

Correlation of Plasma Insulin Concentration with Laminitis Score in a Field Study of Equine Cushing's Disease and Equine Metabolic Syndrome

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ABSTRACT

This study aimed to investigate endocrinologic test values and the response to treatment of two commonly encountered causes of endocrinopathic laminitis, equine Cushing's disease (ECD) and equine metabolic syndrome (EMS), in a veterinary practice setting. In particular, the study aimed to determine whether insulin concentration correlated to the severity of clinical laminitis in horses with EMS or ECD. Twenty-five horses were included in the study and assigned to one of three groups: ECD ($n = 6$), EMS ($n = 10$), and controls ($n = 9$). Blood samples were collected at an initial visit and then at regular intervals for the next 12 months. Plasma concentrations of adrenocorticotropin (ACTH), cortisol, and insulin and serum concentrations of glucose and total thyroxine (T4) were obtained. Horses with ECD had significantly higher plasma ACTH concentrations than EMS horses or controls. Horses with EMS and ECD both had significantly higher plasma insulin concentrations than control horses, which was correlated with the Obel grade of laminitis ($r = 0.63$). After treatment, there was a trend for a reduction in plasma ACTH concentration in horses with ECD. A program of diet and exercise for horses with EMS resulted in reductions in both plasma insulin concentrations and bodyweight, which was variable, depending on the individual. There was a significant correlation between the change in plasma insulin concentration and Obel grade of laminitis ($r = 0.69$). This study has highlighted the importance of baseline plasma insulin concentration as a potential indicator of the susceptibility of horses

to laminitis and the response to a program of diet and exercise.

Keywords: Laminitis; Metabolic syndrome; Cushing's; Insulin

INTRODUCTION

Endocrinopathic laminitis associated with equine Cushing's disease (ECD) or equine metabolic syndrome (EMS) is commonly seen by veterinary practitioners. Of importance to owners and veterinarians is the ability to predict, monitor, and manage the development of laminitis associated with both conditions; however, there has been very little evidence-based medicine to assist them.

ECD (Pituitary pars intermedia dysfunction) is a syndrome of pituitary hyperadrenocorticism caused by dysfunction of the pars intermedia of the pituitary gland.¹ Although the pars intermedia is not usually responsible for most ACTH production, the hormone is still markedly increased in affected horses, and the clinical signs of the syndrome can be attributed principally to its effects. These include: weight redistribution (pot belly and wasted top line caused by muscle catabolism and bulging supraorbital fat), polyuria and polydipsia, susceptibility to infections, and laminitis.¹ Diagnosis using basal adrenocorticotropin (ACTH) has been shown to be sensitive and specific for ECD.^{2,3} However, because of special sample handling requirements, some have advocated use of paired cortisol analysis to diagnose ECD on the basis that horses with ECD should show a reduced diurnal variation.⁴ Treatment for ECD using pergolide has been described by several authors,^{5,6} yet prospective data have been limited.

The term *equine metabolic syndrome* was first used by Johnson in 2002.⁷ It referred to horses that had a history of laminitis, insulin resistance, and a characteristic phenotype of a cresty neck with increased adipose tissue deposits in the withers and dorsal area of the back. What differentiated EMS from horses with ECD was the elevated plasma ACTH concentrations⁸ or altered dynamic endocrine tests⁹ that characterize horses with ECD. Prospective

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treatment of EMS using diet and exercise has been advocated⁷ yet again; few data exist to substantiate this claim.

The ability to recognize the different forms of this condition affects the management protocol and ultimate success of treating laminitis. Trying to explain how insulin resistance plays a role in laminitis, whereas little is known about how horses develop the condition, is challenging. Why some horses with ECD develop laminitis, and others do not, has been a mystery. However, recent evidence by Asplin and colleagues¹⁰ has raised the possibility of insulin as a triggering factor for episodes of laminitis in insulin-resistant horses. Therefore, the primary aims of this study were to determine whether insulin concentration correlated to the severity of clinical laminitis in horses with EMS or ECD and also to observe the effect of a program of exercise and diet on grade of laminitis and serum insulin concentration in EMS horses. The secondary aims of this study were to determine how rapidly pergolide treatment reduces circulating ACTH in horses with ECD and to assess the diagnostic value of measuring morning and afternoon plasma cortisol concentrations in suspected cases of ECD.

MATERIALS AND METHODS

The blood glucose and hormone concentrations of normal, EMS, and ECD horses within a single practice (Homestead Veterinary Hospital in Pacific, MO, near St. Louis) were studied over a 2½-year period. Horses were observed and sampled at their farm/stable by first opinion equine practitioners as part of their routine practice during the period from December 2004 through May 2007. The horses were all located within a 60-mile radius of the practice. The study design aimed to maintain study animals in settings to which they were accustomed, avoiding the potential effect of transport on hormone levels. Horses were classified as having laminitis associated with endocrine dysfunction or control horses that never had laminitis but were living on the same premises and in the same environment as those that did. Their owners consented to the study.

Inclusion Criteria

Horses with laminitis at the time of the initial visit or with a history of endocrinopathic laminitis were selected for the study. Based on clinical findings and initial diagnostic tests, horses were grouped as having ECD, EMS, or normal according to the following parameters:

ECD: plasma ACTH concentration greater than 70 pg/mL (laboratory reference range, 9–35 pg/mL)

EMS: plasma insulin concentration greater than 70 µIU/mL (laboratory reference range, 10–30 µIU/mL) (normal ACTH)

Normal or control: plasma ACTH and insulin concentration within the normal range determined by the laboratory performing the analyses. Control horses

had no history of laminitis and were from the same properties as those with EMS or ECD.

Exclusion Criteria

Horses with laminitis associated with a single limb, gastrointestinal, or systemic disease were excluded. Horses with plasma ACTH concentrations between 30 and 70 pg/mL were excluded to minimize the possibility that elevated ACTH was attributable to conditions other than ECD, such as stress or laminitis pain. Similarly, horses with only mild to moderate elevations in plasma insulin concentrations (between 30 and 70 µIU/mL) were excluded.

Clinical Data Collection

After horses were placed in the study, morning and afternoon samples of blood were obtained at approximately 9:00 AM and 4:00 PM, respectively. Single samples of the blood were again taken at 1, 2, and 3 weeks and 1, 2, 4, 6, 8, 10, and 12 months after recruitment into the study. Plasma concentrations of ACTH, cortisol, and insulin and serum concentrations of glucose and total thyroxine (T₄) were obtained.

Weight, physical condition body score, cresty neck, and soundness were evaluated at each visit. Weights were estimated using a tape measure formula (weight equals heart girth times heart girth, times body length, divided by 330).¹¹ A physical examination of the animal's body condition was performed and a score given based on the Henneke body condition score.¹² Using the cresty neck scoring system described by Carter et al,¹³ excess regional adipose tissue deposited along the dorsal part of the neck scoring greater than 3 Cresty Neck Score was recorded as "cresty." At each examination, the animal was asked to walk and trot if possible to evaluate soundness. Lameness, when present, was ranked using the Obel grading system.¹⁴

Horses with plasma ACTH concentrations greater than 70 pg/mL (ECD horses) were treated with 1 mg pergolide orally per day. EMS horses were placed on low-carbohydrate, reduced caloric intake diets and were placed in dry lots to restrict grass consumption. Because all of the EMS horses were considered overweight (having a body condition score greater than 7),¹² a 20% reduction in caloric intake was recommended along with exercise. All grain-based feeds were eliminated from the diet. All of the EMS horses were withheld from grass and kept in dry lots. A diet of grass hay was fed at a level that reflected a 20% reduction in caloric intake. All horses in the study were encouraged to exercise regularly, with gradually increasing intensity as their health and degree of lameness permitted.

Laboratory Methods

All blood samples were processed according to the protocols of the Diagnostic Endocrinology and Clinical Pathology Laboratories at the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University. Plasma ACTH and cortisol concentration were measured by automated chemiluminescent enzyme immunoassays (Immulite, Diagnostic Products Corporation, Los Angeles, CA) previously validated for horses.^{6,15} Plasma insulin concentration was measured by a double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX) previously validated for horses.¹⁶ Serum T4 concentration was measured by a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) previously validated for horses.¹⁷ Serum glucose concentration was measured by the hexokinase method using a Roche Hitachi 917 Chemical Analyzer (Roche Diagnostics Corporation, Indianapolis, IN).

Analyses

Morning and afternoon plasma cortisol concentrations from the first visit were compared and differences in plasma cortisol concentrations calculated between groups (control horses vs ECD vs EMS). The difference between the plasma cortisol concentration from morning and afternoon sample was divided by the morning value to generate a “mean percent diurnal variation.”⁴ Descriptive statistics were used to define the baseline characteristics of the groups (age, breed, sex, cortisol, glucose, T4, and body weight), and a nonparametric test (Kruskal-Wallis) was used to identify significant differences between groups.

Linear regression analysis was used to determine the relationship between baseline plasma insulin concentration and laminitis score, and the line of best fit was determined. The fitted linear relationship between baseline serum T4 and plasma insulin concentration also was examined. The maximal change in plasma insulin concentration over time was compared with body weight change and laminitis score throughout the trial in the EMS group, using linear regression analysis and determination of the line of best fit, and, where significant, the coefficient of determination (r^2) also was calculated. Changes in plasma insulin, ACTH and cortisol concentrations, serum T4 and glucose concentrations, and body weight over time for horses in each group were examined using a general linear model using repeated measures (SPSS version 12). Results are presented as mean \pm 95% confidence intervals.

RESULTS

Twenty-three horses (over 14-2 hands) and two ponies (total $n = 25$) were included in the study. Based on clinical findings and initial diagnostic tests, the animals were

assigned to one of three groups: ECD ($n = 6$), EMS ($n = 10$), and controls ($n = 9$).

The ECD group had two ponies (one Hackney mix and one mixed breed); an Arabian, a Quarter Horse, a mixed breed horse, and a Connemara. The EMS group had four Tennessee Walking Horses, an Arabian, an Arabian crossbred, three Connemaras and a Connemara–Thoroughbred crossbred. The control group had three Quarter Horses, one Quarter Horse–Thoroughbred, one Tennessee Walking Horse, one Connemara, one Connemara–Thoroughbred crossbred, and two Thoroughbreds.

The mean age for each group was: control horses = 12 ± 7.6 years; ECD = 28.5 ± 8.14 years; and EMS horses = 15.5 ± 5.11 years. Mean body weight was significantly lower ($P < .01$) in horses with ECD (805 ± 85 lb) versus controls ($1,145 \pm 26$ lb) and EMS ($1,111 \pm 30$ lb). However, the ECD group contained the two small ponies in the study. During the study, there was no change in body weight in the control group. However, there was a trend for a decrease in body weight for the EMS group and a trend for an increase in body weight for the ECD group. However, the latter trend was partly attributable to the death of the two ponies after the first 2 months of the study (Fig. 1). A cresty neck (increased adipose tissue in dorsal neck) was present in all of the EMS horses, three of six ECD horses, and none of the control horses.

Mean baseline plasma ACTH concentration was not different between control and EMS horses (22.9 ± 2.2 and 26.6 ± 5.4 pg/mL, respectively), but it was significantly higher in horses with ECD (349.0 ± 161 pg/mL) ($P = .002$). In ECD horses treated with pergolide, there was a decreasing trend for ACTH over time (Fig. 2). However, the two ponies in the ECD group died after 2 months, and a complete dataset was available for only three pergolide-treated horses.

Mean baseline plasma insulin concentration was not different between EMS and ECD horses (53.0 ± 16.3 and 52.0 ± 21.6 μ U/mL, respectively), but together they were significantly greater ($P < .05$) than controls (18.7 ± 3.8 μ U/mL). There was no difference between baseline serum T4 concentration within the group (control, 1.3 ± 0.1 ; EMS, 1.6 ± 0.2 ; ECD, 1.0 ± 0.3 μ g/dL). Both plasma insulin and serum T4 concentration did not change significantly over time. However, there was a weak negative correlation between baseline serum T4 and plasma insulin concentration ($r = -0.22$, $P < .001$) (Fig. 3).

The mean baseline plasma cortisol concentration for ECD and EMS horses was higher (4.0 ± 1.0 and 4.5 ± 1.5 μ g/dL, respectively) than for the control horses (2.6 ± 1.4 μ g/dL), and this did not change significantly over time. The plasma cortisol concentrations from the morning and afternoon baseline samples showed no significant difference between groups in mean percent diurnal variation. The mean percentage difference was as follows:

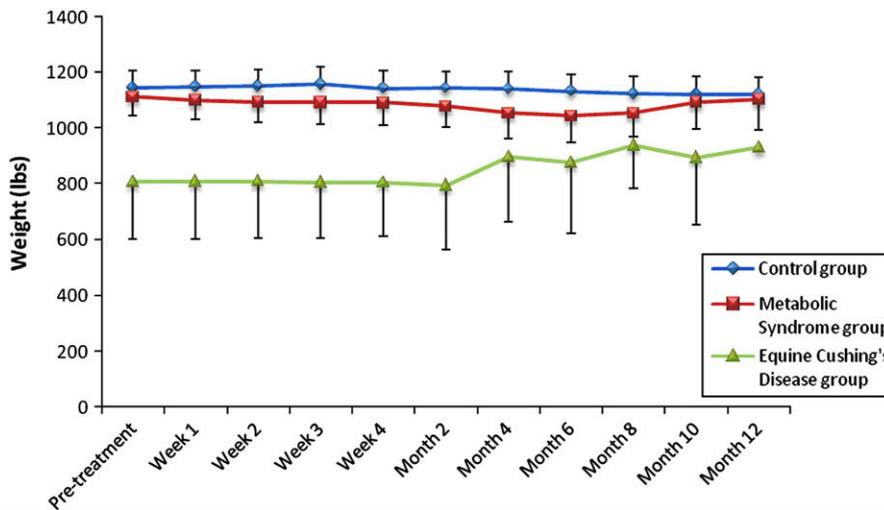


Figure 1. Mean ($\pm 95\%$ CI) bodyweight changes (lbs) over time for the three groups of horses—control, EMS, and ECD. There is a trend for an increase in body weight for ECD horses and a decrease for EMS horses.

control horses, $23.0\% \pm 20.6\%$; EMS horses, $29.2\% \pm 15.8\%$; and ECD horses, $11.5\% \pm 18.9\%$.

The mean baseline serum glucose concentration was as follows: ECD = 155 ± 96.7 mg/dL; EMS horses = 96 ± 7.2 mg/dL; and controls = 95.1 ± 5.0 mg/dL, which was not significantly different between groups and did not change significantly over time.

Six of the EMS horses entered the study with clinical laminitis. Four of the EMS horses entered the study in a normal state but with a history of laminitis, normal ACTH, and insulin greater than $70 \mu\text{IU}/\text{mL}$. Of the ECD horses, four entered the study sound, with a history of laminitis with plasma ACTH above $70 \text{ pg}/\text{mL}$. Two of the ECD horses entered the study with current laminitis and elevated plasma ACTH concentration.

There was a significant correlation between laminitis grade and baseline plasma insulin concentration ($r = 0.63$, $P < .001$) (Fig. 4). When only the ECD and EMS horses were examined, there also was a significant correlation between laminitis grade and baseline plasma insulin concentration ($r = 0.57$, $P = .02$).

The effect of diet and exercise on EMS horses was assessed by comparing the maximal change in plasma insulin concentration with the changes in body weight and laminitis grade. Over a mean duration of 8.0 ± 0.8 months, there was a mean decrease in plasma insulin concentration of $44.4 \pm 128.5 \mu\text{IU}/\text{mL}$, mean decrease in body weight of 56.3 ± 50.8 lbs, and a decrease in laminitis score of 0.7 ± 1.6 Obel grade. Whereas the standard deviations of individual horse response showed substantial variation,

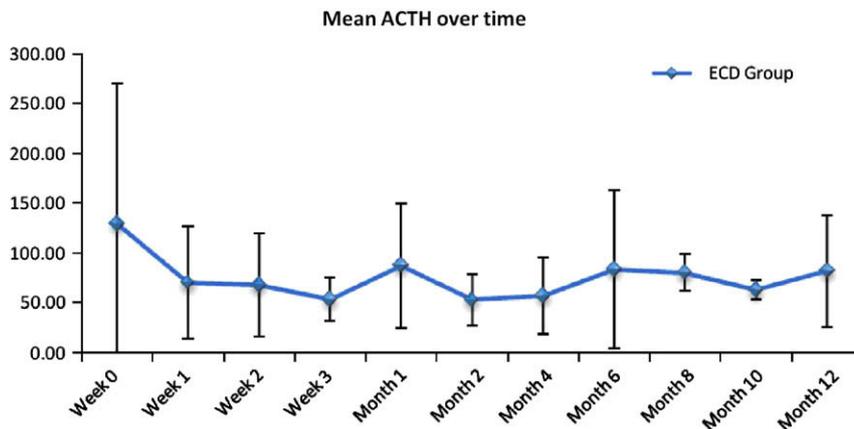


Figure 2. Mean ($\pm 95\%$ CI) plasma ACTH concentration (pg/mL) over time in ECD horses that underwent treatment with pergolide 1 mg per day ($n = 3$). Pergolide treatment tended to decrease plasma ACTH concentrations.

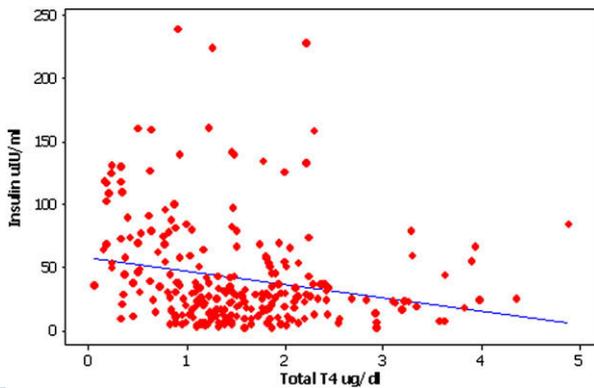


Figure 3. Fitted linear regression showing the inverse relationship between T4 ($\mu\text{g}/\text{dL}$) and plasma insulin concentration ($r = -0.22$).

there was a good correlation between the change in insulin concentration and change in laminitis grade ($r = 0.69$) ($P < .05$). The linear line of best fit had an R^2 of 0.48 (Fig. 5).

DISCUSSION

The main finding of the current study was that plasma insulin concentrations were significantly correlated with laminitis grade, and that a decrease in plasma insulin concentration was significantly correlated to a decrease in laminitis grade. These results support the role of insulin in the pathogenesis of endocrinopathic laminitis as demonstrated by the hyperinsulinemic model in normal ponies.¹⁰ This implies that plasma or serum insulin concentrations

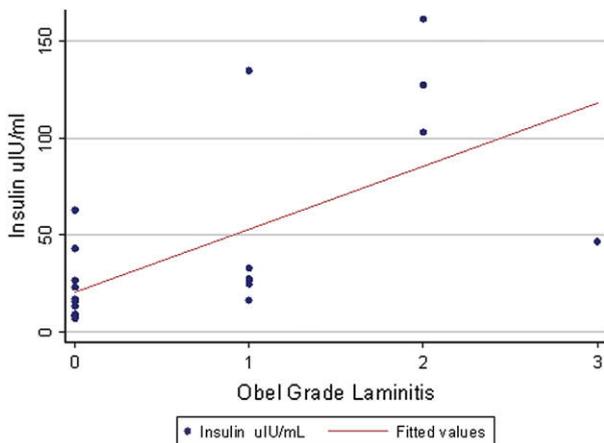


Figure 4. Fitted linear regression showing the relationship between plasma insulin concentration and Obel grade of laminitis in all horses at baseline ($r = 0.63$, $P < .001$). Hyperinsulinemia was associated with increased laminitis severity.

are therefore able to provide important information to the practitioner regarding prediction of laminitis in susceptible horses. Furthermore, the study also showed that a cresty neck score greater than 3 was present in all of the EMS horses and half of the ECD horses and serves as a phenotypic indicator that is associated with insulin resistance and the tendency to develop laminitis.^{13,18}

The correlation between insulin resistance and laminitis was first recognized by Jeffcott et al¹⁹ in 1986. Since then, the association of insulin resistance with endocrinopathic laminitis has been documented by numerous authors.²⁰⁻²³ The onset of laminitis was associated with hyperinsulinemia (mean plasma insulin, $>100 \mu\text{U}/\text{mL}$; normal range, $8-30 \mu\text{U}/\text{mL}$).²⁰ The correlation between insulin concentration and laminitis score in this study may have been greater if the reduction in insulin had resulted in a more rapid recovery of the horses’ laminitis score. Horses with endocrinopathic laminitis have a slow recovery process and, depending on severity, may never achieve a return to complete normality. Clinical laminitis attributable to short-term (55 hours) hyperinsulinemia results in considerable histopathologic damage; the tips of secondary epidermal laminae are elongated and tapered, and the lamellar basement membrane is damaged.¹⁰ It is possible that with more chronic hyperinsulinemia, additive pathology, and worsening clinical laminitis would result. An ideal clinical outcome is more likely if hyperinsulinemia is reversed as early as possible and maintained within normal limits.

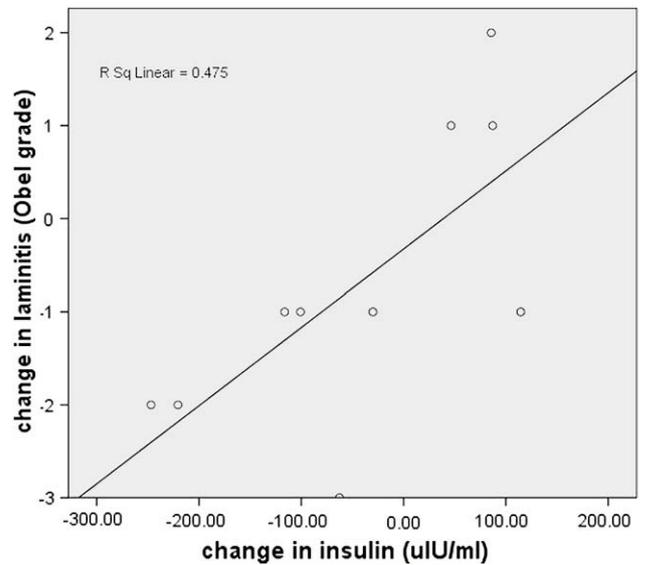


Figure 5. Fitted linear regression showing the relationship between the maximal change in plasma insulin concentration and laminitis grade in EMS horses. Correlation coefficient $r = 0.79$, $r^2 = 0.48$, $P < .05$.

Laminitis grade was significantly correlated with baseline insulin concentration, but more importantly, significantly decreased in response to a decrease in bodyweight and insulin concentration after dietary and exercise management of EMS. Despite this, on their own, the reduction in insulin concentrations and body weight were not significantly different over time. This is likely because of large variations in individual horses and the inability of some horses with laminitis to exercise. Greater weight loss may have occurred if dietary controls were more rigorous. Nevertheless, a reduction in insulin and loss of weight was observed in a number of horses that also improved their laminitis.

The cause of insulin resistance in horses has not been elucidated.¹⁸ In humans, it is caused by a failure of glucose uptake into muscle and adipose tissue by way of the insulin-sensitive Glut 4 transport protein.²⁴ The importance of glucose to the integrity of hoof epidermal laminae *in vitro* was reported by Pass et al.²⁵ They showed that glucose deprivation of hoof laminar explants caused rapid separation of basal cells from the basement membrane, and they therefore suggested that a failure of glucose supply in insulin-resistant horses may be the pathogenesis of laminitis. Inadequate laminar glucose delivery caused by failure of insulin-sensitive Glut 4 transport could cause laminitis by creating conditions similar to the *in vitro* model of Pass et al.²⁵ However, Asplin et al.¹⁰ recently showed that glucose uptake in hoof laminae was insulin independent. Glut 1 receptors predominate in hoof laminae and require no insulin to transfer glucose into cells.²⁶ In people with type 2 diabetes, glucotoxic microvascular lesions develop, presumably because insulin resistance leads to chronic hyperglycemia and hyperlipemia. Similar microvascular lesions may explain how laminitis develops in the horse.⁷ In insulin-resistant, type 2 diabetic humans, pancreatic beta cell production of insulin eventually fails.²⁷ Interestingly, beta cell production of insulin in horses does not usually fail, and hyperinsulinemia persists. Furthermore, as shown by this study, hyperinsulinemia is not concurrent with hyperglycemia, as it is with humans, and in horses hyperglycemia may not adequately explain the link between insulin resistance and laminitis. Only one of the ECD ponies in this study had significant hyperglycemia, hyperinsulinemia, and laminitis, and a link between hyperglycemia and laminitis could not be made. In the EMS group, four of 10 horses had mild elevations of blood glucose associated with the onset of hyperinsulinemic laminitis. Laminitis occurred within 3 days when hyperinsulinemia was experimentally induced in normoglycemic ponies.¹⁰ This new information establishes a direct link between insulin and laminitis, yet does not explain the mechanism by which this occurs and is the subject of further study at The University of Queensland, Australia.

There was an inverse relationship between insulin and T4 in the current study. Although the reason for this is

unknown, this relationship may explain the observation of low serum T4 concentrations in many euthyroid horses with insulin resistance and hyperinsulinemia, as reported by Frank et al.²⁸ Frank also reported that giving levothyroxine sodium reduced insulin resistance and resulted in weight loss in normal horses.²⁸ For many years, practitioners have achieved clinical improvement in overweight horses suffering from laminitis by administering thyroid supplements, and an ongoing study provides support for this approach.²⁹

The diagnostic value of measuring morning and afternoon plasma cortisol concentrations in suspected cases of ECD was not supported by our results, because there were no differences detected between the groups. There was an apparent reduced variation in the ECD group, but it was not significant and not consistent between horses. With larger numbers, we may have been able to detect a lower mean diurnal variation in the ECD group, but the test still would be unlikely to be of clinical value because of the variation between individual horses with ECD.

After treatment with pergolide, the ACTH concentration of the ECD group decreased within 1 week from a mean of over 120 pg/mL to a concentration of approximately 70 pg/mL, where it remained for the rest of the study, except during autumn, when a seasonal peak was observed. Although only a modest number of horses were included in this part of the study, it appeared that the positive effect of the dopamine agonist had a rapid onset. To our knowledge, this is the first study reporting the rapid onset of the therapeutic action of pergolide, albeit with limited numbers. Although the numbers in this study were small, the correlation between laminitis and hyperinsulinemia was important for horses with ECD, similarly to EMS. This supports previous research that contended the usefulness of insulin as a prognostic factor in ECD, where horses with a poor outcome generally succumbed to recurrence or failure to control clinical laminitis.²¹

This study contributes new knowledge to veterinary medicine by showing a positive correlation between hyperinsulinemia and laminitis severity. The correlation between increased levels of insulin and an increase in the laminitis score reported in the study warrants monitoring of insulin levels in horses that have cresty necks and are at risk for development of endocrinopathic laminitis. Although baseline samples may not yield as much information as dynamic tests of insulin resistance,³⁰ and may be of lower value when horses have only mild or marginal increases in plasma insulin concentrations, baseline concentrations can provide a useful screening test for practitioners. Samples should be obtained for insulin determination when horses have not eaten any grain or soluble carbohydrates for 2 to 3 hours. Following this protocol, a single blood sample showing marked hyperinsulinemia enables the practitioner to predict that laminitis may occur or become worse. Efforts to

reduce insulin resistance using a low-carbohydrate diet, exercise, and possibly thyroid supplements may prove effective in preventing hyperinsulinemia and, therefore, laminitis. In this study, the most successful outcomes were cases in which the horse was able to lose a significant amount of weight and get regular exercise. Practitioners can now institute weight loss and exercise programs for the horses and ponies of their clients, confident that decreases in plasma insulin concentration should reflect reductions in clinical laminitis.

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