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Comparison of a 2-step insulin-response test to conventional insulin-sensitivity testing in horses

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Abstract

Equine insulin resistance is important because of its association with laminitis. The insulin-response test is described to diagnose insulin resistance in clinical settings. Practitioners may be reluctant to perform this test because of the time needed for the test and the fear of inducing hypoglycemia. The objective of the study was to compare a 2-step insulin-response test with a complete insulin-response test. A complete insulin-response test was performed on 6 insulin-resistant horses and 6 controls. A 2-step insulin-response test consisting of an intravenous injection of 0.1 IU/kg human insulin and blood glucose determination at 0 and 30 min after injection was performed on the same horses. Times to reach a 50% reduction of glucose baseline were compared between tests and horses. All the horses tolerated both tests well. No significant difference was observed between baseline glucose concentrations of insulin-resistant horses and controls ($P = 0.09$). Time to reach 50% reduction of glucose baseline for controls was not significantly different with the use of the complete insulin-response test or the 2-step test ($P = 0.98$). For insulin-resistant horses, the time to reach 50% reduction of glucose baseline with the use of the 2-step test was significantly longer than for controls ($P = 0.004$). With a cut-off time of 30 min, the 2-step test had the same characteristics as the complete test. The 2-step test provided a safe, rapid, and low-cost method to diagnose insulin resistance in horses in a clinical setting.

Keywords: Insulin; Equine; Diagnostic test; Insulin resistance; Equine metabolic syndrome

1. Introduction

Insulin resistance, which can be caused by a decreased function of insulin receptors, a decrease in pancreatic secretion of insulin in response to a glucose load, or a combination of both, has been shown to involve defects of different steps of the insulin-signaling pathways [1–3].

Insulin resistance is believed to be one of the primary pathophysiologic causes of equine metabolic syndrome, a disorder that affects horses [2]. Clinical signs of equine metabolic syndrome are nonspecific but include obesity, dyslipidemia, abnormal regional adiposity, and a predisposition toward laminitis [4,5].

Insulin resistance may be difficult to diagnose because some horses with the phenotypic appearance of equine metabolic syndrome have normal insulin responses, whereas phenotypically normal horses may be resistant to insulin [6,7]. Direct and indirect methods of varying complexity are currently used for assessing insulin resistance in horses and in people. The direct methods comprise the euglycemic clamp techniques and the insulin suppression test [8–10]. The indirect

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methods include the classical glucose tolerance test or an adapted test such as the minimal model method [9–16]. Fasting hyperinsulinemia and hyperglycemia in the absence of confounding factors provide evidence of insulin resistance in horses suspected of equine metabolic syndrome, but resting concentrations of glucose and insulin are variable and may be normal in horses with insulin resistance [2,17]. In horses, the euglycemic hyperinsulinemic clamp is the "gold standard" to measure insulin resistance, but it requires a research setting [17]. Other protocols have been described in the equine species that are not as labor intensive. These include the ratios between insulin and glucose, the quantitative insulin sensitivity check index (QUICKI) and the homeostasis model assessment of insulin sensitivity (HOMA-IS) [10]. These surrogate tests have been used in some studies [17,18], but their correlation with true insulin resistance as documented by more extensive testing has not been reported in horses. Furthermore, they still require the use of a diagnostic laboratory to determine insulin concentrations.

A combined glucose insulin tolerance test was reported in horses [19] and is recommended by the American College of Veterinary Internal Medicine [2]. The test requires multiple blood samples over several hours and thus may be difficult to perform in the field [2].

Caltabilota et al [20] reported on the use of a complete insulin-response test to diagnose insulin resistance in mares. In that study, it was concluded that a dose of ≤125 mIU/kg of recombinant human insulin could be used safely to differentiate mares with previously diagnosed insulin resistance from normal mares.

The purpose of the study reported here was to compare a rapid 2-step test that characterized the physiological response to an intravenous injection of insulin with the previously described complete insulin-response test. Because the effect of insulin on blood glucose concentrations is immediate in both normal and insulin-resistant horses, it was hypothesized that fewer blood samples over a shorter period of time would be as informative as a complete insulin-response curve.

2. Materials and methods

2.1. Animals

All procedures conducted were approved by the Purdue Animal Care and Use Committee. Twelve adult horses, 6 with insulin resistance and 6 controls (3 geldings and 9 mares) from the Purdue University teaching herd were used. All horses were healthy according to a complete physical examination. Inclusion criteria included absence of disease for ≥ 1 mo and absence of clinical signs of pituitary pars intermedia dysfunction. Horses had a mean age of 16 ± 7 yr (range, 5 to 31 yr). Mean weight was 495 ± 78 kg (range, 364 to 589 kg), and body condition scores were between 3/9 and 9/9. Breeds included Arabian (n = 1), Paint (n = 3), Quarter Horse (n = 3), Standardbred (n = 1), Thoroughbred (n = 1), and Grade (3). The horses were housed in individual stalls on the day before the experiment. Throughout the testing period, horses were fed their regular diet that consisted of mixed-grass hay ad libitum and water ad libitum.

2.2. Study design

One hour before the first day of experiments, an intravenous catheter was aseptically placed in one jugular vein and remained in the vein until the experiment was completed. The catheter was used for insulin injections and for blood collection. Between subsequent day experiments, the catheter was heparin-locked (4.3 mL of 500 IU/mL heparin). For blood collection, 6 mL of blood was taken through the catheter and discarded. An additional sample of 2 mL of blood was then taken and used for measurement. The catheter was then flushed with 12 mL of heparinized saline solution (2 IU/mL heparin in 0.9% NaCl). The horses were randomly assigned to a group to be tested by the complete insulin-response test or the 2-step test first and by the other test the next day.

The complete insulin-response test was performed as described by Caltabilota et al [20]. Regular human recombinant insulin (Humulin R 0.1 IU/kg, Eli Lilly and Company, Indianapolis, IN) was rapidly injected through the intravenous catheter, and blood samples were collected at time 0 (baseline) and 5, 15, 30, 45, 60, 90, 120, 150, and 180 min after insulin injection. A hand-held strip glucometer (Accu-Chek Advantage; Roche, Indianapolis, IN) was used to determine all blood glucose concentrations. The less-precise blood glucose concentration determined via the glucometer was deemed to be sufficient because it was the difference from baseline that was the value of interest rather than the absolute concentration. The same glucometers have been used commonly in previously described protocols designed to diagnose insulin resistance in horses [2,6,19]. Samples were performed in duplicate on a random number of time points. The intra-assay coefficient of variation was 1.8% between duplicate samples and was considered acceptable. On the basis of the complete insulin-response test, a horse was confirmed insulin-sensitive if, after injection of insulin, the blood