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Factors associated with PPID identified during a nationwide PPID awareness initiative

Ireland, J.L.¹, Rendle, D.I.², Durham, A.E.², Turnbull, C.³ and Barrett, E.J.³

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Laboratory assays were performed at the Liphook Equine Hospital Laboratory, and data analyses were performed at the Animal Health Trust. All samples analysed in this study were obtained in line with recognised veterinary practice as defined in The Animals (Scientific Procedures) Act 1986, therefore a Home Office license was not required.

This abstract has not been presented or published previously.

Aims

The aim of this study was to determine factors associated with PPID in a population of horses, ponies and donkeys aged >8 years, during a nationwide laminitis disease awareness campaign in the UK.

Methods

Retrospective analysis of laboratory submissions during a nationwide disease awareness initiative (“Talk about Laminitis”) was undertaken. As treatment status was not available for all animals, only the first laboratory assay was included for individuals with multiple samples. PPID was defined based on ACTH concentration above seasonally adjusted reference ranges (>29pg/ml in non-autumn months and >47pg/ml during autumn). Factors associated with PPID were assessed using logistic regression analysis. The critical probability for descriptive analyses was set at 0.002, following Sidak-Bonferroni correction for multiple comparisons, and 0.05 for logistic regression analyses.

Results

In total, 15,109 unique samples were identified between 30th July 2012 and 30th November 2013. Overall, 58.0% of animals (95% CI 57.2-58.8%) were PPID-positive, and these animals were older (median age 20 years; IQ 17-24 years) compared to PPID-negative animals (median 17 years; IQ 14-20 years) (p<0.001). Median ACTH concentrations were greater in animals exhibiting greater numbers of clinical signs consistent with PPID (p<0.001). Significantly greater proportions of ponies (63.0%; 95% CI 61.8-64.2%) and donkeys (74.2%; 95% CI 65.3-83.1%) were PPID-positive compared to horses (52.1%; 95% CI 50.5-53.6%) (p<0.001).

Median ACTH was greater for animals reported to have current and/or historical laminitis, hypertrichosis, muscle wastage or supraorbital fat deposits (all p<0.001). However, where current and/or historical laminitis was the only clinical sign reported, median ACTH was lower (p<0.001). Median age was greater for animals reported to have hypertrichosis, muscle wastage or recurrent infections (all p<0.001), while median age was lower for animals reported to have no clinical signs, current and/or historical laminitis or polyuria and/or polydyssia (all p<0.001).

On univariable logistic regression, several factors were associated with increased odds of PPID, including season (summer OR 1.33, autumn OR 4.06 and winter OR 2.82, compared to spring), age (OR 1.13), type (pony OR 1.57, donkey OR 2.65, compared to horses), and breed (Arab/Arab cross OR 1.47, unspecified pony breeds OR 1.45, Welsh/Welsh cross OR 1.22, compared to Thoroughbred/Thoroughbred cross) (all p<0.001). Increasing number of clinical signs reported was also associated with increased odds of PPID (OR 1.23, p<0.001). Specific clinical signs associated with increased odds of PPID were hypertrichosis (OR 2.02), supraorbital fat deposits (OR 1.61), muscle wastage (OR 1.26) and current and/or historical laminitis (OR 1.14) (all p<0.001).
Conclusions
Over half the animals sampled during a two-year disease awareness initiative were PPID-positive. Identification of factors and clinical signs associated with increased risk of PPID will be relevant to practising veterinary surgeons.

Acknowledgements
This study was funded by Boehringer Ingelheim. We gratefully acknowledge all participating veterinary practices.
Pathology of natural cases of equine endocrinopathic laminitis: evidence for a chronic preclinical phase with abaxial localization in the hoof wall

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The research was performed at The Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland. The experimental protocol of the present study was approved by the Research Ethics Committee of the University of Helsinki, Finland.

Aims
To describe the histomorphometry and pathological lesions in hoof lamellar tissue of horses and ponies with naturally occurring endocrinopathic laminitis.

Methods
Macroscopic and microscopic lamellar morphology and pathology were described in 14 laminitic ponies and horses that had elevated basal insulin (>20 mIU/L) with reference to 25 age- and breed-matched controls.

Results
The type and severity of lesions noted had no correlation with reported clinical duration, and in at least some cases must have preceded it. Lesions were largely localized abaxially within the lamellar tissue and included apoptotic cell death, as well as lamellar fusion, hyperplasia, and partial replacement with poor quality keratin containing nucleated debris and proteinaceous lakes. The lesions resulted in irregular margins between the inner horn and the lamellar tissue. Acute tearing originated from abaxial region, with minimal associated inflammation. Axially epidermal lamellar tapering was the most frequent morphological observation. The pathology was similar to that described in some models of inflammatory laminitis, and contained features seen in developmental phases of hyperinsulinemic models. These findings support the theory that repeated episodes of subclinical laminitis occur prior to clinical presentation. Additionally, the pathology does not include extensive basement membrane failure seen in some inflammatory models.

Conclusions
This study provides a guide of normal and abnormal morphological variation of the equine hoof lamellae. All cases of endocrinopathic laminitis, regardless of their clinical duration, showed chronic pathology in the abaxial region of the hoof wall, that in all but one horse was correlated with divergent hoof rings. Identification of divergent hoof rings and appropriate endocrine testing before clinical laminitis occurs may offer one possibility for disease prevention.

Acknowledgements
The authors would like to thank Lynn Stevenson and Lynn Oxford from Veterinary Diagnostic Services of University of Glasgow for developing the histological techniques.
Alterations in peripheral cortisol metabolism in horses with metabolic syndrome or pituitary pars intermedia dysfunction

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This study was approved by the University of Edinburgh Ethics committee.

Introduction and Aims

Activation of the hypothalamic-pituitary-adrenal (HPA) axis and altered tissue glucocorticoid action in obesity and metabolic syndrome has been attributed to altered peripheral cortisol metabolism. In human obesity, cortisol clearance is increased with up-regulation of A-ring reductases and down-regulation of cortisol-regenerating 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in liver. The aim of this study was to determine whether abnormalities of glucocorticoid clearance occur in equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID).

Methods

Healthy horses, horses with EMS and horses with PPID were recruited from those attending the clinic of the Royal (Dick) School of Veterinary Studies for euthanasia, with approval from the University of Edinburgh Ethics Committee. Females and castrated males were recruited, reflecting the clinical population in the UK. Each horse underwent a clinical examination and blood was obtained, between 0900h and 1100h, after overnight fasting and following placement of an intravenous cannula for euthanasia. The age, breed, sex, body condition score (out of 5), clinical features of previous laminitis, crest neck score and medical history (specifically history of laminitis and glucocorticoid administration) were recorded. ACTH, cortisol and insulin concentrations were measured and routine haematological and biochemical analyses were carried out.

Samples of urine were taken at post mortem snap frozen and stored at -80°C. Glucocorticoid metabolites were extracted by solid-phase extraction using Bond Elut Nexus cartridges. The extracted steroids were derivatized and then quantified by GC-MS/MS and corrected for creatinine concentration. The ratio (α-THF + THF)/THE was used as an index of 11β-HSD1 activity. 5βTHF /5αTHF and Ulick’s A-ring reduction quotients 5α-THF/F and 5βTHF /F were used as indices of the contributions of 5α- and 5β-reductases. Statistical analysis was performed using Graphpad Prism 4 and SPSS statistics 19. Data from the three groups were analysed using a one-way ANOVA and Bonferroni post-hoc test if normally distributed, or a Kruskal-Wallis and Dunn’s post-hoc test if not normally distributed.

Results

EMS cases had higher body condition scores (P<0.0001) and plasma insulin concentrations (P=0.002) compared to healthy horses or those with PPID. Horses with PPID were older (P=0.01) and had marked elevations in serum ACTH (P=0.0001) but were not obese compared to healthy horses or those with EMS. Plasma cortisol did not differ between the groups. 20β-dihydrocortisol was the most abundant urinary cortisol metabolite in horses. A-ring reduction accounted for less than 20% of urinary cortisol metabolites. Total urinary cortisol metabolite excretion was greater...
in horses with EMS and PPID compared to healthy controls though there were no differences in the metabolite composition. The calculated urinary metabolite ratios ((α-THF + THF)/THE, 5βTHF /5αTHF, 5α-THF/F and 5βTHF /F) did not differ between the three groups.

Conclusions

Unlike humans and rodents, in which A-ring reduction is the main route of cortisol metabolism, the predominant pathway in horses is 20β-oxoreductase with production of 20β-dihydrocortisol. EMS, like PPID, is associated with increased cortisol clearance indicating activation of the HPA axis in this disease. Alterations in peripheral cortisol metabolism may contribute to the pathogenesis of EMS.

Acknowledgements

The authors would like to acknowledge the BBSRC and Zoetis who fund Ruth Morgan’s CASE studentship.
Differences in the Myostatin system between lean and obese Equidae
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Studies were conducted at the University of Liverpool and approved by the University Veterinary Research Ethics Committee. Data have not been presented previously.

Introduction
Skeletal muscle and adipose tissue act in synergy to promote energy balance through the mediation of cross-talking pathways. Cross-talk is achieved through the respective secretion of myokines and adipokines from skeletal muscle and adipose tissue. The recent epidemic in equine obesity has generated a need to identify obesity-prone phenotypes and to explore how cross-talking pathways may be altered in the obese state. The myokine, Myostatin, has been rigorously characterised as a negative-regulator of skeletal muscle mass. Myostatin has been implicated in the development of obesity and corresponding changes in adipogenesis as well as glucose metabolism in other mammalian species.

Aims
To characterise the gene expression of Myostatin and its receptor, activin type IIB (ActRIIB) in skeletal muscles plus adipose tissues, and to evaluate serum myostatin concentrations in lean and obese Equidae.

Methods: Samples of five adipose tissue depots (epicardial, omental, crest, tailhead, and ventro-abdominal retroperitoneal) and four skeletal muscles (Rectus abdominus, Longus colli, Pectoralis profundus, and Pectoralis transversus) were obtained post-mortem from 12, mixed-breed horses and ponies (lean, BCS < 4, n = 6; obese, BCS ≥ 7, n = 6), presented for euthanasia at a commercial abattoir for non-research purposes. Blood samples were obtained at exsanguinations and serum was stored at -20°C. Tissues samples were collected within 30 minutes post-mortem, snap-frozen in liquid nitrogen and stored at -80°C pending RNA extraction. Quantitative real-time PCR was conducted to characterise myostatin and ActRIIB gene expression in the skeletal muscles and adipose tissues from all 12 animals, whilst a solid-phase sandwich ELISA kit, validated for use in Equidae, was used to evaluate serum Myostatin concentrations.

Results
Myostatin mRNA expression was significantly (p<0.05) up-regulated in all skeletal muscles from obese animals compared to levels in lean animals. Concomitantly, ActRIIB was significantly (p<0.05) down-regulated in Longus colli, Pectoralis profundus, and Pectoralis transversus in these obese individuals. Levels of Myostatin transcripts were very low in adipose tissues in both lean and obese animals. However, Myostatin mRNA levels were significantly higher in the crest fat of obese animals compared to lean. No differences were observed for ActRIIB mRNA between lean and obese animals in adipose tissues. Serum Myostatin concentrations were significantly (p<0.01) greater in obese (9198.1 pg/ml +/- 948.2 pg/ml) compared to lean (5064 pg/ml +/- 1103.4 pg/ml) animals and strong positive-associations were...
demonstrated between serum Myostatin concentrations and Myostatin mRNA expression in Rectus abdominus (R2 = 0.69), Pectoralis profundus (R2 = 0.77) and Longus colli (R2 = 0.53) and Pectoralis transversus (R2 = 0.37).

**Conclusion**

These data provide the first demonstration of the differences in the Myostatin system between lean and obese Equidae and highlight the associations between Myostatin and ActRIIB expression and secreted Myostatin in equine obesity. Serum Myostatin concentrations could offer a biological marker for the obesity-prone equine phenotype. Further investigations are warranted to evaluate inter-relationships between breeds as well as weight loss responsiveness.
The correlation of CGIT and IVGTT derived parameters with the euglycaemic hyperinsulinemic clamp parameters in equines

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Presenting author: E. de Graaf-Roelfsema (e.roelfsema@uu.nl).

The study was approved by the committee on animal welfare of the faculty of Veterinary Medicine University Utrecht. The abstract has not been presented previously.

Aim

The objectivity of the study was to examine the correlation between two methods of measuring glucose homeostasis – namely, indices derived from the intravenous glucose tolerance test (IVGTT), the combined glucose insulin tolerance test (CGITT)– and the gold standard method, the hyperinsulinaemic-euglycaemic clamp (HEC) test in equines.

Methods

In total six horses and eight ponies (mean age 10.4 ± 4.7 years, weight 348 ± 208 kg, 8 mares, 6 geldings) were studied. Each animal underwent a HEC, an IVGTT and a CGIT in random order. Glucose metabolism differed between individuals from normal to mild insulin resistant. The clamp derived M-value (amount of metabolized glucose mmol/kg.min-1) and M-to-I ratio (a reflection about the amount of glucose metabolized per unit of insulin in plasma at steady state) were calculated for comparison. Correlations were established using Spearman’s rank correlations.

Results

Both the M-value as the M-to-I ratio were well correlated to the AUC glucose (r = -0.77, -0.67 resp.) and plasma insulin concentration at 120 minutes post glucose administration during the IVGTT (r = -0.77, -0.87 resp.) and plasma glucose levels at 45 minutes post glucose and insulin administration during the CGIT (r = -0.70, -0.77 resp.). The M-to-I ratio was also correlated to the plasma insulin concentration at 45 minutes post glucose and insulin administration during the CGIT (r = -0.66) and plasma basal insulin levels after an overnight fast (r = -0.61).

Conclusions

The results of this study suggest that both the IVGTT and CGIT provide several useful parameters making a valid assessment for glucose homeostasis in horses and ponies under field circumstances possible. The IVGTT may represent the best choice showing the strongest correlation in this study.
Comparison of the in-feed glucose tolerance test and the oral sugar test in horses and ponies
NJ Menzies-Gow. The Royal Veterinary College, London, UK (nmenziesgow@rvc.ac.uk).

This study was carried out under Home Office Licence and approved by the Royal Veterinary College Ethics and Welfare Committee. It follows international, national and institutional guidelines for human animal treatment and complies with relevant legislation in the country in which the study was conducted.

Aims
The aim of the study was to compare the insulin response to the in-feed oral glucose test (OGT) and the oral sugar test (OST) in horses and ponies of unknown insulin sensitivity.

Methods
Non-obese animals (8 ponies [7 mares, 1 gelding; mean±sd age 18±4 yrs] and 5 horses [4 mares, 1 gelding; age 13±5 yrs]) were fasted overnight for eight hours before and throughout testing. At 8am animals were either fed a handful of short-chopped fibre with 1g/kg glucose powder (OGT) or given 0.15ml/kg Karo Light Syrup orally (OST) in a randomised crossover study design with 48 hours between treatments. Blood samples were obtained at 0, 30, 60, 75, 90, 120 and 180 minutes. Insulin concentrations were measured using a previously validated radioimmunoassay. Maximum insulin concentration (Cmax), time to maximum insulin concentration (Tmax) and area under the curve of insulin concentration over time (AUC) were compared using a linear mixed effect model. p<0.05 was considered significant.

Results

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<tr>
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<th>OGT</th>
<th>OST</th>
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<tr>
<td></td>
<td>Cmaxᵢ (mU/l)</td>
<td>Tmaxᵢ (minutes)</td>
</tr>
<tr>
<td>Ponies (n=8)</td>
<td>221 ± 9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>142 ± 53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Horses (n=5)</td>
<td>46.65±17&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>126 ± 58</td>
</tr>
<tr>
<td>All (n=13)</td>
<td>154 ± 16</td>
<td>136 ± 52</td>
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<sup>a-h</sup> Results with the same superscript letter were significantly different (p<0.05).

Using previously defined criteria of insulin resistance (IR), the OGT identified 7/8 ponies as IR whereas the OST identified 5/8 ponies as IR. In contrast, both tests suggested that all horses were insulin sensitive.

Conclusions
The OGT and the OST showed agreement in 11/13 (85%) of animals.

Acknowledgements
This study was funded by and carried out in collaboration with WALTHAM Centre for Pet Nutrition.
Relationship between plasma ACTH and leptin concentrations in horses
Diez de Castro E, Cortés B, López I, Aguilera-Tejero E.

Background and Aims
Seasonal changes in plasma ACTH concentrations have been widely described in horses. An increase in plasma leptin during summer has also been reported, but the cause of this seasonality is not known. Leptin, which is produced by fat tissue, is a good marker of adiposity. Consequently, changes in leptin are likely to occur in parallel to seasonal changes in body weight. In other species a relationship between ACTH and leptin has been described. To our knowledge the association between plasma leptin and plasma ACTH has not been previously studied in horses. Thus the aim of this preliminary study was investigate the relationship between plasma ACTH and leptin levels considering the influence of season, BCS and body weight.

Methods
In Experiment 1, plasma concentrations of ACTH and leptin were measured in 18 horses. In Experiment 2, blood was obtained from six horses twice: once in summer and again in spring. Body weight and body condition score (BCS) were recorded at the time of sampling. ACTH was quantified with a sequential immunometric assay (Immulite) and leptin was measured by radio immune assay (Linco Research RIA kits). A correlation study (Pearson test) between ACTH and leptin values was performed. Paired t-tests were used to compare ACTH and leptin concentrations in Summer and Spring. P<0.05 was considered significant.

Results
In Experiment 1, no correlation was found between plasma concentrations of ACTH and leptin (r=0.13, p=0.6). In Experiment 2, both plasma ACTH and leptin were significantly higher in summer, when the horses weighed more and had higher BCS (see Table below). In these horses leptin was not correlated with ACTH within season but was strongly correlated with ACTH when data from both seasons were considered (r=0.70, p=0.01).

<table>
<thead>
<tr>
<th></th>
<th>BCS</th>
<th>BodyWeight (Kg)</th>
<th>Leptin (ng/dL)</th>
<th>ACTH (pg/mL)</th>
</tr>
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<tr>
<td>Summer</td>
<td>5.83±0.3</td>
<td>466.17±16.9</td>
<td>6.16±0.6</td>
<td>17.14±1.8</td>
</tr>
<tr>
<td>Spring</td>
<td>5.08±0.2</td>
<td>438.00±16.5</td>
<td>2.92±0.6</td>
<td>11.70±0.7</td>
</tr>
<tr>
<td>p</td>
<td>0.017</td>
<td>0.001</td>
<td>0.001</td>
<td>0.006</td>
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</table>

Conclusions
Both plasma ACTH and leptin concentrations were elevated in parallel with increases in body weight and BCS. However, since the increase in body weight was linked to season, further studies are needed to determine the independent influence of adiposity and seasonality on ACTH and leptin.
Resting ACTH as an indicator of Pituitary Pars Intermedia Dysfunction (PPID) in Louisiana horses

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Presenting author: Frank Andrews

Aims

Pituitary par intermedia dysfunction (PPID) is common in older horses (≥15 years of age). Diagnosis is based on clinical signs and endocrine testing. Recently, resting adrenocortical tropic hormone (ACTH) and increased ACTH in response to thyroid releasing hormone (TRH) have been shown to accurately diagnose horses with PPID. It has also been suggested that a normal resting ACTH value can be used to rule out PPID. In addition, there have been regional difference described in the accuracy of resting ACTH at predicting PPID. The purpose of this study was to evaluate resting ACTH value as an indicator of disease in horses with suspected PPID in Louisiana and the surrounding region.

Methods

Resting ACTH and ACTH values after a TRH stimulation test were obtained from samples submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) Endocrinology Laboratory at Louisiana State University. These values were evaluated to determine the sensitivity, specificity, and the frequency of horses with ACTH in the normal reference range resulted in an abnormal stimulation test. The normal reference ranges for resting ACTH and ACTH after TRH stimulation were based on recommendations made by the Equine Endocrine Society, which were ACTH of < 36.0 pg/ml and < 110 pg/ml 10 or 30 minutes after TRH administration.

Results

Resting ACTH and/or ACTH after TRH stimulation tests from thirty-seven horses were obtained from the LADDL Endocrinology Laboratory data base. Of the 37 horses, 21 horses had resting ACTH values within reference range (mean ± SD: 22.5 pg/ml ± 7.1 [range 11.3 – 34.4 pg/ml]) and 16 horses had resting ACTH above reference range (133.9 pg/ml ± 159.3 [range 38.0 – 658 pg/ml]). Of the 21 horses with normal resting ACTH values, 8 horses had normal TRH stimulation tests, 9 horses had abnormal TRH stimulation tests and 4 horses did not have a TRH stimulation test performed. Of the 16 horses that had abnormal resting ACTH values, only 8 horses had stimulation tests performed. Based on the TRH stimulation test as an indicator of disease (n=25), a high resting ACTH value had a sensitivity of 47% and a specificity of 100%. Over 50% of the horses with a normal resting ACTH had abnormal TRH stimulation test indicating PPID.

Conclusions

Based on the this small sample size of horses with suspected PPID, horses with a high resting ACTH value were likely to have PPID, whereas only half horses with normal resting ACTH will likely have PPID. When resting ACTH values are within normal limits and PPID is suspected, a TRH stimulation test is indicated to rule out PPID.
Autumnal changes in basal ACTH of horses with borderline (>19 – 40pg/ml) spring ACTH concentrations

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²Liphook Equine Hospital, Liphook, Hampshire, UK
³Boehringer Ingelheim Vetmedica, Bracknell, Berkshire, UK

Laboratory assays were performed at the Liphook Equine Hospital Laboratory, and data analyses were performed at the Animal Health Trust. All samples were obtained in line with recognised veterinary practice therefore a Home Office license was not required.

This abstract has not been presented or published previously.

Aims

The aim of this study was to evaluate change in ACTH for animals with “borderline” ACTH concentrations during spring/summer when re-tested during autumn months.

Methods

Retrospective analysis of laboratory submissions during a 2013 disease awareness initiative (“Talk about Laminitis”) was undertaken. Horses/ponies sampled during spring/summer (16th March – 1st July), with ACTH concentrations between 19.1-40pg/ml, for which subsequent samples were obtained during autumn (9th September – 31st October) were included. Non-parametric tests were used for statistical analyses.

Results

90 horses/ponies (median age 18 years; IQ 15-21 years) were included, of which 20.0% (n=18; 95% CI 11.7-28.3%) were PPID-positive during spring/summer (ACTH >29pg/ml), and median ACTH concentration was 25.3pg/ml (IQ 22.2-28.6pg/ml). In spring/summer, most animals exhibited one (46.7%; n=42; 95% CI 36.3-57.0%) or two (22.2%; n=20; 95% CI 13.6-30.8%) clinical signs consistent with PPID, with a further 26.7% (n=24; 95% CI 17.5-35.8%) reported to have no clinical signs; however only 15.6% (n=14; 95% CI 8.1-23.0%) were reported to exhibit no clinical signs in both seasons.

ACTH was greater in autumn samples (median 65.8pg/ml; IQ 41.7-129.3pg/ml) compared to spring/summer (p<0.001), and 68.9% of animals (n=62; 95% CI 59.3-78.5%) were PPID-positive (>47pg/ml). Median fold change in ACTH from spring/summer to autumn was 2.52 (IQ 1.50-5.42). Compared to horses (median fold change 2.25, IQ 1.36-3.67), ponies exhibited a greater fold change in ACTH (median 3.48, IQ 1.93-8.18) (p=0.002). Overall, 31.1% of animals (n=28; 95% CI 21.5-40.7%) were PPID-negative in both spring/summer and autumn, 20.0% (n=18; 95% CI 11.7-28.3%) were PPID-positive in both seasons, and 48.9% (n=44; 95% CI 38.6-59.2%) were PPID-negative during spring/summer and PPID-positive during autumn. Horses/ponies with PPID-positive assays during both seasons were older than those with negative paired assays (median 20; IQ 18-24 years vs. median 16; IQ 14-18 years) (p=0.001).

Median fold change was greater for animals that were positive in both seasons (3.01; IQ 2.13-7.47) and those that were negative spring/summer, positive autumn (3.99; IQ 2.56-6.74), compared to those negative in both seasons (1.28; IQ 1.12-1.49). Median fold change was not significantly different between animals that were PPID-positive in both seasons and those that were PPID-negative spring/summer and PPID-positive autumn (p=0.35). There were no significant associations between individual clinical signs reported during either spring/summer or autumn and the...
outcome of paired assays. A greater proportion of animals classed as PPID-negative in spring/summer and PPID-positive in autumn were reported to have ≥1 clinical sign during spring/summer (84.1%; n=37/44; 95% CI 73.3-94.9%), compared to the proportion with negative paired assays (67.9%; n=19/28; 95% CI 50.6-85.2) or those with positive paired assays (55.6%; n=10/18; 95% CI 32.6-78.5%) (p=0.05).

Conclusions
A significant proportion of animals exhibiting clinical sign(s) of PPID, with spring/summer ACTH <29pg/ml, subsequently had autumn ACTH concentrations >47pg/ml, highlighting the importance of repeat testing in animals suspected of having PPID where non-autumn ACTH concentrations are borderline.

Acknowledgements
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Endocrine testing and rapid-slice contrast computed tomography in an eight year old Arabian with clinical neurologic signs

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Aim

Pituitary Pars Intermedia Dysfunction (PPID) is common in older horses (≥ 15 years). Antemortem diagnosis is based on clinical signs and endocrine testing. However, with subacute onset of neurologic signs, computed tomography (CT) might be helpful to determine the size and extent of the tumor. The purpose of this report is to describe history, clinical signs, endocrine testing and use of rapid-slice CT to diagnose a pituitary adenoma in a young horse with progressive neurologic signs.

Methods

History and clinical signs were recorded. Complete blood count (CBC) and biochemical panel were performed in house. Sarcocystis neurona (SN, EPM) testing was performed at Equine Diagnostic Solutions, Lexington, KY, USA. Overnight dexamethasone suppression (DST) and a Thyroid Releasing Hormone (TRH) stimulation tests were performed one week apart. Plasma cortisol and ACTH were measured at the LADDL Endocrinology Laboratory, Baton Rouge, LA. A CT scan (GE lightspeed 16, GE Healthcare, Milwaukee, WI, USA) was performed under general anesthesia with the horse in dorsal recumbency. Contiguous 5 mm thick slices in soft tissue and bone window algorithms were taken before and after administration of iodinated contrast agent (200 ml, 240 mg/ml, IV: Iopomide Ultravist, Bayer Healthcare, Wayne, NJ, USA).

Results

History of progressive (3 months) onset of lethargy, ataxia, head pressing and loss of muscle mass was reported. Clinical signs of low body condition score (4/9), reduced top line, hypertrichosis, ataxia and depression were noted on presentation. CBC was unremarkable and the biochemical panel showed mild hyperglycemia (108 mg/dL). Serology for SN was negative (1:500) for EPM. Baseline cortisol and cortisol 18 hour after administration of dexamethasone (40 μg/kg, IV) was 35.0 ng/mL and 33.1 ng/mL, respectively. ACTH concentration increased from 318 pg/mL at rest to >1,250 pg/mL, 10 and 30 minutes after TRH administration. On rapid-slice CT scan, a rounded, well-defined, smoothly marginated, broad based mass, hyperattenuating (HU 54.3) to surrounding brain parenchyma (HU 32) was noted dorsal to the hypophyseal fossa, measuring 4.6 x 4.6 x 3.8 cm in size. There was focal dorsal displacement of the hypothalamus and focal compression of the third ventricle. There was uniform, moderate enhancement of the mass after contrast administration. An extra-axial pituitary mass consistent with a pituitary macroadenoma was diagnosed.

Conclusion

This is an unusual presentation of a young horse with neurologic signs attributed to a large pituitary adenoma. A rapid-slice CT scan with contrast can help determine the size and extent of the lesion and takes less than 30 minutes to obtain. A large pituitary mass in a young horse with neurologic signs might carry a poor prognosis, as current treatment does not reduce tumor size.
Comparison of Magnetic Resonance Imaging and Histological Scores for Assessing Pituitary Pars Intermedia Enlargement in Horses with Pituitary Pars Intermedia Dysfunction

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The data has not been previously published but it has been accepted for poster presentation at the 2014 ACVIM Forum in Nashville, Tennessee in June, 2014.

Aims
Progressive enlargement of the pituitary pars intermedia (PI) due to hyperplasia, microadenoma (<5 mm), and macroadenoma (>5 mm) formation is the pathologic lesion of pituitary pars intermedia dysfunction (PPID). This study was performed to assess the utility of magnetic resonance imaging (MRI) to detail subgross changes in PI morphology in PPID-affected horses.

Methods
The morphometric PI grading system developed by Miller et al. (2008) was used to grade T2 weighted midline sagittal pituitary gland (PG) MR images and midline sagittal PG histological sections. MRI was performed immediately prior to euthanasia in 21 horses: 7 PPID-affected horses treated with pergolide for 6 months (27±3 yr), 6 untreated PPID-affected horses (24±4 yr), 4 aged non-PPID-affected horses (25±5 yr), and 4 young non-PPID-affected horses (5±2 yr). A diagnosis of PPID was made on the basis of clinical signs and supportive overnight dexamethasone test results. MR images were scored by a radiologist and three internal medicine clinicians and histological sections were scored by a pathologist and a medicine clinician with experience with PG histopathology. Agreement between scorers was assessed by Spearman rank order correlation. PI and total PG areas were measured on MRI (by a radiologist) and histological sections (experienced medicine clinician) and PI/PG ratios were determined. Agreement between mean MRI and histological scores and areas was assessed by Spearman correlations.

Results
PI scores ranged from 1 to 5 but higher scores were overrepresented because the population was skewed toward PPID-affected horses (histological scores: 1 [n=1], 2 [n=3], 3 [n=2], 4 [n=3], and 5 [n=12]). Among PPID-affected horses, histological scores ranged from 3-5 and MRI scores ranged from 2-5. Mean MRI score was highly correlated with mean histological score (r=0.89, p<0.001). Similarly, correlation coefficients comparing histological scores and MRI scores for each clinician ranged from 0.71 to 0.93 (p<0.01 for all). PG weight ranged from 1.7 to 10.1 g and was highly correlated with mean MRI score (r=0.87, p<0.001) and mean histological score (r=0.91, p<0.001). MRI and histological PI/PG ratios were also highly correlated (r=0.90, p<0.001). There was no difference in PG weight between treated (5.3±1.0 g) and untreated PPID-affected horses (5.6±2.8 g). In addition to allowing assessment of PI size, both microadenomas and macroadenomas could be visualized by MRI. Further, cysts and colloid filled follicles (>1 mm) could be appreciated but these structures could not be distinguished from each other. Compared to other sequences, T2 weighted images provided the most contrast between normal and abnormal PI tissue.

Conclusion
MRI can effectively document PG and PI size as well as detail subgross morphologic changes in the PI of PPID-affected horses.
Acknowledgements

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Pharmacokinetics of pergolide following intravenous administration in horses
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The following work was performed in accordance with the ethical guidelines of the participating institutions and relevant legislation.

Objective
Pergolide mesylate is the treatment of choice for equine pituitary pars intermedia dysfunction (PPID) and veterinary preparations are now licensed for this use in a number of countries. Pharmacokinetic properties of the drug have not been established completely in horses and current dosing recommendations are based upon clinical experience. This study aimed to establish the pharmacokinetic properties of the drug following intravenous administration.

Animals- Eight healthy Thoroughbred and Standardbred geldings.

Procedures
Each horse was administered pergolide mesylate IV at 20 µg/kg (equivalent to 15.2 µg/kg pergolide) and blood samples were collected over 48 hours. Pergolide concentrations in plasma were determined using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS) and pharmacokinetic parameters determined using noncompartmental methods.

Results
After IV administration of pergolide mean ± SD clearance, elimination half-life and volume of distribution were 959 ± 492 mL/h/kg, 5.64 ± 2.36 hours and 1.13 ± 0.39 L/kg, respectively.

Conclusions and Clinical Relevance
Results indicate that twice daily dosing may be more appropriate than the once daily dosing that is recommended currently. There is no indication that loading doses of pergolide would be beneficial.
The use of ACTH response to TRH stimulation for monitoring horses with PPID and those receiving long-term pergolide.

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Diagnostic tests for equine pituitary pars intermedia dysfunction are used both in identifying horses affected with PPID and in monitoring response to treatment. Recently, ACTH response to TRH stimulation has been suggested to be a more discriminating test than other currently available testing methods for PPID diagnosis. However, the effect of season and pergolide treatment on ACTH response to TRH stimulation has not been adequately evaluated.

Seventy-five horses with ninety-two sample sets were included in this study. Fifteen horses had clinical signs of PPID and had been previously diagnosed using resting ACTH concentration or overnight dexamethasone suppression. Disease was confirmed at necropsy in all animals that died (14) subsequent to the study. Horses with PPID (n=12) were treated with pergolide at 1-2 mg/horse/day for 4-6 months, with testing occurring at 1 month intervals. Sixty horses without signs of clinical disease were also tested for PPID by ACTH response to TRH stimulation. Twenty-seven tests were performed between Aug 1-Oct 31.

In horses with PPID, there was a significant difference in unstimulated (T0) ACTH concentration at weeks 8, 16, 20 and 24 of pergolide treatment compared to baseline (week 0) ACTH. In comparison, stimulated (T30) ACTH concentration only differed from baseline (T0) at week 24 (Figure 1). In non-PPID horses, both baseline and stimulated ACTH was frequently greater than the cutoffs of 110 pg/ml at 10 minutes and 35 pg/ml at 30 minutes when testing in both the fall and non-fall seasons (Figure 2).

These data suggest that while TRH stimulation may be a better test for diagnosis of disease, further work should be performed to determine if provocative testing is necessary to adequately assess response to treatment. In addition, current reference ranges for ACTH following TRH stimulation should be further examined, particularly in the Fall, to determine optimal diagnostic cut off values in a broader, well defined population.

Figure 1: ACTH before and 30 mins after TRH in 12 PPID horses treated with pergolide mesylate for 24 weeks

Figure 2: TRH testing in non-PPID horses (n=60) in Fall and non-Fall.
**Effect of pergolide on heart rate and behavioural responses to a novel object**

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Presenting author: Cathy McGowan

This research was performed at the University of Liverpool, UK, under Home Office license 40/3072. This data has not been presented or published previously.

**Aims**

This study aimed to examine the behavioural effects of pergolide in normal horses using a novel object test with analysis of defined ethological criteria and telemetric monitoring of heart rate.

**Methods**

Three mares and 3 geldings 536±135 kg were recruited, fed 2.5% BWT grass hay and a commercial nutrient balancer and turned out to pasture for 1 – 2 h per day. After 10 days acclimation, horses were randomly divided into two groups, one group received pergolide (Prascend, Boehringer Ingelheim) at 4 µg/kg; the other received a placebo of identical appearance daily at 15.00 for 2 weeks. Following a washout period of 2 weeks, the groups were reversed and the opposite group received either drug or placebo at the same rate. All investigators were blinded to the medication.

After 1 week of medication horses underwent a novel object test. Horses were fitted with telemetry ECG (Televet, Kruuse) and walked to the test ménage. All horses had been acclimatised to the ménage on 3 occasions prior to the first test, and a further acclimation the week prior to the second test, 4 weeks later. Behaviour and heart rate data were recorded continuously during three consecutive 5 min periods during which horses were individually turned loose in the ménage; Period 1. pre-test; 2. test (a novel object, black plastic or dappled grey plastic horse placed in menage); 3. post-test. Video records were analysed for duration of ‘relaxed’, ‘pacing’ or ‘alert’ behaviours during the pre- and post-test periods and for the period of time ‘foccussed’ vs ‘unfocussed’ on the novel object during the test period. Heart rate was averaged for 15 seconds during 5 periods: end of pre test, initial reaction to novel object, end of novel object test period, the start of the post test period, and end of the post test period. The effect of group and test were analysed using a repeated measures ANOVA with a Greenhouse-Geisser correction.

**Results**

There was no effect of group on any heart rate periods analysed. There was no effect of test in all except the end of the novel object test period where the mean heart rate was significantly higher in test 2 than test 1 (P<0.01), but this was independant of group and there was no significant group-test interaction for any period. Similarly there was no effect of group on any of the recorded behavioural responses. There was no effect of test in all except the pre-test alert proportion which was significantly different between tests (P=0.02), but this was independant of group and there was no significant group-test interaction for any period.

**Conclusions**

Pergolide (Prascend) at 4 µg/kg daily for a week had no detectable effects on heart rate or behavioural response to a

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2nd European Equine Endocrinology Symposium
novel object test in normal horses.

Acknowledgements

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Response of Serum Insulin, Oral Sugar Testing and Body Weight in Horses Treated with Prascend®

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Hyperinsulinemia can occur in horses with a variety of endocrinopathies, including equine pituitary pars intermedia dysfunction (PPID) and equine metabolic syndrome (EMS). Hyperinsulinemia is a known risk factor for development of laminitis. It is unknown if treatment of PPID horses with pergolide mesylate affects serum insulin concentration. Furthermore, many horses are being treated with pergolide mesylate, despite an absence of a diagnosis of PPID. This is especially common in horses with EMS. Therefore the purpose of this study was to determine if treatment of horse with or without PPID with pergolide mesylate (Prascend®) has an effect on serum insulin concentration, insulin response to oral glucose challenge or body weight.

Thirty three horses were enrolled in the study between June 8, 2012 and August 30, 2012. Group 1 included 12 horses with PPID, Group 2 had 13 horses with clinical signs of “EMS” and Group 3 was comprised of 8 control horses of mixed body condition scores. No attempts were made to standardize management among animals; all horses were maintained according to their owner’s standard management practice without change for 8 weeks. At study onset, a baseline oral sugar test (OST) was performed and body weight calculated using a standard formula. Horses in Groups 1 and 2 received Prascend® at 1 mg/horse orally for 8 weeks. At 4 and 8 weeks the OST and body weight calculations were repeated. Outcomes were compared using two way repeated measures ANOVA with time and disease status as factors and insulin, glucose or body weight as outcomes.

Compared to baseline, a significant decrease in weight was observed in pergolide treated horses at 4 and 8 weeks. No difference in weight occurred in the untreated, control horses (Figure 1). There was no effect of pergolide treatment on glucose concentration at rest or 75 minutes after administration of oral sugar as Karo syrup. In contrast, horses receiving pergolide showed a decrease in insulin concentration 75 minutes after oral sugar challenge compared to concentrations prior to pergolide treatment, independent of disease status (Figure 2). This affect was most likely independent of season, as there was no difference in insulin concentrations during OST in untreated control horses.

These preliminary results suggest treatment with pergolide mesylate may have beneficial effects on weight and insulin dynamics, however further work with a larger number of horses is needed to better characterize the effect of pergolide on these metabolic factors.

Figure 1: Body Weight at 0 (white bar), 4 (light purple), and 8 (dark purple) weeks after pergolide treatment (B and C) or in untreated control horses (A). * P<0.05

Figure 2: Oral Sugar Test: Insulin concentration at 0 and 75 mins after Karo syrup, before (white bar) and after (blue bar) 8 weeks of Prascend treatment (B) or no treatment in control horses (A). *P<0.05
Effect of feeding soaked hay for two weeks on dynamic endocrine testing in horses

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This research was performed at the University of Liverpool, UK. Informed consent was obtained from all owners and institutional ethical approval obtained. This data has not been presented or published previously

Aims

This study aimed to determine the effect of feeding soaked hay for 2 weeks, without loss of body mass (BM), on insulin and glucose responses to a combined glucose insulin test (CGIT). A further aim was to determine if this effect was different in previously pastured horses compared with horses already fed soaked hay or straw.

Methods

Ten horses were recruited, four Welsh Section A ponies, one Welsh Section D, two Shetland ponies, one New Forest pony and two cobs; eight mares and two geldings. Four of the horses had already been on either soaked hay (n=3) or straw (n=1) with no or very limited pasture access. The other six horses had been pastured. Horses were managed in large individual stables with an hour of muzzled-grazing daily. All were fed grass hay, calculated to provide 2% of BM (rounded-up to nearest 0.5 kg) as daily dry matter intake and soaked in cold water prior to feeding. A CGIT was performed at recruitment and again 2 weeks later with standard glucose sampling to 150 min and insulin sampled at 0, 45 and 75 min post glucose. Data were analysed using a one-way Wilcoxon matched-pairs signed-ranks test (STATA 10.0).

Results

Mean ± SD body condition score was 7.6/9 ± 0.8 and four horses had had a recent bout of laminitis. Mean BM was not different between recruitment (357.4 ± 155.1 kg) and after 2 weeks of soaked hay (358.4 ± 155.5 kg, P=0.6). Mean glucose area under the curve (AUC) was not different between recruitment (897.3 ± 170.0) and after 2 weeks of soaked hay (997.7 ± 165.5, P=0.2). There was also no difference when horses previously pastured and those previously on soaked hay or straw were examined separately. There was a trend for mean insulin AUC to be lower after 2 weeks of soaked hay (4999.2 ± 3043.1) than on recruitment (6761.5 ± 3857.1) P=0.08. When horses were examined separately, the insulin AUC was significantly lower following 2 weeks soaked hay in the previously pastured horses (P=0.03) but not different in horses previously on soaked hay or straw (P=0.8). Baseline (P=0.03) and 75 min (P=0.02) insulin were lower following 2 weeks soaked hay in the previously pastured horses.

Conclusions

Initial management of endocrine laminitis by veterinary surgeons often involves advising a restricted diet for 2 weeks prior to dynamic endocrine testing. Such management may affect dynamic endocrine testing in horses and recent dietary management should be factored into interpretation of results.
Dry matter losses incurred during hay soaking: Implications for the nutritional management of equine obesity

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Introduction

Nutritional management of equine-obesity and EMS is dependent on evidence-based advice to restrict forage provision and control weight-loss. Soaking decreases the water soluble carbohydrate (WSC) content of grass-hay and improves insulin sensitivity; therefore soaked-hays are increasingly recommended for the management of EMS and obesity. However, when EMS animals were fed soaked-hay at ~1.25% BM as daily DM (pre-soaking), animals lost weight at 1.1% of outset BM weekly, more than doubling weight-losses recorded when horses were fed fresh/’un-soaked’ hay to the same level (Argo et al, 2012) (Fig. 1.).

Aims

To compare the composition and digestibility of fresh/soaked grass-hays to improve nutritional-guidance for the management of EMS.

Methods

After six weeks of dietary restriction to 1.25%BM as hay DM, 6/12 horses which had participated in an earlier study (McGowan et al., 2013), were used to determine the digestibility of the soaked (n =3) and fresh (n =3) hays. Samples of fresh grass-hay (n =6) and hay from the same batch which had been water-soaked overnight (16h, n =6) or by day (7h, n =6) were dried for the measurement of DM content, pooled and ground. Nutrient and gross energy (GE) compositions were determined by proximate analyses. Animals were fed soaked-hay as 2 daily meals (equal-parts long/short-soaked). Apparent digestibilities were determined after total faecal collection over 72h.

Results

Soaking hay; did not alter DM concentrations of GE (17.6 MJ/kgDM), increased ADF (from 30 to 35%), NDF (68 -74%) and crude protein (CP, 8.4-10.6%), decreased water soluble carbohydrate (WSC, from18.2 to 12.2%), and mineral contents (5.7-4.2%). Digestibilities of GE (55.7 ±3.7%), DM (59.8 ±1.6%), Ash (50.7 ±2.1%), NDF (58.0 ±2.2%) and WSC (92.2 ±0.3%) were unaltered by soaking. Conversely, soaking increased the apparent-digestibility of CP (from 55.6 ±4% to 67 ±5%) and ADF (37.5 ±9% to 51 ±4%). However, hay-soaking elicited un-quantified losses of DM. Previous studies report hay ADF and CP as ‘water-insoluble’. When DM losses were retrospectively ‘back-calculated’ from the ADF and CP content of fresh and soaked-hays, daily DM provision decreased from 1.25% to 1.06% and 1.00% respectively. Consequently, DE intakes were estimated to have decreased by ~25%, increasing the severity of DE restriction from the anticipated 81% of maintenance DE requirements (mDER) to 63% (ADF-corrected) and 56% (CP-corrected) mDER.

![Fig 1: Changes in body mass (BM, mean ±SD) for horses/ponies restricted to 1.25%BM as daily DM intake of grass-hay either fed fresh (n=6) or after soaking (n=12). Asterisks denote P<0.001](image)
Conclusions

DM losses from hay-soaking impacted on energy/nutrient provision and increased the severity of dietary restriction which could have severe implications for weight-loss sensitive animals. Evaluating ADF concentrations for hays, pre- and post-soaking could direct nutritional advice for limit fed animals.

Studies were conducted at the University of Liverpool and approved by the University Veterinary Research Ethics Committee. Data have not been presented previously.

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