

Standard Article

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The Effect of Fasting Duration on Baseline Blood Glucose Concentration, Blood Insulin Concentration, Glucose/Insulin Ratio, Oral Sugar Test, and Insulin Response Test Results in Horses

F.R. Bertin, S.D. Taylor, A.W. Bianco, and J.E. Sojka-Kritchevsky

Objectives: Published descriptions of the oral sugar test (OST) and insulin response test (IRT) have been inconsistent when specifying the protocol for fasting horses before testing. The purpose of our study was to examine the effect of fasting duration on blood glucose concentration, blood insulin concentration, glucose/insulin ratio, OST, and IRT results in horses.

Animals: Ten healthy adult horses.

Procedures: Both OST and IRT were performed on horses without fasting and after fasting for 3, 6, and 12 hours. Thus, 8 tests were performed per horse in a randomized order. Blood collected at the initial time point of the OST was analysed for both blood glucose and serum insulin concentrations so that baseline concentrations and the glucose/insulin ratio could be determined. Unless fasted, horses had free-choice access to grass hay.

Results: There was no effect of fasting and fasting duration on blood glucose concentration, serum insulin concentration, glucose/insulin ratio, or the OST. Response to insulin in the IRT was decreased in fasted horses. The effect increased with fasting duration, with the least response to insulin administration after a 12-hour fast.

Conclusions and Clinical Relevance: These data indicate that insulin sensitivity is not a fixed trait in horses. Fasting a horse is not recommended for a glucose/insulin ratio or IRT, and fasting a horse for 3 hours is recommended for the OST.

Key words: Clinical pathology; Endocrinology; Equine; Glucose metabolism.

Insulin resistance is defined as decreased sensitivity to insulin by the body's insulin-dependent processes.¹ Because it is a strong risk factor for the development of laminitis, it is important to know whether horses suffer from disorders in carbohydrate metabolism including insulin resistance.² Documenting insulin resistance in horses, however, can be difficult. The "gold standard" method, the euglycemic hyperinsulinemic clamp, and the frequently sampled intravenous glucose tolerance test are time intensive and require specialized equipment.^{3,4} As such, they are only performed in research settings. Practitioners must rely on a number of screening tests to make a presumptive diagnosis of insulin dysregulation.^{5–9} None of these screening tests has been proven to be accurate in all instances. Horses that are insulin-resistant have exaggerated glucose and insulin responses to PO administration of corn syrup when

Abbreviations:

OS	toral sugar test
IRT	insulin response test
EMS	equine metabolic syndrome

compared to normal horses.⁵ The ability of insulin-sensitive tissue to respond to insulin can be tested directly by means of an insulin response test (IRT)^{8,9} or indirectly by use of the intravenous glucose tolerance test, the combined insulin/glucose tolerance test⁷ or the oral sugar test (OST), although the latter adds an additional layer of complexity by incorporating the enteroinsular axis.

Results of any test must be interpreted in light of the fact that insulin resistance in a single horse is not a fixed trait. An animal's sensitivity to insulin is affected by many factors including diet, adiposity, level of training, drug administration and concurrent disease such as pituitary pars intermedia dysfunction or systemic inflammatory response syndrome.^{10–14} In general, ponies and horses with high body condition score and consuming high-carbohydrate diets are more likely to be insulin-resistant, and some horses with normal body condition score will become increasingly insulin resistant if their weight increases as a consequence of a high carbohydrate diet.¹¹ Similarly, horses that lose weight and adiposity can show improved insulin sensitivity.¹⁵ These findings mirror the situation in human medicine, where improved diet, weight loss, and exercise result in increased insulin sensitivity in individuals with type 2 diabetes mellitus (noninsulin-dependent diabetes mellitus) and pre-diabetic states.¹⁵

In human medicine, diagnostic testing of glucose metabolism is performed on subjects who have fasted for at least 8 hours.¹⁶ Published protocols for the OST and IRT in horses have been inconsistent when specifying whether or not the animals should be fasted before

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN (Bertin, Taylor, Bianco, Sojka-Kritchevsky).

Dr. Bertin's Current address is Lady Davis Institute for Medical Research, 3999 Côte Sainte-Catherine Road, Montréal, QC H3T 1E2, Canada (Bertin).

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Corresponding author: Dr. J.E. Sojka-Kritchevsky, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN 47907; e-mail: sojkaje@purdue.edu.

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the tests are performed, and, if so, for how long. It is often recommended that a horse be given one flake of hay after 10 : 00 pm the evening before testing.¹⁷ As flakes of hay vary in weight, and horses consume feed at different rates, this recommendation allows the possibility that some horses will be tested in the morning after a prolonged fast, whereas others will eat until shortly before testing. The current protocols for insulin sensitivity testing do not mention fasting duration.

The purpose of our study was to determine whether the results of the OST or IRT will differ according to pre-test fasting times in horses. Resting blood glucose concentration and glucose/insulin ratios are other results used to screen horses for the equine metabolic syndrome (EMS). The effect of fasting duration on these measures also was evaluated.

Materials and Methods

Horses

The Purdue University Animal Care and Use Committee approved the study protocol. The study population consisted of 10 adult horses that were donated to the Purdue University Veterinary Teaching Hospital for problems unrelated to their gastrointestinal or endocrine systems. Specifically, their presenting problems included neurologic or behavior issues (5 horses), lameness (1 horse), recurrent airway obstruction (1 horse), heart murmur (1 horse), uveitis (1 horse), and non-healing wound (1 horse). The population consisted of 3 mares and 7 geldings. The age was 14 ± 8 (mean \pm standard deviation) years, and breeds represented were Thoroughbred (5), Quarter Horse (3), Pony of Americas (1), and Arabian (1). Body weights averaged of 468 ± 63 kg and median body condition score was 5 (range 4–7 on a 9-point scale). Seven horses were tested in September, and the remaining 3 in October. All horses were determined to be healthy on the basis of physical examination.

The horses were allowed to acclimate in individual box stalls with access to water and free choice mixed grass hay for at least 3 days before beginning the study. The goal of the study was to investigate whether screening test results would be consistent in a horse regardless of fasting duration and its endocrine disease status. Therefore, no attempt was made to determine whether any of the horses tested was truly insulin resistant using a test such as the frequently sampled intravenous glucose tolerance test. The horses did not receive any grain supplement, but had access to the same batch of hay at all times unless they were muzzled. Both OST and IRT were performed on horses without fasting and after fasting for 3, 6, and 12 hours. Thus, eight tests were performed per horse. The tests were performed daily beginning at 8 : 00 am over a period of 8 days with a washout period of 24 hours between tests. The order of testing in each horse was individually randomized using an on-line random number generator (www.random.org) so that no 2 horses were tested in the same order. Fasting was ensured by applying a tight-fitting muzzle to the horse so that they were unable to ingest any feed or bedding. On the day before the study, a 14 g, 5- $\frac{1}{4}$ inch catheter was aseptically placed in the left jugular vein and sutured in place. Patency was ensured by administration of a concentrated heparin solution (100 U/mL) after the final blood collection on each testing day. Before the first blood collection on any given day, the concentrated heparin solution was withdrawn and discarded, and between blood draws a standard heparinized saline solution (2 U/mL) was used as an irrigation solution. At each time point, 10 mL of blood were withdrawn through the catheter and discarded. Then, 5 mL of blood were

collected into both an evacuated sterile tube and into a tube containing EDTA as an anticoagulant. The blood collected into the EDTA tube was centrifuged immediately, and the plasma was harvested and frozen at -80°C until analysis. The blood collected into the sterile tube was allowed to clot and then the serum was harvested and frozen at -80°C until analysis.

Oral Sugar Test, Baseline Serum Glucose Concentration, Baseline Serum Insulin Concentration, and Glucose/Insulin Ratio

The OST was performed as described previously.⁵ Briefly, after horses were fasted for the pre-determined durations of time, blood was collected for the baseline measurements. These measurements included both serum glucose and insulin concentrations so that the glucose/insulin ratio could be calculated. Immediately after the blood was collected, each horse received 15 mL per 100 kg concentrated light corn syrup^a (158 mg/kg of glucose) PO. Blood again was collected 60 and 90 minutes later. The horses remained muzzled for the 90-minute testing period. After the final sample, the muzzle was removed and the horses were again free to consume hay. Serum was assayed for glucose and insulin at the baseline time point, and for insulin only at the 60-, and 90-minute time points.

Insulin Response Test

The insulin response test was performed using the 2-step insulin tolerance test as described previously⁹ after the horses were fasted for the pre-determined durations of time. A blood sample was collected for baseline serum glucose concentration determination. Horses then received 0.1 IU/kg regular human recombinant insulin^b IV. A second blood sample was collected 30 minutes later. The horses remained muzzled for the duration of the testing period, and the muzzle was removed immediately after the final blood sample was collected. To investigate whether the decrease in insulin sensitivity associated with fasting duration was caused by an increase in stress hormones, serum cortisol concentration was measured using serum collected at baseline during the IRT test.

Constituent Analysis

Serum insulin concentration was determined at the Animal Health Diagnostic Center at Cornell University using a double antibody radioimmunoassay.^c Serum glucose concentration was assayed using an automated clinical chemistry machine^d at the Purdue University Clinical Chemistry Laboratory. Serum cortisol concentration was assayed using a chemiluminescent assay at the Purdue University Clinical Chemistry Laboratory.^e All assays have been validated for use in the horse.^{18,19}

Statistical Analysis

Normal distribution was determined by the Shapiro-Wilk test. Means \pm standard deviations were calculated for normally-distributed data, and medians (range) were calculated for data that did not follow a normal distribution. Baseline serum glucose concentration, insulin concentration and glucose/insulin ratio without fasting or after the pre-determined duration of fasting were compared by means of a 1-way repeated-measures analysis of variance (ANOVA). For the OST, changes in serum insulin concentration induced by PO administration of corn syrup without fasting or after the pre-determined duration of fasting were compared by means of a 2-way repeated-measures ANOVA and Tukey post-hoc test when relevant. For the IRT, changes in serum glucose

concentration induced by IV insulin administration without fasting or after the pre-determined duration of fasting were compared by means of a 2-way repeated-measures ANOVA and Tukey post-hoc test when relevant.

A test result was assigned as either normal or indicative of insulin resistance using previously reported cut-off values.^{5,9,18} For serum glucose concentrations, a result was considered abnormal if above the laboratory reference range. For glucose/insulin ratio, the cut-off used for insulin-resistance was a value <4.5 .¹⁸ A serum insulin concentration >60 $\mu\text{IU/mL}$ after the OST was deemed positive, as was failure of serum glucose concentration to decrease to below 50% of the baseline concentration for the IRT.^{9,17} Categorical data was compared by means of a Fisher's exact test. Commercial software^f was used for analysis, and for each test, significance was defined as $P < .05$.

Results

With 1 exception, all horses tolerated all testing well. One horse developed clinical signs of hypoglycemia including muscle fasciculations and sweating during the IRT after the 6-hour fast. Dextrose was given PO and IV, and the signs resolved. Clinical signs became evident >30 minutes after insulin administration, and thus did not impact the testing protocol.

There was no significant effect of fasting duration on baseline serum glucose and insulin concentrations (Fig 1). Similarly, duration of fasting did not result in a significant difference in glucose/insulin ratios (Table 1). Mean and standard deviation blood glucose/insulin ratio without fasting and after fasting for 3-, 6-, and 12-hours duration were 5.5 ± 3.0 , 6.5 ± 2.1 , 6.7 ± 2.4 , and 8.2 ± 3.0 respectively. Using a ratio of 4.5 as a cut-off value, there was no significant change in the number of horses identified as insulin resistant with

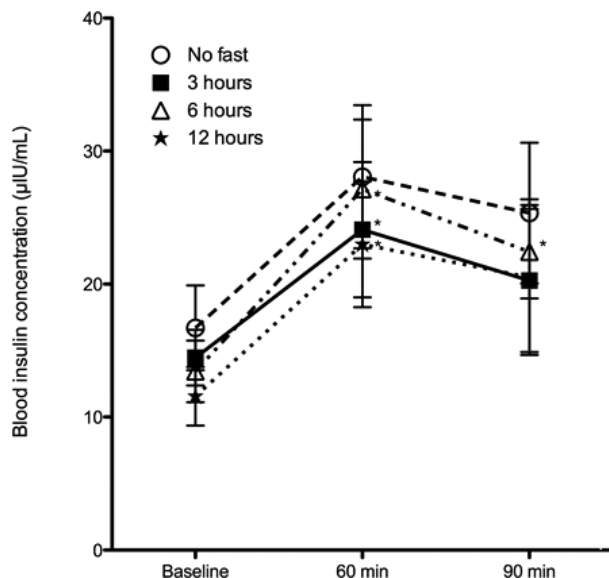


Fig 1. Serum insulin concentration (mean \pm standard deviation, $\mu\text{IU/mL}$) before and 60 and 90 minutes after oral administration of 15 mL/100 kg of concentrated corn syrup in 10 horses after not fasting (open circles) and fasting for 3 (black squares), 6 (open triangles), and 12 (black stars) hours ($*P < .05$ from baseline).

fasting duration. Two horses had ratios <4.5 without fasting, but not after any duration of fasting. One horse's ratio was <4.5 only after a 3-hour fast. A third horse's ratio was <4.5 after the 6- and 12-hour fast, whereas a fourth horse's ratio was <4.5 after baseline, 3-, and 6-hour fasts. All other horses had ratios >4.5 at all time points.

During the OST, corn syrup administration resulted in significant increases in serum insulin concentration only in fasted animals, regardless of the duration of fasting. We failed to find a significant increase in serum insulin concentration after corn syrup administration in non-fasted animals (Fig 1). After 3 hours of fasting, serum insulin concentration was significantly increased only at the 60-minute time point. After 6 hours of fasting, serum insulin concentration was significantly increased at 60 and 90 minutes, but serum insulin concentration at 90 minutes was significantly lower than at 60 minutes. Finally, after 12 hours of fasting, serum insulin concentration was significantly increased only at the 60-minute time point. Using the cut-off value of a serum insulin concentration >60 $\mu\text{IU/mL}$, only 1 of the horses was diagnosed as insulin resistant at 1 time point, without fasting at the 90-minute time point.

During the IRT, insulin administration resulted in a significant decrease in serum glucose concentration, regardless of fasting duration (Fig 2). Although still significantly decreased compared to baseline, when horses were fasted for 12 hours, the serum glucose concentrations were significantly higher than at any other time points. Using the 50% decrease as the cut-off value to diagnose insulin resistance, significantly more horses were identified as insulin resistant after 12 hours of fasting (Fig 2). Again, the classification of individual horses as insulin-resistant or insulin-sensitive was not consistent, depending on how long they were fasted. Baseline serum cortisol concentrations were not affected by fasting duration. Mean \pm standard deviation serum cortisol concentrations after not fasting and fasting for 3-, 6-, and 12-hours were 3.31 ± 1.38 , 3.03 ± 0.65 , 3.73 ± 1.04 , and 3.59 ± 0.93 $\mu\text{g/dL}$, respectively.

Discussion

The results of our study emphasize the importance of considering the specific conditions under which a horse is tested when interpreting screening tests for insulin dysregulation. Doing so is important both when deciding how to conduct a particular screening test and also when interpreting previous studies. For

Table 1. Results of the blood glucose/insulin ratio. IR/IS represents the numbers that were identified as insulin resistant/insulin sensitive using a value of less than 4.5 as the cut off.

	No fast	3 hours	6 hours	12 hours
Mean \pm SD	5.5 ± 3.0	6.5 ± 2.1	6.7 ± 2.4	8.2 ± 3.0
IR/IS	3/7	2/8	2/8	3/7

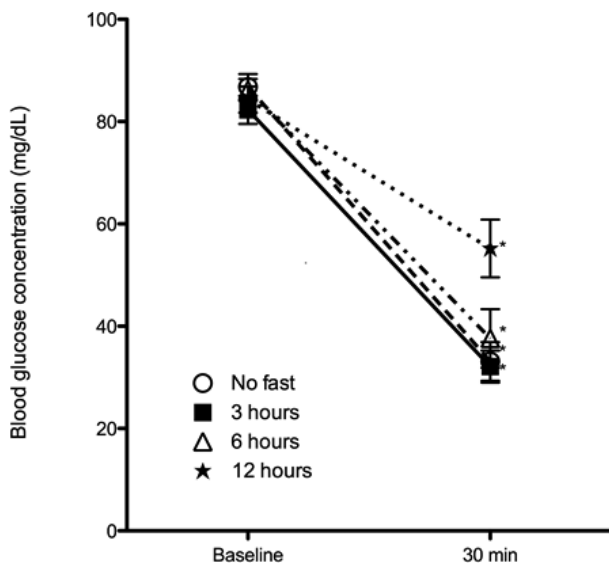


Fig 2. Effect of fasting on values of serum glucose concentration (mean \pm standard deviation, mg/dL) after intravenous administration of 0.1 IU/kg regular insulin in 10 horses after not fasting (open circles) and fasting for 3 (black squares), 6 (open triangles), and 12 (black stars) hours (* $P < .05$ from baseline).

example, previously studied ponies were allowed access to pasture immediately before blood collection, and thus must be considered non-fasted.² We failed to find any significant effect of fasting on glucose/insulin ratios, and the number of horses with glucose/insulin ratios that would identify them as insulin resistant based on current criteria did not increase or decrease with duration of fasting. However, at every fasting duration at least 1 horse I had a blood glucose/insulin ratio less than the cut-off value of 4.5 commonly used in human medicine.²⁰ Although the previous study may have been underpowered to detect an effect, these results suggest that there is an individual response to fasting and that basal concentrations or ratios are unreliable. Different conclusions might have been reached if the subjects included in the previous study had been fasted.²

The longer horses were fasted, the more likely they were to have a smaller decrease in serum glucose concentration after insulin administration. Thus, allowing access to hay while the IRT is performed would result in the highest likelihood of observing insulin sensitivity if it is present. Alternatively, fasting a horse with a high suspicion of insulin resistance would ensure the highest likelihood of uncovering the suspected insulin resistance. Using the criteria that $< 50\%$ decrease in serum glucose concentration is indicative of insulin resistance, the number of horses identified as insulin-resistant increased from 2/10 when horses were tested while eating to 7/10 when they were fasted for 12 hours. Taken together, these results suggest that, in our sample of horses, there is poor agreement among the glucose/insulin ratio, the OST and the IRT. Fasting a horse for a minimum of

3 hours before to the OST is recommended for consistency, because statistically significant increases in insulin concentration after corn syrup administration were only identified when animals were fasted.

Carbohydrate metabolism in horses, as in other mammals, is a complex process that involves appropriate handling of dietary intake; storage of excess energy in the form of glycogen and fat in liver, adipose tissue, and muscle; and, uptake of circulating glucose in response to insulin signalling. The OST and IRT involve different arms of this system, thus results would not be expected to be congruent in every instance. Giving dextrose PO evaluates both the horse's ability to secrete an appropriate amount of insulin from the pancreas and the insulin-dependent tissue's ability to maintain a normal blood glucose concentration. This process may be affected by the presence of incretins in the small intestine, which serve to regulate insulin secretion.²¹ Giving exogenous insulin directly tests the ability of insulin-responsive tissues to increase their uptake of glucose. Therefore, the IRT may be seen as a more direct measure of insulin resistance because it only tests insulin sensitivity whereas the OST may be seen as a more relevant test because it tests the whole axis. Ultimately, the superiority of 1 test over another may depend more on the main risk factor identified to cause laminitis (i.e. insulin resistance vs. hyperinsulinemia). Although the 2 conditions are related, they are not the same and conflicting evidence exists regarding which 1 is more likely to induce laminitis.²²

The goal of our study was not to determine which of the 3 screening methods was more likely to agree with more intensive testing. Rather, we investigated whether a screening test's results were consistent in an individual horse regardless of fasting duration and the horse's endocrine disease status. It appeared to be the case that the OST was more consistent. Because access to hay may increase increase insulin concentrations, fasting a horse for at least 3 hours before the OST should be considered to maximize the chances of unmasking subtle insulin resistance.

The horse that had an adverse reaction to the insulin administration was a 3-year-old Thoroughbred with normal body condition. This type of animal is extremely unlikely to be tested for insulin resistance in a clinical situation. Nonetheless, care should be given when administering insulin to younger animals and those that are well conditioned. The horse responded quickly to dextrose administration.

The horses in our study were not fed grain or concentrate at any time during the study. Hay may contain an important amount of carbohydrate however,²³ and forage availability during our study may have provided sufficient carbohydrate to influence insulin secretion. The hay was not analyzed because the primary research question concerned the effect of fasting, not dietary composition. Similarly, season of the year has been demonstrated to affect some hormone concentrations in horses. Because resting insulin concentration is not increased in normal horses according to season, the time of year that our study was conducted (September

and October) was not thought to be a factor that would have influenced the results, although the horses used in our study were of unknown disease status with regard to endocrinopathy.²⁴

Starvation and very low calorie diets have been shown to cause insulin resistance and diabetes in rodents and humans.^{25–27} The reasons are not well understood, but may relate to alterations in glucose transport function and the effects of increased free fatty acid concentrations on insulin utilization. Stress, as measured by increased cortisol concentrations, does not appear to be an important factor in this phenomenon,²⁷ and serum cortisol concentrations did not change with fasting duration in our study. Previous studies in horses have identified insulin resistance after ≥ 17 hours of fasting.^{12,28} The horses in our study had decreased response to insulin after as few as 6 hours, a much shorter time period. Interestingly, increased fat mobilization can be observed within 4 hours of fasting in horses,²⁹ which suggests that liver glycogen stores are used up quickly in horses or that horses do not store as much hepatic glycogen as do other species. Many horses with EMS are on calorie-restricted diets and may go for long periods of time between meals. These data suggest that withholding feed for more than a few hours will decrease insulin sensitivity, and may be counterproductive to the treatment goals in EMS.

In the absence of comparison to a “gold standard”, our data can only emphasize the fact that insulin sensitivity is not a fixed trait in horses. Whether a horse is fasted or fed and the individual test used to examine insulin dynamics can affect the results. Moreover, the results of our study emphasize why the OST and the IRT must be considered as screening tests for insulin resistance, and why the glucose/insulin ratio determination may not provide the same information as provocative testing. In summary, the longer a horse was fasted, the more likely it was to be classified as insulin-resistant when performing the IRT. Additional testing on known insulin-resistant horses is needed to determine how they would react to fasting before performing tests of their ability to metabolize carbohydrate. Our study design does not permit strong recommendations on testing protocols based on the current results alone; it is simply a starting point. Researchers and clinicians should consider fasting duration when evaluating carbohydrate metabolism in horses, and additional studies are needed to identify which protocol would be the best to use.

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Conflict of Interest Declaration: Authors declare no conflict of interest.

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

Footnotes

- ^a Karo Light syrup, ACH Food Companies, Inc. Cordova TN
 - ^b Humulin R, Eli Lilly and Company, Indianapolis, IN
 - ^c Abbott Cell-Dyn[®] 2000, Abbott Park, IL
 - ^d Diagnostic Systems Laboratories Inc., Webster, TX
 - ^e Seimens Immulite 2000, Malvern, PA
 - ^f Prism, GraphPad Software, Inc. La Jolla, CA
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References

1. Lebovitz HE. Insulin resistance: Definition and consequences. *Exp Clin Endocrinol Diabetes* 2001;109(Suppl 2):S135–S148.
2. Treiber KH, Kronfeld DS, Hess TM, et al. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. *JAVMA* 2006;228:1538–1545.
3. Rijnen KEPM, van der Kolk JH. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. *Am J Vet Res* 2003;64:1260–1264.
4. Toth F, Frank N, Elliott SZB, et al. Optimization of the frequently sampled intravenous glucose tolerance test to reduce urinary glucose spilling in horses. *Equine Vet J* 2009;41:844–851.
5. Schuver A, Frank N, Chameroy KA, Elliott SB. Assessment of insulin and glucose dynamics by using an oral sugar test in horses. *J Eq Vet Sci* 2014;34:465–470.
6. Kronfeld DS, Treiber KH, Geor RJ. Comparison of non-specific indications and quantitative methods for the assessment of insulin resistance in horses and ponies. *JAVMA* 2005;226:712–7719.
7. Eiler H, Frank N, Andrews FM, et al. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. *Am J Vet Res* 2005;66:1598–1604.
8. Caltabillota TJ, Earl LR, Thompson DL, et al. Hyperleptinemia in mares and geldings: Assessment of insulin sensitivity from glucose responses to insulin injection. *J Anim Sci* 2010;88:2940–2949.
9. Bertin FR, Sojka-Kritchevsky JE. Comparison of a 2-step insulin-response test to conventional insulin-sensitivity testing in horses. *Dom Animal Endocrinol* 2013;44:19–25.
10. Murphy D, Reid SWJ, Love S. The effect of age and diet on the oral glucose tolerance test in ponies. *Equine Vet J* 1997;29:467–470.
11. Carter RA, McCutcheon LJ, George LA, et al. Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *Am J Vet Res* 2009;70:1250–1258.
12. Evans JW. Effect of fasting, gestation, lactation and exercise on glucose turnover in horses. *J Anim Sci* 1971;33:1001–1004.
13. Goodpaster BH, Kelley DE, Wing RR, et al. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 1999;48:839–847.
14. Carter RA, McCutcheon LJ, Valle E, et al. Effects of exercise training on adiposity, insulin sensitivity, and plasma hormone and lipid concentrations in overweight or obese, insulin-resistant horses. *Am J Vet Res* 2010;71:314–321.
15. Morgan RA, Keen JA, McGowan CM. Treatment of equine metabolic syndrome: A clinical case series. *Equine Vet J* 2016; 48:422–426.

16. Horowitz M, Edelbrock MAL, Wishart JM, Straathof JW. Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia* 1993;36:857–862.
17. Frank N. Metabolic syndrome. *Vet Clin Equine* 2011;27:73–92.
18. Singh AK, Jian Y, White T, Spassova D. Validation of non-radioactive chemiluminescent immunoassay methods for the analysis of thyroxine and cortisol in blood samples obtained from dogs, cats, and horses. *J Vet Diagn Invest* 1997;9:261–268.
19. Reimers TJ, Lamb SV, Bartlett SA, et al. Effects of hemolysis and storage on quantification of hormones in blood samples from dogs, cattle, and horses. *Am J Vet Res* 1991;52:1075–1080.
20. Legro RS, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1998;83:2694–2698.
21. Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol Rev* 2008;60:470–512.
22. de Laat MA, Clement CK, Sillence MN, et al. The impact of prolonged hyperinsulinemia on glucose transport in equine skeletal muscle and digital lamellae. *Equine Vet J* 2015;47:494–501.
23. <http://www.safergrass.org/pdf/AAEP.pdf>
24. Place NJ, McGowan CM, Lamb SV, et al. Seasonal variation in serum concentrations of selected metabolic hormones in horses. *J Vet Intern Med* 2010;24:650–654.
25. Newman WP, Brodows RG. Insulin action during acute starvation: Evidence for selective insulin resistance in normal man. *Metabolism* 1983;32:590–596.
26. Koffler M, Kisch ES. Starvation diet and very-low-calorie diets may induce insulin resistance and overt diabetes mellitus. *J Diabetes Complicat* 1996;10:109–112.
27. Unsal H, Cotelioglu U. The effects of food restriction on some biochemical parameters and certain bacterial groups in the cecum in Sprague Dawley rats. *Microbial Ecol Health Dis* 2007;19:17–24.
28. Firshman AM, Valberg SJ. Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Vet J* 2007;39:567–575.
29. Frank N, Sojka JE, Latour MA. Effect of withholding feed on concentration and composition of plasma very low density lipoprotein and serum nonesterified fatty acids in horses. *Am J Vet Res* 2002;63:1018–1021.