prior to analysis. Both leptin and adiponectin samples from time 0 were run in duplicate and values were considered valid if the coefficient of variation (CV) of the duplicates were less than 15%.

Statistical Analysis

In order to be included in statistical analysis, horses had to meet strict criteria established in the experimental design. Horses had to start at BCS of 5 ± 1, follow their designated treatment, and follow designated forage-only diets. Horses that failed to meet these criteria were eliminated, resulting in an analysis of 12 horses (GL, n = 3; C, n = 5; LG, n = 4). Normal distribution of data and identification of outliers for both treatment and day were tested using PROC UNIVARIATE of SAS. Physiological state data (i.e. data in which horses were actively gaining, maintaining, or losing weight and condition) were analyzed with repeated measures ANOVA using PROC MIXED of SAS. Here phase, treatment, time, treatment x time, phase x treatment, and phase x treatment x time interaction effects for BCS, BW, RFD, AFD, IS, leptin, and adiponectin across physiological states were evaluated. In each analysis, day 0 values were used as covariates.
(day 130 was a covariate in P2 analysis). Significant main effects were further analyzed using Bonferroni pairwise comparisons in SAS. Additionally, individual treatment group data (i.e. GL, LG, and control groups) were analyzed using PROC MIXED of SAS to determine time effects, over the entire 260-day period, on the aforementioned parameters. Lastly, a one-way ANOVA comparison was conducted using PROC MIXED in SAS at Day 130 to determine if IS, adiponectin, leptin (etc) differed between the “extremes” in body condition score (3, 5 and 7). Dietary energy intake as percentage of DEm requirements consumed (DEm) and average daily weight change (ADWC) using data from P1 and P2 were also evaluated using PROC MIXED of SAS (SAS v 9.1, Cary, NC). Evaluation of linear dependence was examined at day 130 between BCS, BW, RFD, AFD, IS, leptin, and adiponectin using GraphPad PRISM5 (GraphPad Software, Inc., La Jolla, CA) and is expressed as the Pearson correlation coefficient (r). Data are presented as mean ± SEM. Data are considered significant when P < 0.05 and trends are noted when P ≤ 0.10.

Results

Energy Intake and Average Daily Weight Changes

Mean DEm intake for each treatment group was calculated for all of P1 (day 0 to 130) and all of P2 (day 130 to 260), as well as for sub-time periods within each phase (day 0 to 65 and 65 to 130 in P1, and day 130 to 195 and 195 to 260 in P2). To achieve
significant weight and body condition changes mean overall intake in the GL group was 138% of DEm in P1 and 80% of DEm in P2, and 89% of DEm in P1 and 119% of DEm in P2 in the LG treatment group. Control horses were maintained on an average of 109% of DEm through the duration of the study (both P1 and P2). Mean DE intake of GL and LG treatment diets were significantly different from that of the control in both P1 and P2 (P < 0.01). Further details of P1 and P2 sub-time period DEm intake as well as average daily weight change (ADWC) are shown in Table 2.2. Within P1, horses in the GL group consumed a greater percentage of DEm than the control between day 0 and 65, and also between day 65 and 130 (P < 0.01 and P = 0.03, respectively). Dietary intake for horses in the LG group was lower than that of control horses within P1 between days 0 and 65 and 65 and 130 (P < 0.01 and P = 0.01). Evaluation of P2 sub-time periods (following treatment diet reversal) showed lower dietary intake in the GL treatment versus the control between day 130 and 195 and also between day 195 and 260 (P < 0.01), whereas percentage of DEm in the LG group was greater than that of the control from day 130 to 195 and from day 195 to 260 (P < 0.01 and P = 0.02, respectively. Horses in the GL treatment had significant ADWC, such that they gained weight during P1 (P = 0.01) and lost weight during P2 (P < 0.01). In the LG group a weak trend for ADWC was observed in P1, such that horses tended to lose BW (P = 0.10), however, in P2 there was no significant ADWC compared to control horses (P = 0.50).
Body Weight, Body Condition Score & Body Fat Measures

Results of BW and BCS changes are shown in Figure 2.3. Mean and SEM for BW, BCS, RFD, and AFD are shown in Table 2.3. Results of physiological state data using repeated measures ANOVA for BW, under the switch-back design, show significant phase, treatment, treatment x time, and phase x treatment x time interaction effects (P < 0.01), where horses losing weight and condition had significantly lower BW when compared to those gaining or maintaining condition (P < 0.01). Additionally, greater body weights were observed in horses gaining body condition when compared to those horses maintaining body condition (P = 0.01).

Post hoc analysis of treatment x time interaction effects show that in horses losing weight, mean BW at times 2 and 3 (T2 and T3, respectively) were less than at time 1 (T1, P < 0.01), as well as when compared to body weights of horses either gaining or maintaining weight at T2 and T3 (P < 0.01). In horses gaining weight and condition, mean BW at T2 and T3 was greater than at T1 (P < 0.01), however no significant differences in mean BW were seen between T2 and T3 (P = 0.69). Additionally, no significant differences were observed in mean BW at T2 when comparing horses gaining weight to horses maintaining weight (P = 0.16); nor between T2 and T3 in horses losing weight and condition (P = 1.00). No significant differences in mean BW were noted between T1, T2, or T3 in horses maintaining weight and condition (P = 1.00).
Analysis of individual treatment groups show significant time effects on mean BW in the horses in the GL group (P < 0.01) and LG group (P = 0.01), though no effect of time on mean BW was observed in control horses (P = 0.12). In the GL treatment, mean BW at day 65 was greater than at days 0, 195, and 260 (P < 0.01, P = 0.02, and P < 0.01, respectively). Additionally, mean BW at day 130 was also greater when compared to days 0, 130, and 260 (P < 0.01). No significant differences in mean BW were observed in the GL group between days 0 and 260 (P = 1.00). In horses in the LG treatment group, mean BW at day 65 was lower than at days 0 and 260 (P = 0.01 and P = 0.02, respectively). As in the GL group, no significant differences in mean BW between days 0 and 260 were found in the LG horses (P = 1.00).

Analysis of physiological state data showed significant treatment, time, and treatment x time effects on mean BCS (P < 0.01). Horses gaining weight and condition had significantly greater body condition scores when compared to those losing or maintaining condition (P < 0.01). Similarly, mean BCS was lower in horses losing BW when compared to those maintaining BW (P < 0.01). In horses gaining weight, mean BCS at T2 and T3 were greater than at T1 (P < 0.01), as well as when compared to body condition scores of horses either losing or maintaining weight at T2 and T3 (P < 0.01). Interestingly, no significant differences were found between T2 and T3 in horses gaining weight (P = 1.00). Additionally, mean BCS for horses losing weight was lower at T2 and T3 when compared to T1 (P = 0.02 and P < 0.01, respectively). In horses losing weight, mean BCS at T3 was
also lower than at T2 (P = 0.04). No significant differences in mean BCS were noted between T1, T2, or T3 in horses maintaining weight and condition (P = 1.00).

Individual treatment group ANOVA results suggest significant time effects on mean BCS in the GL and LG groups (P < 0.01), as well as the control group (P = 0.04). Horses in the GL treatment had significantly greater body condition scores at day 65 versus days 0, 195, and 260 (P < 0.01, P = 0.04, and P < 0.01, respectively). In addition, BCS in the GL group was greater at day 130 compared to days 0 and 260 (P < 0.01). No significant differences were found between days 0 and 260 (P = 1.00). In the LG group, mean BCS at day 65 was lower than at day 260 (P = 0.04), and tended to be lower than at day 195 (P = 0.05). Mean BCS in the LG group was also lower at day 130 when compared to days 0 and 195 (P = 0.03 and P < 0.01, respectively). No significant differences were seen between days 0 and 260 within the LG treatment (P = 1.00). Control horses had a greater mean BCS at day 65 compared to day 0 (P = 0.04), though no other differences in mean BCS were observed between days.

Evaluation of RFD showed significant phase (P = 0.03), treatment (P = 0.01), treatment x time (P < 0.01), and phase x treatment effects (P = 0.01), where horses gaining weight and condition had significantly greater RFD than those horses losing or maintaining condition (P = 0.02). No significant differences in mean RFD were observed in horses losing BW when compared to those maintaining BW (P = 0.32). Post hoc testing revealed mean RFD at T2 was significantly greater in horses gaining weight versus those horses
losing weight (P = 0.01), while no differences were found at T2 when comparing mean
RFD between horses gaining and maintaining weight and condition (P = 0.20). Mean RFD
in horses gaining weight was also significantly greater than horses maintaining weight at
T3 (P = 0.01). Furthermore, in horses gaining weight, mean RFD was significantly smaller
at T1 versus both T2 and T3 (P = 0.04 and P < 0.01, respectively). No significant
differences in RFD were found between T2 and T3 in horses gaining weight (P = 1.00). In
horses losing weight mean RFD at T1 was significantly higher than at both T2 and T3 (P <
0.01), and no significant differences were observed between T2 and T3 (P = 1.00). No
significant differences in mean RFD were found at T2 when comparing horses losing
weight with horses maintaining weight (P = 0.84). No significant differences in mean BCS
were noted between T1, T2, or T3 in horses maintaining weight and condition (P = 1.00).
Results also suggest that RFD was different between horses gaining weight in P1 versus
P2, where mean RFD was significantly greater in P1 (P = 0.01).

Analysis of individual treatment groups show significant time effects on mean RFD
in GL horses (P < 0.01) and LG horses (P = 0.02). Mean RFD was greater at day 130
versus day 195 (P = 0.04), and tended to be greater than at day 0 (P = 0.06) in the GL
group. Additionally, RFD tended to be greater at day 65 versus day 195 (P = 0.10). No
significant difference between day 0 and 260 RFD was found in the GL group (P = 1.00).
In the LG group, mean RFD at day 130 was significantly smaller than at day 0 (P = 0.02).
No differences were found between days 0 and 260 (P = 1.00) in the LG horses; nor was
there a significant time effect on mean RFD in the control horses (P = 0.70).

Significant treatment and time effects on mean AFD were also observed (P = 0.01, P = 0.02, P = 0.02, and P < 0.01, respectively). Post hoc evaluation showed mean AFD in horses gaining weight was greater than in horses losing weight (P = 0.03) and maintaining weight (P = 0.04). No significant differences in mean AFD were found between horses losing and maintaining weight and condition (P = 1.00). Additionally, mean AFD at T1 was lower than at T2 (P = 0.01), though no significant differences were found between T1 and T3 (P = 0.87), or between T2 and T3 (P = 0.21).

Analysis of individual treatment group AFD showed significant time effects in the GL and control groups (P = 0.04 and P < 0.01), but not in the LG horses (P = 0.13). In horses in the GL treatment group, mean AFD tended to be greater at day 65 when compared to days 130 and 195 (P = 0.10 and P = 0.06, respectively). No significant differences in mean AFD were found between day 0 and 260 (P = 1.00). Interestingly, in the control treatment, mean AFD was greater at day 65 versus days 0, 130, and 195 (P = 0.03, P = 0.02, and P = 0.01, respectively).

*Insulin Sensitivity, Leptin, and Adiponectin*

Table 2.3 shows mean and SEM for IS, leptin, and adiponectin. Insulin sensitivity (IS) between treatment groups is shown in Figure 2.4. Analysis of physiological state data show no significant phase, treatment, time, phase x treatment, or phase x treatment x time
interaction effects on IS (P = 0.43, P = 0.87, P = 0.45, P = 0.22, and P = 0.21, respectively). There was an overall trend for a significant treatment x time interaction effect on IS (P = 0.07). Additionally, no significant effect of time on IS was observed in GL (P = 0.24), LG (P = 0.50), and control horses (P = 0.38) when analyses of individual treatment groups were performed. Analysis of day 130 alone shows no significant effect of treatment on mean IS (P = 0.56).

Mean leptin and adiponectin concentrations for individual treatment groups are shown with RFD in Figure 2.5. Analysis of physiological state data show significant phase effects (P = 0.04), where mean leptin concentration in P1 (days 0 to 130) was greater than in P2 (days 130 to 260). There were no significant effects of treatment, time, treatment x time or phase x treatment on mean leptin concentration in horses (P = 0.47, P = 0.47, P = 0.79, and P = 0.30, respectively). In addition, individual treatment group analyses show no significant effect of time on leptin concentration in the control group (P = 0.44) or in the LG group (P = 0.30). There was an overall trend for significant time effects on mean leptin concentration in the GL group (P = 0.05). No significant effect of treatment group across day 130 was observed (P = 0.24).

Results of analyses of physiological state data show significant phase, time, and treatment x time main effects were found on mean adiponectin concentration (P = 0.01, P = 0.01, and P = 0.03, respectively). Post hoc analysis of time effects on adiponectin show that mean adiponectin concentration at T1 was significantly greater than at T3 (P = 0.01),
and tended to be greater when compared to T2 (P = 0.09). No significant differences were observed between T2 and T3 (P = 0.86). In horses losing weight and condition, mean adiponectin concentration at T3 was significantly higher compared to horses maintaining weight (P = 0.01); however, no differences were observed between the two physiological states at T2, nor between horses gaining weight and those maintaining weight (P = 1.00). Bonferroni pairwise comparisons further revealed no significant differences between T1, T2, and T3 in both horses losing and gaining weight (P = 1.00). Interestingly, mean adiponectin concentration in horse maintaining weight (control group), was significantly greater at T1 versus T3 (P = 0.01). Additionally, mean adiponectin concentration in P1 was significantly greater than during P2 (P = 0.01).

Individual treatment group analyses showed significant time effects on mean adiponectin concentration in the GL (P = 0.01) and control groups (P < 0.01), though no significant effect of time was observed within the LG treatment group (P = 0.13). Mean adiponectin concentration for in horses in the GL treatment group was significantly lower at day 195 versus day 0 (P = 0.01), and tended to be lower at day 195 when compared to day 65 (P = 0.07). Interestingly, the greatest effects of time on mean adiponectin concentration were observed in the control horses, where adiponectin concentration at day 0 was significantly greater than at days 130, 195, and 260 (P < 0.01). Mean adiponectin concentration in the control group was also significantly greater at day 65 versus days 195 and 260 (P = 0.01 and P < 0.01, respectively), and tended to be greater than at day 130 (P =
0.05). Trends for significantly lower mean adiponectin concentration at day 260 versus days 130 and 195 in control horses were also observed (P = 0.06 and P = 0.09, respectively). Treatment group was not observed to have a significant effect on mean adiponectin concentration at day 130 (P = 0.24).

**Pearson Correlations**

Pearson r correlation values identifying relationships between IS, leptin, adiponectin, BW, BCS, RFD, and AFD at day 130 are shown in Table 2.4. Strong positive correlations were identified between RFD and both AFD and BCS (P = 0.01 and P < 0.01, respectively). Additionally, a significant positive relationship was also identified between leptin and IS (P = 0.01). No significant relationships were found between adiponectin and any of the measures of adiposity.

**Discussion**

Previous research has shown an association between obesity (BCS > 7) and insulin resistance in horses (Hoffman, Boston et al. 2003; Vick, Adams et al. 2007). Conversely, decreases in body condition, through caloric restriction, are associated with improved insulin sensitivity (Van Weyenberg, Hesta et al. 2008). The present study differs in that no significant effects of body condition gain or loss on insulin sensitivity were observed, nor
were there significant correlations between IS and different measures of adiposity. It is possible that the effects of adiposity occur only at extremes, over longer periods of time, or in response to high glycemic-index diets.

Though the association between decreased IS and increases in body condition has been established (Hoffman, Boston et al. 2003; Vick, Adams et al. 2007), it remains to be determined if a threshold level of adiposity exists in horses, after which IS begins to decline. For example, Hoffman et al found significant reductions in insulin sensitivity when horses had body condition scores greater than 7 (Hoffman, Boston et al. 2003). In the same study one horse with a BCS of 7.3 ± 0.3 was identified as severely insulin resistant. Similarly, ponies with an initial BCS of 8 or 9 had significantly lower IS versus following body weight and condition loss (Van Weyenberg, Hesta et al. 2008). In the current study, body condition in horses in the GL treatment increased from an average of 4.8 at day 0 to a maximum BCS of 6.9 at day 65 and 6.5 at day 130. Additionally, horses in the LG treatment may not have reached an adequate state of adiposity when diets were reversed (to positive energy balance), given increasing body condition did not have an effect on IS during P2 (BCS 3.6 at day 130 to BCS 5.5 at day 260). As such, it is possible that the changes in IS associated with adiposity occur only at extremes.

Body weight in the GL treatment increased by approximately 12% during P1, while only a 1.2% increase was observed in the LG group during P2. Previous research in humans investigating weight gain in lean, sedentary individuals suggests a 10% increase in
BW results in decreases in fasting insulin concentrations (Kolaczynski, Ohannesian et al. 1996). Though true IS was not assessed by Kolaczynski, results lend some support to the lack of significant decreases in IS observed in the LG treatment group, where BW increases were less than 10% of initial weight. However, results from the Kolaczynski study do not support findings in the GL treatment where BW of horses increased by more than 10% of initial weight, suggesting threshold weight gain (that results in altered IS) may be species specific.

Periods of weight gain in the current study lasted 130 days (approximately 4 months) and it is possible there is an additive effect of time and obesity on insulin and glucose dynamics, such that sustained adiposity for longer durations (> 130 days) are necessary for decreases in IS to occur. For example, Biourge and colleagues found IS decreased in mature cats after 9 ± 2 months of weight gain equating to a 30% increase from initial weight (Biourge, Nelson et al. 1997). Another study in cats observed marked decreases in IS in animals that had gained approximately 44% of initial BW over a period of 9 to 12 months (Appleton, Rand et al. 2001). However, the aforementioned studies indicate changes in IS following active and extreme body weight gain. These studies do not indicate changes associated with smaller increases in adiposity and the subsequent maintenance of weight gain. It is possible that we did not see changes in IS given the relatively short periods of weight gain compared to previous investigations.

Digestible energy intake was initially designed to be fed as 70, 100, and 130% of
DEm (National Research Council 2007) for horses losing, maintaining, and gaining weight and body condition, respectively. Actual dietary intake, however, was fed at an average of 138% of DEm for horses in the GL treatment during P1, and at 80% of DEm during P2. Horses in the LG treatment were fed at 89% of DEm in P1 and 119% of DEm in P2. Increasing dietary energy intake from the initial goal of 70% to between 80 and 89% of DEm requirements in those horses actively losing weight was due to concerns over potential negative problems associated with lack of feed. Though significant body condition losses were observed in the LG treatment during P1 and in the GL treatment during P2 (both physiological states of weight loss), it is possible that the modified dietary energy restriction at 89% and 80% of DEm, respectively, was not extreme enough to elicit significant changes in insulin sensitivity. For example, Van Weyenberg et al fed ponies at 70% of DEm of initial BW, decreasing intake to 50% and 35% of DEm, over an 18-week period and found significant increases in IS with weight loss at 1% of ideal BW per week (Van Weyenberg, Hesta et al. 2008). The same study used ponies with BCS ≥ 8 prior to initiating dietary energy restrictions and reported a dramatic 4-unit decrease from initial BCS to 4 or 5 (Van Weyenberg, Hesta et al. 2008), while the current study only allowed for a 1.4-unit change in BCS in horses in the LG group during P1, and a 1.7-unit change in BCS for horses in the GL group during P2. Here BCS just prior to periods of caloric restriction were 5.0 and 6.5 for LG and GL treatments, respectively. Consequently, it is possible that insulin-sensitizing effects of body condition loss occur only if the horse is
already obese (BCS > 7, (Henneke, Potter et al. 1983)).

Additionally, it is likely insulin-sensitizing effects of body condition loss occur as a result of degree of loss rather than duration over which condition loss occurred. For example, previous studies in mature, neutered cats show rapid (6 wk) reversal of experimentally induced body weight gain (30% of original BW) to initial lean weights results in a complete normalization of IS (Biourge, Nelson et al. 1997). Similar results were found when Hoenig and coworkers reduced excess BW in obese cats by 50% over a longer 6-month period (Hoenig, Alexander et al. 2002). A study in obese ponies suggested IS may increase following as little as 5% loss of BW, though observed improvements in IS may have been confounded by increases in physical activity (Freestone, Beadle et al. 1992). In both the current study and previous work by Van Weyenberg (Van Weyenberg, Hesta et al. 2008), weight loss occurred over a similar 18-wk time period. In contrast to the overall 18% decrease in BW observed previously in ponies (Van Weyenberg, Hesta et al. 2008), results from the current study indicate only an 11% decrease in BW from day 130 in the GL group during P2, and a mere 2% drop in BW from day 0 in the LG group at the end of P1. This suggests improvements in IS due to body condition loss may be affected more by extent of weight loss rather than duration of weight loss, though more research is needed to substantiate these findings.

Furthermore, reductions in insulin sensitivity are associated with high glycemic-index feeds (i.e. diets rich in starch and sugar, (Hoffman, Boston et al. 2003; Pratt, Geor et
al. 2006)), while the present study fed only forage. It is possible that low glycemic-index feeds, such as forages, may not lead to reductions in insulin sensitivity even at greater degrees of body condition. For example, results of a modified FSIGT showed decreases in IS in horses supplemented with a high starch/sugar concentrate compared to both fat/fiber supplemented horses, and control horses maintained on pasture and hay alone (Hoffman, Boston et al. 2003). Hoffman and colleagues noted non-structural carbohydrate (NSC) content of the starch/sugar supplement was three times greater than the fat/fiber supplement at 46% and 14%, respectively (Hoffman, Boston et al. 2003). Though significant decreases in IS were observed in horses fed the high glycemic-index meals, effects of diet on IS may have been influenced by confounding factors such as body condition, where evaluated horses ranged in BCS from 5 to 8 and diets were fed to maintain their current condition. Other early inquires into hay versus hay and grain supplemented diets in healthy horses found horses supplemented with grain showed increases in both glucose and insulin responses to an intravenous glucose tolerance test (Garcia and Beech 1986). However, results from this study did not identify significant differences in plasma insulin-glucose ratios between treatment diets, nor were NSC contents of hay and grain reported. Additional studies in horses comparing supplementation with either high fat/fiber or starch/sugar concentrates have reported 30% (Pratt, Geor et al. 2006) and 37% (Treiber, Boston et al. 2005) decreases in IS in horses fed high starch/sugar supplements with 55% and 49% NSC (DM basis), respectively. In each
of the prior-mentioned studies horse did not differ in BCS, eliminating the confounding factor of body condition.

To date it appears that NSC content of high glycemic-index feeds in studies investigating the effects of diet on IS in horses are well above those which would normally appear in forages (Williams, Kronfeld et al. 2001; Hoffman, Boston et al. 2003; Pratt, Geor et al. 2006). The National Research Council reports forages (grass, mixed grass, legume, and Bermuda grass pasture and hay and alfalfa cubes) contain non-fibrous carbohydrates (NFC) at an average of 14% to 30.8% (DM basis), where the lowest and highest total NFC content is found in fresh Bermuda grass and legume hay, respectively (National Research Council 2007). The NFC component of feeds is comprised of carbohydrates including mono-, di-, and oligosaccharides and starch (National Research Council 2007). It is important to note that NFC and NSC are not interchangeable terms. However, given NSC are a component of NFC, interpretation of NFC composition of feedstuffs can give an indication of NSC values (i.e. the NSC component of a feed should always be lower than the reported NFC value). Laboratory evaluation of cube diets used in this study indicated NFC concentrations were approximately 29, 25, and 15% for High Energy, Bale-in-a-Bag, and Low Energy alfalfa-grass mix hay cubes, respectively, and 17% for fescue hay (all on DM basis). As such, though not calculated, we expect the NSC component of the diets is lower than the aforementioned concentrations. Recently, Hoffman and coworkers established a threshold level of NSC consumption. Regression analysis of glycemic
response in 8 horses fed oat/beet pulp meals in differing ratios to achieve NSC doses ranging from 0.6 to 2.0 g/kg BW, showed that NSC consumption above 0.296 g/kg BW altered glucose response (Hoffman, Haffner et al. 2009).

Leptin, synthesized and secreted by adipose tissue, is believed to function as a regulator of energy intake and body weight homeostasis (Friedman and Halaas 1998; Radin, Sharkey et al. 2009). Previous research in horses suggests a positive association between adiposity and circulating leptin, such that as body fat mass increases so does leptin (Kearns, McKeever et al. 2006). The present study found no significant correlation between leptin concentration and measures of adiposity. It is possible that though significant changes in BCS, BW and RFD were observed, these changes were not enough to elicit a significant effect on circulating leptin. For example, Buff and coworkers found a significant correlation between BCS and leptin concentration in study of 71 Quarter Horses (Buff, Dodds et al. 2002). However, upon evaluation of leptin concentration following a 14-week modification of caloric intake to induce changes in body condition, the authors did not observe significant changes in peripheral leptin concentration. The results were attributed to the lack of extreme change in BCS, where no animal changed more than 2 units (Buff, Dodds et al. 2002). This is similar to the current study in which animals in the GL treatment group underwent a maximum 2.1-unit change in BCS from days 0 to 65 in phase 1 (mean BCS of 4.8 at day 0 to 6.9 at day 65), while animals in the LG treatment gained only 1.9 units in BCS from day 130 to day 260 in phase 2 (mean BCS of 3.6 at day
130 to 5.5 at day 260). Additionally, horses losing weight and condition (LG treatment group in P1 and GL treatment group in P2) showed changes in adiposity equivalent to less than 2 units in BCS, where previous research in horses suggests a change of approximately 4 units in BCS significantly reduces circulating leptin concentration (Van Weyenberg, Hesta et al. 2008).

Previous research in horses has also indicated great variation associated with circulating leptin concentrations (Gentry, Thomspson et al. 2002; Frank, Elliott et al. 2006; Van Weyenberg, Hesta et al. 2008). For example, Van Weyenberg reported leptin concentrations in ponies with a BCS of 8 or 9 ranged from 7.81 to 34.97 ng/mL prior to weight loss, while concentrations after weight loss to BCS of 4 or 5 ranged from 2.49 to 5.04 ng/mL (Van Weyenberg, Hesta et al. 2008). Similar variability was observed by Gentry et al where horses with greater body condition could be grouped as high or low leptin (7-20 ng/mL and <5 ng/mL, respectively) (Gentry, Thomspson et al. 2002). In the current study, leptin concentrations across all groups were less than 6 ng/mL. It may be possible that the findings in the current study are the result of individual animal variation and less extreme changes in adiposity.

Interestingly, there was a strong positive association observed between leptin concentration and IS (r = 0.83, P = 0.01). Previous research suggests leptin exhibits insulin-sensitizing effects through the partitioning of fatty acids (FA) away from intra-muscular triglyceride storage and toward β-oxidation (Dyck, Heigenhauser et al. 2006). It
is believed this occurs via the activation of AMPK, which, ultimately results in decreased activity of the lipogenic enzyme, acetyl CoA carboxylase, and increased translocation of the insulin-sensitive glucose transporter, GLUT4. The result then is improved insulin-stimulated glucose disposal. The mechanisms behind leptin and IS have been demonstrated in rats (Yaspelkis, Ansari et al. 1999; Yaspelkis, Davis et al. 2001), though it they have not been identified in equines. While the current study found a significant correlation between leptin concentration and IS, results of statistical analysis failed to identify significant changes in either IS or leptin as a result of treatment or time effects. This suggests that, while the association between the two parameters (IS and circulating leptin) exists, the relationship may be more complex than initially thought, and may exist independent of changes in adiposity.

Another adipocytokine, adiponectin, has been recognized as an insulin sensitizing agent (Kadowaki and Yamauchi 2005; Radin, Sharkey et al. 2009). The results of the present study appear to support previous studies in horses in which adiponectin is inversely proportional to fat mass (Kearns, McKeever et al. 2006; Gordon, McKeever et al. 2007). For example, mean adiponectin concentration in the GL treatment was significantly lower at day 195 than at day 0 (P = 0.01), and tended to be lower than at day 65 (P = 0.07), when mean RFD and BCS were near or at their maximum (RFD = 7.7 cm and BCS = 6.9). Interestingly, in the GL treatment group, this drop in adiponectin concentration (at day 195) appears after peak body condition is reached (at days 65 and 130), suggesting a
delayed effect of increased adiposity.

Additional results indicate a strong time effect on adiponectin within the control group, such that mean adiponectin decreased throughout the duration of the study with the greatest differences at day 0 (late summer) versus day 260 (late winter/early spring). Previous research in horse and ponies suggest that seasonal variations in other hormones, such as cortisol (Donaldson, McDonnell et al. 2005), exist, though, to our knowledge, none have looked specifically at adiponectin. Other studies suggest seasonal effects on adiponectin in hibernating mammals, with adiponectin expression increasing in summer months, and falling in late fall and winter (Florant, Porst et al. 2004). This evidence is consistent with the function of adiponectin as a hepatic insulin sensitizer during summer months when animals are eating. Our findings support this theory and suggest there may be an unknown adaptive metabolic process occurring as these horses transition from a season of sufficient feed (summer) to what would be a season of limited feed (fall and winter months) in their natural environment. It is necessary to investigate these circannual dynamics further, particularly to determine if in fact circulating adiponectin peaks during spring and summer months.

Currently there is no recommended ideal level of adiposity for insulin sensitivity in horses. Results of this study did not show significant changes in insulin sensitivity, or circulating leptin concentration, associated with changes in adiposity. Subsequent studies need to achieve a greater degree of body condition loss or gain, perhaps over longer
durations, with the possible maintenance of these conditions, to investigate these relationships further. Interestingly, there was a strong, positive relationship found between leptin and IS, which further supports the need for subsequent research to investigate the dynamics and nature of this relationship under extreme body conditions. The current study also found a significant effect of time on adiponectin, indicating a possible seasonal dynamic. Future research needs to address the possibility of circannual regulation, as well as individual animal variation, in these adipocytokines to validate these results. Overall, our results indicate the differences in adiposity achieved were not enough to elicit the alterations in IS seen in other studies. However, it does support the notion that moderate changes in adiposity in horses fed forage-only diets should not increase their risk of developing problems associated with metabolic disturbance. Subsequent research efforts are needed to identify mechanisms behind the regulation of insulin sensitivity, leptin, and adiponectin and their application to individual animal populations.
Table 2.1. Dietary analysis of feeds. All values represented on dry matter basis.

<table>
<thead>
<tr>
<th>Feed Source</th>
<th>High Energy Cubes*</th>
<th>Low Energy Cubes*</th>
<th>Bale-in-a-Bag Cubes*</th>
<th>Tall Fescue Hay**</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (MCal/kg)</td>
<td>2.1</td>
<td>1.7</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>%CP</td>
<td>15.5</td>
<td>15.4</td>
<td>14.1</td>
<td>10.5</td>
</tr>
<tr>
<td>%NFC(^a)</td>
<td>29.0</td>
<td>14.6</td>
<td>25.2</td>
<td>17.0</td>
</tr>
</tbody>
</table>

CP, crude protein; DE, digestible energy; NFC, non-fibrous carbohydrate.

* Idle Acres Farms, Cokato, MN

** Novel-endophyte variety (MaxQ®, Pennington Seed, Madison, GA)

\(^a\) %NFC = 100 – (%CP + %NDF + %EE + %Ash)
Table 2.2. Mean dietary intake as percentage of DE maintenance requirements and ADWC during Phase 1 and Phase 2. Data presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>GL</th>
<th>Control</th>
<th>LG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEm (d0–65)</td>
<td>152.7 ± 3.2*</td>
<td>124.2 ± 2.5</td>
<td>88.5 ± 2.8*</td>
</tr>
<tr>
<td>DEm (d65–130)</td>
<td>122.7 ± 4.0*</td>
<td>106.4 ± 3.1</td>
<td>89.0 ± 3.5*</td>
</tr>
<tr>
<td>ADWC (kg/d)</td>
<td>0.45 ± 0.07*</td>
<td>0.12 ± 0.05</td>
<td>-0.08 ± 0.06 †</td>
</tr>
<tr>
<td><strong>Phase 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEm (d130–195)</td>
<td>76.3 ± 1.5*</td>
<td>100.2 ± 0.4</td>
<td>123.3 ± 1.3*</td>
</tr>
<tr>
<td>DEm (d195–260)</td>
<td>82.7 ± 3.1*</td>
<td>102.6 ± 2.4</td>
<td>115.5 ± 2.7*</td>
</tr>
<tr>
<td>ADWC (kg/d)</td>
<td>-0.53 ± 0.09*</td>
<td>-0.01 ± 0.07</td>
<td>0.15 ± 0.08</td>
</tr>
</tbody>
</table>

ADWC, average daily weight change; DEm, dietary intake as a percentage of digestible energy requirements for maintenance. * P < 0.05 vs control, † P ≤ 0.10 vs control.
Table 2.3. Mean BW, BCS, RFD, AFD, IS, leptin, and adiponectin for horses in the GL, LG, and control groups at day 0, 130, and 260. Horses in the GL treatment group (n = 3) were gaining weight during P1 and losing weight during P2, while horses in the LG treatment (n = 4) were losing weight in P1 and gaining weight in P2. Data are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>GL</th>
<th>Control</th>
<th>LG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D130</td>
<td>D260</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>495.7 ± 15.1</td>
<td>553.3 ± 15.1</td>
<td>493.0 ± 15.1</td>
</tr>
<tr>
<td>BCS</td>
<td>4.8 ± 0.3</td>
<td>6.5 ± 0.3</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>RFD (cm)</td>
<td>4.2 ± 0.8</td>
<td>8.3 ± 0.8</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>AFD (cm)</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>IS (µg/min/kg per µU/mL)</td>
<td>22.5 ± 9.5</td>
<td>31.7 ± 9.5</td>
<td>23.5 ± 9.5</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>1.2 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>6771.2 ± 1350.5</td>
<td>5224.1 ± 1350.5</td>
<td>4709.0 ± 1350.5</td>
</tr>
</tbody>
</table>

AFD, abdominal fat depth; BCS, body condition score; BW, body weight; GL, gain to lose treatment; IS, insulin sensitivity; LG, lose to gain treatment; RFD, rump fat depth.
<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>Leptin</th>
<th>Adipo</th>
<th>BCS</th>
<th>BW</th>
<th>RFD</th>
<th>AFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>--</td>
<td>0.83**</td>
<td>0.35</td>
<td>0.06</td>
<td>-0.40</td>
<td>0.22</td>
<td>-0.17</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.83**</td>
<td>--</td>
<td>0.05</td>
<td>-0.11</td>
<td>-0.38</td>
<td>-0.05</td>
<td>-0.16</td>
</tr>
<tr>
<td>Adipo</td>
<td>0.35</td>
<td>0.05</td>
<td>--</td>
<td>-0.08</td>
<td>0.05</td>
<td>0.35</td>
<td>0.47</td>
</tr>
<tr>
<td>BCS</td>
<td>0.06</td>
<td>-0.11</td>
<td>-0.08</td>
<td>--</td>
<td>0.43</td>
<td>0.84*</td>
<td>0.54</td>
</tr>
<tr>
<td>BW</td>
<td>-0.40</td>
<td>-0.38</td>
<td>0.05</td>
<td>0.43</td>
<td>--</td>
<td>0.39</td>
<td>0.43</td>
</tr>
<tr>
<td>RFD</td>
<td>0.22</td>
<td>-0.05</td>
<td>0.35</td>
<td>0.84*</td>
<td>0.39</td>
<td>--</td>
<td>0.75**</td>
</tr>
<tr>
<td>AFD</td>
<td>-0.17</td>
<td>-0.16</td>
<td>0.47</td>
<td>0.54</td>
<td>0.43</td>
<td>0.75**</td>
<td>--</td>
</tr>
</tbody>
</table>

Adipo, adiponectin; AFD, abdominal fat depth; BCS, body condition score; BW, body weight; IS, insulin sensitivity; RFD, rump fat depth. * P < 0.01, ** P < 0.01.
Figure 2.1. Schematic of experimental design. Phase 1 (P1) of the study in which treatment groups were gaining (BCS 5 to 7), maintaining (BCS 5 to 5), or losing (BCS 5 to 3) weight and condition is shown. Phase 2, with reversal of treatment groups to return to BCS of 5, is also shown. Within individual phases, times (T1, T2, and T3) are noted under day, denoting the switch-back design. Experimental treatment groups (gain to lose, GL; control, C; and lose to gain, LG) are also indicated. Yellow arrows indicate collection times, during which body condition score (BCS) and rump and abdominal fat depth (RFD and AFD, respectively) were assessed; and insulin sensitivity (IS) assessment via the euglycemic-hyperinsulinemic clamp (EHC), blood serum samples for RIA analysis of leptin and adiponectin were collected, and muscle and adipose tissue biopsies were performed.
Figure 2.2. Photograph of locations of RFD and AFD. Locations for rump fat depth (RFD, triangle) and abdominal fat depth (AFD, between dotted lines) ultrasounds are indicated by clipped squares on the horse.
Figure 2.3. Body weight (BW) and BCS in GL (A), control (B), and LG (C) treatment groups, and treatment x time interaction effects (D and E). In figures A through C mean BW is indicated by the teal color, while BCS is indicated by the pink color. Treatment x time interaction effects at times 1, 2, and 3 (T1, T2, and T3, respectively) for the three physiological states are shown for BCS (D) and BW (E). In figures D and E, horses actively gaining weight (in a positive energy balance) are indicated by the blue lines, while horses losing weight (in a negative energy balance) are indicated by the red lines. Horses maintaining weight are indicated by the purple lines. Data shown as mean ± SEM. Figures A-C, paired letters (ex. a vs. b, c vs. d, etc) within a parameter indicate P < 0.05. Figures D-F, paired letters across physiological states, and within time, indicate P < 0.05.
Figure 2.4. Insulin sensitivity in GL (A), control (B), and LG (C) treatment groups, as well as treatment x time interaction effects (D). In figure D, treatment x time interaction effects for the three physiological states at times 1, 2, and 3 (T1, T2, and T3, respectively) are shown. Horses actively gaining weight (in a positive energy balance) are indicated by the blue lines, and horses losing weight (in a negative energy balance) are indicated by the red lines. Horses maintaining weight are indicated by the purple lines. Data shown as mean ± SEM. **No significant differences.
Figure 2.5. Mean adipocytokines (leptin and adiponectin) and rump fat depth (RFD) for GL (A), control (B), and LG (C) treatment groups, as well as treatment x time interaction effects (D – F). In figures A through C mean leptin concentration is indicated by the yellow color, while adiponectin is indicated by the red color. Rump fat depth is indicated by the green color. *Adiponectin is shown at 1:1000 dilution factor in figures A through C. Treatment x time interaction effects at times 1, 2, and 3 (T1, T2, T3, respectively) for the three physiological states are shown for leptin (D), adiponectin (E), and RFD (F). In figures D through F, horses actively gaining weight (in a positive energy balance) are indicated by the blue lines, while horses losing weight (in a negative energy balance) are indicated by the red lines. Horses maintaining weight are indicated by the purple lines. Data is displayed as mean ± SEM. Figures A-C, paired letters (ex. a vs. b, c vs. d, etc) within a parameter indicate P < 0.05. Figures D-F, paired letters across physiological states, and within time, indicate P < 0.05.
References


