Original Research

Investigation of the Effects of a Dietary Supplement on Insulin and Adipokine Concentrations in Equine Metabolic Syndrome/Insulin Dysregulation

Jane M. Manfredia,*, Emma D. Stapley, Jenifer A. Nadeaub, Delia Nashc

Keywords: Resveratrol and leucine Equine metabolic syndrome HMW adiponectin Dietary supplement Insulin dysregulation Oral sugar test

Abstract

High insulin concentrations are a common clinical feature of equine metabolic syndrome (EMS) and insulin dysregulation. Hyperinsulinemia can induce laminitis, so reduction of insulin concentrations in response to an oral challenge should decrease risk. In human studies, diets containing a polyphenol (resveratrol) led to improvements in insulin sensitivity. In rodents, the addition of leucine to a resveratrol supplement caused a decrease in the amount of resveratrol needed to achieve a clinical effect. We hypothesize a supplementation with a low dose of a synergistic polyphenol and amino acid blend including leucine (SPB+L) would improve metabolic health in EMS/insulin dysregulated horses. Fifteen EMS/ID horses received a high or low dose of SPB+L daily for 6 weeks. Insulin during an oral sugar test (OST), body condition score, weight, baseline high-molecular-weight (HMW) adiponectin, triglycerides, nonesterified fatty acids, and tumor necrosis factor alpha were assessed before supplementation (PRE) and after supplementation (POST) via paired Student’s t-tests and a repeated-measures mixed-model analysis of variance (significant at \( P < .05 \)). There were no differences between doses. Horses in the POST group weighed significantly less, had significantly higher baseline HMW adiponectin concentrations, and had significantly lower insulin concentrations at 60- and 75-minute time points (\( P < .05 \)). Insulin concentrations of the horses in the POST group, but not in the PRE group, were lower and similar to results from the study conducted three years before the present study (PRIOR) for 0- and 60-minute time points (\( P < .002 \)). An increased HMW adiponectin level supports increasing insulin sensitivity after supplementation. These results suggest that SPB+L supplementation at either dose leads to improvements in the clinical manifestations of EMS/insulin dysregulation, potentially reducing laminitis risk.

© 2020 Elsevier Inc. All rights reserved.

1. Introduction

Equine metabolic syndrome (EMS), which is characterized by obesity and insulin dysregulation, predisposes horses to development of laminitis: a debilitating and performance-ending disease [1–3]. Hyperinsulinemia, both basal and after an oral challenge, is thought to instigate laminitic episodes as laminitis has been induced in normal horses and ponies exposed to high concentrations of insulin [2,4–7]. While diet has been extensively studied for prevention and/or treatment of EMS/insulin dysregulation-associated laminitis [8–13], dietary supplementation or pharmacologic treatment of EMS/insulin dysregulated (ID) horses has been investigated in a much smaller number of studies [14–19]. Metformin has been of interest, with modest improvements in glucose and insulin concentrations seen in one study, and limited
improvement in others, likely due to poor bioavailability [15,17,20,21]. Short-chain fructo-oligosaccharides (sc-FOSs) have been administered in combination with dietary restrictions, resulting in some success in improving insulin sensitivity [18].

In a previous study in horses, resveratrol (a polyphenol) supplementation alone at a high dose achieved improvements in insulin concentrations after dynamic testing [19]. In studies performed in humans and rats, dietary supplements containing resveratrol without leucine also led to improvements in insulin sensitivity, inflammatory markers, and adipokines [22,23]. However, the large dosage of resveratrol needed to achieve clinical effects is hard to attain in humans [24], making it impractical in horses as well. In rodents, the addition of leucine was found to work synergistically with resveratrol to achieve increased insulin sensitivity with a fraction of the resveratrol dose, potentially due to the fact that both have been shown to target the Sirt1 pathway [24,25]. The addition of leucine also contributed to weight loss [24]. It would be clinically significant if supplementation with a synergistic polyphenol blend plus leucine (SPB + L) would decrease the risk of developing laminitis by blunting insulin peak concentrations and increasing insulin tissue sensitivity via improvement in high-molecular-weight adiponectin concentrations in EMS/ID horses. The synergism between the resveratrol and leucine could also allow for this supplementation to be practically used. The objective of this study was to assess the effects of administration of a low and high dose of a SPB + L dietary supplement on insulin responses to a dynamic challenge in the form of an oral sugar test (OST), as well as on baseline adipokines and an inflammatory biomarker in horses previously diagnosed with EMS/insulin dysregulation.

2. Materials and Methods

2.1. Horses

Fifteen adult horses (6 Arabian horses and 9 Morgan horses; 10 geldings and 5 mares; average age: 10 ± 6 years; average weight: 495 ± 45 kg; average body condition score (BCS): 7 ± 0.8 out of 9) all previously shown to have insulin resistance (determined by an insulin-modified frequently sampled intravenous glucose tolerance test) [26] and/or insulin dysregulation (determined via an OST2) [27] were used. All procedures were approved by the institutional animal care and use committees. All experimentation met the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council of International Organizations of Medical Sciences.

Arabian horses were all present at one site, and the Morgan horses were split as evenly between two sites as possible. Only one Morgan horse had a previous history of lamiotic pain, and she was not symptomatic during the study.

2.2. Study Overview

Horses had OSTs and/or frequently sampled intravenous glucose tolerance tests (FSGTts) performed during the summer three years before this present study (PRIOR). In the present study, in June, horses had a baseline OST performed (before supplementation; PRE) before starting either a low dose (LOW) or high dose (HIGH) of a once-a-day, in-feed SPB + L supplement. At the end of 6 weeks, the OST was repeated for the horses (after supplementation; POST). BCs, history of lamioticis, and weight were recorded before and after supplement administration. Horses were maintained on a typical low nonstructural carbohydrate diet (average nonstructural carbohydrate: 11%) that is suggested for EMS, which included feeding from the same hay batch for several weeks before the study started through the end of the study (so hay changes would not be a confounding factor in our analysis). Horses were fed individually, and feed refusals were looked for after each feeding and would have been weighed if they had been present. (No refusals were noted.)

Horses maintained throughout the study the same amount of turnout and/or light exercise that they were used to performing. Arabian horses were kept in a paddock throughout the study (in the same paddock they are kept year-round), with no forced exercise. Morgan horses were kept in a moderate-size turnout paddock. Only one Morgan horse was used in light lessons during the week, with the same amount of work before and throughout the study.

2.3. OST Procedure

Horses were fasted from 10 PM the night before the test. The morning of the test, intravenous catheters were placed in the jugular vein after subcutaneous injection of 1 ml lidocaine at least an hour before the test, and a baseline blood sample was drawn. All testing was started by 8 AM. Karo light corn syrup (Oakbrook Terrace, IL) was administered as a bolus by mouth (0.25 mL/kg bw), and blood was drawn at 15, 30, 60, 75, 90, 120, 150, and 180 minutes after Karo syrup administration. After being obtained, blood was placed in either serum or lithium heparin tubes, centrifuged, and stored at −80 °C after the removal of plasma and serum.

2.4. Supplement Protocol

After the initial evaluation and OST, horses received 28 g of an SPB + L supplement (InsulinWise, Kentucky Performance Products, Versailles, KY; powder form) of either a LOW (resveratrol 750 mg, leucine 1150 mg) or HIGH (proprietary) dose of synthetic polyphenol in a small amount of nationally produced commercial grain daily for 6 weeks. Horses at each site were randomly assigned to which group they were in, with approximately half at each site with a LOW and half with a HIGH. The horses in the LOW group had an average age of 11.5 years, 7.5 BCS, and 504 kg, 7 geldings, and 1 mare. The horses in the HIGH group had an average age of 10.2 years, 7.5 BCS, and 494 kg, 4 mares, and 3 geldings. Horses were monitored to ensure that they ate the supplement completely on a daily basis.

2.5. Biochemical Evaluation

Baseline levels of high-molecular-weight (HMW) adiponectin (HMW adiponectin ELISA, EMD Millipore, Burlington, MA), leptin (Multi-Species Leptin RIA, EMD Millipore), triglycerides (TGs) (Triglyceride Kit, Sigma Aldrich, St Louis, MO), nonesterified fatty acids (NEFAs) (NEFA Kit, Wako Diagnostics, Richmond, VA), and tumor necrosis factor (TNF) alpha (TNF alpha ELISA, Thermo Fisher, Minneapolis, MN) were measured in all PRE and POST samples (PRE, POST, and PRIOR). All assays have been previously validated in the horse [2,3,12,19]. Interassay and intra-assay controls were performed with CVs of less than 10%. These assays have been regularly used in our laboratory for multiple studies.

2.6. Analysis and Statistics

Differences in weight, BCS, and baseline biochemical markers (HMW adiponectin, leptin, TGs, NEFAs, and TNF alpha) were compared between PRE to POST groups and between HIGH and LOW groups, with a Student’s t test or Wilcoxon signed-rank test (if not normally distributed on the basis of a Shapiro-Wilk normality test). Baseline; peak; 60-, 75-, and 90-minute concentrations; and the area under the curve were analyzed for insulin.
measures mixed-models analysis of variances were used to compare differences in insulin responses between the following groups: HIGH versus LOW, PRE versus POST for HIGH and LOW, and PRIOR versus PRE versus POST within each individual horse. All significance was set at $P < .05$. Horses were classified as either ID or normal based on their responses to the OST, with an insulin level greater than 45 μIU/mL at 60 and/or 90 minutes after corn syrup administration indicating an ID horse.

3. Results

The SPB + L supplement was very palatable, and no adverse effects due to the supplement were noted. One horse developed Potomac horse fever during the period of supplementation (confirmed on polymerase chain reaction) and was treated via intravenous antibiotics. Horses showed statistically significant weight loss ($P = .002$) (Fig. 1) after supplementation, which did not differ between HIGH and LOW groups. There was no significant change in horses’ BCSs or in the presence of regional adiposity. No horses developed laminitis during the study.

Horses in the POST group had increased concentrations of HMW adiponectin as compared with those in the PRE group with SPB + L supplementation ($P = .007$) (Fig. 2); there was no significant difference between LOW and HIGH groups. There were no statistical differences in leptin, TG, NEFA, or TNF alpha concentrations between PRE and POST groups with SPB + L groups with supplementation.

The OST results showed that horses in the POST group had significantly lower insulin concentrations at 60 ($P = .048$) and 75 ($P = .047$) minutes than those in the PRE group, and there was no difference between HIGH and LOW groups (Fig. 3). Ten of 15 horses in the POST group had lower insulin concentrations, with four horses actually recategorized from being ID to normal based on the results of the OST. These horses had insulin profiles similar to those of horses in the top graph of Fig. 4. Five horses (3 Arabian horses and 2 Morgan horses) were nonresponders, similar to the horse pictured in the bottom graph in Fig. 4.
Each horse's insulin response to the three OSTs (PRIOR, PRE, and POST) was compared with the following results: The horses in the PRE group had significantly higher insulin responses than those in both the POST and PRIOR groups, and there was no difference between PRIOR and POST groups at 0 (P = .001) minutes (Fig. 5).

4. Discussion

Significant improvements were seen with regard to body weight and HMW adiponectin and insulin concentrations in this study, which suggests a supportive role of feeding a SPB + L dietary supplement to horses with EMS/insulin dysregulation.

Horses lost weight after the intake of the supplement, which was similar to the results in a rodent study in which a combination of resveratrol and leucine (similar to the SPB + L used in the present study) resulted in weight loss [24] but differed from a previous study in horses that only received resveratrol [19]. This suggests that the addition of leucine contributed to the observed weight loss. Despite the weight loss, no horse had a significant change in the BCS at the end of the study. This implies that visceral adipose depots may have diminished instead of subcutaneous deposits. In humans, visceral adipose depots have been linked to higher incidences of metabolic syndrome (MS) [28–30]. In horses, multiple depots have been investigated, but the nuchal adipose has received the most attention as the adipose reservoir active in EMS [31,32]. With the metabolic improvements seen in this study, further evaluation of the role visceral adipose depots play in EMS/insulin dysregulation is encouraged.

While the exact mechanism by which the SPB + L produced the effects seen is not known for any species, microbiome changes resulting in positively altered lipid metabolism, particularly greater Sirt1 activation, could be contributing [33]. Multiple studies have demonstrated the ability of resveratrol to positively impact the microbiome in humans and rats with obesity and/or MS [33,34], with transplantation of microbiota from resveratrol-fed mice, resulting in decreased obesity and improved insulin sensitivity. With differences apparent in the microbiome between metabolically normal and aged and insulin-dysregulated horses [35,36], restoration of a more normal microbiota could impact metabolism potentially via activation of Sirt1 [25]. The net result of Sirt1 activation is increased mitochondrial biogenesis, increased mitophagy (destruction of compromised mitochondria), and increased mitochondrial fatty acid oxidation [24,25]. Bruckbauer et al [24] report that leucine plays a similar role in Sirt1 activation, suggesting a possible synergy. Although these actions have not been confirmed in equine tissues, there is evidence that Sirt1 is conserved, both structurally and functionally, in vertebrate species [37]. This is supported by the work Serteyn et al [38] did finding significant evidence of mitochondrial dysfunction in muscle biopsies from horses with acute laminitis of both metabolic and inflammatory origin and that of Marycz et al [39–41] who found elevated serum markers of oxidative stress and increased evidence of mitochondrial damage (tailhead adipose tissues, liver). Banse et al [42] noted that increases in the equine BCS were associated with changes indicating increased oxidative stress (increases in markers of mitochondrial biogenesis and a marker of skeletal muscle antioxidant capacity). The present study supports the idea that mitochondrial damage could play a role in the pathogenesis of EMS: marked improvement in the clinical manifestations of EMS was seen after SPB + L supplementation and, theoretically, an increase in mitochondrial function [25].

HMW adiponectin is traditionally described as an insulin-sensitizing adipokine in human and equine literature [43,44]. Horses in this study demonstrated higher concentrations of HMW adiponectin in the POST group, suggesting an insulin-sensitizing effect when coupled with lower insulin concentrations during the OST. This increase in the adiponectin concentration is consistent with increases seen in humans after resveratrol nutraceutical consumption [22]. Whether increasing HMW adiponectin concentrations is the cause or the effect of increased insulin sensitivity is still being debated in the literature [44].

It was somewhat surprising that significant differences were not seen in leptin concentrations as horses in this study lost weight, and leptin typically mirrors body weight changes in the horse, and past studies showed decreased leptin concentration with resveratrol supplementation [19,45,46]. Triglycerides have been used to predict laminitis risk, with thresholds of 57 or 94 mg/dL [2], but in this cohort, only two horses (one Arabian horse and one Morgan horse) had triglyceride concentrations higher than the lower cutoff of 57 mg/dL (the horses had concentrations of 70 mg/dL (Arabian horses) and 56 mg/dL (Morgan horses) at the presupplementation time point, and this was reduced to 35.1 mg/dL in the Arabian horses and 63 mg/dL in the Morgan horses in the POST group. Unlike in a previous abstract investigating a 6-week resveratrol-only supplement trial [19], TG and TNF alpha concentrations were not decreased after SPB + L supplementation. This difference could be explained by either a different dose of resveratrol or breed differences as that study had a more mixed population of horses.

While the OST has some known variability [47], the dramatic decrease in insulin responses seen at all time points in some of the horses extends beyond the reported degree of variation. Clinically, insulin concentrations of ~45 uIU/mL (reduced from the original 60 mL/uIU/mL threshold) at 60, 75, and 90 minutes are now used to determine if a horse is ID, as those time points are associated with insulin peaks after Karo syrup administration in horses [27,47]. Horses in the POST group had statistically significant decreases in insulin peaks at two of the three times, with an influential point (a
severely ID horse), preventing significance at the 90-minute time point. As high insulin concentrations have been associated with laminitis development [6], minimization of insulin response to an oral challenge secondary to dietary supplementation suggests one arm of an approach to prevent laminitis development. It is further clinically encouraging that insulin concentrations after supplementation were decreased to levels seen several years prior because insulin concentrations and the acute insulin response to glucose is known to be exacerbated by aging [11,48,49], although other environmental factors may also play a role. Because interhorse variability of insulin response to the OST can be pronounced and perfect age matching could not be achieved, horses in the PRIOR and PRE groups served as their own controls in this study during the measurements. While most horses had metabolic improvements, some were nonresponders. For this reason, dynamic testing before and after 6 weeks of supplementation is advised.

In conclusion, supplementation with SPB + L had positive impacts on metabolic parameters in horses with EMS/insulin dysregulation. Lowering insulin response to an oral challenge and increasing the HMW adiponectin concentration could provide a clinical benefit by decreasing laminitis risk.

Acknowledgments

The author would like to thank the Michigan State University Horse Teaching and Research Center, the Miner Institute, and the University of Connecticut for the use of their horses.

This project was supported by Kentucky Performance Products (Versailles, KY) and the Boehringer-Ingelheim Veterinary Scholars Program and the MSU Graduate School Fellowship (E. Staplely). Dr Manfredi was supported by a Morris Animal Foundation Research Training Fellowship (D14EQ-401) for part of the PRIOR data collection. Jane Manfredi contributed to conceptualization, methodology, validation, formal analysis, investigation, resources, writing—original draft, writing—review & editing, visualization, supervision, project administration, Emma Stapley contributed to methodology, validation, formal analysis, investigation, resources, writing—original draft, writing—review & editing. Jenifer Nadeau contributed to methodology, validation, investigation, resources, writing—review & editing. Delia Nash contributed to conceptualization, methodology, resources, writing—review & editing, funding acquisition.

References


