Journal of Equine Veterinary Science 33 (2013) 937-941

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Characterization of Glucose Response Curves after Insulin Injection in Sensitive versus Insensitive Mares

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ARTICLE INFO

Article history:
Received 19 December 2012
Received in revised form
28 January 2013
Accepted 30 January 2013
Available online 14 March 2013

Keywords:
Mares
Insulin
Glucose
Insulin sensitivity

ABSTRACT

The glucose responses to intravenous injections of a range of doses of recombinant human insulin were determined for six mares known to be insulin sensitive and six mares known to be insulin insensitive, with the goal of better characterizing the regression lines resulting from the two categories of mares. Insulin doses between 8 and 198 mU of insulin per kg of body weight (mU/kg BW) were administered intravenously between September 13 and 26, 2010, starting with 50 mU/kg BW on the first day. Higher and lower doses were administered on alternate days to obtain percentages of decreases in blood glucose concentrations between 10% and 70%. Linear regression analysis revealed that insulin-insensitive mares have glucose response curves with higher *y* intercepts ($P = .066$), less steep slopes ($P = .0003$), and less goodness of fit ($P = .053$) in addition to the expected greater dose required to produce a 50% reduction in blood glucose concentrations (ED50; $P = .006$), despite the similarities between their body weights and those of insulin-sensitive mares. Linear and nonlinear regression of responses to the 32, 50, and 79 mU/kg BW insulin doses with the overall estimates of ED50 and the natural log of ED50 indicated that the 50 mU/kg BW dose had the greatest coefficient of determination (>0.95). Generally, it appears that estimates of insulin sensitivity based on a single injection of insulin or on multiple injections of insulin are least variable for insulin-sensitive mares.

1. Introduction

Caltabilota et al. [1] reported that the percentage of decrease in serum glucose concentrations in mares after injection of recombinant human insulin could be used to determine insulin sensitivity, with the limitation that multiple data points, preferably three, be obtained between decreases of 10% and 70%, at which point the dose-response curve is linear. Subsequently, Bertin and Sojka-Kritchevsky [2] reported a two-step method based on a single dose of insulin, which compared favorably with the protocol of Caltabilota et al. [1].

In the development of the original technique, Caltabilota et al. [1] noticed that mares with low insulin sensitivities seemed to have less steep dose-response curves. That is, the incremental percentage of decrease in glucose concentrations with each higher dose of insulin in insulin-insensitive mares was not as much as that for insulin-sensitive mares. Caltabilota et al. [1] suggested that three doses of insulin produced the most reliable estimates of insulin sensitivity, even though the response to a single dose of 50 mU per kg of body weight (mU/kg BW) did in fact provide a close approximation to the final sensitivity estimate in most insulin-sensitive mares but less so in mares later diagnosed with insensitivity.

The present experiment was conducted to better characterize the insulin-glucose dose-response curves in mares (a minimum of 5 points) so that differences among horses of differing sensitivities could be assessed. Data from these curves were subsequently used to estimate the predictability of single-injection results compared to those obtained with the multipoint curves.

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http://dx.doi.org/10.1016/j.jevs.2013.01.010

2. Materials and Methods

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the Louisiana State University Agricultural Center. Twelve mares, six known to be insulin insensitive and six known to be insulin sensitive (determined multiple times over several years), were used in this experiment. The mares were between 6 and 16 years of age, weighed between 450 and 650 kg, and had body condition scores of 6 or greater [3]. They were routinely housed on native grass pasture and were brought into an outdoor lot for the experimental procedures. The lot was devoid of feedstuff; however, water was available for consumption ad libitum.

For the first day of the experiment, all mares were moved from pasture to the lot and deprived of feed from approximately 5 PM until 7 AM the next morning (September 13, 2010). Two samples of jugular blood were obtained 10 minutes apart from each mare by venipuncture with a 21-gauge needle with attached syringe, and glucose concentration was estimated in whole blood by a hand-held glucometer (Precision Xtra, Abbott Laboratories; www.abbottdiabetescare.com). Glucometer readings were typically not duplicated, except when an obtained value seemed outside of the expected physiologic range, or when the two samples at the 10-minute interval differed by >10%. Previous assessments with the glucometer used [1] indicated excellent agreement between duplicate readings ($r = 0.98$), and random checking of the single readings in the present experiment confirmed this agreement.

Once the baseline glucose concentration was established, recombinant human insulin (Sigma Chemical Co., St. Louis, MO) was administered to each mare intravenously in saline at 50 mU/kg BW. Injection volume was 0.01 mL/kg BW. Blood samples were then drawn at 40 and 60 min after insulin injection from the jugular vein for glucose concentration measurements by the glucometer.

Percentage decreases in blood glucose concentrations were calculated as described by Caltabilota et al. [1]. First, the glucose concentrations of the two preinsulin injection samples were averaged to obtain a baseline mean. Then, the glucose concentrations for the 40- and 60-minute samples were subtracted from this baseline, and the differences were expressed as a percentage of the baseline. The greatest percentage of decrease was used as the mare's response for that insulin injection.

Mares were returned to pasture after all samples had been drawn and assessed. The entire procedure was repeated on September 15, 17, 20, 22, and 24, with different doses of insulin (8, 12.6, 20, 32, 79, and 125 mU/kg BW). Based on the response to the 50 mU/kg BW dose on day 1, the second injection given was one dose higher (79 mU/kg BW; half the horses, primarily the lowest responders) or lower (32 mU/kg BW; remaining horses). The goal was to obtain at least five decreases in glucose concentrations between 10% and 70%, completing the curve in an alternating manner as much as possible (high then low or low than high, and so forth). The insensitive mares typically needed the highest dose (125 mU/kg BW) to get near a 50% decrease; thus, one higher dose (198 mU/kg BW) was subsequently added on September 26 for those mares as needed.

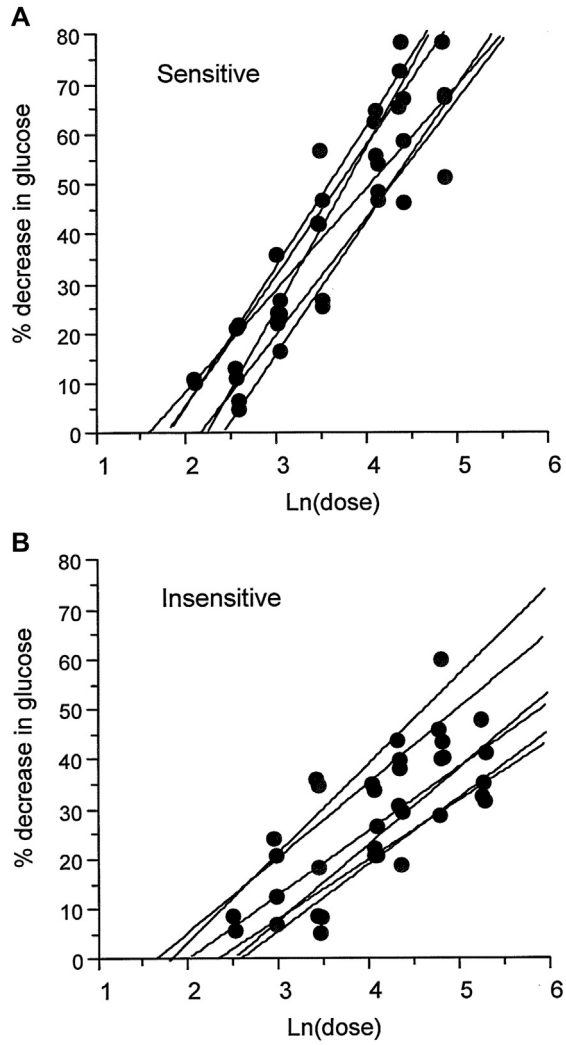

Fig. 1. Combined plots of the percentage decreases in blood glucose concentrations in response to various doses of recombinant human insulin in six sensitive (**A**) and six insensitive (**B**) mares. Each line represents one mare, whereas the mares' individual data points are not differentiated. The degree of parallelism shows the relative agreement (or variability) of the results within each group. Individual regression lines for the sensitive and insensitive mares are based on an average of 6.0 and 5.8 doses per mare, respectively; the range was the same for both groups (5-7 per mare).

Data from each mare were subjected to linear regression analysis [4] in which the x values were the natural log values (ln) of the insulin doses, and the y values were the associated percentage decreases in blood glucose concentrations. The slopes and intercepts for each mare's responses were then analyzed using one-way analysis of variance (ANOVA) [4] with SAS software (SAS Institute, Cary, NC) for comparison between the insulin-sensitive and -insensitive groups. Also, the ln of the effective dose required to produce a 50% decrease in glucose concentrations (ED50) values (lnED50) and the ED50 values were calculated from the individual regression equations as described by Caltabilota et al. [1]. Briefly, the lnED50 value was the calculated x value associated with a y value of 50%, and the ED50 value was the exponent of the lnED50 value.