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5th Global Equine Endocrine Symposium 2023

Proceedings

5th Global Equine Endocrine Symposium

Bern, National Horse Center Switzerland

January 3rd – 5th 2023

Boehringer Ingelheim

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FOREWORD

Dear Delegate,

We are very happy to welcome you to the fifth Global Equine Endocrine Symposium at the National Horse Center in Bern, Switzerland, 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:

- Epidemiology of equine endocrine diseases
- Aetiopathogenesis of pituitary pars intermedia dys- function (PPID) and equine metabolic syndrome (EMS)
- Diagnosis of endocrine diseases
- Treatment and monitoring of PPID
- Management of obesity and EMS
- Endocrinopathic laminitis
- Insulin dysregulation
- Treatment of concurrent endocrine and respiratory conditions
- Steroid use in endocrine disease

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

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Prevalence of Pituitary Pars Intermedia Dysfunction and Insulin Dysregulation in Horse Breeds Classified by Genetic Clade

Rachel Lemcke[1], Kelly Graber[2], Steve Grubbs[2]

[1]Amwell Data Services LLC, Ringoes, NJ, USA
[2]Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

Aims
Understanding of the prevalence of endocrine disorders ("ED") within equids of different genetic backgrounds is limited but may allow for future targeted testing and management strategies.

The objective of this study was to evaluate PPID and/or ID status and endocrine-associated signs within horse breeds separated by genetic clade.

Methods
A retrospective analysis was performed on veterinarian-provided data from a 2016-2020 study in the United States on 6,266 ponies and horses with suspected ED, primarily PPID. Breeds were classified by clade using the parsimony tree by Petersen, et al. 2013. Horses were considered PPID positive if spring endogenous, spring post-TRH-stimulated, or fall endogenous ACTH levels were >35, >110, or >50 pg/mL, respectively. Horses were considered ID positive if basal insulin levels were >20 uIU/mL. Frequency of PPID, ID, and endocrine-associated signs, including laminitis and reduced athletic performance/lethargy, were compared among clades using chi-square or two-way ANOVA statistical tests.

Results
Clade 9 (Quarter Horse and Paint) was omitted due to the high likelihood of misclassification.

ED frequency within clades ranged from 44.44 to 86.06 percent ($p<0.0001$). The highest rates of PPID and ID at 61.52 and 72.22 percent, respectively, both occurred in Clade 3, which included Miniature Horses, Shetland ponies, and dwarf ponies. The lowest rates of PPID and ID were identified at 21.70 and 24.84 percent, respectively, within Clade 2 (Lusitano and Andalusian) and Clade 7 (Percheron and Belgian Draft), respectively. ID rates were higher than PPID in all clades except Clades 7 and 10 (Thoroughbred).

Horses with and without ED averaged a median 19.43 and 17.36 years across clades, respectively. Minimum ages across clades were similar at 4.71 and 5.14 years with and without ED, respectively. For all horses ≤10 years, Clade 7 had the lowest rate of ED at 18.18 percent, Clade 3 had the highest at 80.75 percent, with a median of 58.89 percent across clades.

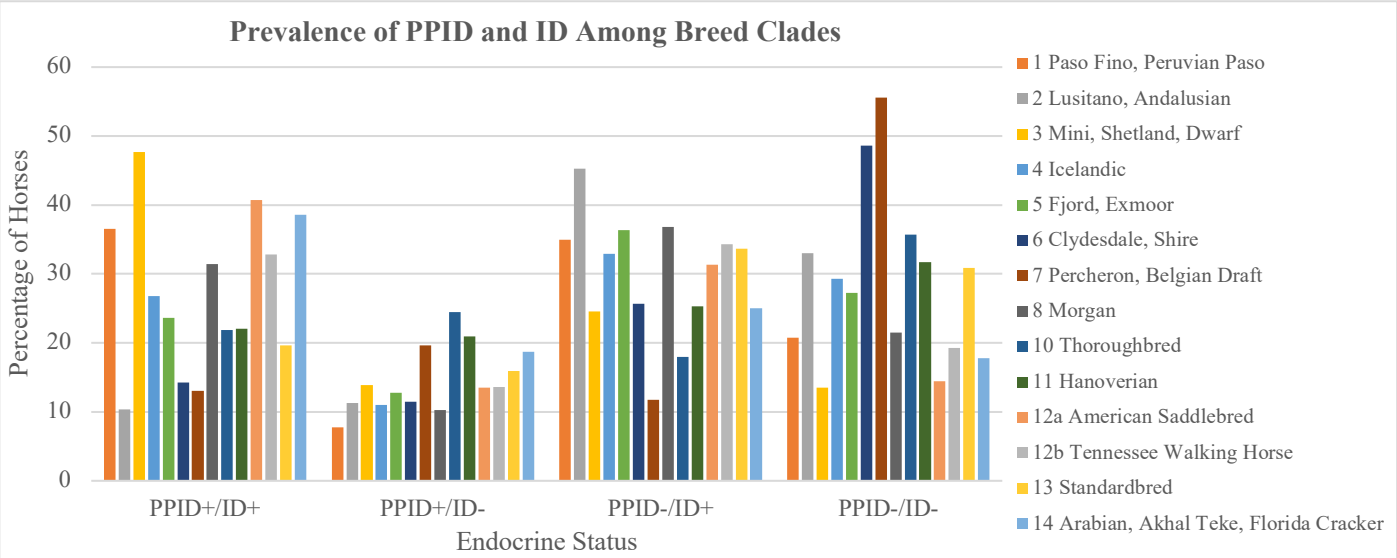
Among horses with ED, the highest and lowest rates of reported laminitis were in Clades 3 and 6, respectively, at 52.58 and 11.11 percent, respectively. Horses with ED only accounted for half of the laminitis cases reported in Clades 6 and 7, versus nearly all the laminitis cases in Clade 3. Laminitis was predominantly correlated with ID ($p<0.0001$) and was more prevalent in Clades 1 and 3 ($p<0.0001$).

Among clades, nearly a quarter to half of horses with ED experienced decreased athletic performance/lethargy. Horses with ED only accounted for 47.37 percent of reported decreased athletic performance/lethargy in Clade 7, versus 86.67 percent of cases in Clade 5.

Conclusions
While this sample population is skewed towards suspected ED, strong differences in PPID and/or ID prevalence are apparent across clades. PPID and ID classification utilized predominately endogenous ACTH and insulin levels, highlighting the continued need for more sensitive, dynamic testing in all breeds. Within this study, ED were identified in >50 percent of horses ≤10 years within 8 of the 13 clades evaluated.

Acknowledgements
The authors would like to acknowledge Boehringer Ingelheim Animal Health USA Inc., Duluth, GA for supporting this work.

		PPID+ & ID+		PPID+ & ID-		PPID- & ID+		PPID- & ID-		Total Qty Horses	ED Prevalence
Clade	Incl. Breeds	Qty Horses	Perc.	Qty Horses	Perc.	Qty Horses	Perc.	Qty Horses	Perc.	N	Perc.
1	Paso Fino, Peruvian Paso	113	36.57	24	7.77	108	34.95	64	20.71	309	79.29
2	Lusitano, Andalusian	11	10.38	12	11.32	48	45.28	35	33.02	106	66.98
3	Miniature Horse, Shetland, Dwarf	472	47.68	137	13.84	243	24.55	134	13.54	990	86.06
4	Icelandic	22	26.83	9	10.98	27	32.93	24	29.27	82	70.73
5	Fjord, Exmoor	13	23.64	7	12.73	20	36.36	15	27.27	55	72.73
6	Clydesdale, Shire	5	14.29	4	11.43	9	25.71	17	48.57	35	51.43
7	Percheron, Belgian	20	13.07	30	19.61	18	11.76	85	55.56	153	44.44
8	Morgan	186	31.42	61	10.30	218	36.82	127	21.45	592	78.55
10	Thoroughbred	260	21.85	291	24.45	214	17.98	425	35.71	1190	64.29
11	Hanoverian	41	22.04	39	20.97	47	25.27	59	31.72	186	68.28
12a	American Saddlebred	130	40.75	43	13.48	100	31.35	46	17.83	319	85.58
12b	Tennessee Walking Horse	243	32.79	101	13.63	254	34.28	143	14.42	741	80.70
13	Standardbred	21	19.63	17	15.89	36	33.64	33	30.84	107	69.16
14	Arabian, Akhal-Teke, Florida Cracker	541	38.62	262	18.70	350	24.98	249	17.77	1401	82.30



Presenting author:
Rachel Lemcke, Amwell Data Services LLC
139 Wertsville Rd., Ringoes, NJ, USA 08551
rachel@amwellds.com

This material has not been presented elsewhere. This work follows national and/or institutional guidelines for humane animal treatment and complies with relevant legislation in the country in which the study was conducted.

Exploring Endocrine Disorders within Warmblood Breeds: Frequency of Pituitary Pars Intermedia Dysfunction and Insulin Dysregulation

Rachel Lemcke[1], Kelly Graber[2], Steve Grubbs[2]

[1]Amwell Data Services LLC, Ringoes, NJ, USA
[2]Boehringer Ingelheim Animal Health USA Inc., Duluth, GA, USA

Aims
Warmbloods are a predominant choice for upper-level equestrian disciplines, particularly dressage and jumping. A rigorous training schedule and careful management are hallmarks necessary for high performance. However, such management is also a primary means to manage endocrine disorders ("ED"). In addition, once retired from competition, highly trained Warmbloods are typically used as schoolmasters, increasing the need for continued athletic performance in older Warmbloods.

The objective of this study was to assess frequencies of PPID and ID in different Warmblood breeds relative to non-breed-specific ("NBS") Warmbloods. The hypothesis was PPID and ID frequencies would be similar between specific-breed and NBS Warmbloods.

Methods
A retrospective analysis was performed on veterinarian-provided data from a 2016-2020 study in the United States on horses with suspected endocrine disorders, primarily PPID. Frequencies of PPID and ID within Warmblood breeds were compared with NBS Warmblood using chi-square statistical tests. Sample sizes for specific-breed and NBS Warmbloods were n=20-186 (median n=59) and n=889, respectively. Horses were considered PPID positive if spring endogenous, spring post-TRH-stimulated, or fall endogenous ACTH levels were >35, >110, or >50 pg/mL, respectively. Horses were considered ID positive if basal insulin levels were >20 uIU/mL. Prevalence of endocrine-associated signs, including regional adiposity, abnormal hair coat, reduced athletic performance/lethargy, and laminitis, were compared using two-way ANOVA. Where applicable, Warmblood breeds were grouped by region of origin for stronger statistical power.

Results
In this study, ED were identified in 55 to 85 percent of specific Warmblood breeds relative to 69 percent of NBS Warmbloods. While endocrine status was not statistically associated with any Warmblood breeds evaluated, ID and lack of ED were more prevalent than PPID (p=0.0001). PPID and/or ID prevalence in specific Warmblood breeds did not statistically differ from NBS Warmbloods (p=0.3286).

In this study, correlation of ED, PPID, and ID presence with age across all Warmbloods evaluated were 0.17, 0.32, and -0.01. The median and maximum ages of specific Warmblood breeds with and without ED were 19 and 17, and 30 and 25 years, respectively. Compared to Warmblood breeds, the NBS Warmblood cohort represented younger and older horses with and without ED, although the median ages were similar.

Frequency of regional adiposity and hair coat changes differed across endocrine status (p=0.0108 and p=0.0004, respectively). Laminitis and decreased athletic performance/lethargy were not statistically associated with any endocrine status or Warmblood breed evaluated.

Conclusions
Though this sample population is skewed, prevalence of PPID and/or ID were similar among specific Warmblood breeds relative to NBS Warmbloods. Hair coat changes were statistically associated with horses with PPID as well as those without ED, suggesting many horses in this study may have undiagnosed PPID. Rates of regional adiposity were lowest in PPID-only horses, further highlighting the need for dynamic testing. Horse owners, caretakers, and their veterinary teams should continue to identify and manage ED in all Warmblood breeds.

Acknowledgements
The authors would like to acknowledge Boehringer Ingelheim Animal Health USA Inc., Duluth, GA for supporting this work, and thank Ramona Goddard for sharing her knowledge of Warmblood breeds.

	PPID+ & ID+		PPID+ & ID-		PPID- & ID+		PPID- & ID-		Total Horses	ED Prevalence
Warmblood Breed	Qty Horses	Perc.	Qty Horses	Perc.	Qty Horses	Perc.	Qty Horses	Perc.	N	Perc.
Belgian Warmblood [a]	4	20.00	3	15.00	4	20.00	9	45.00	20	55.00
Dutch Warmblood [b]	36	23.68	29	19.08	48	31.58	39	25.66	152	74.34
German Warmblood [c]	9	15.25	8	13.56	21	35.59	21	35.59	59	64.41
Hanoverian	41	22.04	39	20.97	47	25.27	59	31.72	186	68.28
Holsteiner	16	27.12	9	15.25	19	32.20	15	25.42	59	74.58
Irish Sport Horse	7	15.56	7	15.56	16	35.56	15	33.33	45	66.67
Oldenburg	22	23.66	13	13.98	31	33.33	27	29.03	93	70.97
Selle Francis	5	18.52	9	33.33	9	33.33	4	14.81	27	85.19
Trakehner	9	23.08	10	25.64	8	20.51	12	30.77	39	69.23
NBS Warmblood	216	24.30	163	18.34	236	26.55	274	30.82	889	69.18

Additional breeds included within cohort: [a]Zangersheide, [b]KWPN, [c]German Riding Pony, Zweibrücker, Rhinelander, Westphalian.

Presenting author:
Rachel Lemcke, Amwell Data Services LLC
139 Wertsville Rd., Ringoes, NJ, USA 08551
rachel@amwellds.com

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Survey of knowledge of Equine Endocrine Diseases by Farriers/Hoof Professionals

Alfredo Sanchez-Londoño, DVM, MS, DACVIM (Large Animal)

Aims: The goal of the survey was to investigate the knowledge that farriers/hoof professionals have about equine endocrine diseases and how they get involved in the management of these diseases. It was also a goal of the survey to find out if farriers/hoof professionals work together with veterinarians on horses affected by laminitis due to these diseases.

Methods: A 16 question web-based survey using Qualtrix XM software (Provo, Utah, USA), was designed to ask questions about general knowledge about equine endocrine diseases and how they are involved in helping owners and affected horses. The survey was approved by the Institutional Review Board (IRB) for the Protection of Human Subjects in Research at Auburn University. The survey was distributed world-wide through farrier associations, farrier magazines and social media. The survey was responded by a total of 230 participants.

Results: From the 230 participants, 179 responded 100% of the survey. Farriers/hoof professionals were familiar with the term PPID (n=141), not familiar (n=37) and no response (n=1). Respondents not familiar with PPID were asked if they recognized the term equine Cushing's disease, all of them (n=37) were familiar with it. When asked about the term EMS, they were familiar (n=167) with it and (n=12) were not familiar with, but when that group was asked about familiarity with the term "easy keeper", all of them recognized that term. The main three clinical signs consistent with PPID, were abnormal haircoat (n=167), cresty neck (n=131) and abnormal hoof rings (n=114), while the most common clinical signs in horses with EMS were obesity (n=160), cresty neck (n=149) and abnormal hoof rings (n=112).

The most common season in which they were presented with laminitic horses was in the spring (n=86), followed by no particular season (n=58). When asked if they worked in conjunction with a veterinarian when managing a horse with laminitis, the majority (n=78) said they would definitely work in conjunction. The main three aspects that a veterinarian can provide in management of horses with laminitis were x-rays (n=109), pain management (n=97) and a collaborative approach to shoeing management (n=75). Farriers/hoof professionals most common way of handling horses with laminitis was trimming (n=150), followed by frog support with pads (n=104) and other (n=97) with the most common being hoof boots, clogs or casting material (n=68). Farriers/hoof professionals also mentioned that it would be very helpful to have nutrition or diet advice from the veterinarian (n= 20) so that their job maintaining horses' feet would be useful. For frequency of rechecks of laminitic horses, the majority (n=50) recommends rechecks every 4 weeks. Foot maintenance is a critical in management of horses affected with PPID or EMS, so a question about owner compliance was asked, and it was considered to be good owner compliance (n=72) followed by average compliance (n=57).

Conclusions: Respondents mentioned that there is the need for more research and education to all the people involved in the management of horses affected by these diseases. A common request by farriers/hoof professionals was that veterinarians should help them with having discussions with the owners about nutrition and management of affected horses.

Acknowledgements: American Farriers Association, American Association of Professional Farriers, Farriers Industry Association for dissemination of survey through their websites and social media pages.

Survey of knowledge of Equine Endocrine Diseases by Horse Owners

Alfredo Sanchez-Londoño, DVM, MS, DACVIM (Large Animal)

Aims: The goal of the survey was to investigate the knowledge that horse owners have about pituitary pars intermedia dysfunction (PPID) and equine metabolic syndrome (EMS), and how it is being diagnosed, treated and managed.

Methods: A 28 question web-based survey using Qualtrix XM software (Provo, Utah, USA), was designed to ask questions about familiarity and knowledge with PPID and EMS. The survey was approved by the Institutional Review Board (IRB) for the Protection of Human Subjects in Research at Auburn University. Survey was distributed world-wide through horse magazines, associations and social media. There was a total of 2227 participants.

Results: From the 2227 participants, 1972 responded 100% of the survey. Questions included years of horse experience, familiarity with equine endocrine terminology, if they had any affected horses with PPID or EMS, and general veterinary care questions. If they had affected horses with PPID or EMS further questions regarding clinical signs, diagnosis, management and treatment were asked. The question about knowledge of equine endocrine diseases was aimed at determining if owners were familiar with the terminology, clinical signs, diagnosis, treatment and management. Out of 1972, 1286 participants were familiar with the term PPID, 679 were not and 7 did not respond. Regarding EMS, participants were familiar with the terminology(n=1636/1972), were not (n=333) and did not respond (n=3). From the 1972 participants, had heard about the diseases but had not had a horse diagnosed with them (n=955), had a horse diagnosed with PPID (n=511), had a horse diagnosed with EMS (n=202) and had a horse diagnosed with both EMS and PPID (n=251).

The most common symptoms recognized by owners of PPID affected horses were abnormal haircoat/delayed shedding (n=422) muscle mass loss (n=281) and weight loss (n=213). In horses affected by EMS, the most common symptoms were abnormal fat deposits/cresty neck (n=132), horse being an “easy keeper” (n=129) and laminitis (n=125). In horses affected with both EMS and PPID the most common symptoms were abnormal haircoat/delayed shedding (n=200), laminitis (n=172) and abnormal fat deposits/cresty neck (n=163). Horses that developed laminitis had x-rays done in 115/154 PPID cases, 91/125 EMS cases and 139/172 cases of combined PPID and EMS. P3 rotation was present in 82/115 PPID, 73/91 EMS and 107/139 EMS/PPID. Pain management, hoof trimming and stall rest were common treatments for laminitis.

Regarding diagnosis, for PPID the three most commonly used diagnostics were baseline ACTH (n=246), clinical signs (n=186) and EMS panel (n=108). For EMS, the three most commonly used diagnostics were clinical signs (n=103), baseline insulin and glucose levels (n=74) and an EMS panel (n=59). For PPID/EMS affected horses, the three most common diagnostics used were baseline ACTH (n=176), baseline insulin and glucose levels (n=138) and an EMS panel (n=122). PPID affected horses were most commonly diagnosed between 20-25 years or age (n=192/511), EMS affected horses between 10-15 years (n=64/202) and PPID/EMS affected horses between 15-20 years of age (n=92/251).

Affected PPID horses are being treated (n=361/511), EMS affected (n=118/202) and PPID/EMS (n=188/251). The most frequently used treatment used in PPID (n=277/511), and PPID/EMS (145/251) affected horses is Prascend ®, while the most common treatment for EMS horses is levothyroxine (Thyro-L®). Regarding success of treatment, owners believed it was successful in PPID horses (n=262/361), EMS horses (n=62/82) and EMS/PPID horses (n=133/188). Owners that are not treating their horse with PPID because there is no need at the time (n=78/135), EMS (n=83/118) and PPID/EMS (n=43/58). Diet changes were also done in PPID (n=288), EMS (n=74) and PPID/EMS (n=176).

Conclusions: Owners want more education on these topics, and also for farriers and veterinarians to have better education to understand the diseases better. Additional research to better understand these diseases in both horses and donkeys. A very important topic that was brought up by horse owners was about nutrition and how to manage affected horses adequately. Many owners also commented that their horse had not been diagnosed in a timely fashion, and that by the time they were diagnosed there were too many problems happening and the horse had to be euthanized.

GEES 2023 Clinical Research Abstract

Dopamine and dopamine D2 receptor expression in the pituitary gland of horses with pituitary pars intermedia dysfunction and their correlation with pituitary histomorphometry

Luoyi Huang, Chiara Palmieri, François-René Bertin

School of Veterinary Science, The University of Queensland, Gatton, Queensland, 4343, Australia

The work follows institutional guidelines for humane animal treatment and complies with conduct of research legislation in the country in which the study was conducted.

Aims: Dopamine D2 receptor (D2r) downregulation is involved in pituitary tumours development in many species; however, its possible role in pituitary pars intermedia dysfunction (PPID) has not been established. We aim to determine the expression of D2r in the pituitary gland of horses with PPID and investigate its association with disease severity assessed by pituitary histomorphometry grades.

Methods: Archived formalin-fixed, paraffin-embedded sagittal sections of pituitary glands from twenty-four horses with PPID and 4 control horses were retrieved. Cases were categorised based on their pituitary gland histomorphometry grade (0 to 5) as described by Miller *et al.*, (2008) and expression of tyrosine hydroxylase (TH) and D2r was assessed by immunohistochemistry. Positive staining areas for 10 randomly selected high magnification fields were averaged for each case and averages were compared between groups using a one-way analysis of variance. $P < .05$ was considered significant.

Results: There was a significant effect of pituitary gland histomorphometry grade on TH expression ($P < .0001$), with significantly lower TH expression in higher grades. There was also a significant effect of pituitary gland histomorphometry grades on D2r expression ($P = .007$); however, significantly higher D2r expression was detected with higher grades.

Conclusions: Lower TH expression in higher pituitary gland histomorphometry grade confirms the pivotal role of dopamine in the pathogenesis of PPID; however, the higher D2r expression indicates that downregulation of the receptor would not be involved in PPID development and supports the use of D2r agonists for the treatment of the disease, even in advanced cases.

Acknowledgement: The authors wish to thank Jo Gordon from the Veterinary Laboratory Services at The University of Queensland for her technical support.

Equine pituitary pars intermedia dysfunction: a spontaneous model of synucleinopathy

J.S. Fortin, A.A. Hetak, K.E. Duggan, C.M. Burglass, H.B. Penticoff, H.C. Schott II. Michigan State University,

Aims: Equine pituitary pars intermedia dysfunction (PPID) is a common endocrine disease of aged horses that appears to have a similar pathophysiology as Parkinson's Disease (PD), in which accumulation of misfolded α-synuclein (α-syn) contributes to degeneration of dopaminergic neurons. While increased levels of α-syn have been found in pars intermedia (PI) tissue of PPID-affected equids, it is unclear if α-syn is also misfolded in the equine PI and whether misfolded α-syn could promote further prion-like self-aggregation and propagation.

Methods: Pituitary glands (PG) were harvested from 15 horses: five young horses (6 ± 3 years, PG histology score 2 ± 1), five aged horses without signs of PPID (29 ± 5 years, PG histology score 4 ± 1), and five aged horses with hypertrichosis and additional signs of PPID (27 ± 4 years, PG histology score 5 ± 0). PI tissue was extracted, sonicated, and lysed to harvest protein and α-syn was immunoprecipitated with rabbit polyclonal anti-α-syn. Thioflavin-T (ThT) dye fluorescence assays (seeding experiments) were performed to evaluate kinetics of *in vitro* fibril formation of recombinant human α-syn incubated with α-syn immunoprecipitated from PPID, aged, and young horses. Finally, equine α-syn was visualized by transmission electron microscopy (TEM) to assess morphology.

Results: ThT dye fluorescence assay seeding experiments confirmed prion-like self-propagation properties of α-syn isolated from PI tissue collected from PPID-affected horses (Figure 1). Detection of α-syn fibrils in PI tissue via TEM was exclusive to PPID-afflicted horses.

Conclusion: These combined animal, bioinformatic, and biophysical studies provide evidence that equine α-syn is misfolded in PPID-affected horses and could contribute to dopaminergic neurodegeneration characteristic of PPID.

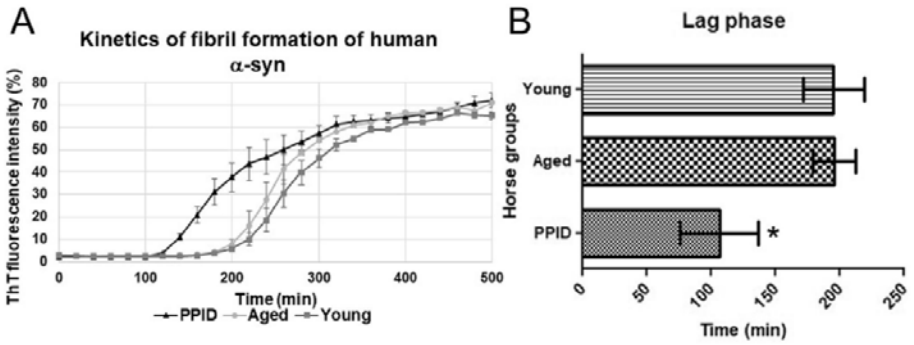

Figure 1. Alpha-synuclein (α-syn) extracted from the pars intermedia of PPID-affected horses has the potential to cross seed recombinant human α-syn. (**A**) Thioflavin T (ThT) fluorescence assay kinetics of human α-syn fibril formation in the presence of equine α-syn isolated from young, aged, and PPID-affected horses. Data represent the mean of the fluorescence intensity with error bars for SEM obtained from three different horses in each group (young, aged, PPID). (**B**) Lag phase time (min) for initiation of human α-syn fibril formation during incubation with equine α-syn extracted from pars intermedia tissue collected from young, aged and PPID-affected horses. The time (min) required for human α-syn fibril elongation (lag phase) is shorter when induced with equine α-syn extracted from PPID-affected horses. Data represent the mean of the lag phase time (min) obtained from five different horses in each group (young, aged, PPID). Data were analyzed by the one-way analysis of variance with Dunnett's multiple comparison post-hoc testing between groups. (*significant difference $p < 0.05$.)

Acknowledgements: The authors thank the horse owners that donated their equids for use in this project. Supported by the American College of Veterinary Internal Medicine and Michigan State University Endowed Research Funds.

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ACTH RESPONSES IN AGED HORSES AND PONIES AFTER 0.5 AND 2.0 µg/kg TRH

Mareike Taube[1], Kerstin Fey[1]
[1] Equine Clinic, Internal Medicine, Justus-Liebig-University Giessen/Germany

Aims:
The TRH-Stimulation test for diagnosing PPID usually is performed by injecting 1mg/TRH per horse (2µg/kg per 500 kg bw). Our group already showed, that ACTH-AUCs and ACTH-Maximum concentrations were not different after 1 or 2 µg/kg TRH in PPID patients. This study aimed to compare ACTH releases after 0.5 versus 2.0 µg/kg TRH in aged (≥15 years) horses and ponies.

Methods:
Eighteen probands (10 geldings, 8 mares, 19.9 ± 4.1 years) of many different breeds were included. All received 0.5 and 2.0 µg/kg TRH (TRH Ferring, Kiel/Germany) in a randomized cross over study with a washout period of 10-14 days. ACTH was measured directly before (0) and 5, 10, 15, 30, 60, 90, 120 minutes after TRH injection. For statistics, a linear mixed model with the fixed effects "sequence" (0.5/2 or 2/0.5), "period" (first or second TRH administration) and "TRH Dose" (0.5 or 2.0 µg/kg) and with the random effect "subject" was used to detect differences for areas under ACTH curves and ACTH peaks.

Results:
Basal ACTH values were 19.2 ± 4.5 pg/ml with a range from 6 to 35 pg/ml. According to the recommendations of the EEG, 13/18 probands showed ACTH values in the same category 10 minutes after TRH administration in both dosages: 2 unlikely (< 100); 3 equivocal (*100-200, in italics in table 1*) and 8 were likely (>200) to suffer from PPID. In 5 horses (**bold**) ACTH releases were different enough after the two TRH dosages for a change in category: a warmblood mare (#15) shifted from unlikely to equivocal and 2 probands from equivocal to likely after 2.0 µg/kg TRH. However, 2 probands, categorized as unlikely after 2.0 µg/kg TRH showed an equivocal ACTH response after 0.5 µg/kg TRH. There were no statistical differences between ACTH-AUCs or in ACTH peaks after 0.5 or 2 µg/kg TRH. Furthermore, there were no significant differences between sequence and period.

Table 1: ACTH (pg/ml) before and 10 min after TRH intravenously.

Patient No.	**Age**	**0 min**		**10 min**	
	years	**0.5 µg/kg TRH**	**2.0 µg/kg TRH**	**0.5 µg/kg TRH**	**2.0 µg/kg TRH**
1	18	16	15	*156*	*171*
2	21	21	24	700	1170
3	16	14	14	***114***	**237**
4	22	11	35	***121***	**384**
5	21	22	30	*110*	*187*
6	19	22	27	***106***	***90***
7	21	26	24	655	447
8	20	21	15	447	265
9	22	24	22	72	86
10	16	18	15	260	287
11	17	22	16	***101***	***91***
12	15	15	17	202	246
13	15	22	20	279	273
14	15	18	17	*141*	*125*
15	23	19	6	43	55
16	21	21	23	***96***	***174***
17	29	13	14	380	527
18	28	27	21	349	255

Conclusion:
Our results indicate, that 0.5 µg/kg TRH might be sufficient for diagnosing PPID.

The Effect of Trailering on Thyrotropin Releasing Hormone Stimulation of Adrenocorticotropic Hormone Concentration in Horses

[1]J.C. Haffner, [1]R.M. Hoffman and [2]S.T. Grubbs
[1]Middle Tennessee State University, Murfreesboro, TN
[2] Boehringer Ingelheim Animal Health USA Inc., Duluth, GA

Aims: Stress is well known to affect the pituitary–adrenal axis and may result in false-positive PPID diagnosis. It has been demonstrated that trailering horses to hospitals to be tested for PPID can affect basal ACTH concentrations. The aim of this study was to determine if trailering affects ACTH concentrations 10 min after a TRH-stimulation test (T10ACTH).

Methods: Ten adult PPID-negative horses were randomized using a 5x5 Latin Square design to avoid effects unrelated to trailering stress. Beginning in February 2020, 5 horses were hauled 40 minutes every 4 weeks using a 5x5 Latin Square design, rotating through 5 trailer positions. Beginning in February 2021, five different horses were treated in the same manner. Blood was collected from each horse prior to loading to determine pre-trailering ACTH (pre-ACTH) concentration. TRH-stimulation was performed at 0, 15, 30, 60, and 120-min post-trailering. As each horse was unloaded at respective post-trailering times, blood was collected before (T0ACTH) and 10 minutes after (T10ACTH) TRH administration. Blood samples were refrigerated, centrifuged, and plasma frozen (-80C) until analysis. A mixed model with repeated measures compared ACTH pre- and post-trailering and after TRH
stimulation, using horse as subject and time as repeated effect. There was no effect of year or day of sampling (P = 0.46), so day and year were removed from the model.

Results:
Average post-trailing T0ACTH concentrations were elevated (P = 0.002) when compared with pre-trailering ACTH. In individual horses, T0ACTH appeared falsely PPID-positive in four normal horses at 0-min, three horses at 15-min, and three horses at 30-min post-trailering.

Post-trailering T10ACTH after TRH-stimulation was not significantly different from pre-trailering T10ACTH at 0-min or 15-min post-trailering, but T10ACTH at 30-min, 60-min, and 120-min post-trailering were significantly (p=0.006) lower. While statistically different, the lower T10ACTH was not clinically relevant, as the concentrations all correctly reflected PPID-negative diagnosis. Only one normal horse appeared falsely PPID-positive based on elevated T10ACTH at 0-min post-trailering.

Conclusions and Clinical Importance: A 40-minute trailer ride resulted in multiple false-positive PPID diagnosis in horses when using basal ACTH for as long as 30-min post-trailering. Except for a single horse, T10ACTH after TRH-stimulation does not appear to be elevated by trailer stress. Veterinarians are recommended to wait at least 30 min after trailering to test horses for PPID when basal ACTH is used for diagnosis.

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

Presenting Author: John C. Haffner DVM
101D MTSU Horse Science Center
314 W. Thompson Lane
Murfreesboro, Tennessee 37129 USA
jhaffner@mtsu.edu

Research performed at: Middle Tennessee State University Horse Science Center, 314 W. Thompson Lane, Murfreesboro, Tennessee 37129 USA

Animal welfare: This study was performed with approval of the MTSU IACUC under protocol number 21-2004 and complies with all relevant regulations

This work was presented as a poster at the 2022 ACVIM Forum in Austin, Texas.

A Combined TRH–Insulin Procedure Identifies Pituitary Pars Intermedia Dysfunction and Insulin Dysregulation in Horses

[1]J.C. Haffner, [1]R.M. Hoffman and [2]S.T. Grubbs
[1]Middle Tennessee State University, Murfreesboro, TN
[2] Boehringer Ingelheim Animal Health USA Inc., Duluth, GA

Aims: Many horses with PPID are also afflicted with insulin dysregulation (ID), characterized by fasting hyperinsulinemia, post-feeding hyperglycemia and hyperinsulinemia, and risk of laminitis. Veterinary and nutritional management of PPID horses varies with ID status, so combined field-testing to diagnose PPID and ID would be ideal.

Combining thyrotropin releasing hormone (TRH) test and insulin tolerance test (ITT) to diagnose PPID/ID in horses was previously reported using T30ACTH to determine PPID status. In North America, T10ACTH is commonly utilized to determine PPID status. The aim of this study was to determine if concentrations of T10ACTH 10-min after TRH stimulation or T30Glucose 30-min after insulin administration were affected when combining the ITT and TRH tests.

Methods: Randomized prospective study. Ten adult horses were used, 5 PPID-negative and 5 PPID-positive. Based on basal insulin concentrations analyzed prior to the study, no horses were considered ID-positive. Horses underwent a TRH-stimulation test, ITT, and a combined TRH/ITT (TRH/insulin in same syringe) using T10ACTH to determine PPID status and T30Glucose for ID status. The TRH-stimulation and ITT were performed as previously described with TRH (1 mg) and insulin (0.1 IU/kg) administered i.v. after basal blood samples were collected. Blood samples collected 10 and 30 min later yielded T10ACTH and T30Glucose used as diagnostics for PPID and ID. Horses with basal (pre-TRH) ACTH >35 pg/mL and >110 pg/mL post TRH-stimulation were considered PPID-positive. A T30Glucose concentration post-insulin administration remaining higher than half the basal glucose concentration (less than 50% decrease) was considered ID-positive. Data were tested for normality using Shapiro-Wilk statistic then analyzed using a mixed model with repeated measures using horse as subject and treatment as repeated effect. Statistical significance was $P \leq 0.05$.

Results: In PPID-positive horses, T10ACTH concentrations were higher (P=0.015) with combined TRH/insulin compared to TRH-only. This difference was not clinically relevant, as the T10ACTH concentrations correctly identified all horses as PPID-positive. In PPID-positive horses, T30Glucose concentrations were lower (P=0.05) in insulin only compared to TRH/insulin. This difference was not clinically relevant, as T30Glucose concentrations indicated ID-negative diagnosis with either insulin-only or combined TRH/insulin treatments. One PPID-positive horse, previously considered ID-negative at the start of the study, tested ID-positive by failing to reach a 50% decrease in T30Glucose when stimulated with either insulin only (33% decrease) or combined TRH/insulin (40% decrease). Compared to basal insulin used for pre-screening, the T30Glucose diagnostic is likely a more reliable assessment of ID.
In PPID-negative horses, T10ACTH concentrations were not different between TRH-only or combined TRH/insulin. No difference (P=0.063) observed in T30Glucose in insulin-only or combined TRH/insulin in PPID-negative horses.

Conclusions: The combined procedure correctly classified PPID and ID status in horses. Combining the TRH stimulation test (using T10ACTH) with the ITT appears to be a valid procedure.

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

Presenting Author: John C. Haffner DVM
101D MTSU Horse Science Center
314 W. Thompson Lane
Murfreesboro, Tennessee 37129 USA
jhaffner@mtsu.edu

Research performed at: Middle Tennessee State University Horse Science Center, 314 W. Thompson Lane, Murfreesboro, Tennessee 37129 USA

Animal welfare: This study was performed with approval of the MTSU IACUC under protocol number 21-2004 and complies with all relevant regulations

This work was presented as a poster at the 2022 ACVIM Forum in Austin, Texas.

One-Week Repeatability of TRH–Stimulation Procedure for Diagnoses of Pituitary Pars Intermedia Dysfunction in Horses

[1]R.M. Hoffman, [1]J.C. Haffner and [2]S.T. Grubbs
[1]Middle Tennessee State University, Murfreesboro, TN
[2] Boehringer Ingelheim Animal Health USA Inc., Duluth, GA

Aims:
This study evaluated if the thyrotropin releasing hormone (TRH) stimulation procedure repeated at weekly intervals yielded reproducible diagnosis of Pituitary Pars Intermedia Dysfunction (PPID) in horses. Previous work in this laboratory found consistent TRH-stimulation results at 4-week intervals. Understanding TRH-stimulation repeatability is useful if a TRH-stimulation test was initiated but unable to be completed due to unforeseen circumstances, or when PPID research protocols require multiple days of testing.

Methods:
The protocol was approved by the Institutional Animal Care and Use Committee. Ten horses (5 PPID-positive and 5 PPID-negative) were haltered and tied at least 5 hours after their last grain meal. Basal blood samples were collected, then 1 mg TRH was given i.v., and blood collected 10 and 30 min after TRH-stimulation. Plasma aliquots were stored at -80C until analysis at the Cornell Animal Health Diagnostic Center, Ithaca, NY. The process was repeated at 1-week intervals for a total of 4 collections. Data were analyzed using a mixed model with repeated measures to compare T0-ACTH, T10-ACTH, T30-ACTH and the percent increase of ACTH after TRH-stimulation, using horse as the subject and Day as the repeated effect. Pearson's correlation coefficients were used to examine relationships between T0-ACTH, T10-ACTH, T30-ACTH on Days 0, 7, 14, and 21. Bland-Altman plots were constructed to compare T0-ACTH, T10-ACTH, T30-ACTH on all subsequent sampling days to Day 0 concentrations.

Results:
In PPID-Negative horses, T0-ACTH was 14.4 ± 0.96 pg/mL, T10-ACTH 31.7 ± 2.29 pg/mL, and T30-ACTH 20.5 ± 2.14 pg/mL, with an ACTH increase of 232 ± 17% at T10 and 151 ± 17% at T30 min after TRH-stimulation. In PPID-Positive horses, T0-ACTH was 289 ± 98 pg/mL, T10-ACTH 950 ± 81, and T30-ACTH 650 ± 108 pg/mL, with an ACTH increase of 1600 ± 357% at T10 and 665 ± 123% at T30 min after TRH-stimulation.

There was no Day effect on T0-ACTH in PPID-Negative (P = 0.97) or PPID-Positive (P = 0.09) horses, or on T10-ACTH in PPID-Negative horses (P = 0.91). In PPID-Positive horses, T10-ACTH was lower on Day 14 than Day 7 (P = 0.005) and Day 21 (P = 0.036). While statistically significant, these differences were not clinically relevant, as ACTH concentrations remained above the PPID-Positive diagnostic cutoff. There was no Day effect on T30-ACTH in PPID-Negative (P = 0.91) or PPID-Positive (P = 0.07) horses.

Bland-Altman plots indicated an average Day bias in PPID-Negative horses of -1.97 pg/mL in T0-ACTH, -0.2 pg/mL in T10-ACTH, and 2.5 pg/mL in T30-ACTH, while Day bias in PPID-Positive horses was 44.3 pg/mL in T0-ACTH, 8.7 pg/mL in T10-ACTH, and 62 pg/mL in T30-ACTH. The Immulite inter-assay CV was 9.3%, which accounts for all observed Day biases.

Conclusions:
Consistent results were obtained when the TRH-stimulation test was repeated at weekly intervals for 21 days.

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

Comparison of Resting ACTH and Dynamic ACTH Evaluation for Pituitary Pars Intermediary Dysfunction During Fall and Spring Seasons in a Single Herd of Horses

[1]R.M. Hoffman, [1]J.C. Haffner and [2]S.T. Grubbs
[1]Middle Tennessee State University, Murfreesboro, TN
[2] Boehringer Ingelheim Animal Health USA Inc., Duluth, GA

Aims:
No definitive reference ranges for the TRH-stimulation test using ACTH 10 minutes after administration of TRH during the Fall are recommended due to high variation and the concern for false positive results. The question remains if evaluating ACTH 30 minutes post TRH-stimulation (T30-ACTH) would be more reliable. Furthermore, whether T30-ACTH is a more sensitive test to identify horses with early PPID during the fall months compared to resting ACTH is unknown. With limited knowledge regarding diagnosis of horses with early PPID, many "early PPID" cases may be undiagnosed. The primary objective of this study was to compare the sensitivity of resting ACTH (T0-ACTH) and T30-ACTH in both Spring and Fall in horses with PPID and without PPID. The secondary objective of this study was to determine fall cut-offs for the T30-ACTH concentrations in this group of horses.

Methods:
The protocol was approved by the Institutional Animal Care and Use Committee. Resting ACTH and TRH-stimulation tests were used to pre-screen PPID status in 30 horses. 15 PPID-positive and 15 PPID-negative were used. TRH-stimulation tests were performed on all horses in September (Fall) and again the following April (Spring). Blood was collected from the jugular vein for resting ACTH (T0-ACTH) into an EDTA-treated tube, followed by 1mg TRH administered intravenously. Thirty minutes after TRH-stimulation, blood was collected (T30-ACTH). All samples were chilled, centrifuged, and plasma aliquots were stored at -80°C until analysis at the Cornell Animal Health Diagnostic Laboratory. Diagnostic ranges for Fall and Spring T30-ACTH were considered using 95% Confidence Intervals, with the upper 95% level of PPID-Negative horses used for the highest Negative diagnostic limit, and the lower 95% level of PPID-Positive horses used for the lowest Positive diagnostic limit.

Results:
Means±SE, 95% Confidence Intervals, Diagnostic Cutoffs, and percent agreement with the initial screening of PPID-Negative and PPID-Positive horses for T0-ACTH and T30-ACTH are summarized in Tables 1, 2 and 3.

Table 1. Fall and Spring Means±SE for T0-ACTH and T30-ACTH, and 95% Confidence Intervals for T30-ACTH.

	FALL ACTH, pg/mL			
	Mean±SE		**T30-ACTH Confidence Intervals**	
Status	**T0-ACTH**	**T30-ACTH**	**Lower 95%**	**Upper 95%**
PPID-Negative	56.8±13.4	98.4±19.9	55.8	141
PPID-Positive	229±63	547±106	320	774
	SPRING ACTH, pg/mL			
Status	**T0-ACTH**	**T30-ACTH**	**Lower 95%**	**Upper 95%**
PPID-Negative	13.8±2.5	21.5±3.9	13.1	30.0
PPID-Positive	129±81	300±107	70.0	530

The PPID-Negative horses showed similar variation around the means for both T0-ACTH and T30-ACTH during Fall and Spring seasons, but in PPID-Positive horses, there was greater variation around the mean in T0-ACTH compared to T30-ACTH during both Fall and Spring, suggesting that T30-ACTH may be a more reliable test.

Table 2. Recommended diagnostic cutoffs for T30-ACTH in Fall and Spring seasons for PPID-Negative and PPID-Positive Horses.

	T30-ACTH, pg/mL, Diagnostic Cutoffs		
	PPID Unlikely	**Equivocal**	**PPID Likely**
FALL	<141	141–320	>320
SPRING	<30	30–70	>70

Table 3. Percentages of true and false results for individual horses using T0-ACTH and T30-ACTH.

		% Correct Diagnosis		**% Change to Equivocal**		**% False Diagnosis**	
	Season	T0-ACTH	T30-ACTH	T0-ACTH	T30-ACTH	T0-ACTH	T30-ACTH
PPID-Negative at pre-screening	Fall	27%	80%	53%	20%	20%	0%
	Spring	53%	80%	47%	20%	0%	0%
PPID-Positive at pre-screening	Fall	53%	60%	47%	33%	0%	7%
	Spring	33%	53%	53%	47%	13%	0%

Conclusions:
Diagnostic cutoffs were established for T30-ACTH in Fall and Spring seasons. Compared to T0-ACTH, the T30-ACTH may be a more reliable test during Fall and Spring seasons.

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

Seasonal Effects on Insulin and ACTH

Andy E Durham, Liphook Equine Hospital, Liphook, UK, GU30 7JG.
andy@theleh.co.uk

Aims

To investigate seasonal and breed effects on insulin and ACTH and their possible interactions.

Methods

This study was a retrospective analysis of laboratory data from Liphook Equine Hospital. Cases were included where submissions requested resting insulin and/or insulin measured between 60-90 mins following oral administration of 0.45 mL/kg Karo Light Syrup. Plasma ACTH was also measured in some of these cases. Median insulin concentrations were calculated and compared for every month.

Results

In total there were 26,999 submissions requesting resting insulin analysis, of which 14,727 also had plasma ACTH measured. There were 5,570 submissions requesting insulin analysis following Karo Light syrup, of which 1,605 also had plasma ACTH measured.

Basal ACTH values showed the expected seasonal changes as previously published with the highest values in September and the lowest values in April (Fig 1a). Resting insulin showed significant monthly variability ($P<0.0001$) with the highest values in December, January and February, and the lowest values in July, August and October (Fig 1b). Similarly, post-Karo insulin showed significant monthly variability ($P<0.0001$) and was highest in December, January and February with the lowest values in August, September and October (Fig 1c). The circannual pattern was broadly similar for all breeds except for resting insulin values in Thoroughbreds which had higher summer and lower winter values.

Median monthly values for resting and post-Karo insulin concentrations were significantly correlated ($r= 0.89$, $P<0.001$), whereas those for ACTH were significantly inversely correlated with resting insulin ($r= -0.65$, $P=0.026$) and post-Karo insulin ($r= -0.77$, $P=0.005$). (Figure 2).

Conclusions

Circannual changes in resting and post-Karo insulin were remarkably similar and showed higher values through the winter months and the lowest values through the summer and autumn. This could reflect a physiologic adaptation to increasing sugar ingestion through the grazing season being associated with a diminishing β-cell response. Alternatively, seasonal changes in peripheral insulin sensitivity (with secondary effects on resting insulin concentrations) could lie behind this observation. The concept of seasonal changes in insulin sensitivity would make sense in evolutionary terms with the grazing season being typified by relative insulin sensitivity (promoting energy storage) followed by relative insulin resistance in the winter (facilitating mobilisation of energy stores). The finding that ACTH concentrations were inversely correlated with insulin concentrations could be coincidental or perhaps support a *pars intermedia*-derived insulin-sensitising factor driving the seasonal changes.

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

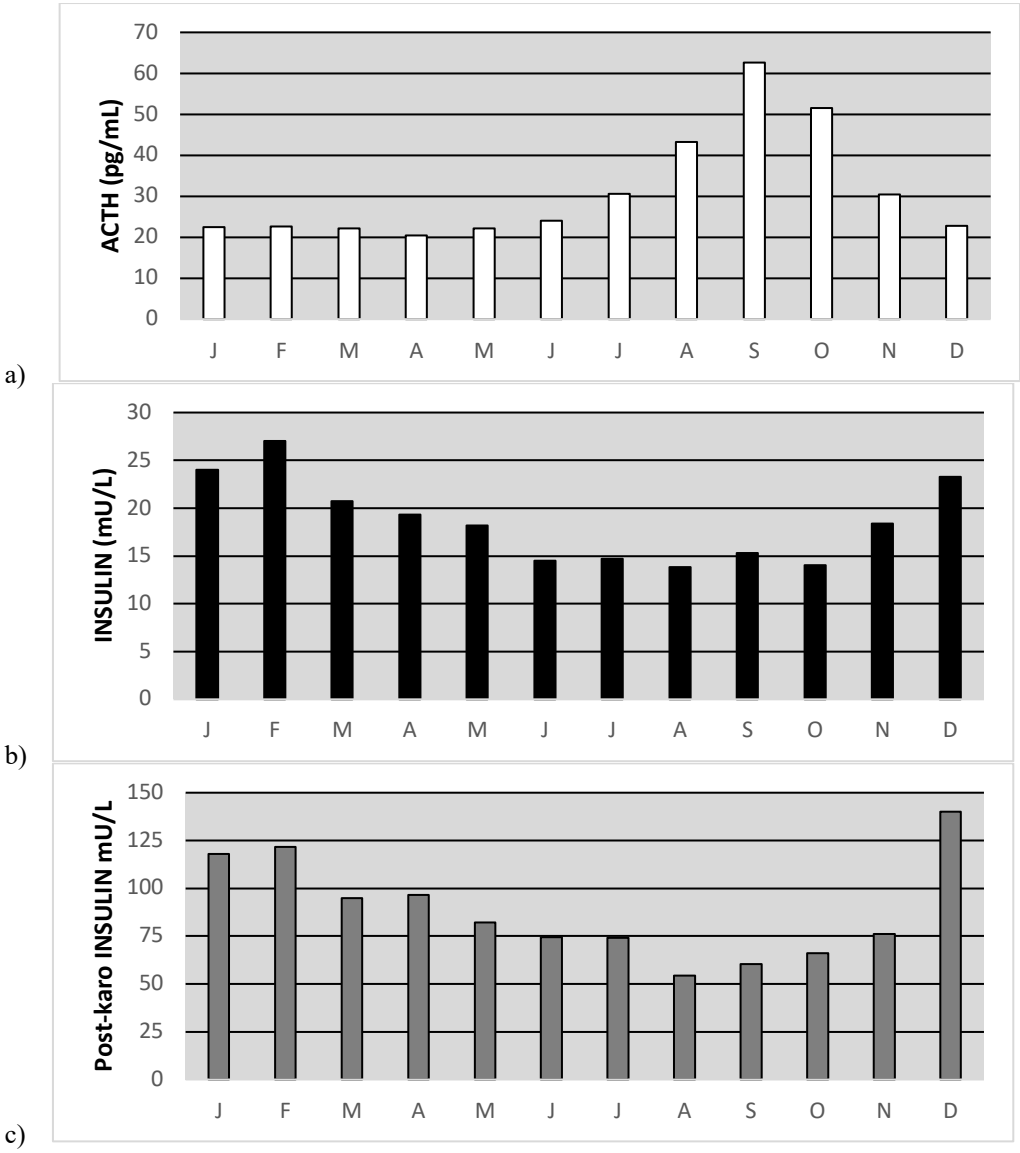

Figure 1. Median monthly values of a) ACTH, b) resting insulin, and c) post-Karo insulin including all breeds.

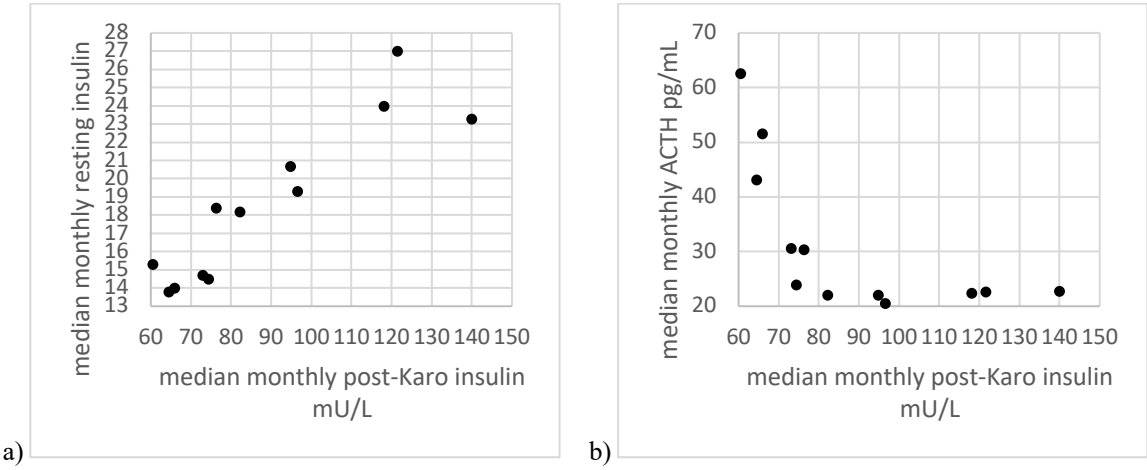

Figure 2. Comparison of median monthly post-Karo insulin responses with a) resting insulin and, b) basal ACTH.

Insulin dysregulation is associated with high autumnal ACTH concentrations in aged horses and ponies with no clinical signs of pituitary *pars intermedia* dysfunction (PPID)

Fang Li[1], Robert Spence[1], Melody de Laat[1], Patricia Harris[2], Johanna Sonntag[3], Nicola Menzies-Gow[4], Andy Durham[5], Simon Bailey[6] and Martin Sillence[1]

[1]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Australia; [2]Equine Studies Group, Waltham Petcare Science Institute, Waltham-on-the-Wolds, UK; [3]Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany; [4]The Royal Veterinary College, North Mymms, UK; [5]Liphook Equine Hospital, Forest Mere, UK; [6]Melbourne Veterinary School, The University of Melbourne, Parkville, Australia

Presenting author: Fang Li (f32.li@hdr.qut.edu.au)

Animal ethics: This study was approved by the Queensland University of Technology Animal Care and Ethics Committee (Approval #1900001168) and was conducted in compliance with applicable Australian law.

Aims

High concentrations of adrenocorticotropic hormone (ACTH) are used as a diagnostic indicator of pituitary *pars intermedia* dysfunction (PPID), which is commonly observed in senior horses and ponies. However, ACTH can be influenced by other factors, such as stress or excitement. Furthermore, in about one-third of cases, PPID coexist with insulin dysregulation (ID). Here we describe an observational study, which aimed to determine if there is a relationship between ACTH and ID in senior horses and ponies with no clinical signs of PPID.

Methods

Eleven horses and ten ponies (two mares, nineteen geldings; all > 15 years-old), were selected from three local herds maintained by the Riding for the Disabled Association in south-east Queensland. Animals were excluded if they were on pergolide treatment, or had clinical signs of PPID (hypertrichosis, abnormal fat distribution, muscle wasting, hyperhidrosis, signs of chronic laminitis, polyuria/polydipsia). The animals were weighed, clinical examinations were performed, and blood samples were collected, in autumn (April, southern hemisphere) 2021 and 2022. Blood was collected by jugular venepuncture at approximately 7.30 am, before feeding, for the analysis of plasma ACTH. An oral glucose test (OGT) was then performed by feeding a mixture of 0.3% BW Lucerne chaff, 0.75% BW D-glucose, and 200 g wheat bran. Two hours after feeding this mixture, a second blood sample was collected to measure serum insulin. Both hormones were assayed by a commercial laboratory (Vetpath, WA, Australia), using an Immulite 2000xpi chemiluminescent assay. The data were $\log_{10}$ transformed, then analysed using Spearman's correlation test. The results for each animal were compared against seasonally adjusted diagnostic cut-off values for PPID (> 90 pg/mL) and ID (> 84 µU/mL), based on published data.

Results

Despite uniformity in age, insulin concentrations, measured post-OGT, showed considerable variation across the cohort, ranging from 12 to 642 µIU/mL. Similarly, ACTH ranged from 19 to 847 pg/mL. Based on their insulin concentrations, 8/21 animals (38%) were diagnosed with ID in 2021, and 9/21 (43%) had ID in 2022. Seven animals (33%) had ACTH concentrations > 90 pg/mL, which exceeds the equivocal zone for PPID diagnosis (in animals with clinical signs), based on the latest recommendations of the Equine Endocrinology Group (2021).

There was a significant, positive, linear correlation between ($\log_{10}$) plasma ACTH and serum insulin (post-OGT) in both 2021 ($r = 0.449$; $P < 0.05$) and 2022 ($r = 0.497$; $P < 0.05$). Despite the absence of PPID clinical signs, in both years, five animals with ID (four ponies and one horse) exceeded the ACTH threshold. In contrast, only two animals without ID (one pony and one horse) exceeded this threshold in each year.

Conclusions

- Insulin dysregulation was positively associated with high autumn ACTH concentrations in a cohort of horses and ponies with no clinical signs of PPID
- These observations warrant further studies to elucidate the interaction between breed, insulin, ACTH and PPID

Acknowledgements

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Lumbar Vertebral Bone Density is Decreased in Horses with Pituitary Pars Intermedia Dysfunction

A.C. Colbath, J.S. Fortin, C. Panek, F.B. Vergara-Hernandez, T.N. Johnson, C.M. Burglass, C.A. Robison, A.A. Logan, N.A. Nelson, B.D. Nielsen, H.C. Schott II. Michigan State University

Aims: To assess whether equids with PPID have decreased bone mineral density (BMD) in weight-bearing bones (third metacarpus and metatarsus) or non-weight-bearing bones (lumbar vertebrae) in comparison to aged horses without PPID. Further, the force to induce fracture of the fourth lumbar vertebrae was compared between aged healthy horses and aged, PPID-affected horses.

Methods: Five equids (four horses and one pony) with PPID and six aged and four young control horses without PPID were studied. PPID was diagnosed based on the presence of clinical signs, supportive thyrotropin releasing hormone (TRH) stimulation test results, and histologic scoring of the pituitary gland (PG). The lumbar vertebral column, right front third metacarpus (MC3), left hind third metatarsus (MT3), and PG were removed after euthanasia. BMD was determined using computed tomographic imaging of regions of interest (ROI) in each bone compared to a density phantom. Serum concentrations of parathormone (PTH), vitamin D3 (D3), and osteocalcin (OC) were also compared between groups. Finally, biomechanical testing was performed on the fourth lumbar vertebrae from aged controls and PPID-affected groups to determine force at fracture, displacement at fracture, strain, and Young's modulus. Data were analyzed using a one-way ANOVA and Tukey's multiple comparisons test.

Results: BMD of cortical and trabecular regions of the third, fourth, and fifth lumbar vertebrae were lower (P<0.01) in PPID-affected equids as compared to aged and young controls (**Figure 1**). In contrast, no differences were found in BMD of cortical or trabecular ROIs of MC3 and MT3 between PPID-affected equids and aged and young controls. Serum concentrations of PTH, D3 and OC varied within groups and no differences between groups were found. No differences in force at fracture, displacement at fracture, strain, and Young's modulus of the fourth lumbar vertebrae was found between aged controls and PPID-affected groups, although power for these analyses was low due to small sample sizes.

Conclusions: Pathologic fractures can develop in human and canine patients with Cushing's disease and have also been described in PPID-affected equids. We have documented lower BMD of non-weight bearing lumbar vertebrae in PPID-0affected equids. Potential mechanisms contributing to loss of BMD with PPID warrant further investigation.

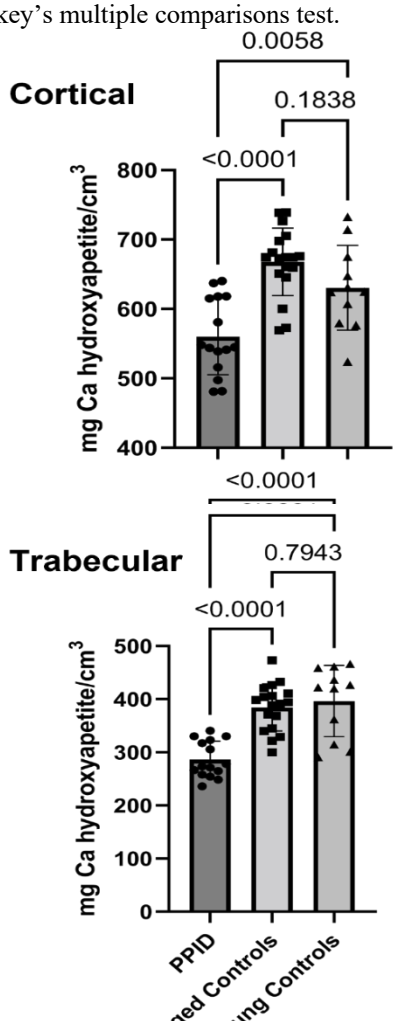

Figure 1. Cortical and trabecular bone mineral density (BMD, mg Ca hydroxyapatite/cm^3) of the third, fourth and fifth lumbar spinal vertebrae in horses with pars pituitary intermedia dysfunction (PPID), aged controls, and young controls. Cortical BMD was significantly lower in PPID versus aged controls (<0.0001) and young controls (P=0.0058). Likewise, trabecular bone density was significantly lower in PPID versus aged controls (<0.0001) and young controls (<0.0001). No difference was observed in BMD between aged and young controls. Data points for bar graphs represent measurements for all three vertebrae with bar height indicating overall mean and standard deviation for BMD.

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Plasma immunoreactive β-endorphin concentrations and spontaneous blink rate as an index of stress, measured in a population of aged horses and ponies with and without PPID

M Erdody,[a] N. Galinelli,[a] NJ Bamford,[a] S Mackenzie [a], T Warnken[b], PA Harris,[c] MN Sillence[d], SR Bailey[a].

[a]Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, Australia.
[b]Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany.
[c]Equine Studies Group, Waltham Petcare Science Institute, Melton Mowbray, Leicestershire, UK.
[d]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Queensland Australia.

Presenting author: Madi Erdody (merdody@student.unimelb.edu.au)

Animal ethics: This study was approved by the University of Melbourne Animal Ethics Committee (Approval #23234) and was conducted in compliance with applicable Australian laws.

Aims:
Beta-endorphin is a neuropeptide that is derived from the C-terminal region of the pro-opiomelanocortin (POMC) peptide secreted by the pituitary gland. It is an endogenous opioid, reducing stress and pain, and may be increased in equids with pituitary pars intermedia dysfunction (PPID), although it has not previously been correlated with ACTH levels in PPID cases. Spontaneous blink rate has been explored as a non-invasive tool to determine stress levels in horses and has been found to correlate with blood cortisol concentrations. This study aimed to correlate the plasma levels of β-endorphin with ACTH in horses and ponies being screened for PPID, and also compare these values with spontaneous blink rate.

Methods:
Thirty-one horses and ponies >15 years old were evaluated at two nearby properties in Victoria, Australia. The sample population was intended to include potential cases of PPID (i.e. not a random sample). Animals were sampled in late May and early June 2022 (equivalent to late November and early December in the Northern hemisphere). The horses and ponies were evaluated for clinical signs of PPID; baseline samples for measurement of ACTH (Immulite 1000) and β-endorphin were obtained and a TRH stimulation test was performed. A radioimmunoassay for β-endorphin was developed based on a polyclonal anti-human β-endorphin antibody. A subjective demeanour score was used in the assessment of the horses and then, to determine the spontaneous blink rate, a video from the left eye was recorded over a 5-minute period. Horses were classified as PPID or non-PPID based on their basal and/or TRH-stimulated ACTH concentrations, plus various clinical signs (including hypertrichosis, weight loss, polyuria/polydipsia, evidence or history of laminitis). Spearman correlations were performed between ACTH, β-endorphin values, blink rate and demeanour score. Data was compared using a Mann-Whitney test, with the significance level set at $P<0.05$.

Results:
There were 10 horses classified with PPID. Basal ACTH showed a strong correlation ($r=0.97$; $P<0.001$) with immunoreactive β-endorphin plasma levels. Immunoreactive β-endorphin values were significantly greater in PPID cases (1571 ±480 pg/ml) than non-PPID cases (543 ±176 pg/ml; $P=0.02$). Blink rate was positively correlated with demeanour score ($r=0.48$; $P=0.01$); however, immunoreactive β-endorphin was not significantly correlated with either blink rate ($P=0.27$) or demeanour score ($P=0.61$). No significant differences were detected in blink rate or behaviour score between PPID and non-PPID groups.

Conclusions:
The correlation between basal ACTH and immunoreactive β-endorphin suggests that it may be a useful adjunct in the assessment of PPID, although further validation is required to determine the post-translational forms of β-endorphin detected by the assay. Spontaneous blink rate may be a useful non-invasive index of stress, but there was no evidence of increased stress or altered demeanour in the PPID cases.

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Funded by the Australian Research Council (LP180101000), Boehringer Ingelheim Vetmedica GmbH and the Waltham Petcare Science Institute.

Muscle atrophy scores in a population of aged horses and ponies with and without PPID

M Erdody[a], S Mackenzie[a], N Galinelli[a], NJ, Bamford[a], T Warnken[b], MN Sillence[c], SR Bailey[a], PA Harris[d]

[a]Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, Australia
[b]Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany
[c]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Queensland Australia
[d]Equine Studies Group, Waltham Petcare Science Institute, Melton Mowbray, Leicestershire, UK.

Presenting author: Pat Harris (pat.harris@effem.com)

Animal ethics: This study was approved by the University of Melbourne Animal Ethics Committee (Approval #23234) and was conducted in compliance with applicable Australian laws.

Aims:
Muscle atrophy has been reported in horses suffering from pituitary pars intermedia dysfunction (PPID). An increase in proteolysis has been suggested as the underlying mechanism, and an elevation of muscle atrophy markers has been described in recent papers. Recently, a muscle atrophy scoring system has been developed and published, including a pilot study in a group of horses with PPID. However, this scoring system has not previously been evaluated in pony breeds. This study aimed to evaluate the muscle atrophy scoring system (vs. adiposity) in aged horses and ponies being screened for PPID, to compare between PPID and non-PPID animals.

Methods:
Thirty-one animals (18 ponies and 13 horses; >15 years old) were evaluated at two nearby properties in Victoria, Australia. Animals were evaluated in late May and early June 2022 (equivalent to late November and early December in the Northern hemisphere). PPID diagnosis was established, based on clinical signs (including hypertrichosis, weight loss, polyuria/polydipsia, evidence or history of laminitis), plus baseline ACTH (Immulite 1000) and a TRH stimulation test. Body condition score (BCS 1-9; Kohnke-modified Henneke scale) and cresty neck score (CNS 0-5 Carter scale) were assessed, and the muscle atrophy scoring system was also used, which yields a scale from 4 (no atrophy) to 16 (severe atrophy). Scores were compared between PPID and non-PPID animals using a Mann-Whitney test, and Spearman's correlation was used to examine the correlation between muscle atrophy score and BCS/CNS (significance level $P < 0.05$).

Results:
Seven ponies and five horses were diagnosed with PPID. Ten of the twelve PPID cases showed some evidence of muscle atrophy, with a median atrophy score of 7 (range 4-9). Fifteen of 19 non-PPID animals showed no signs of muscle atrophy (median score 4), while one horse (a 32-year-old mare) had marked atrophy (score of 11) and gradual weight loss of unknown cause; and three other horses showed mild signs of atrophy. There were no significant differences in median (range) BCS in PPID vs. non-PPID animals (6 [5-7.5] vs. 5.9 [4-8]), or in CNS (3 [2-4] vs. 3 [1-4]). The age distribution of the two groups was also not significantly different (median [range] of the PPID group was 20 [16-32] years and non-PPID group was 19 [15-32] years) (all comparisons $P>0.05$).

Conclusions:
The results confirm that muscle atrophy is a common feature of PPID; and that the scoring system developed in horses is also applicable to ponies, including Shetland/miniature ponies. The majority of non-PPID animals (of a similar age to the PPID group) showed no evidence of atrophy, indicating that old age *per se* is not typically associated with muscle loss in healthy animals. As the PPID animals had similar BCS and CNS to the non-PPID cohort, there appears to be no loss of adiposity due to this condition (assuming they are otherwise healthy). Rather, PPID appears to be associated with a specific loss of muscle tissue. These findings warrant further investigation and may have important implications for optimising the nutrition of horses and ponies with PPID.

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Factors influencing owner decision-making regarding the management and treatment of pituitary pars intermedia dysfunction

Authors: R.C. Tatum[1], C.M. McGowan[1] and J.L. Ireland[1]
Address: School of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Wirral, CH64 7TE, UK
Presenting Author Email: joirel@liverpool.ac.uk

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. Written informed consent was obtained from all participating horse owners. The study was granted ethical approval by the University of Liverpool's Committee on Research Ethics (reference number VREC667).

Aims: This study aimed to understand the context in which owners think about PPID and make decisions about their horse's management and treatment, as well as how owners perceive quality of life (QoL) in horses with PPID.
Methods: Individual semi-structured in-depth interviews were conducted with ten owners of PPID cases, registered with the University of Liverpool Equine Practice. Interviewees were participants in a previous cross-sectional questionnaire study. To ensure diverse experiences and viewpoints, questionnaire responses were used to purposively select owners based on characteristics such as PPID treatment(s) used, reported co-morbidities, length of ownership and premises type on which they kept their horse/pony. An interview topic guide was developed to direct conversations, using open questions to encourage meaningful discussion around PPID and its treatment. Data were analysed using thematic analysis, where interview transcripts were coded using line-by-line and section coding, and themes were actively searched for by reviewing the coded data to identify unifying features and key differences.
Results: Interviewees had owned/cared for their horses for a median of 16 years, which had been diagnosed with PPID between 2 months and 4 years prior to interview. Six overarching themes were identified; disease tangibility, balancing management and treatment complexities, owners being experts in their own horse, having a horse-centred approach, the vet-owner relationship and how health and happiness go 'hand in hand'. Themes demonstrated how decisions were influenced by the impact of PPID on the horse's daily life and visible changes observed by the owner. These perceptions were built upon and framed by owners' understanding of PPID, their in-depth knowledge of what is "normal" for their horse and the vet-owner relationship. Many owners found it difficult to quantify PPID as they did not see their horse as 'ill'. Owners described their familiarity with more advanced or obvious signs associated with PPID, such as a curly overgrown coat and laminitis. However, there was a lack of recognition of more subtle clinical signs. Owners' goals were to ensure their horse was both happy and healthy, and that their daily life was, at least in some way, influenced by what the horse itself wanted (as perceived by the owner). Factors such as the owner's relationship with their vet and complexities of treating co-morbidities were taken into account when reaching these goals. A complex mixture of emotional attachment, experience and knowledge of their horse underpinned how owners perceived their horse's health and therefore influenced the decisions made around treatment and management.
Conclusions: This study provides insight into owner decision-making around PPID treatment, which is complex and influenced by a multitude of factors. Decisions were driven by what owners thought was best for their horse, and enabled the best QoL possible. However, this could be confounded by conflicting needs. To improve compliance with treatment recommendations, it is important that veterinarians communicate clearly, and consider the wider context of the owner's situation, including current management strategies, presence of co-morbidities and how they perceive their horse's QoL.
Acknowledgements: We gratefully acknowledged all participating horse owners.

Long-Term Response of Equids with Pituitary Pars Intermedia Dysfunction to Treatment with Pergolide

H.C. Schott II, J.R. Strachota, J.V. Marteniuk, K.R. Refsal. Michigan State University

Aims: Limited data exist documenting long-term responses of equids with pituitary pars intermedia dysfunction (PPID) to pergolide treatment. Our objective was to describe clinical response, medical problems, outcome, and owner satisfaction with pergolide treatment of PPID-affected equids over 0.6-11 years.

Methods: After completion of the field efficacy study for Prascend™, 28 horses and two ponies were enrolled in an extended pergolide treatment study (15 receiving 2 µg/kg, PO, q24h and 15 receiving 4 µg/kg, PO, q24h). Equid owners were interviewed every 3 months and equids were re-evaluated after 2.5, 3, 3.5, 4.5, 5.5, 6.5, 9.5, and 12.5 years of treatment. Equid owners also completed an on-line survey 10 years after enrolling in the study.

Results: After 2.5 years of treatment, all owners reported clinical improvement and endocrine test results were normal in 79% of equids. After 5.5 years, owners of 13 surviving equids reported continued clinical improvement and 75% had normal endocrine test results. After 9.5 years of treatment, only 2/6 of surviving equids had normal endocrine test results. 7/15 equids had a dosage increase to 4 µg/kg, PO, q24h (maximum study dose) from 1.7 to 4.7 years of the study. Medical problems documented over the course of the study included gastrointestinal disturbances (colic, diarrhea), loss of body condition (attributed to dentition and ageing), laminitis, arthritis, and worsening of hypertrichosis. Of interest, a decrease in appetite (the most common adverse effect reported in the open field clinical efficacy study) was reported for only three (10%) equids in the extended use study. Over the course of the study, 29 of 30 equids died (n=5) or were subjected to humane euthanasia (n=24) between 1.5 and 11 years after onset of treatment. Median survival time during the extended use study was 3.3 years, ranging from 0.6-10.5 years. Euthanasia was performed due to complications of PPID, specifically laminitis, in five equids while death in the remaining 24 equids was attributed to disorders associated with advancing age (acute colic [n=6], poor dentition and wasting [n=5], acute neurological disorders [n=3], sudden death [n=5]), arthritis [n=3], and catastrophic fractures after falling on ice [n=2]).

In response to the on-line survey, 71% and 70% strongly agreed and 25% and 30% agreed that treatment with Prascend™ improved their equids quality of life and prolonged lifespan, respectively. 87% of owners either agreed or strongly agreed that they would provide lifelong treatment if they had another equid with PPID. However, medication cost would be a factor: 26% and 57% of owners were willing to pay an annual cost of $500 or $1000, respectively, while only 17% of owners would be willing to pay $1500 or more annually.

Conclusion: Long-term treatment of PPID-affected equids with pergolide produces clinical improvement in nearly all affected animals, normalization of endocrine test results in some cases, and high owner satisfaction. Further, this case series provides evidence that some horses may respond favorably long-term to a low pergolide dose, rather than a need for a progressive increase in dose over time in all PPID-affected equids.

Acknowledgements: The authors thank the dedicated equid owners that participated in this long-term study, Sue Wismer and Lauren Houston for assisting with data collection, and Boehringer-Ingelheim Animal Health USA, Inc., Duluth, GA for providing PRASCEND for the participants in this study.

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The hypothalamic-pituitary-adrenal gland axis response to vasopressin stimulation test in healthy and critically ill foals

K. Dembek[1], E. Elder[1], H, Robertson[1], M. Edwards[1], K. Johnson[2], N. Brown[3], D. Wong[2]

[1] College of Veterinary Medicine, North Carolina State University
[2] College of Veterinary Medicine, Iowa State University
[3] Hagyard Equine Medical Institute

Presenting author:
Katarzyna Dembek, DVM, PhD, DACVIM, College of Veterinary Medicine, 1060 William Moore Dr, Raleigh, NC 27606, kdembek@ncsu.edu

This prospective clinical study was performed at North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina. The study was approved by the Institutional Animal Care and Use Committee of North Carolina State University, the Clinical Research Advisory Committee of the College of Veterinary Medicine NCSU and adheres to the principles for the humane treatment of animals in veterinary clinical investigations as stated by the American College of Veterinary Internal Medicine and National Institutes of Health guidelines.

Aims: Sepsis is the main cause of death in newborn foals. The hypothalamic-pituitary-adrenal gland axis (HPAA) is the key regulator of the stress response to sepsis. Hypothalamic hormones such as corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) control the pituitary gland to release adrenocorticotropic hormone (ACTH), which then stimulates the adrenal gland to produce cortisol. Relative adrenal insufficiency (RAI) also known as critical illness related corticosteroid insufficiency (CIRCI) occurs in both human and equine patients and refers to the impairment of the HPAA during times of critical illness resulting in abnormal cortisol production, cellular activity, and metabolism. Studies have shown that septic foals with evidence of HPAA dysfunction are more likely to develop multiple organ dysfunction syndrome and are less likely to survive to discharge than septic foals with an intact HPAA. AVP has been implicated as the primary adrenocorticotropin-releasing hormone in horses giving it the potential to assess HPAA function more effectively in this population. However, no studies evaluated the adrenocortical and pituitary response to AVP stimulation test in septic foals with suspected HPAA dysfunction. Due to the lack of current information available regarding the complete endocrine response of newborn foals to critical illness, particularly the response to AVP, investigation is warranted. We hypothesized that administration of AVP would stimulate a rise in systemic ACTH and cortisol in healthy foals, and no clinically adverse effects would be detected. We also proposed that cortisol and ACTH response would be decreased in critically ill foals compared to healthy foals, and that the diminished response would be associated with increased mortality rate and severity of disease.
Methods: HPAA function was assessed in 12 healthy foals utilizing three doses of AVP (2.5, 5, 7.5 IU), administered between 24-48h of age in this randomized cross-over study. Hospitalized foals (n=18) were <7 days old and received 2.5 or 5 IU of AVP on admission. Cortisol, ACTH, and CRH were measured at 0 (baseline), 15, 30, 60, and 90 minutes after AVP administration with immunoassays. A fold increase 15 and 30 minutes from baseline was calculated for cortisol and ACTH concentrations.
Results: All doses of AVP resulted in a significant increase in cortisol concentration and a dose-dependent increase in ACTH concentration over time in both groups. ACTH and cortisol concentration increased 15 and 30 minutes after all doses of AVP compared to baseline in healthy and hospitalized foals ($P<0.01$). Cortisol and ACTH response to AVP administration (2.5 and 5 IU) at 30 and 15 minutes was lower in critically ill foals compared to healthy foals suggesting HPAA dysfunction ($P<0.05$). There was no effect of AVP on endogenous CRH.
Conclusions: Administration of AVP is safe and results in a significant rise in ACTH and cortisol in both healthy and hospitalized foals. A stimulation test with 2.5 and 5 IU of AVP can be considered for HPAA assessment in critically ill foals.
Acknowledgements: Thank you to the clinicians and staff at NCSU, ISU, and Hagyard Equine Medical Institute for the sample collection, and access to medical records.

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A single nucleotide polymorphism in the *POMC* gene is associated with key aspects of equine metabolic syndrome in horses

C. M. Cash, D. M. Fitzgerald, P. J. Prentis and M. A. de Laat

School of Biology and Environmental Science, Queensland University of Technology, Brisbane, 4001, Queensland, Australia

Presenting author: Melody de Laat (melody.delaat@qut.edu.au)

Use of cells and hair follicles in this study was granted ethical approval (QUT University Animal Ethics Committee; 1800000144).

Aims: Key components of equine metabolic syndrome (EMS) can include insulin dysregulation, increased adiposity and altered adipokine and triglyceride concentrations. The aetiology of EMS involves both environmental and genetic factors, although genetic causes remain largely unidentified. In other species, variations in genes involved in the leptin-melanocortin system, such as proopiomelanocortin (POMC), have been strongly associated with obesity and metabolic disease. The aim of this study was to investigate the *POMC* gene for variants that could be associated with biomarkers of EMS.

Methods: Initially, genomic DNA was extracted from white blood cells collected from horses of known EMS status (positive $n = 14$; negative $n = 16$). Hybridised capture was used to generate a reference sequence for the exons of the *POMC* gene in the healthy animals, which the unhealthy animals were aligned against (in addition to the reference genome (EquCab3.0)). Subsequently, in a separate population of horses ($n = 34$; various breeds including miniature horses) the identified variant was genotyped using DNA from hair follicles, and phenotypic and biochemical markers of EMS measured. Variables were compared with ANOVA on ranks or chi-square tests.

Results: A synonymous single nucleotide polymorphism (A>G) was identified on exon 1 with a homozygous A genotype (healthy reference sequence), a heterozygous AG genotype and a homozygous G genotype. The genotype groups did not differ in age, breed, height, body condition score or blood glucose, triglyceride or ACTH concentrations. However, post-prandial serum insulin concentrations were marginally higher ($P = 0.05$), and basal adiponectin concentrations lower ($P = 0.02$), for the GG genotype (Table 1). The GG genotype also had a higher-than-expected proportion of animals with ID ($P = 0.03$), as well as a CNS of 3 ($P = 0.03$). The potential for a sex predisposition warrants investigation in a larger population (Table 1).

Conclusions: The GG genotype appears to be associated with increased risk of EMS, compared to the AA genotype, in horses. These findings require investigation in a larger population that includes pony breeds, so that multivariate modelling and determination of allele frequency can aid in validating associations between the variant and EMS.

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Table 1: The sex distribution and selected phenotypic and biochemical markers of EMS (reported as median [IQR]) in 34 horses that were genotyped for a POMC gene variant.

Genotype	***AA***	***AG***	***GG***	***P value***
n	18	11	5	-
Sex (G; M)	15 G; 3 M	5 G; 6 M	4 G; 1 M	0.08
CNS (0-5)	1 [1-2]	1 [0-2]	3 [1-3]	0.03
Basal serum insulin (µIU/mL)	2.5 [2-21]	2 [2-7]	6 [2-118]	0.5
Post-prandial serum insulin (µIU/mL)	42.5 [21.8-107]	20 [13-65.7]	141 [57-270]	0.05
Basal adiponectin (ng/mL)	5.25 [2.58-7.8]	2.2 [1.7-4.38]	0.9 [0.53-3.93]	0.02

Key: CNS; cresty neck score, G; gelding, M; mare

Hepatic insulin clearance in equine metabolic syndrome

Holly Payne[1], Laura Scott[1], Jacqueline Poldy[2], John Keen[2], Alexandra Malbon[2] and **Ruth Morgan[1,2]**

[1] SRUC, Roslin Institute Building, Easter Bush Campus, EH25 9RG

[2]University of Edinburgh, Easter Bush Campus, EH25 9RG

Presenting Author: Ruth Morgan

This work was approved by the local animal ethics committee and adheres to the animal ethical research guidelines for the UK.

Aims:

Equine metabolic syndrome (EMS) is often characterised by marked and sustained elevation of insulin, a major risk factor for laminitis. Data from humans suggest that reduced hepatic clearance of insulin is a significant factor driving hyperinsulinaemia (Najjar & Perdomo 2019) and is particularly associated with non-alcoholic fatty liver disease (NAFLD). In this study we address the hypothesis that impaired hepatic insulin clearance contributes to the pathogenesis of hyperinsulinaemia in horses. We first determined whether NAFLD was a feature of equine hyperinsulinaemia and then quantified the expression/activity of insulin clearance proteins (CEACAM1 and Insulin Degrading Enzyme).

Methods:

Tissue samples were collected at post-mortem from clinically well-characterised horses/ponies with (n=7) and without (n=9) hyperinsulinaemia associated with EMS. Horses without endocrine disease were euthanased for unrelated orthopaedic disease and only included if they did not have any history/clinical signs of liver disease. EMS was defined as a body condition score >3.5, fasting basal insulin >20mlU/L, current/history of laminitis, plasma ACTH within seasonal reference range, and an absence of histological changes to the pituitary. In addition to endocrine testing (ACTH and serum insulin, Immulite), serum biochemistry was conducted to quantify liver enzymes. Liver tissue was fixed in 10% formalin, paraffin-embedded and 5 µm sections stained with haematoxylin and eosin (H&E) and immunohistochemically for CEACAM1. Each H&E stained section was scored by two blinded observers using an equine liver disease scoring system (Durham *et al* 2003) and a human NAFLD scoring system (Bedossa *et al* 2012). In frozen liver sections, triglycerides (Abcam, Cambridge, UK) in whole homogenates and Insulin Degrading Enzyme (IDE) activity in the hepatic cytosol (SensoLyte® 520 IDE Activity Assay Kit) were quantified. Data were tested for normality and appropriate statistical tests chosen.

Results:

Cumulative NAFLD scores (control 1.71+/- 1.1 v EMS 2.5 +/- 2.0), were not significantly different between the groups, however 2/7 horses with EMS were classified as having NAFLD (score >4) compared to 0/9 control animals. Hepatic triglyceride content was not significantly different between the groups. There was no correlation between NAFLD score and basal insulin measurement.

CEACAM1 was identified in all horses by immunohistochemistry and initial analysis suggests that CEACAM1 staining is reduced in horses with hyperinsulinaemia; further analysis is required to confirm these results. Insulin degrading enzyme activity was significantly decreased in horses with hyperinsulinaemia compared with controls (controls 2.6 +/- 0.1 v EMS 2.2 +/- 0.2 activity/mg protein).

Conclusions:

These preliminary data suggest that whilst hepatic triglyceride accumulation and fatty-liver disease are not features of equine hyperinsulinaemia, there are differences in insulin clearance proteins. The apparent reduction in insulin clearance capacity could contribute to the development, worsening or persistence of hyperinsulinaemia in EMS and represents a potential therapeutic target. Insulin is also cleared via renal pathways and further work is required to determine if any hepatic changes are compensated for by other tissues.

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Effect of dopamine depletion on insulin sensitivity and insulin response to a glycaemic meal in Standardbred horses

N. Galinelli[a], M Erdody[a], NJ Bamford[a], J. Sonntag[b], PA Harris[c], MN Sillence[d], SR Bailey[a]

[a]Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, Australia
[b]Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany
[c]Equine Studies Group, Waltham Petcare Science Institute, Melton Mowbray, Leicestershire, UK
[d]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Qld, Australia
Presenting author: Nicolas Galinelli

Animal ethics: This study was approved by the University of Melbourne Animal Ethics Committee (Approval #20600) and was conducted in compliance with applicable Australian laws.

Background: Low dopamine concentrations have been associated with the pathophysiology of Pituitary Pars Intermedia Dysfunction (PPID), and about one-third of PPID cases are insulin dysregulated. In other species, dopamine levels appear to affect insulin secretion and tissue insulin sensitivity, as do dopamine agonist and antagonist drugs. However, to date, studies with these drugs in horses have not shown a significant effect. Alpha methyl para-tyrosine (AMPT) is a reversible inhibitor of tyrosine hydroxylase, the rate-limiting enzyme involved in the synthesis of dopamine. AMPT has been shown to reduce dopamine concentrations in other species, but its effects have not been studied in horses.

Aims: To determine the role of dopamine and the effect of short-term dopamine depletion in controlling tissue insulin sensitivity and the insulinemic response to a glycaemic meal in horses.

Methods: Six adult gelding Standardbred horses were selected, with an average age of 16 years (± 5.2 SD),. Horses were treated with either placebo or a single dose of AMPT (40 mg/kg orally) in a randomized cross-over design, 14 days apart. Dopamine reduction was demonstrated by an acute increase in plasma prolactin (measured by radioimmunoassay). The horses were given an oral glucose test (OGT; 1g glucose/kg BW) and insulin sensitivity was measured by an IV frequently sampled insulin and glucose test (FSIGT) with a 2-week wash out period between procedures. The FSIGT was initiated with a bolus of glucose (300 mg/kg BW), followed by a dose of insulin (20 mIU/kg BW) after 20 min. Blood glucose was measured using a validated hand-held glucometer, and plasma insulin was measure using an Immulite 1000 chemiluminescent assay. For the OGT, the peak responses (C_{max}) and area under the curve (AUC) for glucose and insulin were compared using Wilcoxon matched-paired rank tests. For the FSIGT the Minimal Model (MiniMod) software was used to determine insulin sensitivity (SI), acute insulin response to glucose (AIRg) and glucose effectiveness (Sg). The significance was set at $p < 0.05$.

Results: AMPT produced a prolactin peak at 28.3 ± 1.4 ng/ml (mean ± SEM), which was not observed following the placebo treatment. Dopamine suppression did not affect SI ($p = 0.75$) nor AIRg ($p = 0.31$). However, the glucose disposal (Sg) was decreased by AMPT (0.017 ± 0.005 vs 0.029 ± 0.01 min^-1, mean ± SEM; $p = 0.023$). Following the OGT, the AMPT caused an increase in the insulin peak and the AUC (11019 ± 5387 vs 7663 ± 3904 µIU/ml/min; $p < 0.05$) when compared with the placebo.

Conclusions: No evidence was found for dopamine influencing insulin sensitivity. This differs from other species, but it is consistent with previous work in horses. The Sg is defined as the ability of glucose to enhance its own clearance independently of insulin, but further work in necessary to understand its physiological significance. The results of the OGT suggest that AMPT may have a local effect by inhibiting dopamine release from peripheral sites such as the stomach, which then affects the pancreas. Further work is warranted to investigate this mechanism.

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Presenting author: E J Knowles e.j.knowles@gmail.com, Bell Equine Veterinary Clinic, Mereworth, Kent, ME18 5GS

The study was carried out at the Royal Veterinary College, Hawkshead Lane, Hatfield AL9 7TA

Ethical Approval: The study was approved by the Royal Veterinary College Animal Welfare and Ethical Review Board. and conducted under a UK Home Office Licence. The study complies with all relevant UK legislation.

Previous publication: The present data analysis has not been previously published. The underlying data were used to in a risk analysis for laminitis development published in the Equine Veterinary Journal. A limited part of the present analysis will be presented as a poster at the ECEIM Congress October 2022.

Word count excluding title and authors: 498 words

Factors associated with the insulin response to an oral sugar challenge in a cohort of ponies

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

Aims: The study aimed to: 1) describe associations between serum insulin concentration at 60 minutes during an oral sugar test (OST) ([insulin]T60), and physical, management and signalment factors to determine whether these factors could be used to select animals for assessment of insulin dysregulation; 2) to combine these factors with basal metabolic markers into a statistical model to characterise-better OST insulin responses.

Methods: Physical examinations and OSTs (0.3ml/kg Karo syrup) were performed on a cohort of ponies every 6 months (autumn and spring) for ≤4 years. Physical factors included body condition score (BCS) (1-9), cresty neck score (CNS), clinical signs of PPID and evidence of divergent hoof growth. Factors associated with [insulin]T60 were determined using two pre-specified linear mixed-effect models. Model-1 included season, physical, signalment and management variables as fixed-effects and pony and premises identifiers as random-effects. Model-2 included all factors from Model-1 and basal plasma concentrations of ACTH, adiponectin, triglycerides and glucose as fixed effects. ACTH was dichotomised based on seasonally-adjusted reference ranges and an interaction between a positive ACTH and season was included in the final model. Variables were selected based on practical factors and to reduce correlation between fixed-effects. Residuals were assessed for normality.

Results: 1167 samples from 311 ponies on 23 premises were included. Mean (±SD) [insulin]T60 (µIU/ml) was 71.8 (±91.6). Factors with statistically significant ($p<0.05$) associations with [insulin]T60 (effect estimate, 95% confidence interval (µIU/ml)) were:
1) Model-1: season (autumn) (10.9, 3.3-18.6), Welsh / Welsh X (compared with 'other breeds') (29.4, 6.3-52.5), bulging supraorbital fat pads (22.2, 9.4-35), BCS (12.8, 6-19.6) per unit of BCS, age (4.5, 3-6) per year, hypertrichosis (-35.2, -6.4- -64.1) and the duration/ intensity of exercise (-2.8, -0.5- -5.1)) per unit of composite exercise score.
2) Model-2: season (autumn) (14.7, 6.5-22.8), Welsh / Welsh X (compared with 'other breeds') (23.9, 3.7-44.2), bulging supraorbital fat-pads (21.9, 9.9-34), BCS (11.1, 4.7-17.6) per unit of BCS, age (3.4, 2.1-4.8) per year, hypertrichosis (–35.9, -8.1- -63.6), 'positive' basal ACTH (35.8, 21.8-49.9), basal glucose (39.4, 32.8-46) per mmol/l, basal triglycerides (27.5, 11.7-43.3) per mmol/l and basal adiponectin (-0.7, -0.2- -1.2) per µg/ml. Associations with sex, CNS, a 'pot belly', divergent hoof growth, and a turnout composite score were not significant in either model. The interaction between a 'positive ACTH' and season was not significant. Nakagawa's R-squared values and within-pony ICC were:
1) Model-1: 0.13 (marginal/fixed-effects) and 0.7 (conditional/ full model), ICC=0.65.
2) Model-2: 0.24 (marginal/fixed-effects) and 0.7 (conditional/ full model), ICC=0.61

Conclusions: A small proportion of the variation in [insulin]T60 was explained by the physical, signalment, seasonal or management factors studied. Selection of ponies for diagnostic testing on this basis requires caution. Model-2 explained a higher proportion of the variation in [insulin]T60 but substantial variation in [insulin]T60 resulted from within-pony factors not characterised in the present models. The effect of hypertrichosis may be biased by few affected animals. Exercise and BCS are modifiable factors associated with [insulin]T60.

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

Insulin response to short-term transportation stress in horses: Effects of age and insulin dysregulation.

E.T. Jacquay[1], P.A. Harris[2], A.A. Adams[3]

[1]MARS Equestrian Scholar, M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, United States of America
[2]Equine Studies Group, Waltham Petcare Science Institute, Waltham-on-the-Wolds, Leicestershire, England, United Kingdom
[3]MARS Equestrian Research Fellow, M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, United States of America

Presenting author: Erica T. Jacquay, 1400 Nicholasville Rd, Lexington, KY, 40546, Erica.Jacquay@uky.edu

Aims: To understand the endocrine response to short-term transportation stress in horses of different ages and insulin dysregulation status.

Methods: Study #1: Twelve healthy, non-pregnant mares of mixed light breed were categorized by age into young (n = 6, 2 ± 1 years) and aged (n=6, 22 ± 1 years) groups. Horses were transported in a livestock trailer on a round trip journey of approximately 1.5 hours in the morning directly from pasture in groups of 4; necessitating 3 separate trips over 2 weeks, with 2 aged and 2 young horses on each trip. Blood and saliva were collected 1 month prior to transport, 24 hr before transportation and on the day of transportation: 1 hr before loading plus 15min, 30 min, 1 hr, 2 hr and 4 hr after unloading. Study #2: Fourteen non-pregnant, non-PPID mares of mixed age and breed were age-matched and grouped as insulin dysregulated (ID, n=7) and non-insulin dysregulated (non-ID, n=7). Horses were transported in groups of 3-4 on 4 separate trips over 2 weeks under the same conditions as study #1. Blood and saliva were collected 24 hours before transport and on the day of transportation: 1 hr before loading, directly after unloading plus 15min, 1 hr, 3 hr and 24hr post transportation. An oral sugar test (OST) was performed 24 hr pre-transportation and 3 hr post-transportation. For both studies serum insulin analysis was done via RIA, while plasma ACTH and serum cortisol were performed using a chemiluminescent immunoassay system (Cornell University). Salivary cortisol and insulin were determined through ELISA (Salimetrics, LLC.).

Results: For both studies serum and salivary cortisol increased in response to transportation with peak cortisol directly post-transport; however, cortisol responses were not different between aged vs young or ID vs non-ID horses. In study #1 plasma ACTH was increased in aged horses compared to young (P=0.007) with an effect of transportation (P<0.001), but no age x transportation effect. In study #1 insulin increased in response to transportation in aged horses with a more exaggerated insulin response from 1 hr to 3 hr post-transportation (P<0.001). For study #2 serum insulin was increased in ID horses compared with non-ID horses (P<0.0001) with an effect of transportation (P=0.02), whereas salivary insulin only had an effect of insulin status (P=0.03). ID horses had an increase in insulin response to OST both pre- and post-transportation (P<0.05) and while there was not a statistical difference in insulin responses to the OST for non-ID horses' post vs pre-transportation the mean T60 insulin was 56.6 ± 9.9 uU/mL, which is above the cutoff for ID diagnosis. Serum glucose increased in both ID and non-ID horses in response to transportation (P=0.003).

Conclusions: There were no differences in cortisol responses to short-term transportation in horses regardless of age or insulin status; however, aged and ID horses' insulin responses increased after short-term transportation. Performing an OST in non-ID horses or collecting basal insulin on aged horses' post-transportation could cause potentially misleading results for ID diagnosis.

Acknowledgements: This study was funded by MARS EQUESTRIAN™.

This study was conducted at the University of Kentucky's Department of Veterinary Science North Farm in Lexington, Kentucky, USA. It was approved by the Institutional Animal Care and Use Committee under protocol #2021-3854.

This material has not been presented or published.

The assessment of equine insulin dysregulation using a glycaemic carbohydrate pellet

M. A. de Laat[a#], T. Warnken[bc#], J. Delarocque[c], D. B. Reiche[b], A. J. Grob[c], K. Feige[c], H. B. Carslake[d], A. E. Durham[e], M. N. Sillence[a], K. E. Thane[f], N. Frank[f], J. Brojer[g], S. Lindase[g] and J. Sonntag[b]

[a]School of Biology and Environmental Science, Queensland University of Technology, Brisbane, 4001, Queensland, Australia

[b]Boehringer Ingelheim Vetmedica GmbH, Binger Straße 173, 55216 Ingelheim, Germany

[c]Clinic for Horses, University of Veterinary Medicine, Hannover, Germany

[d]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, United Kingdom

[e]Liphook Equine Hospital, Liphook, United Kingdom

[f]Department of Comparative Pathobiology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA. USA

[g]Department of Clinical Sciences, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden

[#] These authors contributed equally to this work

Presenting author: Melody de Laat (melody.delaat@qut.edu.au)

The work undertaken in this study was approved by the relevant Animal Ethics Committee in each location.

Aims: Dynamic testing is recommended to diagnose insulin dysregulation (ID), but test inconsistency and poor acceptance impair clinical management. This study used specially formulated glycaemic pellets as a novel carbohydrate source during an oral glucose test (OGT). The aims were to determine 1) the palatability of the pellets, 2) the glucose and insulin responses to their administration and 3) the serum insulin threshold for the diagnosis of ID when administering the pellet.

Methods: The study was undertaken in five locations (Australia, Germany, Sweden, UK, USA) using adult horses (n = 92) and ponies (n = 65) of any sex or breed. Pellets were offered for free intake over 10 min at 0.5 g/kg BW soluble carbohydrate. Blood samples were collected for measurement of insulin and glucose concentrations before, and hourly for 3h after, the pellets were offered. Pellet intake time and behavioural responses to the pellets were determined to assess palatability. The diagnostic threshold was calculated using two clusters (high and low insulin) by complete linkage hierarchical clustering.

Results: The pellets were palatable to 84% of animals with ponies more (P = .004) willing to accept them than horses. The speed of intake (4 [3-6] min) was positively associated with acceptance (P < .0001). Peak glucose (6.6 [5.8-7.8] mmol/L) and insulin (40.5 [19-99.8] µIU/mL) responses to the pellets occurred at 2h. The corresponding diagnostic threshold for ID was calculated to be 83 µIU/mL.

Conclusions: The glycaemic pellets were found to be a palatable source of carbohydrate for the OGT.

Acknowledgements: This study was funded by Boehringer Ingelheim Vetmedica GmbH. The authors acknowledge the Riding for the Disabled Associations at Anstead, Arundel Park and Sunshine Coast in Australia and the Animal Research Centre at the Swedish University of Agricultural Sciences.

Elin Svonni, Sanna Lindåse, Johan Bröjer

- Presenting author: Elin Svonni, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, 750 07 Uppsala, Sweden; email: elin.svonni@slu.se
- 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.

Postprandial insulin responses to feeding forage with different carbohydrate content in horses with moderate to severe insulin dysregulation – preliminary results from an ongoing study

Aims: Dietary management is the most important strategy to prevent excessive postprandial hyperinsulinemia in horses with insulin dysregulation (ID). Forage with low water-soluble carbohydrate (WSC) content (< 10 %) is recommended to horses with ID. However, little is known about the postprandial insulin responses in horses with ID. The aim of this study was to evaluate the insulin responses to feeding forage with different WSC content in horses and ponies with moderate to severe ID.

Methods: This is an ongoing study using privately owned horses and ponies, previously diagnosed with ID by referring veterinarians using an oral sugar test (OST; Dansukker glucose syrup 0.2 mL/kg). Horses were enrolled if OST insulin concentrations were > 100 µIU/mL based on blood samples obtained between 60 and 90 minutes. The horse owners had introduced new dietary strategies as recommended by treating veterinarians prior to participation in the study. Horses were excluded if they had an ongoing acute episode of laminitis or if they had PPID.

The study consisted of a clinical visit to obtain baseline data. On the third day of the clinical visit a 180 minutes OST (Dansukker glucose syrup 0.5 mL/kg) with eight samplings was conducted. In the morning of the fourth day, the horses were subjected to a meal tolerance test (MTT) and were fed 0.4 kg dry matter forage/100 kg body weight of their own regular diet. Blood samples were collected at baseline (fasting samples), and then at 30 minute intervals up to 300 minutes.

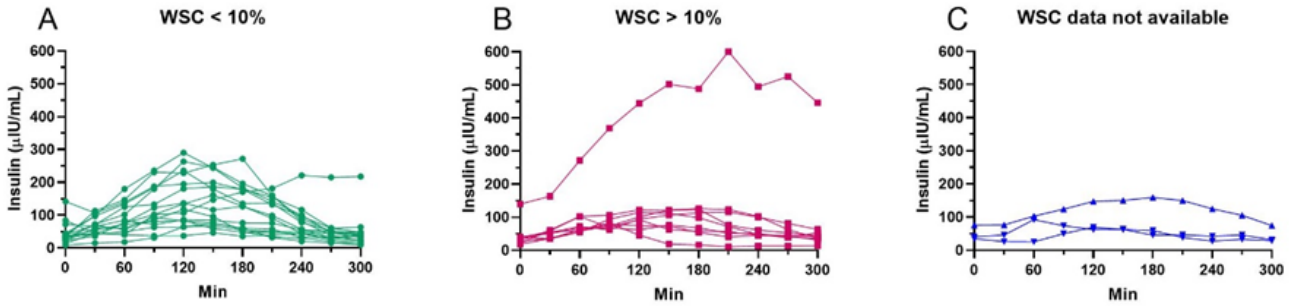
Results: The majority of the horses (25/28) had a history of at least one episode of laminitis. The horses were fed hay (14/28), haylage (13/28) or a combination of hay and haylage (1/28). Most of the forage used (25/28) were analyzed for WSC content. The WSC content was < 10% on a dry matter basis for 16/28 forages used and > 10% for 9/28 forages used.

The median insulin concentration at 90 minutes from the OST performed during the clinical visit was 216.8 µIU/mL (103.6 – 836.6). Maximal insulin concentration (insulin C_{max}) from the MTT exceeded 200 µIU/mL for six of the horses (21%) and was between 150 and 200 µIU/mL for four of the horses (14%) of the horses (Fig. 1). There was no linear correlation between insulin C_{max} from the MTT and the WSC content in the fed forage (P = 0.95). The insulin $C_{90\ min}$ from the OST was positively correlated to the insulin C_{max} from the MTT (P = 0.0002).

Conclusions: Preliminary results from this ongoing study suggests that the current dietary recommendation of feeding forage with < 10% WSC content is not always enough to avoid postprandial hyperinsulinemia beyond the level at risk to induce laminitis in horses with moderate to severe ID. Therefore, it is advisable to evaluate the insulin response after feeding in horses with ID.

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

Figure 1. Insulin concentrations after feeding horses forage (0.4 kg dry matter/100 kg body weight) with a WSC content < 10% on a dry matter basis (A), > 10% (B) and with unknown WSC content (C).



Insulinemic responses to small meals of forage pellets in insulin dysregulated vs non-insulin dysregulated horses

E. Macon[1], B. Perron[1], P. Harris[2], M. Greiter[1], and A. Adams[3]
[1]Department of Veterinary Science, M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA
[2]Equine Studies Group, Waltham Petcare Science Institute, Waltham-on-the-Wolds, Leicestershire, England, United Kingdom
[3]MARS EQUESTRIAN Research Fellow, M.H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, United States of America

Presenting author: Brittany Perron; Brittany.Perron@uky.edu

All procedures described were approved by the University of Kentucky Institutional Care and Use Committee (#2019 - 3395).

This material is original and has not been presented elsewhere.

Aims:

To investigate the effect of meal feeding forage pellets with different non-structural carbohydrates and crude protein contents on the insulin responses in horses with insulin dysregulation compared to metabolically, healthy adult horses.

Methods:

Fifteen adult horses were metabolically categorized, on the basis of their response to an oral sugar test into either, insulin dysregulated (ID; n=8; 16.1 ± 2.2 yrs; BCS 7.5 ± 1.1) or non-insulin dysregulated (NID; n=7; 17.0 ± 2.8 yrs; BCS 6.4 ± 0.7). The randomized crossover study was conducted over 5 weeks, preceded by a one-week acclimation period. Five dietary treatments were fed at a rate of 1.0 g/kg BW/meal once a week so that all horses received each diet on one occasion. Diets were 1) a positive control: oat groats (OG; 14.7% CP & 59.7% NSC DM), 2) a negative control: a low nonstructural carbohydrate pellet (LNSC; 12.8% CP & 5.4% NSC DM; Buckeye TM, MARS USA), 3) timothy hay pellets (TH; 9.5% CP & 10% NSC DM), 4) alfalfa hay pellets (AH; 16.3% CP & 9.8% NSC DM), and 5) timothy/alfalfa hay pellets (TAH; 17.2% CP & 9.8% NSC DM). Blood samples were collected immediately pre-feeding and, 60 (T60), 90 (T90), and 120-minutes (T120) postprandially. All diets were analyzed by wet chemistry (Equi-Analytical) and blood samples were analyzed for insulin by RIA (Cornell AHDC lab). Data were analyzed using Minitab software using a mixed effects model with explanatory factor of metabolic group, dietary treatment, and horse (random) with response variables of peak insulin and AUCi.

Results:

Peak insulin for ID horses was higher ($P<0.05$) for all dietary treatments compared to NID peak insulin, except for LNSC ($P=0.06$). ID horses' peak insulin was higher for OG (230.2 ± 38.2 µIU/mL) compared to all other feedstuffs ($P<0.01$). Similarly, NID peak insulins were higher for OG (34.6 ± 2.75 µIU/mL) compared to LNSC, AH, and TH ($P<0.01$). In addition, TAH peak insulin for NID horses was higher than AH and TH ($P<0.01$). ID horses AUCi were different for all diets compared to NID AUCi ($P<0.01$). ID AUCi was higher for OG compared to all other dietary treatments ($P<0.01$). For NID horses, AUCi was higher for OG compared to AH and TH ($P<0.04$).

Conclusion:

Although ID horses insulin values were greater than NID, their postprandial insulin responses to forage pellets were similar. Hay pellets had varying levels of protein, but nearly identical NSC content further proving that protein is not the main driver of the postprandial insulinemic response. ID horses have an exacerbated response to high NSC feedstuffs but do not have an exacerbated postprandial insulinemic responses to forage pellets (NSC ≤10%) when fed in small quantities (1 g/kg BW). Current studies are underway to examine the insulin responses of ID horses to long-stem forage fed in larger amounts.

Acknowledgements:

This study was funded by Morris Animal Foundation.

Comparison of published cut-off values for blood insulin concentrations using a newly developed web app

Julien Delarocque[1]*, Karsten Feige[1], Harry B. Carslake[2], Andy Durham[3], Tobias Warnken[1#]
[1] Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Bünteweg 9, 30559 Hanover, Germany
[2] Institute of Infectious, Veterinary and Ecological Sciences, University of Liverpool, Leahurst, Cheshire CH64 7TE, UK
[3] The Liphook Equine Hospital, Forest Mere, Liphook, Hampshire GU30 7JG, UK
* Presenting author, julien.delarocque@tiho-hannover.de
[#] Current address: Boehringer Ingelheim Vetmedica GmbH, Binger Straße 173, 55218 Ingelheim, Germany

The following research was conducted in accordance with the respective national legislation of the authors. Part of the samples was obtained from experiments approved by the authors' respective home university and/or state office for animal protection. The remaining samples were collected for diagnostic purposes. Informed consent was obtained from the owners.

Aims: To provide means of comparing blood insulin concentrations obtained using different insulin immunoassays to overcome the strictly assay-specific relevance of published reference ranges and cut-off values.

Methods: Blood insulin concentrations were measured with at least two different insulin immunoassays in parallel in samples belonging to a diverse equine population. Polynomial models of the first (linear) or second degree were selected by 10-fold cross-validation to optimize their predictive performance, avoid overfitting, and enable conversion of the insulin concentrations from one assay to another. The resulting models were published as a web app available at www.equine-insulin-converter.org. A selection of published cut-off values for diagnostic tests of insulin dysregulation was converted using this tool to allow for their comparison.

Results: The ADVIA Centaur insulin chemiluminescent immunoassay (CLIA), Beckman Coulter insulin radioimmunoassay (RIA), Immulite 1000 CLIA, Immulite 2000 CLIA, Immulite 2000 XPi CLIA, Mercodia equine insulin enzyme-linked immunosorbent assay (ELISA), and Millipore porcine insulin RIA were compared in pairs of assays with a median number of 70 samples (range: 36–179) available for comparison. The obtained models had good predictive performance (median $r^2 = 0.94$, range: 0.70–0.996) without obvious overfitting. When converted to a common reference (Mercodia equine insulin ELISA), the published cut-offs for basal insulin concentrations ranged from 9.5 to 46 µIU/mL (median: 28.2 µIU/mL). At 120 min of oral glucose test protocols, the median cut-off for insulin was 164.5 µIU/ml (range: 110–199 µIU/mL).

Conclusions: Our web app can be used to gain insight into the differences between some insulin immunoassays or compare values from clinical cases to published reference ranges and cut-offs. Even when selecting studies with similar methodologies, the comparison between published cut-offs can be hampered by many factors, starting with the metrics used to select the optimal cut-off, the prevalence of insulin dysregulation in the studied population and the reference method to identify insulin-dysregulated individuals. Nevertheless, a reasonable agreement can be observed among cut-offs after conversion to a common reference assay. Although its limitations need to be acknowledged before using it for clinical decision-making, this app may facilitate the detection of equids affected by insulin dysregulation and prone to laminitis when the assay used cannot be selected by the clinician themselves.

Parts of the results will be presented at the ECEIM Congress 2022. Data from two additional insulin assays were added in the present version, which focuses on the implications for diagnostic tests of insulin dysregulation rather than the development of the app itself.

Validation and method comparison for a point-of-care lateral flow assay measuring equine whole blood insulin concentrations

Emily H Berryhill, Naomi S Urbina, Sam Marton, William Vernau, Flavio H Alonso

Corresponding author: Emily H Berryhill, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, One Garrod Dr, Davis, CA 95616, USA. eberryhill@ucdavis.edu

Research was conducted at the University of California, Davis, with additional sample analysis performed at the Cornell Animal Health and Diagnostic Center. The study received approval from the Institutional Animal Care and Use Committee (protocol #20751) and followed guidelines for humane animal treatment.

Aims - The Wellness Ready Equine Insulin Test (WRT) is a stall-side lateral flow assay that measures equine insulin in whole blood. This study aimed to validate the WRT and compare performance to a reference radioimmunoassay (RIA). It was hypothesized that the WRT would show acceptable precision, accuracy, linearity, and association with insulin concentrations obtained using the RIA.

Methods - Equine whole blood was collected after fasting, feeding, or oral sugar testing to obtain insulin concentrations spanning the assay's working range. Three samples were obtained with anticipated insulin concentrations of 139 – 278 pmol/L [20 - 40 μIU/mL] (low), 278 – 417 pmol/L [40 - 60 μIU/mL] (intermediate), and >417 pmol/L [>60 μIU/mL] (high). Twenty replicates were performed for each sample using kits from 1 lot number and 1 WRT reader to determine intra-assay precision. Ten replicates were performed using kits from 2 additional lots and multiple readers to determine inter-assay precision. To determine linearity, 5 dilutions (3 replicates per dilution) were created from blood with an insulin concentration <139 pmol/L [<20 μIU/mL] combined with blood with a concentration approaching 695 pmol/L [100 μIU/mL] (representing lower and upper limits of dynamic range, respectively). For method comparison, 99 whole blood (WRT) and corresponding plasma samples (RIA) with concentrations ranging from <139 pmol/L [<20 μIU/mL] to >695 pmol/L [>100 μIU/mL] were tested in duplicate.

Intra- and inter-assay precision were estimated using mean, standard deviation, and coefficient of variation (CV). Linearity was assessed by plotting measured against expected concentrations at each dilution, with results analyzed by weighted linear regression. Bias of the WRT versus the RIA was assessed using Passing-Bablok linear regression, Spearman correlation, and Bland-Altman plots. Assay clinical sensitivity, specificity, accuracy, and total observed error (TE_O) were determined at insulin cut-offs used for diagnosing insulin dysregulation, using the RIA as the gold standard.

Results - Tested insulin concentrations ranged from <139 to >695 pmol/L [<20 and >100 μIU/mL]. The WRT intra-assay CVs at low, intermediate, and high concentrations were 13.3%, 12.9%, and 15.3%, respectively. Inter-assay CVs were 15.9%, 11.0%, and 11.7%, respectively. Weighted linear regression showed $R^2 = 0.98$, slope 1.02, and y-intercept 14.4 pmol/L [2.08 μIU/mL]. The Spearman correlation coefficient (r_s) was 0.90 (95% CI 0.85-0.94). The WRT concentrations averaged 10.4% higher than the RIA, with mean bias of 25.9, 26.1, and 26.7 pmol/L [3.74, 3.76, 3.84 μIU/mL] for cut-offs of 312, 347, and 451 pmol/L [45, 50, and 65 μIU/mL]. Clinical sensitivity varied from 87-95%, specificity from 92-96%, accuracies from 91-95%, and TE_O 28-30.4%, determined by the 3 clinical cut-offs.

Conclusions - The WRT performed adequately regarding analytical precision and linearity and showed good association with insulin concentrations measured with the RIA. Specific reference ranges are indicated to further aid WRT interpretation.

Acknowledgements - We acknowledge Wellness Ready Labs for financial support; Thomas Kwan for statistical analysis; Judy Edman, Emily Phenix, Brittany Smith, Hannah Labrie-Smith, Maria Gonzalez for technical support; the UC Davis Center for Equine Health for providing horses; and the Cornell Animal Health and Diagnostic Center for reference assay testing.

Plasma high molecular weight adiponectin concentrations in a population of aged horses and ponies with and without PPID

S. Mackenzie[a], N Galinelli,[a], M Erdody,[a] NJ Bamford,[a] MN Sillence[b], T Warnken[c], PA Harris[d], SR Bailey[a]

[a] Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, Australia.
[b] School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Queensland Australia
[c] Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany
[d] Equine Studies Group, Waltham Petcare Science Institute, Melton Mowbray, Leicestershire, UK.

Presenting author: Skye Mackenzie (samackenzie@student.unimelb.edu.au)

Animal ethics: This study was approved by the University of Melbourne Animal Ethics Committee (Approval #23234) and was conducted in compliance with applicable Australian laws.

Background:
Old age in equids may be associated with the onset of insulin resistance (IR), although the causal mechanism of this phenomenon is not well understood. Pituitary *pars intermedia* dysfunction (PPID) is also a very common condition in this cohort, with up to 21% of horses and ponies over 15 years affected. Among PPID cases, a significant proportion may be insulin dysregulated and this may lead to a high risk of laminitis. Adiponectin is an adipokine hormone with anti-inflammatory and insulin sensitising properties; and low plasma levels of adiponectin have been associated with IR and laminitis risk.

Aims: The aim of this study was to correlate plasma adiponectin levels with insulin sensitivity, basal ACTH and TRH response tests in a population of horses and ponies >15 years old, with and without PPID.

Methods:
Thirty-five horses and ponies, >15 years old, were evaluated at two nearby properties in Victoria, Australia. The sample population was intended to include potential cases of PPID (i.e. not a random sample). The horses and ponies were clinically evaluated for signs of PPID, and basal blood samples were taken for measurement of plasma high molecular weight (HMW) adiponectin (Merck High Molecular Weight Adiponectin ELISA), along with a combined insulin tolerance test and TRH stimulation test. Animals were sampled in late May and early June (equivalent to late November and early December in the Northern hemisphere). Insulin sensitivity was graded as % reduction of blood glucose concentration and correlated against adiponectin concentrations.

Results:
Of the 35 horses and ponies evaluated, 12 were diagnosed with PPID based on basal and/or TRH-stimulated ACTH concentrations plus various clinical signs (including hirsutism, weight loss, polyuria/polydipsia, evidence or history of laminitis). Overall, 27 were insulin resistant and 8 were not insulin resistant, using a cut-off of 50% reduction in blood glucose following the administration of insulin. Of the 12 PPID cases, 10 were insulin resistant, including 2 with diabetes mellitus. Plasma concentration of HMW adiponectin in PPID cases was 9.63 ±2.99 µg/ml (mean ± SEM) and in non-PPID animals was 6.01 ±1.01 (P=0.17). Furthermore, there was no apparent correlation between adiponectin and insulin sensitivity in this aged population.

Conclusions:
A high proportion of animals overall in this population were IR, probably due to age, although in some cases it may have been related to their PPID status. Adiponectin levels did not appear to correlate with IR in this population. Therefore, age-related IR may potentially have a different aetiology to diet-induced IR. The finding that adiponectin levels did not differ between PPID and non-PPID animals suggests that this adipokine does not play a significant role in the pathogenesis of this condition.

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Evaluation of adiponectin and serum amyloid A concentrations in equine diet-induced insulin dysregulation

NJ Bamford,[a] SI Jacob,[b] PA Harris,[c] ME McCue,[d] SR Bailey[a]

[a] Melbourne Veterinary School, University of Melbourne, Parkville, Victoria, Australia.
[b] College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA.
[c] Equine Studies Group, Waltham Petcare Science Institute, Melton Mowbray, Leicestershire, UK.
[d] College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA.

Presenting author: Nicholas Bamford; n.bamford@unimelb.edu.au

Background: Reduced adiponectin concentrations have been associated with insulin dysregulation in equids and low-grade systemic inflammation is a potential cause of insulin resistance in other species. Previous diet studies performed at Michigan State University (MSU) and the University of Melbourne (UM) yielded intriguing results, where a high glucose diet fed for 7 or 20 weeks, and a high starch diet fed for 7 weeks, improved tissue insulin sensitivity, assessed using a frequently-sampled intravenous glucose tolerance test. In contrast, a high starch diet fed for 20 weeks resulted in tissue insulin resistance.

Aims: To investigate relationships among plasma adiponectin concentrations, systemic inflammation and tissue insulin sensitivity in equids.

Methods: Plasma and serum samples from the two previous diet studies at MSU and UM were analysed for plasma high molecular weight adiponectin and serum amyloid A (SAA) concentrations using validated assays. The animals included in this extension study were those from MSU fed either a high sugar (n=16) or high starch (n=16) diet for 7 weeks, and those from UM fed either a high sugar (n=6) or high starch (n=12) diet for 20 weeks. Samples that had been stored at -80°C from weeks 0 and 6 (both MSU and UM) and weeks 14 and 20 (UM only) were analysed. Statistical analysis was performed using mixed model ANOVA to account for variables including diet, breed, institution and time.

Results: Plasma adiponectin concentrations were not significantly different from baseline values over the study period for the high sugar and high starch diets at MSU and the high sugar diet at UM (all $P>0.05$). In contrast, there was a decrease in plasma adiponectin concentrations to <50% of baseline by week 20 for the high starch diet at UM ($P<0.05$). SAA concentrations were not significantly different from baseline over the study period for the high sugar and high starch diets at MSU (both $P>0.05$), while SAA concentrations were increased approximately 5-fold by week 20 in both the high sugar and high starch groups at UM (both $P<0.05$).

Conclusions: Feeding of a high starch diet for 20 weeks resulted in reduced tissue insulin sensitivity alongside decreased adiponectin and increased SAA concentrations. However, these changes were not observed when a high starch diet was fed for 7 weeks, or a high sugar diet was fed for 7 or 20 weeks. Further investigation of the roles of adiponectin and systemic inflammation in equine insulin dysregulation is warranted.

Acknowledgements: This work was supported by funding from the Morris Animal Foundation, Australian Research Council, Waltham Petcare Science Institute, and Michigan State University.

Animal ethics: These studies were approved by the University of Melbourne Animal Ethics Committee (Approval #1011918.2) and the Michigan State University Institutional Animal Care and Use Committee (Approval #11/14-178-00) in compliance with applicable national laws.

Effects of experimentally induced insulin dysregulation on adiponectin concentrations in metabolically healthy, insulin-sensitive ponies

Marine Barnabé[1*], Jonathan Elliott[2], Pat Harris[3], Nicola Menzies-Gow[1]

[1]Department of Clinical Sciences and Services, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, UK

[2]Department of Comparative Biomedical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, UK

[3]Equine Studies Group, Waltham Petcare Science Institute, Freeby lane, Waltham-on-the-Leics, LE 14TRT, UK

**Presenting author (mbarnabe@rvc.ac.uk).*

Statement: 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).

Aims: This study aimed to investigate the effects of two forms of experimentally induced insulin dysregulation (tissue insulin resistance and hyperinsulinemia) on circulating adiponectin concentrations in insulin-sensitive ponies.

Methods: Two forms of short-term, reversible insulin dysregulation were induced in healthy, native-breed ponies in the UK (n = 6; four mares, two geldings; 6-18 years; 210-420 kg). Ponies had no previous history of laminitis and showed normal basal insulin concentrations and normal insulin responses to an oral sugar test. Tissue insulin resistance was induced via intravenous administration of dexamethasone (0.08 mg/kg) with blood samples collected every 15 min over 3 h. Fourteen days later, hyperinsulinemia was induced for 9 h via euglycemic-hyperinsulinemic clamp, with blood samples collected every 30 min. Serum insulin and plasma adiponectin concentrations were measured using validated assays and gene expression (adiponectin receptors [AdipoR] 1 and 2, insulin receptor, insulin-like growth factor 1 receptor [IGF-1R]) in whole-blood was assessed via qPCR. Finally, whole-blood was incubated with 10, 100, and 1000 ng/mL dexamethasone for 3 h at 37 °C to investigate its direct effects on AdipoR1 and IGF-1R gene expression.

Results: Dexamethasone-induced tissue insulin resistance did not alter circulating insulin or adiponectin concentrations at any time-point, but significantly upregulated AdipoR1 (two-fold, $P<0.01$) and IGF-1R (four-fold, $P<0.05$) expression at 150 and 180 min. *Ex vivo* incubation of whole-blood with dexamethasone did not cause similar upregulation, confirming the observed changes were not a direct effect of dexamethasone on leucocytes. There was no change in adiponectin concentrations or gene expression associated with induced hyperinsulinemia (serum insulin: 689.08 ± 172.36 mIU/mL).

Conclusions: Short-term induced hyperinsulinemia and tissue insulin resistance did not affect circulating adiponectin concentrations in metabolically healthy, insulin-sensitive ponies. However, tissue insulin resistance was associated with upregulation of two receptors linked to adiponectin signalling. The effect of longer-term insulin dysregulation (including excessive insulin responses to non-structural carbohydrates) on adiponectin signalling requires further research.

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GEES 2023 Clinical Research Abstract

Investigation of plasma leptin concentrations in association with laminitis or obesity status in ponies

Brianna L. Clark[1], Allison J. Stewart[1], Kate L. Kemp[1], Nicholas J. Bamford[2], François-René Bertin[1]

[1.]School of Veterinary Science, The University of Queensland, Gatton, Queensland, 4343, Australia

[2.]Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, 3010, Australia

The work follows institutional guidelines for humane animal treatment and complies with conduct of research legislation in the country in which the study was conducted.

Aims: Leptin is an adipokine released from adipose tissue; however, there are inconsistent reports of associations between increased leptin concentrations with laminitis and obesity status in horses and ponies. This study aimed to investigate plasma leptin concentrations in association with laminitis or obesity status in a cohort of ponies.

Methods: Cross-sectional study of 143 Shetland and Welsh ponies. Laminitis was defined as active (modified-Obel score >1) or historical (previously diagnosed by a veterinarian). Generalised and regional obesity was assessed using body condition and cresty neck scores and defined as > 6/9 or > 2/5, respectively. Plasma leptin concentrations were measured in all ponies by radioimmunoassay. Descriptive and interferential statistics (t-tests or Mann-Whitney tests, as appropriate) were performed with significance accepted at $P < 0.05$.

Results: Forty ponies were classified as laminitic including 13 with active laminitis. No significant differences in media plasma leptin concentrations between laminitic, 11 ng/mL (IQR 8.1 – 14 ng/mL), and non-laminitic ponies, 8.8 ng/mL (IQR 6.1 – 15 ng/mL), was detected ($P = 0.11$), including when only considering those with active laminitis ($P = 0.80$). Obesity was diagnosed in 101 ponies. No significant differences in median plasma leptin concentrations between obese, 10.4 ng/mL (IQR 7.0. – 14.8 ng/mL), and non-obese ponies, 8.0 ng/mL (IQR 5.8 – 13.4 ng/mL), was detected ($P = 0.10$).

Conclusions: In this cohort of ponies, no associations between plasma leptin concentration and laminitis or obesity status were detected. The clinical utility of measuring plasma leptin concentration remains to be determined.

Acknowledgement: The authors with to thank the owners who volunteered their ponies for this study.

The sodium-glucose cotransporter-2 inhibitor velagliflozin decreases basal plasma insulin concentrations in horses with moderate-severe insulin dysregulation

K. Thane[1], R. Voth[2], R. Klee[3], T. Warnken[3], N. Frank[1]

[1]Department of Comparative Pathobiology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA. USA.

[2]Boehringer Ingelheim Animal Health USA Inc, Duluth, GA, USA

[3]Boehringer Ingelheim Vetmedica GmbH; Ingelheim am Rhein, Germany

Presenting author: Kristen Thane
200 Westboro Road, North Grafton MA 01536
kristen.thane@tufts.edu

This work followed national guidelines for humane animal treatment (Cummings School of Veterinary Medicine CSRC Protocol #002-20)
This study was funded by Boehringer Ingelheim Animal Health USA Inc.

Aims: Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are a promising new therapy to treat hyperinsulinemia in horses with insulin dysregulation (ID). This study aimed to investigate the efficacy of velagliflozin, a novel SGLT2i, to decrease insulin concentrations in horses with ID.

Methods: Thirty-seven privately owned adult horses with moderate-severe ID (oral sugar test [OST] insulin >75 µIU/mL) were enrolled. Eighty-nine percent of enrolled horses had a history of prior laminitis. Horses were randomly assigned to placebo (n=19) or active drug (n=18) for the first 20 weeks of the trial (Wk2-20); subsequently, all horses received active drug for an additional 20 weeks (Wk22-40). Physical examination and blood sample collection to measure basal insulin concentrations (INS_b) and other hematologic parameters were performed at 0, 2, 4, 8, 12, 20, 22, 24, 28, 32, and 40 weeks. Blood samples were analyzed at a commercial veterinary laboratory. All data are reported as median [range]. Preliminary associations between insulin and glucose at baseline were made using Spearman correlation. Kolmogorov-Smirnov testing was used for preliminary comparison of insulin concentration between treatment groups at each study visit with significance set at $P = 0.05$.

Results: The population included 9 Miniature horses, 10 ponies, and 18 horses, aged 21 years [3-29], with body condition score (BCS) 6/9 [4-8/9], and cresty neck score (CNS) 3/5 [0-4/5]. Pre-study OST insulin concentrations at 60 and 90 minutes were 197 µIU/mL [76-1316] and 255 µIU/mL [88-1517], respectively. Prior to initiating drug administration, Day 0 INS_b, glucose, and triglyceride (TG) concentrations were 191 µIU/mL [19-877], 107 mg/dL [70-182], and 77 mg/dL [36-358], respectively. Day 0 INS_b and glucose concentrations were moderately correlated ($r = 0.54$, $P < .001$). Day 0 INS_b was not correlated with either BCS ($P = .23$) nor CNS ($P = .77$). Day 0 INS_b did not differ significantly between placebo (193 µIU/mL [19.5-824]) and velagliflozin (188 µIU/mL [37.9-877]) treatment groups ($P = .43$). Median insulin concentration measured at each study visit from Weeks 2-20 was significantly lower in the velagliflozin treatment group compared with the placebo treatment group ($P < .05$). Median insulin concentration measured at each study visit from Weeks 22-40 was not significantly different between groups. Six horses (16%) exhibited laminitis within 6 weeks of study enrollment; 5/6 (83%) were receiving placebo and one horse in the velagliflozin group had a recurrence of laminitis on Day 5. All horses exhibited an increase in serum TG concentrations during velagliflozin treatment. Development of even marked hypertriglyceridemia was not accompanied by clinical abnormalities (lethargy, anorexia).

Conclusions: Velagliflozin significantly decreased basal plasma insulin concentrations in horses with moderate-severe ID. SGL2i medications offer a novel, robust therapeutic option for treating horses with hyperinsulinemia.

Sanna Lindåse, Katarina Nostell, Siv Hanche-Olsen, Ingunn Risnes Hellings, Constanze Fintl, Johan Bröjer

- Presenting author: Sanna Lindåse, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, 750 07 Uppsala, Sweden; email: sanna.lindase@slu.se
- 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

Short-term effects of canagliflozin on postprandial glucose and insulin responses – preliminary results from an ongoing randomized, double-blind, placebo-controlled study

Aims: Reducing excessive hyperinsulinemia is a cornerstone in preventing laminitis in horses with insulin dysregulation (ID). There is a need for complementary pharmacological treatments that efficiently decrease the postprandial hyperinsulinemia in ID horses, especially in cases that are refractory to dietary management. The aim of this study was to compare the short-term effects of two different doses of canagliflozin versus placebo on the glucose and insulin responses after an oral sugar test in horses with ID.

Methods: This is an ongoing randomized, double-blind, placebo-controlled study in horses with ID conducted at two centers in Sweden and Norway. Only preliminary results from the Swedish part of the study are reported here. Privately 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 for at least 1 month before enrollment.

Horses were randomized to either once-daily oral treatment with 0.6 mg/kg canagliflozin (n = 9), 1.2 mg/kg canagliflozin (n = 8) or placebo (n = 9). The study consisted of an initial 5-day phase for obtaining baseline data, a 3-week double-blind treatment phase at home and a 5-day follow-up period similar to the initial baseline period but with double-blind treatment. Horses were subjected to an OST (0.5 mL/kg Dansukker glucose syrup) in the morning of the third day on both clinical visits. Blood samples were collected at baseline (fasting blood samples), and at 15, 30, 60, 90, 120, 150 and 180 minutes after oral sugar administration.

Results: Maximal insulin concentration (insulin C_{max}), area under the plasma insulin vs time curve (insulin AUC_{0-180}) and fasting glucose concentrations from the OST significantly decreased at 3-weeks of canagliflozin treatment (0.6 mg/kg and 1.2 mg/kg) when compared with placebo, but no difference was found between the two doses of canagliflozin (Table 1). The geometric least squares mean insulin response (AUC_{0-180}) for canagliflozin treated horses (0.6 mg/kg and 1.2 mg/kg) were on average 35 % of the geometric least squares mean insulin response (AUC_{0-180}) for the placebo treated horses. Seven out of 17 horses (41%) treated with canagliflozin had insulin C_{max} concentrations < 65 µIU/mL, the cut off for diagnosing ID with the OST (Dansukker glucose syrup), whereas all placebo treated horses had insulin C_{max} concentrations > 65 µIU/mL.

Conclusions: Preliminary results from this ongoing study demonstrate that treatment with canagliflozin efficiently decreases the postprandial insulin response after an OST in ID horses. The small study population might explain the lack of difference between the two dosages of canagliflozin. None of the horses developed fasting hypoglycemia during treatment with canagliflozin at 0.6 or 1.2 mg/kg. Taken together, canagliflozin is a promising drug for treatment of ID in horses that requires future studies.

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

Table 1. Comparison of oral sugar test (OST) glucose and insulin parameters in horses after a 3-week double-blind treatment phase with 0.6 mg/kg canagliflozin, 1.2 mg/kg canagliflozin or placebo. Adjusted means for baseline values are presented as least squares means ± SEM or geometric least squares means with 95% confidence interval. Least squares means with different superscript letters within row differ at P < 0.05.

Parameter	Placebo (n=9)	Canagliflozin 0.6 mg/kg (n=9)	Canagliflozin 1.2 mg/kg (n=8)
Insulin AUC_{0-180} (µIU/mL × min)	32 894[a] (24 091 – 44 913)	12 031[b] (8760 – 16 525)	10 901[b] (7804 – 15 227)
Insulin C_{max} (µIU/mL)	285.5[a] (205.1 – 397.4)	101.7[b] (72.8 – 142.2)	92.4[b] (64.9 – 131.5)
Fasting plasma glucose (mmol/L)	5.5 ± 0.2[a]	4.7 ± 0.2[b]	4.7 ± 0.2[b]

Johan Bröjer, Elin Svonni, Sanna Lindåse

- Presenting author: Johan Bröjer, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, 750 07 Uppsala, Sweden; email: johan.brojer@slu.se
- 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

Short-term effects of canagliflozin on β-cell function in horses with insulin dysregulation – preliminary results from an ongoing randomized, double-blind, placebo-controlled trial

Aims: There is a need for complementary pharmacological treatments to reduce excessive hyperinsulinemia in horses with insulin dysregulation (ID) in order to efficiently prevent laminitis in these horses. Sodium-glucose transporter 2 (SGLT2) inhibitors are a new potential treatment option for horses with ID that decrease postprandial plasma glucose concentrations and thereby reduce the postprandial insulin response. Preliminary results from our research group suggest that the decreased postprandial insulin response seen in horses treated with the SGLT2 inhibitor canagliflozin cannot solely be explained by decreased postprandial glucose response. The aim of this study was to compare short-term effects of two different doses of canagliflozin versus placebo on the β-cell sensitivity to glucose (β-cell function) in horses with ID.

Methods: This is an ongoing randomized, double-blind, placebo-controlled study in horses with ID conducted at two centers in Sweden and Norway. Only preliminary results from the Swedish part of the study are reported here. 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 were randomized to either once-daily oral treatment with 0.6 mg/kg canagliflozin (n = 9), 1.2 mg/kg canagliflozin (n = 8) or placebo (n = 9). The study consisted of an initial 5-day phase to obtain baseline data, a 3-week double-blind treatment phase at home and a 5-day follow-up period similar to the initial baseline period but with double-blind treatment. Horses were subjected to an oral sugar test (day three), a meal tolerance test (day four) and a graded glucose infusion test (GGI) adapted for use in horses (day five). The GGI was performed to measure the horses' β-cell sensitivity to glucose and consisted of a stepwise increase in continuous glucose infusion rates over 240 minutes. The slope for the linear dose-response relationship between plasma glucose and plasma insulin was used as an index to quantify the β-cell sensitivity to plasma glucose for each horse and occasion.

Results: The β-cell sensitivity to glucose was significantly decreased at 3-weeks of canagliflozin treatment when compared to placebo ($P \leq 0.0002$), but no difference was found between the two doses of canagliflozin ($P = 0.98$). The β-cell sensitivity to glucose geometric least square means with 95% confidence interval after treatment was 18.6 (15.3 – 22.5), 19.0 (15.5 – 23.3) and 36.6 (30.2 – 44.4) mIU/mmol for canagliflozin 0.6 mg/kg, canagliflozin 1.2 mg/kg and placebo respectively.

Conclusions: Preliminary results from this ongoing study demonstrate that the β-cell sensitivity to glucose decreased on average by 50% in canagliflozin treated horses. Thus, the efficient decrease in postprandial insulin hypersecretion in ID horses treated with canagliflozin is attributed to a reduced β-cell function and to a lesser extent to a diminished postprandial glucose response.

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

Effects of *ex vivo* hormonal exposure on oxidative responses in equine leukocytes

Sarah A. Vaughn,[1] Londa J. Berhagus,[1] David J. Hurley,[2] Kelsey A. Hart[1]
[1]Department of Large Animal Medicine and [2]Department of Population Health, University of Georgia College of Veterinary Medicine, Athens GA, USA, khart4@uga.edu

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

This abstract/research has not previously been presented.

Aims. Obesity and insulin dysregulation (ID) are associated with inflammatory dysregulation and increased oxidative stress in other species. Hormones, such as insulin, leptin, and hormones from the hypothalamic-pituitary-adrenal (HPA) axis, play key roles in cellular glucose metabolism, which produces reactive oxygen species (ROS). Oxidative damage to the hypothalamus also plays a key role in the pathogenesis of pituitary pars-intermedia dysfunction (PPID). Increased circulating concentrations of these hormones and exaggerated oxidant responses *in vivo* have been demonstrated in ponies. These hormones can directly impact leukocyte function in other species, but their effects on equine leukocytes are unknown. Increased plasma concentrations of these hormones or altered sensitivity to them at the leukocyte level could result in breed-related differences in oxidant responses that could impact endocrine pathogenesis. Our **objective** was to determine the effects of *ex vivo* exposure to adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), insulin, or leptin on ROS production from leukocytes isolated from horses and ponies. **We hypothesized that these hormones induce oxidant responses from equine leukocytes, with exaggerated responses in leukocytes from ponies.**

Methods. Blood was collected from 10 apparently healthy Quarter horses and 9 Welsh ponies for isolation of neutrophils and peripheral blood mononuclear cells (PBMCs) via density gradient centrifugation. No animals met criteria for PPID diagnostic based on resting ACTH concentrations. Cells were incubated with media (negative control), microbial antigens (positive control), or physiologically relevant concentrations of ACTH, α-MSH, leptin, or insulin for 2 hours. Induced ROS production was quantified with a validated fluorometric assay, and compared within and between groups and among stimulants using repeated measures ANOVA ($P<0.05$).

Results. There was no significant effect of breed on basal, microbial-induced, or hormone-induced ROS production from neutrophils ($P=0.418$) or PBMCs ($P=0.510$), but in neutrophils, the interaction between breed and stimulant significant ($P=0.041$). Hormone exposure did not induce significant ROS production compared to basal levels from horse or pony neutrophils ($P=0.082$-0.622) or horse PBMCs ($P=0.090$-0.228) In pony PBMCs, however, exposure to ACTH, α-MSH, or leptin induced a significant increase in ROS production ($P=0.004$-0.032), and results after exposure to insulin approached significance ($P=0.072$).

Conclusions. Hormones associated with equine endocrine diseases induce *ex vivo* pro-oxidant responses in equine leukocytes, but specific effects are cell- and hormone-dependent. Breed differences in oxidative activity may warrant further investigation in the pathogenesis and consequences of equine endocrine disease.

Acknowledgements: We would like to acknowledge UGA VTH clients and personnel for their assistance with animals for this work, and the Morris Animal Foundation (D20EQ-035) for funding support.

Effects of commercially available equine antioxidant supplements on plasma oxidative status in equids with PPID or ID.

Sarah A. Vaughn,[1] David J. Hurley,[2] Kelsey A. Hart[1]
[1]Department of Large Animal Medicine and [2]Department of Population Health, University of Georgia College of Veterinary Medicine, Athens GA, USA svaughn@uga.edu

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

This abstract/research has not previously been presented.

Aims. Oxidative damage to the hypothalamus is important in the pathogenesis of pituitary pars intermedia dysfunction (PPID). Obesity and insulin dysregulation (ID) are also associated with increased oxidative stress in other species. Anti-oxidants may present a potential approach to slow progression of these important equine diseases. RRR-α-tocopherol (vitamin E, VITe) is an anti-oxidant vitamin that increases in plasma and cerebrospinal fluid after oral administration in horses. Resveratrol is a polyphenol with anti-oxidant effects that crosses the blood brain barrier and may have neuroprotective effects in other species, and that modulates insulin responses in some horses. The anti-oxidant effects of these compounds have not been evaluated in equine endocrine disease. The **objective** of this study was to compare systemic oxidative status in equids with PPID or ID before and during treatment with VITe and resveratrol in combination (VITe/RESV). **We hypothesized that oral administration of VITe/RESV decreases plasma oxidative stress in equids with endocrine dysfunction.**

Methods. Client-owned equids with PPID (n=10) or ID (n=6) housed on their home farm and on their typical diet were used. PPID animals were treated with pergolide mesylate for ≥ 3 weeks prior to enrollment. In a randomized crossover design, animals received VITe/RESV treatment [10 IU/kg of water-dispersible RRR-α-tocopherol and 28g of a resveratrol-containing powder] or tap water (placebo) once daily for 6 weeks, with a ≥28-day washout before the alternate treatment. Plasma reactive oxygen metabolites (dROM) and anti-oxidant capacity (PAC) were measured on day 0 and after 3 and 6 weeks of each treatment using a commercial photometric analytical system. Linear mixed models and ANOVA were used to examine treatment effects within and between groups ($P<0.05$).

Results. Plasma dROMs or PAC did not differ between horses with PPID or ID at any time point (P=0.170-0.932). In equids with PPID, dROMs and PAC prior to or after 6 weeks of treatment were comparable between placebo and VITe/RESV (P=0.664-0.845). In the middle of the treatment period, animals treated with 3 weeks of VITe/RESV had 20% lower dROMs and 16% lower PAC than animals treated with placebo, with P values that approached statistical significance (P=0.069, P=0.057 respectively). There were no significant differences in oxidative parameters between placebo and VITe/RESV treatment in ID animals at any time point (P=0.560-0.799).

Conclusions. We did not document a profound anti-oxidant effect of VITe/RESV treatment on plasma oxidative status in horses with PPID or ID. VITe/RESV treatment may transiently alter plasma oxidative status in some horses with PPID, but further study is needed to determine if this effect is consistent in a larger group of animals. If these compounds have an overall systemic antioxidant effect, it does not appear to be due to an increase in PAC, so investigations into other anti-oxidant mechanisms is also warranted in these populations.

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NOTES

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