

## Seasonal Variation in Serum Concentrations of Selected Metabolic Hormones in Horses

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**Background:** Determination of adrenocorticotrophic hormone (ACTH) concentration is a commonly used test in the evaluation of endocrine causes of equine laminitis, but the concentration in healthy horses can be high at certain times of year, which alters the specificity of the ACTH test.

**Objective:** To determine if circulating concentrations of ACTH, cortisol, glucose, insulin, and thyroxine vary month to month in healthy horses and in horses with equine metabolic syndrome (EMS).

**Animals:** Nine healthy adult horses were studied on their farm/stable over the course of 1 year. After the diagnosis of EMS, 10 laminitic horses residing at the same farm/stable were also studied.

**Methods:** Prospective study of healthy and laminitic horses. Plasma/serum samples were analyzed for concentrations of hormones and glucose.

**Results:** ACTH was the only analyte to show a discrete seasonal pattern, with concentrations in healthy and EMS horses frequently outside of the reference range (9–35 pg/mL) in August through October. Insulin was elevated ( $>40 \mu\text{IU/mL}$ ) in EMS horses during most months and median serum glucose was generally higher in EMS horses (100 mg/dL, range, 76–163 mg/dL) than in controls (94 mg/dL, range, 56–110 mg/dL), but no seasonal patterns for insulin or glucose were found.

**Conclusions and Clinical Importance:** An increased ACTH concentration in horses in late summer or autumn should be interpreted with caution. In contrast, insulin concentration is maintained within the reference range throughout the year in healthy horses, thus an increased insulin concentration at any time of year should raise suspicions of EMS, ECD, or both.

**Key words:** Adrenocorticotrophic hormone; Horse; Insulin; Season.

The accurate diagnosis of equine endocrinopathic disorders associated with laminitis—equine Cushing's disease (ECD) and equine metabolic syndrome (EMS)—is a challenging proposition for practitioners because the plasma concentrations of hormones key to the assessment, such as adrenocorticotrophic hormone (ACTH), vary in healthy horses at different times of year.<sup>1</sup> A substantial proportion of healthy horses and ponies have plasma ACTH concentrations above the established reference range in the month of September.<sup>1</sup> Therefore, it is recommended that there be careful consideration of seasonal changes in ACTH concentrations and the establishment of seasonal reference ranges.<sup>2</sup> To that end, the present study sought to extend current understanding<sup>1,3</sup> by measuring ACTH concentrations in healthy horses for 12 consecutive months to determine more precisely, which months are associated with increased ACTH concentrations. Because tests other than for ACTH are often used to differentiate between ECD, EMS, and other endocrinopathic conditions,<sup>4</sup> we also monitored the concentrations of insulin, thyroxine (T4),

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### Abbreviations:

ACTH	adrenocorticotrophic hormone
ECD	equine Cushing's disease
EMS	equine metabolic syndrome
T4	thyroxine

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cortisol, and glucose to determine if there is seasonal variation in these variables.

We also sought to determine if similar seasonal hormone patterns are apparent in laminitic horses. To determine if the effects of season on hormone and glucose concentrations are modulated in EMS horses, we measured ACTH, insulin, T4, cortisol, and glucose concentrations in EMS horses from the same farms/stables as the healthy horses. These assessments were made during the routine course of evaluation, treatment, and follow-up for 10 EMS horses.

The principal aim of this study was to assess the variation in circulating concentrations of hormones and glucose over a 12-month period in healthy and EMS horses that were maintained on their farm/stable.

## Materials and Methods

### Subjects

Nine healthy horses under the care of a single practice (Homestead Veterinary Hospital, Pacific, MO, near St Louis) were studied from December 2004 to May 2007. The healthy group had 2 Quarter Horses (QH), 2 Thoroughbreds (TB), 1 Tennessee Walking Horse (TWH), 1 Palamino, 1 Connemara (Conn), 1 Conn  $\times$  TB, and 1 QH  $\times$  TB. None of the healthy horses had any history of laminitis or endocrinopathies and their median age was 12.0 years (range, 4–23 years). Median initial body mass was 509 kg (range, 477–592 kg) and 5 of 9 control horses (56%) had an initial body

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condition score, on a 9-point scale, of 7 or greater. One of 9 (11%) had a cresty neck score, on a 5-point scale, of 3 or greater, which is indicative of regional adiposity. The history provided by the owner included no laminitis, and the initial blood work matched a normal blood profile. Physical exam revealed no abnormalities of the feet. Healthy horses for this study were selected because they resided on the same farm/stable as EMS horses that were monitored and treated at frequent and regular intervals by one of the authors and his associates.<sup>5</sup>

The EMS horses studied were determined to be laminitic at the time of their initial visit or had a history of endocrinopathic laminitis. Their classification was based on the presence of abnormal body fat, cresty neck, high body condition score in all horses, and the lack of any other etiologic laminitis factors. The breeds of the EMS group were 4 TWH, 3 Conn, 1 Conn × TB, 1 Arabian, and 1 Arabian Cross. Only horses with initial insulin values above the laboratory reference range were included in the study. For EMS horses, plasma insulin concentration was >70 µIU/mL (reference range, 10–40 µIU/mL) and plasma ACTH was within the reference range (9–35 pg/mL). Insulin sensitivity was not evaluated in these animals, thus this study was limited to a subpopulation of EMS horses with clear evidence of resting hyperinsulinemia. Pain and distress associated with laminitis were unlikely causes of hyperinsulinemia in the EMS horses, because each of these animals had an increased insulin concentration when signs of laminitis were absent during the course of this study. The EMS horses and their data were included in an earlier report,<sup>5</sup> where their treatment and monitoring were previously described in detail. Briefly, EMS horses were placed on a low carbohydrate diet, with the elimination of grain-based feed and grass. Overweight EMS horses were kept in a dry lot and their caloric intake was reduced by approximately 20%. All horses were encouraged to exercise regularly as the health and lameness allowed. We recognized that the diet and exercise regime might mask any potential seasonal effects on hormone and glucose concentrations in the EMS horses studied, but their laminitic history warranted treatment.

The median age of the 10 EMS horses was 16.0 years (range, 4–22 years). Median initial body mass was 493 kg (range, 462–578 kg), and all of the EMS horses (100%) had an initial body condition score of 7 or greater and a cresty neck score of 3 or greater.

Animals were cared for according to the principles outlined by the NIH Guide for the Care and Use of Laboratory Animals and by accepted veterinary practice principles.

### *Sampling and Assay Methods*

Horses were entered into the study during various months of the year, depending on when EMS horses were first identified as affected by a combination of clinical assessment and hormone testing—resting and fasting ACTH, insulin, cortisol, and T4. Healthy horses recruited as controls underwent an identical evaluation to rule out subclinical endocrinopathies. Blood samples were taken in the morning hours (08:00–11:00 AM CST). Horses were allowed grass hay overnight, but feed was withheld on the mornings when samples were obtained. For all horses, blood samples were obtained at 1–3 weeks and 1, 2, 4, 6, 8, 10, and 12 months after recruitment into the study. The recruitment and sampling scheme meant all horses were not sampled in every month of the calendar year. Samples were drawn into tubes containing ethylenediaminetetraacetic acid to assay for plasma ACTH, insulin, and cortisol and plain glass tubes to assay for serum T4, and glucose. Samples were chilled on ice followed by separation of plasma and serum within 2 hours of collection. Plasma and serum samples were stored at –20°C until analyzed at the Diagnostic Endocrinology and Clinical Pathology Laboratories within the New York State Animal Health Diagnostic Center, Cornell University.

All assays used had been previously validated for horses.<sup>6–8</sup> ACTH and cortisol concentrations were measured by automated chemiluminescent enzyme immunoassays,<sup>a</sup> and insulin concentration was measured by a double antibody radioimmunoassay.<sup>b</sup> Total T4 concentration was measured by a solid-phase radioimmunoassay,<sup>a</sup> and glucose concentration was measured by the hexokinase method with a Roche Hitachi 917 Chemical Analyzer.<sup>c</sup>

### *Statistical Analysis*

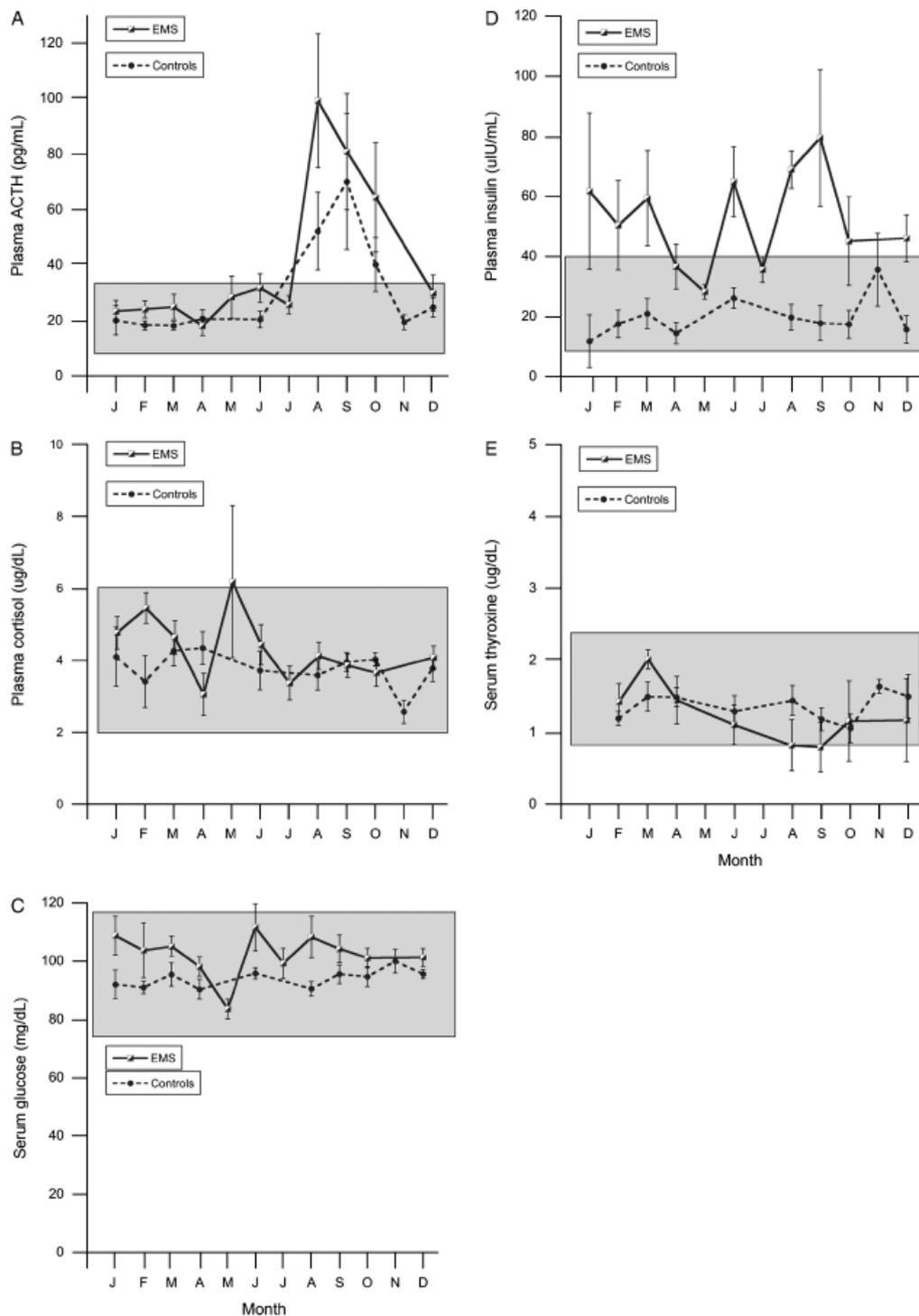
Results were analyzed with a commercial statistical program.<sup>d</sup> Natural log transformation of ACTH and insulin data was required to approximate a normal distribution. Untransformed data were used for graphical representation. The effect of group, month, and any group-month interaction on the concentrations of 4 hormones and glucose was determined for control and EMS horses by a mixed model repeated measures autoregression. One control and 4 EMS horses treated with levothyroxine were excluded from the analysis of T4 data. Whereas administration of levothyroxine may affect endogenous serum insulin concentration,<sup>9</sup> inclusion or exclusion of levothyroxine-treated horses did not appear to affect the patterns of month-to-month variation in the present study. Therefore, the insulin data presented are inclusive for all control and EMS horses.

Our sampling regime was originally designed to determine the rate of response to pergolide treatment in ECD horses,<sup>5</sup> thus the sampling times were highly variable for individual horses and as a result, some months were only represented by 1 or 2 horses in a particular group (Controls: May, July; EMS: November). Therefore, only months for which values from 3 or more horses in both the control and EMS groups were available were included in the analyses. When a sample was obtained on multiple days in a given month from a single individual, the first sample of the month was used for analysis and graphical representation of data. Data are presented as means±SD.

## **Results**

### *ACTH*

There was no effect of group on mean plasma ACTH ( $P = .9$ ) and no group-month interaction. Mean plasma ACTH concentration demonstrated a significant effect of month ( $P < .0001$ ) in control and EMS horses (Fig 1A). Pairwise comparisons of months showed that ACTH values during August, September, and October were significantly higher than all other months ( $P < .05$ ). The lowest plasma ACTH was found in April, which was significantly lower than in June, August, September, October, and December (Fig 1A;  $P < .05$ ). The greatest proportion of horses having an ACTH concentration above the reference range (9–35 pg/mL) was in September for controls (4 of 7) and for EMS horses (7 of 8). Moreover, the EMS group had a substantial number of horses in August (4 of 7), in September (3 of 8), and in October (3 of 9) with ACTH concentrations above the cutoff for Cushing's disease (70 pg/mL), whereas no more than 2 of 7 control horses had such high ACTH concentrations in August and in October. The proportion of horses with plasma ACTH concentrations above the 70 pg/mL cutoff was not statistically different between control and EMS horses (Fisher's exact test,  $P > .05$ ).



**Fig 1.** Serum or plasma concentrations of metabolic hormones and glucose during different months of the year in control (healthy) or equine metabolic syndrome (EMS) horses. Monthly sample sizes for control horses were 3, 7, 7, 8, 1, 7, 2, 7, 7, 7, 3, and 7, and for EMS horses were 5, 8, 9, 7, 3, 8, 4, 7, 8, 9, 2, and 8, from January to December, respectively. However, only months with 3 or more horses in a group were included in the statistical analyses and plotted here. Shaded rectangles indicate laboratory reference ranges. (A) Plasma adrenocorticotropic hormone (ACTH) concentrations, (B) plasma cortisol concentrations, (C) serum glucose concentrations, (D) plasma insulin concentrations, and (E) serum thyroxine concentrations. *Note:* One control and 4 EMS horses were treated with levothyroxine and were excluded from the study, leaving neither group with 3 or more animals in January.

### Cortisol

Mean plasma cortisol concentration was within the reference range throughout most months in control and

EMS horses with no effect of group or month (Fig 1B). One horse with EMS had an unusually high cortisol concentration in May (10.2  $\mu\text{g}/\text{dL}$ ), which raised the mean

for the group just above the upper limit of the reference range (6.0 µg/dL) and explains the large variance.

### Glucose

Mean serum glucose concentration was uniformly within the reference range throughout all months in control and EMS horses; however, there was a significant effect of group, with EMS horses ( $102.2 \pm 2.5$  mg/dL) having a significantly greater mean glucose than control horses ( $93.7 \pm 2.9$  mg/dL) ( $P < .001$ ) (Fig 1C).

### Insulin

Mean plasma insulin concentration showed significant effects of both group ( $P < .0001$ ) and month ( $P < .03$ ) but no group-month interaction ( $P = .5$ ). EMS horses had mean insulin values significantly greater than normal horses and often above the reference range. This was to be expected, as one of the inclusion criteria for EMS horses was an increased insulin concentration at the time of the initial evaluation. Mean plasma insulin concentration was uniformly within the reference range throughout all months in control horses (Fig 1D). Insulin concentrations varied significantly among months, but no pattern was apparent and no single month was consistently different from most of the others (Fig 1D).

### T4

Mean serum T4 concentration was consistently within the reference range throughout most months in control and EMS horses (Fig 1E), but the T4 concentration for EMS horses in September was just below the lower limit of the reference range (0.85–2.40 µg/dL). There was no effect of group or any group-month interaction. However, there was an effect of month where March showed significantly higher serum T4 values than the other months except for November and December.

## Discussion

The results of the present study demonstrate that plasma ACTH concentration is affected by season, with increased concentrations observed in healthy horses during August to October inclusive. Horses with EMS showed similar seasonal elevations in ACTH, but the elevations were not substantially different from controls. It is now necessary to consider the implementation of seasonal reference ranges for equine ACTH,<sup>2</sup> however, larger sample sizes will be needed. At the very least, clinicians must interpret a high ACTH concentration in autumn with caution unless an appropriate reference range is available.

Serum insulin concentration showed considerable variability between individuals in the EMS group and from month-to-month within individuals. However, insulin did not show similar seasonal variation as ACTH. The seasonal undulations in plasma insulin concentration in EMS horses are difficult to interpret because individuals were first diagnosed and initiated treatment at different times of year. Moreover, differences in com-

pliance with management recommendations regarding feed and exercise probably contributed to the high degree of variation within and between months. Because samples were drawn at the farm/stable, tight controls on diet and stress-related factors were difficult to implement, which may have contributed to insulin variability. The absence of tight controls on exercise and diet may explain why insulin concentrations for individual EMS horses might or might not remain above the reference range and why body mass did not decline significantly.<sup>5</sup>

There were no seasonal variations in plasma cortisol or serum glucose concentrations. However, our samples were drawn at one time of day, and the effects of season and group may have been detected if multiple samples were collected at different times of day.<sup>10</sup> Overall serum glucose was generally higher in horses with EMS that were hyperinsulinemic than in normal horses, despite being within the normal reference range. This might reflect reduced insulin sensitivity in EMS horses in combination with a failure of hyperinsulinemia to adequately compensate.

Mean serum T4 concentrations for EMS horses were at or just below the lower limit of the reference range in August and September. Therefore, additional studies are warranted to determine if serum T4 concentrations can be suppressed in EMS horses during autumn. This is especially important because supplemental levothyroxine is being considered and used in the treatment of EMS.<sup>9</sup>

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## Footnotes

<sup>a</sup> Immulite, Diagnostic Products Corporation, Los Angeles, CA

<sup>b</sup> Diagnostic Systems Laboratories Inc., Webster, TX

<sup>c</sup> Roche Diagnostics Corporation, Indianapolis, IN

<sup>d</sup> SAS version 9.1, SAS Institute, Cary, NC

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