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Metabolic and inflammatory responses to the common sweetener stevioside and a glycemic challenge in horses with equine metabolic syndrome

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ABSTRACT

Extracts derived from the leaves of the stevia plant (stevioside) are commonly used as sweeteners for humans and horses. Stevioside appears to be safe for human consumption, including for individuals with insulin dysregulation. In the horse, the safety or metabolic effects of stevioside on normal animals or on those with metabolic dysfunction are unknown. Furthermore, the inflammatory response to a glycemic challenge or to stevioside in horses is not well defined. Therefore, the objective of this study was to measure the effects of stevioside and a glycemic challenge on insulin, glucose, and inflammatory responses in horses with a common metabolic dysfunction (equine metabolic syndrome or EMS) compared with non-EMS controls. To accomplish this, 15 horses were selected; 8 EMS and 7 age-matched controls. An oral sugar test was performed using Karo corn syrup (karo) or stevioside in a random crossover design. Horses were given 0.15 mL/kg body weight of karo or its equivalent grams of sugar in stevia dissolved in water. Blood samples were collected by jugular venipuncture before administration of either stevia or karo and at 60 and 240 min after administration. Serum was used for glucose and insulin determination and plasma for isolation of peripheral blood mononuclear cells (PBMCs) for inflammatory cytokine analysis via flow cytometry and reverse transcription PCR (RT-PCR). Stevia appeared to stimulate lower glycemic and insulinemic responses when compared to karo, in particular in EMS horses. EMS and control horses had inverse inflammatory responses to administration of either stevia or karo with EMS horses having a proinflammatory response ($P \leq 0.05$). These data provide evidence as to why horses with EMS may be predisposed to developing laminitis, potentially as a result of an exaggerated inflammatory response to glycemic and insulinemic responses. Furthermore, the data provide new avenues for exploring mechanisms behind the syndrome, in particular when using a glycemic challenge.

1. Introduction

Stevioside, a glycoside derived from the leaves of the stevia plant, has been increasingly used as a sweetener for both horses and humans [1]. It appears in a range of equine products, such as veterinary pastes, supplements, and anthelmintics. Stevioside can even be found in equine products intended for use in the insulin resistant animal. However, the safety and efficacy of stevioside in the metabolically normal or metabolically dysfunctional animal is unknown. For humans, stevioside is primarily used as a nonglycemic sugar replacer and is classified as

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Generally Recognized as Safe by the United States food and drug administration [2]. Research indicates that stevioside is safe for human consumption, including for individuals with hypertension and metabolic dysfunction [3]. Stevioside may even be metabolically beneficial in individuals with metabolic disorders as it has been shown to increase insulin sensitivity in insulin resistant rats [4] and to positively influence glucose and insulin dynamics in people [5–8]. Humans with metabolic dysfunction typically suffer from hyperglycemia and hyperinsulinemia. Considering that stevioside stimulates insulin release from the pancreas [7], it can therefore have a potentially positive effect in humans by lowering circulating glucose concentrations. However, horses with metabolic dysfunction, unlike humans with metabolic syndrome, typically have circulating glucose concentrations within the normal range, but are hyperinsulinemic. Thus, increasing the amount of circulating insulin in the horse may actually exacerbate existing insulin dysregulation (ID) [9] in the horse with metabolic syndrome (equine metabolic syndrome, or EMS). Even with the increasing use of stevia in equine products, the effects of stevioside on glucose and insulin metabolism in the horse have yet to be determined. The oral sugar test (OST), a common dynamic test used to determine glycemic and insulinemic responses to a simulated high carbohydrate meal, is typically carried out using a bolus of Karo corn syrup (karo) as a sugar source. However, no research has characterized the inflammatory response to a glycemic challenge with either karo or stevia. Considering that increases in inflammatory markers such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) have been observed postprandially in people with metabolic dysfunction [10] and in horses [11], as well as the prevalence of stevioside in the equine market, it is of importance to understand its metabolic and inflammatory effects on normal and metabolic syndrome horses. Therefore, the experiment was designed not only to measure the effects of the OST using both stevioside and karo on insulin and glucose responses but also inflammatory responses in horses with EMS compared with non-EMS controls.

2. Materials and methods

2.1. Horse selection and EMS determination

Eight EMS horses and 7 controls were selected from the University of Kentucky, Department of Veterinary Science herd. EMS was defined by the criteria established in the 2010 American College of Veterinary Internal medicine consensus statement [12]. In brief, EMS was characterized by ID [9], general or regional adiposity, and a history of or predisposition to laminitis. All EMS horses were predisposed to laminitis given their increased adiposity and ID status, with 4 of the 8 selected EMS horses having a documented history of laminitis. A portable agriculture scale (model 700, Tru Test Inc, Mineral Wells, TX) was used to establish body weight, which was used to determine the amounts of stevia and karo to administer to each horse for the OST. Body condition score was determined and averaged between 3 trained investigators (S. E., M. M., and B. R.) using the 1 to 9 Henneke scoring system [13] with 1 representing an extremely emaciated animal and 9 representing an extremely obese animal. The same 3 trained individuals scored and averaged neck crests via the 0 to 5 cresty neck score (CNS) system established by Carter et al [14] with 0 representing no neck crest and 5 representing a crest so large it permanently droops to one side of the neck. Blood collection for serum and plasma samples was carried out via jugular venipuncture. To ascertain the presence of ID, the OST was performed similar to previously described [15]. Briefly, morning serum samples were collected, and oral sugar was administered in the form of 0.15 mL/kg body weight of karo. A second serum sample was collected 60 min post karo administration. A fasting insulin level of >20 μIU/mL was considered indicative of hyperinsulinemia, and an increased insulin (>60 μU/mL) 60 min post administration of karo was considered diagnostic of ID [16]. All OST sampling was performed between 8 AM and 12 AM for all sampling time points. Horses were not fasted before sampling. Screening results from the OST along with phenotypic data are presented in Tables 1 and 2. Of the 8 EMS horses, 3 were of mixed breed, 1 was a Thoroughbred cross, 1 horse was a Paint, 1 a Morgan, 1 a Warmblood, and 1 a Standardbred cross. Of the 7 control horses, 4 were Thoroughbreds, 2 were of mixed breed, and 1 was a Quarter Horse. Of the 7 control horses, 2 were of mixed breed, 4 were Thoroughbreds, and 1 was a Quarter Horse.

Horses were also screened 1 mo before the start of study via thyrotropin releasing hormone (TRH) stimulation and low-dose dexamethasone suppression testing [17,18] to ensure that none were affected by pituitary pars intermedia dysfunction (PPID). TRH stimulation testing was carried out as previously described [19,20] at least 2-wk before the first sampling time point, but no further than 2 mo before sampling. In brief, a baseline blood sample (0800, EST) was taken via jugular venipuncture into EDTA containing tubes. Following this, a 1 mL dose of TRH dissolved to 1 mg/mL in 0.9% saline (Sigma-Aldrich, St. Louis, MO) was administered intravenously (IV). Ten min post TRH injection, a second blood sample was taken. Tubes were kept on ice for transport to the laboratory and immediately centrifuged and

Table 1
Characterization of EMS and non-EMS control horses.

Group	(Insulin; μIU/mL) 60 min post oral sugar administration	BCS	CNS	Wt (Kg)
EMS	75 ± 6	6.8 ± 0.3	3.0 ± 1.1	569 ± 88
Control	31 ± 4	6.4 ± 0.1	1.2 ± 1.0	610 ± 56
P value	*<0.001*	*0.278*	*0.009*	*0.307*

Abbreviations: BCS, body condition score; CNS, cresty neck score; EMS, equine metabolic syndrome; Wt, weight.
Italicized values represent statistical significance in the form of *P* values.

Table 2
Characterization of EMS and non-EMS control horses analysis on ranks.

Group	Baseline serum (insulin; μIU/mL)	Age
EMS	44 (95% CI: 26–51)	12 (95% CI: 10–13)
Control	16 (95% CI: 13–18)	14 (95% CI: 12–15)
P value	*<0.001*	*0.054*

Abbreviation: EMS, equine metabolic syndrome.
Italicized values represent statistical significance in the form of *P* values.