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Equine Cushing's Disease / Equine Metabolic Syndrome: A Practitioner Field Study.

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Introduction

Endocrinopathic laminitis associated with equine Cushing's disease (ECD) or equine metabolic syndrome (EMS) is commonly seen by veterinary practitioners. The ability to recognize the different forms of this condition affects the management protocol and ultimate success of treating laminitis. Of importance to owners and veterinarians is the ability to predict, monitor and manage the development of laminitis associated with both conditions, yet, to

date, there has been very little evidence-based medicine to assist them. Trying to explain how insulin resistance plays a role in laminitis, while little is known about how horses develop the condition, is challenging. Why some horses with ECD develop laminitis, while others do not, is a mystery.

ECD (pituitary pars intermedia dysfunction) is a syndrome of pituitary hyperadrenocorticism caused by dysfunction of the pars intermedia of the pituitary.[1] While the pars intermedia is not usually responsible for the majority of 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 due to muscle catabolism and bulging supraorbital fat), polyuria and polydipsia, susceptibility to infections and laminitis.[1]

The term equine metabolic syndrome was first used by Johnson in 2002.[2] 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 concentrations that characterize horses with ECD.[3]

Donaldson et al.[4] reported that circulating ACTH concentrations and responses to dexamethasone suppression tests in normal horses differed when sampled in January than in September. Thus, we investigated whether seasonal differences in hormone concentrations also existed in EMS and ECD horses. By including a cohort of normal horses in our regular sampling regimen, we extended the findings of Donaldson et al. and determined if circulating concentrations of glucose and several metabolic hormones fluctuated month by month over the course of a year.

The blood glucose and hormone concentrations of groups of horses within a single practice were studied over a 2_-year period. The study consisted of normal, EMS and ECD horses that were observed and sampled at their farm/stable by equine practitioners as part of their routine practice. The study design had the advantage of maintaining study animals in settings to which they were accustomed, thus avoiding the potential for transport-induced modulation of hormone levels. They were classified as horses with 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.

Objectives

1) Determine if hormone concentration correlated to the severity of clinical laminitis.

2) Determine how rapidly Pergolide treatment reduced circulating hormone levels, especially adrenocorticotropin (ACTH), in horses affected by ECD. When did clinical signs improve relative to the reduction of circulating ACTH concentrations?

3) Assess the diagnostic value of measuring morning and afternoon plasma cortisol concentrations in suspected cases of ECD.

4) Determine if there were seasonal variations in hormone concentrations in Pergolide-treated horses with ECD and EMS.

5. Determine if there were seasonal variations in hormone concentrations in normal, healthy horses.

Methods and materials

Twenty-three horses and two ponies were recruited from the practice area of Homestead Veterinary Hospital in Pacific, Mo., near St. Louis, during the period from December 2004 through May 2007. The horses were all located within a 60-mile radius of the practice. Based on clinical findings and initial diagnostic tests, animals were assigned to one of three groups: ECD (four horses and two ponies), EMS (10 horses) and controls (nine horses).

Plasma values for ACTH, cortisol and insulin and serum values for glucose and total thyroxine (T4) were evaluated over a 12-month period.

Inclusion criteria

Horses with laminitis at the time of the initial visit or with a history of endocrinopathic laminitis were selected for the study.

From an initial analysis, horses were grouped as having ECD or EMS, or as normal, according to the following parameters:

ECD — ACTH concentration greater than 70 pg/mL (normal 9 to 35 pg/mL).
EMS — Insulin concentration greater than 70 mlU/ml (normal ACTH).
Normal — 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 plasma ACTH concentrations between 30 and 70 pg/mL were excluded to minimize the possibility that elevated ACTH was due to conditions other than ECD, such as stress or laminitis pain.

Clinical data collection

After horses were placed in the study, morning and afternoon samples of blood were obtained. Single samples of the blood were taken again at one week, 2 weeks, 3 weeks, one month, 2 months, 4 months, 6 months, 8 months, 10 months and 12 months. Plasma values for ACTH, cortisol and insulin and serum values for glucose and total thyroxine (T4) were obtained.

Weight, physical condition, body score and soundness were evaluated at each visit. Weights were estimated using a weight tape formula (weight equals heart girth times heart girth, times body length, divided by 330).[5] The presence of a cresty neck was noted and lameness was ranked using the Obel grading system.[6]

Horses with elevations of ACTH greater than 70 pg/mL (ECD horses) were treated with 1mg 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. Since all of the EMS horses were considered overweight, a 20 percent reduction in caloric intake was recommended along with exercise. 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.

ACTH and cortisol were measured by automated chemiluminescent enzyme immunoassays (Immulite®, Diagnostic Products Corporation, Los Angeles, CA) previously validated for horses.[7,8]

Insulin was measured by a double antibody radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX) previously validated for horses.[9]

Thyroxine was measured by a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) previously validated for horses.[10]

Glucose was measured by the hexokinase method using a Roche Hitachi 917 Chemical Analyzer (Roche Diagnostics Corporation, Indianapolis, IN).

Analyses

Morning and afternoon plasma cortisol values from the first visit were compared and differences in cortisol levels calculated between groups (control horses vs. ECD vs. EMS).

Descriptive statistics were used to describe the baseline differences between groups (age, breed, sex, bodyweight) and non-parametric tests (Kruskal-Wallis) used to describe differences between groups.

Correlations between insulin and laminitis score were made using the Pearson correlation test on both baseline time points and any time point throughout the study. The correlation between T4 and insulin also was examined.

The maximal change in insulin over time was compared to bodyweight change and laminitis score throughout the trial in the EMS group using the Pearson correlation test, and, where significant, the sum of squares (r2) also was calculated.

Changes in insulin, ACTH, T4 and bodyweight over time for horses in each group were examined using one-way ANOVA.

The effect of season on ACTH was examined using one-way ANOVA. Where significant differences were found, post hoc analyses using Tukey's and Fishers post hoc analysis were used.

Results

Twenty-three horses (over 14-2 hands) and two ponies (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 the 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 crossbred, 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 yrs.; ECD = 28.5 ± 8.14 yrs.; and EMS horses = 15.5 ± 5.11 yrs.

Mean bodyweight was significantly lower ($P<0.01$) in horses with ECD (805 ± 85 lb) versus controls (1145 ± 26 lb) and EMS (1111 ± 30 lb). However, the ECD group contained the two small ponies. During the study, there was no change in bodyweight in the control group. There was a trend for a decrease in body weight for the EMS group and a trend for an increase in bodyweight for the ECD group. However, the latter trend was partly due to the death of the two ponies after the first two 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 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 pmol/L). In ECD horses treated with Pergolide, there was a decreasing ACTH trend (Fig 2). However, the two ponies in the ECD group died after two months, and a complete dataset was available for only three Pergolide-treated horses.

Mean baseline insulin concentration was not different between EMS and ECD horses (53.0 ± 16.3 and 52.0 ±21.6 mlU/ml respectively), but together they were significantly greater ($P<0.05$) than controls (18.7 ± 3.8 mlU/ml). There

was no difference between baseline T4 within the group (control 1.3 ± 0.1; EMS 1.6 ± 0.2; ECD 1.0 ± 0.3 ug/dL). Both insulin and T4 did not change significantly over time.

The mean baseline cortisol level for ECD and EMS horses was higher (4.03 ± .99 and 4.49 ± 1.53 ug/dL respectively) than the control horses (2.58 ± 1.39 ug/dL). The cortisol values from the morning and afternoon baseline samples showed no significant difference in the mean percent diurnal variation. The mean percentage difference was: control horses, 22.97± 20.58 percent; EMS horses, 29.16 ± 15.79 percent; and ECD horses, 11.45 ± 18.92 percent.

The mean baseline value for blood glucose was: ECD = 115 ± 107.7 mg/dL; EMS horses = 95 ± 9.8 mg/dL; and controls = 93.5 ± 8.11 mg/dL.

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 mlU/ml. Of the ECD horses, four entered the study sound with a history of laminitis with ACTH above 70 pg/mL. Two of the ECD horses entered the study with current laminitis and elevated ACTH.

There was a significant correlation between laminitis grade and baseline insulin concentration ($r = 0.63$, $P<0.001$) as well as between laminitis grade and insulin at any point throughout the study ($r = 0.62$, $P<0.001$) (Fig 3).

When only the ECD and EMS horses were examined, there also was a significant correlation between laminitis grade and baseline insulin ($r = 0.57$, P 0.02).

There was a weak negative correlation between baseline T4 and insulin concentration ($r = -0.22$, $P<0.001$) (Fig 4).

The effect of diet and exercise on EMS horses was assessed by comparing the maximal change in plasma insulin concentration to 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 ± 128.5 mIU/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. While the standard deviations of individual horse response show great variation, there was a good correlation between the change in insulin concentration and change in laminitis grade ($r = 0.69$) ($P<0.05$). The linear line of best fit had an R2 of 0.48 (Fig 5).

When ACTH concentration was examined over time, there was a significant ($P<0.01$) effect of month. This effect was significant in each group, and when all the groups were pooled. Post hoc analysis of seasonal variation showed that ACTH was significantly greatest in September, with elevations also evident in both August and October. Control horses showed the same overall pattern as the whole group combined, but, interestingly, EMS horses showed an earlier peak in ACTH concentration in August, and ECD horses had a significant difference in ACTH concentration only in September (Fig 6).

Discussion

The main finding is that insulin concentrations are correlated quite strongly to laminitis grade.

Weight loss in EMS horses did not result in significantly lower insulin, despite an overall trend for this outcome. This is likely due to large variations in

individual horses and the inability of horses with laminitis to exercise. Greater weight loss may have occurred if dietary controls were more rigorous. Nevertheless, decreasing the plasma insulin concentration of EMS horses appeared to reduce the severity of laminitis.

The correlation between insulin resistance and laminitis was first recognized by Jeffcott in 1986.[11] Since then, the association of insulin resistance with endocrinopathic laminitis has been documented by numerous authors.[12,13,14,15]

The study shows that the cresty neck in all of the EMS horses and half of the ECD horses serves as a phenotypic indicator that is associated with insulin resistance and the tendency to develop laminitis.[16]

The onset of laminitis was associated with hyperinsulinaemia (plasma insulin > 100 mlU/m; normal range = 8 to 30 mlU/ml). The correlation 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 due to hyperinsulinaemia results in considerable histopathological damage: the tips of secondary epidermal laminae are elongated and tapered and the laminar basement membrane disintegrates.[17]

It is predicted that additive pathology and worsening clinical laminitis would result with the increasing chronicity of hyperinsulinaemia. A better clinical outcome is likely if hyperinsulinaemia is permanently reversed as early as possible.

The cause of insulin resistance in horses has not been explained.[15] In people, it is caused by a failure of glucose to enter cells by way of the insulin sensitive Glut 4 transport protein.[18] The importance of glucose to the integrity of hoof epidermal laminae was reported by Pass et al.[19] They showed a lack of in vitro glucose caused separation of hoof laminae and suggested that a failure of glucose supply could result in laminitis in insulin resistant horses. Inadequate laminar glucose delivery because of failure of insulin sensitive Glut 4 transport could cause laminitis by creating conditions similar to the *in vitro* model of Pass et al.

Recently Asplin et al. showed glucose uptake in hoof laminae was insulin independent. Glut 1 receptors predominate in hoof laminae and require no insulin to transfer glucose into the cells.[20] In people with type 2 diabetes, glucotoxic microvascular lesions develop, presumably because insulin resistance leads to chronic hyperglycemia and hyperlipemia. It has been speculated that similar microvascular lesions may explain how laminitis develops in the horse.[2] In insulin resistant, type 2 diabetic, humans, pancreatic beta cell production of insulin eventually fails.[21] Interestingly, beta cell production of insulin in horses does not fail and hyperinsulinaemia persists. However, as shown by this study, hyperinsulinaemia is not concurrent with hyperglycemia, as it is with people, and in horses a link between hyperglycemia and laminitis does not seem plausible. Only one of the ECD ponies in this study had significant hyperglycemia, hyperinsulinaemia and laminitis, and a link between hyperglycemia and laminitis could not be made. In the EMS group, four out of 10 horses had mild elevations of blood glucose associated with the onset of hyperinsulinaemic laminitis. Laminitis occurs within 3 days when hyperinsulinaemia is experimentally induced in normoglycemic ponies.[17] This new information establishes a direct pathway between insulin and laminitis. How insulin initiates laminitis without causing overt pathology elsewhere in the

body remains a mystery and is the subject of further study at The University of Queensland, Australia.

There was an inverse relationship between insulin and T4 that may explain why horses with insulin resistance and hyperinsulinaemia have low thyroid levels with normal thyroid function, as reported by Frank.[22] Frank also reported that giving levothyroxine sodium reduced insulin resistance and resulted in weight loss in normal horses.[22] 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.[23]

Weight loss in EMS horses did not result in a significant decrease in plasma insulin. However, a reduction in insulin and loss of weight was observed in a number of horses that also improved in their laminitis grade.

The diagnostic value of measuring morning and afternoon plasma cortisol concentrations in suspected cases of ECD is uncertain from our results since 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 due to the variation between horses with ECD.

After treatment with Pergolide, the ACTH concentration of the ECD group significantly decreased within one week (Fig 2). A modest number of horses were included in this part of the study, but 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.

The seasonal elevation of ACTH in all groups in August and September adds additional information that is significant in testing horses for ECD. Donaldson first reported elevations in ACTH in normal horses and ponies during September in 2004.[4] The results of the present study indicate that using the ACTH concentrations in August or September may not differentiate ECD horses from normal horses. Homestead Veterinary Hospital has put the findings of Donaldson et al.[4] and the present study into practice. If ACTH levels are elevated in August or September in horses with laminitis, the hospital will start the horse on Pergolide for three months and then retest the ACTH level after withdrawal of the drug for 14 days. It is interesting that ECD horses on Pergolide had a significant increase in ACTH in September. Yet, no laminitis occurred and insulin levels remained in the normal range.

The physiologic role of elevated ACTH concentrations in the fall is interesting. Presumably, activation of hypothalamic, dopaminergic inhibition results in increased appetite, weight gain, increased fat in the body and thickening of the hair coat.[4] These changes prepare the horse for survival in the coming winter. However, in domestic horses, food supplies are not limited and are often increased by well-meaning owners, raising the possibility of a laminitic episode. Laminitis is seen so frequently that it often is referred to as "winter laminitis" in these horses. Perhaps these individuals were meant to have a period of reduced caloric intake during the winter, bringing them into spring in a thin condition with reduced insulin resistance. This would enable them to ingest large amounts of high-sugar spring grass without developing laminitis.

The correlation between increased levels of insulin and an increase in the laminitis score reported in the present study warrants monitoring of insulin levels in horses that have cresty necks and are prone to develop endocrinopathic laminitis. The usefulness of insulin as a prognostic factor in ECD has been reported by McGowan et al.[14] Samples should be obtained for insulin determination when horses have not eaten any grain or soluble carbohydrates for two to three hours. Following this protocol, a single blood sample showing hyperinsulinaemia enables the practitioner to predict that laminitis will occur or become worse. Efforts to reduce insulin resistance using a low carbohydrate diet, exercise and possibly thyroid supplements may prove effective in preventing hyperinsulinaemia and, therefore, laminitis. Drugs that treat type 2 diabetes in humans by increasing insulin sensitivity may prove to be helpful to the horse. 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.

Endocrinopathic laminitis is challenging for veterinary practitioners to treat and prevent, and more research is needed to better understand its pathophysiology.

This study contributes new knowledge to veterinary medicine by showing a positive correlation between hyperinsulinaemia (insulin resistance) and laminitis severity. Practitioners now can institute weight loss and exercise programs for the horses and ponies of their clients confident that decreases in plasma insulin should ameliorate clinical laminitis.

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Figure 1: Bodyweight changes over time for the 3 groups of horses, control, EMS and ECD. There is a trend for an increase in bodyweight for ECD horses and a decrease for EMS horses.

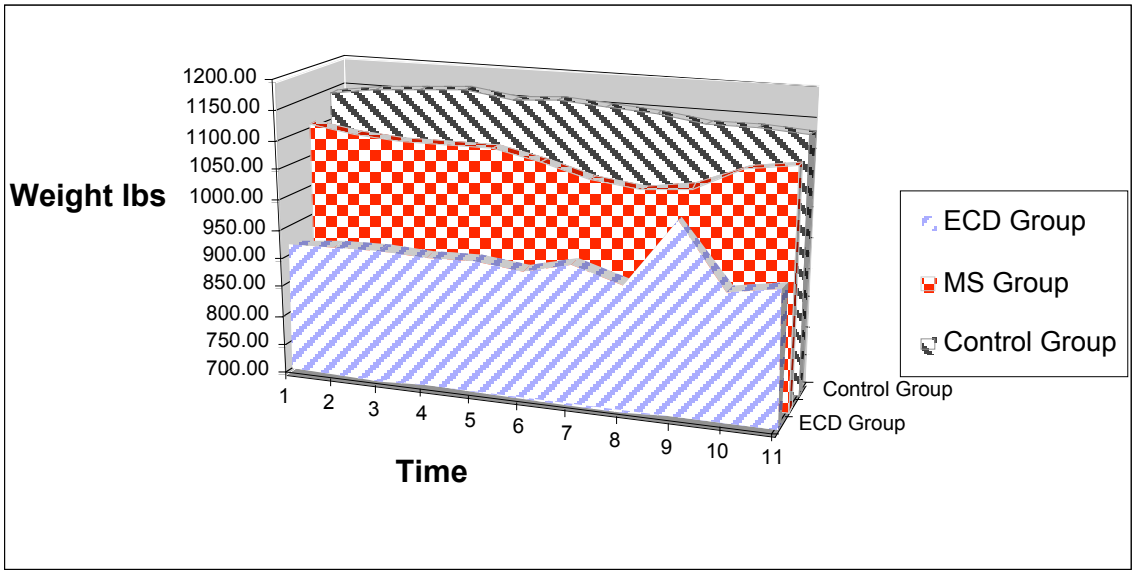

Figure 2: ACTH concentration (pg/ml) over time in ECD horses that underwent treatment with Pergolide 1mg per day (n=3). Pergolide treatment tended to decrease plasma ACTH concentrations.

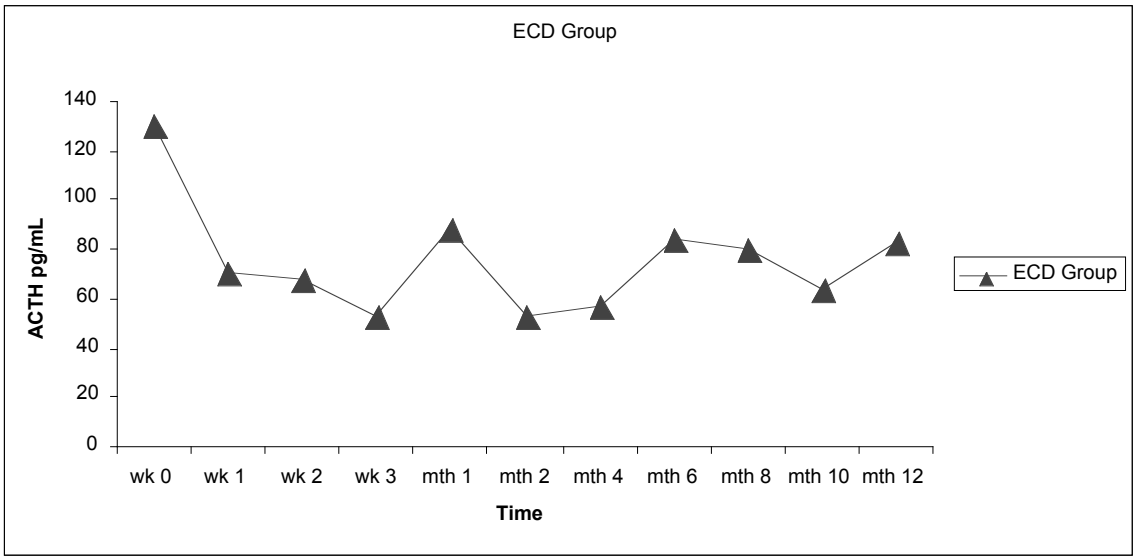

Figure 3: Scatterplot and line of best fit for correlation between plasma insulin concentration and Obel grade of laminitis in all horses at all time points (r = 0.62, P<0.001). Hyperinsulinaemia was associated with increased laminitis severity.

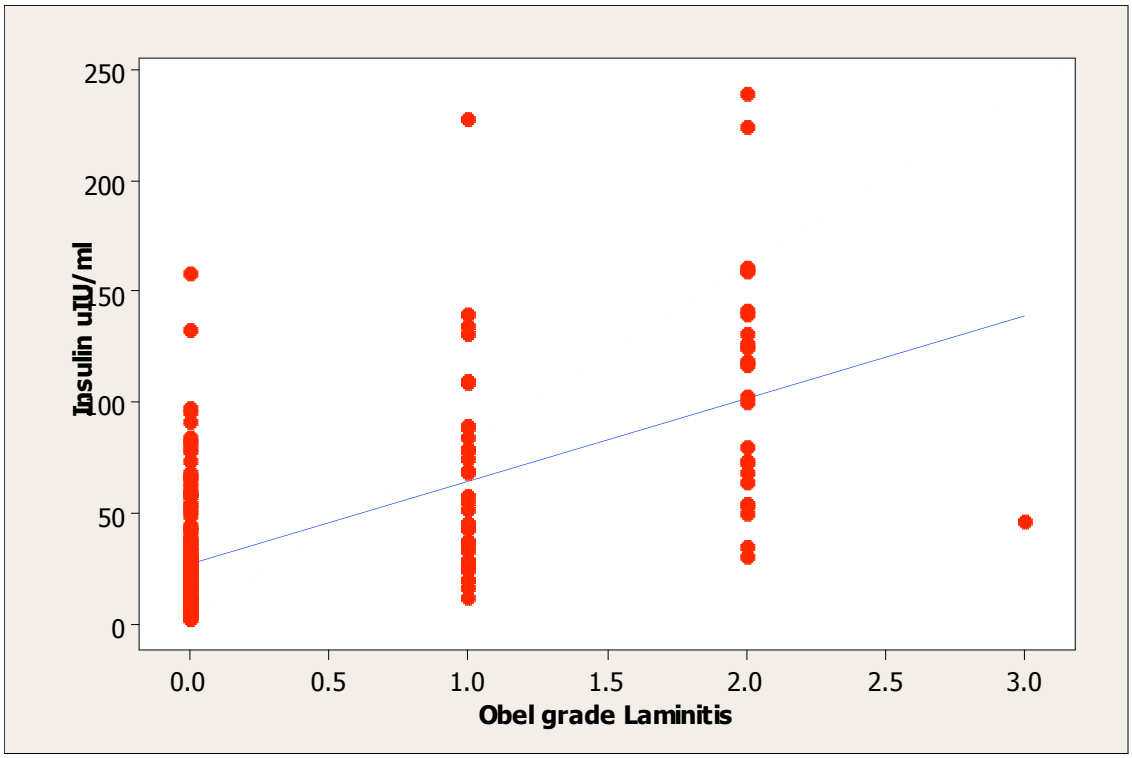

Figure 4: Correlation between T4 (ug/dl) and plasma insulin concentration was inverse.

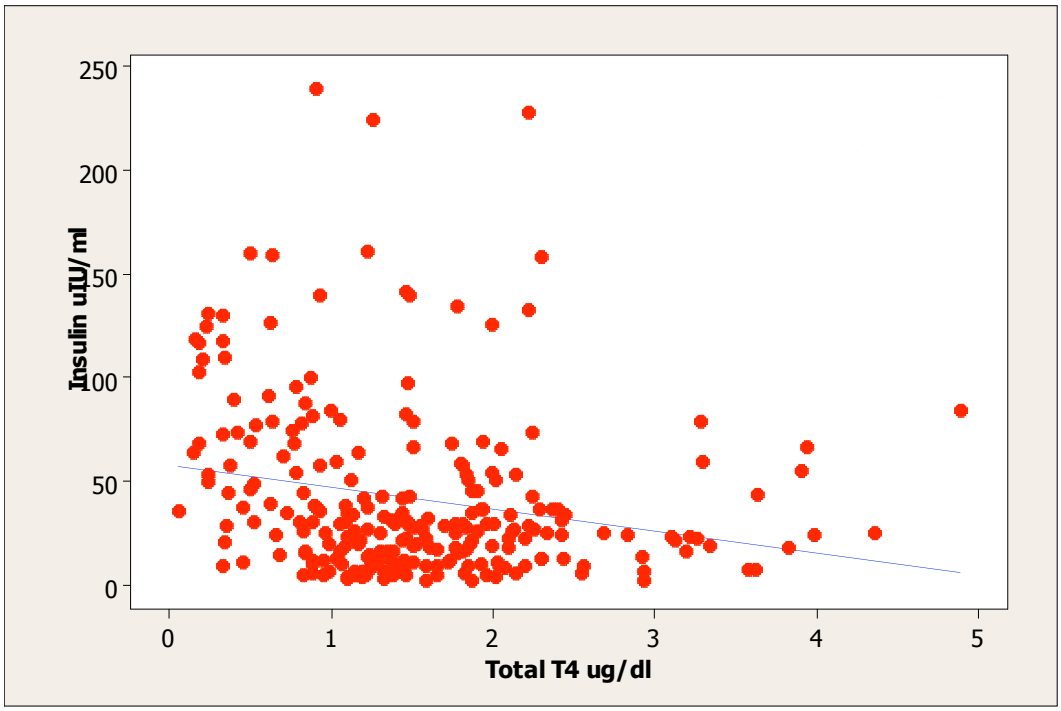

Figure 5: Correlation between the maximal change in insulin and laminitis grade in EMS horses. Correlation coefficient r = 0.79, r^2 = 0.48, P<0.05.

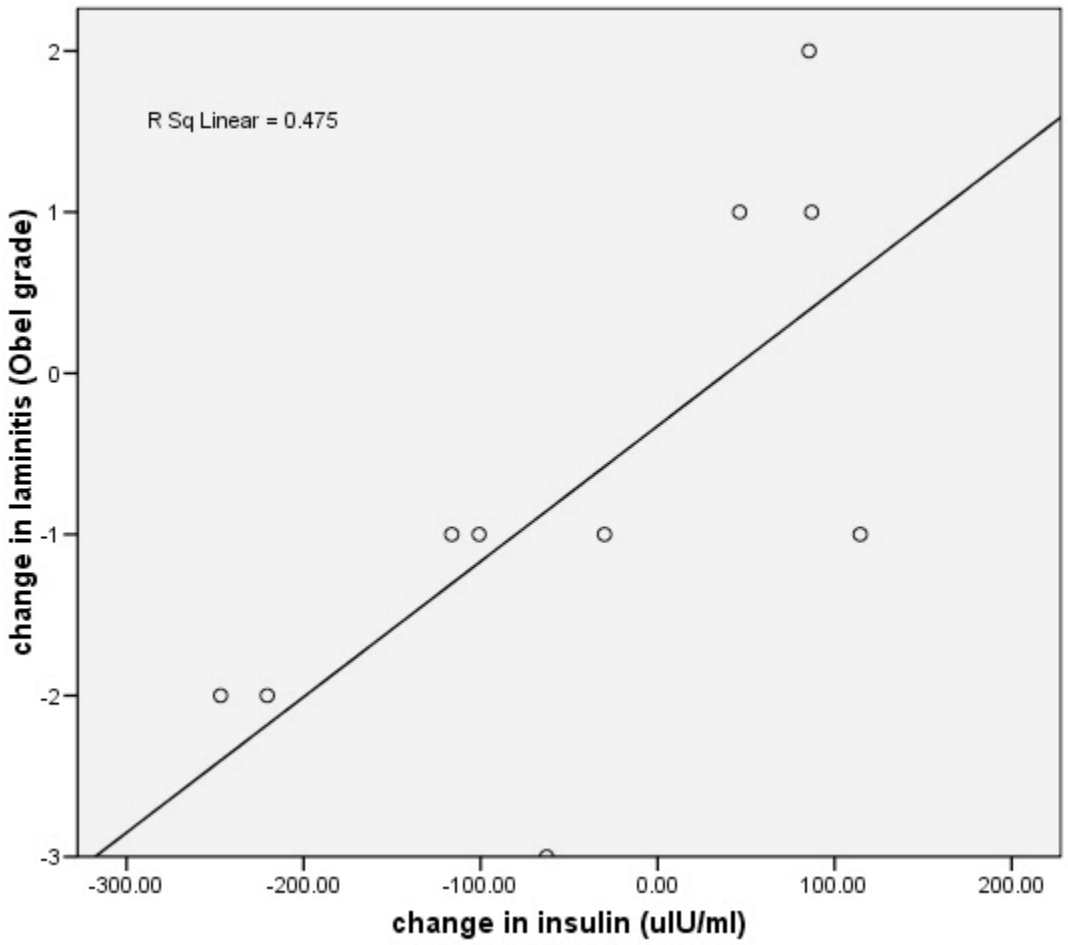

Figure 6: Seasonal variations in ACTH concentration (pg/ml) for the 3 horse groups (Control, EMS and ECD). Monthly data irrespective of year are shown pooled. ACTH concentrations were highest in August, September and October.