Proceedings

6th Global Equine Endocrine Symposium

Ocala, World Equestrian Center Florida

January 7th – 9th 2025

Boehringer Ingelheim

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FOREWORD

Dear Delegate,

We are very pleased to welcome you to the sixth Global Equine Endocrinology Symposium at the World Equestrian Horsecenter (WEC), Ocala, USA, hosted by Boehringer Ingelheim.

As a community within the equine world, we are dedicated to raising awareness, to better understanding and to developing a standard of care for horses suffering from EMS, PPID and other misunderstood endocrinopathies to all equine stakeholders.

Our program this year will focus on a number of areas:

- Equine metabolic syndrome (EMS) pathophysiology
- EMS diagnosis
- EMS therapeutics
- Pituitary pars intermedia dysfunction (PPID) pathophysiology and diagnosis
- PPID management
- Adipokines and equine endocrine dysfunction
- Future directions for equine endocrine research

We hope that the symposium will succeed at bringing the scientific community together and further drive research and knowledge about endocrine diseases.

Finally yet importantly, we hope that you enjoy this symposium.

Sincerely,
Boehringer Ingelheim & the Scientific Committee

Boehringer Ingelheim

CONTENTS

Epidemiological Evaluation of Equine Metabolic Syndrome in Arabian Horses and their Subgroups

Jennifer Hesser[1], Audrey Johnston[1], Brianna Young[1], **Elaine Norton[1]**

University of Arizona, Department of Animal and Comparative Biomedical Sciences[1]

Background: Equine metabolic syndrome (EMS) is influenced by both genetic and environmental factors with specific breeds being more susceptible to EMS. While Arabians are recognized as a high-risk breed for EMS, there is a lack of large-scale epidemiological studies assessing their susceptibility across the breed. Further, the Arabian horse is considered a versatile breed; however, ideal body condition, breeding, and management varies across disciplines, leading to distinct subgroups and potentially influencing EMS risk.

Aims: Our aim was to estimate prevalence and individual and environmental risk factors influencing EMS in the Arabian horse breed and within the breed's subgroups.

Methods: 591 Arabians (70 stallions, 398 mares, and 123 geldings) with a median age of 10 years (range 5-30 years) were enrolled across 43 farms between April and July. Phenotypic data included basal insulin, non-esterified fatty acids (NEFA), triglycerides (TG), insulin concentrations post oral sugar test at 60 and 90 minutes (INS-OST), body condition score (BCS), and cresty neck score (CNS). Horses were categorized based on management and owner reported subgroups: English (n=91), Western (n=56), leisure (n=78), lesson (n=37), halter (n=81), broodmare (n=195), or breeding stallion (n=25). Binary outcome variables were defined as obesity (BCS≥8), fasting hyperinsulinemia (basal insulin >20μIU/mL), post-OST hyperinsulinemia (max INS-OST >45μIU/mL), and hypertriglyceridemia (TG>43mg/dL). Prevalence of each binary outcome variable was calculated across our cohort and within subgroups. Stepwise regression was used to analyze EMS quantitative measurements, with farm as a random effect and sex, age, CNS, BCS, sampling month, state, and subgroup as predictors. Mixed model analyses were performed, and the percentage of phenotypic variation explained by each predictor was calculated. Pairwise comparisons of the estimated marginal means (emmeans) were used to identify significant differences between categorical predictors (subgroup, sex, and sampling month) at a Tukey-corrected p-values <0.05, and marginal R^2 and partial correlation coefficients were calculated to estimate the percent of phenotypic variation explained by each explanatory variable. All methods were approved by the University of Arizona IACUC (#2021-0819)

Results: Across our cohort, 25% were obese (BCS≥8), 58% had a CNS of ≥3, 15% had fasting hyperinsulinemia, 25% had post-OST hyperinsulinemia, and 19% had hypertriglyceridemia. Within subgroups, the prevalence of obesity and post-OST hyperinsulinemia were highest in broodmares (32.7% and 23.1%) and horses in English disciplines (26.3% and 18.7%). Breeding stallions had the highest prevalence of a CNS ≥3 (67.1%) but hypertriglyceridemia, hyperinsulinemia, and post-OST hyperinsulinemia were absent in this group. Subgroup (R^2=5-10%), month sampled (R^2=0.01-5%) and CNS (R^2=0.5-3%) were significantly associated with all quantitative outcome variables. Pairwise comparisons of the emmeans revealed that sex was significantly associated with INS-OST with stallions having an average of 6.2 μIU/mL lower insulin concentrations compared to mares (p=0.008).

Conclusion: These results confirm that Arabians are a high-risk breed for EMS with differences in prevalence and risk across subgroups and sex, supporting more frequent monitoring and intensive management in this breed.

Acknowledgements: The authors would like to acknowledge all the owners and trainers which participated in this study as well as USDA-NIFA (award #2023-67016-40110) for funding to support this project.

Postprandial hyperinsulinaemia in Icelandic horses is associated with an increase in pancreatic beta cell mass and beta:alpha cell ratio

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Aims:
Postprandial hyperinsulinaemia is an important component of insulin dysregulation (ID). In animal models of metabolic syndrome, beta cell mass may be increased. The proportion of glucagon-expressing cells within islets is reduced in insulin resistant (IR) horses (Newkirk et al, 2017); but the association between postprandial hyperinsulinaemia and overall beta and alpha cell mass has not been investigated. The aim of this study was to correlate cell mass with postprandial insulin responses, as well as IR.

Methods:
In a separate study, twelve Icelandic horses (age range 15-29 years old) underwent an oral glucose tolerance test (OGTT; 0.5 g/kg glucose via nasogastric tube) and a standardised meal challenge (SMC; 0.5 g/kg glycaemic pellets) to characterise their insulin responses; and an insulin response test (IRT; 0.1 IU human recombinant insulin, IV). Post-mortem pancreatic samples prepared for immunohistochemistry for other reasons were subsequently repurposed for this study.

Beta cells and alpha cells were identified by positive immunostaining for insulin and glucagon, respectively. To objectively quantify the relative cell mass, immunostained slides were digitised and converted to binary black and white images, with white pixels representing the immunopositive cell mass. At x20 magnification, the ratio of white:black pixels (the percentage of the field represented by positive staining cells) was calculated using image analysis software (ImageJ). Five separate fields (including a total of 24-37 islets per animal) were evaluated from each slide giving a mean value for each animal. Spearman correlation assessed the relationship between peak or AUC postprandial insulin concentrations and beta and alpha cell mass. Cell mass was also compared between IR and non-IR animals using an unpaired t-test.

Results:
Of the 12 ponies, 3 were determined to be IR on the basis of the IRT and 11 had peak insulin responses >50 μIU/mL after oral glucose administration. One animal was neither hyperinsulinaemic nor IR (therefore not ID).

Beta cell mass (BCM: % of the tissue field) was positively correlated with both peak insulin and insulin AUC following the oral glucose challenge ($p=0.012$; $r_s=0.71$) whilst the alpha cell mass (ACM) was negatively correlated ($r_s=-0.72$ and -0.60 for peak insulin and AUC, respectively). SMC peak insulin was correlated with BCM ($r_s=0.71$) and ACM ($r_s=-0.71$). With only 3 IR animals, no significant effect of IR was apparent.

Conclusions:
Postprandial hyperinsulinaemia is associated with an increase in BCM and a decrease in ACM. This may be a contributing factor to hyperinsulinaemia and the risk of laminitis. The cause of changes in endocrine cell mass requires further investigation.

References:
Newkirk et al. (2017). Immunohistochemical expression of insulin, glucagon, and somatostatin in pancreatic islets of horses with and without insulin resistance. AJVR 79(2):191-198.

Acknowledgements:
The authors are grateful to the University of Veterinary Medicine, Hannover, for providing the metadata and slides relating to these horses and also to Dr Pat Harris who provided consultancy advice on the interpretation of the current study.

Ethics statement:
All experimental procedures were approved by the State Office for Consumer Protection and Food Safety in accordance with German Animal Welfare Law (Ref:33.19-42502-04-18/3006).

Effect of an oral glucose test on glucagon secretion in horses with insulin dysregulation

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This study was approved by The University of Queensland Animal Ethics Committee approval number SVS/153/19.

Aims: Glucagon is a counter regulatory hormone of insulin. Its concentration is increased in pre-diabetic people and associated with pancreatic hyperactivity. As equine insulin dysregulation (ID) is considered a pre-diabetic condition, the aim of the study was to evaluate glucagon secretion in response to an oral glucose test (OGT) in horses.

Methods: Sixteen light breed horses, including 7 with ID, identified based on insulin concentration during an OGT and insulin sensitivity index from a modified frequently sampled intravenous glucose tolerance test. All horses underwent an OGT with insulin, glucagon and glucose concentrations measured at 0, 60, 90 and 120 min after dextrose administration and compared overtime and between groups with a linear mixed effect model.

Results: There was a significant effect of dextrose administration on glucagon concentration ($p<0.0001$) with a significant decrease detected at 60, 90 and 120 min (effect size: -0.220 [95% confidence interval (CI): -0.351--0.089] pg/mL, $p=0.0002$, -0.274 [95% CI: -0.406--0.143] pg/mL, $p=0.0001$ and -0.277 [95% CI: -0.414--0.141] pg/mL, $p=0.0001$, respectively). No effect of ID was detected at any timepoint ($p=0.8$). The Glucagon/Insulin ratio was significantly decreased by dextrose administration ($p<0.0001$ for all comparisons), and this decrease was more severe in horses with ID ($p<0.001$). The Glucagon/Glucose ratio was significantly decreased by dextrose administration ($p<0.0001$ for all comparisons); however, no effect of ID was detected ($p=0.7$).

Conclusions: Unlike what is described in pre-diabetic people, glucagon dysregulation does not appear to be a feature of ID suggesting an absence of α-cell hyperactivity in horses.

Active ghrelin, GLP-1 and insulin secretion over 24 hours in ponies

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This study complied with national guidelines for humane animal treatment and with relevant legislation in Australia. The study was approved by the institution's Animal Ethics Committee. The abstract has not been presented or published previously.

Aims

The hormone ghrelin contributes to metabolic disturbance in several mammalian species. As ghrelin is involved in glucose metabolism, in part by moderating appetite and food intake, it might be implicated in the aetiopathogenesis of equine metabolic syndrome. This study aimed to determine the concentration range of the active form of ghrelin in ponies throughout one day/night cycle and relate this to insulin and GLP-1 concentrations measured concurrently.

Methods

Eight mixed breed ponies were blood sampled 2 hourly for 24 hours from an indwelling jugular catheter, with additional samples collected at 9am and 5pm shortly after feeding. Blood was placed into p800 (plasma) vacutainer tubes for ghrelin and GLP-1 measurement and into clot activator (serum) tubes for insulin determination. Active ghrelin and active GLP-1 were measured using a radioimmunoassay and an ELISA (respectively), previously validated for use in horses. Insulin was measured by a commercial laboratory (Immulite 2000XPi). A one-way repeated measures ANOVA or ANOVA on ranks (where data were not normally distributed), were used to identify changes in individual hormone concentration over time, and two-way repeated measures ANOVA was used to detect hormone interactions. Post-hoc pairwise comparisons were performed with Bonferroni's t-test. Either Pearson's (continuous) or Spearman's (ordinal) correlation coefficients were used to assess associations between other variables. Graphpad Prism 10.2.3 and Sigmaplot v15 were used for data analyses and $P \leq 0.05$ was considered significant.

Results

The median (IQR) plasma active [ghrelin] over 24 hours was 9.9 (6.6-14.3) pg/mL with individual values occurring over a range of 19.7 pg/mL. The median (IQR) C_{max} was 18.6 (12.2-20.4) pg/mL and C_{min} was 5.7 (4.1-8.7) pg/mL. The active [ghrelin] changed over time ($P = 0.04$), decreasing after eating, with differences between the fed and unfed state. For example, 2am vs. 12pm ($P = 0.002$) and 2am vs. 8pm ($P = 0.001$). The post-feed nadirs were recorded ~3 hrs after eating. The secretory patterns of active GLP-1 and insulin were similar to each other and generally opposite to that of active [ghrelin], showing a significant difference in the early morning when active [ghrelin] peaked ($P \leq 0.01$), and at midday when [GLP-1] and [insulin] were high ($P < 0.05$). Active $[ghrelin]_{Cmax}$ correlated ($P = 0.035$) with active $[GLP\text{-}1]_{Cmax}$, but not with $[insulin]_{Cmax}$. Active $[ghrelin]_{Cmax}$ also positively correlated with girth measurement ($P = 0.04$), while the active $[ghrelin]_{Cmin}$ was positively correlated with age ($P = 0.05$).

Conclusions

There was a large variation in individual active [ghrelin], with the range measured here lower than the concentrations previously published in horses. Ghrelin decreased after eating, with no evidence of a diurnal rhythm. The secretory pattern of active ghrelin opposed both GLP-1 and insulin, with a peak when fasted and a post-prandial nadir. A relationship between fasted active [ghrelin] and post-prandial active [GLP-1] is plausible. Relationships between active [ghrelin], phenotype and metabolic disease in equidae deserve further investigation.

Acknowledgements

The authors thank Poppy Sibthorpe, Danielle Fitzgerald and the Translational Research Institute, Brisbane.

Effect of Day and Time on Pasture Nonstructural Carbohydrates and Insulin in Horses with Insulin Dysregulation.

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All procedures described were approved by the University of Kentucky Institutional Care and Use Committee (#2017 - 2720).

This material is original and has not been presented elsewhere.

Aims: In the early summer, when cool season pasture has gone past the early flush period, current recommendations are to consider restricting grass turnout for animals prone to laminitis to early mornings. This study therefore evaluated changes in early summer pasture non-structural carbohydrate (NSC) and insulinemic response in insulin dysregulated (ID) horses by day and time.

Methods: Seven mixed-breed, mixed-sex adult horses (Mean±SD; 16.0±3.8yrs) were group housed in the same paddock (Kentucky Cool season grass) with *ad libitum* access to pasture and water. All horses were classified as ID (*n*=7), based on history and diagnostic testing, using basal insulin and oral sugar tests. The same horses and paddock with green, active growth vegetation were utilized in each of two phases in June 2024. In Phase 1, peripheral blood and pasture samples were collected and daily environmental temperatures (DT) recorded during the same morning hours (between 0800 and 0900) for five consecutive days. The following week in Phase 2, peripheral blood and pasture samples were collected and the environmental temperature (ET) recorded on the same day at two different time points: 0800 (AM) and 1500 (PM). Blood samples were analyzed for insulin by AIA (Tosoh: University of Kentucky). Representative samples of pasture forage were collected via sampling forage in a "W" pattern in the paddock. Immediately following collection forage samples were stored at -20°C prior to being shipped on ice to be analyzed by NIR (Equi-analytical). A one-way RM ANOVA (Phase 1) and a t-test (Phase 2) were used to analyze the data in GraphPad Prism. Statistical significance was considered at $P<0.05$. Data is presented as Mean±SD.

Results: All horses remained clinically healthy throughout the study. In Phase 1 there was no correlation between DT and pasture NSC but there was a numerical decline in the NSC (DT and NSC % being, 19, 22, 20, 21°C and 15.3, 12.5, 11.5, 11.4, 10.1 %DM respectively). The decrease in pasture NSC over the five days was associated with changes in serum insulin concentrations, which were significantly higher ($P<0.05$) on day 1 (216.90±188.90µIU/mL) than day 4 (128.90±87.53µIU/mL), and day 5 (124.20±97.13µIU/mL). In Phase 2 ET and NSC both increased from AM (24°C, 9.5%DM) to PM (32°C, 13.4%DM). Serum insulin concentrations also increased significantly ($P=0.03$) from AM (78.17±44.94µIU/mL) to PM (101.00±50.97µIU/mL).

Conclusion: There was great individual variation in the insulin responses to the same pasture in grazing ID horses. The study confirmed the NSC increases that can occur from morning to mid-afternoon in cool season pastures even under warm/hot conditions which are reflected in the insulin concentrations. Changes in morning pasture NSC can also change significantly from day to day; and even in early summer the morning NSC% may result in undesirable insulin concentrations in some grazing ID animals. As the NSC changes can occur rapidly, and the insulin response is highly individual, it is important to monitor pasture NSC to provide an indication of its potential safety for grazing but more importantly to frequently monitor individual insulin responses especially in high-risk ID horses.

Acknowledgements: The authors would like to acknowledge the University of Kentucky, Gluck Equine Research Center, the farm staff at the C. Little Oran Research Farm, and Lincoln Memorial Richard A. Gillespie College of Veterinary Medicine for their support of this project.

Postprandial increases in cytokines in ponies associated with hyperinsulinaemic responses: potential role of inflammatory mediators

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Aims:
A link between insulin dysregulation (ID) and inflammation in equids has been speculated; and the cytokine IL-1β has been shown to increase postprandially in horses fed a meal containing 1.2 g/kg BW starch and sugar. The aims of this study were: 1) to measure postprandial levels of a large array of cytokines and chemokines following a starch-rich meal given to ID ponies; and 2) to determine whether IL-1β receptors are present on equine pancreatic beta cells.

Methods:
Sixteen horses and ponies (age range 15-32 years; body weight 130-412 kg) were fed a meal containing 1.1g/kg BW starch; and serial blood samples were taken pre- and for 5 hours post-prandial. Four ponies exhibiting the greatest insulinaemic responses (peak 256-480 μIU/mL; 'higher responders') were compared with four breed-matched ponies exhibiting lower insulinaemic responses (peak 115-171 μIU/mL; 'lower responders'). A panel of 23 cytokines and chemokines were measured in plasma samples using a multiplex ELISA (Milliplex magnetic bead panel; Merck). Cytokine concentrations were compared between higher and lower responders using a 2-way repeated measures ANOVA, with $P<0.05$ considered significant.

In a subsequent study, pancreatic tissue samples were aseptically collected post-mortem, from six horses and ponies that were euthanised for non-research reasons. Immunohistochemistry was used to determine the presence of IL-1β receptors in pancreatic tissue, using a rabbit anti-human IL1-b receptor primary antibody.

Results:
Significant increases in IL-1β were observed postprandially in both groups of ponies; but the peak concentration (at 2.5 hrs) was significantly greater in the higher responder group (38.3 ±2.5 pg/ml; mean ±SD; vs. 16.5 ±2.0 pg/ml at Time 0) compared with the lower responder group (peak 26.3 ±2.2 pg/ml; $P=0.0002$). An increase in other cytokines was not detected in the lower responder group; but in the higher responder group significant increases in IL-18 (baseline 15.9 ±2.5 pg/ml to peak 33.6 ±4.5 pg/ml), IL-10 (baseline 46.8 ±7.7 pg/ml to peak 75.2 ±32.4 pg/ml) and IL-6 (baseline 3.8 ±2.0 pg/ml to peak 30.5 ±6.8 pg/ml) were also observed in addition to IL-1β. Immunohistochemistry demonstrated the presence of IL-1β receptors on beta cells in pancreatic islets.

Conclusions:
A postprandial increase in IL-1β was detected in the plasma of ponies following a starch-rich meal. Furthermore, ponies with higher insulinaemic responses demonstrated a broader inflammatory response, with an increase in other cytokines, suggestive of monocyte/macrophage activation. The presence of IL-1β receptors on pancreatic beta cells suggests that this cytokine may contribute to the stimulation of insulin production in equids, as in other species.

Acknowledgements:
The feeding study was funded by the Australian Research Council (LP180101000), The University of Melbourne, Queensland University of Technology, Boehringer Ingelheim Vetmedica GmbH, Racing Analytical Services, Liphook Equine Hospital and the Waltham Petcare Science Institute.

Animal ethics:
All studies were conducted according to the Australian Code for the Care and Use of Animals for Scientific Purposes, with the approval of the University of Melbourne Animal Ethics Committee (ID 23234).

Investigation of arginine and related metabolites during an oral glucose test

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The study was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (file number 33.19-42502-04-23-00366) and was conducted in accordance with applicable German laws and institutional guidelines for humane animal treatment.

Aims: Arginine is the precursor of nitric oxide (NO), the major vasodilator in the body. Since there is evidence of vascular dysfunction in horses with insulin dysregulation and since arginine has been found to decrease following glucose administration, this study aimed to describe the response of arginine and indicators of NO bioavailability (nitrite and nitrate) to an oral glucose test (OGT) in relation to the metabolic status.

Methods: An OGT was performed in 22 fasted horses and ponies by administering 1 g/kg bodyweight of glucose via a nasogastric tube and taking blood samples at 0, 60, 120, and 180 min. Blood glucose was measured using a handheld glucometer, insulin and arginine were measured using ELISAs, and nitrite and nitrate were measured using colourimetric assays. The effect of time during the OGT and area under the curve for insulin ($AUC_{insulin}$) or glucose ($AUC_{glucose}$) on arginine, nitrate and nitrite was analysed using mixed linear models.

Results: Arginine showed a significant decrease during the OGT ($\beta_{0\text{-}120\ min} = -16.78 \pm 3.21\ \mu mol \cdot L^{-1}$, $p < 0.0001$). Nitrite but not nitrate increased significantly with $AUC_{glucose}$ ($\beta = 0.018 \pm 0.005\ mmol \cdot min^{-1}$, $p = 0.015$) with no difference between basal and stimulated samples. There was no significant association between $AUC_{insulin}$ and arginine, nitrite or nitrate.

Conclusions: Hyperinsulinemia and hyperglycaemia were confirmed to affect arginine metabolism in horses, with effects on the nitrate-nitrite-nitric oxide pathway that remain to be fully elucidated but are likely to play a role in vascular dysfunction associated with insulin dysregulation.

Effect of dexamethasone administration on insulin response to oral carbohydrate challenge in horses

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This study was approved by The University of Queensland Animal Ethics Committee approval number SVS/334/20.

Aims: Dexamethasone is used to create an experimental model of insulin resistance; however, its impact on insulin secretion is unclear. This study aimed to assess the response to an oral carbohydrate challenge in dexamethasone-treated horses.

Methods: Eight Standardbreds (5–13 years old, 467–548 kg) received 0.08 mg/kg of dexamethasone intramuscularly every 48 hours for 15 days. Oral glucose tests (OGT) were conducted before treatment (day 1) and on days 8 and 15. Glucose, insulin, total and active glucagon-like peptide-1 (tGLP-1 and aGLP-1), and glucose-dependent insulinotropic polypeptide (GIP) were measured at baseline and at intervals up to 240 min after OGT. Results were analysed using a mixed-effects linear regression model.

Results: After 8 days of dexamethasone, significant increases in areas under the curve (AUC) and maximum concentrations (Cmax) of glucose (effect size: +28833.4 [95% confidence interval (CI) 25495.1-32171.7] mg/dL*min, +139.1 [95% CI: 124.0-154.1] mg/dL, both $p<0.0001$), insulin (+53172.77 [95% CI: 38845.9-67499.7] µIU/mL*min, +297.6 [95% CI: 214.6-380.8] µIU/mL, both $p<0.0001$), tGLP-1(+399.5 [95% CI: 12.1-786.9] pmol/L*min, +2.58 [95% CI: 0.23 4.93] pmol/L, both $p<0.05$), and GIP (+13479.4[95% CI: 8108.9-18849.9] pg/mL*min, +65.56[95% CI: 40.98-90.16] pg/mL, both $p<0.0001$) were detected post-OGT. These effects were however blunted by day 15, with glucose, insulin, and aGLP-1 AUC and Cmax significantly lower than on day 8, and tGLP-1 and GIP AUC and Cmax not different from day 1. No horse developed clinical laminitis.

Conclusions: Dexamethasone increased insulin secretion after an oral carbohydrate challenge, but the effect was transient and partially reversed by day 15. While dexamethasone induces insulin resistance consistently over prolonged periods, its effect on insulin secretion seems temporary.

Acknowledgements: This work was funded by a grant from the Grayson Jockey Club Research Foundation.

Effect of dexamethasone administration on insulin-independent glucose disposal and glucose absorption

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This study was approved by The University of Queensland Animal Ethics Committee, approval number SVS/334/20.

Aims: Dexamethasone is used to create experimental models of insulin dysregulation. This study aims to quantify the effect of dexamethasone treatment on insulin sensitivity (SI), glucose effectiveness (whole-body insulin-independent glucose disposal, GE), and glucose absorption (Glucose GI half-life).

Methods: Eight Standardbreds (5–13 years old, 467–548 kg) received 0.08 mg/kg of dexamethasone intramuscularly every 48 hours for 15 days. Oral glucose tests (OGT) were conducted before treatment (day 1) and on days 8 and 15. SI, GE, and glucose absorption were estimated using a mathematical model of glucose metabolism during OGT. Results were analyzed using a mixed-effects linear regression model.

Results: Before treatment with dexamethasone (day 1), the insulin sensitivity (SI) was estimated to be 8 10^{-4}.mU/L^{-1}.min^{-1} (95% CI: 6.2 – 9.9 10^{-4}.mU/L^{-1}.min^{-1}). After 8 days of dexamethasone treatment, SI was significantly (P<0.001) reduced by 92.5% (SI: 0.6 10^{-4}.mU/L^{-1}.min^{-1}; 95% CI: 0.3 – 0.9 10^{-4}.mU/L^{-1}.min^{-1}). Though SI was still significantly reduced (P<0.001) by day 15 of treatment, we observed partial recovery (SI: 3.4 10^{-4}.mU/L^{-1}.min^{-1}; 95% CI: 2.3 – 4.4 10^{-4}.mU/L^{-1}.min^{-1}). GE was estimated to be 0.022 min^{-1} (95% CI: 0.021 – 0.023 min^{-1}) at day 1. After 8 days of treatment, GE remained unchanged. By day 15, GE was significantly reduced (P=0.004) by 20% (GE: 0.018 min^{-1}; 95% CI: 0.015 – 0.020 min^{-1}). Glucose GI half-life was 64.6 minutes (95%: 59.5 – 69.6 min) at day 1. After 8 days of treatment, the dexamethasone GI half-life was significantly reduced (P=0.021; Glucose GI half-life: 57.1 min; 95% CI: 54.0 – 60.2 min). By day 15, glucose GI half-life was further reduced (P<0.001; Glucose GI half-life: 51.5 min; 95% CI: 47.5 – 55.5 min).

Conclusions: Dexamethasone effectively reduced insulin sensitivity throughout the treatment period. Somewhat surprisingly, after 15 days of treatment, GE was also reduced. Furthermore, a significant reduction in the index of glucose absorption was observed. Our current data indicates that in horses, besides the detrimental effects on insulin sensitivity, dexamethasone treatment also causes progressive reductions in both GE and glucose absorption.

Acknowledgments: This work was funded by a grant from the Grayson Jockey Clube Research Foundation

Factors associated with leptin concentration in ponies at risk for Equine Metabolic Syndrome.

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This study was approved by The University of Queensland Animal Ethics Committee approval number SVS/210/20 and University of Georgia CVM Clinical Research Committee.

Aims: Hyperleptinaemia is reported as a component of Equine Metabolic Syndrome (EMS); however, there are inconsistencies in its clinical value. The aim was to investigate the association between leptin concentration and obesity, insulin dysregulation and laminitis.

Methods: Cross-sectional study of 211 Shetland and Welsh ponies sampled in spring and autumn. Morphometric information (height, body condition score [BCS], cresty neck score) and laminitis status were recorded. Insulin dysregulation was defined if ponies had resting or post-oral sugar test hyperinsulinaemia, or insulin resistance (IR) after an insulin tolerance test. Leptin concentrations were measured with radioimmunoassay. Factors associated with leptin concentrations were assessed with a univariable analysis followed by stepwise backward multivariable analysis. Significance was set at $p < 0.2$ to be considered for the model and < 0.05 to remain in the model.

Results: One-hundred-and-thirty-two ponies were insulin-dysregulated, including 98 hyperinsulinaemic and 86 insulin-resistant ponies. Median age was 9 years (range 1-30), 113 ponies had laminitis and obesity was identified in 60 ponies in autumn and 59 in spring. Leptin concentrations were higher in autumn than in spring (2.88 ± 0.05 *vs.* 2.69 ± 0.05 ng/mL, $p = 0.004$). In autumn, variables significantly associated with leptin concentration included breed (Welsh estimate 0.88, SE 1.05), age (0.99, SE 1.01), sex (male 0.97, SE 1.05) and IR (IR 1.11, SE 1.05) while in spring, they included BCS (1.09, SE 0.02) and basal insulin (1.16, SE 0.04).

Conclusions: Leptin concentration appear weakly and inconsistently associated with EMS traits, questioning the clinical value of measuring leptin.

Acknowledgments: Students who helped sample and pony owners.

Effect of Seasonal Changes in Pasture Nonstructural Carbohydrates on 24-hour Insulin Responses in Horses with Insulin Dysregulation.

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All procedures described were approved by the University of Kentucky Institutional Care and Use Committee (#2022 - 4118).

This study comprises three 24-h phases, Phase 1) late summer 2023- pasture, was presented at ACVIM by the same authors under the title "Effect of Changes in Pasture Water Soluble Carbohydrates in Horses With and Without Insulin Dysregulation." Phases 2) spring 2024-pasture and 3) spring 2024-drylot with low NSC hay, have not been presented elsewhere.

Aims: 1) To examine changes in pasture non-structural carbohydrate (NSC) content and insulinemic responses in insulin dysregulated (ID) and non-ID (NID) horses over a 24-h grazing period across two seasons (late summer 2023 and spring 2024). 2) 24hrs post the spring study to evaluate insulin responses over 24-h in a drylot with *ad libitum* access to low NSC hay (<10%DM basis) in the same ID horses.

Methods: Twelve adult horses (Mean±SD; 19.0±3.04yrs) were classified as either ID (n=6) or NID (n=6), based on history and diagnostic testing. The study comprised three 24-h phases: Phase 1: on late summer 2023 pasture, Phase 2: on spring 2024 pasture, and Phase 3: following Phase 2 in a drylot. During the 24-h grazing periods all horses were group housed in a paddock with *ad libitum* access to pasture and water. The same paddock was utilized each season and horses were moved there 24hrs before sample collection. Peripheral blood and pasture samples were collected every 2 hours from 0700hrs. In Phase 3, the 6 ID horses were removed from pasture after Phase 2 completed and group housed on a drylot with *ad libitum* access to low NSC hay. Starting the following morning (0700hrs) peripheral blood samples were collected every 2 hours for the 24-h period. Blood samples were analyzed for insulin by AIA (Tosoh: University of Kentucky), cortisol by RIA (Cornell AHDC lab), and glucose by glucometer. All forage samples were analyzed by NIR (Equi-analytical). Data was analyzed in GraphPad Prism, a two-way RM ANOVA was performed with variables of metabolic status and time. Statistical significance considered at $P<0.05$.

Results: No clinical signs of laminitis were seen. In Phase 1 NSC peaked at 15.4% (1900hrs) and then slowly decreased to 7.5% (0500next day). Serum insulin concentrations increased significantly in ID horses from baseline (0700hr: 101.2±53.4µIU/mL) to that recorded at 1500 (187.3±78.7µIU/mL), 1700 (203.0±89.0µIU/mL), 1900 (226.1±86.0µIU/mL), 2100 (216.3±88.7µIU/mL), and 2300 (215.8±84.2µIU/mL) ($P<0.01$) and then decreased. In Phase 2 the pasture NSC levels ranged from 19.0-24.7% and resulted in ID horses' insulin remaining consistently high (602.8±308.3µIU/mL). 4 of the 6 NID animals also showed a significant response over the day (234.0±184.7 µIU/mL). Insulins remained at 33.9±10.2µIU/mL in the other 2 NID. Cortisol showed apparent circadian changes, with differences observed by time ($P<0.01$), but not metabolic status. In Phase 3, within 24-h of being housed on a drylot, with low NSC hay, all but one ID horses' insulin had significantly reduced ($P=0.04$) and remained low over the subsequent 24hrs (74.7±66.2µIU/mL). During the 24-h grazing periods, glucose was different by time and metabolic status ($P<0.01$).

Conclusion: Changes in pasture NSC content and grazing animal metabolic status can occur rapidly, necessitating repeated monitoring (especially insulin) and/or careful access restriction. There is significant individual variability in insulin responses to changes in pasture NSC in both ID and NID horses. Grazing on high pasture NSC can markedly increase insulin levels in NID horses. Drylots with a low NSC hay are effective in lowering insulin responses in most ID horses.

Acknowledgements: Funding was provided by Mars Petcare and the University of Kentucky, Department of Veterinary Science. The authors would like to acknowledge the Farm Staff at the C. Little Oran Research Farm for their support of this project.

A comparison of six immunoassays, including the Tosoh AIA-360, for quantification of insulin in equine samples

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Approved by the University of Pennsylvania Institutional Animal Care and Use Committee protocol number 807119.

Aims: Radioimmunoassay (RIA) is the gold standard for accurately quantifying equine insulin in blood samples; however, rapid turnaround is clinically desirable since hyperinsulinemia is an emergency in horses. We aimed to compare 5 different immunoassays to the RIA and to validate the Tosoh AIA-360 for quantification of insulin in equine serum.

Methods: A total of 100 blood samples from 83 patients hospitalized at the University of Pennsylvania's New Bolton Center, submitted to the clinical laboratory for routine insulin measurement (either resting or post carbohydrate challenge), were centrifuged, separated and frozen in aliquots for analysis using 6 methods: an RIA (Cornell Animal Health Diagnostic Center); an ELISA (Mercodia); three separate chemiluminescent assays (Immulite 2000 and Immulite 2000XPi [Siemens] and Cobas e 411 [Roche]) and an automated fluorescence enzyme immunoassay (AIA-360, Tosoh Bioscience). Pearson's correlation, Bland-Altman plots and Passing–Bablok linear regression were used to compare between methods. Precision of the AIA-360 was further evaluated by performing intra- and inter-assay replicates (10 each, 4 reagent lots) and linearity was determined with 5 serial dilutions of 5 separate samples using the manufacturer's diluent.

Results: Insulin concentrations measured using RIA ranged from 11.7-318 µIU/ml: reported analyses and results were limited to samples <100 µIU/ml (72 total), the important interpretive range. Correlation with RIA was high for the ELISA ($r=0.96$, $p<0.001$); Cobas e ($r=0.98$, $p<0.001$) and AIA-360 ($r=0.91$, $p>0.001$), but poor for the Immulite 2000 ($r=0.15$, $p=0.25$) and the Immulite 2000 XPi ($r=0.14$, $p=0.25$) in the <100 µIU/ml range. Bland-Altman bias (95% limits of agreement) was 31.3 (1.6-60.9) µIU/ml for the RIA vs Cobas and 16.7 (1.8 to 35.1) µIU/ml for RIA vs AIA-360. For AIA-360 the Passing–Bablok slope was 1.2 and the intercept 11.9 µIU/ml. The intra-assay CVs for the AIA-360 were 2.8%, for low (<20 µIU/ml) 1.3% for medium (20-80 µIU/ml) and 1.3% for high (>100 µIU/ml) insulin concentration samples, and the inter-assay CVs ranged from 2.4% to 8.6% in samples with concentrations ranging from 9.2 µIU/ml to 302 µIU/ml. The mean +/- s.d. recovery on dilution was 104 +/- 5.6%.

Conclusions: Correlation with the RIA was excellent for the ELISA, Cobas e411 and AIA-360. The large bias would hamper clinical use of the Cobas. The precision and linearity of the AIA-360 was excellent, and the assay is suitable for clinical use with appropriate reference ranges.

Repeatability of an oral sugar test and a meal tolerance test in insulin dysregulated horses and ponies

Kira Casén, Sanna Lindåse, Elin Svonni, Siv Hanche-Olsen, Johan Bröjer

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- The research was performed at the Equine Clinic at the Animal University Clinic, Swedish University of Agricultural Sciences, Sweden.
- All horse owners provided written informed consent and the trial protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden

Aims: Insulin dysregulation (ID) is commonly diagnosed using an oral sugar test (OST). Basal insulin sampling during the postprandial phase after feeding forage has gained increased interest as ancillary testing for ID, but the diagnostic technique has not been evaluated in horses. Therefore, the aim of the study was to investigate the repeatability of an OST and a standardized meal tolerance test (MTT) using forage.

Methods: Thirteen privately owned horses and ponies diagnosed with ID were enrolled (hereafter collectively referred to as horses). The horses were investigated at two consecutive occasions at the equine clinic with a 3-week washout period at home between investigations. The horses were allowed to adapt to the clinical environment for 2 days prior to investigations. On both visits, horses were subjected to a 300-minute OST (0.5 mL/kg Dansukker glucose syrup) (day 3) and a 300-minute MTT using the horses' own forage (0.4 kg on dry matter basis/100 kg of body weight) the next day.

Results: The overall insulin responses to the OSTs and MTTs respectively, did not differ between tests (Fig. 1). The coefficient of variations (CV) with 95% confidence interval for area under the plasma insulin concentration vs time curve (AUC) were 30.6% (23.3 – 36.5) and 25.9% (19.6 – 31.0) for the OST and MTT respectively. Median time to insulin peak concentration was 120 min for the first and second OST, and 150 min for the first and second MTT.

Conclusions: The repeatability of an OST using a higher dose of glucose syrup and a MTT based on forage is acceptable in ID horses and ponies. Recommended sampling time points are 120 minutes for the OST and 150 minutes for the MTT. The repeatability for the MTT is expected to increase for an individual horse if different batches of forages are used with repeated sampling.

Acknowledgements: Funded by the Swedish-Norwegian Foundation for Equine Research.

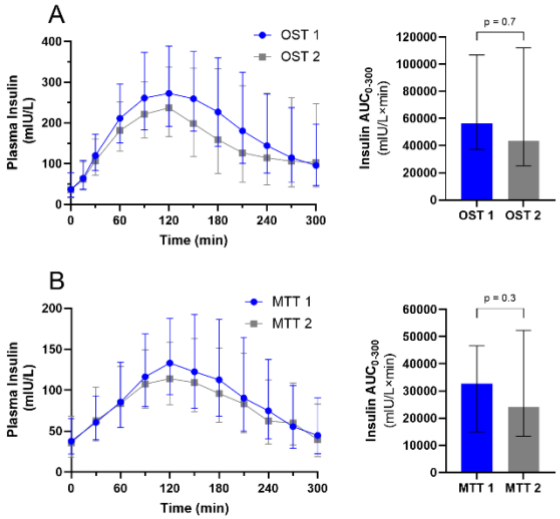

Figure 1. Plasma insulin concentration for the oral sugar test, (OST; Dan sucker glucose syrup 0.5 mL/kg body weight) (Figure 1A) and the meal tolerance test (MTT; 0.4 mg forage on dry matter basis per 100 kg body weight) (Figure 1B). Data are presented as geometric means with 95% confidence interval. The corresponding area under the plasma insulin concentration vs time curves are displayed to the right. Data are presented as median and interquartile range.

Relationship between serum and salivary insulin concentrations in horses with and without insulin dysregulation following an oral sugar test.

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Aims: To compare serum and salivary insulin responses of horses with and without insulin dysregulation (ID) following an oral sugar test (OST) using different analytical methods.

Methods: Fourteen adult, age-matched horses (14-27 years) of mixed breeds were categorized based on 60-minute (T60) post-OST insulin concentrations as ID (n = 7, T60 OST insulin > 100 μU/mL) and non-ID (n=7, T60 OST insulin <40 μU/mL). Horses were group housed on dry lots and forage plus grain-fasted in the morning of the study prior to the OST and sample collection. Initial blood and saliva samples were collected at T0, before performing an OST by administration of 0.15 mL/kg BW karo syrup, with subsequent samples collected at 60-, 90-, 120- and 180-minutes post-OST. Saliva was collected using a cotton swab (Salimetrics LLC) held in the horse's mouth under the tongue with a hemostat for 60 seconds. The horses' mouths were rinsed with 60 cc of water 10 minutes prior to the T60 saliva collection to remove any residual karo syrup. Serum insulin analysis was done via RIA (Cornell University) and automated immunoassay (AIA, Tosoh). Salivary insulin concentrations were determined through ELISA (Salimetrics) and AIA. Repeated measures ANOVA were performed to determine differences between time and ID status within each sample type and analytical method, with Bonferroni adjustments for multiple comparisons. Pearsons correlations coefficients were then calculated between different assays and sample types.

Results: Serum insulin concentrations were significantly elevated in ID vs non-ID horses at all timepoints ($P<0.05$), regardless of assay used. Peak serum insulin for ID horses post-OST was at T90 for both AIA (218.4 ± 50.0 μU/mL) and RIA (188.7 ± 42.1 μU/mL). There was a strong positive correlation between RIA and AIA for serum insulin concentrations ($r=0.98$, $P<0.001$, $Y = 1.093*X - 15.76$). Conversely, salivary insulin was highest in ID horses at T180 for AIA (316.3 ± 242.7 μU/mL) and ELISA (5982.6 ± 7002.6 pg/mL), with a difference between ID and non-ID horses only at T180 using ELISA ($P=0.04$). There was no correlation between salivary insulin and serum insulin for either method; however, the two methods for analyzing salivary insulin (AIA and ELISA) had a strong positive correlation ($r=0.97$, $P<0.001$).

Conclusions: The strong positive correlation between serum insulin concentrations measured by RIA and AIA methods indicates these methods are highly comparable. However, salivary insulin was not correlated with serum insulin and did not consistently differ between ID and non-ID animals, suggesting it may not be reliable as a standalone diagnostic for ID. Further research is needed to determine if salivary insulin could serve as a diagnostic tool for ID as there could be a delayed response past T180, although a further time delay might reduce the practicality of using saliva as a non-invasive diagnostic method.

Acknowledgements: Funding provided by MARS Equestrian™ and Dr. Adams' Department of Veterinary Science support.

This study was conducted at the University of Kentucky's Department of Veterinary Science Woodford Farm in Lexington, Kentucky, USA. It was approved by the Institutional Animal Care and Use Committee under protocol #2022-4063.

This material has not been presented or published.

Fibrosis scoring and comparison of histologic and ultrasonographic characteristics of the nuchal ligament adipose in non-obese and obese horses.

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Aims: Fibrosis of adipose tissue and adipocyte hypertrophy are considered key structural features of unhealthy adipose in multiple species, including horses. In human medicine, a fibrosis of adipose tissue (FAT) histologic scoring system has been validated as a predictor for weight loss resistance. No equivalent system exists in horses to standardize histologic assessment of equine adipose fibrosis. Similarly, stall-side measures to assess adipose phenotype are limited with no descriptions of the ultrasonographic changes associated with an unhealthy adipose phenotype. The current study aims to develop and evaluate interobserver repeatability of an equine fibrosis of adipose tissue (eFAT) histologic scoring system in nuchal adipose tissue. Additionally, correlations between the ultrasonographic characteristics of nuchal adipose and the histologic features of the same deposit, historic weight gain, body condition, and age were explored.

Methods: 10 university-owned mares were used for this study and were weighed monthly while under the same management conditions. Mares were subdivided as obese or non-obese, aged or mature, and having gained weight or remained static over the course of the study. Ultrasound images and punch biopsies of adipose tissue were collected from the nuchal adipose deposit for all mares. Biopsies were assigned equine fibrosis of adipose tissue (eFAT) scores by four observers, including an anatomic pathologist. Adipocyte diameter was measured manually and using an adipocyte measurement software (Adiposoft). Relative echogenicity and heterogeneity were assessed via histograms. Data was tested for normality and variables analyzed by Mann-Whitney U, Spearman rank correlation and Friedman rank tests as applicable. Significance was set at P=0.05.

Results: The eFAT scores showed excellent interobserver repeatability (P=0.353) but were not associated with age, body condition, or weight gain. Adiposoft performed poorly in the nuchal deposit. Adipocyte area was greater in horses with static weight (P=0.0159) and there was a negative correlation between adipocyte area and percentage weight gain (P=0.0202). Ultrasound image intensity was negatively correlated with crest depth (P=0.019). Standard deviation of ultrasound image intensity was negatively correlated with body condition score (P=0.019). Intensity was significantly higher in non-obese horses (P=0.0317). The mode of US image intensity was negatively correlated with the standard deviation of adipocyte size (P=0.049).

Conclusions: eFAT scoring offers a repeatable assessment of adipose fibrosis in the nuchal deposit but further evaluation was limited by sample size. Adiposoft should not be used in the nuchal deposit. Histologic and morphologic changes are associated with changes in nuchal adipose tissue ultrasonographic echotexture. Both eFAT and adipose ultrosonography may offer robust and repeatable tools for assessing the phenotype of equine adipose tissue. ↘

Acknowledgements: Funding for this project was provided through a grant from the University of Wisconsin-Madison Companion Animal Fund.

This abstract was presented at the 2024 American College of Veterinary Internal Medicine Forum in Minneapolis, MN on June 6, 2024 as an oral abstract titled Novel Fibrosis Scoring and Automated Histopathology Offer a OneHealth Approach to Equine Adipose Tissue Abnormalities and an associated abstract was published in the proceedings.

All animal use was approved by the University of Wisconsin – Madison Animal Care and Use Committee (protocol V006476-R01).

Effect of sirolimus on insulin dynamics in a dexamethasone-induced model of insulin dysregulation

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This study was approved by The University of Queensland Animal Ethics Committee approval number SVS/334/20.

Aims: A single intravenous dose of sirolimus suppresses the insulin response to oral glucose challenge in healthy horses. We aimed to assess the effect of 2 different oral sirolimus dosages on the insulin response to oral glucose challenge in horses with dexamethasone-induced insulin dysregulation.

Methods: Twenty-four healthy Standardbreds (3-18 years old, 422–598 kg) received 0.08 mg/kg of dexamethasone intramuscularly every 48 hours for 15 days. Starting on day 8, the horses were randomly divided into 3 groups to be treated with either sirolimus at 0.03 mg/kg *per os s.i.d* (SIRO3; n=8), sirolimus at 0.06 mg/kg *per os s.i.d.* (SIRO6; n=8) or a placebo (n=8) for a further 7 days, in addition to dexamethasone. An oral glucose test (OGT) was conducted on day 15. Glucose, insulin, total and active glucagon-like peptide-1 (tGLP-1 and aGLP-1), and glucose-dependent insulinotropic polypeptide (GIP) were measured at baseline and at intervals up to 240 min after OGT. Results were analyzed using a mixed-effects linear regression model.

Results: At the 15-day OGT both the area under the curve (AUC) and maximum concentration (Cmax) of insulin were decreased in SIRO6 compared to placebo (mean [95% confidence interval] effect size -7031.2 [-13299.5 to -762.9] mIU/mL*min, P=0.03; -43.9 [-86.1 to -1.6] mIU/mL, P=0.04). The AUC and Cmax of GIP were also both decreased in SIRO 6 compared to placebo (effect size -16897.4 [-25549.9 to -8244.9] pg/mL*min P<0.001; -76.1 [-132.6 to -19.6] pg/mL, P=0.008). Similar significant decreases in GIP were noted in SIRO3 vs. placebo; however, there were no significant differences between SIRO3 and placebo for the insulin measurements.

Conclusions: Oral sirolimus decreased the insulin response to carbohydrate in a dexamethasone-induced model of insulin dysregulation, but only at the 0.06-mg/kg-dose. Further evaluation of its effects in naturally occurring insulin dysregulation are warranted.

Acknowledgements: This work was funded by a grant from the Grayson Jockey Club Research Foundation

Long term treatment with canagliflozin in insulin dysregulated horses – preleminary results from an ongoing study evaluating efficacy and side effects in horses treated for 2 years

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Aims: Short-term treatment with SGLT2 inhibitors has shown to decrease the postprandial insulin response in insulin dysregulated (ID) horses, but there is a need for studies evaluating long-term treatment efficacy and side effects. The aim was to investigate the effects on postprandial insulin responses as well as the serum triglyceride (TG) and glutamate dehydrogenase (GLDH) concentrations in ID horses treated with canagliflozin for up to two years.

Methods: The study is an ongoing open-label, single-center study. Privately owned horses previously diagnosed with ID were included. Horses were subjected to an oral sugar test (OST) and a meal tolerance test (MTT) at baseline (BL) (n=16) and after one (n=16) respectively two years (n=9) of treatment with once daily canagliflozin (0.4 mg/kg).

Results: The postprandial insulin responses decreased with canagliflozin treatment and remained low for two years (Fig. 1A). For the OST, the average insulin responses were 29.3% and 37.6% of the BL response at one and two years respectively. The corresponding data for the MTT was 28.2% and 33.7%. There was no difference in GLDH concentrations at any time point compared to BL. Some individuals developed elevated TG concentrations during canagliflozin treatment (Fig. 1B) but showed no clinical signs of hyperlipemia.

Conclusion: Preliminary results from this ongoing study indicate that canagliflozin treated horses have sustained decrease in postprandial insulin responses over two years without severe side effects related to hypertriglyceridemia. Thus, canagliflozin is a promising drug for long-term treatment of ID horses.

Acknowledgements: The study was funded by the Swedish-Norwegian Foundation for Equine Research.

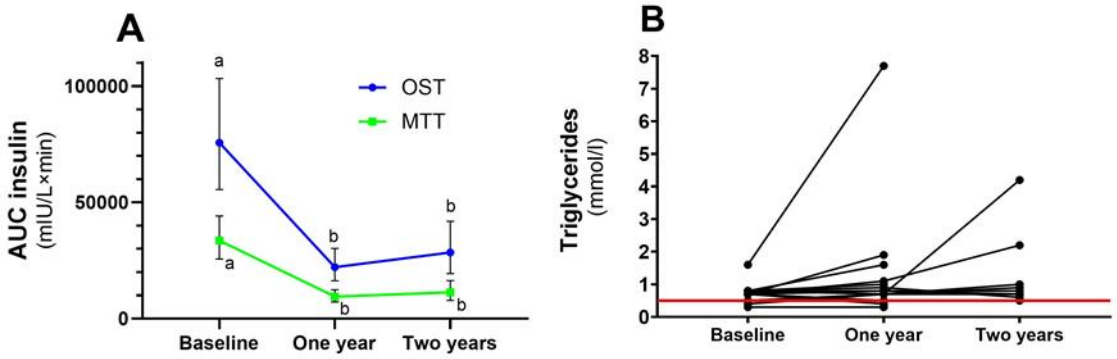

Figure 1. A Geometric least square mean insulin response (AUC_{0-240}) during an oral sugar test (OST) and a meal tolerance test (MTT) at baseline, and after one and two years of canagliflozin treatment. Different superscript letters indicate significant difference ($P < 0.05$) between time points within a diagnostic test (OST vs MTT). **B** Serum triglyceride concentrations for individual horses at baseline (n=16), and after one (n=16) and two years of treatment (n= 9), red line represents upper reference limit (0.5 mmol/L).

Canagliflozin in insulin dysregulated horses: sustained reduction in insulin response over 12 weeks with reduced dose of canagliflozin

Johan Bröjer, Elin Svonni, Katarina Nostell, Sanna Lindåse

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- The research was performed at the Equine Clinic at the Animal University Clinic, Swedish University of Agricultural Sciences, Sweden.
- All horse owners provided written informed consent and the trial protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Aims: Short-term treatment with SGLT2 inhibitors has shown to decrease the postprandial insulin response in insulin dysregulated (ID) horses. The aim of the study was to evaluate the postprandial insulin response during prolonged treatment (12 weeks) with a reduced dose of the SGLT2 inhibitor canagliflozin in ID horses and ponies.

Methods: Following a 4-week randomized, placebo-controlled, double-blind study at a single-center in Sweden evaluating the treatment effect of canagliflozin, participants were offered to participate in an open-label extension study for 12 weeks in which all participants received active treatment. Client owned horses and ponies previously diagnosed with ID within the last 6 months using an oral sugar test (OST) were enrolled. Horses were excluded if they were < 4 years of age, had an ongoing acute episode of laminitis, had PPID, had systemic disease other than ID or if they had been exposed to grass pasture during the last month before enrollment.

Horses and ponies were randomized to either once daily oral treatment with canagliflozin (CAN 0.6 mg/kg; n=10 or 1.2 mg/kg; n=10) or placebo (PBO; n = 10) (**Fig. 1**). The study consisted of an initial 5-day phase to obtain baseline data, a 24-day double-blind treatment phase at home and a 5-day follow-up phase similar to the initial baseline phase but with double-blind treatment. The study was extended with an open-label study for an additional 12 weeks where all participating horses received once daily active treatment (CAN 0.4 mg/kg) followed by a follow-up period similar to the initial baseline evaluation. Horses were subjected to an OST using Dan Sukker glucose syrup 0.5 mL/kg (day three) and a meal tolerance test (MTT) based on the horses' own forage (day four) on each 5-day evaluation period.

Results: Only per-protocol data are reported in the result section. Twenty-four horses completed the whole study. The geometric least square (LS) mean insulin responses (Insulin $AUC_{0\text{-}240}$) during the OST (**Fig. 2A**) and the MTT (**Fig. 2B**) decreased after 4 weeks of canagliflozin treatment (CAN 0.6 and CAN 1.2). After additional 12 weeks of treatment with a reduced dose of CAN (0.4 mg/kg) all treatment groups had comparable geometric LS mean insulin responses (Insulin $AUC_{0\text{-}240}$) during the OST and the MTT (Fig. 2A and 2B) as for the 4 week treatment period with canagliflozin (CAN 0.6 and 1.2).

Conclusion: Compared with placebo, canagliflozin (CAN 0.6 and 1.2) decreased the insulin response after 4 weeks of treatment. The treatment effect was sustained over 12 weeks of treatment with a reduced dose of canagliflozin (CAN 0.4).

Acknowledgements: Funded by the Swedish-Norwegian Foundation for Equine Research.

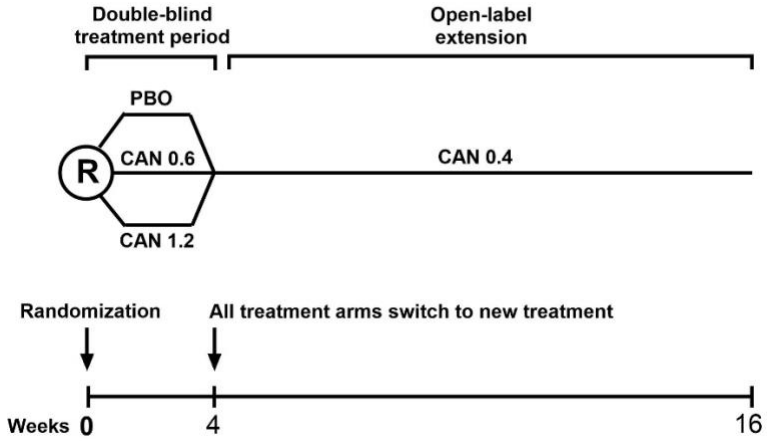

Figure 1. Overview of study design. PBO, placebo; CAN 0.6, canagliflozin 0.6 mg/kg once daily; CAN 1.2, canagliflozin 1.2 mg/kg once daily; CAN 0.4, canagliflozin 0.4 mg/kg once daily.

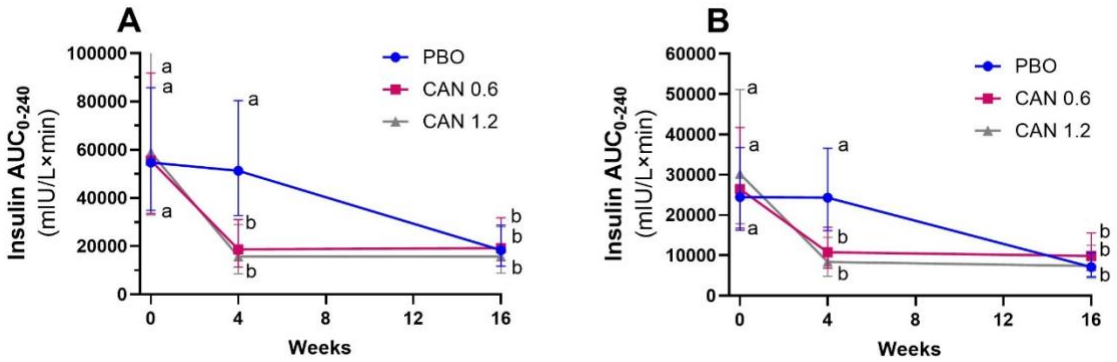

Figure 2. Geometric least square means and 95% confidence interval for the area under the insulin vs time curve at 0 to 240 minutes (Insulin $AUC_{0\text{-}240}$) during an (A) oral sugar test (Dan Sukker, 0.5 mL/kg PO) and during a (B) meal tolerance test (horses own forage 0.4 kg on dry matter basis per 100 kg body weight). PBO, once daily oral treatment with placebo during double-blind phase (n=10); CAN 0.6, once daily oral treatment with canagliflozin 0.6 mg/kg during double-blind phase (n=8); CAN 1.2, once daily oral treatment with canagliflozin 1.2 mg/kg during double-blind phase (n=6). Different superscript letters indicate significant difference ($P<0.05$) between time points or between treatment groups.

Blood pressure in horses with insulin dysregulation after short-term treatment with canagliflozin

Katarina Nostell, Sanna Lindåse, Elin Svonni, Johan Bröjer

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- The research was performed at the Equine Clinic at the Animal University Clinic, Swedish University of Agricultural Sciences, Sweden.
- All horse owners provided written informed consent and the trial protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden

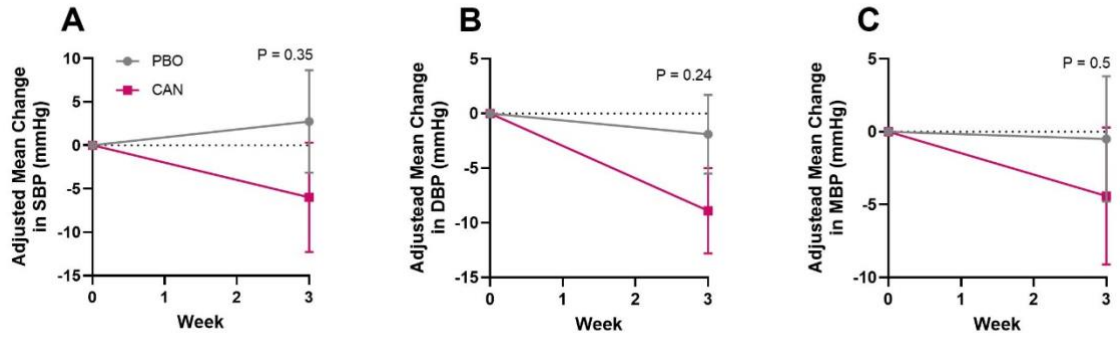
Aims: Short-term treatment with SGLT-2 inhibitors decreases blood pressure in people with type 2 diabetes. The aim was to investigate the impact of a 3-week treatment with canagliflozin (CAN) on blood pressure in insulin dysregulated (ID) horses and to study effects on electrolyte and fluid balance.

Methods: This was a single-centre, randomised, double-blind, placebo-controlled, parallel design study evaluating glucose and insulin responses during an oral sugar test. Here we report the effect of CAN treatment on blood pressure, fluid balance and serum electrolytes. Baseline data were collected over an initial 3-day period from 16 privately owned ID horses and ponies. On the morning of the third day serum electrolytes, serum proteins and PCV were analysed along with measurement of blood pressure using the HDO-technique. Horses were then randomly assigned to either once daily oral treatment with 0.6 mg/kg CAN or placebo (PBO) for a 3-week double-blind treatment period at home, which was followed by a 3-day follow-up period similar to the initial baseline period but with double-blind treatment.

Results: The systolic-, diastolic- and mean arterial blood pressure did not decrease significantly in CAN treated horses compared to PBO (Fig 1A-C). Plasma volume and electrolytes did not differ between treatments.

Conclusions: Compared to humans, reduction in blood pressure was not observed after short-term CAN treatment, but the study might have been underpowered to detect differences between treatments. Additionally, there were no transient changes in plasma volume or electrolytes as observed in humans with short-term treatment with CAN.

Figure 1. Adjusted least square mean differences with 95% confidence interval in blood pressure after treatment with canagliflozin (CAN) and placebo (PBO) for (**A**) systolic- (SBP), (**B**) diastolic- (DBP) and (**C**) mean arterial blood pressure (MAP). Values of P<0.05 are considered as statistical significant.



Acknowledgement: Funded by the Swedish-Norwegian Foundation for Equine Research.

Short-term metabolic responses following treatment with dapagliflozin or ertugliflozin in horses with hyperinsulinaemia.

Sundra, T.S, Knowles, E.J., Rendle, D.E., Kelty, E., Lester,G. and Rossi, G.

Aims: To: 1) report dapagliflozin use for the management of hyperinsulinaemia in client-owned equids and compare outcomes with similar cases treated with ertugliflozin. 2) report changes in lipoprotein profiles and other metabolic markers in cases treated with dapagliflozin or ertugliflozin .3) investigate whether plasma triglyceride concentration ([triglycerides]) after 30 days of SGLT2-inhibitor treatment are predicted by clinicopathological findings at days 0 or 7 or associated with other day-30 changes.

Methods: Analysis of clinical records and stored serum from equids with hyperinsulinaemia that received dapagliflozin (0.02mg/kg, (n=34)) or ertugliflozin (0.05mg/kg (n=24)) PO SID and from which blood was sampled pre-treatment (day 0) and after 7 and/or 30 days of treatment. Serum insulin concentration ([insulin]) was analysed by chemiluminescent immunoassay and routine biochemical parameters by automated colorimetric methods. The proportions of each major lipoprotein class (very-low, low- and high-density lipoprrpotein) were estimated using plasma electrophoresis and densimetry. Within-horse changes, correlations between biochemical analytes and differences between treatments were assessed using Wilcoxon signed-rank, Spearman's rank correlation coefficient (rho) and Mann-Whitney U tests, respectively.

Results: Between days 0 and 30 modified Obel lameness grade (scale 0-12) reduced in all treated horses from (median (inter-quartile-range)) 6 (4-10) to 2 (0-2) ($p<0.05$) and estimated body weight (kg) reduced from 476 (281-585) to 457 (255-553) ($p<0.05$). Selected clinicopathological changes are shown in Table 1 Differences between ertugliflozin and dapagliflozin groups in these parameters were not significant at days 0, 7 or 30 and pooled results are reported. Some parameters showed marked inter-individual variation. [Insulin] was lower than day 0 in all horses at day 7 and in 46/48 (96%) of horses on day 30 but increased between days 7 and 30 in 13/36 (36%) of horses with available data. Serum gamma glutamyl transferase (GGT) activity exceeded the laboratory reference range (LRR) in 7/58 (12%) horses on day 0 and 8/49 (16%) on day 30 (maximum 278 IU/ml), of which 4 were within the LRR on day 0. [Triglycerides] for all cases are shown in Figure 1. At day 30, 10/48 (21%) cases had [triglycerides] > 2.0mmol/l (maximum = 10.8mmol/l).
One dapgliflozin treated horse showed signs of inappetence and colic consistent with clinical hyperlipemia ([triglycerides] = 8.7mmol/l). Day 30 [triglycerides] was associated ($p<0.05$) with day 0: basal [insulin] (rho=0.47), [triglycerides] (rho=0.42) and %VLDL (rho=0.34), day 7: [triglycerides] (rho =0.45) and, day 30: [total cholesterol] (rho=0.67), %HDL (rho=-0.432) and %VLDL (rho=0.708). Complications were reported to dapagliflozin (n=13, 38.2%) and ertugliflozin (n=7, 29.2%) and included polyuria/polydipsia (n=12), lethargy (n=3) and intermittent diarrhoea (n=2).

Conclusions: Dapagliflozin is associated with metabolic changes including reductions in [insulin] and lameness comparable to ertugliflozin although further clinical and safety data are needed. Triglyceride and lipoprotein profile changes following SGLT2-inhibitor treatment were usually minor, with occasional marked hypertriglyceridemia. Metabolic changes were consistent with the mobilisation of adipose tissue and hepatic conversion to lipoproteins as the predominant pathway and ketogenesis as a minor pathway. Higher pre-treatment [insulin] and [triglycerides] were weakly associated with day 30 [triglycerides] and may inform clinical management.

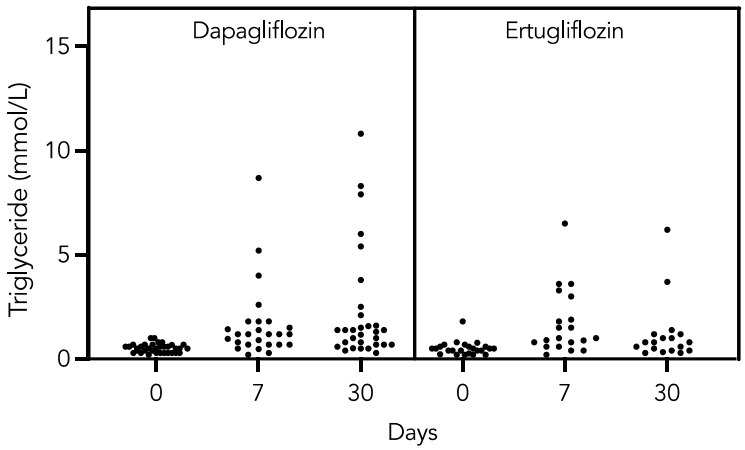
Acknowledgments: owners of included horses.

Table 1: Median (Inter-quartile range) values for clinicopathological markers on day 0 (immediately pre-treatment and days 7 and 30 of treatment. Day 7 or day 30 values that differ significantly from day 0 are bolded ($p<0.05$*, $p<0.01$**)

Analyte	Day 0	Day 7	Day 30
Insulin (uIU/ml)	170 (92-280)	**41.5 (24.8 - 131)****	**28.7 (14.5 – 90.0)****
Triglycerides (mmol/l)	0.5 (0.3-0.6)	**1.2 (0.7 - 1.8)****	**1.0 (0.6 - 1.56)****
Cholesterol (mmol/l)	2.36 (2-2.6)	**2.65 (2.28 – 3.11)***	**2.91 (2.49 – 3.69)****
B-HB (umol/l)	0.22 (0.17-0.27)	**0.267 (0.21 – 0.31)***	**0.30 (0.24 – 0.35)****
NEFA (mmol/l)	0.08 (0.05-0.13)	**0.23 (0.15-0.35)****	**0.17 (0.07-0.38)***
GGT (IU/ml)	21 (16.5-31.5)	23.0 (17 - 32.5)	**24.8 (18.4 – 38.0)***
%VLDL	11.2 (7.4-14.6)	**12.4 (9.55 – 16.4)***	**12.3 (9.88 – 16.8)****
% LDL	34.8 (28.7-40.7)	**40.5 (30.8 – 45.8)***	36.7 (29.5 – 44.3)
%HDL	50 (47.3-61)	**45.0 (38.4 – 51.7)****	**50.0 (41.0 – 54.8)***

B-HB= beta-hydroxy butyrate, NEFA = non-esterified fatty acids, GGT = gamma glutamyl transferase, VLDL= very-low-density lipoprotein, LDL = low-density lipoprotein, HDL = high-density lipoprotein.

Figure 1: Serum triglyceride concentrations for all included horses.



Short-term effects of canagliflozin on fasting- and postprandial glucagon concentrations in ID horses – results from a multi-center, randomized, double-blind, placebo-controlled, parallel-design study

Sanna Lindåse, Siv Hanche-Olsen, Elin Svonni, Katarina Nostell, Johan Bröjer

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- The research was performed at the Equine Clinic at the Animal University Clinic, Swedish University of Agricultural Sciences, Sweden.
- All horse owners provided written informed consent and the trial protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Aims: Treatment with SGLT2 inhibitors is associated with an increase in plasma glucagon concentrations in humans. The aim of this study was to compare short-term effects of the SGLT2 inhibitor canagliflozin (CAN) vs placebo (PBO) on fasting- and postprandial plasma glucagon concentrations in insulin dysregulated (ID) horses.

Methods: This was a randomized, double-blind, placebo-controlled, parallel-design study performed at two centers (Sweden and Norway). Privately owned horses diagnosed with ID were enrolled. Horses were randomized to either once-daily oral treatment with 0.6 mg/kg CAN (n=14), 1.2 mg/kg CAN (n=13) or PBO (n=13). The study consisted of a 5-day baseline sampling phase, a 24-day double-blind treatment phase at home and a 5-day follow-up sampling phase with double-blind treatment. Horses were subjected to a 300-minute oral sugar test (0.5 mL/kg Dansukker glucose syrup) on both clinical visits.

Results: Fasting glucagon concentrations and insulin/glucagon ratios differed between CAN treated horses (0.6 and 1.2 mg/kg) and PBO but no differences were found between the two CAN doses. Areas under the plasma concentration vs time curve (AUC) for glucagon were higher in horses treated with 1.2 mg/kg CAN compared to 0.6 mg/kg CAN and PBO whereas AUC insulin/glucagon ratios differed between all three groups (Figure 1).

Conclusions: Short-term CAN treatments increase fasting glucagon concentrations and induce a dose dependent postprandial hypersecretion of glucagon. This underscores the importance of identifying the lowest effective dose of CAN for treating ID in horses.

Acknowledgements: Funded by the Swedish-Norwegian Foundation for Equine Research.

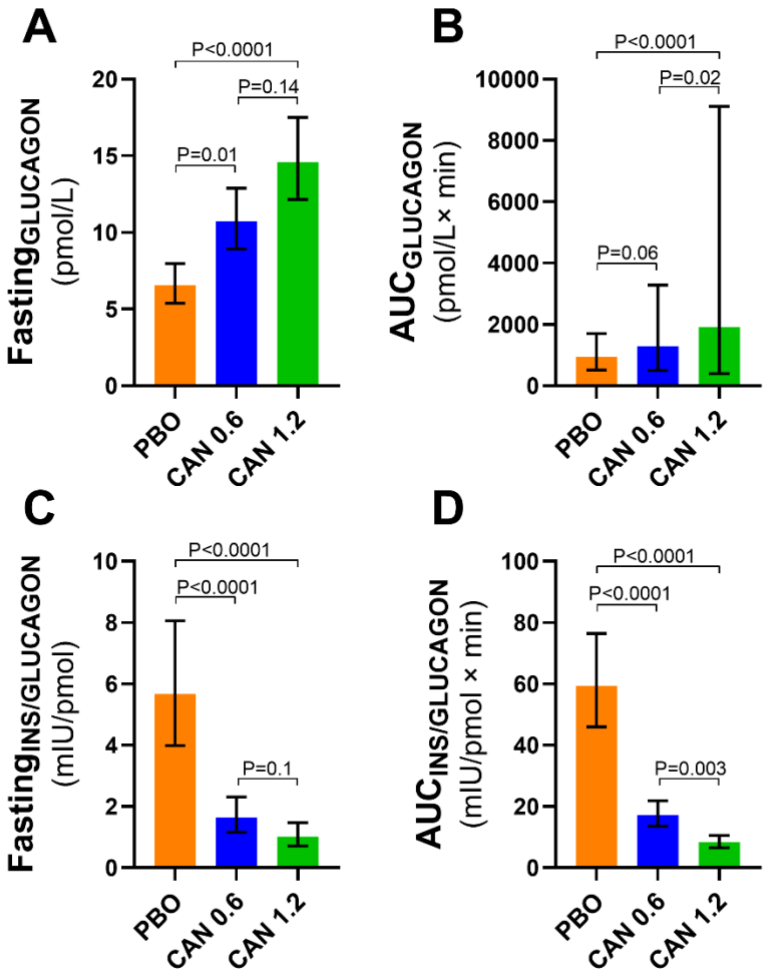

Figure 1. Comparison of fasting glucagon concentrations (A), oral sugar test derived glucagon responses (B) and insulin/glucagon ratios (C and D) in horses after a 4-week double-blind treatment phase with 0.6 mg/kg CAN (n=14), 1.2 mg/kg CAN (n=13) or PBO (n=13). Data are presented as geometric least squares means with 95% confidence interval. Values of P<0.05 were considered as statistical significant. AUC, area under the plasma concentration vs time curve; PBO, once daily oral treatment with placebo; CAN 0.6, once daily oral treatment with canagliflozin 0.6 mg/kg; CAN 1.2, once daily oral treatment with canagliflozin 1.2 mg/kg.

Effect of ertugliflozin, a sodium-glucose cotransporter-2 inhibitor, on glucose and insulin dynamics in healthy horses receiving dexamethasone

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The study was approved by the Institutional Animal Care and Use Committee (protocol #23690).

AIMS
The objective of the study was to evaluate the effect of ertugliflozin on insulin and glucose dynamics in healthy horses with concurrent dexamethasone administration. We hypothesized that dexamethasone would cause insulin dysregulation and that coadministration of ertugliflozin would mitigate dexamethasone-induced increases in glucose and insulin.

METHODS
This was a crossover randomized controlled trial utilizing 7 healthy research horses with normal baseline ACTH and Oral Sugar Tests (OSTs). Horses were randomly allocated to receive dexamethasone (0.06 mg/kg intravenously q48 hours) (DEX group), or ertugliflozin (0.05 mg/kg *per os* q24 hours) plus dexamethasone (SGLT-2 group) for 7 days. After a washout period, the groups were inverted. OSTs and Insulin Tolerance Tests (ITT) were performed before and after treatment. Additional timepoints were collected for glucose measurement during the OSTs for area under the curve (AUC_{Glu}) calculation. Whole blood glucose and insulin were analyzed using an AlphaTRAK 3 glucometer and Wellness Ready Insulin Tests, respectively. Insulin concentrations <20.0 µIU/mL or >99.9 µIU/mL were treated as 20 or 100 µIU/mL. Aliquots of serum from each timepoint were saved for analysis at a reference laboratory.

Statistical analyses were performed using commercial software, with a P-value <0.05 considered significant. Normal data are presented as mean ± standard deviation and non-normal as median (range).

RESULTS
Five geldings and 2 mares 13 ± 5 years of age were included. Preliminary results revealed increased basal insulin concentrations in both groups post-treatment (DEX pre-20 (20.0-21.9) µIU/mL versus post-treatment 37.9 (33.8-67.7) µIU/mL, P=0.02; SGLT2 pre- 20.9 ± 2.4 µIU/mL versus 36.2 ± 16.3 µIU/mL post-treatment, P=0.03). Basal glucose increased in both groups (DEX pre- 99.4 ± 8.4 mg/dL versus 124.3 ± 18.9 mg/dL post-treatment, P=0.006; SGLT2 pre- 95.9 ± 9.7 mg/dL and post treatment 117.6 ± 14.03 mg/dL, P=0.01). No differences in post-treatment insulin or glucose were found between the DEX and SGLT2 groups (P=0.23 and P=0.56, respectively).

There was no difference in the Δ $insulin_{T60-0}$ between baseline and post-treatment OST results within the DEX or SGLT2 groups (P=0.30 and P=0.08, respectively), There was no difference in post-treatment OST Δ $insulin_{T60-0}$ or in post-treatment OST AUC_{Glu} between DEX and SGLT2 groups.

The change in glucose concentrations from T=0 to T=30 min during the ITT decreased to 27.7 ± 10.4% after treatment in the SGLT2 group compared to 60.7 ± 9.6% at baseline (P=0.0004) and was lower than the DEX group ($T=30_{Post}$ 54.8 ± 6.1%; P<0.0001). There was no difference pre or post within the DEX group.

CONCLUSIONS

The preliminary results suggest induction of insulin dysregulation in healthy horses receiving dexamethasone at 0.06 mg/kg intravenously every other day based on basal insulin and glucose. However, the co-administration of ertugliflozin did not mitigate insulin dysregulation. This study was limited by small sample size and utilization of point-of-care assays. Additional study is warranted.

ACKNOWLEDGEMENTS

Grant support was provided by the UC Davis Center for Equine Health. The UC Davis Clinical Laboratory Services and the Cornell Animal Health and Diagnostic Center are acknowledged for sample analysis. Multiple students are thanked for their hard work.

Prospective cohort study of clinical and adrenocorticotropic hormone changes in healthy horses and pituitary pars intermedia dysfunction cases over a 12-month period

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The research reported in this abstract was undertaken at the Gluck Equine Research Center, University of Kentucky, USA. The study followed national and institutional guidelines for humane animal treatment. This research involved secondary analysis of data from a previous project for which ethical approval was granted by the University of Kentucky's Institutional Animal Care and Use Committee (IACUC #00708A2004). This abstract has not been presented previously.

Aim: To compare clinical signs and adrenocorticotropic hormone (ACTH) in healthy horses and horses with pituitary pars intermedia dysfunction (PPID) over a 12-month period.
Methods: Data from a cohort study evaluating the effect of season on thyrotropin-releasing hormone (TRH) stimulation tests (Adams *et al.*, 2023) were included in this secondary data analysis study. Sixty-three horses were assigned to control (n=17), subclinical PPID (n=21) and clinical PPID (n=25) groups, based on clinical history and endocrine tests. No horses received PPID treatment during the study. Clinical examinations, basal ACTH and TRH stimulation tests were performed monthly for 12 months. Semi-quantitative systems were used to score hypertrichosis, abnormal fat distribution and epaxial muscle wasting (0=normal to 3=severe) and body condition score (BCS) was recorded using the Henneke scale. Non-parametric methods were used to test statistical significance of differences in clinical and endocrine variables between months and between groups.
Results: Differences between groups were identified in every month for hypertrichosis score ($p \leq 0.01$) and percentage increase in ACTH from basal at 10 min ($p \leq 0.03$) and 30 min post-TRH ($p \leq 0.02$). The percentage of monthly examinations where hypertrichosis was observed (score ≥1/3, where 1 indicates regional hypertrichosis) was associated with PPID status ($p<0.001$): medians 17%, 42% and 92% for control, subclinical and clinical PPID groups, respectively. Scores for hypertrichosis (Fig.1), fat distribution, BCS and bodyweight differed between months in all three groups (all $p<0.001$). Hypertrichosis scores were higher, while fat distribution and BCS scores were lower, during winter/early spring. Weight loss was associated with PPID status ($p=0.04$): 65% of subclinical and 64% of clinical PPID cases lost weight over the study period compared to 29% of controls. Muscle wasting score differed between months in the subclinical PPID group ($p<0.001$), but not in the control or clinical PPID groups. Hypertrichosis score was positively associated with basal and TRH-stimulated ACTH concentrations (Fig.2). Percentage increase in ACTH from basal at both 10 min and 30 min post-TRH differed between months in all three groups (all $p<0.001$). Positive percent agreement between a positive TRH stimulation test at the start of the study and subsequent basal ACTH results remained high throughout the study for the clinical PPID group (≥86%) but varied across months in the subclinical PPID group (12-84%), with only 50% of these horses having a positive basal ACTH test at the end of the study.
Conclusions: This study has provided valuable information about PPID progression in untreated horses. Clinical monitoring may be complicated by seasonal changes in haircoat and fat accumulation that occur in both PPID cases and healthy horses. However, increasing severity of hypertrichosis was associated with increased ACTH. Muscle atrophy scoring may be particularly useful for monitoring progression in subclinical cases.
Acknowledgements: We thank the University of Kentucky Department of Veterinary Science farm crew and Dr Fernanda Cesar for their assistance and Morris Animal Foundation for funding the original study.

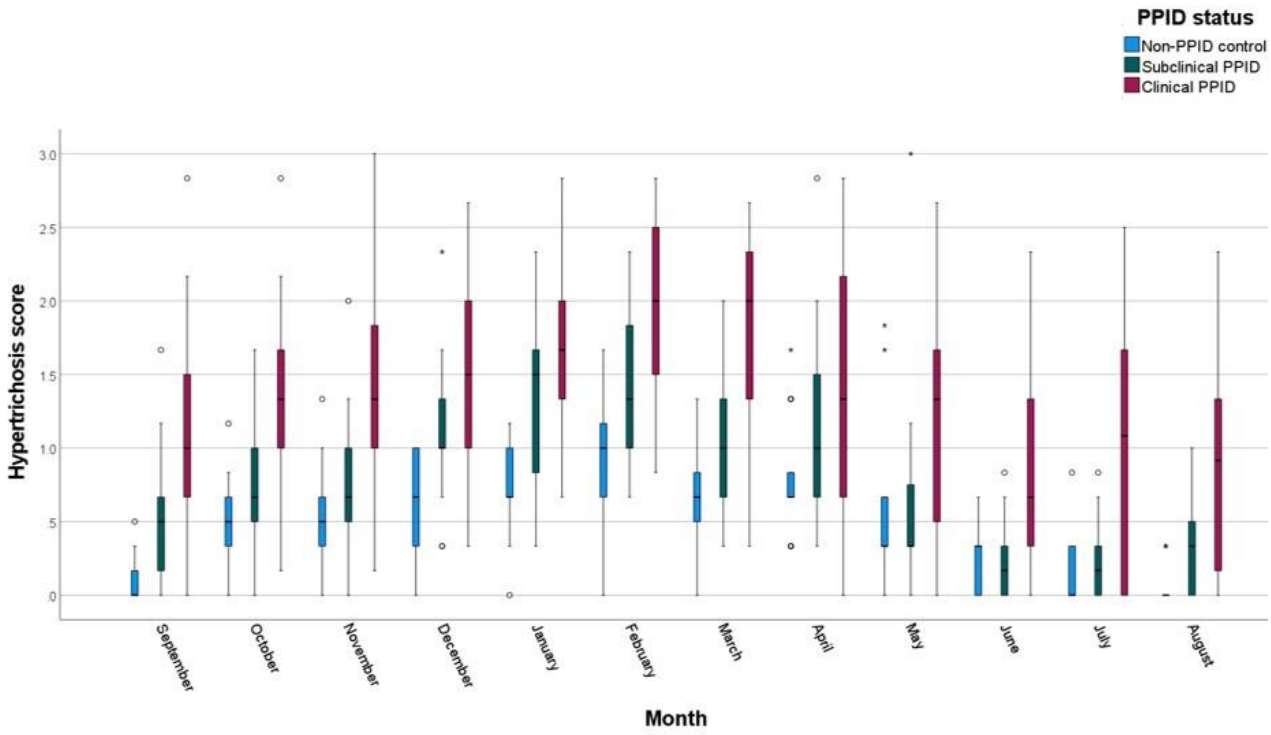

Figure 1: Box and whisker plot of hypertrichosis scores for each group over a 12-month period.

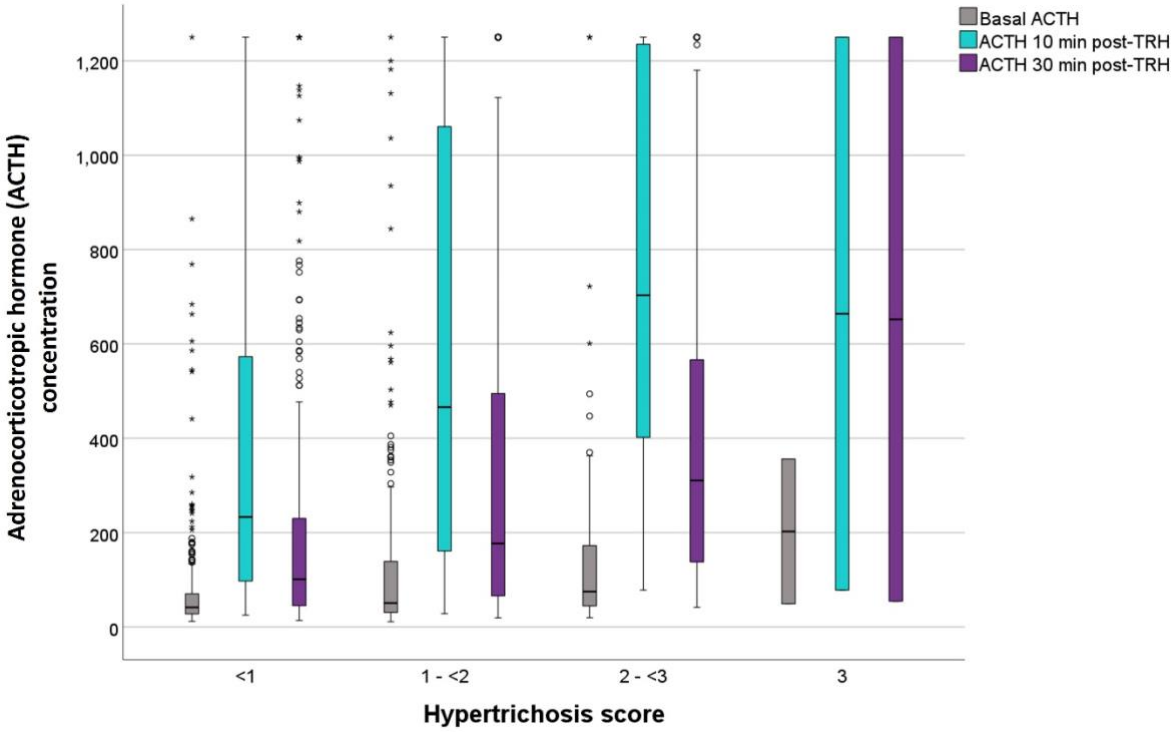

Figure 2: Box and whisker plot of ACTH concentrations by hypertrichosis score.

Characterization and comparison of fecal microbiota in age-matched control and horses with pituitary pars intermedia dysfunction

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Abstract

Aims: Parkinson's disease is associated with alterations of the fecal microbiota suggesting an involvement of the gut-brain axis in the development of the disease. Considering the similarities between pituitary pars intermedia dysfunction (PPID) and Parkinson's disease, this study aimed to investigate the effect of PPID on horse fecal microbiota.

Methods: Prospective control study involving 9 horses with PPID and 13 age-matched control horses. Fecal samples were collected bimonthly and microbial analysis was performed using 16S rRNA sequencing to determine the relative abundance at genus and phylum levels, assess alpha and beta diversity and identify core microbiota.

Results: Compared to control horses, horses with PPID (median [95% confidence interval]) showed decreased abundances of *Rikenellaceae* RC9 gut group (9.4 [8.3 – 10.4]% vs. 10.8 [9.6 – 11.7]%, P = 0.02), *Christensenellaceae* R-7 group (2.1 [1.8 – 2.3]% vs. 2.5 [2.4 – 2.8]%, P = 0.03), and NK4A214 group (0.51 [0.48 – 0.55]% vs. 0.56 [0.54 – 0.59]%, P = 0.03), and increased *Treponema* abundance (0.63 [0.54 – 0.69]% vs. 0.55 [0.42 – 0.60]%, P = 0.04). There was a significant effect of PPID on beta diversity (P = 0.004), while alpha diversity only varied with months (P = 0.001). Six unique genera were identified in PPID horses and 12 in control horses.

Conclusions: The fecal microbial composition is distinctly altered in horses with PPID and the changes appear similar to the ones described with Parkinson's disease. These findings highlight the significance of the microbiota-gut-brain axis in the pathogenesis of PPID and could suggest new methods for therapeutic intervention.

Acknowledgements: The Australian Companion Animal Health Foundation, UQ RTP Scholarship, Keith Mackie Lucas Scholarship.

Ethical animal research: The study protocol was approved by The University of Queensland Animal Ethics Unit (2022/AE000462)

Statement:
The abstract has not been published nor presented in other meetings previously.

Markers of calcium metabolism in horses and ponies with and without pituitary pars intermedia dysfunction.

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This study used archived samples; an animal ethics approval was waived by Purdue University Animal Ethics Committee.

Aims: Calcium is crucial for dopamine release, and dysregulation of calcium metabolism markers like parathyroid hormone (PTH) and 25-hydroxyvitamin D, along with low calcium levels, are identified as Parkinson's disease risk factors. Given the connection between Parkinson's in humans and pituitary pars intermedia dysfunction (PPID), this study aims to assess calcium metabolism markers in horses.

Methods: Archived serum samples from 45 client-owned horses and ponies (ages 5-28) were retrieved. PPID diagnosis was based on clinical signs and seasonally adjusted ACTH concentrations for resting or post-thyrotropin-releasing hormone stimulation tests. Ionized calcium, PTH, and 25-hydroxyvitamin D levels were compared between PPID and control groups, considering season (fall vs. non-fall) and breed-type (pony vs. horse), with significance set at p=0.05.

Results: Thirty animals were classified as PPID (16 ponies), and 15 as controls (5 ponies) with 10 animals sampled in the fall, 35 in other seasons. A significant correlation between ACTH and ionized calcium was detected in healthy animals (r=0.72 [95% CI: 0.25-0.92], p=0.008), but not in those with PPID (p=0.6). Ponies with PPID had higher PTH levels than horses with PPID (effect size: +8.7 ± 3.9 pmol/L, p=0.03). PTH concentrations tended to be higher in fall than in other seasons (+18.18 ± 10.42 pmol/L, p=0.05). A significant correlation between ACTH and 25-hydroxyvitamin D was detected in healthy animals (r=0.60 [95% CI: 0.03-0.87], p=0.04), but not in those with PPID (p=0.9). Ponies with PPID also had higher 25-hydroxyvitamin D concentrations in fall than in other seasons (+5.5 ± 3.1 nmol/L, p=0.04).

Conclusions: Despite limitations from small sample size and breed/seasonal effects, trends in calcium dysregulation associated with PPID were observed. Calcium might play a role in PPID pathophysiology, warranting further investigation.

Evaluation of plasma catecholamine concentrations in older horses with or without pituitary pars intermedia dysfunction

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Aims:
Dopamine (DA) is both a neurotransmitter and a hormone. It has several functions within the central (CNS) and peripheral nervous systems and is the precursor of adrenaline (A) and noradrenaline (NA). In horses and ponies with pituitary pars intermedia dysfunction (PPID), dopaminergic neurons are degenerated, hypothalamic control over this part of the pituitary gland is impaired, and hormones derived from pro-opiomelanocortin are overproduced. Plasma concentrations of DA have been reported to be lower in horses with PPID than in those without PPID; however, the methodology was not ideal and other catecholamines were not measured. The aims of this study were to measure plasma catecholamine concentrations in horses and ponies with and without PPID, using a more robust methodology (high performance liquid chromatography with electrochemical detection; HPLC-ECD), and to explore the relationship between plasma DA and plasma adrenocorticotropic hormone (ACTH) concentrations in these animals.

Methods:
Thirty-two horses and ponies aged >15 years were evaluated. Clinical signs were recorded and a standard thyrotropin releasing hormone (TRH) stimulation test was performed to classify individuals as PPID or non-PPID. Catecholamines (DA, A and NA) were quantified in plasma using HPLC-ECD (Chromsystems), and ACTH was measured by chemiluminescent immunoassay (Immulite 1000). Data were evaluated for normality, and values were log transformed prior to analysis. Plasma DA and ACTH concentrations were compared using Pearson's correlation. Plasma NA or A concentrations that were below the limit of detection (LOD) were assigned half the LOD value to enable statistical comparison. Plasma catecholamine concentrations were compared between PPID and non-PPID animals using an unpaired *t*-test or Mann-Whitney test. Statistical significance was set at $p < 0.05$.

Results:
Nineteen animals were classified as non-PPID and 11 as PPID only. Two were classified as PPID with concurrent diabetes mellitus and were excluded from further analysis. The PPID group had lower plasma concentrations of DA (median, 106 pg/ml [range 26 - 202] vs 141 pg/ml [8.1-374], $p = 0.037$) and NA (median, 4.7 pg/ml [range 4.7 - 92.1] vs 21 pg/ml [4.7 - 195], $p = 0.013$), whereas A did not differ between the groups ($p = 0.67$). There was a moderate negative correlation between plasma DA and baseline ACTH concentrations ($p = 0.04$, $r^2 = 0.47$).

Conclusions:
Plasma DA concentrations were lower in horses with PPID compared with horses of a similar age without PPID. Plasma NA concentrations were also lower in individuals with PPID compared to those without PPID. The correlation between plasma DA and ACTH indicates that the reduction in dopaminergic activity in the CNS is mirrored in the periphery. These findings demonstrate that plasma catecholamine concentrations are lower in animals with PPID, and this could help to explain some of the clinical signs typically attributed to PPID, such as alterations in demeanour.

Acknowledgements:
Funded by Waltham Petcare Science Institute in collaboration with The University of Melbourne.

Ethics Statement:
All studies were conducted according to the Australian Code for the Care and Use of Animals for Scientific Purposes, with the approval of the University of Melbourne Animal Ethics Committee.

Abstract Information

- The format for the abstract should be as follows: Aims, Methods, Results, Conclusions, Acknowledgements.

- The abstract must be written and presented in English. Use single line spacing and Times New Roman 10-point font; do not indent paragraphs. Simple tables, graphs and figures may be included. Abstracts must not exceed ***500 words*** excluding all titles, tables and figures.

- Closing date of submissions ***(15th August)***

Influence of breed, season and pituitary pars intermedia dysfunction on plasma β-endorphin concentrations in horses and ponies

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Aims: Plasma adrenocorticotropic hormone (ACTH) concentration is routinely measured for the diagnosis and monitoring of pituitary pars intermedia dysfunction (PPID). However, interpretation of results can be challenging due to variability with factors such as breed and season. β-endorphin is a peptide hormone produced by the pituitary pars intermedia from cleavage of pro-opiomelanocortin. Factors that can alter plasma β-endorphin concentrations have not been well characterised. This study aimed to evaluate the influence of breed, season and PPID status on plasma β-endorphin concentrations in horses and ponies.

Methods: The study population comprised healthy age-matched Thoroughbred horses (n=40), Shetland ponies (n=40) and Welsh ponies (n=40), plus Welsh ponies with PPID (n=20), located in Victoria, Australia. Paired blood samples were collected from all animals within 2 weeks of the autumn equinox and subsequent spring equinox. Plasma ACTH and β-endorphin concentrations were determined using chemiluminescent immunoassay and radioimmunoassay, respectively. Data were log transformed and analysed using a two-way ANOVA for repeated measures, to evaluate the effects of breed and season, with P<0.05 considered significant.

Results: Mirroring seasonal differences in ACTH concentrations, higher β-endorphin concentrations were found in autumn compared to spring (P<0.001). Breed differences were also apparent among healthy horses and ponies, with higher autumnal β-endorphin concentrations in Shetland ponies compared with Thoroughbred horses (P<0.001) and Welsh ponies (P<0.001), and higher in Welsh ponies compared with Thoroughbred horses (P<0.001). Welsh ponies with PPID demonstrated markedly higher β-endorphin concentrations compared with healthy Welsh ponies in both autumn and spring seasons (P<0.001).

Conclusions: Higher plasma concentrations of β-endorphin were found in ponies with PPID compared with healthy ponies. Seasonal and breed effects were observed in healthy horses and ponies, with higher plasma concentrations of β-endorphin during autumn versus spring and in pony breeds versus Thoroughbred horses. Whether measurement of plasma β-endorphin concentration could assist in the diagnosis and monitoring of PPID warrants further investigation.

Acknowledgements: Funded by a University of Melbourne Early Career Researcher grant. The authors thank Alix Rao (University of Melbourne Assay Centre) and Fiona Armour (U-Vet Clinical Pathology Service) for performing laboratory assays.

Animal ethics: This study was approved by the University of Melbourne Animal Ethics Committee in compliance with applicable national laws (ID: 1914774.1).

Preliminary validation of point-of-care assay for equine adrenocorticotropic hormone (ACTH)

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This work was conducted under the direction and oversight of the University of Georgia College of Veterinary Medicine Institutional Animal Care and Use Committee to ensure compliance with institutional and national guidelines for humane animal treatment.

This abstract/research has not previously been presented.

Aims. Quantification of plasma adrenocorticotropic hormone (ACTH) concentrations is currently the primary diagnostic test for equine Pituitary Pars Intermedia Dysfunction (PPID). Accurate equine ACTH measurement typically requires careful sample handling, timely separation of plasma, and overnight shipment of chilled plasma to an outside lab for analysis. A point-of-care (POC) assay for equine ACTH (eACTH) recently became commercially available, which could address some of these concerns, but this test has not been independently validated. Thus, our **objective** was to compare the POC-eACTH assay against an accepted chemiluminescent immunoassay used globally for equine ACTH measurement. **We hypothesized that the POC-eACTH assay would show acceptable agreement with concentrations obtained using the chemiluminescent immunoassay in horses with and without previously diagnosed PPID.**

Methods. Twenty-eight mixed-breed horses aged 9-35 years were used. Thirteen horses had a previous diagnosis of PPID based on clinical signs and resting or TRH-stimulated ACTH concentrations and were being treated with pergolide; the remaining 15 animals were classified into a non-PPID group based on previous ACTH concentrations, age, and absence of clinical signs of PPID. EDTA-anticoagulated blood was collected from all animals on the same days in February, April, and July, and ACTH concentrations were assessed each month in fresh, chilled plasma by both the POC-eACTH assay and via the chemiluminescent immunoassay. Bias of the POC system was assessed using Spearman correlation analysis and Bland-Altman plots.

Results. For the entire data set, there was good association between the POC-eACTH and chemiluminescent immunoassay results (r = 0.91, P < 0.001), with the POC-eACTH reading an average of 9.2 pg/ml lower than the chemiluminescent immunoassay (bias -9.2 ± 40.9 pg/ml). When data from non-PPID animals were examined separately, comparably good association between the two methods (r = 0.93, P < 0.001) was found and bias improved by 53% (4.9 ± 7.9 pg/ml). In contrast, when animals with PPID were evaluated separately, association between the two methods remained strong (r = 0.89, P < 0.001) but the bias increased by 280% (-25.5 ± 55.4 pg/ml). When data from each sample month was examined separately in the entire study population and in PPID horses alone, association between the POC-eACTH and chemiluminescent immunoassay remained significant and strong (r = 0.83 – 0.96, P < 0.001 for all comparisons).

Conclusions. Overall, there was good overall agreement between the POC-eACTH and the chemiluminescent immunoassay in both non-PPID and PPID animals and over the winter, spring, and summer months, though assay bias was increased in PPID animals. Some individual animals having very disparate results between the two methods. Further evaluation of the linearity and precision of the POC-eACTH assay in equine plasma samples, and of the agreement between the POC-eACTH assay and the chemiluminescent immunoassay during the autumn months and in PPID animals is warranted to further assess the clinical utility of this new platform.

Acknowledgements: We would like to acknowledge the UGA CVM equine farm personnel for their assistance with animal care and handling for this work, and Zomedica® Corporation for funding support.

Feeding Grain Before TRH-Stimulation Did Not Affect ACTH, T3 or T4 Concentrations in Horses

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Aims: The thyrotropin releasing hormone (TRH) stimulation test is a dynamic test for PPID. Published studies have suggested that horses should not be fed grain within 12 h of testing but have not evaluated TRH testing in grain-fed vs hay-only in PPID positive (PPID+) and negative (PPID-) horses. The hypothesis was that feeding grain and its associated insulin response would not affect ACTH before (T0-ACTH), 10 min after (T10-ACTH), or thyroid hormone (T3 and T4) responses after TRH-stimulation.

Methods: Randomized prospective study. Six PPID+ and 6 PPID- adult horses were used. The protocol was approved by the Institutional Animal Care and Use Committee. All horses were fed prairie grass hay as the base diet, or hay plus grain concentrate meeting NRC requirements, in a crossover design with a 2-week testing interval. Previous work in this laboratory found consistent basal ACTH and TRH-stimulation results at 2-week intervals and peak postprandial insulin 2 h after feeding. Thus, 2 h after eating Hay-only or Hay+Grain diet, blood samples were collected for T0-ACTH, insulin, serum T3 and T4. The TRH-stimulation used 1 mg TRH i.v. Blood samples were collected 10 min after TRH-stimulation for T10-ACTH diagnostic for PPID, then 120 and 240 min later for T3 and T4 analysis, respectively. Horses with T0-ACTH>35 pg/mL and T10-ACTH>110 pg/mL were considered PPID-positive. Greater than 1.5-fold increases in T3 and T4 concentrations were considered evidence of thyroid gland response to TRH-stimulation and excluded primary hypothyroidism. A mixed model with repeated measures analyzed the effect of grain feeding on T0-ACTH, T10-ACTH, postprandial insulin, T3 and T4.

Results: Postprandial insulin was higher (P = 0.008) when horses were fed Hay+Grain (47.2 ± 4.0 µIU/mL) compared to Hay-only (15.7 ± 1.9 µIU/mL). PPID status did not affect insulin within diet (P = 0.94). Compared to Hay-only, Hay+Grain did not affect T0-ACTH (P=0.22), T10-ACTH (P=0.09), or the percent increase in ACTH (P=0.18) in PPID- or PPID+ horses. No change in PPID classification was observed comparing grain-fed vs hay-only in PPID- or PPID+ horses. Postprandial thyroid hormone concentrations before TRH-stimulation were 43.8 ± 4.3 ng/dL for T3 and 1.06 ± 0.14 µg/dL for T4. Thyroid hormone concentrations indicated normal responses to TRH-stimulation, with post-TRH peaks in T3 (224 ± 14.5 ng/dL) at 120 min and T4 (2.36 ± 0.14 µg/dL) at 240 min. Compared to PPID+ horses, PPID- horses had higher T3 (P = 0.012) and T4 (P = 0.003), but diet had no effect (P = 0.22) on T3 or T4.

Conclusions: Based on these results, despite higher postprandial insulin response, with no effect on ACTH or thyroid hormone response, horses may be fed grain prior to TRH stimulation testing for PPID and thyroid evaluation.

Acknowledgments: This study was supported by Boehringer Ingelheim Animal Health USA, Duluth, GA, and the John C. Miller Chair of Equine Reproductive Physiology.

The effect of pergolide mesylate on blood thyroid concentrations and thyroid function in horses

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This study was approved by Purdue University Animal Ethics Committee approval number 1222002329.

Aims: Pergolide is labelled for the treatment of pituitary pars intermedia dysfunction (PPID) and is over 90% protein bound. As such, it could cause a decrease in thyroid hormone concentrations by displacing them off circulating proteins. The aim of the study was to determine the effect of pergolide administration on the thyroid function of horses.

Methods: Six light breed horses (17–24 years old, 530–599 kg) received 1 mg of pergolide mesylate orally once a day for 5 days. Total thyroxine (tT4) was measured daily from day 0 to 11 (before, during and after pergolide treatment). Thyrotropin-releasing hormone (TRH) stimulation tests were conducted on days 0 and 6. Total triiodothyronine (tT3), tT4 and free thyroxine by equilibrium dialysis (fT4) were measured at baseline, 2 hours (tT3) and 4 hours (tT3, T4 and fT4) after TRH administration. Effect of pergolide administration on thyroid hormone concentration was determined by analyses of variance, with $p < 0.05$ considered significant.

Results: No effect of pergolide administration was detected on tT4 during and after treatment ($p = 0.4$). Administration of TRH resulted in a significant increase in tT3 (size effect: +165.8 [95% confidence interval (CI): 109.4-222.2] ng/dL, $p<0.0001$), tT4 (+1.162 [95% CI:0.7135-1.610] µg/dL, $p<0.0001$), fT4 (+1.195 [95% CI: 0.7195-1.670] µg/dL, $p<0.0001$). There was, however, no significant effect of pergolide on any thyroid hormone (tT3, $p=0.2$, tT4: $p=0.7$, fT4: $p=0.5$).

Conclusions: Pergolide has no detected effect on thyroid hormone concentrations and thyroid gland function in horses. Protein-bound agents do not necessarily affect blood T4 concentrations.

Farrier and other hoof care providers' awareness of, and approaches, to equine endocrine diseases

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The research reported in this abstract was undertaken at the University of Liverpool, United Kingdom. The study did not include animal participants, therefore international, national, or institutional guidelines for humane animal treatment are not applicable. Informed consent was obtained from all participating farriers and hoof care providers. The study was granted ethical approval by the University of Liverpool's Committee on Veterinary Research Ethics (reference number VREC1406). This abstract has not been presented previously.

Aims: Farriers and other hoof care providers (HCPs) play a vital role in treating endocrinopathic laminitis, but information about their approaches is limited. This study aimed to explore farrier/HCPs' understanding, and involvement in management, of equine endocrine disease.
Methods: A questionnaire, adapted from a previous study (Sanchez-Londoño, 2023), was designed using JISC Online Surveys. It included predominantly close-ended questions regarding participants' current role, awareness and knowledge of endocrine diseases and approach to managing laminitis. Study invitations were emailed directly to members of The Farriers Registration Council and four equine podiatry/natural hoof care associations and shared by the British Farriers & Blacksmiths Association and Equine Podiatry Association via social media.
Results: 189 responses were received, 80.4% of which were from farriers, and 97.4% of respondents were UK-based. Median farriery/hoof care experience was 20 years. All respondents were familiar with the term equine metabolic syndrome (EMS) while 77.8% were familiar pituitary pars intermedia dysfunction (PPID) and 77.2% were familiar with endocrinopathic laminitis. Familiarity with PPID was associated with profession (100% of HCPs compared to 72.5% of farriers; $p<0.001$), and 97.6% of respondents unfamiliar with PPID (n=41/42) were familiar with the term Equine Cushing's disease. Signs most frequently considered to be clinical signs of PPID were abnormal hair coat (91.0%), delayed/incomplete shedding (86.2%) and acute laminitis (66.1%), while respondents most frequently considered cresty neck (83.6%), obesity (80.4%) and acute laminitis (73.5%) to be signs of EMS. Most respondents were very likely to recommend clients sought veterinary attention in horses with signs of PPID (73.5%) or EMS (73.4%), specifically for endocrine tests (mentioned by 36 respondents in free text responses), and other recommendations included dietary (55.0%) and exercise (16.2%) advice. Trimming (66.7%), frog support with pads (66.1%) and heartbar shoes (56.6%) were the most commonly used approaches in laminitis cases and 58.7% would not change their approach in cases diagnosed with PPID and/or EMS. Approaches were associated with profession: only farriers used nail-on shoeing methods and pour-in sole support, more farriers used frog support than HCPs (77.5% and 18.9%, respectively) while more HCPs than farriers used hoof boots (86.5% and 28.5%, respectively) (all $p<0.001$). 55.3% of respondents recommended rechecks within 4 weeks for laminitis cases, and 49.5% estimated that 76-100% of their clients followed their recommended recheck frequency, rating overall client adherence to hoof care recommendations in horses with PPID and/or EMS as excellent (14.9%) or very good (36.2%). Most respondents worked in conjunction with a veterinarian (always 20.2% or very often 45.3%), with radiographs (94.7%) and pain management (90.5%) the veterinary procedures most frequently considered to be important in the management of horses with laminitis.
Conclusions: Awareness of EMS and PPID amongst farriers and HCPs was high. Although diagnosis of an endocrine disease did not alter hoof care approaches by the majority of respondents, they valued veterinary involvement for diagnosis and treatment of the underlying endocrine cause of laminitis.
Acknowledgements: We gratefully acknowledge participating farriers and HCPs, and the BFBA and EPA for assistance with survey dissemination.

Insulin and glucose concentrations increase similarly post intra-articular injection of triamcinolone acetonide in metabolically normal and Pars Pituitary Intermedia Dysfunction horses

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The research was performed at the same address as the presenting author.

This work follows international, national, and institutional guidelines for humane animal treatment and complies with relevant legislation in the country in which the study was conducted.

This work has not been presented at any state, national or international conferences.

Aims: Corticosteroids are a commonly used, inexpensive intra-articular (IA) treatment for osteoarthritis which may increase the risk for laminitis in horses due, in part, to hyperinsulinemia. Horses without insulin dysregulation experience increases in insulin and glucose post-injection, but responses in horses with Pars Pituitary Intermedia dysfunction (PPID) remain unknown. The aim of this study was to determine the effect of a single intra-articular (IA) dose of triamcinolone acetate (TA) on blood insulin and glucose concentrations in metabolically normal horses compared to those with PPID. This study hypothesized that there would be a greater increase in insulin and glucose in PPID horses than metabolically normal horses and that this increase would take longer to return to baseline.

Methods: Five metabolically normal horses and eight PPID horses without insulin dysregulation (as assessed by an oral sugar test) received 18 mg of TA into one middle carpal joint. PPID status was determined by a Thyrotropin Releasing Hormone stimulation test. Insulin and glucose concentrations were evaluated at baseline and 4, 6, 8, 24, 48, and 72 hours following IA corticosteroid injection. Normality was assessed using a Shapiro-Wilk Test. Differences from baseline for each group were evaluated using a repeated measures ANOVA with Dunnett's multiple comparison testing, differences between groups were evaluated using a two-way repeated measure ANOVA (significant at $P<0.05$).

Results: In metabolically normal horses, blood insulin concentration post IA TA injection was elevated at 6hr ($P=0.005$), 8hr ($P=0.01$), and 24hr ($P=0.002$) compared to baseline, with the peak at 48hr (Figure 1A). Blood glucose concentration post IA TA injection in metabolically normal horses was elevated at 6hr ($P=0.03$), 24hr ($P=0.002$), and 48hr ($P=0.05$) compared to baseline, with the peak at 24hr (Figure 1B). Blood insulin concentration post IA TA injection in PPID horses was elevated at 6hr ($P=0.01$), 8hr (0.04), 24hr ($P=0.003$), 48hr ($P=0.03$), and 72hr ($P=0.03$) compared to baseline, with the peak at 48hr (Figure 2A). Blood glucose concentration post IA TA injection in PPID horses was elevated at 6hr ($P=0.02$), 8hr ($P=0.004$), 24hr ($P=0.0001$), 48hr ($P\leq0.0001$) and 72hr ($P=0.0007$) compared to baseline, with the peak at 24hr (Figure 2B). There was no difference in insulin (Figure 3) or glucose (Figure 4) concentrations post IA TA injection between metabolically normal and PPID horses at any timepoint.

Conclusions: Insulin and glucose concentrations modestly increase in both metabolically normal and PPID horses. PPID horses experience longer increases in insulin and glucose than metabolically normal horses; however, this increase is not greater than those experienced by metabolically normal horses.

Acknowledgement: This study was funded by the Michigan Alliance for Animal Agriculture.

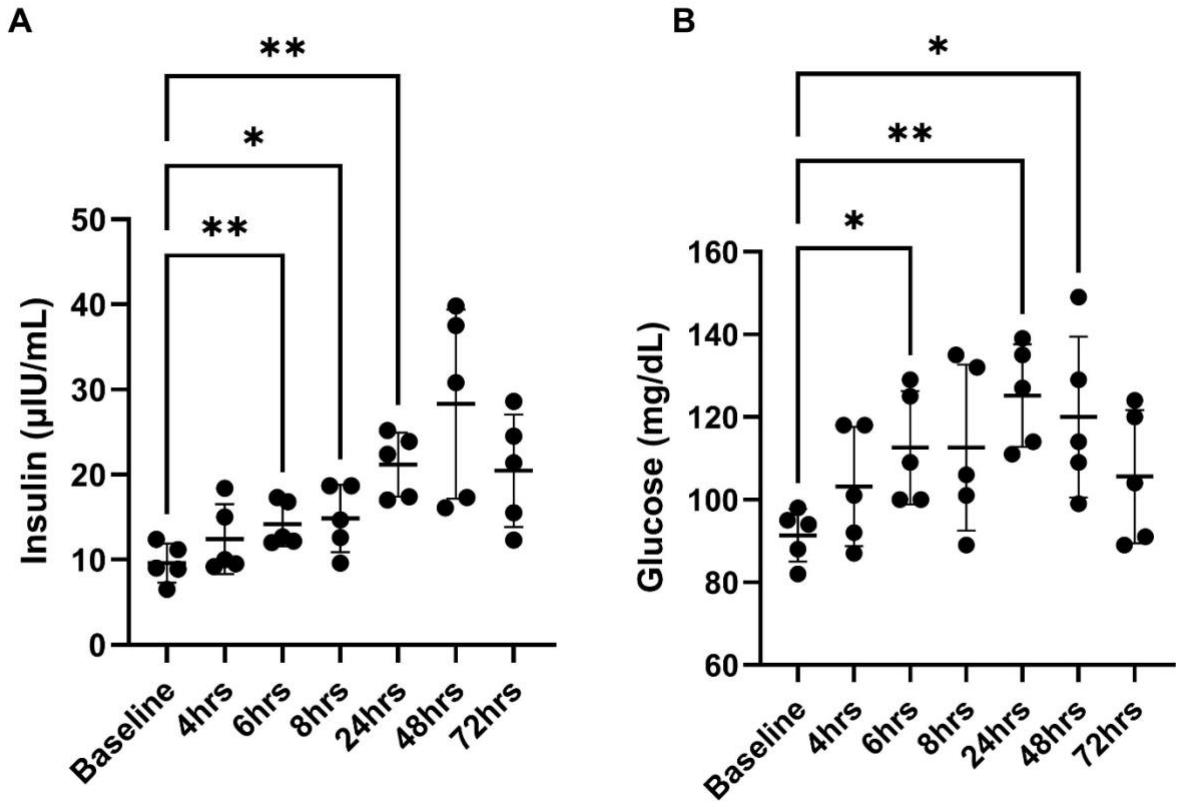

Figure 1: Insulin (uIU/mL, A) and glucose (mg/dL, B) concentrations post intra-articular triamcinolone acetonide in metabolically normal horses. *=P<0.05, **=P<0.01

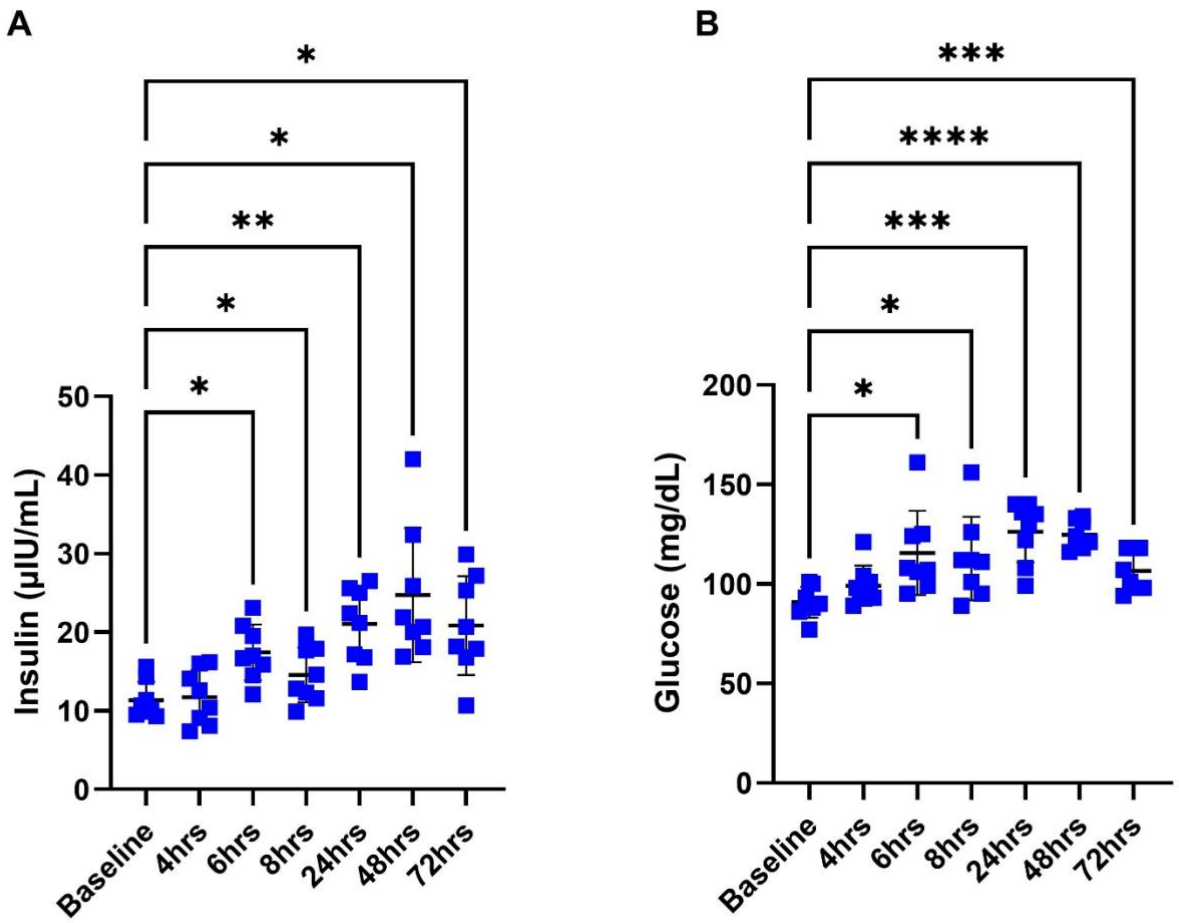

Figure 2: Insulin (uIU/mL, A) and glucose (mg/dL, B) concentrations post intra-articular triamcinolone acetonide in horses with Pituitary Pars Intermedia Dysfunction *=P<0.05, **=P<0.01, ***= P<0.001, ****=P<0.0001

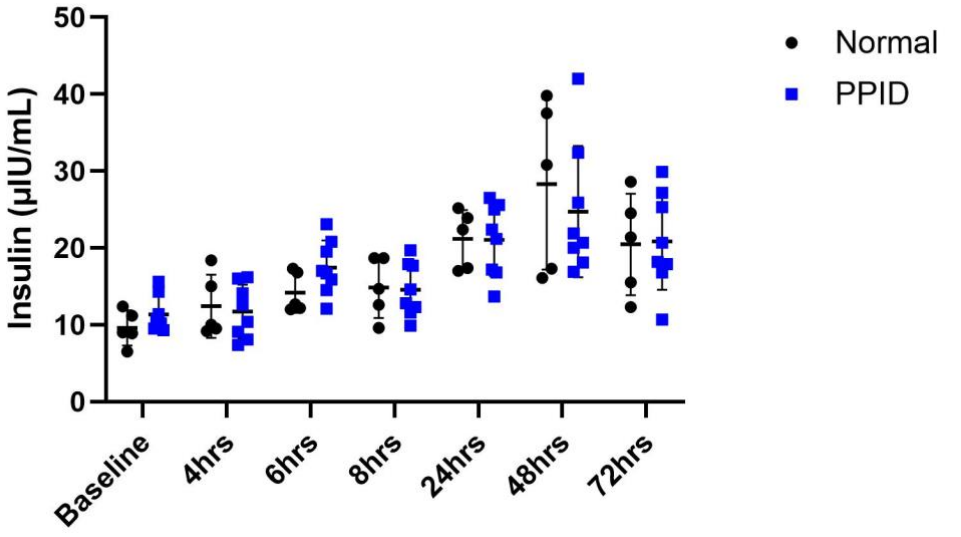

Figure 3: Insulin (uIU/mL) concentrations post intra-articular triamcinolone acetonide in metabolically normal (black circles) horses and horses with Pituitary Pars Intermedia Dysfunction (blue squares).

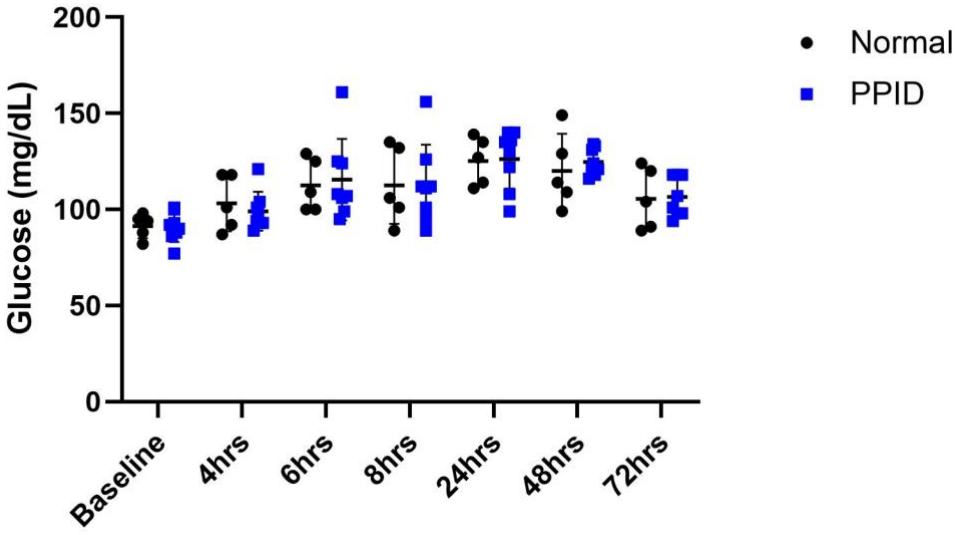

Figure 4: Glucose concentrations (mg/dL) post intra-articular triamcinolone acetonide in metabolically normal (black circles) horses and horses with Pituitary Pars Intermedia Dysfunction (blue squares).

Results of an international survey of owners' knowledge regarding PPID: Identifying areas for owner education

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Aims:
Owners' understanding of pituitary pars intermedia dysfunction (PPID) and their ability to make appropriate management decisions are crucial factors in maintaining the quality of life of affected horses. Assessing owners' knowledge and understanding of PPID will provide information that will help veterinarians, researchers and allied professionals to target and design owner education more efficiently.

The main aim of this study was to investigate the ability of owners to recognise PPID and to assess their level of understanding about this condition. Where owners were actively managing animals with PPID, the factors influencing owners' decisions about management, veterinary involvement and treatment of this condition were also investigated.

Methods:
An online survey was distributed worldwide. Questions were asked about factors that impact decisions related to the management of horses, the role of veterinarians, and factors influencing the management of a horse with PPID. Data was cleaned removing the responses from owners who had completed less than 50% of the answers. Descriptive analysis was performed, followed by inferential statistical analysis using SPSS software (IBM SPSS). Where responses required a ranking (e.g. relative importance of clinical signs), the frequency of responses in each ranking category were compared according to self-declared owners understanding of PPID. The Shapiro-Wilk test was used to assess normality of continuous variables, and parametric or non-parametric statistical tests (for nominal and ordinal variables) were used, as appropriate.

Results:
A total of 1344 responses were collected, with 1143 responses meeting the inclusion criteria. Responses were received from Europe (49%; 554/1143), Australia and New Zealand (32%; 365/1143), and North America (18%; 202/1143). Respondents were grouped based on their self-declared understanding of PPID, with 43% (443/1024) classified as having an 'incomplete understanding' and 57% (581/1024) classified as having a 'good understanding'. Respondents who declared a good understanding of PPID rated laminitis as the most important clinical sign ($P=0.016$) followed by susceptibility to infections ($P<0.001$). Respondents who declared an incomplete understanding of PPID rated long and curly hair as the most indicative clinical sign ($P=0.001$). Veterinarians were selected as the main source of information for general health and management decisions (66%; 629/969). Other sources of information for general health and management were different between owners in the 'good understanding' and 'incomplete understanding' groups. Both groups selected veterinarians as their main source of information. However, nutritionists, trainers and farriers ($P=0.008$, $P=0.001$ and $P=0.001$) were ranked as more important sources of information for owners in the 'incomplete understanding' group, while in the 'good understanding' group, the second most selected category was represented by scientific papers ($P=0.001$).

Conclusions:
There is considerable scope for education of horse owners regarding PPID, since almost half of respondents declared an incomplete understanding of this condition. Being aware of what horse owners know about PPID will help to inform future education strategies, in order to optimise health outcomes for affected animals.

Acknowledgements:
Dr. Galinelli received a PhD scholarship funded by the Australian Research Council.

Ethics statement:
This study was approved by the University of Melbourne Human Ethics Committee in compliance with applicable national laws.

Factors associated with total adiponectin concentrations in ponies

Knowles, E.J., Harris, P.A., Elliott, J. and Menzies-Gow, N.J.

Aims: Hypoadiponectinaemia may contribute to insulin dysregulation (ID) development. The present study aimed to describe epidemiological associations between total plasma adiponectin concentration ([adiponectin]) and physical, management and metabolic markers in ponies.

Methods: Physical examinations were performed and blood collected from non-laminitic ponies every 6 months (autumn and spring) for ≤4 years. Assessed physical variables included body condition score (BCS) (1-9), cresty neck score (CNS), clinical signs of PPID and evidence of divergent hoof growth. Metabolic markers included plasma concentrations of: total adiponectin, ACTH, triglycerides and glucose and serum concentrations of insulin at basal levels ([insulinT0]) and 60 minutes after oral sugar administration ([insulinT60]). Adiponectin was measured by immunoturbidometric assay, insulin by immunofluorescent assay and ACTH by chemiluminescent immunoassay.

Factors associated with [adiponectin] were determined using linear mixed-effect models with physical and metabolic factors as fixed effects and random effects for each pony and premises. ACTH was dichotomised based on seasonally-adjusted reference ranges. For illustrative (rather than iterative) purposes univariable analysis was performed for all variables. A prespecified multivariable model to include factors considered as (markers of) biologically plausible contributors to [adiponectin] was built. Residuals were assessed for normality.

Results: Ponies (n=372) from 24 premises provided 1883 samples for inclusion. Mean (±SD) [adiponectin] (µg/ml) was 17.4 (±12). Univariable analysis revealed significant ($p<0.05$) positive associations between [adiponectin] and: the Cob/CobX breed/type and withers-height; negative associations were revealed with: the Shetland/ ShetlandX breed/type, CNS, BCS, [insulinT60] and the presence of a pot belly and divergent hoof growth. Significant associations were not shown with season, age, sex, turnout or exercise scores, the presence of bulging-supra-orbital fat pads or concentrations of ACTH, glucose, triglycerides or [insulin]T0.

Multivariable model factors with significant positive associations with [adiponectin] (effect estimate, 95% confidence interval (µg/ml), p) were: Cob/CobX breed/type (compared with 'other breeds') (4.85, 1.2 to 8.51, $p=0.009$), withers height (0.22, 0.10 to 0.33, $p<0.001$) for every cm of height, and a turnout composite score (0.24, 0.01to 0.47, $p=0.039$) for each score level. Significant negative associations with [adiponectin] (effect estimate, 95% confidence interval (µg/ml), p) were: age (-0.29, -1.48 to -0.32, $p=0.002$) per year and BCS (-2.16, -2.92 to -1.4, $p<0.001$) for each unit of BCS, Associations with season, sex, CNS, bulging supra-orbital fat pads, a 'pot belly', an exercise composite score, a 'positive ACTH' and the interaction between season and positive ACTH were not statistically significant. Nakagawa's R-squared values and within-pony intra-class correlation (ICC) were: 0.12 (fixed-effects) and 0.76 (full model), ICC = 0.73.

Conclusions: Within-pony variation in [adiponectin] was modest. The fixed effects in the present model explain a small proportion of [adiponectin] variation and included modifiable and non-modifiable factors. In contrast to another similar study, lower [adiponectin] was associated with obesity. Differences in study findings may reflect within-individual changes revealed by the present longitudinal analysis or the use of different assays. Further investigation into the pathogenesis of hypoadiponectinaemia and obesity and associations between height, ID and [adiponectin] are warranted.

Acknowledgements: MARS Petcare UK and The Mellon Trust for study funding. Veterinary students for data collection assistance.

Total and high molecular weight adiponectin does not change in response to intra-articular steroids in horses

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This work follows international, national, and institutional guidelines for humane animal treatment and complies with relevant legislation in the country in which the study was conducted.

This work has not been presented at any state, national or international conferences.

Aims: In humans, insulin, glucose, and total adiponectin increased post-injection of corticosteroids. Adiponectin increased more in metabolically normal versus abnormal humans, likely as a compensatory response because systemic adiponectin is insulin-sensitizing. In metabolically normal horses, insulin increased after intra-articular steroid administration, but it is unknown if adiponectin increases as well. Greater concentrations of total or high molecular weight (HMW) adiponectin (the form thought to be most insulin sensitizing) could blunt the insulin response, thus mitigating the risk of development of hyperinsulinemia associated laminitis. The aims of this study were to determine the systemic total and HMW adiponectin responses to intra-articular corticosteroids in metabolically normal and insulin dysregulated horses. The study hypothesized intra-articular corticosteroid injections would lead to increased total and HMW adiponectin concentrations in metabolically normal but not insulin dysregulated horses post-injection.

Methods: Eleven adult horses had an oral sugar test performed before injection of 18 mg of triamcinolone acetonide (TA) into one middle carpal joint. Blood samples were collected before injection and at 6 other time points for up to 72 hours post-injection. For total adiponectin, 6 metabolically normal, 4 insulin dysregulated horses were compared. For HMW adiponectin, 4 metabolically normal (including one not assessed for total adiponectin) and 3 insulin dysregulated horses were evaluated. Total and HMW adiponectin concentrations were measured using ELISAs. Statistical analyses included Shapiro-Wilk tests and a repeated measures ANOVA with Tukey's post-hoc testing or non-parametric equivalent, with significance set at $p<0.05$.

Results: The mean total adiponectin did not change from baseline (580.29±79.98 ng/ml) to 72 hours (579.92 ±3.32 ng/ml) post intra-articular TA or between metabolically normal and insulin dysregulated horses (588.98±3.17 ng/ml and 588.07±7.65 ng/ml respectively) (Figure 1). Mean H1MW adiponectin did not change from baseline (2081.63±2689.20 ng/mL; 12891.12±2538.15ng/mL) to 72 hours (2215.70±2551.48ng/mL; 12488.7±2779.41ng/mL) post intra-articular TA in metabolically normal or insulin dysregulated horses respectively (Figure 1).

Conclusions: Horses do not possess the compensatory mechanism present in humans that elevates adiponectin levels after corticosteroid administration, potentially increasing their susceptibility to hyperinsulinemia associated laminitis.

Acknowledgements: This study was funded by the Michigan Alliance for Animal Agriculture and the MSU Spellbound Equine Endocrine Research Fund

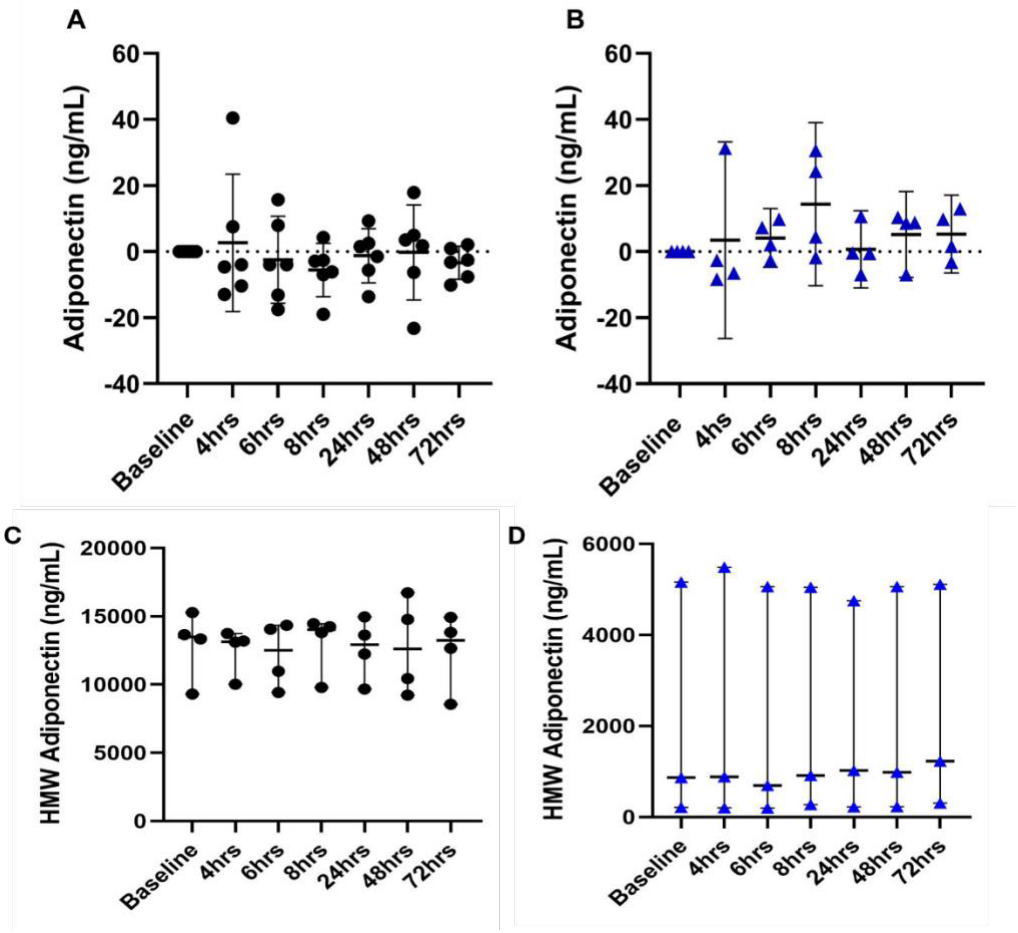

Figure 1. Total (A and B) (percent change from baseline, n=10) and High Molecular Weight (HMW) (C and D) Adiponectin concentrations (mean +/- standard deviation, n=7) over time after 18 mg of intra-articular steroid administration in metabolically normal (left column, black circles) and insulin dysregulated (right column, blue triangles) horses.

Daily fluctuation in high molecular weight adiponectin concentrations in ponies

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This study complied with national guidelines for humane animal treatment and with relevant legislation in Australia. The study was approved by the institution's Animal Ethics Committee. The abstract has not been presented or published previously.

Aims: Total and high molecular weight (HMW) adiponectin concentrations are negatively associated with regional adiposity in horses and ponies with equine metabolic syndrome. Further, low total adiponectin concentrations have been associated with an increased risk of HAL. The HMW multimer of adiponectin is thought to be the most biologically active form, but less data are available for this multimer in association with equine metabolic disease. The aim of this study was to determine the temporal profile of HMW adiponectin concentration in ponies over one full day/night cycle.

Methods: Blood samples were collected from 8 adult, mixed-breed ponies (4 male; 4 female) using an indwelling i.v. catheter every 2 hours for 24 hours, with additional samples collected at 9am and 5pm. The ponies were fed twice in the 24-hour period (soybean hulls, lucerne chaff and grassy alfalfa hay at ~ 8:30am and grassy alfalfa hay only at ~4:30pm). All husbandry procedures were consistent before and during the study. All ponies underwent a clinical examination (including being weighed on an electronic scale) and were assigned a body condition score (BCS; 9-point scale) and cresty neck score (CNS; 5-point scale) 2-4 weeks before the study. Plasma (EDTA) HMW adiponectin concentrations were measured using an ELISA previously validated for equine plasma. Timepoints were compared with one-way repeated measures ANOVA and a Bonferroni post-hoc test. Correlations were assessed with Spearman's test.

Results: The median (IQR) BCS and CNS of the cohort were 5 (5-6) and 1 (0.25-1.75) respectively. Mean (± se) adiponectin concentrations fluctuated over time ($P < 0.001$) with higher concentrations during the morning (C_{max} of 14.9 ± 4.17 µg/mL at 2 am). The nadir was 9-fold lower and occurred at 5pm (C_{min} of 1.59 ± 1.16 µg/mL; 5pm vs 2am $P < 0.001$). The $AUC_{0\text{-}24h}$ for HMW adiponectin was not correlated with either BCS ($r = -0.46$, $P = 0.23$) or CNS ($r = -0.62$, $P = 0.09$).

Conclusions: Adiponectin concentrations fluctuated throughout the day/night period in this pony cohort, thus the selection of a consistent sampling time by veterinarians might be important. This differs to previous reports of minimal daily fluctuations in adiponectin concentrations in female horses. Thus, investigation of the diurnal variation in both total and HMW adiponectin in a larger cohort comprising a range of horse and pony breeds is warranted.

Acknowledgements: The authors thank Kate Andrews, Poppy Sibthorpe, Fang Li and Christina Cash for technical assistance.

The effect of pasture-induced obesity on insulin sensitivity and circulating total adiponectin concentrations in native breed ponies

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This research was performed at the Royal Veterinary College, Hawkshead Lane, North Mymms, Herts. AL9 7TA. UK

This work follows international, national, and institutional guidelines for humane animal treatment and complies with relevant legislation in the country in which the study was conducted (UK). It was approved by the Royal Veterinary College Animal Welfare Ethical Review Board (AWERB) and performed under a Home Office Project Licence (PP5634400).

Aims

To investigate the effect of pasture-induced weight gain followed by maintained obesity on insulin sensitivity and circulating total adiponectin concentrations in native-breed ponies.

Methods

Native breed ponies (n=7; three mares, four geldings; 5–14 years) with ideal body condition score (BCS=4.5–5.5/9), no history of laminitis, and normal basal insulin concentration and insulin response to an oral sugar test (OST) were allowed to graze spring/summer pasture for 12 weeks until they became obese (BCS 7/9) and then were maintained at BCS 7/9 for another 12 weeks. Morphometric (weight, heart and belly girth, rump width, BCS, cresty neck score [CNS]) and metabolic parameters (basal [insulin], insulin tolerance test [ITT], [insulin] 60min post OST [T60 OST], and total adiponectin concentration ([adiponectin]) were determined fortnightly. Pasture conditions were concurrently visually assessed and scored according to pasture height and vigour each on a scale of 1 to 5 with scores added to create a combined score.

Results

Morphometric measurements increased in all ponies over weeks 0-12; mean±SD BCS increased from 4.9±0.4 at week 0 to 6.9±0.6 at week 12. Basal [insulin] did not change over the study; T60 OST [insulin] was significantly higher at weeks 14, 16 and 20 compared to week 0; tissue insulin sensitivity (ITT result) was significantly lower at weeks 2-6 and 12-20 compared to week 0; and [adiponectin] was significantly lower weeks 10–22 compared to week 0. All ponies were classified as insulin dysregulated (ID) on least one occasion (mean 7, range 1-11/12 occasions) and 6/7 ponies became/remained hypoadiponectinaemic ([adiponectin]<7.9µg/mL; 4 at week 0, 2 from week 10 and 1 from week 12). Mean basal [insulin] was significantly positively correlated with pasture scores (Pearson's r=0.620, P=0.03). Combined pasture scores were a significant variable in repeated measures models of adiponectin and ITT results, but not basal or T60 OST [insulin]; very low (3/10) and high (8–9/10) pasture scores were associated with tissue insulin resistance, and low [adiponectin] was associated with a low pasture score. BCS and CNS were significantly negatively correlated with [adiponectin] (Spearman's r=-0.9, P<0.001 for both) but not with basal [insulin], T60 OST [insulin] or ITT results. Using repeated measures models, BCS was significantly associated with basal [insulin], T60 OST [insulin], ITT result and [adiponectin].

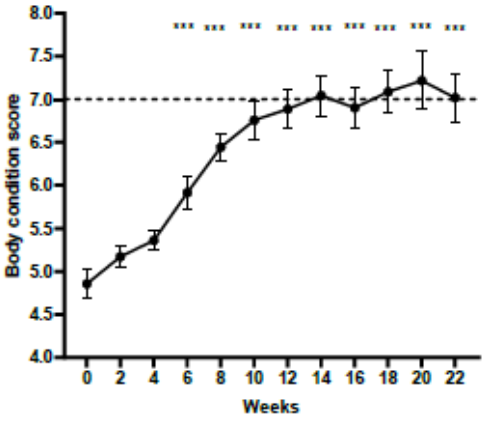
Conclusions

Insulin sensitivity status varied throughout the study, sometimes changing from week to week. Tissue insulin resistance and post-OST hyperinsulinemia were the most common manifestations of ID, and each occurred without the other two manifestations at certain time-points. Basal hyperinsulinemia was only observed on three occasions and never without at least one other manifestation of ID. Individual ponies responded differently to spring/summer pasture in terms of weight gain and changes in insulin sensitivity, despite all being insulin sensitive at the start of the study, but all ponies demonstrated hypoadiponectinaemia. Grazing conditions were associated with tissue insulin resistance, basal [insulin] and [adiponectin] with both short, stressed grass and very long lush grass being associated with lower tissue insulin sensitivity.

Acknowledgements

This work was funded by Waltham Petcare Science Institute.

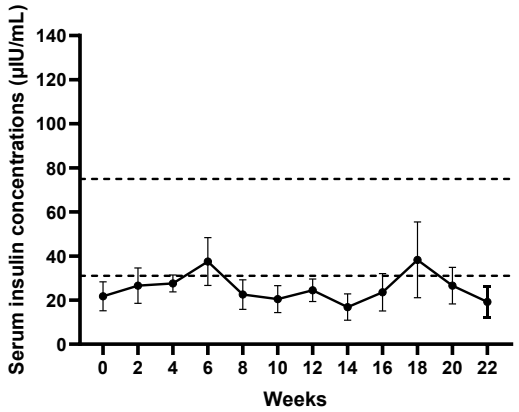
Figure 1: Mean ± SEM body condition score from weeks 0 to 22 for native breed ponies at pasture (n=7) sufficient to induce (weeks 0-12) and then maintain (weeks 13-22) obesity (BCS≥7/9).



***P<0.0001 compared to week 0

Figure 2: Serum insulin concentrations at T0 (basal; A) and T60 (B) after administration of 0.45ml/kg Karo Light corn syrup (OST) and blood glucose concentrations 30 minutes after administration of insulin (ITT; C) in native breed ponies (n=7) at pasture sufficient to induce (weeks 0-12) and then maintain (weeks 13-22) obesity (BCS≥7/9).

A



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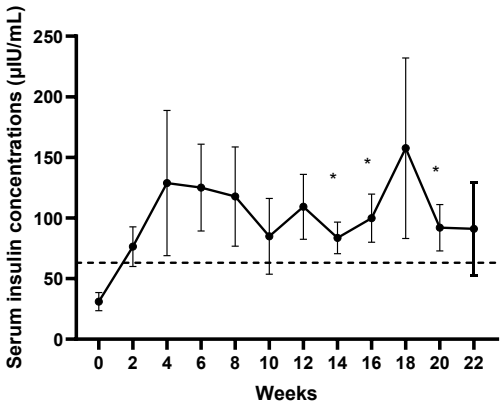

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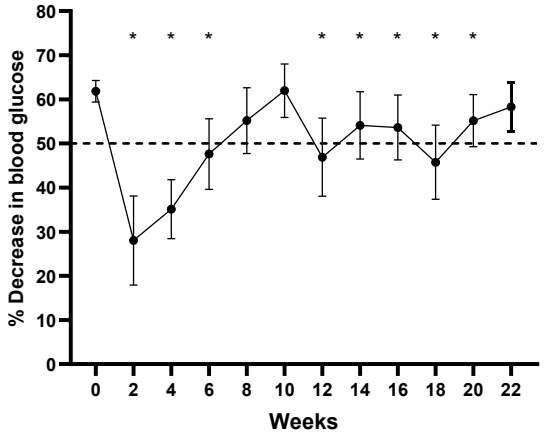
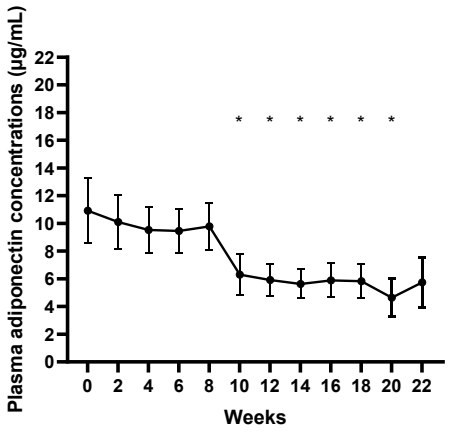


*P<0.05 compared to week 0.

Dotted lines represent diagnostic threshold values of T0=71µiu/ml and T60 OST=63µiu/ml for values measured using Immulite 2000 xpi and <50% decrease in blood glucose concentration (ITT) as suggestive of ID.

Figure 3: Plasma total adiponectin concentrations weeks 0-22 in native breed ponies (n=7) at pasture sufficient to induce (weeks 0-12) and then maintain (weeks 13-22) obesity (BCS≥7/9).



*P<0.05 compared to week 0.

Pergolide increases plasma adiponectin concentrations in ponies and horses via stimulation of dopamine receptors on adipocytes

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Aims:
Adiponectin is an adipokine hormone with insulin-sensitising and anti-inflammatory properties. In equids hypoadiponectinaemia has been correlated with an increased risk of hyperinsulinaemia-associated laminitis. In other species, dopamine receptors on adipocytes mediate the increased expression of adiponectin, although this has not previously been demonstrated in equids. The dopamine receptor agonist, pergolide mesylate, is used for the treatment of pituitary pars intermedia dysfunction (PPID) and acts principally on D_2 receptors (but may also act at D_1 receptors).
The aims of this study were: 1) to investigate the effects of pergolide on plasma adiponectin concentrations in ponies and horses with and without PPID; and 2) to measure the expression of D_2 and D_1 receptors in equine adipose tissue.

Methods:
Sixteen horses and ponies >15 years old, either with or without PPID (n=8 in each group), were included in a randomised cross-over study. Four animals per group received pergolide (2 µg/kg, orally) daily, while the others received no treatment for 4 weeks, followed by a 4-week washout period before treatments were reversed. Plasma samples were assayed for high molecular weight (HMW) adiponectin (Merck High Molecular Weight Adiponectin ELISA) and concentrations were compared using a Wilcoxon test, with $P<0.05$ considered significant.

In a subsequent study, adipose tissue samples were aseptically collected post-mortem, from six horses and ponies that were euthanised for reasons unrelated to this research. Samples of adipose tissue from the neck crest, tailhead and mesenteric regions were lysed, and protein extracted for Western blotting analysis. The expression of D_1 and D_2 receptors were compared across the 3 regions by densitometry using image analysis software (ImageJ).

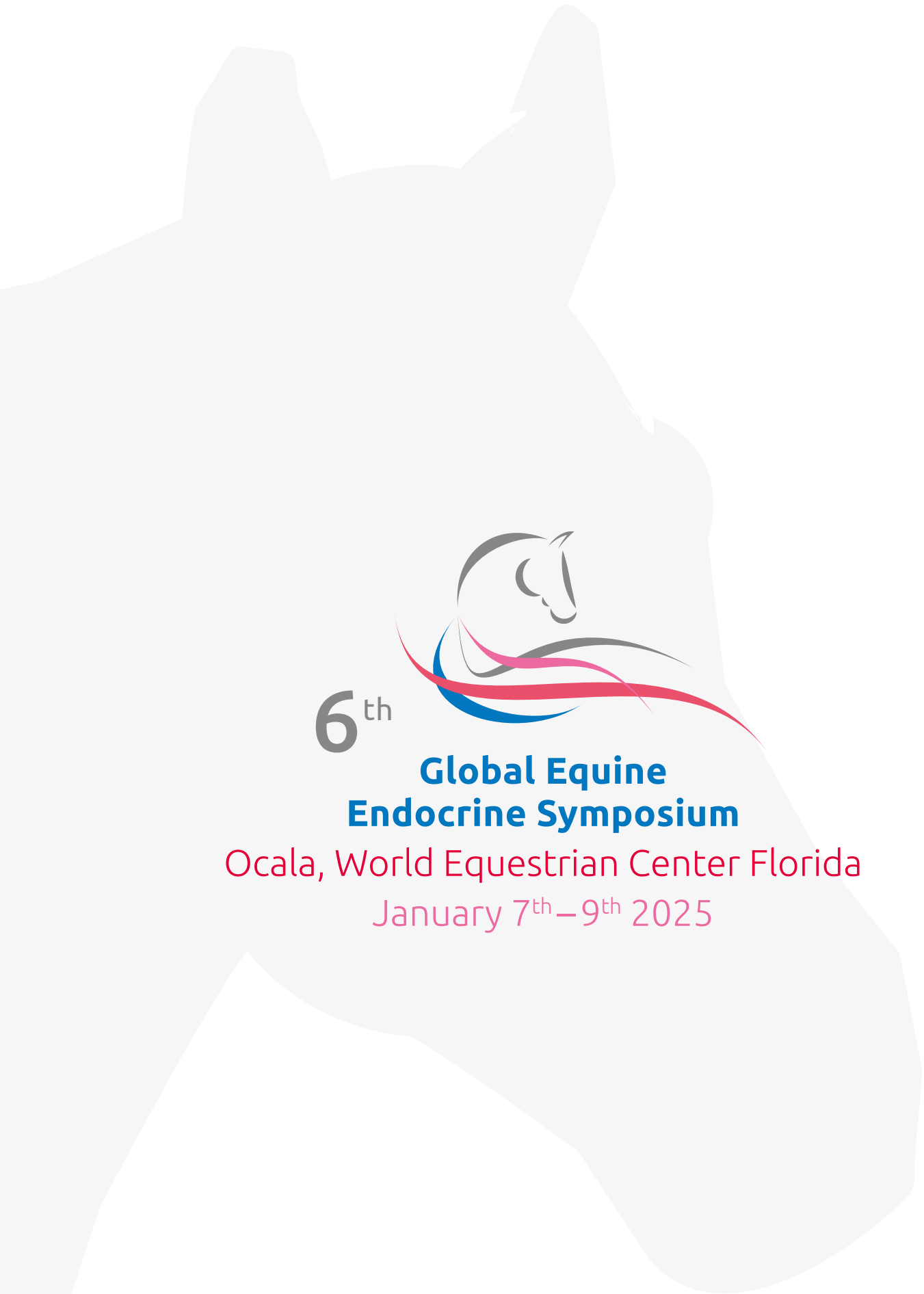
All studies were conducted according to the Australian Code for the Care and Use of Animals for Scientific Purposes, with the approval of the University of Melbourne Animal Ethics Committee.

Results:
There were no significant differences in pre-treatment plasma adiponectin concentrations (µg/ml) between PPID and non-PPID groups (median 5.0 [range, 0.2-42.6]) for PPID group and 5.8 [1.9-21.4] for non-PPID group, P=0.95). Regardless of PPID status, plasma adiponectin concentrations were 1.4-fold (95% CI 1.2-2.0) higher following pergolide treatment (5.0 [0.2-42.6]) pre-treatment and 8.3 [0.2-63.4] post-treatment, P=0.001); whereas plasma adiponectin concentrations did not change over time when animals were not treated (P=0.76).

Adipocyte D_2 receptors were present in equine adipose tissue, with similar abundance in all three regions examined. The D_1 receptor subtype only showed a feint band on Western blot and its possible presence requires further investigation.

Conclusions:
Pergolide treatment increases plasma adiponectin concentrations, likely acting directly on D_2 receptors on adipocytes. This may have several beneficial effects which warrant further investigation.

Acknowledgements:
Funded by the Australian Research Council (LP180101000), The University of Melbourne, Queensland University of Technology, Boehringer Ingelheim Vetmedica GmbH, Racing Analytical Services, Liphook Equine Hospital and the Waltham Petcare Science Institute.



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