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Original Research

Plasma Lipidomic and Inflammatory Cytokine Profiles of Horses With Equine Metabolic Syndrome

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ABSTRACT

Equine metabolic syndrome (EMS) is a growing problem in the equine industry, particularly considering it is a risk factor for the development of laminitis. Equine metabolic syndrome is similar to metabolic syndrome in humans, which is associated with abnormal circulating plasma lipid concentrations. Thus, our objectives were to characterize the plasma lipid profiles, or lipidome, of horses with EMS compared to non-EMS controls and to further characterize the inflammatory state of these horses. Twenty-three horses of mixed breed and sex were selected. Of these, 14 were classified as EMS and 9 as non-EMS controls. Equine metabolic syndrome was determined by insulin resistance, general or regional adiposity, and a history of or predisposition to laminitis. Fasting serum and plasma samples were collected via jugular venipuncture. Serum samples were used to determine insulin, leptin, triglyceride, cholesterol, and nonesterified fatty acid concentrations. Heparinized plasma samples were used to isolate peripheral blood mononuclear cells for inflammatory cytokine determination and ethylenediaminetetraacetic acid plasma to analyze lipidomes. Equine metabolic syndrome horses had increased serum leptin and triglycerides. Plasma lipidomic analysis indicated that EMS horses had elevated triacylglycerides, diacylglycerides, monoacylglycerides, and ceramide compared to control horses. They had lower plasma sphingomyelins, suflatide, and choline plasmalogens. Peripheral blood mononuclear cell analysis for cytokine protein concentration via flow cytometry and gene transcription via real-time polymerase chain reaction showed no differences between the two groups; however, high variability may have influenced results. These data demonstrate that EMS horses have differences in their plasma lipidome compared to controls, similar to what has been observed in humans with metabolic syndrome.

1. Introduction

Obesity rates are rising not only in the human population, but in equids as well [1–4]. Increased adiposity is one of the defining characteristics of equine metabolic syndrome (EMS). Equine metabolic syndrome was classified in 2010 in an ACVIM consensus statement as insulin resistance, general, or regional adiposity and a history of or predisposition to laminitis [5,6]. Equine metabolic syndrome is similar to metabolic syndrome (MetS) in humans, which is categorized by three or more of the following: visceral obesity, hypertriglyceridemia, glucose intolerance, low high-density lipoprotein cholesterol, or hypertension [7,8].

Increased inflammation as well as altered lipid profiles have been associated with obesity in humans and mice [9–11]. Considering that abnormal lipid profiles may be a

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contributing factor to altered cell signaling or increased inflammation [9], as well as the influence of certain lipids on insulin resistance [12,13], it is becoming increasingly important to examine possible connections between plasma lipid profiles, or lipidomes, and metabolic dysfunction. Lipidomic analysis is a relatively new field, but is providing promising new avenues for exploring questions related to metabolic dysfunction [14].

In humans, over 600 diverse molecular species make up the plasma lipidome [15]. It can be categorized into six primary mammalian lipid categories: fatty acyls, prenols, sterols, sphingolipids, glycerophospholipids, and glycerolipids. Changes in lipidome composition may affect human health [15,16]. Differences in plasma lipidomes have been observed in humans and mice that experience obesity or hypertension. For example, changes in ether lipid or plasmalogen concentrations have been shown to be associated with atherogenic status and hypertension [17–19]. In particular, decreases in plasma ether phosphatidylethanolamines and phosphatidylcholines are indicative of hypertension in middle aged men [16] and patients with coronary artery disease with significant stenosis had a decrease in serum choline plasmalogens compared to patients without significant stenosis [18]. Additionally, differences in lipid composition between individuals who exercise regularly and those who consume a high fat diet have been demonstrated [20].

Research is also indicating a role of the immune response in lipid metabolism. The immune system has been shown to have the ability to recognize lipids as antigens [21]. One notable instance of this is the stimulation of T lymphocytes by CD1 antigen-presenting cells. CD1 cells recognize both foreign and self-lipids, such as phosphoglycerolipids and glycosphingolipids as stimulatory antigens [21–23]. Likewise, elevated levels of certain lipids have been shown to impact cell signaling, including activation of NF-κB and programmed cell death [24–26].

Work in humans and mice has allowed for analysis of the lipidome in obese, hypertensive, or normal individuals, as well as those with metabolic dysfunction [27,28]. However, no published work has been done to classify the lipidome of horses with EMS. Existing data in the horse have allowed for characterization of EMS and explored connections to laminitis, but have yielded conflicting results, particularly in regards to inflammation [29]. Some studies have shown differences in inflammatory cytokines such as gene expression of interleukin 1β (IL-1β) and IL-6 in equine nuchal adipose tissue in EMS horses versus controls [30] and an increase in circulating plasma and serum concentrations of tumor necrosis factor alpha (TNF-α) in horses with EMS [31] as well as in ponies with a history of pasture-associated laminitis [32]. However, others have shown a trend for circulating plasma TNF-α concentrations to be lower in obese horses compared to controls as well as a decrease in gene expression of IL-1β and IL-6 in the peripheral blood mononuclear cells (PBMCs) of obese horses [29]. Conflicting results may in part stem from the fact that many studies were carried out on ponies or on horses with an induced form of hyperinsulinemia [31,32]. Not only that, but it is unclear if many were tested for confounding factors, such as pituitary pars intermedia dysfunction (PPID), which can influence insulin/glucose dynamics.

Considering the negative effects of obesity and insulin dysregulation, in particular the increased risk of laminitis in horses, it is important to examine contributing mechanisms to the disorder. Thus, the objective of this study was to investigate and characterize the plasma lipidome as well as the inflammatory status of horses with naturally occurring EMS compared to non-EMS controls.

2. Materials and Methods

2.1. Horse Selection and Sample Collection

Twenty-three horses of mixed sex and breed were selected from the University of Kentucky's Department of Veterinary Science herd. Of these horses, n = 14 were classified as EMS (mean 13 ± 4 years) and n = 9 were non-EMS controls (mean 13 ± 3 years). There was no significant difference in age between EMS and control horses. Of the horses classified as EMS, three were Standardbred or Standardbred crosses, three of mixed breed, and three were paints. In addition, one EMS horse was a Morgan, one a draft, one a warmblood cross, one a Walking horse, and one a Thoroughbred. Of the control horses, two were Quarter horses, three were Thoroughbreds, and four were of mixed breed. Ten of the 14 EMS horses were mares and four geldings. Seven of the controls were mares, and the remaining two were geldings. All horses were housed at the University of Kentucky's Main Chance or Woodford farm facilities, maintained on a similar all forage diet of free choice mixed grass hay and minimal pasture, and had access to water and a mineral block ad libitum. Horses were acclimated to their respective pastures for at least 2 months before sampling. All materials and methods were approved by the Institutional Care and Usage Committee of the University of Kentucky.

Equine metabolic syndrome was determined by the following criteria from the 2010 ACVIM consensus statement, insulin dysregulation, general or regional adiposity, and a history of or predisposition to laminitis. To ascertain the presence of insulin dysregulation, an oral sugar test was performed following overnight fasting. In brief, fasting serum sample collection was followed by PO administration of 0.15 mL/kg of Light Corn syrup (Karo; ACH Food Companies, Cordova, TN) and a second serum sample collected 1 hour later [5,6]. All blood collection was carried out via jugular venipuncture. After centrifugation, aliquots of serum samples were kept at −20°C until analysis. A fasting insulin level of >20 μIU/mL was considered indicative of hyperinsulinemia, and an increased insulin (>60μU/mL) 60 minutes postadministration of oral sugar was defined as insulin resistance [5,6]. The presence of either hyperinsulinemia or insulin resistance was considered positive for insulin dysregulation. Obesity and adiposity were determined by body condition score (BCS) and cresty neck score (CNS). Body condition score was established and averaged between three trained investigators using the 1 to 9 Henneke scoring system [33] with a 1 representing an extremely emaciated animal and a 9 representing an extremely obese animal. Investigators were blinded to which horses were positive for insulin dysregulation. The same trained individual's scores were averaged for CNS via the 0 to 5 system