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Relationships of Body Condition, Blood Glucose and Insulin Concentration of Grazing Horses

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RELATIONSHIPS OF BODY CONDITION, BLOOD GLUCOSE AND INSULIN CONCENTRATION OF GRAZING HORSES

A Thesis
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In Partial Fulfillment
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Master of Science

By
Ashley E. Monfort
May 2007
RELATIONSHIPS OF BODY CONDITION, BLOOD GLUCOSE AND INSULIN CONCENTRATION OF GRAZING HORSES

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RELATIONSHIPS OF BODY CONDITION, BLOOD GLUCOSE AND INSULIN CONCENTRATION OF GRAZING HORSES

Ashley Monfort May, 2007 39 pages

Directed by: Charles E. Anderson, Elmer Gray, and Jenks Britt

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A recent study has reported that blood glucose levels and founder in horses consuming forage/concentrate mixed diets are positively correlated (Pass et al., 1998). Other studies have reported body fat and insulin resistance are also positively correlated in horses and humans (Hoffman et al., 2003; Boshell et al., 1968; DeFonzo et al, 1991; Kahn et al., 2000). Few studies have monitored these relationships in horses consuming forage only diets, even though the incidence of grass founder is quite high in obese horses and ponies.

Four thin, four moderate, and four obese horses were grouped two mares and two geldings per group in a completely randomized design. They were allowed six months to adapt to an all forage diet consisting of free choice access to a mixed grass pasture. The pasture consisted of primarily Fescue with limited additional quantities of Bermuda Grass and Blue Grass. Following the adaptation period, blood samples were collected at four-hour intervals, during a twelve-hour grazing period to determine effects of body condition, sex, and time of sampling on blood insulin and glucose concentration.

Blood glucose analysis was done utilizing the Ultrasmart monitor. Accuracy verification was obtained by dual sample analysis with the Vetest 8008 animal glucose...
monitor. Results indicate the Ultrasmart monitor is a highly accurate and effective method of analyzing blood glucose concentrations in the horse.

No significant blood glucose variation due to time of sampling, sex, or body condition was observed. Sex of animal and time of sampling had no significant effect on circulating insulin concentration. Mean blood glucose concentration for all horses consuming the pasture diet was 77 mg/dl. Results also indicated that the mean normal blood glucose concentration of horses consuming this grass pasture diet was similar to the mean of 74.7mg/dl reported for horses consuming forage/concentrate mixed diets (Williams et al., 2001). Blood insulin concentrations of the moderate horses were not different from those of the thin or fat horses. They were however significantly lower, in the thin horses than the obese horses. Therefore, fatter horses secreted greater quantities of insulin while maintaining normal blood glucose levels.

In a second study, Glycosylated Hemoglobin (HBA1c) measurements of the aforementioned horses using a human blood meter was attempted. The technique was not effective for measuring horse blood and was abandoned. Further research in this area may provide an effective mechanism for evaluating long term blood glucose and insulin chemistry.
Chapter 1

INTRODUCTION

In its natural habitat, the horse was a roaming animal surviving on an all forage diet and was successful due to the constant grazing pattern that was always available. Upon domestication, man gradually decreased the amount of time allowed to graze on pasture, and substituted stall confinement, which introduced periodic intake of food. Stall confinement proved to be time consuming and costly. Stall confinement seemed to be more beneficial for the owner but deficient in meeting the caloric intake needs of the horse. In order to maintain the feeding frequency, grain was introduced to increase caloric intake, which, in turn, resulted in metabolic changes.

Digestive conditions and metabolic changes have precipitated a frenzy of research pursuits involving feed types and their impact on metabolism. Studies have been conducted on insulin and glucose in horses being fed different types of diets (Stull and Roediek, 1988, 1994). These diets were studied to determine their effects on metabolic changes. The high starch corn diet precipitated a higher spike in insulin during the onset of exercise when compared to the alfalfa hay diet.

The two most common equine diets are all forage and mixed grain/forage diet fed twice daily. Williams, et al. (2001) agreed that horses on the diets supplemented with grain have higher spikes of glucose and insulin directly after ingestion when compared with horses consuming diets filled with fiber.

Research also supports the fact fatter horses have higher insulin and glucose spikes after feed consumption (Johnson et al., 2004). Obesity has also been validated as a
causative factor in the development of type II diabetes and insulin resistance in humans. (Defonzo et al., 1991; Boshell et al., 1968; Kahn et al., 2000)

Limited research has focused on the relationships between fat, blood chemistry, and physiology of horses consuming an all forage diet. It has been widely accepted that fatter horses have a tendency to founder more frequently than thin horses. Harris et al. (2006) suggested that a preventative measure for founder was to limit the factors associated with the development of insulin resistance. Research has suggested that these variables of obesity, excessive glucose intake, insulin resistance, and type II diabetes may be dependent on one another but further studies are needed to validate these relationships.

It has been widely recognized that founder occurs most frequently when horses have free choice access to lush pasture. Hintz (2000) reported that in the spring the incidence of laminitis is at 6% while in the winter it is only about 2% in the Northeast, Western, and Central Regions of the US. Founder has also been known to occur with ingestion of excessive quantities of grain, which are high in starch. It has been theorized that extreme fluctuations of blood glucose and insulin may cause founder (Pass et al. 1998).

Glycosylated hemoglobin (HBA1c) is a well established method of determining long term blood glucose concentrations in diabetic patients (Koenig et al., 1976). HB1Ac accurately measures abnormal hemoglobin formation due to elevated blood glucose levels in humans during the 90 days preceding the test. Therefore, it is an excellent indicator of elevated glucose concentrations in type II diabetics and perhaps in horses. The HB1Ac human meter has not been validated for use in testing horse blood but would
allow veterinarians to easily and economically monitor long term fluctuations in blood glucose of the horse if it could be validated.
Chapter 2

LITERATURE REVIEW

Insulin and Glucose in Non Diabetics vs. Diabetics

Many studies have shown a positive correlation between ingestion of food and increased circulating glucose concentrations, in humans. (Sinha et al., 1996; Shen, 1970). Sinha et al. (1996) compared blood glucose and insulin in individuals of different size and different health states. The study compared lean, obese and type II diabetic human subjects. They defined obesity using the body mass index (BMI). Male subjects having a BMI of 27.3 and female subjects possessing a 27.8 or greater were classified as obese. Blood glucose was found to average 100.4, 103.3, and 196.1mg/dl, respectively, for the thin, obese and, type II diabetics consuming identical diets. Blood insulin concentrations were 14.4, 25.6, and 30.7μU/mL for the thin, obese, and diabetics, respectively. Shen et al. (1970) reported similar results and found that glucose levels were 52% higher in diabetics than non-diabetics. These studies imply that diabetic subjects displayed substantially higher levels of blood glucose and insulin than fat “normal” humans and that obese individuals produced greater blood glucose and insulin concentrations than thin subjects. These studies verify that individuals with type II diabetes and obese “normal” humans appear to exhibit insulin resistance.

The relationship between obesity and susceptibility to type II diabetes and susceptibility to insulin resistance has been widely studied in humans (Boshell et al., 1968; DeFonzo et al., 1991; Kahn et al., 2000). Boshell et al. (1968) indicated 37.75% of the normal weight group and 59.30% of the obese subjects displayed above normal blood glucose levels through glucose tolerance tests. They evaluated five patients after infusion
of glucose: three normal weight non-diabetics, and two obese diabetics, and discovered a
difference between blood glucose and insulin between the two groups. Glucose averaged
176mg/dl for non-diabetics, and 321mg/dl for the obese diabetic group. Insulin averages
were 131μU/mL and 555 μU/mL for the normal, and the obese groups; respectively. The
results of the study validate the fact that diabetics can have extreme blood glucose and
insulin levels. They evaluated weight loss and its effect on insulin and glucose by
comparing insulin and glucose changes in obese non-diabetics and obese diabetics. They
found through glucose tolerance tests, that when adipose cell size decreases from weight
loss, insulin sensitivity increased, and glucose levels dropped, showing that insulin
resistance declined with loss of excess weight. (Boshell, 1968). These results led to
questions about the interrelationships between the effects of obesity on blood chemistry
and metabolic disorders in animals and humans.

**Insulin Resistance**

Insulin resistance and type II diabetes have been extensively studied in humans.
This is due to the disease being so prevalent and the fact that it can often be prevented or
its severity decreased through a proper balance of exercise, weight, and diet control. It is
estimated that by the year 2025, 300 million people will be diagnosed with diabetes.
(WHO, 1998). Biddinger and Kahn, 2006 reported 27% of adults in the United States
have an insulin resistant syndrome, and up to 50% of severely overweight children
exhibit insulin resistance. Insulin resistance occurs when normal concentrations of insulin
produce a less than normal biological response (decline in blood glucose concentration)
(Kahn, 1978). Reaven (1988) found that type II diabetes occurs when excess insulin is
constantly circulating through the blood stream. These many studies have confirmed the
positive correlation that exists between excessive glucose intake, elevated blood glucose concentrations, obesity, insulin resistance and type II diabetes in humans.

Normally, when food is consumed, glucose rapidly enters the blood stream resulting in a spike in circulating glucose levels. In response, the pancreas is stimulated to secrete additional insulin, which in turn acts to drive glucose into the skeletal muscle cells and therefore lower blood glucose levels. A rise in the level of glucose should be followed by a rise in the level of insulin permitting the glucose to be processed and blood glucose concentration to quickly return to and remain at normal levels. This mechanism regulates the amount of free glucose and insulin in the blood stream at any given time. Ogata et al. (2007) studied this feedback mechanism in diabetics and non-diabetics by monitoring their blood glucose continuously throughout the day. They concluded that the constantly elevated blood glucose levels exhibited by diabetic patients was due to an impaired negative feedback regulation. If this mechanism is disturbed, the animal may become insulin resistant. When glucose is circulating in the blood at high rates, insulin is also released at high rates, in order to reduce the amount of glucose to a normal level. The insulin overload reduces sensitivity of insulin yielding insulin resistance. When this occurs the glucose will not be utilized by the skeletal muscle cells, resulting in excess blood glucose and insulin circulating in the blood (Reaven 1988). Insulin resistance has been linked with many harmful health conditions, including obesity (DeFonzo et al., 1991; Seidell, 2000). Akiyana et al. (1996) found that rats fed twice the calorie intake of the control rat diets gained twice as much weight as the control group during the 27-day feeding period. Rats fed a higher caloric diet were diagnosed as insulin resistant through intragastric glucose loading tests. This study implies that excess calories will increase
blood glucose concentrations circulating in the blood stream and leads to insulin resistance.

Numerous studies have examined possible causes of the onset of insulin resistance and type II diabetes. A major study by Martin et al. (1992) found that the development of type II diabetes was detected before onset occurs, by testing insulin-dependent and insulin-independent glucose uptake; dysfunctions in these mechanisms indicate that the person is a prime candidate for type II diabetes. One side effect of type II diabetes is inflammation. Festa et al. (2000) study compared body fat, inflammation, and insulin sensitivity in insulin resistant individuals. One third of those tested had impaired glucose tolerance, indicating that there is chronic sub clinical inflammation present, as part of insulin resistance. Rossi et al. (1998), evaluated cutaneous blood flow in type I diabetics with an average HBA1c of 8.3%. HBA1c, Patients who had been diagnosed with diabetes for more than eight years suffered with vasoconstriction, resulting in limb amputations. Vasoconstriction seen in humans prior to limb amputations may be similar to the mechanism that causes vasoconstriction in limbs of animals that have the condition founder.

Insulin resistance in humans has been associated with other illnesses such as cardiovascular disease and conditions such as obesity. More recent research findings have resulted in recommendations to eat a diet high in complex carbohydrates and fiber (Anderson et al., 2004; Segasothy and Phillips, 1999), and eating foods with low glycemic index reduces insulin and blood glucose levels (Anderson et al., 2004) in order to prevent the onset of type II diabetes. Insulin resistance can be controlled or reversed by modification of lifestyle to reduce waist circumference (Seidell, 2000). In an overview of
many diabetic studies Reaven (2005) stated that many studies indicate that a moderate amount of weight loss can decrease insulin resistance and improve insulin sensitivity, thereby decreasing the chances of type II diabetes.

**HBA1c How It Works**

Glycoslated hemoglobin is an accurate method of determining the average blood glucose concentrations in humans during an extended two to three month period. As glucose enters red blood cells hemoglobin becomes glycated. Glucose is held for the life span of the red blood cell. The life span of red blood cells are approximately 120 days. The more glucose that is present in the blood, the more glycated the hemoglobin becomes. Consistently higher blood glucose levels lead to higher amounts of abnormal cells therefore giving higher HBA1c readings. McDonald and Davis (1979) found that the use of the fractions of hemoglobin, primarily HBA1c, could be used to monitor long term blood sugar.

The normal HBA1c reading for a non-diabetic adult human, the average is five percent; humans displaying values above seven percent are classified as diabetics, and uncontrolled diabetics may range up to 13% (Koenig, 1976). Koenig et al. (1976) discovered a correlation between the percentage of HBA1c and range of blood glucose levels. They found that diabetic patients averaged 343mg/dl, five times normal, blood glucose levels and an HBA1c of 9.8% before treatment. After insulin treatment they exhibited a blood glucose level of 84mg/dl as well as a drop in the HBA1C to 5.8%. He created a standard for HBA1C levels and the blood glucose values that correlate with those percentages. Koenig also found that there were no differences in the readings between test subjects that were prescribed medication to control their condition and those
who had received no treatment. Ferrell et al. (1984) found that there were no significant HBA1c differences based on the length of time that the test subject had been ill. Bunn et al. (1976) found that the C component of hemoglobin had a life span of approximately 120 days indicating that the test results were valid indicators for a three-month period. The enzyme immunoassay test was studied in the 1990’s, to evaluate how it would fare as an HBA1c monitor. Using immunoassay, John et al. (1993), found that the HBA1c in diabetics was significantly higher than that of non-diabetics. Diabetics showed an average of 6.86%, while non-diabetics showed an average low of 3.46%. This information is useful in scrutinizing how well blood glucose levels have been moderated by patients controlling their medication and glucose intake.

The Inview HBA1c meter is currently validated only for use in humans but may be a reliable diagnostic tool in monitoring diabetes control in the animal if validated. The Inview HBA1c monitor has been proven to be 99% accurate, when used properly to test human blood. The monitor is certified by the National Glycohemoglobin Standardization Program. HBA1c monitors have proven to be an inexpensive and easy method to measure long term blood sugar control. This benefit has allowed medical doctors to more accurately check a patient’s glucose monitoring skills and adapt treatment programs accordingly.

**Equine Digestive System**

The horse digestive system is suitable for grazing. When horses were roaming grazing animals, they likely experienced fewer metabolic health problems than they do today. The constant intake of food allowed for continuous levels of blood glucose levels and decreases the amount of peaks and valleys that may cause metabolic disorders.
Humans have changed the normal roaming and grazing process. The horse is no longer an all pasture animal but is primarily “stalled”. The horse was periodically confined and its rhythms of feeding changed from continuous grazing to being subjected to interval feeding. This change in feeding methods has caused changes in the digestive tract response to slug feeding, resulting in problems such as ulcers and colic. Clarke et al. (1990) found that when an animal is fed twice daily, it increases the speed at which it ingests the food. This rapid intake triggers many quick responses in the gut, finally leading to great amounts of food being fermented quickly. The fast fermentation changes the normal gastrointestinal microflora. Clarke (1990) further stated that in a normal continuous feeding program is implemented, this system of quick responses were less likely to happen; therefore, lowering the chances of gastrointestinal upset. The type of feed also needs to be closely evaluated. Researchers at Ohio State evaluated different commonly used equine diets to determine whether the glycemic index of certain foods would alter endocrine hormones. Seven horses of normal body weight were subjected, to four test diets: corn, barley, oat groats or a diet of a 50% glucose solution. Blood was collected at thirty-minute intervals, for four hours after ingestion, to monitor glucose spikes. They found that all of the diets had a similar glycemic index. The glycemic index is a measure of effect of a given feed on blood glucose concentrations after ingestion. Low glycemic index foods produce little changes in blood glucose concentrations than high ranked foods. The glycemic index is very important because it can be used to moderate spikes in glucose, lowering the occurrence of illnesses related to metabolic changes. (Jose-Cunilleras, 2004).
Equine Body Condition Score

Henneke et al. (1983) derived a numerical scoring system to evaluate body condition in horses. The numerical system ranged from one to nine. Horses were scored by palpation and visualization of six external anatomical sites. Differences in fat on the neck, shoulders, tail head, withers, ribs, and loin were used to determine the numerical score. A score of one denotes an animal with the bone structure easily visible, hipbones protruding, and ribs easily visible. Moderately fleshy horses score a five with their shoulders and neck blend in smoothly, ribs cannot be seen but were palpable, back was level and soft fat over tail head. Extremely fat horses receive a score of nine these horses have bulging fat over the neck, tail head, behind the shoulder, and the withers, along with ribs that were not palpable and an obvious crease down the back.

Insulin and Glucose Levels in the Non Insulin Resistant Horse

Stull et al. (1988) studied different types of regular equine diets in order to determine their effects on the digestive tract as well as their effects on endocrine hormones. In this study, animals were allowed to eat one of four common equine diets. These diets consisted of 100% alfalfa, 50% corn with 50% alfalfa, 100% corn, and 90% corn with 10% corn oil. Their findings indicated that diets of alfalfa and the 90% corn and 10% corn oil gave results comparable to the basal glucose level of 94.2 mg/dl. Diets consisting of corn and corn with alfalfa produce significantly higher glucose concentrations (142.6mg/dl) at the highest peak. Stull and her research team also found that insulin concentrations differed between the four diets being studied. In comparison to the basal insulin (4.7 μU/mL) Stull’s findings indicated that the alfalfa and the corn diet were twelve times higher. Corn alone produced approximately seven times higher than
the basal amount. The alfalfa diet and the corn/corn oil diet produced slightly higher than the basal amount, but were not significantly different from pre-feeding levels.

Williams et al. (2001) used baseline values of 74.7 mg/dl for blood glucose, and 5.86 μU/mL for insulin in mares being fed a pelleted common feed, a starch filled diet, or a fiber filled diet, to determine the changes in insulin and glucose after parturition. The starch and sugar diet yielded higher glucose and insulin levels than diets filled with fiber and fat. Studies have also been conducted on glucose and insulin levels on a forage diet supplemented with grain.

Arana et al. (1989) compared changes in insulin and glucose when feeding barley, corn, oats, and sweet feed. They also compared frequently fed alfalfa hay in different forms, including cubed, chopped, long, or pelleted. The test subjects' pre-feeding averages were 93 mg/dl for glucose and 3 μU/mL for insulin. When comparing hay diets to the grain diets, the grain diets produced an average of 20 mg/dl higher blood glucose than that of the hay diets.

**Horse Insulin Resistance**

Research has been more recently focused on the effects of insulin resistance in the horse and its connection with founder, obesity, and other metabolic disorders. Insulin resistance in the horse is defined as insulin insensitivity at the cell surface, which regulates glucose available inside the cell, or from insulin ineffectiveness due to disruption of glucose metabolism in the cell (Kronfeld, 2005). Johnson (2004) reported that feeding high glycemic index rations, such as corn, during long periods of physical inactivity promoted the development of obesity.
Hoffman (2003) conducted studies to chart insulin and glucose variations based on differences such as diet, size, and breed of the horse. Using thoroughbred geldings, he studied obesity and diet, two of the main factors thought to be connected to insulin resistance and found that obese horses rely on glucose mediated disposal of glucose. Insulin sensitivity was lower in obese horses when compared to thin horses. Grain based diets were also found to decrease insulin sensitivity in horses.

Lowering the body condition score of an animal, while feeding the animal a diet lower in starch, would decrease chances that the animal would become insulin resistant. Frank et al. (2006) compared obese horses diagnosed with insulin resistance with moderately conditioned healthy horses. Researchers compared glucose, insulin, and lipid concentrations. Resting insulin levels were as much as twelve times higher in the horses in the insulin resistant group; indicating that body fat and circulating insulin levels were positively correlated with insulin resistance. Vick et al. (2007) found that insulin sensitivity decreased as percentage of fat increased. They also recognized that inflammatory factors in the horse increased as obesity increased, therefore linking obesity insulin resistance, and inflammation in the hoof; otherwise known as laminitis.

Harris et al. (2006) summarized findings on laminitis, and developed steps to countermeasure laminitis as experienced by pasture-fed horses. Her recommendations included limiting insulin resistance, by replacing starch and sugar with fat and fiber, monitoring weight, and maintaining regular exercise. These are the same recommendations to decrease type II diabetes in the human. A second recommendation was to avoid intakes of rapidly fermenting material, and lowering changes in blood flow,
by having good pasture management, periodic grazing, adding certain types of forages like Timothy, and not allowing animals to graze on stubble.

**Founder or Laminitis**

The condition known as founder or laminitis results from an allergic reaction, which causes vasodilatation. Swelling results from vasoconstriction due to inflammation of the tissue and a lack of space for swelling provided by the hard outer covering of the hoof. This causes a lack of blood flow to the supportive tissue in the foot. If the vasoconstriction continues, the supportive tissues in the foot die, due to lack of blood flow. As these supportive structures die, the last bone of the horse’s foot known as the P3 starts to lose support and begins to rotate forward and down. This rotation can be slight but can also be drastic, sometimes rotating downward as much as 90 degrees. The rotation may result in the bone penetrating the sole of the hoof leaving the horse permanently damaged, and in many cases requiring euthanization. The likelihood of reoccurrence of founder increases after the animal has previously suffered from this condition (Kronfeld, 2006). Kane (2000) completed research illustrating how prevalent this condition is. He found that facilities housing three or more horses have had at least one case of lameness in the previous twelve-month period.

There is limited knowledge regarding the cause or cure of this condition, more research is being conducted exploring these two issues. There are many different theories that are currently being tested. It has been widely recognized that there are a few specific conditions, which precipitate the onset of founder: over grazing of lush pasture, excess ingestion of carbohydrate and stress are the primary conditions believed to increase with the onset of founder. Inflammation occurs with all three of these causes of founder. In
certain regions of the United States there were increased reports of lameness during the spring and summer months, as compared to the winter months, when pasture is not as readily available (Kane, 200). Hintz (2000) reported that of the total of lameness issues that were reported in his study, 45.6% of them were due to grazing lush pastures. Hoffman et al. (2001) found that fermentable carbohydrates were more prevalent in grains than in forages. A later study found fermentable carbohydrate pasture levels were higher in the spring, when founder is the most prevalent, than in the fall. The research suggested the two components, hydrolysable carbohydrates and fermentable carbohydrates, in the two feedstuffs, could be a possible cause of laminitis.

Pollit (1998) found that 16-40 hours following a carbohydrate overload, there was vasodilatation in hooves of horses marked as laminitis positive, as well as a rise in hoof temperature (Pollit and Davies, 1998) and Pass et al. (1998), found that hoof explants were dependent on glucose, possibly indicating that laminitis and intake and regulation of glucose may be related. Jeffcott et al. (1986) had suggested this same concept, but his findings were not as definitive. They found that laminitic ponies were more intolerant to glucose loading than non-laminitic ponies. He also discovered that fat and laminitic horses had a minimal response to insulin response tests, indicating that they may also be insulin resistant. Field and Jeffcott (2005) stated that decreased insulin effectiveness caused thromboxane A2 activity, which led to vasoconstriction.

**HB1Ac in the Horse**

HB1Ac testing in the horse would easily allow the owner or veterinarian to monitor long term trends in blood glucose concentration. The long-term blood glucose evaluation would allow comparisons of glucose over time allowing the early detection of
insulin resistance and perhaps conditions associated with it. When insulin fluctuations are detected early, insulin resistance may be prevented and more easily treated. The ease and the cost of the test would allow owners to be more proactive in their animal’s health. The lowering of insulin resistance and glucose fluctuations may prevent or treat founder.

**Objectives**

Objectives of the present study were to compare blood glucose and insulin concentrations in horses, with different body condition score, consuming an all pasture diet. Effects of time of sampling and sex were also tested.

In a side study, a human HB1AC meter was utilized in order to examine the accuracy in measuring abnormal hemoglobin formation, in the horse. The accuracy of the HB1Ac monitor was also evaluated in a cow and cat. Diabetic and non-diabetic dog’s blood was also analyzed with the HB1Ac monitor to validate accuracy.
Chapter 3

MATERIALS AND METHODS

The present study was conducted to determine the effect of body fat on blood glucose and insulin concentrations present in normal healthy horses consuming adlibitum quantities of mixed grass pasture. The study was conducted on the Western Kentucky University farm in Bowling Green, Kentucky. The blood collection for the insulin and glucose testing took place in August of 2006. All horses were property of Western Kentucky University. The horses had previously been used in the equitation program for the university. All horses were assigned an identification number that was kept throughout the entire study. All values reported as not significant or significant are at the p.05 level.

Horses

Twelve disease free, healthy, aged quarter horses ranging in age from six to twenty two, were given a six-month period to adapt to an all forage diet. The horses were separated into three test groups: fat, moderate, and thin using the body condition scoring system established by Henneke et al. (1983). Horses possessing a body condition score of two or three were classified as thin; those horses having a body condition score of four, five or six were classified as moderate; and horses having a condition score of seven or eight were classified as fat or obese. For humane reasons, horses with body condition scores of one, extremely thin, and nine, extremely fat, were not used. Each group was composed of two mares and two geldings. The test subjects were given free choice access to a 14.49 hectare mixed grass pasture before and throughout the duration of the study.
No forced exercise was administered during the three months prior to, and during the collection period.

**Pasture**

Prior to the study the pasture was treated with Weedmaster (dicamba), Milestone (aminopyralid), and Crossbow (triclopyr) to eradicate as many weeds as possible. The pasture had also been fertilized (N-80, P-46, K-60) to ensure proper growth. A test square was randomly thrown nine times in different areas of the pasture. Vegetative pasture cuttings were taken away from the fence line in areas with maximum growth and subjected to nutrient analysis. These results are presented in Table 1.

The pasture was open and contained no shade. The horses had access to clean, fresh water, and trace mineralized salt blocks.

**Blood Sampling**

Blood was drawn via veinapuncture using a 10cc syringe tipped with a 20 gauge needle. Samples were collected at 7h, 11h, 15h, and at 19h for one day following the adaptation period. Alternating sides of the neck were used with successive samples to eliminate stress and anxiety associated with veinapuncture. The blood sampling required less then five minutes per horse. The horses remained calm and were easily caught for subsequent collection periods. After sampling, horses were immediately released and allowed to continue grazing. Approximately ½ cc of blood sample was placed on an Accuview ultrasmart blood glucose meter test strip and analyzed to determine the whole blood glucose concentration of each sample. The remainder of each blood sample was placed into test tubes that were not treated with any type of anticoagulant. After the last collection the blood was immediately centrifuged for five minutes. After the blood had
been successfully separated, the serum was pipetted off and placed in a pre-marked test tube then frozen at -1° C. A total of 48 serum samples were analyzed.

**Sample Analysis**

1 cc of each serum sample was analyzed by the Cornell University Endocrinology Lab. The Diagnostic System Laboratories (Webster, Texas) radio immunoassay system, validated for use in the equine, was utilized to determine numerical insulin concentrations as per the techniques described by Eigenmann et al., (1984). The blood from the same collection was used to verify the accuracy of Accusmart with the Vettest 8008, a machine validated for producing accurate insulin results in the animal. The Vettest 8008 requires serum for glucose evaluation. Readings from the two machines varied slightly due to the different states of the blood. The Accusmart used fresh blood whereas the Vettest8008 used serum samples. Numerical values for glucose and insulin were analyzed using a completely randomized design for comparison of body condition, sex, and sampling time.

**Blood Sampling for HBA1c**

In order to test abnormal hemoglobin formation in these horses’, blood samples were subjected to analysis in the HB1Ac meter in an attempt to determine if any of the test subjects had long term elevated glucose concentrations. The A1CNOW meter, from Metrika (Sunnyvale, CA), was calibrated for human use only. When the whole blood was introduced to the meter, directly from the syringe, the monitor showed an error reading. In order to get an accurate response, blood was placed in test tubes treated with different types of anticoagulants. During the first test, the blood was placed directly in the meter. A second evaluation involved placing blood in the tube and waiting for thirty minutes. Validation testing on this particular meter was also conducted on regular and diabetic cats
and dogs. Blood was also collected from a heifer in order to assess if the blood could be analyzed by utilizing the human handheld machine used by humans. The blood from the horse, cow, and cat was never processed correctly.
RESULTS AND DISCUSSION

Each horse maintained the same body condition score and remained healthy throughout the duration of the study.

Glucose

Blood glucose concentrations for all the test animals are presented in Table 2. Glucose concentration ranged from 56 mg/dl to 94 mg/dl. Average glucose readings for thin, moderate and obese horses were 79, 75.9, 77.90 mg/dl; respectively. All these values were similar to those of non-diabetic humans (Shen, 1970; Sinha, 1996) and fell within the expected range for the normal adult horse. These findings indicate that differences associated within or between body condition groups were not significant. Normal blood glucose in the horse falls between 60-120 (Stull et al. 1988; Williams, 2001; Hoffman, 2003). Numbers above this level are considered abnormal. The values in the abnormal animal can be in excess of 300 mg/dl. Boshell (1968). The abnormal animal should be tested after ingestion of different feed types for a period of time to assess the changes in glucose and insulin levels. Based on the results from prior studies, obese horses would be expected to produce higher insulin than the thin horses. Thus, obese horses may be prime candidates for insulin resistance. In the present study, the obese group tended to have slightly higher blood glucose concentrations when compared to the horses that were in the moderately fleshy group or the thin group. Glucose levels among and between all test subjects however did not differ significantly. Analysis for variation due to the sex of the test subject yielded similar results. Mares had average blood glucose of 75.09 mg/dl and the geldings averaged 78.75 mg/dl. These values are presented in Table 3. There was no
significant difference between the glucose concentrations of the castrated males and females. Therefore, the sex of the animal did not affect circulating insulin and blood glucose levels in the grazing horse. It appears therefore female steroid hormones patterns have no effect on blood glucose and insulin concentrations.

Blood glucose concentrations were also evaluated to determine the effect of sample collection time. The collection time means were as follows: 7h- 74.67, 11h-75.75, 15h- 79.00, and 19h- 78.25mg/dl. Values are presented in Table 4. There were no significant differences due to collection times throughout the day. Thus continuous consumption of all pasture diets produces minimal fluctuations in blood glucose concentration in the adult horse. These data indicate that continuous grazing of grass pastures produce minimal blood glucose fluctuations in the adult horse, especially when compared with animals consuming grain supplemented diets.

Insulin

Insulin results ranged from 1.59uIU/ml to 27.22uIU/ml. When the insulin data were analyzed by sex, mares averaged 11.33 uIU/ml, while geldings averaged 9.13uIU/ml. Values are presented in Table 5. Averages fell so close that there was no significant difference in the level of insulin circulating in the blood stream between mares and geldings therefore insulin production was not affected by female steroid hormone interactions.

Collection time means were as follows: 7h- 9.91, 11h-7.46, 15h- 11.10, and 19h-12.47 uIU/ml. Values are presented in Table 6. Analysis showed no significant differences accredited to time of collection. It was noted that all horses were within the normal range for insulin after ingestion of food. Average insulin concentrations for thin, moderate, and
obese animals were 5.80, 11.10, 13.80 μIU/ml; respectively. These values are presented in Table 7. Blood insulin concentrations of the moderate group were not different from those of the thin or fat horses. Circulating insulin levels were however significantly lower, in the thin horses than the obese horses. Therefore, fatter horses secreted greater quantities of insulin in order to maintain normal blood glucose levels and higher body condition scores. There was no evidence of insulin resistance exhibited by these horses. The elevated insulin concentration associated with the obese horses is in agreement with reports by Hoffman (2003). These results also indicate that skeletal muscle cellular receptors of obese horses are being constantly bathed in excessively high levels of insulin. These horses may therefore be possibly predisposed to insulin resistance and therefore predisposed to metabolic disorders such as laminitis.

Our study indicates that horses exhibiting obese body condition scores should be carefully monitored when grazing grass pastures. Their weight and body condition should be kept in the moderate range in order to increase insulin sensitivity thereby, lowering the animal’s susceptibility to founder and other metabolic disorders.

HBA1c

Glycosylated hemoglobin values were not derived for horses in this study because the Inview A1c+ meter appears to have species specific accuracy. While the test accurately assesses HBA1c values in humans no values were derived for the horses, cow, or cat in this study and repeatability of derived dog values was questionable. If the technique for monitoring horse hemoglobin were to be perfected it would provide a cost effective tool for evaluating long term blood glucose concentrations in the horse. HBA1c tests might allow for early recognition of a predisposing laminitis factors and other
metabolic disorders associated with elevated blood glucose and insulin concentrations and insulin resistance.

Blood glucose analysis was done utilizing the Ultrasmart monitor. Accuracy verification was obtained by dual sample analysis with the Vetest 8008 animal glucose monitor. Results indicate the Ultrasmart monitor is a highly accurate and effective method of analyzing blood glucose concentrations in the horse.
Chapter 5

IMPLICATIONS

Blood glucose for all the test subjects fell within the normal range. The positive correlation between body condition score and blood insulin concentrations appears to be due to the fact that more insulin is being secreted by fat horses to maintain normal blood glucose levels than by thin horses. Obese horses may be more prone to insulin resistance over time and therefore should be closely monitored and placed on a reduced calorie diet and exercise regimen to return to a moderate body condition and increase insulin sensitivity.

It appears that moderately conditioned and even thin horses have more desirable blood chemistry profiles than obese horses. We do therefore recommend that horses be maintained at moderate body condition scores in order to moderate blood glucose and insulin concentrations.

The Ultrasmart monitor that is validated for humans accurately assessed blood glucose concentrations in the horse. Therefore these portable hand-held devices offer researchers, veterinarian, and horse owners a much cheaper, faster and equally accurate alternative for determining horse blood glucose concentration when compared with normal laboratory testing procedures.

Further testing may provide for development of an accurate HBA1c test for horse blood. Refinement of this technique would provide a valuable management tool for the early diagnosis of insulin resistance and therefore associated conditions in the horse.
Table 1

<table>
<thead>
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<th>Pasture Analysis of Air Dried Pasture Cuttings</th>
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</tr>
<tr>
<td>ADF</td>
<td>35.9</td>
</tr>
<tr>
<td>ADP</td>
<td>0.7</td>
</tr>
<tr>
<td>DM</td>
<td>90.1</td>
</tr>
<tr>
<td>NDF</td>
<td>59.7</td>
</tr>
<tr>
<td>AV Protein</td>
<td>20.7</td>
</tr>
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<td>DP Protein</td>
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<td>TDN</td>
<td>60.2</td>
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<td>Energy</td>
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<tr>
<td>Relative Feed Value</td>
<td>94.9</td>
</tr>
<tr>
<td>Phosphorus</td>
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<td>Calcium</td>
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<td>Potassium</td>
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</tr>
<tr>
<td>Magnesium</td>
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Table 2
Blood Glucose Concentrations of Horses with Different Body Condition Scores

<table>
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<th></th>
<th>THIN</th>
<th>MODERATE</th>
<th>FAT</th>
</tr>
</thead>
<tbody>
<tr>
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<td>GELDING</td>
<td>MARE</td>
</tr>
<tr>
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<td>68</td>
<td>84</td>
</tr>
<tr>
<td>H2</td>
<td>68</td>
<td>79</td>
<td>77</td>
</tr>
<tr>
<td>H3</td>
<td>79</td>
<td>73</td>
<td>84</td>
</tr>
<tr>
<td>H4</td>
<td>80</td>
<td>76</td>
<td>66</td>
</tr>
<tr>
<td>H5</td>
<td>82</td>
<td>82</td>
<td>88</td>
</tr>
<tr>
<td>H6</td>
<td>78</td>
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</tr>
<tr>
<td>H7</td>
<td>69</td>
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<td>81</td>
</tr>
<tr>
<td>H8</td>
<td>74</td>
<td>74</td>
<td>76</td>
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<td>80.25</td>
<td>72.63</td>
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<tr>
<td></td>
<td>76.94&lt;sub&gt;a&lt;/sub&gt;</td>
<td>75.88&lt;sub&gt;a&lt;/sub&gt;</td>
<td>75.88&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values with the same subscripts are not different (p>.05)
Table 3

Blood Glucose Concentrations of Grazing Horses Based on Gender of the Test Subject

<table>
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<tr>
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<th></th>
</tr>
</thead>
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<td>MODERATE</td>
<td>FAT</td>
<td>THIN</td>
</tr>
<tr>
<td>H1</td>
<td>H2</td>
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<td>H6</td>
<td>H9</td>
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<td>84</td>
<td>68</td>
<td>81</td>
</tr>
<tr>
<td>80</td>
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<td>81</td>
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<td>75.09(a)</td>
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</table>

Values with the same subscripts are not different (p > .05)

![Glucose Concentration Based on Gender](image)
Table 4
Blood Glucose Concentrations of Grazing Horses Based on Collection Time

<table>
<thead>
<tr>
<th>BCS</th>
<th>SEX</th>
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<th>11h</th>
<th>15h</th>
<th>19h</th>
</tr>
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<td>82</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2</td>
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<td>66</td>
<td>78</td>
<td>74</td>
</tr>
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<td>80</td>
<td>74</td>
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<td>H5</td>
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<td>66</td>
<td>88</td>
<td>81</td>
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<tr>
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<td>68</td>
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</tr>
<tr>
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<tr>
<td></td>
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<td>80</td>
</tr>
<tr>
<td>Fat</td>
<td>MARE</td>
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<td>85</td>
<td>77</td>
<td>81</td>
</tr>
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<td></td>
<td></td>
<td>H10</td>
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<td>H12</td>
<td>74</td>
<td>80</td>
<td>67</td>
<td>69</td>
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</tbody>
</table>

* Values with the same subscripts are not different (p>0.05)

Graph: Glucose Concentrations Based on time of Collection
- 7h
- 11h
- 15h
- 19h

Horse Identification
1 2 3 4 5 6 7 8 9 10 11 12
Glucose mg/dl
Table 5

Blood Insulin Concentrations of Grazing Horses due to Gender of the Test Subject

<table>
<thead>
<tr>
<th>MARES</th>
<th></th>
<th>GELDINGS</th>
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<th></th>
</tr>
</thead>
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<td>FAT</td>
<td>THIN</td>
<td>MODERATE</td>
</tr>
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<td>H5</td>
<td>H6</td>
<td>H9</td>
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<td>12.64</td>
</tr>
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<td>1.59</td>
<td>14.83</td>
<td>4.07</td>
<td>6.70</td>
</tr>
<tr>
<td>2.39</td>
<td>6.82</td>
<td>21.72</td>
<td>7.17</td>
<td>9.45</td>
</tr>
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<td>10.91</td>
<td>25.03</td>
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<td>7.49</td>
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<sup>a</sup>Values with the same subscripts are not different (p>.05)
Table 6

Blood Insulin Concentrations of Grazing Horses Based on Time of Collection

<table>
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<tr>
<th>BCS</th>
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<th>3pm</th>
<th>7pm</th>
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</thead>
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<td>3.51</td>
<td>2.39</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2</td>
<td>2.59</td>
<td>1.59</td>
<td>6.82</td>
<td>8.66</td>
</tr>
<tr>
<td></td>
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<td>5.94</td>
<td>9.63</td>
<td>7.90</td>
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<td>21.72</td>
<td>24.19</td>
</tr>
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<td></td>
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<td>4.07</td>
<td>7.17</td>
<td>10.91</td>
</tr>
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<td>11.97</td>
<td>7.96</td>
</tr>
<tr>
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<td>6.57</td>
<td>6.89</td>
<td>6.39</td>
</tr>
<tr>
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<td>MARE</td>
<td>H9</td>
<td>12.64</td>
<td>6.70</td>
<td>9.45</td>
<td>25.03</td>
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<tr>
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<td></td>
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</tbody>
</table>

\( ^a \) Values with the same subscripts are not different (p>0.05)

Insulin Concentration Fluctuations over Time

![Insulin Concentration Fluctuations over Time](image)
### Table 7
Blood Insulin Concentrations of Grazing Horses based on Body Condition Scores

<table>
<thead>
<tr>
<th>THIN MARE</th>
<th>MODERATE MARE</th>
<th>GELDING</th>
<th>FAT MARE</th>
<th>GELDING</th>
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<tbody>
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<td>H5 27.22</td>
<td>H6 7.56</td>
<td>H9 12.64</td>
<td>H11 14.77</td>
</tr>
<tr>
<td>H2 2.59</td>
<td>H7 6.66</td>
<td>H8 2.72</td>
<td>H10 14.77</td>
<td>H12 20.69</td>
</tr>
<tr>
<td>H3 7.31</td>
<td>H3 3.51</td>
<td>H4 7.37</td>
<td>H3 6.70</td>
<td>H4 2.18</td>
</tr>
<tr>
<td>H6 8.62</td>
<td>H7 24.19</td>
<td>H7 6.70</td>
<td>H7 9.45</td>
<td>H7 12.76</td>
</tr>
<tr>
<td>H7 7.90</td>
<td>H8 7.96</td>
<td>H8 11.97</td>
<td>H8 25.03</td>
<td>H8 23.99</td>
</tr>
<tr>
<td>H8 6.51</td>
<td>H9 6.39</td>
<td>H9 11.97</td>
<td>H9 25.03</td>
<td>H9 20.82</td>
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<tr>
<td>H9 2.39</td>
<td>H10 24.19</td>
<td>H10 7.96</td>
<td>H10 25.03</td>
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<td>H11 6.39</td>
<td>H11 25.03</td>
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<tr>
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<td>H12 24.19</td>
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<td>H12 25.03</td>
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<tr>
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<td>5.80a</td>
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</table>

Values with the different subscripts are different (p<.05)

![Graphs showing insulin levels for Thin, Moderate, and Obese conditions](image-url)


Stull, C. L., and A. V. Rodiek. 1994. Effects of postprandial interval and feed type on substrate availability during exercise. CATI.


