

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

L.K. Dunbar, K.A. Mielnicki, K.A. Dembek, R.E. Toribio, and T.A. Burns

Background: Several tests have been evaluated in horses for quantifying insulin dysregulation to support a diagnosis of equine metabolic syndrome. Comparing the performance of these tests in the same horses will provide clarification of their accuracy in the diagnosis of equine insulin dysregulation.

Objectives: The aim of this study 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).

Animals: Twelve healthy, light-breed horses.

Methods: Randomized, prospective study. Each of the above tests was performed on 12 horses.

Results: 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 cut-off 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%, whereas CGIT ($[\text{insulin}]_{45} > 100 \mu\text{IU/mL}$) sensitivity and specificity were 28.5% and 100%, respectively. Area under the glucose curve ($\text{AUC}_{\text{g0-120}}$) was significantly correlated among the OST, CGIT, and FSIGTT, but Bland–Altman method and Lin's concordance coefficient showed a lack of agreement.

Conclusions: Current criteria for diagnosis of insulin resistance using BIC and the OST are highly specific but lack sensitivity. The CGIT displayed better sensitivity and specificity, but modifications may be necessary to improve agreement with minimal model analysis.

Key words: Equine metabolic syndrome; Insulin dysregulation; Laminitis.

Documentation of insulin dysregulation in horses is considered a key component in the diagnosis of equine metabolic syndrome (EMS), which currently is defined by an American College of Veterinary Medicine Consensus Statement to include insulin dysregulation, increased adiposity or generalized obesity, and a predisposition to laminitis.¹ This definition recently has been modified to include dyslipidemia (including hypertriglyceridemia) and adipokine dysregulation (hyperleptinemia) with or without obesity, causing a predisposition to laminitis.^{2,3} The underlying pathophysiology relating EMS, insulin dysregulation, and laminitis is not completely understood, but hyperinsulinemia is a known risk factor for pasture-associated laminitis.⁴ Furthermore, laminitis recently has been experimentally induced by infusion of supraphysiologic concentrations of insulin.^{5,6} Therefore, insulin dysregulation is likely to be involved in the pathogenesis of pasture-associated laminitis. Insulin dysregulation may include overall

Abbreviations:

| | |
|---------------------|--|
| AIR _g | acute insulin response to glucose |
| AUC _g | area under the glucose curve |
| CGIT | combined glucose-insulin test |
| EMS | equine metabolic syndrome |
| FSIGTT | frequently sampled insulin-modified intravenous glucose tolerance test |
| IR | insulin resistant |
| IS | insulin sensitive |
| OST | oral sugar test |
| S _I | insulin sensitivity |
| S _G | glucose effectiveness |
| PP-D _{glu} | positive phase duration of the glucose curve |
| [Ins] ₆₀ | insulin concentration at 60 minutes |
| [Ins] ₄₅ | insulin concentration at 45 minutes |

tissue insulin resistance, excessive hyperinsulinemia, or other undetermined mechanisms. Current understanding suggests that 1 of these factors may be more important to the pathogenesis of EMS and pasture-associated laminitis in different horses (ie, there may be multiple mechanisms that result in the same phenotype 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,⁷ stress, drugs (eg, $\alpha 2$ -agonists,

From the Ohio State University College of Veterinary Medicine, Columbus, OH (Dunbar, Mielnicki, Dembek, Toribio, Burns).

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Corresponding author: T.A. Burns, The Ohio State University College of Veterinary Medicine, 601 Vernon L. Tharp St., Columbus, OH 43210; e-mail: burns.402@osu.edu.

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corticosteroids),⁸ and feeding,^{1,9} and therefore may not be well-correlated with insulin sensitivity.⁹ Furthermore, IR horses rarely may develop inadequate compensatory insulin secretion, or type II diabetes mellitus, which may not be detected by screening tests.² In addition, proxy measurements of insulin sensitivity may be calculated based on glucose and insulin concentrations, and these proxies have been correlated with gold standard tests for insulin resistance in humans¹⁰ and horses and shown to have high specificity but low sensitivity.¹¹ 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.¹⁰ Minimal model analysis of the FSIGTT was chosen as the gold standard in our study because of its feasibility and physiologic estimation of insulin-dependent and insulin-independent glucose dynamics, although higher variation within subjects has been reported.¹² However, these gold standard tests are not practical for use in clinical cases because of the equipment, time, and cost necessary to perform them. Other dynamic tests have been developed, including the oral sugar test (OST)^{2,13} and combined glucose and insulin test (CGIT).⁸ These tests are increasingly used by practitioners as estimates of insulin dysregulation, and although the OST quantifies postprandial hyperinsulinemia and insulin dysregulation in response to PO glucose, its results have been used to estimate insulin sensitivity in horses and ponies.^{13,14} The CGIT is a measure of whole body insulin resistance by determining the individual's response to IV dextrose and insulin. Although the clinical utility of these tests is improved over the HEC and FSIGTT, their performance has not been critically evaluated in the same cohort of horses.

The objective of this study was to directly compare the results of 4 tests of insulin dysregulation, to determine the degree of agreement among these tests when performed on the same cohort of horses, and to determine the sensitivity and specificity of BIC, the OST, and CGIT when compared to the gold standard FSIGTT.

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) =

$(\text{girth}^2 \times \text{length [cm]}) \div 11900$ for dosage calculations.¹⁵ 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 system¹⁶ and the CNS.¹⁷ 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 OST was performed as previously described.¹³ Briefly, a blood sample was collected by direct jugular venipuncture at time 0. Light corn syrup^a 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.¹⁸ 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.⁸ An IV catheter^b was placed in a jugular vein the night before testing to minimize the 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^c (150 mg/kg IV) immediately followed by regular insulin^d (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.¹⁹ Two jugular venous catheters^b 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^c (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^c infusion. Regular insulin^d (0.1 U/kg, IV) diluted in 3 mL 0.9% sodium chloride solution was administered 20 minutes after the 50% dextrose^c infusion. Blood glucose concentration was measured at all time points, and serum insulin concentration 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, 100, 120, 150, and 180 minutes after 50% dextrose^c administration.

Blood Glucose and Insulin Concentrations

Blood glucose concentrations were measured with a portable glucometer^e validated for use in horses.²⁰ Blood samples were collected in EDTA^f and silicone-coated tubes^g 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^h validated for use in horses.^{21,22}

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 (AUC_{g0-120}) was calculated for the OST, CGIT, and FSIGTT. The CGIT parameters calculated included positive phase duration of the glucose curve ($PP-D_{glu}$)ⁱ and insulin concentration at 45 minutes ($[Ins]_{45}$). Insulin and glucose data from the FSIGTT were analyzed using minimal model analysis with computer software.^j Calculated parameters included insulin sensitivity (S_I), glucose effectiveness (S_g), acute insulin response to glucose (AIR_g), and disposition index (DI).^{23,24}

Quantitative variables were assessed for normality using the D'Agostino & Pearson omnibus normality test. Insulin resistant (IR) was defined as S_I less than 1.0×10^{-4} L/mU/min from minimal model analysis.²⁵⁻²⁷ 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 μ IU/mL, insulin concentration >60 μ IU/mL at 60 or 90 minutes during the OST, and $PP-D_{glu} >45$ minutes or $[Ins]_{45} >100$ μ IU/mL during the CGIT.¹⁸ The AUC_{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.²⁸ Bias is calculated as the mean difference between the 2 methods, and the 95% limits of agreement 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 .^{29,30} 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).^{31,32} It represents the proportion of observed agreement after accounting for agreement expected by chance alone.³² 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.^k

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 ($S_I <1.0 \times 10^{-4}$ L/mU/min) and 5 horses as IS ($S_I >1.0 \times 10^{-4}$ L/mU/min). Basal insulin concentration classified all horses as IS using currently recommended diagnostic criteria for IR (>20 μ IU/mL).¹⁸ The OST also classified all horses as IS (insulin concentration <60 μ IU/mL at 60 and 90 minutes).¹⁸ 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,¹⁸ which resulted in classification of 9 individuals as IR and 3 as IS. When categorized using $[Ins]_{45} >100$ μ IU/mL¹⁸ 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 $PP-D_{glu}$. 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 ($P < .05$), but values were not significantly different between IR and IS horses for the OST ($P = .34$). The AUC_{g0-120} values were significantly correlated for the FSIGTT, CGIT, and OST (Table 2). However, Lin's

Table 1. Descriptive statistics and minimal model parameters of study horses (mean \pm SD) and IR and IS horses (median and range).

| | All Horses (mean \pm SD) | IS Group (Median and Range) | IR Group (Median and Range) |
|---|----------------------------|-----------------------------|-----------------------------|
| Age (years) | 13.42 \pm 4.32 | 9 (7-17) | 13 (10-24) |
| Body Weight (calculated - kg) | 581 \pm 65 | 537.5 (534-698) | 590 (485-650) |
| BCS | 5.96 \pm 1.03 | 4.75 (4.5-6.5) | 6.5 (4.5-8) |
| CNS | 2.17 \pm 0.81 | 1.5 (1-2.5) | 2 (1-4) |
| BIC (μ IU/mL) | 5.71 \pm 3.16 | 2.76 (2.62-5.47) | 6.97 (2.319-12.6) |
| Basal [Glucose] (mg/dL) | 105.9 \pm 6.86 | 97.83 (96-107.3) | 108 (96.3-116.3) |
| FSIGTT AUC_{g0-120} (mg/dL \times min) | 22,175 \pm 4,187 | 18,678 (16,797-21,301)* | 23,906 (20,343-31,222)* |
| CGIT AUC_{g0-120} (mg/dL \times min) | 14,864 \pm 3,668 | 13,071 (9,877-14,535)* | 16,421 (11,342-22,015)* |
| OST AUC_{g0-120} (mg/dL \times min) | 15,644 \pm 1,453 | 14,715 (12,990-16,470) | 15,750 (15,045-18,975) |
| S_I (L/min/mU $\times 10^{-4}$) | 1.384 \pm 1.256 | 1.683 (1.47-3.84)* | 0.5929 (0.131-0.708)* |
| AIR_g (mU/L/min)* | 198 \pm 123.6 | 84.92 (83.28-245.9) | 253 (44.5-449.7) |
| S_g (min ⁻¹ $\times 10^{-2}$) | 1.28 \pm 0.57 | 1.55 (1.42-2.47)* | 0.84 (0.62-1.59)* |
| DI $\times 10^4$ | 205.9 \pm 182.8 | 190 (157.9-674.6)* | 95.6 (26.4-188.2)* |

*Indicates significant difference between IR and IS groups ($P < .05$).

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; AIR_g , acute insulin response to glucose; S_g , glucose effectiveness; DI, disposition index.

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 ($[Ins]_{45} > 100 \mu IU/mL$ and $PP-D_{glu} > 45$ minutes) and the FSIGTT (Table 4).

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 $PP-D_{glu}$ from the CGIT had high sensitivity but low specificity. Using $[Ins]_{45}$, the CGIT displayed not only 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.¹³ 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.³³ 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. In addition, 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.¹³ This cut-off value subsequently has been published in review articles,^{18,34} and is widely used clinically. Cut-off criteria for the CGIT were similarly determined based on an initial study in normal horses⁸ and arbitrary cut-off values later were used to determine insulin sensitivity.^{1,13,35} However, validation of these values has not been performed using formal statistical analysis (ie, 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 (eg, 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, clinically 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 interday coefficient of variation $14.1 \pm 5.7\%$) compared to minimal model analysis of the FSIGTT (average interday 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.¹² The degree of insulin dysregulation required to predispose horses to the development of laminitis

Table 2. Area under the glucose curve from 0–120 minutes comparisons for the FSIGTT, CGIT, and OST.

| | Comparison of FSIGTT to CGIT | Comparison of FSIGTT to OST | Comparison of CGIT to OST |
|-------------------------------|------------------------------|-----------------------------|---------------------------|
| Linear correlation | | | |
| Pearson's r | 0.7527* | 0.6564* | 0.5883* |
| Lin's Concordance Coefficient | 0.6655 | 0.4652 | 0.3863 |
| Bland–Altman analysis | | | |
| Bias (mg/dL × min) | 1,766 | 15,431 | –779.9 |
| 95% LOA | (–3,216, 6,748) | (9,674, 21,189) | (–6,756, 5,196) |

*Indicates significant linear correlation ($P < .05$).

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

Table 3. Sensitivity, specificity, positive predictive value, and negative predictive value of BIC, the OST, and CGIT compared to gold standard (FSIGTT).

| | Sensitivity | Specificity | Positive Predictive Value (PPV) | Negative Predictive Value (NPV) |
|--|-------------|-------------|---------------------------------|---------------------------------|
| BIC | 0% | 100% | 0% | 41.7% |
| OST | 0% | 100% | 0% | 41.7% |
| CGIT _{PP-D_{glu} > 45 min} | 85.7% | 40% | 66.7% | 66.7% |
| CGIT _{[Ins]₄₅ > 100 μIU/mL} | 28.5% | 100% | 100% | 50% |

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 4. Cohen's Kappa coefficients assessing agreement with gold standard (FSIGTT).

| Agreement with FSIGTT | Cohen's Kappa | 95% CI for Kappa |
|--|---------------|------------------|
| BIC | 0 | [-0.48, 0.48] |
| OST | 0 | [-0.48, 0.48] |
| CGIT _{PP-D_{glu} > 45 min} | 0.27 | [-0.31, 0.85] |
| CGIT _{[Ins]₄₅ > 100 μIU/mL} | 0.25 | [-0.25, 0.75] |

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.

currently is unknown. It is also unclear how parameters derived from minimal model analysis (eg, S₁) correlate to the risk of clinical or subclinical laminitis. The cut-off used in this study to define IR horses was S₁ < 1.0 × 10⁻⁴ L/mU/min in concordance with previous studies.²⁵⁻²⁷ In a previous study, S₁ of 1 laminitic pony was 0.089 × 10⁻⁴ L/mU/min, and the lowest reference quintile for 46 healthy horses in another study ranged from 0.14 to 0.78 × 10⁻⁴ L/mU/min.¹¹ The S₁ in human subjects with normal glucose tolerance was 2.0 ± 0.25 × 10⁻⁴ L/mU/min, 1.11 ± 0.18 × 10⁻⁴ L/mU/min in subjects with impaired glucose tolerance, and 0.67 ± 0.17 × 10⁻⁴ L/mU/min in subjects with noninsulin-dependent diabetes mellitus.³⁶ These reports suggest that the value used in this study to define IR in horses may be appropriate, but further investigation into how S₁ 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 nonstructural carbohydrates. In addition, 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 nonstructural 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 affected horses. In addition, peak insulin and glucose concentrations after a PO sugar challenge have been shown to vary significantly among individual horses,¹⁴ 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 (ie, 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.^{4-6,37} 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.

Footnotes

- ^a Karo Light Corn Syrup, ACH Food Companies, Inc, Oakbrook, IL
^b Terumo SURFLO EFTE IV Catheter 14G × 2", Terumo Medical Corp, Somerset, NJ
^c Dextrose 50% Injection, VetOne, MWI, Boise, ID
^d Humulin R, Eli Lilly and Company, Indianapolis, IN
^e AlphaTRAK blood glucose monitoring system meter, Abbott Animal Health, Chicago, IL
^f K₂ EDTA BD Vacutainer tubes, Franklin Lakes, NJ
^g Silicone-coated BD Vacutainer tubes, Franklin Lakes, NJ
^h Coat-A-Count insulin RIA, Siemens Medical Solutions Diagnostics, Los Angeles, CA
ⁱ Woltman, et al. Comparison of the combined glucose insulin tolerance test to Minimal Model insulin sensitivity in ponies and horses. *J Vet Intern Med* 2014;28;1115 [ABSTRACT]
^j MINMOD MILLENIUM Minimal Model Software, MINMOD, Inc, Los Angeles, CA
^k GraphPad Prism 6, GraphPad Software, La Jolla, CA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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