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Original Research

Comparison of Insulin Sensitivity of Horses Adapted to Different Exercise Intensities

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ABSTRACT

Diets high in concentrates and soluble carbohydrates are associated with reduced insulin sensitivity in horses. Exercise training could protect against diet-induced insulin resistance. The objective of this study was to determine the intensity of exercise training required to affect insulin sensitivity in stabled horses fed a diet high in concentrates but moderate in soluble carbohydrates. In all, 31 stabled horses underwent three different exercise regimens: turnout, light exercise, and moderate exercise, while being fed a diet containing 60% concentrate. Blood was sampled monthly and analyzed for insulin. Insulin sensitivity was assessed using basal insulin concentrations and calculated insulin sensitivity (reciprocal of the square root of insulin) and compared across months by analysis of variance with repeated measures. Insulin sensitivity (reciprocal of the square root of insulin) was higher during periods of moderate and light physical activity as compared with turnout. These results indicate that turnout alone may not be adequate to improve insulin sensitivity in horses fed high amounts of concentrate.

1. Introduction

Exercise has been shown to improve insulin sensitivity in horses [1,2]; however, it is unclear what exercise intensity is required for this improvement. In young Standardbred horses, light exercise training (4 weeks) was shown to be as effective in improving insulin sensitivity as long-term training (18 weeks) with moderate to high intensity [3]. A better understanding of the effect of different exercise regimens on insulin sensitivity is needed to develop effective management strategies and exercise recommendations by which owners may reduce insulin resistance-related health risks in their horses.

Horses fed diets rich in soluble carbohydrates have an increased risk of developing insulin resistance, obesity [4-6], and colic [7]. Diets with moderate amounts of soluble carbohydrates have been shown to avoid decreases in insulin sensitivity [8]. Basal proxies are a noninvasive and cost-effective tool to evaluate insulin sensitivity in large populations and have been used to determine insulin sensitivity in horses and other species [8-10]. The basal proxy reciprocal of the square root of insulin (RISQI) reflects the degree of chronic compensatory hyperinsulinemia that has been negatively correlated to insulin sensitivity, as determined by the more rigorous minimal model [10].

The objective of the current study was to compare different exercise intensities and their effects on insulin sensitivity and basal insulin of horses fed a high cereal-based diet.

2. Materials and Methods

The study was performed at the Second Cavalry and Guard Regiment of the Brazilian Army, located in the city of Rio de Janeiro, during the year of 2007. A total of 31

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mixed breed army horses were weighed on an analytic scale (Coimma, São Paulo, Brazil), scored for body condition score (BCS) [11] by two independent scorers, and sampled for blood by venipuncture between 2:00 and 3:30 AM after 8 hours of fasting. Sampling was performed monthly during 9 consecutive months (February to October of 2007). The study was approved by the Animal Care and Use Commission of the Federal Rural University of Rio de Janeiro.

2.1. Diet and Management

Horses were maintained in individual box stalls measuring 2 × 2.5 m^2, unless they were turned out or exercised. All horses were fed individually in stalls: 6 kg commercial concentrate (Royal horse, Brazil) divided into three daily feedings plus 4 kg of Bermuda grass hay divided in two feedings (Table 1). The afternoon hay feeding was done on the field during turnout months. The feeding regimen was consistent with the Brazilian Army guideline book [12]. Roughage feeding was restricted because of low forage availability and quality in this region. High amounts of concentrate were fed to meet nutrient requirements.

Diet was offered in amounts independent of the horses' body weight or work. Hay and concentrate were sampled every 2 months and analyzed for nutritional content by proximate analysis (dry matter, fat, ash, crude protein, acid detergent fiber, neutral detergent fiber). Hydrolyzable carbohydrates (CHO-H) were analyzed by extraction with hot water and amylolytic enzymes [13] and represented disaccharides, some oligosaccharides, and starch [14]. Nonfibrous carbohydrates were estimated by subtracting the sum of crude protein, fat, ash, and neutral detergent fiber from 100 [15]. Rapidly fermentable carbohydrates ($CHO\text{-}F_R$) were calculated by subtracting CHO-H from nonfibrous carbohydrates; however, in the $CHO\text{-}F_R$ fraction, organic acids may be included [15].

2.2. Physical Activity

Exercise intensity and turnout time are described in Table 2. Exercise schedule was based on military instruction and guard activities. Before sampling started, horses were turned out for 8 hours a day in dirt or low grass areas (November of the previous year to January of 2007; Table 2). Horses walked about 1 km to reach the paddock (45,000 m^2) where they were turned out. During turnout period each paddock had from 30 to 70 horses. Turnout in April to June was performed in the same way. Exercise regimen changes were implemented at the beginning of the month, when applicable. In February, horses were exercised at a light intensity (Table 2) and were walked for 60 minutes three times a week, and according to National Research Council (NRC; 2007), this activity may include trot and canter too. This activity was part of the normal military activity where horses needed to be prepared to walk in the city. This level of activity also prepares the soldiers who never rode before. In March, activity was increased to a moderate intensity and horses were walked and trotted for 90 minutes three times a week, and according to NRC (2007), it includes some walking, mostly trotting, and some canter during 3 to 5 hours a week. During this month, soldiers were trained to trot and horses were prepared to work on guard activities in the city. In the months of April, May, and June, horses were turned out because the Guard Regiment was involved in other activities. In July, horses were worked again at a moderate intensity (NRC, 2007), most of the time at walk and about 40% of the time at trot (Table 2) for 5 days a week for 90 minutes. This exercise intensity was part of the normal guard activities for horses that worked on guard rounds in the city. Activities were stopped in November, when horses were turned out again. The exercise regimen was applied to all horses.

Table 1
Daily feeding schedule of a group of Brazilian army horses

Time	Commercial Concentrate (Kg)	Bermuda Hay (Kg)
4:00 AM	2	–
11:00 AM	–	2
1:00 PM	2	–
4:00 PM	–	2
5:30 PM	2	–

Concentrate was composed of crimped oats, linseed meal, soybean meal, wheat bran, grass hay, molasses, cracked corn, soybean oil, probiotics, mineral and vitamin premix, calcium carbonate, and salt.

2.3. Blood Sampling, Handling, and Analysis

Blood samples were collected between the eighth and 19th of each month. Horses had free access to water during the night and their last meal of grain (Table 1) was consumed by 6:00 PM the night before sampling. Monthly jugular blood samples were drawn between 2:00 and 3:30 AM into heparinized vacutainer tubes and fluorized tubes (BD Vacutainer evacuated blood collection tube, Fisher HealthCare, Chicago, IL) before the morning feeding at 4:30 AM. Tubes were placed on ice water and centrifuged at 3,000*g* for 10 minutes within 30 minutes of collection. Plasma was removed, maintained at 4°C for 4 hours until frozen at −20°C for further analysis.

Insulin was determined by radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA) previously validated for equine insulin [16]. The proxy for insulin sensitivity was calculated from basal plasma concentrations of insulin (mU/L) as follows: $RISQI = 1/\sqrt{\text{basal insulin concentration}} = \text{basal insulin concentration}^{-0.5}$ [10].

2.4. Statistics

Insulin and insulin sensitivity (RISQI) were compared across months with exercise intensity as a main effect by analysis of variance with repeated measures. For statistical analysis, plasma insulin and RISQI values were log-transformed because of their non-normal distribution. Multiple comparisons were made by Fisher least square means analysis. Subject age, gender, and weight were included as covariates in the analysis of variance. Spearman rank correlations were calculated with age, BCS, and weight to determine their effect on insulin and insulin sensitivity. Bimonthly hay sample nutritional content was analyzed by linear trends analysis. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC). Results are presented as means and SEM.

Table 2
Monthly physical activity of a group of Brazilian army horses

Physical activity				
Months	Type	Duration	Frequency	Intensity
November 2006–January 2007	Turnout	8 hours	4 days per week	Turnout
February	Walk	60 minutes	3 days per week	Light
	Turnout	6 hours	3 days per week	
March	Walk and trot	90 minutes	3 days week	Moderate
	Turnout	8 hours	2 days week	
April, May, and June	Turnout	8 hours	4 days week	Turnout
July–October	Walk and trot	90 minutes	5 days week	Moderate
November–January 2008	Turnout	8 hours	4 days per week	Turnout

3. Results

3.1. Horse Demographics and Sampling Days

Of the 31 horses, 14 were sampled during all 9 months and 17 were sampled on 8 of 9 months. Age of the horses varied between 6 and 23 years (12.5 ± 0.3 years), with no age difference between months. Horses were not tested for Cushing disease, but no horses demonstrated clinical signs of the disease. Gender of the horses consisted of 68% geldings and 32% mares, with no difference between months. Weights and BCS did not vary among months ($P = .99$). Average weight was 427 ± 2.9 kg (range: 305 to 513 kg) and BCS was 5.0 ± 0.1 (range: 3 to 7). Blood samples were collected on February 8, March 8, April 10, May 10, June 15, July 10, August 15, September 19, and October 15.

3.2. Diet and Management

Feed analysis is shown in Table 3. No variation was found in the bimonthly concentrate or hay composition and data for each were pooled. Horses were fed their concentrate in stalls and they generally consumed all concentrate and hay offered. Hay consumption was not controlled individually during turnout months when fed in the afternoon. Grass intake was considered insignificant because the paddock consisted primarily of dirt. Concentrate intake based on the horses' body weight was 1.40% ± 0.01% (range: 1.16% to 1.96%) and average hay intake 0.95% ± 0.01% as fed (range: 0.78% to 1.31%).

3.3. Weight, BCS, Concentrate Percentage, and Gender

BCS was negatively associated ($r = -0.21$, $P = .030$) with RISQI, whereas it was positively associated with insulin concentrations ($r = .16$) and age ($r = .17$) ($P < .010$). Concentrate percentage fed based on body weight (range: 1.16% to 1.96%) was not a significant factor in any analysis.

3.4. Physical Activity

The intra-assay and interassay coefficient of variation of insulin duplicate samples were 8% and 11%, respectively. The majority of the results, however, were within the lowest level of the standard curve of the insulin assay. Overall, basal fasting insulin concentration was higher ($P = .013$) in months where horses received only turnout (log estimate = 0.78 ± 0.04; insulin mean = 6.05 ± 0.50 μU/mL) than in months where horses underwent light exercise (log estimate = 0.59 ± 0.06; insulin mean = 3.9 ± 0.42 μU/mL) and lowest ($P < .001$) with moderate exercise intensity (log estimate = 0.33 ± 0.03; insulin mean = 2.12 ± 0.15 μU/mL) (Fig. 1). Insulin sensitivity was lower ($P = .013$) in turnout horses (log estimate = −0.39 ± 0.018; RISQI mean = 0.40 ± 0.02) than lightly exercised horses (log estimate = −0.23 ± 0.023; RISQI mean = 0.59 ± 0.03) and also lower ($P < .001$) than moderately exercised horses (log estimate = −0.13 ± 0.014; RISQI mean = 0.74 ± 0.05) (Fig. 2). Insulin sensitivity was higher ($P < .001$) in moderately exercised horses as compared with lightly exercised horses.

Insulin and RISQI were not significantly different between the months of February, March, and April (Figs. 1, 2). Insulin and RISQI were lower in March, as compared with May and June ($P < .014$), and higher than July, August, September, and October ($P < .008$) (Figs. 1, 2). Although there was only a trend for a difference in insulin and RISQI between February and March ($P = .067$), and March and April ($P = .068$), overall turnout insulin and RISQI were different ($P \leq .013$) from light exercise intensity (Figs. 1, 2).

Table 3
Means and SEM for the pooled hay and grain chemical analysis

Feedstuff	DM	Energy (Mcal/kg)	CP	Ash	Fat	NDF	ADF	NFC[a]	CHO-H[b]	CHO-F_R[c]
Hay	89 ± 0.2	1.94 ± 0.05	6.8 ± 0.8	5.3 ± 0.5	1.9 ± 0.3	70.7 ± 2.0	36.0 ± 1.0	3.8 ± 0.6	0.24 ± 0.6	2.76 ± 0.7
Grain	91 ± 0.1	3.3 ± 0.05	17.6 ± 0.6	9.1 ± 0.02	7.5 ± 0.1	29.7 ± 0.3	9.9 ± 0.3	27.2 ± 0.02	16.6 ± 0.05	10.6 ± 0.05

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, nonfibrous carbohydrate; CHO-H, hydrolyzable carbohydrate; CHO-F_R, rapidly fermentable carbohydrate.

[a]Nonstructural carbohydrate, NFC = 100−ash−CP−fat−NDF.

[b]Analyzed directly using extraction with hot water and amylolytic enzymes [13].

[c]CHO-F_R = NFC−CHO-H.

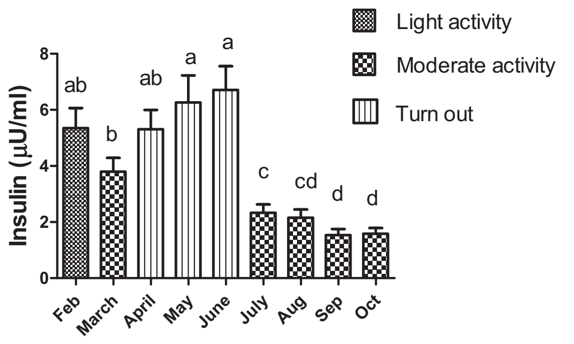

Fig. 1. Monthly means and SEM for insulin. Bars with different superscripts differ ($P < .050$).

4. Discussion

This study found that exercise intensity can affect insulin sensitivity in horses, and that turnout alone may not be sufficient to improve insulin sensitivity. The majority of the horses in this study were young and not obese, thus lowering the risk of insulin resistance. Horses were fed about 0.95% of their body weight as forage, which is close to the minimum recommended forage intake [17]. Consequently, concentrate intake was high, which can predispose to insulin resistance [2,4,18,19]. However, lower CHO-H (166 g/kg DM) and CHO-F_R (106 g/kg DM) content of the concentrate fed in this study could have reduced the likelihood of the horses to develop insulin resistance, as has been shown to occur with fat- and fiber-based diets [14]. The low insulin values reported in this study are also likely to be because of fasting before the sampling.

Although supplied feed did vary during the study, BCS and weights did not. It is possible, however, that during turnout months, horses did not consume all 2 kg of offered hay, which is estimated to contain about 3.9 Mcal, thus reducing the energy intake.

Despite low insulin concentrations, this study showed that exercise was associated with lower circulating insulin concentrations, suggesting a positive effect on insulin sensitivity. In human beings, regular moderate physical activity (40% to 55% of their peak oxygen consumption) has been shown to improve insulin sensitivity [20,21]. In horses, exercise has also been shown to improve insulin sensitivity in previously inactive obese and lean mares [1], untrained Standardbred horses [3], and lean Standardbred horses [2]. However, in the lean Standardbreds [2], insulin did not reflect this difference in insulin sensitivity, possibly because of small sample size and individual variability in basal values. Insulin sensitivity returned to pretraining levels in mares within 9 days after training stopped [1], indicating that training should be continuous to maintain improved sensitivity. In obese horses that are likely to be insulin-resistant, feed restriction in addition to exercise might be necessary to improve insulin sensitivity; however, one study found that 4 weeks of low intensity exercise followed by 4 weeks of higher intensity exercise did not improve insulin sensitivity, although it reduced obesity moderately [18].

In the current study, moderate exercise improved insulin sensitivity within 15 days (Fig. 2; July [turnout] as compared with August [moderate exercise]). Differences in insulin concentration between light exercise (Fig. 1; February) and moderate exercise (March) were not evident. Insulin concentration in March during moderate exercise done three times a week was still higher than insulin concentration in the months of moderate exercise intensity in July to October, where horses were exercised 5 days a week (Fig. 1). Exercise at moderate intensity may need to be frequent to improve insulin sensitivity.

The results of the current study suggest that continuous moderate exercise performed at least 5 days a week can reduce the risk of insulin resistance in horses in as little as 15 days. From the current study, it is not clear for how long insulin sensitivity will remain the same if exercise is not undertaken. Although horses receiving turnout were not compared with horses with no turnout in this study, it is unlikely that horses turned out on pasture will significantly increase their heart rate and oxygen consumption to prompt metabolic adaptations.

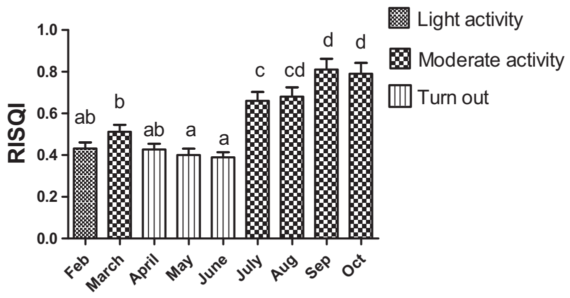

Fig. 2. Monthly means and SEM for reciprocal of the square root of insulin. Bars with different superscripts differ ($P < .050$).

5. Conclusions

Moderate exercise intensity performed 5 days a week seems to benefit insulin sensitivity, even in insulin-sensitive horses. Turnout may not be sufficient to improve insulin sensitivity, but an exercise regime comparable with light or moderate intensity exercise, as performed in the study (e.g. walking horses for 60 minutes five times a week), may be recommended as a management strategy to improve insulin sensitivity. This should be tested in an at-risk population of obese horses.

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