PREVALENCE, RISK FACTORS AND SEASONALITY OF PLASMA INSULIN CONCENTRATIONS IN NORMAL HORSES IN CENTRAL OHIO

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree of Masters of Science in the Graduate School of the Ohio State University

By

Jill D. Muno, BA, BS, DVM

***************

The Ohio State University

2009

Master’s Examination Committee:
Dr. Laurie Gallatin, Advisor
Dr. James Belknap
Dr. Raymond Geor
Dr. Kenneth Hinchcliff

Approved by

Advisor
Graduate Program in Veterinary Clinical Sciences
ABSTRACT

Insulin resistance in horses is a potent predisposing factor for laminitis although the causal mechanisms for this association have not been elucidated. Obesity is a common occurrence in horses and is increasingly recognized as a risk factor for insulin resistance. However, risk factors for proxy measures of insulin resistance in horses have not been exhaustively documented. Additionally, some tests of endocrine function in horses, including the dexamethasone suppression test and plasma adrenocorticotropic concentration have seasonal variation. Measuring the concentrations of insulin and glucose in plasma or serum of feed-restricted horses is readily achieved in the field situation and is considered a useful screening test for insulin resistance. The aim of this study was two-fold, first to determine the prevalence and risk factors for hyperinsulinemia in clinically normal, adult horses and second, to determine if those concentrations vary with season.

For the first part of the study, a convenience sample of 300 horses (138 mares, 143 geldings and 19 stallions; 4 to 30 years of age) was drawn from 18 farms that partially comprised the Ohio State University field service population. Plasma insulin and glucose were measured after a 10-12 hour period of grain and grass withholding. Measures of body condition score, height, weight tape and neck circumference as well as pertinent history and management practices were obtained at that time. A univariable logistic
regression and a multivariable logistic regression with a random effect of farm were used to estimate odds ratios.

The prevalence of hyperinsulinemia, defined by a resting insulin concentration >15 μU/L, was 22.3% (67/300). A number of factors including breed, age, body condition score, girth circumference, not feeding grain and lack of access to pasture were associated with hyperinsulinemia in the univariable analysis. Multivariable analysis revealed that increasing age (odds ratio per year of increasing age 1.1, 95% CI 1.03-1.17) and body condition score (odds ratio 2.43, 95% CI 1.6-3.7) increased the risk for hyperinsulinemia while access to pasture (odds ratio 0.34, 95% CI 0.14-0.86) decreased the risk. Breed, sex, exercise, and measures of neck and girth circumference were not associated with risk of hyperinsulinemia.

This study demonstrates that when accounting for common management factors, only indirect measures of obesity and increasing age are independently associated with increased risk of hyperinsulinemia.

For the second part of the study, twenty-nine healthy horses, as determined by physical examination, were evaluated at three-month intervals over a one year period. Plasma insulin and glucose concentrations were measured after a 10-12 hour period of grain and grass withholding. Body condition score, height, weight tape and neck circumference were measured at the same time. Friedman’s test and Wilcoxon’s signed rank test were
used to compare the overall and individual differences between the four seasons in the values of insulin and glucose. The type 1 error rate was 5%.

There was an effect of season on the concentration of both insulin and glucose (P<0.001). The concentration of insulin in summer was lower (median 4.6 μU/L, range 1.2 to 22.0) than any of the other three months (P<0.001). There was no significant difference between any of the other seasons (autumn 13.4, range 4.3 to 271.7; winter 13.6, range 5.3 to 401.7; spring 12.5, range 2.0 to 412.4). The concentration of glucose in autumn was less than in any of the other three seasons (P<0.001), and the concentration in summer was less than in spring (p=0.041).

This study demonstrates that an apparent seasonal variation exists in the plasma concentrations of both insulin and glucose in normal horses. This information may modify the interpretation of laboratory findings indicative of insulin resistance.
Dedicated to my little brother, Ty.
ACKNOWLEDGMENTS

I wish to thank Dr. Ken Hinchcliff for sticking with me on the project and for helping me with study design, interpretation of results and editing of the thesis and presentations.

Thank you to Dr. Ray Geor for participating in the Master’s committee and for performing the sample analysis.

Thank you to Dr. Laurie Gallatin for her help and support during the design and data collection, and thank you to Dr. Garry Anderson for performing the statistics.
VITA

April 19, 1977…………….Born, Downers Grove, IL

May 1, 2000………………….B.A. Biology, North Central College
                        Naperville, IL

June, 2002…………………...B.S. Veterinary Clinical Science
                        College of Veterinary Medicine
                        University of Illinois

June, 2004…………………...D.V.M. College of Veterinary Medicine
                        University of Illinois

2004-2005………………….Intern, University of Missouri

2006-2009………………….Graduate Research Associate
                        The Ohio State University
                        Department of Veterinary Clinical Sciences

2006-2009………………….Equine Ambulatory Resident
                        The Ohio State University
                        Department of Veterinary Clinical Sciences

FIELD OF STUDY

Major Field: Veterinary Clinical Sciences

Area of Emphasis: Equine Ambulatory Medicine
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2. Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Definition of Insulin Resistance</td>
<td>4</td>
</tr>
<tr>
<td>2.3 Insulin and Glucose Regulation</td>
<td>5</td>
</tr>
<tr>
<td>2.4 Pathophysiology of Obesity</td>
<td>7</td>
</tr>
<tr>
<td>2.5 Insulin resistance and Laminitis</td>
<td>8</td>
</tr>
<tr>
<td>2.6 Quantification of Insulin Resistance</td>
<td>9</td>
</tr>
<tr>
<td>2.7 Seasonal Variation and Metabolic Disorders</td>
<td>12</td>
</tr>
<tr>
<td>2.8 Assessment of Body Condition Score</td>
<td>13</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Descriptive Statistics</td>
<td>25</td>
</tr>
<tr>
<td>3.2</td>
<td>Univariable Analysis of Risk Factors</td>
<td>26</td>
</tr>
<tr>
<td>3.3</td>
<td>Multivariable Analysis of Risk Factors</td>
<td>28</td>
</tr>
<tr>
<td>4.1</td>
<td>Mean Values for Each Season</td>
<td>39</td>
</tr>
<tr>
<td>4.2</td>
<td>Individual P Values Between Seasons</td>
<td>40</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>3.1</td>
<td>BCS Agreement</td>
<td>29</td>
</tr>
<tr>
<td>4.1</td>
<td>RISQI Compared by Season</td>
<td>39</td>
</tr>
<tr>
<td>4.2</td>
<td>Plasma Insulin Concentrations Compared by Season</td>
<td>40</td>
</tr>
<tr>
<td>4.3</td>
<td>Glucose Compared by Season</td>
<td>41</td>
</tr>
<tr>
<td>4.4</td>
<td>RISQI Relationship to BCS and Season</td>
<td>42</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Metabolic syndrome is a relatively common disease in humans. Metabolic syndrome is described by a set of findings associated with obesity including, hyperinsulinemia, dyslipidemia and the accelerated development of atherosclerosis. Research into the pathophysiology of the condition is ongoing, but at this point several key attributes of the disease are known. As adipose tissue deposits increase in the body, other tissues of the body become less responsive to insulin. This leads to a decreased ability of insulin to produce its typical effects i.e. the transport of glucose and other nutrients into insulin sensitive cells and the cessation of release of glucose from the liver. Reduction in adiposity improves sensitivity to insulin.

Obesity in the horse has long been linked to chronic laminitis which is more common when the animal is not in work and has access to a steady caloric intake such as lush pasture. Equine veterinarians have long recognized a typical phenotype of an at risk horse consisting of obesity with abnormal fat deposition especially in the crest of the neck, abdomen and over the rump. These horses are predisposed to the development of laminitis, especially when nutrition is abundant. They often resemble the phenotype of the typical horse affected with pituitary pars intermedia dysfunction (PPID), but without some of the typical clinical signs such as hirsutism. Additionally, these
overweight animals are often younger than most PPID horses and when challenged with a dose of dexamethasone, are typically able to suppress cortisol production normally. Therefore the need arises to further characterize and diagnose this disease process which causes significant morbidity in horses and ponies. Much research has been done that elucidates the pathophysiology of this disease. Most of this work is being done in a research setting using tests that are much too expensive and time-intensive for use in the field. This constraint necessitates the development of a method of evaluating horses at risk for this syndrome in a practical and affordable manner. The focus of this research project was two-fold; first to evaluate a large number of animals from different farms, being fed different rations and used for many disciplines to attempt to identify risk factors associated with basal hyperinsulinemia and, second, to evaluate the seasonal variation in plasma concentrations of insulin and glucose in horses managed in a fixed environment.
As is the case in humans and domesticated animals such as dogs and cats, obesity is a common and increasing problem in horses. Just as has been shown in humans, insulin resistance (or insensitivity) has been associated with obesity in horses and the disease process, which typically includes subclinical or chronic laminitis, has been labeled with several terms including peripheral Cushing’s syndrome, equine metabolic syndrome (EMS), syndrome X and prelaminitic metabolic syndrome (PLMS). The condition that all these names describe is characterized by obesity, insulin resistance (or insensitivity) and the predisposition to develop laminitis.

The group of horses most often affected by this syndrome of obesity and chronic laminitis typically embodies several general characteristics. Many breeds and adults of both sexes are affected and horses tend to be middle aged to older. These horses have a characteristic body type in that they tend to be overweight to obese, with regional deposition of adipose tissue at the crest of the neck, abdominal region and over the tailhead and croup. A multicenter study conducted in 2001 found that horses with both acute and chronic laminitis were more likely to have a “cresty” neck conformation and both groups were also less likely to have been in recent exercise. Individuals are
generally thought of by their owners as “easy keepers” meaning they require very little concentrates and forage to maintain their body weight and it can be difficult to reduce their body condition. Additionally, although it can go unnoticed for long periods of time by owners, these horses are predisposed to develop chronic laminitis.

Insulin resistance can generally be defined as the inability of target tissue to respond appropriately to normal concentrations of the hormone. The insensitivity of tissues to the action of insulin leads to a state of glucose intolerance which implies that return of blood glucose to resting concentrations is delayed after a grain or other high sugar meal. In a normal, healthy animal, the hyperglycemia associated with a meal stimulates the pancreatic beta cells to release insulin which in turn stimulates the uptake and utilization of glucose by cells. When glucose intolerance and insulin insensitivity (or resistance) develops, the tissues (both peripheral and central i.e. liver) are not responsive to the action of insulin; beta cells do not release enough insulin, or both. Chronically inhibited insulin action leads to persistent elevations in glucose and increased triglyceride production in the liver. Genetically predisposed humans develop type-2 diabetes after prolonged periods of insulin resistance. Diabetes mellitus does not appear to be a common sequel in horses with insulin resistance but it is unknown why this is so.

Insulin is an important regulator of glucose metabolism. It is an anabolic hormone which increases the storage of glucose, fatty acids and amino acids. The hormone is secreted from the β cells of the endocrine pancreas as a preprohormone and then cleaved to its active form before being released from the islet cell. Insulin receptors are found on many cells in the body, but are essential for those tissues which require the
hormone in order to utilize glucose. The insulin receptor consists of two alpha subunits which are extracellular and bind the hormone and two beta subunits, which span the cell membrane. Binding of insulin initiates a cascade of events involving tyrosine kinase which leads to autophosphorylation of the receptor, speeding translocation of glucose transporters into the cell membrane.7

Glucose enters most cells via carrier-mediated facilitated diffusion. The carriers which are involved in this type of glucose transport are called GLUT 1-7. Their affinity for glucose is variable and each appears to have evolved for a specific purpose. GLUT-1 and GLUT-3 appear to be ubiquitous and mediate basal glucose transport. GLUT-2 is present in the liver and pancreas and mediates bidirectional transport depending on concentrations of glucose. GLUT-4 is regulated by insulin and is present in skeletal and cardiac muscle, adipose tissue, and possibly other types of tissues as well. When insulin binds to receptors on insulin-sensitive cells, vesicles rapidly transport GLUT-4 to the cell membrane where it is inserted. When the action of insulin stops, the portions of the cell membrane containing the GLUT-4 receptors are endocytosed. The other GLUT transporters that are not insulin-sensitive appear to remain in the cell membrane.7

Many factors stimulate or inhibit insulin secretion, but plasma glucose concentration exerts feedback control with a high level of precision and is likely the most important factor in its regulation. Glucose acts directly on the β cells of the pancreas to increase secretion of insulin. The response of these cells to glucose occurs in two phases. In the first and immediate phase, glucose enters the cells via the GLUT-2 receptors causing a cascade of events which results in depolarization of the β cell and the
exocytosis of secretory granules containing ready-made insulin. The second phase occurs more slowly, producing a prolonged phase of insulin release. This phase involves a second messenger, glutamate, which primes a second pool of secretory granules for release.7

The end effects of insulin receptor stimulation lead to transport and storage of nutrients. Within 3-5 minutes of activation of the insulin receptor, about 80% of the body’s cells greatly increase their uptake of glucose. The cell membranes of these cells also become more permeable to amino acids, potassium and phosphate ions. Over the next 10-15 minutes, many intracellular metabolic enzyme activities are altered and over the next several days changes occur in the rates of protein metabolism. Insulin causes changes in the liver which lead to an increase in glycogen storage.8

The important metabolic role of insulin becomes disordered in the presence of obesity. A decrease in the ability of insulin to move glucose into tissues and to decrease its release from hepatocytes occurs and is termed insulin resistance.7 Insulin resistance can be generally defined as the inability of tissues to respond appropriately to normal concentrations of the hormone. In humans this disease process has been named the “Metabolic Syndrome.”9 Findings with this syndrome include hyperinsulinemia, elevated circulating concentrations of triglycerides and accelerated development of atherosclerosis.10 Large depots of fat act as endocrine tissues, secreting hormones that affect fat metabolism and act on muscle and liver to increase insulin resistance. These hormones, called adipokines, include leptin, TNF-α, adiponectin and resistin.7
In horses, obesity has been purported to be linked to both insulin resistance and glucose intolerance. Glucose intolerance refers to a delay in the resolution of post-feeding hyperglycemia.\textsuperscript{2} In normal animals, hyperglycemia associated with feeding causes the release of insulin from pancreatic $\beta$ cells which leads to the uptake of glucose into many of the body’s tissue and its storage in the liver. Insulin resistance implies that the body’s tissues do not respond appropriately to the hormone.\textsuperscript{2}

Although obesity is a well-recognized risk factor for the development of insulin resistance in humans, other factors such as age, genetics and reduced physical activity exist as well. Proposed mechanisms for the development of insulin resistance include a reduced number of receptors, a decrease in affinity of receptors for insulin or an abnormality in intracellular signaling. As the actions of insulin are inhibited over time, persistant elevations in plasma glucose and an increase in triglyceride production in the liver occurs. Increased concentrations of circulating triglycerides are thought to worsen insulin resistance and hasten the development of atherosclerosis in people.\textsuperscript{2}

Laminitis is a common cause of morbidity and mortality in horses and is an area of increasing research concentration in recent years. A connection between insulin resistance and laminitis in horses has been the focus of research in recent years.\textsuperscript{5,11-15} Therefore, more clearly defining the risk associated with obese body condition and insulin resistance in the equine species could lead to clearer recommendations about the management of these animals and the prevention and treatment of laminitis associated with this process.
Laminitis in horses can be caused by many diseases including carbohydrate overload, acute inflammatory conditions, and diseases leading to endotoxemia including colic and metritis. A common feature of the pathogenesis of laminitis in horses is the separation of the secondary epidermal and dermal lamellae of the hoof. The separation occurs between the epidermal basal cells and their basement membrane. The pathogenesis of laminitis consists of three distinct stages. In the pre-laminitic stage, damage to the cellular components is occurring, but no outward signs of laminitis are evident. The acute stage of laminitis occurs when the horse shows signs of pain and the chronic stage commences when the coffin bone detaches and rotation occurs.

Insulin insensitivity has been shown as early as the 1980s to be related to the pathogenesis of laminitis in ponies. Many authors have investigated the link involving diet, endocrine status and laminitis in horses and ponies. Recently, a study involving ponies predisposed to laminitis found that compared to control ponies, the laminitis predisposed ponies were insulin resistant and were more sensitive to feeding of plant fructan or administration of dexamethasone. In a case report involving a hyperlipemic, laminitic pony with a normal body condition score, a specific quantitative method (FSIGT) was used to diagnose uncompensated insulin resistance.

Several methods of assessing insulin resistance in horses have been reported and these can be separated into nonspecific indicators and quantitative methods. Nonspecific indicators are weaker than quantitative methods, but are less expensive and easier to perform on a large number of horses or in a field service situation. While both plasma or blood insulin and glucose concentrations are nonspecific indicators of IR, they
are easily measured in the clinical field situation and would be helpful to practitioners if proven to be a valid measure of insulin resistance in obese horses. In a study of 7 obese adult horses and 5 nonobese mares, higher resting serum insulin concentrations and lower resting glucose-to-insulin ratios were found in obese, insulin resistant horses compared with nonobese horses. This suggests that these measures could be used as a screening test for insulin resistance in horses. Quantitative methods, including the frequently sampled intravenous glucose tolerance test (FSIGT) or the euglycemic-hyperinsulinemic clamp method are time and labor intensive and can only be practically done on small numbers of animals.

In humans, the euglycemic-hyperinsulinemic clamp is considered the “gold standard” for measuring insulin resistance. This technique requires a constant rate infusion of both insulin and glucose. The glucose is “clamped” at a specified concentration by infusing a variable rate against a fixed rate of insulin. Once a steady state is reached, the rate of glucose administration needed to keep the glucose at the same concentration is measured. This technique is time-consuming, labor-intensive and expensive. An alternative to this technique is the insulin modified frequently sampled intravenous glucose tolerance test (FSIGT). This technique is also time-consuming and expensive, but does not require the titration of insulin and glucose infusions. The FSIGT consists of an initial dose of insulin and then a bolus dose of glucose. The blood glucose concentration is then measured about 25 times over the following three hours. A computer program using a minimal model analysis is then used to calculate several parameters of insulin resistance.
Proxies and reference quintiles determined with insulin and glucose concentrations could be used to screen for insulin sensitivity and beta-cell responsiveness in horses as similar proxies are used in human medicine for this purpose. This characterization could lead to identification of horses and ponies needing special management to avoid laminitis and measures to institute to increase insulin sensitivity. Specifically, Treiber et al developed several proxies including RISQI (reciprocal of the square root of insulin) and MIRG (modified insulin-to-glucose ratio) which can be used to determine insulin sensitivity and beta cell responsiveness, respectively. Reference quintiles were then developed using the minimal model of the glucose/insulin regulatory system which is based on data from an FSIGT. The minimal model provides measures of four parameters including insulin sensitivity, glucose effectiveness, and acute insulin response to glucose and disposition index. By applying the minimal model to 46 healthy horses, researchers were able to develop reference quintiles.

The early identification of horses prone to insulin resistance would help practitioners institute measures to reverse the disease and potentially halt or prevent the damaging effects of chronic laminitis. Just as in human beings, appropriate diet and exercise are the crux of improving insulin sensitivity. Therefore maintaining horses at an ideal body condition and feeding them diets low in sugar and non-structural carbohydrates and higher in fiber and fat, should decrease the risk of developing insulin resistance, especially in horses prone to this condition. Additionally, short-term exercise periods of only 7 days was shown to increase insulin sensitivity in obese mares, but this effect dissipated by 9 days after exercise ceased. A study involving insulin
resistant ponies found that both rested and exercised ponies fed a balanced diet improved their insulin sensitivity and lost weight. In the exercised ponies, a marked improvement in insulin sensitivity occurred in the first two weeks of conditioning which was significantly better than in the rested ponies. The conditioned ponies continued to show an improvement in insulin sensitivity even six weeks after the exercise period was discontinued. Since both groups of ponies showed improved insulin sensitivity, additional studies are needed to determine if a reduction in weight alone caused the improvement or if exercise adds an additional benefit.

The effect of season on insulin resistance has not been widely evaluated. It has long been noted by equine veterinarians that laminitis in obese ponies and horses has a seasonal variation and is more common in the spring and summer months. This may be due to an increase in nonstructural carbohydrate content of pasture. Tests of endocrine function to evaluate pituitary pars intermedia dysfunction (PPID) have been shown to have seasonal variation. In one study using healthy horses and ponies, plasma adrenocorticotropic hormone concentrations and plasma cortisol concentration after dexamethasone suppression tests were significantly higher in September than in January. A recent investigation compared laminitis-prone and normal ponies in the summer and winter. Serum insulin and plasma triglyceride concentrations were significantly higher in the laminitis-prone ponies compared with control ponies in the summer, but no significant difference was found between the two groups in the winter. Additionally, the proxy measurements, RISQI and MIRG, were indicative of insulin resistance in the laminitis-prone ponies in the summer, but were not significantly
different between the two groups in the winter. All ponies in this study were of normal body condition (<7/9).11

The assessment of an individual horse’s body condition or fitness level is a qualitative assessment. Several methods have been described previously. The association between body condition score and body fat was used to create a systematic method of determining body condition score.33 Additionally, a method for determining mean neck circumference has been used to assess condition in horses. Mean neck circumference was found to correlate most closely with elevated insulin levels.23 An additional method to assess condition involves using ultrasound to measure back fat thickness.34 A recent investigation combined several morphometric measurements as well as the Henneke BCS system to classify horses and ponies which were overweight (BCS≥7) or obese (BCS≥8), and to describe a system for neck crest adiposity.35

Although many recent investigations have focused on assessing insulin resistance in individual horses, most studies have used very labor-intensive diagnostic tests in small numbers of animals. These tests are not practical in the field situation or for a large-scale investigation. Additionally, few studies have sampled large numbers of animals to determine what “normal” value ranges of insulin are in the horse. Several risk factors, such as obese body condition and neck circumference have been linked to insulin resistance; however, few large-scale investigations have been conducted to further characterize risk factors in the general equine population. Finally, seasonality of other endocrine tests such as the low dose dexamethasone suppression test have been noted, but
it is unknown whether season may play a role in the presence of insulin resistance or the normal insulin concentration in horses.
CHAPTER 3

PREVALENCE AND RISK FACTORS FOR ELEVATED INSULIN CONCENTRATION IN HEALTHY HORSES IN CENTRAL OHIO

Introduction

There is a syndrome in horses comprised of obesity or regional adiposity, insulin resistance and predisposition to chronic or subclinical laminitis.³,⁵ Because the syndrome has some, albeit limited, similarity to metabolic syndrome in humans, the term equine metabolic syndrome has been adopted by some authors.² The cardinal feature of the syndrome is insulin resistance. Insulin resistance implies that tissues are unable to respond appropriately to normal concentrations of circulating hormone, with the result that insulin concentrations in blood are elevated, even after a prolonged period of feed withholding.²

Although pasture-associated laminitis is reported to be the most common cause of laminitis in horses¹⁶, the prevalence of insulin resistance and associated risk factors which would put an animal at risk to develop the disease are unknown. This lack of information is in part due to the difficulty and expense of performing definitive tests for diagnosis of insulin resistance in large numbers of animals. For screening purposes, single sample tests and proxies are used in human medicine and are more practical in a field situation.⁵ However, few published studies regarding insulin and glucose
concentrations in large numbers of horses exist and normal ranges for the measurements vary.

Some risk factors for the development of metabolic syndrome in horses have been reported. Anecdotally, horses with areas of increased adipose tissue deposition evident over the crest of the neck, or tailhead, obese horses and middle aged to older horses have been suspected of being at risk. Average neck circumference and body condition score were significantly correlated with an abnormal combined glucose-insulin test. \(^{23}\) Ponies with current or previous episodes of laminitis have higher body condition scores, are older and have higher plasma insulin concentrations than do healthy ponies. \(^{5}\)

The purpose of the study reported here was to evaluate the prevalence of elevated plasma insulin concentration in a population of healthy horses and to identify risk factors associated with increased insulin concentrations in those horses. The hypothesis was that risk factors for hyperinsulinemia would be identified and that these would be body condition score, neck circumference, grain-feeding and a lack of exercise.

Methods and Materials

The study design used was a prospective, observational study. A convenience sample of 300 horses were examined. Fifty-one healthy horses were selected from the university teaching herd. The University Institutional Laboratory Animal Care and Use Committee approved this study and horses were treated in accordance with National Institutes of Health Institutional Animal Care and Use guidelines. The remaining horses were client-owned and written consent authorizing study participation was obtained from each client or agent. This study was conducted in accordance with the guidelines of the
Eighteen farms participated in the study. Farms were identified from the Ohio State University Equine Field Services clientele which are located within a 35 mile radius of Columbus, Ohio. Farms that participated included boarding stables with fewer than ten to more than 30 horses, as well as privately owned breeding herds and the Ohio State University’s teaching herd. Letters requesting voluntary participation were sent to barn managers and consent forms were obtained for each horse involved in the study. Horses were included if they were at least four years of age, were not of pony or draft breeding, were greater than 14.2 hands, were not currently diagnosed or being treated for a metabolic disease, did not have clinical signs of Cushing’s disease or laminitis and had not had an episode of laminitis in the last three months. Horses were not fed grain, pasture or treats for 12 hours prior to sampling, but were allowed access to hay (type and amount were not restricted and were determined by the individual owner or farm) and water. Sampling was performed at a time that was typically just prior to feeding.

Blood was collected from the jugular vein of each horse into an evacuated glass tube containing sodium heparin. Height was measured in hands at the withers with a standard measure stick and then converted to centimeters. The girth circumference was measured in centimeters just behind the withers. A weight tape was then used to estimate body weight\(^a\). Neck measurements were performed as previously described.\(^{23}\) Briefly, the neck length was determined and then the circumference of the neck was measured at 25, 50 and 75% of length. Average neck circumference was then calculated using these

\(^a\) Purina Mills
three measurements. Body condition score of each horse was evaluated independently by two investigators using a scale of 1-9. The scoring system was standardized between the two investigators prior to beginning sampling. The body condition score used for statistical analysis was the average of these two estimates. Agreements between the two scores were assessed and 23/300 or 7.67% were within the 95% limits of agreement. (Figure 3.1)

The agent or owner were questioned either at the time of sampling, or later via telephone as to the signalment, pregnancy status, any medications, supplements or current treatment for disease. The type and amount of both hay and grain fed were recorded as well as hours per day in turnout and whether or not the horse had regular access to pasture. The use of the horse (pleasure, broodmare, stud, dressage, hunter/jumper, etc.) was recorded and the level of exercise (none, light, moderate, heavy or very heavy) was determined using the standard NRC definitions.

Tubes containing blood were placed on ice until processing which occurred within three hours of collection. The blood was centrifuged at 1,500 x g for 10 minutes. The plasma was then collected and placed in plastic vials, and stored at -80˚C until all samples were analyzed as a batch.

Plasma glucose concentration was assayed enzymatically by use of a commercial kit and an automated analyzer (CX5 Chemistry Analyzer, Beckman Coulter Inc., Fullerton, CA). Plasma insulin concentrations were measured by use of a commercial radioimmunoassay previously validated for use in equine plasma (Coat-A-Count Insulin, Diagnostic Products Corp, Los Angeles, CA). All analyses were performed in duplicate.
Mean intra-assay coefficient of variations were 0.48% and 5.7% for glucose and insulin, respectively. Mean inter-assay coefficients of variation were 4.2% for glucose and 6.8% for insulin.

Statistical Analysis- Risk factors were screened, using a univariable logistic regression model with a random effect of farm, for further modelling with a multivariable model including a random effect of farm.37 The criterion for inclusion was a likelihood ratio test (LRT) P value < 0.25. A backward elimination procedure was then used to build the final multivariable model, with a LRT P value >0.05 used to remove variables from the model. The Stata (Stata version 10.1 for Windows, StataCorp, College Station TX) command **xtlogit** was used to build the model. Individual variables were then reentered into the final multivariable model, one at a time, to assess if confounding was present as assessed by a meaningful change in the odds ratios. Linearity of the effect of age and body condition score was assessed by fractional polynomials38 and the addition of a quadratic coefficient. An interaction between age and body condition score was also assessed.

Goodness-of-fit was assessed using a logistic regression with a fixed effect of farm and the Hosmer and Lemeshow goodness-of-fit test. A plasma cut-off value of 15 mU/L was used to define hyperinsulinemia based on the personal experience of one researcher in this field (Ray Geor, personal communication). A classification table was used to determine the sensitivity and specificity, with a cut-off probability value to define a positive or negative horse being the observed prevalence of 0.22 for insulin > 15 mU/L. The area under the receiver operating characteristic curve was used as a measure of
discrimination for positive and negative horses with respect to the insulin concentration being > 15 mU/L. The maximum area under the curve is 1.0, whilst an area of 0.5 has no predictive ability at all.

Results

Three hundred horses from 18 farms were included in the study. Horses in the study population were between 4 and 30 years of age, mean age was 11.5 ± 5.67 (mean ± 1 SD) and consisted of several breeds (83 Thoroughbred, 95 Quarter Horses, 51 of Warmblood breeding, and 71 of other breed) and both sexes (143 geldings, 138 mares and 18 stallions).

The maximum value for plasma insulin concentration was 601.04 mU/L, while the minimum value was 1.12 mU/L. The prevalence of hyperinsulinemia, defined by a resting insulin concentration >15 mU/L, was 22.3% (67/300). Mean age of horses in the insulin <15 mU/L group was 10.9 years (SD, 5.5 years, range, 4-30 years), and mean age of horses in the insulin >15 mU/L group was 13.5 years (SD, 5.9 years, range, 4-29 years). Average body condition score of horses in the insulin <15 mU/L group was 5.9 (SD, 1.0, range, 2.5-8.75) and average body condition score of horses in the insulin >15 mU/L group was 6.8 (SD, 1.0, range, 4.25-8.5). Girth circumference, average neck circumference and height were measured for all subjects and were not significantly different between groups. (Table 3.1)

Univariate analysis of risk factors for hyperinsulinemia included the farm as a random effect. The following factors were not statistically different between the two
groups; breed, sex, pregnancy status, girth circumference, average neck circumference, feeding of grain, level of exercise and hours of turnout per day. Factors which were statistically different between groups were, age (p=0.002), body condition score (p<0.0001) and whether or not subjects had access to pasture (p=0.009). (Table 3.2)

Variables which were statistically significant in the univariate model were placed into a multivariate model which also included farm as a random effect. In this model, subject’s risk of having a plasma insulin level >15 mU/L increased 1.1 times for every increase in year of age (95% CI 1.03-1.17, Wald p value=0.005), increased 2.43 times for each whole number increase in body condition score (95% CI 1.60-3.70, Wald p value <0.0001) and decreased by 0.34 times if they were allowed access to pasture (95% CI 0.14-0.86, Wald p value=0.023). (Table 3.3)

Discussion

Results of this study demonstrate that older horses and those with higher body condition scores are more likely to have elevated plasma insulin concentrations, while horses with access to pasture are less likely to have a plasma insulin concentration greater than 15 mU/L. Other investigators have set a cut-off value for normal insulin concentration at 20 or 30 mU/L, but in some cases, subjects have been allowed access to pasture prior to sampling.40 The establishment of any reference range or cut-off value for insulin concentrations in horses at this point is somewhat arbitrary and requires further investigation.
In a study which measured the feed-restricted plasma insulin concentrations of 14 Morgan horses and 21 Thoroughbreds which were non-obese, the median insulin concentrations were 3.4 mU/L (95% reference range, 2.7-8.2) and 2.9 mU/L (95% reference range, 2.3-12.0) respectively. An additional investigation comparing hyperleptinemic and normal mares found that the mean insulin concentration of normal mares was 11.4 mU/L while that of the hyperleptinemic mares was 20.4 mU/L. Hyperleptinemia has been associated with insulin resistance. All mares (hyperleptinemic and normal) in this study that were on a diet that consisted solely of hay had a mean insulin concentration of 8.2 mU/L in a 36-hour sampling period (no feed restriction) while the mares being fed grain/hay meals or maintained on pasture were higher at 18.7 mU/L and 20.7 mU/L, respectively. The group being fed hay most closely resembles our study group which was held off all concentrates and pasture, but allowed access to hay prior to sampling. An investigation comparing obese, insulin resistant horses (OB-IR) and nonobese mares, found that insulin concentrations overlapped between the two groups, with the median value and range for the OB-IR group being 50.5 µU/ml (17.0-93.4) and that of the nonobese group being 9.1 µU/ml (6.4-21.1). The term µU/ml is equal to mU/L. That study used the CGIT to identify insulin resistant horses. However, if proxy values had been applied, a cutoff value of 20 or 30 µU/ml would miss some horses in the insulin resistant group. However, with a lower cutoff value of 15 µU/ml, such as was used in the present study, all insulin resistant horses would be identified but some in the nonobese group would be considered insulin resistant when in fact, they are not. Authors of the study recommended the use of a
challenge test such as the combined glucose insulin test (CGIT) for horses suspected of being insulin resistant, yet falling in this middle range.\textsuperscript{42}

Data on several common potential risk factors were collected for all horses in the present study; however only body condition score, age, and access to pasture were found to be significantly related to an insulin concentration above the cutoff value. A syndrome of obesity, regional adiposity and insulin resistance has long been recognized by equine practitioners. Therefore, it is reasonable that increasing body condition score be associated with risk of elevated insulin concentration.\textsuperscript{2} An association between obese body condition score and neck width and insulin resistance has been reported in horses.\textsuperscript{23} Additionally, several papers have reported on the link between obesity and insulin resistance in horses\textsuperscript{1,29} and ponies.\textsuperscript{5,11,30} Evidence exists that reduction of weight in ponies, with or without exercise, improves insulin sensitivity.\textsuperscript{30} Although a “cresty neck” has often been reported as associated with or a risk factor for insulin resistance, neck circumference measurements were not found to be a risk factor for an insulin concentration greater than the cutoff value in this group of horses. In an investigation where ponies were grouped by their laminitis history (not laminitic, previously laminitic or clinically laminitic), body condition score, cresty neck score, and insulin were predictors of the development of laminitis, but the cresty neck score could not be validated on its own since all ponies with a high score also had an obese body condition.\textsuperscript{44} In the present study, an average neck circumference previously shown to be associated with obese, insulin resistant horses was used,\textsuperscript{42} however, recently, a cresty
neck scoring system\textsuperscript{35} has been developed which could be a more accurate way of evaluating this characteristic and warrants further study.

Ageing is associated with increased incidence of metabolic syndrome in humans as it is associated with loss of muscle mass and an increase in body fat, both of which can increase insulin resistance.\textsuperscript{10} Few studies of horses have evaluated the effect of age on insulin concentrations, however one group did note that all horses in their obese, insulin resistant group were greater than 10 years of age, and suggested that time may be required for environmental factors to alter glucose metabolism.\textsuperscript{42} As in humans, older horses are often less physically active and have a higher percentage of body fat than do their younger counterparts, both of which could contribute to a higher insulin concentration.

Access to pasture decreased the risk for an insulin concentration higher than 15mU/L. A horse was considered to have access to pasture if it was regularly (daily) turned out in a grassy area. Possible explanations for this finding are that horses with regular access to pasture are allowed more exercise or that they are fed less high-calorie concentrate rations. However, the feeding of grain as well as level of exercise were recorded for all subjects but were not found to be a significant independent risk factor for an elevated insulin concentration. The NSC (nonstructural carbohydrate) content of the pasture was not evaluated; however, since the sampling was performed in the fall months, this could have affected the results. Grass pastures typically have their highest carbohydrate content in the spring, coinciding with periods of active growth and therefore
have been associated with the presence of elevated insulin concentration and the development of laminitis in horses and ponies.\textsuperscript{45,46}

Although a nonspecific indicator of insulin resistance,\textsuperscript{22} elevated insulin concentration after a period of feed-withholding is a useful screening test for insulin resistance in horses. Other risk factors, such as advanced age, obese body condition score and a lack of regular access to pasture should be considered when interpreting results. Horses with insulin concentrations greater than 15 mU/L but less than 30 mU/L might benefit from additional testing such as CGIT. For horses that are insulin resistant, caloric reduction to reduce body condition as well as increased access to pasture and regular exercise are important husbandry changes. Access to pasture may be contraindicated in obese, insulin resistant horses which have had a previous episode or have clinical evidence of a previous episode of laminitis.

In summary, an evaluation of 300 normal, healthy horses ranging in age between 4 and 30 years, had a prevalence of hyperinsulinemia, defined as greater than 15 mU/L, of 22.3% (67/300). Risk factors found to be significant for the presence of elevated insulin concentration were a higher body condition score, increased age, and lack of access to pasture. These findings have implications for the identification and management of obese and insulin resistant horses.
<table>
<thead>
<tr>
<th></th>
<th>Insulin &lt;15mU/L</th>
<th></th>
<th>Insulin &gt;15mU/L</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.9*</td>
<td>4-30</td>
<td>13.4*</td>
<td>4-29</td>
</tr>
<tr>
<td>BCS (1-9)</td>
<td>5.9*</td>
<td>2.5-8.75</td>
<td>6.7*</td>
<td>4.25-8.5</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>189.6</td>
<td>164-218</td>
<td>193.2</td>
<td>174-222</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>101.2</td>
<td>88.3-118.7</td>
<td>102.1</td>
<td>87.3-115.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.41</td>
<td>147.96-184.15</td>
<td>160.29</td>
<td>149.86-177.8</td>
</tr>
</tbody>
</table>

Table 3.1: Descriptive statistics, * indicates significant difference between groups (P<0.05)
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Number insulin &gt; 15 (%)</th>
<th>Odds Ratio (95%CI)</th>
<th>P value</th>
<th>Odds Ratio (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoroughbred</td>
<td>12/83 (14.5)</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Quarter Horse</td>
<td>16/95 (16.8)</td>
<td>1.20 (0.53-2.70)</td>
<td>0.66</td>
<td>2.07 (0.76-5.65)</td>
<td>0.15</td>
</tr>
<tr>
<td>Warm blood</td>
<td>17/51 (33.3)</td>
<td>2.96 (1.27-6.88)</td>
<td>0.012</td>
<td>2.78 (0.87-8.85)</td>
<td>0.08</td>
</tr>
<tr>
<td>Other</td>
<td>22/71 (31.0)</td>
<td>2.65 (1.20-5.86)</td>
<td>0.016</td>
<td>3.17 (1.12-8.97)</td>
<td>0.03</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelding</td>
<td>27/143 (18.9)</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>38/138 (27.5)</td>
<td>1.63 (0.93-2.86)</td>
<td>0.087</td>
<td>2.39 (1.17-4.88)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stallion</td>
<td>2/19 (10.5)</td>
<td>0.51 (0.11-2.32)</td>
<td>0.38</td>
<td>0.85 (0.15-4.67)</td>
<td>0.85</td>
</tr>
<tr>
<td>Age (year)</td>
<td>67/300 (22.3)</td>
<td>1.08 (1.03-1.13)</td>
<td>0.002</td>
<td>1.08 (1.02-1.14)</td>
<td>0.011</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63/267 (23.6)</td>
<td>Referent</td>
<td></td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4/33 (12.1)</td>
<td>0.45 (0.15-1.32)</td>
<td>0.14</td>
<td>0.40 (0.10-1.53)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 3.2: Univariable analysis of risk factors for plasma insulin concentration > 15 mU/L, * median unbiased estimate from exact logistic regression, bolded p-values are likelihood ratio statistic p-values and unbolded p-values are Wald test p-values
Table 3.2 Continued

<table>
<thead>
<tr>
<th>Body Condition Score</th>
<th>67/300 (22.3)</th>
<th>2.21 (1.65-2.97)</th>
<th>&lt; 0.0001</th>
<th>2.31 (1.55-3.44)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girth circumference (cm)</td>
<td>67/300 (22.3)</td>
<td>1.05 (1.02-1.09)</td>
<td>0.003</td>
<td>1.05 (1.00-1.10)</td>
<td>0.03</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td>0.18</td>
<td></td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Moderate to high</td>
<td>12/76 (15.8)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>15/72 (20.8)</td>
<td>1.40 (0.61–3.25)</td>
<td>0.43</td>
<td>2.53 (0.87-7.38)</td>
<td>0.09</td>
</tr>
<tr>
<td>None</td>
<td>40/152 (26.3)</td>
<td>1.90 (0.93-3.89)</td>
<td>0.08</td>
<td>1.87 (0.63-5.55)</td>
<td>0.26</td>
</tr>
<tr>
<td>Pasture</td>
<td></td>
<td>0.009</td>
<td></td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34/111 (30.6)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33/189 (17.5)</td>
<td>0.48 (0.28-0.83)</td>
<td>0.009</td>
<td>0.33 (0.14-0.78)</td>
<td>0.011</td>
</tr>
<tr>
<td>Risk Factor</td>
<td>Number insulin &gt; 15 (%)</td>
<td>Coefficient (± SE)</td>
<td>Odds Ratio (95%CI)</td>
<td>Wald P value (LRT P value)</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>----------------------------</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>67/300 (22.3)</td>
<td>0.091 (± 0.032)</td>
<td>1.10 (1.03-1.17)</td>
<td>0.005 (0.004)</td>
<td></td>
</tr>
<tr>
<td>Body Condition Score (1-10)</td>
<td>67/300 (22.3)</td>
<td>0.890 (± 0.213)</td>
<td>2.43 (1.60-3.70)</td>
<td>&lt; 0.0001 (&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>Pasture</td>
<td></td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34/111 (30.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33/189 (17.5)</td>
<td>-1.068 (±0.470)</td>
<td>0.34 (0.14-0.86)</td>
<td>0.023 (0.020)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3: Final multivariable model, including random effect of farm, of risk factors for plasma insulin concentration > 15 mU/L in 300 horses.
Figure 3.1: Limits of agreement between two assessments of body condition score. Size of circle is dependent on number of evaluations.
SEASONAL VARIATION STUDY

Introduction

Seasonal variability of some tests of endocrine function occurs in horses and ponies. The dexamethasone suppression test (DST) and endogenous plasma adrenocorticotropic hormone (ACTH) concentration for detection of PPID (pituitary pars intermedia dysfunction) are less accurate in autumn than in the spring or summer months. Recent studies regarding seasonal variation of insulin resistance and the risk for development of pasture-associated laminitis have confirmed clinical observations by veterinarians and owners that ponies and horses are more susceptible to bouts of pasture-induced laminitis during the spring and summer months. This increased susceptibility has been suggested to be related to higher content of carbohydrate in pasture at these times. Identification of ponies with a prelaminitic profile prior to the grazing season successfully predicted the development of laminitis in 11/13 ponies.

Although it has been shown that the nutritional content of feed affects insulin sensitivity in horses, few studies regarding the seasonality of measures of insulin resistance in normal horses exist. The soluble carbohydrate and specifically, the fructan content of pastures has been shown to display seasonal changes and so it would be logical
to infer that this would have concomitant effects on insulin and glucose concentrations in horses grazing those pastures. A previous investigation has identified and described the seasonal variation of insulin and glucose concentrations and their relationship to pasture carbohydrate content. 47

The purpose of the study reported here was to evaluate plasma insulin and glucose concentrations in normal horses over a one year period. The hypothesis was that plasma insulin and glucose concentrations would vary seasonally.

Methods and Materials

Thirty horses that resided at the university-owned research farm were used for the seasonal variation study. These individuals were chosen randomly from three groups of horses at the farm. Samples were collected at four time points; autumn (September 5th), winter (December 12th), spring (March 26th) and summer (June 3rd).

Subjects were not fed pasture, grain or treats for 12 hours prior to sampling. They were allowed free-choice access to hay and water. Girth circumference, weight tape, neck circumference and body condition score were measured at each sampling date. The girth circumference was measured just behind the withers and a standard weight tape was then used to estimate weight b. Neck measurements were performed as previously described. 23 Briefly, the neck length was determined and then the circumference of the neck was measured at 25, 50 and 75% of length using centimeters. Average neck circumference was then calculated using these three measurements. Body condition score of each horse was independently evaluated by two observers on a scale of 1-9. 33 The average body condition score was then determined for each horse.

b Purina Mills
Blood was collected from each horse from the jugular vein into a 10ml sodium heparin tube. The blood was placed on ice until processing which occurred within three hours of collection. The blood was centrifuged at 1,500 x g for 10 minutes. The plasma was then collected and placed in plastic vials, and stored in a -80°C freezer until all samples had been collected and could be sent to the laboratory for analysis.

Plasma glucose concentration was assayed enzymatically by use of a commercial kit and an automated analyzer (CX5 Chemistry Analyzer, Beckman Coulter Inc., Fullerton, CA). Plasma insulin concentrations were measured by use of a commercial radioimmunoassay previously validated for use in equine plasma (Coat-A-Count Insulin, Diagnostic Products Corp, Los Angeles, CA). All analyses were performed in duplicate. Mean intra-assay coefficient of variations were 0.48% and 5.7% for glucose and insulin, respectively. Mean inter-assay coefficients of variation were 4.2% for glucose and 6.8% for insulin.

Statistical analysis- Insulin concentration was not normally distributed within season as shown by the Shapiro-Wilk test for normality. Insulin was transformed to the reciprocal of the square root of insulin concentration, $RISQI [mU/L]^{-0.5}$, as this represented a proxy for insulin resistance. A linear mixed model was used to compare the means of a response variable between the four seasons, with a fixed effect of season and a random effect of horse. A fixed effect of body condition score was later included to assess if this affected the effect of season. Two-tailed p-values <0.05 were considered significant. Stata (StataCorp, College Station, TX) for Windows, version 10.1, and the xtmixed command were used for analyses.
Results

There was an effect of season on both RISQI and glucose concentration (P<0.001) (Table 4.1). The RISQI in summer was greater than any of the other three seasons (Figure 4.1) (P<0.001), therefore the insulin concentration was lower (Figure 4.2) whilst there was no significant difference between any of the other seasons (all P>0.61). Adjusting for body condition score did not alter these conclusions. The concentration of glucose in autumn was less than in any of the three other seasons (Figure 4.3) (all P<0.003), and the concentration in spring was greater than in both winter (P=0.040) and summer (P=0.028). This conclusion was not changed when body condition score was included in the model (Table 4.2).

Both season and body condition score were included in a linear mixed model and were associated with RISQI. Both were statistically significant (P<0.001). The RISQI values in summer were still high even after taking into account the overall association between RISQI and body condition score and the low body condition scores in summer. Twenty of 29 RISQI values are above the regression line, indicating that even at a given body condition score the RISQI value is generally above the predicted value based on body condition score (Figure 4.4).

Horse 121 had a very high value of glucose in spring (244, the next highest value over all seasons was 141 mg/dL and the overall mean was 98 mg/dL). Conclusions were similar when this horse was excluded from the analysis, however spring was no longer statistically different from winter (P=0.16) or summer (P=0.065).
Twenty eight of 29 horses had their lowest insulin value in summer. Twenty three of 29 horses had their lowest glucose value in autumn (Tables 4.3 and 4.4).

**Discussion**

Results of this study demonstrate that plasma insulin and glucose concentrations in normal horses display seasonal variation. This finding has implications for the diagnosis and treatment of horses predisposed to insulin resistance and the equine metabolic syndrome.

Proxies for insulin resistance have been used in human medicine to aid in the detection of patients at risk for metabolic syndrome. Similar proxies have been developed for use in equine medicine. The RISQI or the reciprocal of the square root of insulin concentration is calculated as a proxy for insulin resistance. Because the insulin concentration is in the denominator of the equation, higher insulin results in a lower number for the RISQI.

Insulin concentrations in grazing horses have previously been shown to have a seasonal variation whereas those of horses fed only hay throughout the year did not vary. In that study, horses were kept on either grass pasture or fed hay only (control horses) during the 36 hour measurement period. The sampling was performed hourly over a 36 hour period and the insulin concentration was an average of three values taken in the morning on day one. Values for the grazing horses were highest in April and lowest in August, October and January which were not significantly different from each other. The control horses did not have any significant difference in mean insulin concentrations over the one-year study period. The plasma insulin concentrations were
significantly correlated to the non-structural carbohydrate and sugar content of the pasture in April, May and January. Although the variation of insulin was similar between that study and the present one, the methods differed in that horses were sampled over a 36 hour period and were not held off feed prior to sampling while in the present study; one blood sample was taken from feed-restricted subjects.

An additional investigation which compared control ponies to laminitis prone ponies found that the insulin concentrations were not statistically different between the two groups during the winter months, but during the summer months, the laminitis prone ponies had significantly higher insulin concentrations than the control ponies. This study did not compare the winter insulin to that of the summer insulin in either group. However, the graphs of the insulin concentrations show that the overall numbers were lower for both groups in summer than in winter. It is not possible to know if this difference is statistically significant, however, since the study did not look at this question.

Similar to our findings for glucose concentrations displaying seasonal variability, the average glucose concentrations for horses grazing pasture were found to be highest in the spring months of April and May and lowest in October. The control horses, which were fed only hay throughout the year, did not have a significant change in glucose concentration.

It has been shown that decreasing the body weight or improving physical conditioning improves insulin sensitivity in ponies. In the present study, RISQI was highest (therefore insulin concentration lowest) in summer compared with the other three
seasons (Figure 4.1). Body condition score (BCS) was also significantly lower in summer than in the other three seasons. Season was associated with both BCS and RISQI; however after accounting for the effect of BCS on RISQI, there is still an important and significant effect of season on RISQI (Figure 4.2). A study in which laminitis-prone ponies were compared to control ponies in the winter and in the summer found that both groups of ponies had a decrease in body weight in the summer as well as a decrease in the neck circumference and crest thickness. The authors suggested that a change in metabolism or activity in the summer as compared to the winter may contribute to the seasonal variation of measures of insulin resistance.

The increase in RISQI seen in the summer is likely related to both the improvement in body condition score of the subjects and to an effect of season. Although there was an improvement in body condition score in summer, an effect of season on RISQI was also present which was independent of the change in body condition score. We did not determine a reason for seasonal variation. This issue warrants further investigation, but could be related to photoperiod, diet, or other seasonal variation in metabolism.

The implications associated with a seasonal fluctuation of glucose and insulin concentrations affect both the management and diagnosis of horses prone to obesity and a metabolic-type syndrome. The variability of this measure must be taken into account when determining a patient’s risk of having or developing the disease.
Table 4.1: Mean values from 29 horses sampled at each of four seasons, means with different superscripts within column are significantly different (P<0.05), Insulin concentration was transformed to RISQI for statistical analysis, BCS=Body Condition Score.

<table>
<thead>
<tr>
<th>Season</th>
<th>Insulin (mU/L) Mean ± SE</th>
<th>RISQI(mU/L)$^{-0.5}$ Mean ± SE</th>
<th>Glucose(mg/dL) Mean ± SE</th>
<th>BCS Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>37.42 ± 12.43</td>
<td>0.286$^a$ ± 0.024</td>
<td>88.0$^a$ ± 2.5</td>
<td>7.1$^a$ ± 0.2</td>
</tr>
<tr>
<td>Winter</td>
<td>39.58 ± 14.37</td>
<td>0.272$^b$ ± 0.023</td>
<td>98.7$^b$ ± 1.6</td>
<td>7.2$^b$ ± 0.2</td>
</tr>
<tr>
<td>Spring</td>
<td>49.97 ± 17.21</td>
<td>0.272$^a$ ± 0.028</td>
<td>106.2$^c$ ± 5.2</td>
<td>7.2$^a$ ± 0.2</td>
</tr>
<tr>
<td>Summer</td>
<td>6.43 ± 1.14</td>
<td>0.533$^b$ ± 0.042</td>
<td>98.2$^b$ ± 1.9</td>
<td>6.5$^b$ ± 0.2</td>
</tr>
<tr>
<td></td>
<td>RISQI</td>
<td>RISQI and BCS</td>
<td>Glucose</td>
<td>Glucose and BCS</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------</td>
<td>---------------</td>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Overall</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Winter vs autumn</td>
<td>0.62</td>
<td>0.74</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>Spring vs autumn</td>
<td>0.61</td>
<td>0.69</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Summer vs autumn</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.001</td>
</tr>
<tr>
<td>Spring vs winter</td>
<td>0.99</td>
<td>0.95</td>
<td>0.040</td>
<td>0.039</td>
</tr>
<tr>
<td>Summer vs winter</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.89</td>
<td>0.59</td>
</tr>
<tr>
<td>Summer vs spring</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.028</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 4.2 individual P values between seasons, bolded values are significant (P<0.05)
Figure 4.1: RISQI variation by season in 29 horses. * indicates statistical significance, p<0.05, RISQI= reciprocal of the square root of insulin.
Figure 4.2. Plasma insulin concentrations in 29 horses sampled on 4 occasions over one year.
Figure 4.3: Plasma glucose concentration in 29 horses sampled on 4 occasions over one year. * indicates statistical significance, p<0.05
Figure 4.4: RISQI values in summer are high even after taking account of the overall association between RISQI and body condition score and the low body condition scores in summer. Twenty of 29 summer RISQI values are above the regression line, indicating that even at a given body condition score the RISQI value is generally above the predicted value based on body condition score during the summer season. RISQI = reciprocal of the square root of insulin, BCS = body condition score.
REFERENCES


