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Stress response as a contributing factor in horses with laminitis

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ABSTRACT

Background: Laminitis is a complex and debilitating disease of horses. Numerous predisposing factors contribute to laminitis development, however the exact pathogenesis remains undetermined. Serum T4, cortisol, and histamine are components of the innate stress response and could play a causative or contributory role. Stress hormone concentrations in laminitis are largely unknown.
Objective: To evaluate parameters associated with stress response in horses with laminitis, and compare these to healthy horses and horses with gastrointestinal (GI) disease.
Methods: Thirty-eight adult horses presenting for non-medical conditions, GI abnormalities, or clinical laminitis were prospectively enrolled. Horses were assigned to the appropriate disease group (healthy, GI disease, and laminitis) and had blood drawn on presentation to the hospital. Samples were analyzed for plasma endogenous adrenocorticotrophic hormone (eACTH), serum cortisol, serum thyroid hormone, and plasma histamine.
Results: Stress hormone concentrations were significantly different between horses in the laminitis and GI disease groups. Plasma histamine levels were highest in horses with laminitis, compared with GI disease and controls. Both horses with laminitis and GI disease had increased plasma eACTH when compared to healthy horses. Horses with GI disease had higher serum cortisol concentrations than horses with laminitis or controls. Serum T4 was lower in horses with GI disease than in horses with laminitis and controls.
Conclusions: Horses with laminitis had relative increases in both plasma histamine and eACTH concentrations. Serum T4 and cortisol concentrations of horses with laminitis did not differ significantly when compared to healthy horses. The role of stress hormones in equine disease warrants further investigation.

Keywords: Hormones; gastrointestinal diseases; histamine; adrenocorticotropic hormone

INTRODUCTION

Laminitis is a complex and debilitating disease process involving the hoof wall in horses that can lead to recurrent episodes of lameness and ultimately euthanasia. Numerous mechanisms leading to lamellar failure have been proposed, but the exact pathogenesis of laminitis remains unknown. Sepsis/Systemic Inflammatory Response Syndrome, underlying

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Conflict of Interest
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endocrinopathy, ingestion of toxic substances such as black walnut, or overwhelming unilateral weight bearing on a support limb are the main predisposing factors for the development of laminitis in horses [1].

Various stress hormones, such as endogenous adrenocorticotrophic hormone (eACTH) and cortisol, have been reported in disease states associated with the development of laminitis such as ischemic intestinal injury [2], equine metabolic syndrome (EMS), and equine pituitary pars intermedia dysfunction [3]. It is well known that horses with complex gastrointestinal (GI) diseases are at a higher risk for health complications, including laminitis [4]. Direct association between these previously measured hormone concentrations and laminitis has not been determined. The same holds true for other related endogenous compounds, such as thyroid hormone (T4), which is known to be decreased in various disease states [5]. Histamine, in addition to being an important mediator of inflammation, has also been reported to play a role in the stress response of other species [6] and increase in plasma concentration has been reported in horses with laminitis [7].

Overall, there are limited reports evaluating stress hormones in clinical cases of laminitis [3,7,8]. Therefore, our objectives were to evaluate a panel of stress hormones in horses with laminitis and compare them to horses with GI disease and those free of disease to better determine a stress profile associated with equine laminitis. We hypothesized that the characteristics of the physiologic stress response would be similar between horses with laminitis and those with GI disease. However, we anticipated that horses with clinical laminitis would have a unique stress hormone profile compared to horses with GI disease or controls.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee (#05-164A-01). Adult horses > 2 yr of age were prospectively enrolled, with informed client consent, between the months of April and August by sequential admission. Three types of cases were enrolled, including: 1) horses with clinical and radiographic evidence of laminitis (n = 14), 2) horses presenting for primary GI disease (n = 12), and 3) healthy horses (n = 12), presented for non-medical conditions such as pre-purchase examination or routine dental work.

Signalment and primary clinical diagnosis was recorded for all cases. Horses were assigned to one of the three groups based on presentation. Blood samples were collected via venipuncture of the left jugular vein. Serum and plasma were separated respectively and stored in plastic vials frozen at –70°C until analysis. Concentrations of plasma eACTH, serum cortisol, and serum thyroid hormone (thyroxine, T4) were determined by chemiluminescent immunoassay (Immunite 1000; Siemens AG, Germany). Plasma histamine was determined by immunoassay (Bertin Parhma, France) following validation in equine plasma.

All continuous variables underwent Shapiro-Wilks testing for normality, and those that displayed normal distribution were reported as median and interquartile range. Statistical analysis was performed using SAS version 9.1 (SAS Institute, USA). Significant differences identified were further investigated through individual comparisons using the least squares mean test. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 38 horses, of varied breeds, including American Quarter Horse (n = 15), American Paint Horse (n = 6), Arabian (n = 6), Warmblood (n = 4), Thoroughbred (n = 2), Tennessee Walking Horse (n = 1), Morgan (n = 1), Shire (n = 1), Appaloosa (n = 1), and English Riding Pony (n = 1), were enrolled. The age of horses ranged from 3 to 26 yr of age, with a median age of 11 yr. Each group had the following sex distributions: group 1: laminitis - 8 geldings, 5 mares, and 1 stallion; group 2: GI - 5 mares, 5 geldings, and 2 stallions; and group 3: control - 7 mares and 5 geldings. Of the horses in the laminitis group, nine had chronic laminitis and five were acute cases with no prior history of laminitis. Of the acute laminitis cases, three developed laminitis while hospitalized for treatment of an orthopedic issue, while the other two were admitted within 2 d of developing signs following ingestion of spring grass. The GI group included nine surgical colic cases and three colitis cases with no causative agent identified. The control group was composed of four mares presented with sick companion foals and eight horses presented for routine lameness workup. Median hospitalization length was 3 d for the laminitis group, 4 d for the GI disease group, and 7 d for the control group.

All horses across the three groups had all analytes measured. There were no missing samples. The results are summarized in **Table 1**. Horses in both the laminitis and GI disease groups showed alterations in stress hormone levels relative to healthy horses. Serum T4 was significantly lower in horses with GI disease than in horses with laminitis and controls (**Fig. 1A**). Plasma eACTH was significantly greater in horses with laminitis and GI disease than in controls (**Fig. 1B**). Serum cortisol was significantly higher in horses with GI disease than in horses with laminitis or controls (**Fig. 1C**). Plasma histamine levels were also significantly higher in horses with laminitis than in horses with GI disease and normal horses (**Fig. 1D**).

DISCUSSION

The results of this study indicate differences in stress response hormones (eACTH, cortisol, T4 and histamine) between horses with laminitis and those with GI disease or healthy controls. Horses with laminitis had higher plasma concentrations of histamine than were present in the other groups. Horses affected by GI diseases had higher plasma cortisol and lower serum T4 concentrations, than horses with laminitis or healthy controls. However, plasma eACTH concentrations were increased in both laminitic horses and those with GI disease when compared to healthy controls. Although this is a preliminary, high-level, survey looking at the concentration of stress hormones at a singular point in time, we believe these findings show promise for use of stress hormone levels in the diagnosis or surveillance for laminitis as a complication in hospitalized patients. Further research into the individual hormone, specifically histamine, profile over time is warranted. Additionally, investigation into the effect of primary disease severity and chronicity deserves consideration in future work.

Table 1. Peripheral indices of stress in 38 horses

Test	Reference range	Healthy group	Gastrointestinal disease group	Laminitis group	*p* value
Thyroxine (T4) (mg/dL)	1.0–2.5	1.85^{a} (1.43–2.40)	0.90^{b} (0.90–0.90)	1.90^{a} (1.00–2.23)	< 0.001*
eACTH (pg/mL)	18–25	14.15^{b} (9.63–19.83)	32.70^{a} (16.50–46.60)	22.70^{a} (16.25–37.70)	0.033*
Cortisol (mg/dL)	3.6–4.5	4.45^{a} (2.53–5.80)	8.20^{b} (6.60–12.50)	4.45^{a,b} (3.60–8.40)	0.004*
Histamine (pg/mL)	4.6–11.14	6.76^{b} (5.35–7.59)	7.16^{b} (5.14–8.82)	7.95^{a} (7.21–9.48)	0.045*

All values reported as mean (interquartile range).
eACTH, endogenous adrenocorticotrophic hormone.
*Values with a *p* value < 0.05.
Superscript letters indicate results of each analyte between groups made by the least squares mean test.

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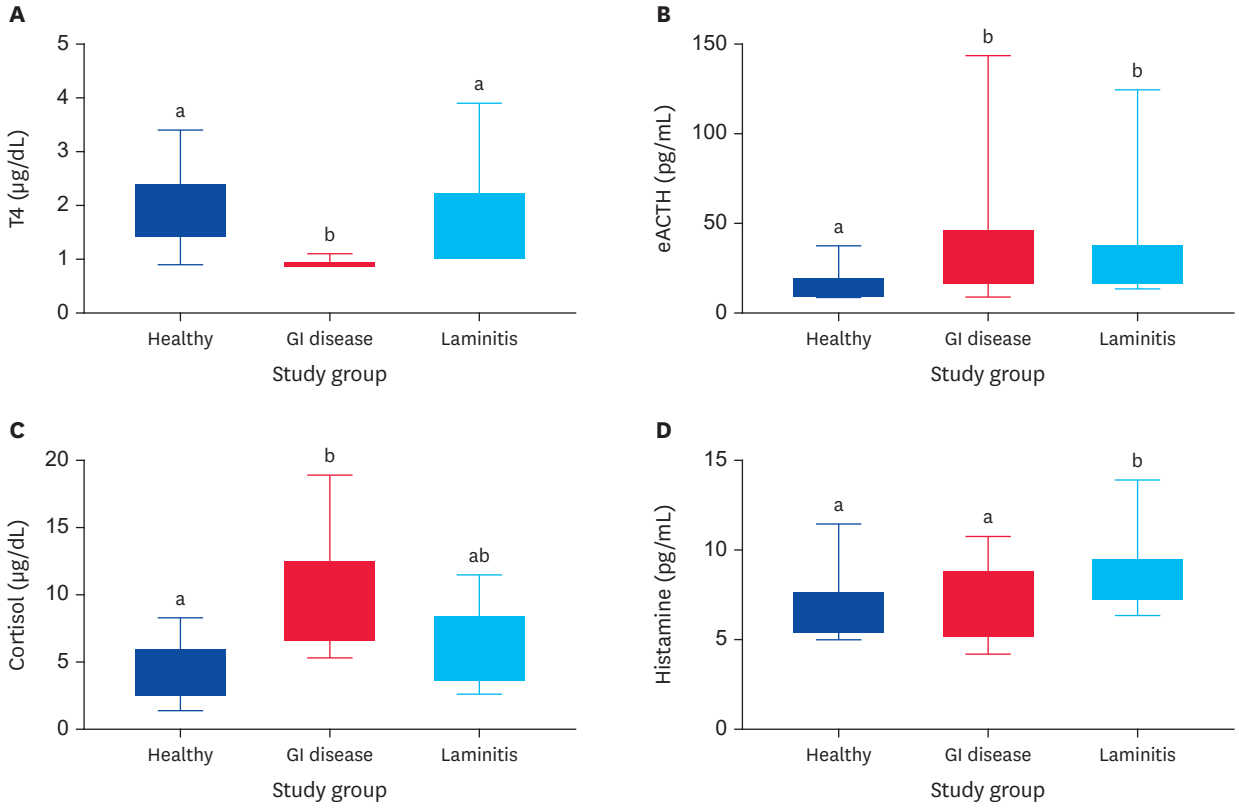

Fig. 1. Stress hormone concentrations in healthy horses, horses with GI disease, and horses with laminitis. (A) Serum T4, (B) plasma eACTH, (C) serum cortisol, (D) plasma histamine are represented.
[a,b]Statistical significance between groups with $p < 0.05$ are indicated by letter designation.
GI, gastrointestinal; eACTH, endogenous adrenocorticotrophic hormone.

Increases in plasma eACTH and serum cortisol in horses with acute laminitis and acute GI disease has been reported [8]; however, in that study there were no differences noted between laminitis and GI groups. In the present study, horses with laminitis and GI disease had increases in plasma eACTH concentrations when compared to healthy controls. Unlike the previous report [8], laminitic horses in the present study did not demonstrate a difference in serum cortisol concentrations. One reason for this finding may be related to the chronicity of disease as the majority of horses in the present study had chronic laminitis, while the previous study only evaluated acute cases. A study by Rietmann et al. [6] measured cortisol levels in laminitic horses more than 16 h after the onset of lameness, and found no changes in levels when compared to healthy horses. Horses with more chronic conditions might not have increased serum cortisol due to the short half-life and rapid secretion of this hormone following stimulus. Another reason could be due to cortisol insufficiency associated with illness, resulting in a damped cortisol response in horses with certain disease states. Although this is an established condition in many species, there is limited information specifically related to horses [9,10]. Horses with GI disease having plasma eACTH concentrations above the normal reference interval have been reported to be 6 times less likely to survive, suggesting that eACTH could be useful in evaluating disease severity. Stress associated with the actual disease response and pain likely predispose these horses to increases in eACTH. Although using eACTH concentrations as a solo marker to differentiate between disease states may not be useful; further evaluation of outcome correlated to eACTH concentrations could provide useful information in the future.

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Serum T4 concentration was not different between laminitic horses and healthy control horses. This finding is similar to previous results where ponies currently demonstrating laminitis had no difference in total or free T4 concentrations [11] and another report where no difference in serum T4 concentration was noted between horses with EMS and laminitis when compared with non-affected controls [3]. Serum T4 concentrations were, however, found to be decreased in the present study in horses with GI disease. Decrease in serum T4 concentrations has been reported in sick horses, with more substantial decreases in those with worsened disease states [5]. Results from that study suggest that serum T4 concentrations alone might not be beneficial in the assessment of a stress response in laminitic horses, but when evaluated in a panel could help differentiate stress associated with severe disease from laminitic pain.

Histamine, produced by mast cells, has been reported to play a role in the stress response of other species [6] as the release of corticotropin releasing hormone can induce the degranulation of mast cells under stress conditions [12]. Histamine producing bacteria have also been isolated from the rumen of cattle fed grain and the cecum of horses [12]. Although histamine can be produced and released by numerous cells within the GI tract [13] there was no increase in plasma concentrations of histamine in horses with GI disease in the present study. There was, however, a significant increase in plasma histamine concentration in the horses with laminitis in this study when compared to healthy controls. Plasma histamine concentrations have been previously reported to be increased in horses with laminitis [7]. Cattle with chronic laminitis, but not acute laminitis have also been reported to have increases in plasma histamine concentrations [14]. Though histamine is a major player in the acute inflammatory response it can also have an effect on chronic inflammation [13]. This was seen reflected in our results, where all horses with chronic laminitis and 3 of the 4 with acute laminitis had increases in plasma histamine. Histamine was the only hormone found in this study to be significantly different in laminitic horses compared to both controls and horses with GI disease. As such, it presents the best prospect for evaluation as a biomarker for clinical use in the diagnosis of, or surveillance for, laminitis. This study is limited in its evaluation of only a single time point concentration, and therefore further evaluation of the plasma histamine profile in both acutely and chronically laminitic horses is necessary. Additionally, the role of histamine in the stress response of the horse is incompletely understood, including with regards to its role in the laminitis disease process; further research is similarly warranted to elucidate this, which may also improve its prospects as a clinically useful biomarker for laminitis.

Blood based testing allows non-invasive evaluation of potential biomarkers associated with disease. One limitation of this study is that blood samples were collected at only one time point on admission to the hospital. There was no control for time of day or time of year. Time of year can affect eACTH concentrations, increasing during late summer and fall [15]. Both cortisol [16] and T4 [17] have been reported to have diurnal effects. As a result, the findings associated with these hormones could be affected by confounding factors. Another limitation of this study relates to the clinical nature of the study. Performing studies that utilize naturally occurring disease allows for direct interpretation on clinical cases, but they carry intrinsic variability related to the severity and/or duration of the disease state. However, this preliminary prospective study provides valuable insight on application of a stress hormone profile in laminitis and related disease states, relative to control, in a clinical population that represents the population of interest. Despite the limitations of sample size and patient variability, distinct patterns of stress hormone alterations were observed in

horses with laminitis. This finding elucidates the need for future study in this area. Another consideration is that single analyte evaluation may not be as useful in discrimination between health and disease because this diagnostic testing approach often lacks sensitivity and specificity [18]. Evaluation of multiple analytes simultaneously allows an approach that combines various biological pathways in concert, which is more reflective of complex disease processes such as laminitis. However, from this study histamine shows the greatest promise for distinct differences when specifically evaluating laminitis and therefore its inclusion in a multivariate analysis would be recommended for future research.

This study demonstrated derangements in stress hormones in horses with laminitis. These included increases in histamine and eACTH concentrations relative to healthy horses. Furthermore, when compared to horses with GI disease, horses with laminitis were found to have a higher serum T4, lower cortisol, and higher histamine concentrations. These findings suggest that the profile of stress hormones associated with laminitis characteristically differs from that seen in horses with GI disease. Further research is warranted to better understand the role of stress hormones in laminitis.

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