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Factors Affecting the Glucose Response to Insulin Injection in Mares: Epinephrine, Short- and Long-Term Prior Feed Intake, Cinnamon Extract, and Omega-3 Fatty Acid Supplementation

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ABSTRACT

Five experiments were conducted with mares to better define factors that might affect the assessment of insulin sensitivity via direct insulin injection, and to then apply this method of assessing insulin sensitivity to trials which tested two potential supplements for improving poor insulin sensitivity in horses. The experiments assessed the effects of the following: (1) previous administration of epinephrine, (2) overnight feed deprivation versus hay or pasture consumption, (3) 10-day acclimatization to hay in a dry lot versus pasture grazing, (4) cinnamon extract supplementation, and (5) fish oil supplementation on insulin sensitivity. Mares of known high and low insulin sensitivities were used in the first three experiments, whereas mares with low insulin sensitivities were used in the supplement trials. Epinephrine administration increased blood glucose concentrations ($P < .05$) and prevented the insulin-induced decrease in blood glucose concentrations in both sensitive and insensitive mares. Overnight feed deprivation decreased ($P < .06$) insulin sensitivity relative to overnight ad libitum access to hay, and both regimens resulted in reduced insulin sensitivity relative to overnight pasture availability; sensitive and insensitive mares responded similarly except when kept on pasture ($P = .0854$). Ten days of hay consumption in a dry lot reduced ($P < .05$) insulin sensitivity in insensitive mares, but not in sensitive mares, relative to pasture grazing. Supplementation with cinnamon extract or fish oil had no effect on insulin sensitivity of mares with known low insulin sensitivity under the conditions of these experiments.

1. Introduction

We have shown previously that insulin sensitivity could be detected in horses by direct insulin injection [1]. In the previous study [1], mares with hyperleptinemia were found to be less insulin-sensitive than mares with normal or low leptin concentrations, as was predicted by the fact that hyperleptinemic horses also have elevated insulin concentrations [2] and an exaggerated insulin response to administered glucose [3].

Poor insulin sensitivity in horses has been associated with laminitis and metabolic syndrome [4-6]. Exercise has been reported to improve insulin sensitivity in horses [7,8], and both cinnamon extract [9] and omega-3 fatty acid (through fish oil) consumption [10,11] improves insulin sensitivity in various species.

The series of experiments reported herein were conducted to better define some factors that might affect the assessment of insulin sensitivity, as described by Caltabilota et al. [1], and to then apply this method of assessing insulin sensitivity to trials which tested two potential supplements for improving poor insulin sensitivity in horses. Specifically, the first three experiments assessed the effects of elevated epinephrine (as would occur in stressed horses) and previous feed intake in the

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short- (feed deprived, overnight hay, and pasture) and long-terms (10 days of pasture vs. hay in a dry lot) on the insulin-induced decrease in glucose concentrations in horses. The second objective was to determine whether supplementation with cinnamon extract or fish oil would improve the insulin sensitivity of hyperleptinemic (insulin-insensitive) mares.

2. Materials and Methods

All procedures were approved by the Animal Care and Use Committee of the LSU Agricultural Center. Light horse mares aged between 13 and 23 years from our resident herd in Baton Rouge, LA, were used. They weighed between 463 and 648 kg, and had body condition scores [12] between 5 and 8. They were routinely maintained on native grass pastures in the spring, summer, and fall, and were supplemented with native grass hay (round bales) in winter as needed to maintain body condition. All mares had been previously categorized with regard to mean leptin concentration (relative to other mares; ie, either hyperleptinemic or normal [3,13]) and insulin sensitivity (sensitive or insensitive as determined by their responses to insulin injection [1]).

2.1. Experiment 1

Experiment 1 was designed to determine the effect of preinjection with epinephrine on the glucose response to a single dose of recombinant human insulin [1] in sensitive versus insensitive mares. Four insulin-sensitive and four insulin-insensitive mares, as described by Caltabilota et al. [1], were used. The experiment was performed as a single switch-back, with two mares within each sensitivity group exposed to epinephrine on the first day (December 5, 2009; the rest received saline), and the other mares received epinephrine on the second day (December 7, 2009). Epinephrine was administered i.v. at a dose of 5 μg/kg body weight (BW) [14] in saline at a volume of 0.01 mL/kg; control injections were the same volume of saline only. For each treatment day, mares were brought in from pasture and were deprived of feed overnight (approximately 13 hours) in a dry lot paddock but had ad libitum access to water. Injections were administered at approximately 08 AM the next morning. Jugular blood samples were collected for glucose determination through a hand-held glucometer [1,15] (Precision Xtra, Abbott Laboratories, Abbott Park, IL) at −10 minutes and 0 minute relative to epinephrine or saline injection. Subsequent samples were collected at 20 minutes (followed by the insulin injections), and 30, 40, 60, and 80 minutes. Insulin was administered i.v. after the 20-minute sample at 50 mU/kg BW for sensitive mares and 125 mU/kg BW for insensitive mares. These doses had been found earlier [1] to produce decreases of approximately 50% in blood glucose for the mares in the respective categories. Once sampling had been completed, mares were returned to pasture.

Blood glucose concentrations were analyzed by analysis of variance (ANOVA; SAS Institute, Inc, Cary, NC) as a replicated 2 × 2 Latin square design with a 2 × 2 factorial arrangement of treatments (epinephrine treatment and insulin sensitivity category).

2.2. Experiment 2

Experiment 2 was designed to test the effects of overnight feed intake on the glucose response to a single injection of human recombinant insulin in sensitive versus insensitive mares. The experiment was conducted as a replicated 3 × 3 Latin square, with the 3 treatments being (1) overnight feed deprivation, (2) overnight ad libitum access to grass hay, and (3) overnight in the pasture; water was available for ad libitum consumption in all cases. Six insulin-insensitive and six insulin-sensitive mares were used. Within each phase, two mares of each category were managed overnight as described in the treatments, and then tested the following morning. The test days were July 16, 18, and 20, 2010. All mares were returned to pasture no later than noon on each test day. For overnight feed deprivation, mares were brought in from the pasture at approximately 7 PM the day before and kept in a dry lot with ad libitum access to water. For the hay-fed treatment, mares were brought in from pasture the same way, but placed in a dry lot with ad libitum access to grass hay and water overnight. For the pastured group, mares were left in the pasture until the morning of testing, and were brought up at approximately 7 AM.

On each day of testing, mares were tethered loosely inside an open-sided barn at 7 AM, and two blood samples were drawn by jugular venipuncture 10 minutes apart. Insulin was administered i.v. at a dose of 50 mU/kg BW for sensitive mares and 125 mU/kg BW for insensitive mares. Subsequent blood samples were collected at 40 and 60 minutes after insulin injection [1]. Glucose concentration was determined in all blood samples with the glucometer described in experiment 1. The maximum percentage decrease in blood glucose concentrations was calculated for each mare on each occasion by first averaging the two preinsulin blood glucose concentrations. The blood glucose values at 40 and 60 minutes were subtracted from this mean, and the net decrease was then expressed as a percentage of the preinjection mean. The largest percentage decrease in the two concentrations (at 40 and 60 minutes) was used as the data point for that mare on that occasion.

When all phases were complete, the percentage decreases were analyzed by ANOVA as a replicated (four squares) Latin square design. Treatment effects were arranged as a 2 × 3 factorial (two sensitivity categories and three overnight feeding regimens). Differences between means were assessed by the least significant difference test [16].

2.3. Experiment 3

Experiment 3 was conducted in October and November 2010, as a replicated 2 × 2 Latin square design to test the effect of long-term feeding regimen (10 days) on the percentage decrease in blood glucose concentrations after a standard dose of insulin in sensitive versus insensitive mares. Procedures were similar to those in experiment 2, except that mares were acclimated for 10 days to either native grass hay fed in a dry lot with ad libitum access to water, or maintenance on pasture with ad libitum access to