Evaluation of Diagnostic Tests for Insulin Dysregulation in Adult Light-Breed Horses

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By
Laura K. Dunbar, B.S., D.V.M.

Graduate Program in Comparative and Veterinary Medicine

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Master’s Examination Committee:
Assistant Professor Teresa A. Burns, Advisor
Associate Professor Ramiro E. Toribio
Associate Professor James K. Belknap
Abstract

Several tests have been evaluated in horses for quantifying insulin dysregulation in order to support a diagnosis of Equine Metabolic Syndrome (EMS). Comparing the performance of these tests in the same horses will provide clarification of their accuracy in the diagnosis of equine insulin dysregulation. The objective of the following investigations was to evaluate the agreement between basal serum insulin concentrations (BIC), the oral sugar test (OST), the combined glucose-insulin test (CGIT), and the frequently sampled insulin-modified intravenous glucose tolerance test (FSIGTT) in twelve healthy, light-breed horses. Each of the above tests was performed once, in randomized order on 12 adult horses, and the results were compared.

Minimal Model analysis of the FSIGTT was considered the reference standard and classified 7 horses as insulin resistant (IR) and 5 as insulin sensitive (IS). In contrast, BIC and OST assessment using conventional cutoff values classified all horses as IS. Kappa coefficients, measuring agreement among BIC, OST, CGIT, and FSIGTT were poor to fair. Sensitivity of the CGIT (positive phase duration of the glucose curve > 45 minutes) was 85.7% and specificity was 40%, while CGIT ([insulin]_{45} > 100 \mu IU/ml) sensitivity and specificity were 28.5% and 100%, respectively. Area under the glucose curve (AUC_{g0-120}) was significantly correlated among the OST, CGIT, and FSIGTT, but Bland-Altman method and Lin’s concordance coefficient showed lack of agreement. These results suggest that current criteria for diagnosis of insulin dysregulation using BIC
and the OST are highly specific but lack sensitivity. The CGIT displayed better sensitivity and specificity; however, modifications may be necessary to improve agreement with Minimal Model analysis.

Proxy measurements of insulin sensitivity calculated from fasting insulin and glucose concentrations have been correlated to Minimal Model analysis of the FSIGTT in horses and humans, but correlation with several morphometric parameters measured in the same cohort of equids has not been reported. Morphometric measurements (body condition score [BCS], cresty neck score [CNS], calculated body weight [BW], mean neck circumference [MNC], retroperitoneal fat depth [RFD], and tailhead fat depth [TFD]) were obtained from 12 horses in which Minimal Model analysis was performed. Proxy measurements of insulin resistance (reciprocal of the square root of insulin [RISQI], modified insulin to glucose ratio [MIRG], quantitative insulin sensitivity check index [QUICKI], insulin to glucose ratio [I:G], and homeostasis model assessment [HOMA]) were calculated.

BCS, CNS, TFD, and MNC were significantly correlated with proxy measurements. However, no significant correlation was observed between morphometric data and insulin sensitivity ($S_I$), and no significant difference was observed between insulin resistant (IR) and insulin sensitive (IS) horses with respect to morphometric parameters or proxies. Significant correlations were observed between Minimal Model parameters and proxy measurements.

Many morphometric measurements correlated with proxy measurements of insulin sensitivity, and proxies were significantly correlated to $S_I$. CNS displayed the
strongest correlation to proxies, consistent with its reported utility in identification of horses at risk for EMS.
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Vita

2004………………………………………………………………Pittsford-Mendon High School, Pittsford, NY
2008………………………………………………………………B.S., Animal Science, Clemson University, Clemson, SC
2012………………………………………………………………D.V.M, Cornell University, Ithaca, NY
2012-2013…………………………………………………………..Intern, Rhinebeck Equine, LLC, Rhinebeck, NY
2013-2016…………………………………………………………..Resident, Equine Internal Medicine, The Ohio State University, Columbus, OH

Fields of Study

Major Field: Comparative and Veterinary Medicine
Table of Contents

Abstract ........................................................................................................................................... ii

Acknowledgements .................................................................................................................... v

Vita ................................................................................................................................................. vii

List of Tables ............................................................................................................................ x

Chapter 1: Introduction ............................................................................................................ 1

1.1: Equine Metabolic Syndrome ................................................................................................. 1

1.2: Insulin dysregulation and laminitis ....................................................................................... 4

1.3 Assessment of insulin dysregulation ..................................................................................... 7

1.4 Comparison of dynamic tests for insulin sensitivity ............................................................... 14

1.5 Morphometric measurements of obesity and adiposity ...................................................... 16

Chapter 2: Evaluation of four tests for insulin dysregulation in adult light-breed horses ........... 18

2.1 Materials and Methods ........................................................................................................ 18

2.2 Results .................................................................................................................................. 22

2.3 Discussion ............................................................................................................................ 23

Chapter 3: Comparison of morphometric data and proxy measurements of insulin resistance to Minimal Model analysis in adult light-breed horses ..................................................... 34

3.1 Materials and Methods ........................................................................................................ 34

3.2 Results .................................................................................................................................. 36
3.3 Discussion ..................................................................................................................37
Chapter 4: Summary .......................................................................................................48
References ......................................................................................................................50
List of Tables

Table 2.1. Descriptive statistics and Minimal Model parameters of study horses (mean ± SD) and IR and IS horses (median and range)..............................................................29

Table 2.2. Area under the glucose curve from 0-120 minutes comparisons for the FSIGTT, CGIT, and OST..................................................................................................................31

Table 2.3. Sensitivity, specificity, positive predictive value, and negative predictive value of BIC, the OST, and CGIT compared to gold standard (FSIGTT).................................32

Table 2.4. Cohen’s Kappa coefficients assessing agreement with gold standard (FSIGTT).........................................................................................................................33

Table 3.1. Morphometric parameters recorded on 12 adult light-breed horses........41

Table 3.2. Calculation of proxy measurement of insulin resistance....................42

Table 3.3. Morphometric data, proxy measurements of insulin resistance, and Minimal Model parameters in all study horses (mean ± SD), IS, and IR horses (median and range).........................................................................................................................43

Table 3.4. Correlation of proxy measurements of insulin sensitivity to Minimal Model parameters (S₁, AIRg).................................................................45

Table 3.5. Linear correlations between morphometric data and proxy measurements of insulin resistance.........................................................46
1.1 Equine Metabolic Syndrome

Equine Metabolic Syndrome (EMS) is currently defined by the American College of Veterinary Medicine Consensus Statement to include insulin dysregulation (measured by basal hyperinsulinemia or abnormal glucose and insulin responses to intravenous or oral glucose and insulin), increased adiposity or generalized obesity, and a predisposition to laminitis. This definition has recently been modified to include several other phenotypic abnormalities including dyslipidemia (including hypertriglyceridemia) and adipokine dysregulation (hyperleptinemia and decreased adiponectin). Additional factors that may or may not contribute to the EMS phenotype include hypertension and systemic inflammation. Hyperinsulinemia currently is thought to be of primary importance in the development of EMS and subsequent laminitis. Investigation into the other metabolic and phenotypic abnormalities associated with EMS and laminitis risk has been driven by similarities between EMS and the human metabolic syndrome (MetS), which includes a group of abnormalities including obesity, dyslipidemia, glucose intolerance, and hypertension, which are associated with an increased risk of cardiovascular disease and type 2 diabetes mellitus.

Obesity has been included in the definition of EMS from the time when it was first recognized, and was a useful predictor of laminitis in ponies. In addition, measurements of regional fat accumulations have been associated with insulin resistance
and the development of pasture-associated laminitis in horses and ponies.\textsuperscript{5-7} Further, obesity and body fat percentage have been inversely associated with insulin sensitivity in horses.\textsuperscript{8} Regional adiposity, specifically mean neck circumference (MNC), as well as BCS, have been correlated with area under the insulin and glucose curves during the combined glucose and insulin test (CGIT) in a group of horses,\textsuperscript{7} while BCS (\(\geq 7\)), cresty neck score (CNS) (\(\geq 4\)), and neck circumference: height ratio (\(> 0.71\)) were useful in predicting clinical laminitis in a group of ponies.\textsuperscript{5} The association between obesity, regional adiposity, and laminitis is clear based on multiple studies. However, it is not yet clear whether obesity or regional adiposity is a causal factor in EMS or simply a marker of the associated metabolic abnormalities, in which case, it may not be appropriate to use obesity or regional adiposity measurements as a diagnostic criterion or in assessing response to treatment, as it may be an effect of the metabolic abnormalities rather than a cause.\textsuperscript{3} While a recent case series showed that a reduction in BCS due to calorie restriction and exercise caused decreases in basal insulin concentrations (BIC) and insulin responses during a CGIT,\textsuperscript{9} recent work found that morphometric measurements and BCS did not discriminate between ponies previously diagnosed with laminitis and without a history of laminitis, which the authors suggested was caused by persistence of metabolic abnormalities despite weight loss.\textsuperscript{3} Studies have shown varied results on decreasing insulin sensitivity through weight gain. Insulin sensitivity was not different in thoroughbred geldings after controlled weight gain, but decreased in response to a high starch and sugar diet compared to a high fat diet.\textsuperscript{10} In another study, diet-induced weight gain (maintenance of a BCS \(\geq 7\)) caused a decrease in insulin sensitivity, resulting in hyperinsulinemia and hyperleptinemia in horses.\textsuperscript{11} The interaction between obesity,
adiposity, and the metabolic abnormalities associated with EMS is not well-defined, but obesity and measures of increased adiposity have been associated with hyperinsulinemia, hyperleptinemia, dyslipidemia, and increases in inflammatory cytokines,\(^7,8,12\) and these metabolic abnormalities have also been associated with clinical laminitis in ponies.\(^5\) It appears that a combination of genetic and environmental factors are involved in determining insulin sensitivity, dyslipidemia, and predisposition to laminitis.

Other metabolic aberrations included in the MetS are dyslipidemia and adipokine dysregulation. Plasma triglyceride concentrations were significantly higher in a group of previously laminitic (PL) and clinically laminitic (CL) ponies, compared to non-laminitic (NL) ponies, although non-esterified fatty acids (NEFA) and glucose concentrations were not different. Plasma triglyceride concentrations, along with BCS, reciprocal of the square root of insulin (RISQI), and modified insulin to glucose ratio (MIRG) differentiated PL from NL ponies.\(^6\) In another study, although plasma triglyceride concentrations distinguished PL from NL ponies, they were not useful in predicting clinical laminitis.\(^5\) Evidence of dyslipidemia was also found in a group of obese, insulin resistant horses when compared to non-obese horses, including increased NEFA, very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) concentrations.\(^7\) Studies in humans and horses have demonstrated that adipose tissue plays an active role in energy homeostasis and insulin regulation through the secretion of adipokines. Leptin is an adipokine important in modulating body weight and satiety. In humans, increased adiposity is proportional to increases in plasma leptin concentrations and reduced insulin sensitivity, which may be related to leptin resistance. Leptin was significantly correlated with fat mass, resting insulin and area under the insulin and glucose curves during a
CGIT in horses. Additionally, positive correlations between BCS, leptin, glucose, and insulin concentrations were found in donkeys. Adiponectin is inversely related to adiposity in humans, and decreased in humans with insulin resistance, which has also been shown in horses. The association between adipokines and dyslipidemia appears important in identifying the metabolic phenotype, but the association with laminitis is unclear.

1.2 Insulin dysregulation and laminitis

The underlying pathophysiology relating EMS, insulin dysregulation, and laminitis is far from being clear, but hyperinsulinemia is a known risk factor for pasture-associated laminitis; further, laminitis recently has been experimentally induced through induction of supraphysiologic concentrations of insulin. Therefore, insulin dysregulation is likely to be involved in the pathogenesis of pasture-associated laminitis. Insulin dysregulation may include overall tissue insulin resistance, postprandial hyperinsulinemia, abnormal energy disposal, and other undetermined mechanisms. It is our current understanding that one or more of these factors may be more important to the pathogenesis of EMS and pasture-associated laminitis in different horses (i.e., there may be multiple mechanisms that result in the same phenotype of insulin dysregulation). Hyperinsulinemia may be caused by increased insulin secretion or decreased insulin clearance, both of which may either contribute to or be a result of insulin resistance. Decreased tissue sensitivity has long been thought to result in compensatory increases in insulin secretion, but decreased hepatic clearance of insulin is also associated with insulin resistance. Low connecting-peptide (C-peptide) to insulin ratios have been detected in horses with EMS. C-peptide is a portion of the proinsulin prohormone that is cleaved
before secretion from pancreatic β cells. It is released in equal amounts to insulin but has a longer half life in circulation, so concentrations of C-peptide reflect pancreatic secretion and are used to evaluate hepatic clearance of insulin.²

Postprandial hyperinsulinemia recently has been identified in horses and ponies that have normal glucose and insulin responses to IV glucose and insulin challenge, suggesting that other factors may play a larger role in insulin dysregulation in these individuals.² Incretin hormones are secreted by intestinal cells in response to ingested sugars to increase insulin secretion from β cells and delay gastric emptying. Therefore, increases in their secretion or delay in their degradation may lead to postprandial hyperinsulinemia. Hyperinsulinemia itself may be either a cause or consequence of insulin resistance, as chronic hyperinsulinemia may lead to receptor downregulation or desensitization, leading to decreased insulin sensitivity. Additionally, obesity may contribute to or be associated with insulin resistance, but may also result from insulin dysregulation and its effects on lipid metabolism.²

The association of insulin dysregulation and pasture-associated laminitis (PAL) has been identified through observational studies showing positive relationships between several metabolic abnormalities (including hyperinsulinemia and hyperleptinemia), obesity, and neck crest adiposity and the development of PAL.⁵ When pasture is rich in non-structural carbohydrates, these carbohydrates may be delivered to the hindgut for rapid fermentation, which could lead to the development of laminitis. However, the increased susceptibility of certain horses and ponies (i.e. those with EMS) to the development of PAL has lead to observations of the direct effect of prolonged hyperinsulinemia on the laminae. The hyperinsulinemic euglycemic clamp (HEC)
method produces prolonged hyperinsulinemia while maintaining constant blood glucose concentrations through simultaneous infusion of glucose. The HEC produces supraphysiologic insulin concentrations and results in laminitis after 48 hours, even in insulin sensitive horses. The hyperinsulinemia model of laminitis causes lengthening of the secondary epidermal lamellae (SEL) and variable separation of the lamellar basement membranes from the dermis of the third phalanx.\textsuperscript{16,19,20} Similar histopathological findings were found in cases of naturally-occurring endocrinopathic laminitis, although more chronic changes were observed, including irregular SEL fusion and replacement with keratinized tissue, separation of epithelial islands, and increased numbers of apoptotic cells.\textsuperscript{21} Additionally, changes in the epidermal lamellae after induction of laminitis with hyperinsulinemia is similar, but different from the oligofructose model, particularly with respect to leukocyte infiltration, suggesting that induction of laminitis via hyperinsulinemia is less inflammatory than via oligofructose or black walnut extract.\textsuperscript{22} Another study also showed a lack of inflammatory events in laminitis associated with high carbohydrate feeding in ponies.\textsuperscript{23}

Aside from the induction of laminitis via the HEC, the pathogenesis of hyperinsulincic laminitis is not understood. Alterations in glucose metabolism have been hypothesized to play a role in the development of laminitis, although recent studies have suggested that glucose transport into the lamellae is primarily insulin-independent, and therefore, insulin resistance may not be a requirement for induction of laminitis. However, differences in expression of various glucose transport proteins (GLUTs) have been shown during metabolic dysfunction in horses, suggesting that alterations do occur with systemic aberrations of glucose and insulin concentrations.\textsuperscript{24} Another hypothesis is
that insulin mediates vascular dysfunction within the lamellae through its actions on nitric oxide (NO) and endothelin-1 (ET-1). The observation of increased mitosis and cellular proliferation within the laminae has implicated altered growth factor signaling as a possible mechanism for hyperinsulinemic laminitis. Although the insulin receptor was only found within endothelial cells and not within the laminar basal epithelial cells (LBEC), the insulin-like growth factor-1 (IGF-1) receptor was found in many cell types within the laminae, including the LBEC. The IGF-1 receptor has significant homology to the insulin receptor and therefore binds insulin at two-fold lower affinity than the insulin receptor. However, the IGF-1 receptor is capable of binding insulin at supraphysiologic concentrations, as in the hyperinsulinemic model of laminitis. The IGF-1 receptor has similar, but not identical downstream effects to the insulin receptor, causing the IGF-1 receptor to exert greater mitogenic and antiapoptotic effects, while the insulin receptor initiates downstream signaling resulting in metabolic regulation. The IGF-1 receptor is also a tyrosine kinase receptor, and its activation leads to phosphorylation of insulin receptor substrate (IRS) proteins, as in the insulin receptor. These proteins activate signaling via the phosphatidylinositol-3-kinase (PI3K), which, along with mammalian target of rapamycin complex 2 (mTORC2) activation, ultimately leads to phosphorylation of AKT, promoting cell survival, inhibits apoptosis, and promotes cellular proliferation. IRS proteins also activate parallel pathways including RAS, RAF, and mitogen-activated protein kinase (MAPK) isoforms including extracellular signal-regulated kinases (ERKs), p38, and c-jun N-terminal kinase (JNK), which promote gene transcription leading to cellular proliferation. These downstream signaling events may lead to the cellular proliferation associated with hyperinsulinemic
laminitis and, ultimately laminar failure. Additionally, the IGF-1 receptor has been characterized in horses undergoing an HEC, and the authors found IGF-1 receptor downregulation during the HEC, suggesting its activation during this model of laminitis. Current work continues to focus on the hyperinsulinemic model of induction of laminitis, its differences from other mechanisms of laminitis induction, and pathophysiology.

1.3 Assessment of insulin dysregulation

Quantifying an individual horse’s degree of insulin dysregulation, risk of laminitis, and establishing a diagnosis of EMS can provide a rationale for encouraging compliance with often inconvenient dietary, management, and medical interventions that promote weight loss and improved insulin sensitivity. Current strategies for diagnosis include a clinical suspicion of the EMS phenotype and screening tests based on fasting insulin concentrations. However, serum insulin and glucose concentrations may be influenced by many factors including sampling time, stress, drugs (e.g. α2-agonists, corticosteroids), and feeding, and therefore may not be well-correlated with insulin sensitivity. Furthermore, IR horses rarely may develop inadequate compensatory insulin secretion, or type II diabetes mellitus, which may not be detected by screening tests.

Fasting insulin concentrations have been widely used in large epidemiological studies to assess laminitis risk in ponies and horses due to the ease of obtaining a single blood sample. Fasting insulin concentrations have been well correlated with obesity, adiposity, and laminitis risk, but were not useful in screening for ponies at risk for laminitis on pasture. Insulin concentrations typically fell within the reference range even in ponies predisposed to laminitis. However, a later study found that serum insulin and leptin
values were useful in predicting laminitis in a group of ponies using an insulin cut-off value of > 32 µIU/ml.\textsuperscript{5} The reason for this discrepancy is unknown, however, laminitic ponies had significantly higher serum insulin values on the grass diet compared to a hay diet, so differences in diet may alter results.\textsuperscript{32} Fasting hyperinsulinemia currently is defined as a fasting insulin concentration greater than 20 µIU/ml in horses.\textsuperscript{33} Proxy measurements of insulin sensitivity also may be calculated based on glucose and insulin concentrations, and these proxies have been correlated to gold standard tests for insulin resistance in humans\textsuperscript{34} and horses, and shown to have high specificity but low sensitivity.\textsuperscript{35} Proxy measurements have also been shown to be predictive of laminitis, particularly in association with obesity and hypertriglyceridemia.\textsuperscript{6} Gold standard laboratory tests for insulin resistance include the hyperinsulinemic-euglycemic clamp (HEC) method and the frequently-sampled insulin-modified intravenous glucose tolerance test (FSIGTT) with minimal model analysis, which both provide a quantitative assessment of insulin and glucose dynamics.\textsuperscript{34} However, these gold standard tests are not practical for use in clinical cases because of the equipment, time, and cost necessary to perform them.

*The Hyperinsulinemic-Euglycemic Clamp*

The HEC requires infusion of IV insulin at a predetermined rate, resulting in hyperinsulinemia. While IV dextrose is concurrently administered at varying rates to maintain blood glucose concentration within a narrow range (euglycemia). After steady state is achieved, hyperinsulinemia suppresses hepatic glucose production and increases glucose disposal in skeletal muscle and adipose tissue. Since hepatic glucose production is suppressed, the glucose infusion rate is equal to the glucose disposal rate. The glucose
disposal rate is then used to estimate insulin sensitivity. Glucose disposal rate was found to be lower in obese mature mares compared to feed restricted or lean mares, and lower in ponies compared to Dutch Warmblood horses, consistent with their predisposition to IR and laminitis. Glucose disposal rate also increased after 7 days of light exercise, and decreased to pre-exercise values after 9 days without exercise. Fasting insulin concentrations in the same horses were not indicative of a change in insulin sensitivity, suggesting that the glucose disposal rate calculated during the HEC is a more sensitive indicator of insulin sensitivity changes compared to fasting insulin concentrations. Although the HEC is regarded as one of the gold standard tests for insulin sensitivity and an accurate way to quantify insulin and glucose dynamics, one criticism of this method is that glucose disposal rates are calculated at nonphysiologic insulin concentrations, which might not make comparison of this method to others feasible. Additionally, the FSIGTT allows for calculation of additional parameters including the effect of glucose on its own disappearance from plasma and the acute insulin response to a glucose bolus, which is the effect of insulin on glucose within the first ten minutes after insulin administration. However, the repeatability of the HEC was superior to the FSIGTT (interday coefficients of variation 14% and 24%, respectively).

The Frequently-Sampled Insulin-modified Intravenous Glucose Tolerance Test

The frequently-sampled insulin-modified intravenous glucose tolerance test (FSIGTT) is an indirect measure of insulin sensitivity through minimal model analysis. IV dextrose is administered, followed by exogenous insulin 20 minutes after the glucose bolus, and glucose and insulin concentrations are measured at frequent intervals. The minimal model uses mathematical modeling to describe first the factors that determine
the restoration of plasma glucose after the initial dextrose injection (including the effect of glucose itself as well as the effect of endogenous insulin to restore euglycemia). The second equation of the minimal model represents the movement of insulin from the plasma into the interstitial compartment (i.e. where it acts to skeletal muscle and adipose to increase glucose uptake and decrease lipolysis, respectively).\textsuperscript{38} Insulin sensitivity ($S_I$) can then be defined as the effect of insulin to catalyze the disappearance of glucose from plasma, and calculated as the partial derivative of insulin and glucose concentrations upon net glucose disappearance. One advantage of the minimal model is that additional parameters can be calculated, including glucose effectiveness ($S_g$), the acute insulin response to glucose (AIR\textsubscript{g}), and the disposition index (DI). $S_g$ is defined as the effect of glucose on its own disappearance, independent of insulin’s effect. AIR\textsubscript{g} is defined as the first-phase insulin response to the increase in glucose, and DI is the ability of the pancreatic beta cells to compensate for insulin resistance by increasing beta cell responsiveness to maintain normal glucose tolerance. This is now considered the hyperbolic law of glucose tolerance, in which a hyperbolic curve represents the ability of the $\beta$ cells to upregulate the secretion of insulin in response to increasing insulin resistance.\textsuperscript{38} The FSIGTT has been applied to horses in many studies previously. It was first performed by Hoffman \textit{et al.} using dosages for dextrose and insulin from human studies,\textsuperscript{12} and later optimized by Tóth \textit{et al.} to reduce urinary glucose spillover, thus making $S_g$ and $S_I$ more accurate estimates, as the minimal model does not account for urinary glucose loss. Tóth \textit{et al.} found that AUC\textsubscript{g} reached a plateau at dextrose doses $\geq$ 200 mg/kg. Therefore, doses at or above this level are not necessary for performing the FSIGTT in horses, and lower doses are preferable to maintain accuracy. Interestingly,
the investigators found that increasing insulin dosages linearly decreased $AUC_g$, but no significant differences were found in any other variables, including $AUC_i$, $S_I$, $S_g$, $AIR_g$, and $DI$. Dosages for dextrose and insulin were selected for the current study based on these findings (150 mg/kg 50% dextrose IV and 0.1 U/kg regular insulin IV).

The Combined Glucose-Insulin Test

Other dynamic tests have been more recently developed in horses, including the oral sugar test (OST)\textsuperscript{2,40} and combined glucose and insulin test (CGIT).\textsuperscript{30} The CGIT was originally developed by Eiler et al. as a combination of the traditional IV glucose tolerance test and insulin sensitivity test, which would provide more information than the individual tests, but less technically demanding and more useful for use by practitioners. The test is a measure of whole body insulin sensitivity by determining the individual’s response to IV dextrose and insulin. Dextrose is administered at 150 mg/kg IV, which was found to increase plasma glucose concentrations by 100% and reach the proposed renal threshold and transport maximum. This bolus is immediately followed by 0.1 U/kg regular insulin IV, which causes an approximately 300-times increase in insulin concentration and induces a negative phase in the glucose curve in normal horses.\textsuperscript{30} The CGIT has been used to characterize insulin sensitivity in various studies, using arbitrary cut-off values including the duration of the positive phase of the glucose curve and insulin concentration at 45 minutes.\textsuperscript{7,9,41} A positive phase duration greater than 45 minutes or an insulin concentration greater than 100 µIU/ml at 45 minutes after dextrose and insulin administration is considered consistent with insulin resistance.\textsuperscript{33} The repeatability of the CGIT also has been assessed in two breeds (Icelandic and Standardbred horses). The authors found low repeatability of the glucose curve
parameters of the CGIT, and higher repeatability of the insulin curves,\textsuperscript{42} in agreement with a more recent study.\textsuperscript{9} They cautioned against using cut-off values from the glucose curve alone as criteria for diagnosing insulin resistance in horses. Additionally, the authors proposed future studies measuring the accuracy of the CGIT against the FSIGTT and HEC, as they has only assessed repeatability.\textsuperscript{42} Another assessment of the CGIT looked at the effect of season on CGIT parameters, as variation in endogenous hormones as well as dietary changes occur across seasons, which may affect the outcome of testing the same individual. This study showed repeatability across season for the parameters used to diagnose insulin resistance, although this study used duration of the positive phase of the glucose curve greater than 45 minutes or failure of insulin to return to baseline by 75 minutes as criteria for insulin resistance.\textsuperscript{43}

*The Oral Sugar Test*

The oral sugar test was most recently developed in order to evaluate the impact of intestinal absorption of sugar on insulin and glucose responses, as many horses of the EMS phenotype are initially diagnosed due to an episode of laminitis associated with ingestion of sugar or starches on pasture. While fasting insulin concentrations have been used to determine resting hyperinsulinemia, they appear to be an insensitive marker of insulin resistance, and glucose and insulin responses to a controlled oral sugar load may better characterize the physiologic circumstances leading to PAL in horses and ponies with EMS. Additionally, postprandial hyperinsulinemia has been found in horses with normal responses to IV glucose and insulin tolerance tests.\textsuperscript{2} The OST was first described by Schuver et al., in which 0.15 ml/kg corn syrup was administered orally via 60-ml syringe, which was estimated to provide 150 mg/kg dextrose-derived digestible sugars.
Plasma glucose and serum insulin concentrations were then measured at various time points after corn syrup administration. Values were compared between an EMS group and a control group, as well as between the OST and results of an IV glucose tolerance test on the same individuals. AUC_g and AUC_i values of the OST were higher for EMS compared to control horses, and positive correlations were found between AUC_g and AUC_i values of the IV glucose tolerance test and the OST. Cut-off values recommended for the oral sugar test include a serum insulin concentration greater than 60 µIU/ml at 60-90 minutes after oral sugar administration.

Proxy measurements of insulin sensitivity

Proxy measurements of insulin sensitivity are widely used in human medicine and correlate well with quantitative measurements of insulin sensitivity. The use of proxy measurements of insulin resistance is attractive as a simple and practical way to determine insulin sensitivity based on the insulin and glucose concentrations of a single sample. The glucose-to-insulin ratio (G:I) and insulin-to-glucose ratio (I:G) are the simplest methods of estimating insulin sensitivity and insulin secretion, respectively. These have been correlated with quantitative assessments of insulin sensitivity in humans measured by the hyperinsulinemic-euglycemic clamp (HEC) and to insulin secretion by the hyperglycemic clamp method. Homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) correlate well with direct measures of insulin resistance via the HEC and minimal model analysis of the FSIGTT in humans. QUICKI may correlate better with insulin sensitivity derived from the HEC in comparison to insulin sensitivity from minimal model analysis or HOMA. QUICKI also appears to be a better surrogate of insulin sensitivity assessment over a wide range of
insulin sensitivities, including subjects with significant insulin resistance, obesity and hypertension. Reciprocal of the square root of insulin (RISQI) and Modified insulin-glucose ratio (MIRG) have previously been correlated with results of insulin resistance testing in healthy horses, and reference quintiles were created for interpretation of these proxy measurements in comparison to minimal model analysis. Both RISQI and MIRG were found to adequately predict SI and AIR\textsubscript{g}, respectively, and differentiate between compensated and uncompensated insulin resistance. Additionally, these proxies were found to improve with dietary adaptation of one horse to a low glycemic diet, suggesting that they may be useful for serial monitoring response to dietary and management interventions. RISQI, QUICKI, and modified insulin to glucose ratio for ponies (MIRGP) were also able to distinguish between normal and previously laminitic ponies, although MIRG was not accurate in predicting laminitis in this and another study. Additionally, these proxies were not useful in predicting individual laminitis susceptibility, suggesting that they may not be applicable for use in clinical cases. Many of the proxy measurements have not been compared directly in a group of insulin resistant (IR) and insulin sensitive (IS) horses, although reference quintiles were created in a group of normal horses.

1.4 Comparison of dynamic tests for insulin sensitivity

Dynamic tests of insulin dysregulation have been minimally compared directly in horses. A recent study compared the HEC, FSIGTT, and oral glucose tolerance test (OGTT) in a group of healthy standardbred horses. Estimates of insulin sensitivity from the HEC and FSIGTT were highly correlated, although not equivalent. Additionally, calculated indices estimating insulin sensitivity, as well as area under the curve, peak
insulin concentration, and insulin concentration at 120 minutes were well correlated with the HEC and FSIGTT estimates of insulin sensitivity in healthy horses. The authors recommended that future studies focus on comparing results among individuals with varying body condition scores and insulin sensitivities.\textsuperscript{47}

The OST and the insulin response to dexamethasone test (IRDT) were compared to the HEC in a group of insulin sensitive quarter horses of varying body condition scores.\textsuperscript{48} No association was found between variables obtained from these three testing methods, suggesting that differences in components of insulin regulation including β cell response to glucose, enteral glucose absorption, and tissue insulin sensitivity might contribute to overall insulin regulation to varying degrees. The various dynamic tests for insulin dysregulation likely reflect and test for different components of insulin and glucose homeostasis.\textsuperscript{48} This could easily account for variation in these testing modalities, and it is still unclear which component is most important and which testing method is most accurate in determining EMS and laminitis susceptibility in ponies and horses.

When the OST was initially developed, its results were compared to an intravenous glucose tolerance test (IVGTT). Area under the glucose and insulin curve values were positively correlated between the OST and IVGTT, and higher in EMS horses compared to control horses.\textsuperscript{40}

The OST and the in-feed oral glucose test (OGT) have also recently been compared in 8 horses and 5 ponies.\textsuperscript{49} The study found that the OGT identified a larger number of ponies as insulin resistant than the OST, and all horses were classified as insulin sensitive. Without a direct measure of insulin sensitivity, the study was unable to determine which test more accurately identifies ponies and horses as insulin sensitive or
insulin resistant. Additionally, previously used but unvalidated cut-off values were used to classify the horses and ponies, which may or may not be accurate. The authors recommended further work to establish which test is more accurate in determining insulin sensitivity in horses and ponies in a clinical setting, and the establishment of breed-specific reference ranges or cut-off values may be useful.49

A small number of studies have been performed that directly compare dynamic methods of testing for insulin and glucose dysregulation. The results of these studies indicate that further work is necessary to validate and standardize insulin dysregulation testing. However, the currently available tests vary in their clinical feasibility, making certain tests impractical for clinical practice. Additionally, further work in defining normal reference ranges and validated cut-off values is necessary for both quantitative tests of insulin sensitivity and clinically-feasible tests of insulin dysregulation. The study reported here aims to directly compare four methods of insulin dysregulation testing, including one method of quantitative insulin sensitivity testing (the FSIGTT), and examine how the tests classify the same horses in terms of insulin sensitivity using currently established criteria.

1.5 Morphometric measurements of obesity and adiposity

Morphometric measurements of adiposity have been used to characterize horses at risk for EMS, providing a means of monitoring risk and response to treatment. BCS has been widely used to assess obesity in horses and ponies.50 However, objective morphometric parameters may be a more reliable assessment of obesity, and certain measurements may differentiate regional adiposity from generalized obesity. Ponies with increased BCS and cresty neck score (CNS) were more likely to be hyperinsulinemic, but
the same association was not found in horses. Additionally, laminitic episodes in ponies were significantly associated with CNS and BCS, suggesting that these scoring systems are useful in the assessment of laminitis risk in ponies, but it is not known whether this applies to light-breed horses as well. Previous studies have also found that BCS correlated closely with AUCg of the CGIT. Mean neck circumference (MNC) was also well correlated to AUCi during the same test, suggesting that MNC might be an additional measurement useful in identifying horses at risk for insulin resistance.

Ultrasonic measurements of subcutaneous fat depth can be used to predict percent body fat in horses and ponies, although these calculations have been based on studies in a limited number of horses and ponies, and fat depth measurements have not been standardized. Additionally, fat measurements may vary across different breeds. However, the measurement of ultrasonographic fat depth is attractive as an easily obtained and quantitative assessment of adiposity in horses and ponies. Its use as an adjunct in the diagnosis of EMS and predisposition to laminitis is unknown at this time, but serial monitoring of individual equids may provide additional information and a more objective assessment than BCS.
Chapter 2: Evaluation of four diagnostic tests for insulin dysregulation in adult light-breed horses

2.1 Materials and Methods

Experimental Design

All experimental procedures were approved by the OSU Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Twelve light-breed horses owned by The Ohio State University College of Veterinary Medicine and housed at the college teaching and research farm were studied in a prospective, randomized experimental study. All horses were housed on pasture with access to grass hay ad libitum with no concentrate feeding. The horses were placed in a stall the night before testing and allowed access to free-choice grass hay and water overnight; a muzzle was applied the next morning 2 hours before testing. Body weight was calculated using a formula for estimation of body weight from girth and body length measurements: body weight (kg) = (girth^2 * length [cm]) / 11900 for dosage calculations. Body condition score (BCS) and cresty neck score (CNS) were recorded as the average of 2 observers (LD and TB) based on the Henneke scoring system and the CNS. Each of the 12 horses was assigned an order of testing by use of a random
number generator. Testing took place in 3 sessions (with the OST, CGIT, or FSIGTT performed in each of the 12 horses during each session). Each testing session took place over a period of 2 days (6 horses were tested per day), with a period of 8-12 days between testing sessions. Horses were placed in stalls the night before testing and returned to the herd between tests. Testing took place during a 3-week period from April to May 2014.

**Insulin Sensitivity Testing**

The oral sugar test (OST) was performed as previously described.\textsuperscript{40} Briefly, a blood sample was collected by direct jugular venipuncture at time 0. Light corn syrup\textsuperscript{i} was administered PO using a dosing syringe at a dosage of 0.15 ml/kg body weight, which is estimated to contain 150 mg/kg glucose-based digestible carbohydrates.\textsuperscript{33} Subsequent blood samples (6-12 ml per time point) were collected by direct jugular venipuncture at 30, 60, 90, and 120 minutes after administration of light corn syrup for measurement of blood glucose and serum insulin concentrations.

The combined glucose-insulin test (CGIT) was performed as described previously.\textsuperscript{30} An IV catheter\textsuperscript{ii} was placed in a jugular vein the night before testing to minimize stress of catheter placement on test results. Blood samples (6-12 ml per time point) were collected from and dextrose and insulin administered through the IV catheter, which was maintained patent by irrigation with heparinized saline after collection of each sample. A minimum of 10 ml blood was collected and discarded before each sample collection. After baseline blood sample collection, 50% dextrose solution\textsuperscript{iii} (150 mg/kg IV) immediately followed by regular insulin\textsuperscript{iv} (0.1 U/kg IV) diluted in 3 ml 0.9% sodium chloride solution was administered rapidly over 1-2 minutes. Blood samples were
collected at baseline (time 0), and 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, and 120 minutes post-dextrose and -insulin administration. Blood glucose concentration was measured at all time points, and serum insulin concentration was measured at time 0 and 45 minutes.

The FSIGTT was performed as described previously. Two jugular venous catheters were placed the night before testing. One catheter was utilized for blood collection, and the other was used for dextrose and insulin administration. Blood samples were collected 10, 5 and 1 minute before infusion of 50% dextrose solution (150 mg/kg, rapidly IV) at time 0. Blood samples were collected (6-12 ml per time point) at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after 50% dextrose infusion. Regular insulin (0.1 U/kg, IV) diluted in 3 ml 0.9% sodium chloride solution was administered 20 minutes after the 50% dextrose infusion. Blood glucose concentration was measured at all time points, and serum insulin concentration was measured 1 minute before, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 120, 150, and 180 minutes after 50% dextrose administration.

**Blood Glucose and Insulin Concentrations**

Blood glucose concentrations were measured with a portable glucometer validated for use in horses. Blood samples were collected in EDTA and silicone-coated tubes and remained on ice until centrifugation for harvesting of plasma and serum. Plasma and serum samples were stored at -80°C until analysis. Serum insulin concentrations were measured using a commercially-available radioimmunoassay validated for use in horses.

**Data Analysis**
Basal insulin and glucose results were determined by calculating the mean of the baseline insulin and glucose concentrations measured before each test. Area under the glucose curve concentration (AUC\textsubscript{g0-120}) was calculated for the OST, CGIT, and FSIGTT. The CGIT parameters calculated included positive phase duration of the glucose curve (PP-D\textsubscript{glu})\textsuperscript{ix} and insulin concentration at 45 minutes ([Ins]\textsubscript{45}). Insulin and glucose data from the FSIGTT were analyzed using minimal model analysis with computer software.\textsuperscript{x} Calculated parameters included insulin sensitivity (S\textsubscript{I}), glucose effectiveness (S\textsubscript{g}), acute insulin response to glucose (AIR\textsubscript{g}), and disposition index (DI).\textsuperscript{38,56}

Quantitative variables were assessed for normality using the D’Agostino & Pearson omnibus normality test. Insulin resistant (IR) was defined as S\textsubscript{I} < 1.0 \times 10^{-4} \text{ L} \cdot \text{mU}^{-1} \cdot \text{min}^{-1} from minimal model analysis.\textsuperscript{18,57,58} Cut-off values for each test to classify horses as IR or insulin sensitive (IS) were selected based on those used clinically; IR was defined as a BIC > 20 \text{ µIU/ml}, insulin concentration > 60 \text{ µIU/ml} at 60 or 90 minutes during the OST, and PP-D\textsubscript{glu} > 45 minutes or [Ins]\textsubscript{45} > 100 \text{ µIU/ml} during the CGIT.\textsuperscript{33}

The AUC\textsubscript{g0-120} values were compared among the OST, CGIT, and FSIGTT using Pearson’s linear correlation, Bland-Altman method of differences, and Lin’s concordance coefficient. The Bland-Altman method is used to compare 2 quantitative test results without considering 1 method a gold standard.\textsuperscript{59} Bias is calculated as the mean difference between the 2 methods, and the 95% limits of agreement (LOA) are defined as the range in which 95% of the differences between 2 methods are found. Lin’s concordance correlation coefficient also measures agreement between 2 continuous variables and is considered more robust than linear correlation measures in assessing agreement. Poor agreement is indicated by a concordance coefficient < 0.9, whereas almost perfect
agreement is indicated by a coefficient > 0.99. Characteristics (age, calculated body weight, BCS, CNS), basal insulin and glucose concentrations, $AUC_{g0-120}$, and calculated parameters from the FSIGTT ($S_I$, $AIR_g$, $S_g$, and DI) were compared between IR and IS horses using Mann Whitney U test, because values within IR and IS groups were not normally distributed. Categorical outcomes (IR,IS) were assessed for agreement using Cohen’s Kappa, which is a measure of agreement (0.8-1.0 indicating almost perfect agreement, 0.6-0.8 substantial agreement, 0.4-0.6 moderate, 0.2-0.4 fair, 0.0-0.2 slight, and <0.0 poor agreement). It represents the proportion of observed agreement after accounting for agreement expected by chance alone. Sensitivity, specificity, positive predictive and negative predictive values also were calculated for the BIC, OST and CGIT using the FSIGTT minimal model analysis as the gold standard. Statistical analysis was performed using commercial statistical software. 

2.2 Results

The horses consisted of 2 mares and 10 geldings of various breeds (4 Warmbloods, 3 Thoroughbreds, 2 Quarter Horses, and 1 each American Saddlebred, Appaloosa, and Standardbred). Descriptive statistics and minimal model parameters are summarized in Table 1. When comparing results of the tests in classifying individual horses, minimal model analysis of the FSIGTT classified 7 horses as IR ($S_I < 1.0 \times 10^{-4} \text{ L}\cdot\text{mU}^{-1}\cdot\text{min}^{-1}$) and 5 horses as IS ($S_I > 1.0 \times 10^{-4} \text{ L}\cdot\text{mU}^{-1}\cdot\text{min}^{-1}$). Basal insulin concentration classified all horses as IS using currently recommended diagnostic criteria for IR (> 20 µIU/ml). The OST also classified all horses as IS (insulin concentration < 60 µIU/ml at 60 and 90 minutes). Results of the CGIT varied depending on the cut-off value used to define IR. Using the $PP-D_{glu}$, horses with $PP-D_{glu} > 45$ minutes were
classified as IR, which resulted in classification of 9 individuals as IR and 3 as IS. When categorized using [Ins]$_{45}$ >100 µIU/ml as the cut-off, 2 horses were classified as IR and 10 as IS by the CGIT. When using both criteria together, results were the same as when using PP-D$_{\text{glu}}$. To evaluate these tests between groups of horses, AUC$_{g0-120}$ values were correlated among tests and compared between IR and IS horses. The AUC$_{g0-120}$ values were significantly different between IS and IR horses for the FSIGTT and CGIT (p<0.05), but values were not significantly different between IR and IS horses for the OST (p = 0.34). The AUC$_{g0-120}$ values were significantly correlated for the FSIGTT, CGIT, and OST (Table 2). However, Lin’s concordance coefficients among FSIGTT and CGIT, FSIGTT and OST, and CGIT and OST were poor (Table 2). Bland-Altman analysis was performed to evaluate agreement among AUC$_{g0-120}$ values for the OST, the CGIT, and FSIGTT. Differences were normally distributed, and analysis showed large bias and poor agreement among the tests (Table 2). Using minimal model analysis of the FSIGTT as a gold standard, sensitivity, specificity, and positive and negative predictive values were calculated for each test and summarized in Table 3. Cohen’s Kappa coefficients reflected poor agreement between BIC and OST and fair agreement between both cut-off values of the CGIT ([Ins]$_{45}$ > 100 µIU/ml and PP-D$_{\text{glu}}$ > 45 minutes) and the FSIGTT (Table 4).

2.3 Discussion

Evaluation of currently recommended tests for insulin dysregulation in our study yielded variable diagnostic results when performed in the same group of adult light-breed horses. Based on clinically used cut-off values, the 4 common diagnostic tests for insulin dysregulation evaluated in this study displayed poor agreement in classifying horses as IR
or IS. The BIC and OST were highly specific but displayed poor sensitivity. The PP-D$_{\text{glu}}$
from the CGIT had high sensitivity but low specificity. Using [Ins]$_{45}$, the CGIT displayed
greater sensitivity than BIC and the OST, but also maintained moderate specificity.

Previous studies have demonstrated significant correlations between AUC$_g$ and
area under the insulin curve values of the OST and an IV glucose tolerance test.$^{40}$ In
another study, indices from the oral glucose tolerance test, the HEC, and insulin-modified
FSIGTT also were well-correlated but not equivalent and demonstrated poor
concordance.$^{47}$ Our study supports these results and further compares diagnostic
agreement among these tests in classifying horses as IR or IS. The high degree of
diagnostic variability among tests observed in this study suggests that currently utilized
cut-off values for these tests require refinement to improve agreement with minimal
model analysis. Additionally, frequently used testing modalities (BIC and the OST) may
not detect insulin resistance in horses unless severe.

The currently utilized cut-off value for the OST is based on a preliminary study
including 10 EMS horses and 8 control horses, in which the criteria for EMS included a
BCS $\geq 7/9$, regional adiposity or both along with CGIT or FSIGTT results consistent with
insulin resistance within the past 6 months.$^{40}$ This cut-off value subsequently has been
published in review articles,$^{33,64}$ and is widely used clinically. Cut-off criteria for the
CGIT were similarly determined based on an initial study in normal horses$^{30}$ and
arbitrary cut-off values later were used to determine insulin sensitivity.$^{7,3}$ However,
validation of these values has not been performed using formal statistical analysis (i.e., by
generation of receiver operator characteristic curves).
Limitations of our study include small sample size and variation within the study population. Horses in the study had a wide range of insulin sensitivities, which allowed comparison of IR and IS horses. This also produced large variation in indices of insulin sensitivity, which likely affected the degree of correlation among the results of the different tests. However, variation within the study population should not affect direct comparison among tests performed on the same individuals. Furthermore, significant correlations among the $AUC_{g0-120}$ values from the dynamic tests (OST, CGIT, and FSIGTT) were observed. Additional comparison of substantially IR individuals such as ponies or predisposed breeds may have provided different results, because horses at the extremes of insulin dysregulation (e.g., severely IR, hyperinsulinemic, or very IS) may have generated better agreement among test results. However, the objective of our study was to determine agreement in light-breed horses and evaluate the performance of dynamic testing in horses that may have normal resting insulin concentrations.

Seven horses in the study were classified as IR based on minimal model analysis of the FSIGTT. This test was chosen as the gold standard because it is relatively easy to perform, more feasible, and correlates well with the HEC method. The HEC was shown to have improved repeatability in healthy horses in quantitative assessment of insulin sensitivity (average inter-day coefficient of variation $14.1 \pm 5.7\%$) compared to minimal model analysis of the FSIGTT (average inter-day coefficient of variation, $23.7 \pm 11.2\%$), although these results were found using the original protocol rather than the insulin-modified FSIGTT performed in this study. Similar variation has been reported in studies of humans and cats. The degree of insulin dysregulation required to predispose horses to the development of laminitis currently is unknown. It is also unclear how parameters
derived from minimal model analysis (e.g., $S_l$) correlate to the risk of clinical or subclinical laminitis. The cut-off used in this study to define IR horses was $S_l < 1.0 \times 10^{-4}$ L·mU⁻¹·min⁻¹ in concordance with previous studies.¹⁸,⁵⁷,⁵⁸ In a previous study, $S_l$ of 1 laminitic pony was $0.089 \times 10^{-4}$ L·mU⁻¹·min⁻¹, and the lowest reference quintile for 46 healthy horses in another study ranged from $0.14$ to $0.78 \times 10^{-4}$ L·mU⁻¹·min⁻¹.³⁵ The $S_l$ in human subjects with normal glucose tolerance was $2.0 \pm 0.25 \times 10^{-4}$ L·mU⁻¹·min⁻¹, $1.11 \pm 0.18 \times 10^{-4}$ L·mU⁻¹·min⁻¹ in subjects with impaired glucose tolerance, and $0.67 \pm 0.17 \times 10^{-4}$ L·mU⁻¹·min⁻¹ in subjects with non-insulin-dependent diabetes mellitus.⁶⁵ These reports suggest that the value used in this study to define IR in horses may be appropriate, but further investigation into how $S_l$ values from the FSIGTT correlate with clinical or subclinical laminitis is needed. The use of an arbitrary cut-off value for defining IR in horses may have affected the calculation of sensitivity and specificity of the other tests evaluated in our study.

Results of our study suggest that the OST is poorly sensitive and does not provide greater diagnostic utility for detecting insulin resistance in horses than does BIC, although it may be useful in quantifying hyperinsulinemia and insulin dysregulation. The OST is an attractive test for insulin dysregulation in that it is dynamic and mimics physiologic conditions in which a PO glucose load leads to stimulation of the enteroinsular axis, which may play a role in altered insulin and glucose responses to a meal high in non-structural carbohydrates. Additionally, it is easy to perform clinically and does not require placement of an IV catheter. However, using current diagnostic criteria (insulin concentration $> 60$ µIU/ml between 60 and 90 minutes), the OST performed similarly to a single BIC in this group of horses. Lowering the diagnostic cut-
off value to 45 μIU/ml classified 1 horse with excessive hyperinsulinemia, improving the OST’s sensitivity from 0% to 14% and maintaining 100% specificity for estimating insulin resistance. Reasons for this discrepancy may be inherent differences between testing a horse’s response to a PO versus IV glucose load. The OST can determine whether a horse exhibits an inappropriate insulin response to a high non-structural carbohydrate diet, whereas the CGIT and FSIGTT are likely more direct measures of tissue insulin sensitivity. At this time, it is unknown which of these mechanisms plays a larger role in EMS, and this may vary among EMS horses. Additionally, peak insulin and glucose concentrations after a PO sugar challenge have been shown to vary significantly among individual horses, which may affect these test results and make creating a cut-off value difficult. In our study, many horses never reached peak insulin and glucose concentrations within the 120-minute sampling time, and peak concentrations could not be evaluated. These values could be evaluated in the future to add to the diagnostic utility of the OST.

The results of our study suggest that further research comparing results of dynamic tests for insulin dysregulation is needed, including determination of diagnostic cut-off values that maximize sensitivity and specificity for detecting insulin dysregulation. In clinical practice, false negative results (i.e., IR horses that are classified as IS) may place the horse in question at greater risk of pasture-associated laminitis, whereas a false positive result may result in dietary and management changes for weight reduction and pasture access restriction that are unnecessary (although not directly harmful). One could argue that maximizing the sensitivity of any screening test for
insulin dysregulation would be most appropriate, because laminitis can be a life-threatening consequence of this condition.\textsuperscript{15-17,19} The sensitivity of currently recommended insulin dysregulation testing appears to be quite low, and further investigation of testing methods that will be useful for practitioners is necessary.

In conclusion, commonly used tests for insulin dysregulation appear to produce variable results in the assessment of insulin sensitivity in horses, in that the results of a single test often do not accurately classify horses as IR or IS. Additional studies are required to determine the most useful tests for insulin dysregulation and to identify appropriate cut-off values for defining insulin resistance, postprandial hyperinsulinemia, and their association with risk of laminitis.
Table 2.1. Descriptive statistics and minimal model parameters of study horses (mean ± SD) and IR and IS horses (median and range)

*Indicates significant difference between IR and IS groups (p < 0.05)
Table 2.1 continued

SD, standard deviation; IS, insulin sensitive; IR, insulin resistant; BCS, body condition score; CNS, cresty neck score; FSIGTT, insulin-modified frequently-sampled IV glucose tolerance test; AUC$_{g0-120}$, area under the glucose curve from 0 to 120 minutes; BIC, basal insulin concentration; CGIT, combined glucose and insulin test; OST, oral sugar test; S$_i$, insulin sensitivity; AIRg, acute insulin response to glucose; S$_g$, glucose effectiveness; DI, disposition index
Table 2.2. Area under the glucose curve from 0-120 minutes comparisons for the FSIGTT, CGIT, and OST

* Indicates significant linear correlation (p< 0.05)

FSIGTT, insulin-modified frequently-sampled IV glucose tolerance test; CGIT, combined glucose and insulin test; OST, oral sugar test; LOA, limits of agreement

<table>
<thead>
<tr>
<th>Linear Correlation:</th>
<th>Comparison of FSIGTT to CGIT</th>
<th>Comparison of FSIGTT to OST</th>
<th>Comparison of CGIT to OST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s r</td>
<td>0.7527*</td>
<td>0.6564*</td>
<td>0.5883*</td>
</tr>
<tr>
<td>Lin’s Concordance Coefficient</td>
<td>0.6655</td>
<td>0.4652</td>
<td>0.3863</td>
</tr>
<tr>
<td>Bland-Altman analysis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bias (mg/dl x min)</td>
<td>1,766</td>
<td>15,431</td>
<td>-779.9</td>
</tr>
<tr>
<td>95% LOA</td>
<td>(-3,216, 6,748)</td>
<td>(9,674, 21,189)</td>
<td>(-6,756, 5,196)</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Positive Predictive Value (PPV)</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>BIC</strong></td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>OST</strong></td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>CGIT_{PP-D_{glu}&gt;45~min}</strong></td>
<td>85.7%</td>
<td>40%</td>
<td>66.7%</td>
</tr>
<tr>
<td><strong>CGIT_{[Ins]_{45}&gt;100~mU/ml}</strong></td>
<td>28.5%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2.3. Sensitivity, specificity, positive predictive value, and negative predictive value of BIC, the OST, and CGIT compared to gold standard (FSIGTT).

BIC, basal insulin concentration; OST, oral sugar test; CGIT, combined glucose and insulin test; FSIGTT, insulin-modified frequently-sampled IV glucose tolerance test; PP-D_{glu}, positive phase duration of the glucose curve; [Ins]_{45}, insulin concentration at 45 minutes.
<table>
<thead>
<tr>
<th>Agreement with FSIGTT</th>
<th>Cohen’s Kappa</th>
<th>95% CI for Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIC</td>
<td>0</td>
<td>[-0.48, 0.48]</td>
</tr>
<tr>
<td>OST</td>
<td>0</td>
<td>[-0.48, 0.48]</td>
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<tr>
<td>CGIT&lt;sub&gt;PP-D_{glu}&gt;45 min&lt;/sub&gt;</td>
<td>0.27</td>
<td>[-0.31, 0.85]</td>
</tr>
<tr>
<td>CGIT&lt;sub&gt;[Ins]_{45}&gt;100\mu IU/ml&lt;/sub&gt;</td>
<td>0.25</td>
<td>[-0.25, 0.75]</td>
</tr>
</tbody>
</table>

Table 2.4. Cohen’s Kappa coefficients assessing agreement with gold standard (FSIGTT)

FSIGTT, insulin-modified frequently-sampled IV glucose tolerance test; CI, confidence interval; BIC, basal insulin concentration; OST, oral sugar test; CGIT, combined glucose and insulin test; PP-D_{glu}, positive phase duration of the glucose curve; [Ins]_{45}, insulin concentration at 45 minutes
Chapter 3: Comparison of morphometric data and proxy measurements of insulin resistance to Minimal Model analysis in adult light-breed horses

3.1 Materials and Methods

Experimental Design

All experimental procedures were approved by the OSU Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Twelve healthy, adult, light-breed horses owned by The Ohio State University College of Veterinary Medicine were enrolled in a prospective, randomized study in which insulin resistance was assessed by minimal model analysis of the insulin-modified frequently sampled IV glucose tolerance test (FSIGTT), the CGIT, and the oral sugar test (OST). Horses were housed on pasture with access to grass hay ad libitum, and no concentrate feed. Horses were housed in individual stalls, and IV catheters were placed the night before testing. Grass hay was provided the night before testing and a muzzle was placed two hours before initiation of insulin resistance testing.

Insulin-modified Frequently Sampled IV Glucose Tolerance Test

The insulin-modified frequently sampled IV glucose tolerance test (FSIGTT) was performed as optimized by Tóth et al.\textsuperscript{39} Two jugular venous catheters were placed the night prior to testing. One catheter was utilized for blood collection and the other designated for insulin and dextrose administration. Blood samples were collected 10, 5 and 1 minute before administration of 50% dextrose solution (150 mg/kg
rapidly IV at time 0) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minutes after 50% dextrose\textsuperscript{iii} infusion. Regular insulin\textsuperscript{iv} (0.1 U/kg, IV) diluted in 3 ml 0.9% sodium chloride solution was administered 20 minutes after the 50% dextrose\textsuperscript{iii} infusion. Blood glucose was measured at all time points, and serum insulin was measured at 1 minute before, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 120, 150, and 180 minutes after 50% dextrose\textsuperscript{iii} administration. Blood glucose was measured with a portable glucometer\textsuperscript{v} validated for use in horses\textsuperscript{53} immediately after collection. Blood samples were collected in EDTA\textsuperscript{vi} and silicone-coated tubes\textsuperscript{vii} and remained on ice until centrifugation for harvesting of plasma and serum. Plasma and serum samples were stored at -80°C until analysis. Serum insulin concentrations were measured using a commercially-available radioimmunoassay\textsuperscript{viii} validated for use in horses.\textsuperscript{54,55}

\textit{Morphometric Measurements}

Morphometric measurements were obtained including BCS,\textsuperscript{50} calculated body weight (BW),\textsuperscript{52} retroperitoneal fat depth (RFD), tailhead fat depth (TFD),\textsuperscript{67} CNS,\textsuperscript{51} and MNC as described previously.\textsuperscript{51} Briefly, BCS and CNS were determined as the average of scores by two observers (LD and TB) based on the Henneke body condition scoring system\textsuperscript{50} and CNS as outlined by Carter et al.\textsuperscript{51} Body weight was calculated based on the formula for estimation of body weight in kilograms.\textsuperscript{52} RFD and TFD were performed ultrasonographically as described,\textsuperscript{67} and final measurements were calculated as the mean of three measurements obtained by the same observer (LD). MNC was performed by one of the investigators (LD) as described.\textsuperscript{7,51} Complete descriptions of the morphometric parameters are outlined in table 3.1.
Proxy Measurements of Insulin Resistance

Basal insulin and glucose concentrations were obtained by averaging the baseline glucose and insulin concentrations from three dynamic tests of insulin sensitivity performed in each horse. Basal insulin and glucose concentrations were used to calculate proxy measurements of insulin resistance, including HOMA, QUICKI, RISQI, MIRG, and insulin to glucose ratio (I:G) as described previously.\textsuperscript{35} Calculations for proxy measurements of insulin resistance are shown in table 3.2.

Data Analysis

FSIGTT insulin and glucose data were analyzed using Minimal Model analysis with computer software\textsuperscript{x} and calculated parameters included insulin sensitivity (S_I), glucose effectiveness (S_g), acute insulin response to glucose (AIR_g), and disposition index (DI).\textsuperscript{38,56} Normality was assessed using the D’Agostino & Pearson omnibus normality test. Nonparametric testing (Mann Whitney U) was used to compare morphometric measurements and proxy measurements of insulin resistance between IR and IS horses. Linear correlation between morphometric measurements, proxy measurements, and insulin sensitivity (S_I) from Minimal Model analysis was assessed by Pearson’s correlation coefficient (r). Linear correlation between proxy measurements of insulin resistance and morphometric data was assessed using Pearson’s correlation. Statistical analysis was performed using commercial statistical software\textsuperscript{x}.

3.2 Results

Morphometric data, proxy measurements, and minimal model parameters are summarized in table 3.3. Morphometric data and proxy measurements of insulin sensitivity were normally distributed. When data were grouped by IR and IS horses, the
data were not normally distributed. No significant difference was observed between IR and IS horses with respect to morphometric parameters or proxy measurements of insulin sensitivity. No significant correlations were observed between morphometric data and $S_i$ derived from Minimal Model analysis. However, significant linear correlations were observed between proxy measurements and parameters from Minimal Model analysis. BIC ($r = -0.6364$), QUICKI ($r=0.6617$), RISQI ($r = 0.6349$), HOMA ($r = -0.6404$), and I:G ($r = -0.6279$) were significantly correlated to $S_i$ ($p < 0.05$) (table 3.4). BCS, CNS, TFD, and MNC were also significantly correlated to proxy measurements (table 3.5).

3.3 Discussion

In the current study, there were no significant differences between IR and IS horses with respect to morphometric parameters or proxy measures of insulin sensitivity. Additionally, morphometric parameters were not well correlated with $S_i$ as estimated from minimal model analysis. This is in contrast to previous studies, which have shown significant correlations between morphometric parameters (BCS and MNC), $AUC_g$, and $AUC_i$ from the CGIT, and between morphometric measurements (BCS and CNS) and basal insulin, glucose, triglyceride, and leptin concentrations in both horses and ponies. However, horses with a BCS greater than or equal to 7 were not more likely to be hyperinsulinemic than horses with lower BCS, and horses with a CNS greater than or equal to 3 were not more likely to be hyperinsulinemic than horses with a lower CNS. In ponies, however, higher BCS and CNS did increase the likelihood of hyperinsulinemia. Further, BCS, CNS, and neck circumference to height ratio were shown to have diagnostic accuracy in predicting laminitis in ponies. The lack of a relationship between morphometric parameters and $S_i$ found in this study might be a reflection of the dietary
management of the horses in the study, as concentrate feeds were not routinely fed, or, as has been found in other studies, may reflect a difference between ponies and horses. Additionally, it may indicate that in some IR horses, morphometric assessments of obesity and regional adiposity are not accurate in predicting degree of insulin resistance.

Four morphometric characteristics (BCS, CNS, MNC, and TFD) were significantly correlated to all proxy measurements of insulin sensitivity, including BIC. Three out of these four morphometric measurements are considered measures of regional adiposity rather than generalized obesity (CNS, MNC, and TFD), suggesting that measures of regional adiposity may be more closely associated with insulin resistance. Several previous studies have documented an association between neck crest adiposity and hyperinsulinemia,\textsuperscript{4,7,51} which is consistent with the current findings. Additionally, CNS consistently showed the strongest association with all proxies (table 3.5), supportive of its utility in assessing horses for insulin resistance and risk of pasture-associated laminitis. However, a previous study in ponies found that all clinically laminitic ponies showed both generalized and localized obesity,\textsuperscript{5} which did not support measurements of the neck crest or regional fat deposits over measures of overall obesity, such as BCS. Results of the current study suggest that the same may not be true in light-breed horses.

Proxy measurements of insulin sensitivity were not different between IR and IS horses; moderate, significant correlations were detected between proxies and minimal model estimates of $S_1$ and AIRg. This is consistent with previous reports\textsuperscript{35,46} and suggests that proxies might be useful for screening populations, performing large-scale studies, or monitoring response to treatment through trends over time. However, their utility in diagnosing individuals is likely limited to severely affected animals. Moderate
correlations were detected among all proxy measurements, and there was no single proxy measurement that displayed superior correlation to SI. Therefore, this study could not determine whether certain proxy measurements are more useful in detecting insulin resistance in horses.

Limitations of this study include the small sample size. Five horses were classified as IS and 7 as IR, which limited our ability to detect differences between IR and IS horses with respect to morphometric and proxy measurements. Therefore, although there was no significant difference with respect to these parameters between IR and IS horses in this study, the study was underpowered to detect a difference, and a large-scale study including more horses may have found detectable differences. Additionally, there was wide variation in these measurements, particularly among IR horses, which further limited our ability to detect statistically significant differences between groups. The variation in morphometric parameters among the IR horses might be indicative of the difficulty in detecting insulin resistance in horses based on phenotypic characteristics. However, the proxy measurements in this group are directly derived from basal insulin and glucose concentrations. All horses classified as IR in this study based on minimal model analysis had normal basal insulin concentrations (< 20 µIU/ml), but an abnormal response to exogenous dextrose and insulin during the FSIGTT, which may be the reason that a significant difference between IR and IS groups was not found.

Performing the same study in a group of with more severely IR horses may have detected a difference between groups with respect to both morphometric and proxy measurements. Future studies correlating proxy measurements of insulin sensitivity to
Minimal Model analysis in both healthy and EMS-affected light-breed horses are needed. Additionally, at this time, the degree of $S_I$ (or lack of $S_I$) placing horses at risk for pasture-associated laminitis is unknown, and studies determining the degree of $S_I$ and corresponding morphometric and proxy measurements that place light-breed horses at risk for laminitis are needed.

In conclusion, further studies are necessary to determine if there are significant differences in morphometric parameters and proxy measurements of insulin sensitivity between IR and IS horses. However, significant correlations between BCS, CNS, MNC, and TFD and proxy measurements were observed. CNS displayed the strongest correlation to all proxy measurements, suggesting that it may be the most useful parameter for monitoring risk of hyperinsulinemia, insulin dysregulation, and predisposition to EMS in light-breed horses.
| **BCS** | Evaluated on a scale of 1 to 9, as described by Henneke et al. |
| **CNS** | Evaluated on a scale from 0 to 5, as described by Carter et al.: |
| | 0: No visual appearance of a crest and no palpable crest |
| | 1: No visual appearance of a crest, but slight filling palpable |
| | 2: Noticeable appearance of a crest, but fat deposited evenly from poll to withers. Crest easily cupped in one hand and bent from side to side. |
| | 3: Crest enlarged and thickened, so fat is deposited more heavily in the middle of the neck than toward poll and withers, giving a mounded appearance. Crest fills cupped hand and begins losing side-to-side flexibility. |
| | 4: Crest grossly enlarged and thickened, and can no longer be cupped in one hand or easily bent from side to side. Crest may have wrinkles/creases. |
| | 5: Crest is so large it permanently droops to one side. |
| **BW** | Calculated as described by Carroll and Huntington, 1988: |
| | Weight (kg) = \( \frac{\text{Girth}^2 \times \text{length (cm)}}{1190} \) |
| **MNC** | Neck circumference measured perpendicular to a line from the poll to the withers at 0.25, 0.50 and 0.75 of the distance from the poll to the withers and the mean circumference of these three measurements is calculated. |
| **RFD** | Measured via transcutaneous ultrasonography using a linear probe positioned on midline transversely at the level of the umbilicus. |
| **TFD** | Measured via transcutaneous ultrasonography using a linear probe positioned parallel to the vertebral column, just lateral and cranial to the first tail hairs |

Table 3.1. Morphometric parameters recorded on 12 adult light-breed horses

BCS, body condition score; CNS, cresty neck score; BW, calculated body weight in kilograms; RFD, retroperitoneal fat depth; TFD, tailhead fat depth; MNC, mean neck circumference
<table>
<thead>
<tr>
<th>Index</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOMA</strong></td>
<td>$\frac{[\text{glucose}] \times [\text{insulin}]}{22.5}$</td>
</tr>
<tr>
<td><strong>QUICKI</strong></td>
<td>$\frac{1}{\log ([\text{glucose}] \times [\text{insulin}])}$</td>
</tr>
<tr>
<td><strong>RISQI</strong></td>
<td>$\frac{1}{\sqrt{[\text{insulin}]}}$</td>
</tr>
<tr>
<td><strong>I:G Ratio</strong></td>
<td>$\frac{[\text{insulin}]}{[\text{glucose}]}$</td>
</tr>
<tr>
<td><strong>MIRG</strong></td>
<td>$\frac{(800-0.3 \times ([\text{insulin}]-50)^2) \times ([\text{glucose}]-30)}{([\text{glucose}]-30)}$</td>
</tr>
</tbody>
</table>

Table 3.2. Calculation of proxy measurement of insulin resistance

HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; RISQI, reciprocal of the square root of insulin; I:G, insulin to glucose; MIRG, modified insulin to glucose ratio
Table 3.3. Morphometric data, proxy measurements of insulin resistance, and Minimal Model parameters in study horses (mean ± SD) as well as IS and IR horses (median and range) (Continued)

<table>
<thead>
<tr>
<th></th>
<th>All horses (mean±SD)</th>
<th>IS Group (median and range)</th>
<th>IR Group (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>13.42 ± 4.32</td>
<td>9 (7-17)</td>
<td>13 (10-24)</td>
</tr>
<tr>
<td><strong>Body Weight</strong></td>
<td>581 ± 65</td>
<td>537.5 (534-698)</td>
<td>590 (485-650)</td>
</tr>
<tr>
<td><strong>BCS</strong></td>
<td>5.96 ± 1.03</td>
<td>4.75 (4.5-6.5)</td>
<td>6.5 (4.5-8)</td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td>2.17 ± 0.81</td>
<td>1.5 (1-2.5)</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td><strong>RFD (cm)</strong></td>
<td>2.67 ± 1.26</td>
<td>1.60 (0.74-3.4)</td>
<td>3.23 (0.93-4.70)</td>
</tr>
<tr>
<td><strong>TFD (cm)</strong></td>
<td>1.60 ± 0.86</td>
<td>1.24 (0.38-2.18)</td>
<td>1.94 (0.64-3.25)</td>
</tr>
<tr>
<td><strong>MNC (cm)</strong></td>
<td>94.7 ± 5.1</td>
<td>96 (89-101)</td>
<td>94.3 (85-101)</td>
</tr>
<tr>
<td><strong>Basal [Insulin]</strong></td>
<td>5.71 ± 3.16</td>
<td>2.76 (2.62-5.47)</td>
<td>6.97 (2.319-12.6)</td>
</tr>
<tr>
<td><strong>Basal [Glucose]</strong></td>
<td>105.9 ± 6.86</td>
<td>97.83 (96-107.3)</td>
<td>108 (96.3-116.3)</td>
</tr>
<tr>
<td><strong>HOMA</strong></td>
<td>27.5 ± 16.6</td>
<td>16.3 (11.9-26.1)</td>
<td>33.3 (11.0-65.1)</td>
</tr>
<tr>
<td><strong>QUICKI (1/ (mg/dl·µIU/ml))</strong></td>
<td>0.37 ± 0.03</td>
<td>0.39 (0.36-0.41)</td>
<td>0.35 (0.32-0.42)</td>
</tr>
<tr>
<td><strong>I:G ratio</strong></td>
<td>0.053 ± 0.03</td>
<td>0.037 (0.027-0.051)</td>
<td>0.061 (0.022-0.11)</td>
</tr>
<tr>
<td><strong>RISQI (1/µIU/ml)</strong></td>
<td>0.46 ± 0.12</td>
<td>0.52 (0.42-0.62)</td>
<td>0.38 (0.28-0.66)</td>
</tr>
<tr>
<td><strong>MIRG</strong></td>
<td>2.7 ± 0.89</td>
<td>2.25 (1.76-2.25)</td>
<td>2.91 (1.55-4.41)</td>
</tr>
<tr>
<td>**S₁ (l/min/mU x 10⁻⁴)</td>
<td>1.384±1.256</td>
<td>1.683 (1.47-3.84)*</td>
<td>0.5929 (0.131-0.708)*</td>
</tr>
<tr>
<td>**AIRg (mU/L * min⁻¹)</td>
<td>198 ±123.6</td>
<td>84.92 (83.28-245.9)</td>
<td>253 (44.5-449.7)</td>
</tr>
<tr>
<td>**S₂ (min⁻¹ x 10⁻²)</td>
<td>1.28 ± 0.57</td>
<td>1.55 (1.42-2.47)*</td>
<td>0.84 (0.62-1.59)*</td>
</tr>
<tr>
<td><strong>DI x 10⁴</strong></td>
<td>205.9 ± 182.8</td>
<td>190 (157.9-674.6)*</td>
<td>95.6 (26.4-188.2)*</td>
</tr>
</tbody>
</table>
Table 3.3 continued

*Indicates significant difference between IR and IS groups (p<0.05)

SD, standard deviation; IS, insulin sensitive; IR, insulin resistant; BCS, body condition score; CNS, creesty neck score; RFD, retroperitoneal fat depth; TFD, tailhead fat depth; MNC, mean neck circumference; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; I:G, insulin to glucose; RISQI, reciprocal of the square root of insulin; MIRG, modified insulin to glucose ratio; SI, insulin sensitivity; AIRg, acute insulin response to glucose; Sg, glucose effectiveness; DI, disposition index
### Table 3.4. Correlation of proxy measurements of insulin sensitivity to Minimal Model parameters ($S_I$, AIRg)

* Indicates significant linear correlation

$S_I$, insulin sensitivity; AIRg, acute insulin response to glucose; QUICKI, quantitative insulin sensitivity check index; RISQI, reciprocal of the square root of insulin; HOMA, homeostasis model assessment; I:G, insulin to glucose; MIRG, modified insulin to glucose ratio
Table 3.5. Linear correlations between morphometric data and proxy measurements of insulin resistance

(Continued)
Table 3.5 continued

<table>
<thead>
<tr>
<th></th>
<th>Comparison with HOMA:</th>
<th>Comparison with BIC:</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>0.6444*</td>
<td>0.6447*</td>
</tr>
<tr>
<td>BW</td>
<td>0.2797</td>
<td>0.2604</td>
</tr>
<tr>
<td>CNS</td>
<td>0.8536*</td>
<td>0.8557*</td>
</tr>
<tr>
<td>MNC</td>
<td>0.6466*</td>
<td>0.6498*</td>
</tr>
<tr>
<td>RFD</td>
<td>0.4811</td>
<td>0.477</td>
</tr>
<tr>
<td>TFD</td>
<td>0.7334*</td>
<td>0.7447*</td>
</tr>
</tbody>
</table>

*Indicates significant linear correlation (p<0.05)

BCS, body condition score; BW, body weight (kg, calculated); CNS, cresty neck score; RFD, retroperitoneal fat depth; TFD, tailhead fat depth; MNC, mean neck circumference; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; I:G, insulin to glucose; RISQI, reciprocal of the square root of insulin
Chapter 4: Summary

The diagnosis of insulin dysregulation as an aid in diagnosing horses with EMS remains difficult despite a growing body of studies on the subject. There are many tests available for diagnosing insulin dysregulation currently in practice. Previous studies show significant correlation between variables obtained from these tests, and between proxy measurements and morphometric measurements of adiposity. However, the values used to classify horses as IR or IS have primarily been determined arbitrarily, based on studies in other species. This study further examined the agreement between these tests for insulin dysregulation and how they classified horses using currently published criteria. The sensitivity of BIC and OST was low in comparison to the gold standard FSIGTT, while the CGIT was slightly more sensitive.

Proxy measurements of insulin sensitivity and morphometric measurements of adiposity in horses are practical, easy ways to estimate insulin sensitivity for the general practitioner, and might be useful to determine response to treatment, although this remains to be determined. This study examined the correlation of proxy measurements of insulin sensitivity with results of the gold standard FSIGTT (Si), and found that significant correlations were observed between proxy measurements and Si. However, morphometric measurements of adiposity performed in the same group of horses did not correlate with Si, suggesting that phenotypic characteristics may not be predictive of insulin sensitivity in some horses. A few morphometric measurements
(BCS, CNS, MNC, and TFD) did, however, correlate with proxy measurements of insulin sensitivity, including basal insulin concentrations. CNS consistently exhibited the strongest correlation with proxy measurements, suggesting that evaluation of the neck crest might be the most useful morphometric assessment of horses to predict their risk for insulin dysregulation.

The results of these studies provide evidence that future investigations into insulin dysregulation testing are needed. Studies determining the relationship between insulin dysregulation and laminitis will be useful in defining the level of insulin dysregulation that increases the likelihood of equine metabolic syndrome associated laminitis (EMSAL) in horses and ponies. Additional studies examining the pathogenesis of EMSAL also are necessary to determine whether tissue insulin resistance, postprandial hyperinsulinemia, or other metabolic derangements are more important in the determining risk of EMSAL. These studies underline the need for future work in this area, as the most useful test(s) for determining insulin dysregulation are not known.
References


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i Karo® Light Corn Syrup, ACH Food Companies, Inc, Oakbrook, IL

ii Terumo SURFLO® EFTE IV Catheter 14G x 2", Terumo Medical Corp., Somerset, NJ

iii Dextrose 50% Injection, VetOne®, MWI, Boise, ID

iv Humulin R, Eli Lilly and Company, Indianapolis, IN

v AlphaTRAK blood glucose monitoring system meter, Abbott Animal Health, Chicago, IL

vi K₂ EDTA BD Vacutainer® tubes, Franklin Lakes, NJ

vii Silicone-coated BD Vacutainer® tubes, Franklin Lakes, NJ

viii Coat-A-Count insulin RIA, Siemens Medical Solutions Diagnostics, Los Angeles, CA


x MINMOD MILLENIUM Minimal Model Software, MINMOD, Inc, Los Angeles, CA
GraphPad Prism 6, GraphPad Software, La Jolla, CA