THE EFFECT OF CHANGES IN BODY CONDITION ON INSULIN SENSITIVITY IN HORSES

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Introduction

Insulin sensitivity is defined as the ability of insulin to facilitate glucose uptake into target tissues such as skeletal muscle and adipose, while insulin resistance is the inability of insulin to exert its effect at these tissues. Insulin resistance has been linked to several equine conditions including laminitis[1,2] and hyperlipemia[3]. Therefore it is of interest to investigate factors that affect insulin sensitivity in horses. It has been shown that dietary energy source, specifically diets rich in starch and sugar, negatively affect insulin sensitivity[4,5]. In contrast, exercise conditioning appears to increase tissue sensitivity to insulin[6]. An association between insensitivity to insulin and obesity has been reported in horses[7]. The nature of how insulin sensitivity (IS) is related to weight gain and obesity in horses is not fully understood, nor is there a recommendation for an ideal level of adiposity for insulin sensitivity. There are several methods available to quantify insulin sensitivity in horses. The euglycemic-hyperinsulinemic clamp (EHC) is the "gold-standard" method to quantify whole body insulin sensitivity in human research[8] and has proven useful in equine studies[9]. The objective of the present study was to determine how changes in body condition (adiposity), achieved through the modification of forage-only dietary energy intake (to avoid confounding factors such as DE source[4,5]), affect insulin sensitivity in the horse. It was hypothesized that an increase in body condition would result in a decrease in IS, with the opposite being true for a decrease in body condition.

Materials and Methods

North Carolina State University's Animal Care and Use Committee approved all procedures. Seventeen mature gelding horses, 3-14 yr of age (8.1 ± 4.4 yr), were used in this study that was conducted in the fall and early winter of 2008. Breeds consisted mostly of Quarter Horses, Quarter Horse crosses, and Quarter Horse types, either Paint or Appaloosa (QH, n = 9). Other breeds included Arabian crosses (Arabx, n = 3), Thoroughbred or Thoroughbred crosses (TB, n = 4), and Warmbloods (WB, n = 1). Horses were maintained on endophyte-free fescue pasture and supplemented with free choice, round-bale timothy-orchard grass mix hay for approximately 25 weeks prior to commencing the study. Horses were moved from pasture to 12 x 36 dry-lot stalls 6 weeks prior to day 0 collections. Horses were fed grass-alfalfa mix hay cubes (1.9 MCal DE/kg, 14.1% CP, DM basis, Bale-in-a-Bag, Idle Acres, Cokato, MN) to achieve a moderate body condition score (BCS) of 5 (1-9 Henneke scale)[10] prior to start of the study. Horses were blocked by age (young 3-4 yr, mid 5-9 yr, and old 10-14 yr) and randomly assigned to one of three treatment groups (weight gain or loss, n = 6 each, and maintenance, or control, n = 5) and fed to gain (fleshy, BCS = 7), maintain (moderate, BCS = 5), or lose (thin, BCS = 3) weight and body condition for a period of approximately 130 days. Weight changes were assessed weekly via a digital livestock scale (Smart Scale 200, Gallagher Group Ltd., Hamilton, New Zealand). Body condition score[10], percentage body fat (assessed with ultrasound)[11], and insulin sensitivity via the EHC[8,9] were assessed at days 0, 65 and 130 (described below).

Diets

Weight and body condition changes were achieved by feeding of one of three types of grass-alfalfa mix hay cube products (Idle Acres, Cokato, MN). Horses gaining or losing weight were fed High End Mix (2.1 MCal DE/kg, 15.5% CP, DM basis) or Cube Lite (1.7 MCal DE/kg, 15.4% CP, DM basis), respectively. Control horses were maintained on Bale-in-a-Bag cubes (see above). Cubes were fed as a percentage of DE maintenance requirements as specified by the National Research Council[12] with goal DE intakes at 70, 100, and 130% of maintenance for the lose, control, and gain groups, respectively. Horses were fed only cubes 3 times per day for 10 weeks, after which flaked endophyte-free fescue hay (2.2 MCal DE/kg, 10.5% CP, DM basis) was added to the diet as 20% of total DE per day. Horses were then fed 3 times per day with cubes fed at morning and midday meals and hay fed at the evening meal only. Daily hay and cube feed refusals (orts) were collected separately and weighed. Horses had free access to fresh water and a vitamin/mineral supplement (Nature's Essentials Free Balance 12:12, Purina Mills, Gray Summit, MO). Two horses in the gain treatment were supplemented with vegetable oil to provide 3.51 MCal DE per day due to lack of weight gain on forage alone.

Measures of Adiposity

Body condition score (1-9 Henneke scale)[10] was assessed at days 0, 65, and 130 by one or two trained persons, blind to treatment. If both persons assessed BCS the scores were averaged. Percentage body fat was determined based on ultrasounds of rump and abdominal fat at days 0, 65, and 130. The same individual performed all evaluations. Rump fat was measured in a 12 x 12 cm square centrally located between the top of the croup, point of the hip, and point of the buttock. Abdominal fat location was determined by counting five ribs spaces caudal to cranial, from the last rib, at a height between the ventral midline and stifle. Ultrasounds at each location were performed bilaterally and measured in cm. Percent fat was determined using rump fat depth in the equation described by Kearns et al[11].

Euglycemic-Hyperinsulinemic Clamp

Feed was withheld for 12 hours prior to the EHC. The morning of the clamp, horses were weighed and two indwelling 14 G, 13.3 cm catheters (Angiocath, BD, Franklin Lakes, NJ) were placed in each jugular vein, following subcutaneous local anesthesia with mepivacaine hydrochloride (2% Carbocaine®-V, Pharmacia & Upjohn Co., Pfizer Inc., New York, NY). Insulin infusate was prepared by mixing 2 ml of each horse's serum (to prevent binding or absorption of insulin to plastic surfaces) with 5 ml human recombinant DNA insulin (Humulin®R, 100 U/ml, Hospira Inc., Lake Forest, IL) and 493 ml saline solution (0.9% Sodium Chloride Injection, USP, Hospira Inc., Lake Forest, IL). After collection of baseline blood samples, a priming insulin bolus in the amount of 18 mU/kg BW was administered to initiate the clamp and an infusion of insulin via controlled-rate infusion (CRI) pump (Vet-Pro VIP 2000, Caesarea Medical Electronics Ltd., Lichtenstein, Germany) at a rate of 3 mU/min/kg BW was started and maintained for a minimum of 120 min. Concurrently, a variable rate infusion of glucose (50% w/v Dextrose Injection, Hospira Inc., Lake Forest, IL) using a CRI pump was initiated for maintenance of euglycemia, defined as whole blood glucose concentration of 90 mg/dl ± 10%. Blood samples (3 ml) were collected at 5-min intervals throughout the clamp for determination of blood glucose concentration by precision glucometer (One Touch Ultra Mini, Life Scan Inc., Milpitas, CA). If blood glucose deviated from euglycemia, the glucose infusion rate (GIR) was adjusted. Additional blood samples (17 ml) were collected every 15 min. Both 10

ml additive-free and 7 ml K2EDTA (10.8 mg) tubes (Vacutainer, BD, Franklin Lakes, NJ) were allowed 20 min to clot, centrifuged for 30 min at 1000 x g, and serum and plasma collected and stored at -20°C until further analysis.

Analysis of Serum Insulin and Quantification of Insulin Sensitivity

Insulin concentration was assessed using commercially available radioactive immunoassay kits (Coat-a-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). The ratio of mean GIR during euglycemia to mean insulin concentration was calculated as an index of IS[5].

Statistical Analysis

Only data from horses meeting criteria established in the experimental design were used for statistical analysis. Horses were required to have an initial BCS of 5 ± 1, follow their designated treatment, and follow designated forage-only diets; therefore 12 horses (gain n = 3, control n = 5, lose n = 4) were included in statistical analysis. One-way repeated measures ANOVA using PROC MIXED of SAS (version 9.1; SAS Institute, Cary, NC) was calculated on DE intake, IS, BW, rump fat depth (RFD), abdominal fat depth (AFD), % body fat (%BF), % change in body weight (%BW) and body condition score (BCS) to identify differences due to time (day) or treatment. Day 0 BW was used as a covariate in analysis of day 65 and 130 BW. Data are presented as mean ± SD, and significance was detected when $P < 0.05$ and trends when $P \leq 0.10$.

Results

Energy Intake

Digestible energy intake was initially designed to be fed as 70, 100, and 130% of maintenance energy requirements for lose, control, and gain groups, respectively. However actual mean intake from day 0 to 130 was approximately 89, 115, and 138% of DE maintenance requirements[12]. In the gain group, mean DE intake as a percent of maintenance requirements from day 0 to day 65 was 153%, and 123% from day 65 to 130. Mean DE intake in the control group from day 0 to 65 was 124%, and 106% from day 65 to 130. The lose treatment stayed at 89% of maintenance DE requirements for both the day 0 to 65 and day 65 to 130 time periods. Significant differences in DE intake between both the gain and lose groups versus the control group were found between day 0 and 65 ($P < 0.01$) and day 65 and 130 ($P < 0.05$). Figure 1 shows the percent body weight change in treatment groups versus their initial body weight at day 0. Overall, horses in the gain treatment gained a significant percent of initial BW versus control horses by day 130 ($P = 0.01$), while percent BW loss from day 0 was not significant in the lose treatment ($P = 0.11$).

Body Weight, Body Condition Score & Body Fat

Results of body weight, BCS, and body fat changes are shown in Table 1. Using initial BW as a covariate, significant treatment effects were observed with mean gain treatment weight being different from mean treatment weight for the lose group ($P < 0.05$). There was an overall trend toward significantly greater BW in the gain versus control groups ($P = 0.07$). Horses in the lose treatment had significantly lower body weights than the control group ($P = 0.04$). Body condition scores for both the gain and lose treatment groups were significantly different from control horses ($P < 0.05$). Overall, significant differences in mean RFD were detected in the gain group versus both control ($P = 0.02$) and lose ($P < 0.01$) groups. Significant treatment effects on mean AFD were detected between gain versus control ($P = 0.03$) and gain versus lose ($P = 0.01$) groups, however, there were no significant difference in mean RFD or AFD in the lose treatment

compared to the control. Average %BF was significantly different between the gain and control groups (P = 0.02), though no overall significant differences were detected in lose versus control treatments (P = 0.20).

Insulin Sensitivity

Mean insulin sensitivity (IS) across the 130 days is shown in Figure 2. There was a trend for significant treatment by day interaction (P = 0.05) such that IS was significantly higher in horses within the gain group at day 130 versus day 65. There were no significant differences in IS within time or treatment in both lose and control groups, nor were there significant differences between these groups. Day 0 IS was not significantly different between any of the treatment groups (P = 1.00).

Discussion

The results of the present study differ from several previous studies in which insulin sensitivity is lower in horses with higher body condition scores[4,7]. In the present study, horses in the gain treatment achieved a maximum BCS of 6.9 at day 65. It is possible that negative effects of adiposity occur only at extremes and perhaps over longer periods of time. For example, Hoffman et al[4] found significant reductions in insulin sensitivity when horses had body condition scores greater than 7. It is likely that significant reductions in insulin sensitivity do not occur unless the horse is obese. It is also possible that there is an additive effect of time and obesity on insulin sensitivity, such that a longer duration of time (more than 130 days) is required to have a negative impact. Furthermore, reductions in insulin sensitivity are associated with high glycemic-index feeds (i.e. diets rich in starch and sugar)[4,5], while the present study fed only forage. It is possible that low glycemic-index feeds, such as forages, may not lead to reductions in insulin sensitivity even at greater degrees of body condition. Though dietary energy intake in the lose group was significantly different from that of the control, it was not extreme enough to elicit significant reductions in body condition and weight, which may explain why significant effects of body composition loss on insulin sensitivity were not detected. Subsequent studies need to achieve a greater degree of body condition loss or gain, perhaps over longer durations, to substantiate these effects on insulin and glucose dynamics.

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Tables and Figures

Table 1. Mean ± SD body weight (BW), body condition score (BCS), rump fat depth (RFD), abdominal fat depth (AFD) and percent body fat (%BF) across treatments.

	Treatment						
	Control			Gain		Lose	
	Day 0	Day 65	Day 130	Day 65	Day 130	Day 65	Day 130
BW (kg)	483.5 ± 38.0	513.8 ± 44.6	513.0 ± 46.4	537.3 ± 31.1	553.3 ± 19.7	465.5 ± 69.4	478.3 ± 64.8
BCS	4.8 ± 0.5	5.3 ± 0.6	5.2 ± 0.5	6.9 ± 0.6 * †	6.5 ± 0.5 * †	4.2 ± 1.3 *	3.6 ± 0.5 * †
RFD (cm)	4.5 ± 0.9	4.2 ± 1.5	3.8 ± 1.6	7.7 ± 1.5	8.3 ± 1.5 *	2.8 ± 2.9	1.2 ± 0.2
AFD (cm)	1.1 ± 0.4	1.4 ± 0.4	0.7 ± 0.2	2.7 ± 0.6	1.2 ± 0.6	1.0 ± 0.7	0.5 ± 0.1
% BF	27.3 ± 4.7	25.4 ± 8.1	23.3 ± 9.0	44.4 ± 8.4	48.1 ± 8.4 *	17.7 ± 16.0	9.0 ± 1.3

* $P < 0.05$ within day vs. control.
† $P < 0.05$ vs. day 0.

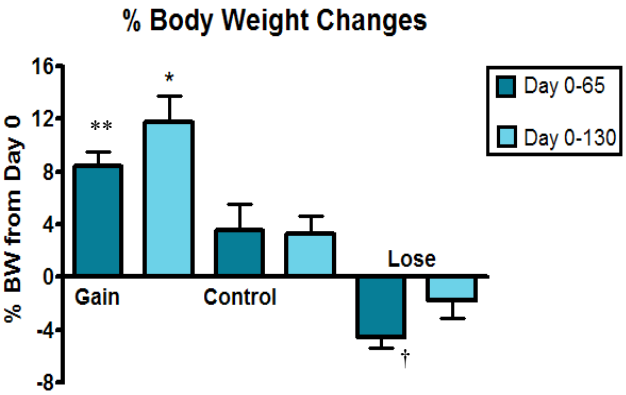

Figure 1. Percent body weight (%BW) changes from initial BW at day 0. Data presented as mean ± SEM.
* P < 0.05 vs. lose and control day 130.
** P < 0.01 vs. lose day 65.
† P < 0.05 vs. control

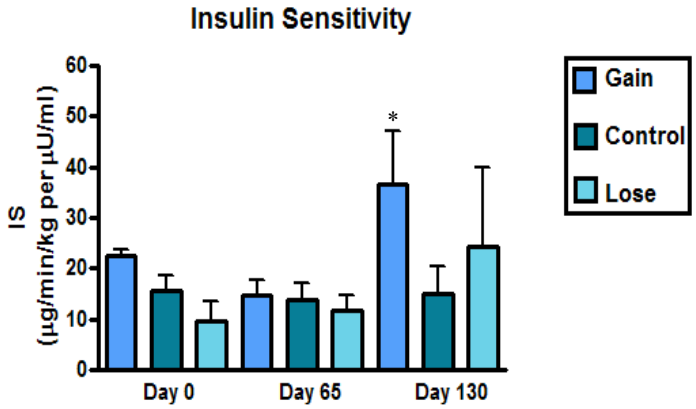

Figure 2. Insulin sensitivity (IS) at days 0, 65, and 130 in gain, control, and lose treatment groups. Data presented as mean ± SEM.
* P < 0.10 within treatment vs. day 65.