Dorothy Russell Havemeyer Foundation

Equine Geriatric Workshop II

3rd Equine Endocrine Summit

November 17-20, 2014

Middleburg, Virginia

Workshop Program of Events

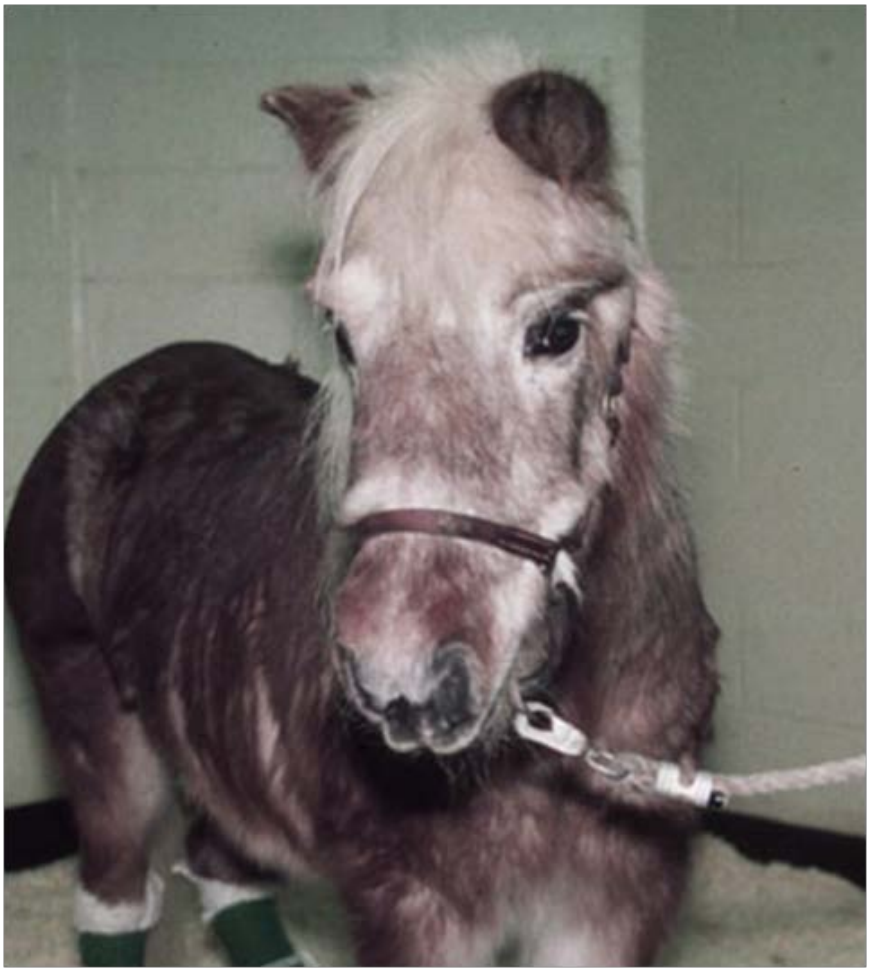
Sunday, November 16 – Arrive	
6:00	**Reception**
7:30	***Dinner***

Monday, November 17 – PPID Pathophysiology	
8:00 – 9:00	***Breakfast***
9:00 – 9:45	Keynote – Dianne McFarlane – PPID: What is it? What is it not? What remains to be learned? *(45 minutes)*
9:50 – 10:10	Ramiro Toribio – A perspective on steroid precursors and neurosteroids in equine diseases. *(20 minutes)*
10:15 – 10:30	Dianne McFarlane – Is the metabolically thrifty horse at greater risk of PPID? *(15 minutes)*
10:35 – 10:50	Melinda Freckleton – PPID: Reality and results in a primary care ambulatory practice.
10:55 – 11:10	***Break***
11:15 – 11:30	Hal Schott – Alternations in small intestinal morphology and carbohydrate absorptive capacity in equids with PPID. *(15 minutes)*
11:35 – 11:50	Betta Breuhaus – Thyroid hormone and thyrotropin concentrations and responses to TRH in horses with PPID compared to age-matched normal horses. *(15 minutes)*
11:55 – 12:10	Amanda Adams – Does equine PPID affect immune responses to vaccination? *(15 minutes)*
12:15 – 12:45	Discussion
12:45 – 2:00	***Lunch***

Speed abstracts – 8 minutes/6 slides – PPID Testing	
2:00 – 2:10	Dave Rendle – Single and paired measurements of adrenocorticotropic hormone for the diagnosis of PPID. *(8 minutes)*
2:10 – 2:20	Andrew Durham – Further observations of seasonality of pars intermedia secretory function in 30,000 horses and ponies. *(8 minutes)*
2:20 – 2:30	John Haffner – Comparison of resting and dynamic adrenocorticotropic hormone following administration of thyrotropin-releasing hormone during autumn and non-autumn seasons in horses. *(8 minutes)*
2:30 – 2:40	Ann Chapman – Evaluation of season variation in adrenocorticotropin concentration in response to thyrotropin-releasing hormone in ponies. *(8 minutes)*
2:40 – 2:50	Dave Rendle – How do we interpret high adrenocorticotropic hormone concentrations in young horses? *(8 minutes)*
2:50 – 3:45	Discussion
3:45 – 6:00	***Free time***
6:00	***Dinner***

Tuesday, November 18 – PPID Treatment and Other Information	
8:00 – 9:00	***Breakfast***
9:00 – 9:30	Keynote – Hal Schott – Treatment of PPID. *(30 minutes)*
9:35 – 9:50	Dave Rendle – Short-term responses to pergolide and effects of sample timing in horses with PPID. *(15 minutes)*
9:55 – 10:10	Dave Rendle – Effects of pergolide mesylate on plasma ACTH concentration in horses with PPID. *(15 minutes)*
10:15 – 10:45	***Break***
Speed abstracts – 8 minutes/6 slides	
10:50 – 11:00	Jon Fletcher – Evaluation of resting ACTH concentration as a screening test for PPID in 23 Louisiana horses. *(8 minutes)*
11:00 – 11:10	Dianne McFarlane – Pharmacokinetics and pharmacodynamics of pergolide mesylate after chronic oral administration in horses with PPID. *(8 minutes)*
11:10 – 11:20	Frank Andrews – Neurologic signs, endocrine testing and rapid-slice contrast computed tomography in an 8 year old Arabian. *(8 minutes)*)
11:20 – 11:30	Hal Schott – Comparison of magnetic resonance imaging and histological scores for assessing pituitary pars intermedia enlargement in horses with pituitary pars intermedia dysfunction. *(8 minutes)*
11:30 – 11:40	Jo Ireland – Factors associated with PPID in donkeys identified during a nationwide PPID awareness initiative. *(8 minutes)*
11:40 – 11:50	Nicholas Frank – Effects of fasting and the oral sugar test on thyrotropin-releasing hormone stimulation test results in horses. *(8 minutes)*
11:50 – 12:30	Discussion
12:30 – 2:00	***Lunch***
Equine Metabolic Syndrome	
2:00 – 2:30	Molly McCue – Keynote on Equine Metabolic Syndrome *(30 minutes)*
2:35 – 2:50	Janice Sojka-Kritchevsky – The effect of fasting length on baseline blood glucose, insulin, glucose-insulin ratio, oral sugar test, and intravenous insulin response test values in horses. *(15 minutes)*
2:55 – 3:10	Javier Mendoza, Ramiro Toribio – Blood glucose homeostasis in donkeys: intravenous glucose and combine glucose-insulin testing. *(15 minutes)*
3:15 – 3:30	Michelle Coleman – Lipid profiling in laminitic, obese, and healthy horses. *(15 minutes)*
3:35 – 4:00	Discussion
4:00 – 6:00	***Free Time***
6:00	***Dinner***

Wednesday, November 19 – Other Geriatric Research	
8:00 – 9:00	***Breakfast***
9:00 – 9:30	Keynote – Mary Rose Paradis – What we learned from the first geriatric workshop. *(30 minutes)*
9:35 – 9:50	Amanda Adams – Interleukin-6: A predictor of health status in geriatric horses? *(15 minutes)*
9:55 – 10:10	Melissa Siard – Comparison of inflammation, nutritional status, muscle mass, pituitary function, and age in geriatric horses. *(15 minutes)*
10:15 – 10:30	Karen Malinowski – HSP70 and HSP90 in whole blood and skeletal muscle in young and aged Standardbred mares. *(15 minutes)*
10:30 – 10:45	***Break***
10:45 – 11:00	Heidi Banse – Transcription regulation of skeletal muscle proteolysis in underweight aged horses *(15 minutes)*
11:05 – 11:20	Kristine Urschel – Activation of the signaling pathways regulation muscle protein synthesis and degradation in aged horses. *(15 minutes)*
11:25 – 11:40	Ana Pacheco – Age-effects on blood gas, spirometry, airway reactivity, and bronchoalveolar lavage fluid cytology in clinically healthy horses. *(15 minutes)*
11:45 – 12:00	Jo Ireland – Prevalence of and risk factors for recurrent airway obstruction in geriatric horses and ponies. *(15 minutes)*
12:20 – 12:35	Kirstin Bubeck – Anesthesia and the older horse. *(15 minutes)*
12:40 – 2:00	***Lunch***
2:00 – 2:15	Betta Breuhaus – Thyroid hormone concentrations in aging horses. *(15 minutes)*
2:20 – 2:35	Ken McKeever – Aging alters thermoregulation and cardiovascular function in Standardbred horses *(15 minutes)*
2:40 – 2:55	Jennifer Mahon, Mary Rose Paradis – Comparison coagulation testing in non-geriatric and geriatric horses with/without PPID. *(15 minutes)*
3:00 – 3:30	Discussion
3:30 – 4:30	Wrap-up session
4:30 – 6:00	***Free Time***
6:00	***Closing Dinner***
Thursday, November 20, Departure	



Welcome to the Dorothy Havemeyer Workshop. Because the endocrine problem, pituitary pars intermedia dysfunction is a large part of equine geriatric medicine, it was felt that the combining of the 3rd Equine Endocrine Summit and the Equine Geriatric Workshop 2 would provide an opportunity for clinicians and researchers to share the newest findings or works in progress on the subject of the older horse. This workshop will concentrate on the endocrine disorders of the older horse and on pathogenesis, diagnosis, and treatment of diseases of aging. The Foundation is conducting the workshop with generous support from Boehringer Ingelheim. Drs. Mary Rose Paradis, Dianne McFarlane and Hal Schott are the workshop planners and facilitators. We would like to thank both the Dorothy Havemeyer Foundation and Boehringer Ingelheim for bringing us together to explore the problems of the geriatric horse.

Mary Rose Paradis, DVM, MS DACVIM(LAIM)
Dianne McFarlane, DVM, PhD DACVIM(LAIM)
Hal Schott, DVM, PhD DACVIM(LAIM)

Abstract Table of Contents

Keynote lecture - PPID: What is it? What is it not? What remains to be learned?

Dianne McFarlane, DVM, PhD, DACVIM

Equine pituitary pars intermedia dysfunction continues to be a disease of much interest to horse owners, clinicians and researchers alike. This talk will review our current understanding of PPID, explore the strength of the evidence on which we have built this understanding and will discuss potential research directions to pursue as we go forward in our attempt to better understand this important equine disease.

PPID is a disease of old horses which results from a loss of ability to regulate hormonal output from the pars intermedia. It is not increased PI hormonal output that defines the disease, it is loss of regulation. The function of any gland is to facilitate coordinated systemic responses to maintain homeostasis. It is therefore expected that hormonal output will vary with changes in environment. Understanding the physiological role of the PI is essential for accurate diagnostic test interpretation since under certain situations the PI should be hyperresponsive, secreting increased amounts of α-MSH, β-end, CLIP and ACTH. Based on a relatively unbiased sample set of 154 horses with postmortem disease confirmation, the sensitivity and specificity of plasma ACTH for diagnosis of PPID was 70-80%, suggesting incorrect test results occur 20-30% of the time. The high percentage of false positive results is likely a consequence of "physiological" causes of increased ACTH, while the high percentage of false negatives may reflect the inability of current diagnostic tests to detect early PPID. It is important to consider the limitation of plasma ACTH concentration when used as a diagnostic test for inclusion into a research study. Similar data is lacking for the TRH stimulation test. Until this data is available, caution should be exercised in use of the TRH stimulation test as the sole inclusion criteria into research trials.

An example of physiological response of the PI that affects diagnostic testing is season. In 2004, we first reported that plasma concentration of equine PI hormones increased in the autumn. This finding led to a series of studies revealing an effect of season on diagnostic test results, pituitary histology and appearance of clinical signs. While the actual physiological role of the PI in seasonal adaptation remains speculative at this time, several studies have described exaggerated seasonal flux in thrifty horses, suggesting that the PI may coordinate seasonal metabolic regulation. We have also shown that in the horse, plasma α-MSH increases following endotoxin administration, similar to what is observed in other species, suggesting a role of the PI in inflammatory response. As we adopt new diagnostic testing strategies it is important to determine how PI function will influence the results. For example, evidence is accumulating that thrifty horses have a more dynamic TRH response than non-thrifty horses and the established reference range is not appropriate for this population. A more complete understanding of PI function would lead to a greater ability to discriminate disease with current and future diagnostic tests.

Key Etiologic and Pathophysiologic Points

The underlying cause of periventricular neurodegeneration is currently under investigation however, it is likely that there are more than one set of risk factors that lead to PPID. Oxidative stress and accumulation of intraneuronal misfolded proteins are two mechanisms that have been implicated, although confirmatory data is still necessary. Although it has been suggested that equine metabolic syndrome is a risk factor for PPID, evidence is lacking. It does appear that these two conditions can occur concurrently in horses. As a result there are at least two distinct clinical syndromes observed in horses with intermediate lobe dysfunction (Table 1), the syndrome observed in horses with PPID only and that of horses with both PPID and EMS. The importance of distinguishing between these two disease presentations is that laminitis appears only to affect those animals with concurrent EMS. Furthermore, preliminary data indicates that in horses with the combined syndrome, the risk of hyperinsulinemia is greater than in horses with either EMS or PPID alone.

TABLE 1: Clinical signs of PPID

Clinical Signs of PPID with EMS	Clinical Signs of PPID without EMS
Haircoat and shedding abnormalities	Haircoat and shedding abnormalities
Regional adiposity	Muscle wasting
Laminitis	Behavioral changes, lethargy
Polydipsia/ Polyuria	Exercise intolerance
Exercise intolerance	Secondary infections
Abnormal reproductive cycling	Abnormal reproductive cycling
Anhidrosis/Hypohidrosis/Hyperhidrosis	Anhidrosis/Hypohidrosis/Hyperhidrosis
Secondary infections	Neurologic impairment
Behavioral changes, lethargy	
Neurologic impairment	

A Perspective on Steroid Precursors and Neurosteroids in Equine Diseases

KA Dembek[1], BS Barr[2], NM Slovis[3], KA Hart[4], AJ Stewart[5], RE Toribio[1]

[1]College of Veterinary Medicine, The Ohio State University, Columbus, OH; [2]Rood and Riddle Equine Hospital, Lexington, KY; [3]Hagyard Equine Institute, Lexington, KY; [4]University of Georgia, Athens, GA; [5]Auburn University, Auburn, AL

A number of equine pathologies have been associated with abnormal concentrations of adrenocortical steroids. Some of the signs in horses with pituitary pars intermedia dysfunction (PPID) have been attributed to increased cortisol concentrations. It also has been proposed that cortisol secretion by adipocytes may play a role in equine metabolic syndrome. In critically ill foals, inappropriate cortisol and aldosterone secretion has been associated with severity of disease and mortality. Of interest, a number of steroidal factors from the adrenal cortex and the brain are known to promote neurogenesis and neuronal plasticity, modulate the hypothalamic-pituitary-adrenal axis (HPAA), alter neuronal connectivity and sensory information, modify behavior, and regulate immune function. However, these factors have been ignored in veterinary medicine. Recent work from our group and others in healthy and sick foals raises questions on whether steroid precursors and neurosteroids are involved in the development of pathologies of geriatric horses, horses with a deregulated energy metabolism, behavioral disorders, as well as other pathologies. In a series of studies we provide evidence that in addition to gluco- and mineralocorticoids (cortisol, aldosterone), multiple steroid precursors and neuroactive steroids (allopregnanolone, pregnenolone, progesterone, 17α-hydroxyprogesterone, dehydroepiandrosterone [DHEA], and androstenedione) are associated with various neonatal illnesses. Moreover, sick foals display different steroid profiles during hospitalization and in response to ACTH stimulation when compared to healthy foals. These findings could be used as a justification to investigate their role in the pathogenesis of diseases of adult horses. For example, brain-derived neurotropic factor (BDNF), which regulates hypothalamic neurons that integrate nutritional (glucose, insulin, leptin), stress (CRH, AVP, HPAA), metabolic (TRH), reproductive (GnRH), and seasonal (dopamine) signals, is under the control of neuroactive steroids via NMDA, GABA, and cannabinoid receptors. One may speculate that neurosteroid imbalances could contribute to hypothalamic dysfunction in geriatric (e.g. dopaminergic failure) and obese (leptin resistance) horses. It is also possible that abnormal secretion of steroid precursors (e.g. DHEA, androstenedione) could be influencing organ function (integument, immunity, energy) or that dysfunctional cells (e.g. adipocytes) metabolize precursors into active steroids. It is apparent that this area of equine endocrinology needs to be explored.

Is the Metabolically Thrifty Horse at Greater Risk of PPID?

J Fredrick[1], D McFarlane[2], F Yang.[2] [1]Center for Veterinary Care, Millbrook, NY; [2] Oklahoma State University

Horses with a thrifty phenotype are at risk for obesity, hyperinsulinemia, and laminitis; a collection of clinical signs known as equine metabolic syndrome (EMS). It is suspected that horses with EMS are at greater risk for developing PPID, although the exact mechanism by which EMS may lead to PPID is of yet unexplored. In previous work, we found that thrifty ponies had a greater seasonal increase in plasma concentrations of PI hormones. Therefore, we hypothesized that genetically thrifty horses have an increased pituitary intermediate (PI) lobe response to stimulation and that over time this hyperactivity results in periventricular dopaminergic neuronal injury and ultimately, PPID. As a first step to test this hypothesis, thyroid releasing hormone (TRH) stimulation testing in thrifty horses was compared to that in metabolically normal horses. The thrifty group included 8 mustangs with normal hair coats, a median body condition score (BCS) of 7, and a mean age of 11.25 yrs. Six had a history of laminitis. The normal group was comprised of thoroughbred horses (n=9) with a median BCS of 5, mean age of 10.4 yrs, and no history of laminitis or abnormal hair coats. Oral sugar testing revealed a greater insulin response in the thrifty group (mean serum insulin @ 75 mins: 70.9 ± 21.9 µIU/ml vs 37.2 ± 17 µIU/ml, $P<0.01$), as well as a difference in baseline insulin (34.2 ±3.6 µIU/ml vs 17 ±4.3 µIU/ml, $P<0.01$). TRH stimulation testing was performed in July and October, with ACTH measured at 0, 10 and 30 minutes after TRH. One thrifty horse had an ACTH markedly above the reference range on all 6 samples; results from this animal were excluded from analyses. ACTH response was analyzed using two way repeated measures ANOVA with animal as the subject, and breed and time as factors. When compared to normal horses, thrifty horses had greater ACTH release following TRH stimulation when tested in October ($P=0.03$) but not in July ($P=0.09$). Thrifty horses may have a greater PI response to stimulation, particularly in the fall. Further studies are needed to investigate the association of thriftiness and damage to the periventricular neurons to determine if chronic hyperactivity of the PI leads to PPID.

PPID: Reality and Results in a Primary Care Ambulatory Practice

Melinda Freckleton, DVM

Equine pituitary pars intermedia dysfunction (PPID) is a problem commonly encountered and managed on the farm in equine ambulatory practice. Analysis of the medical records of the over 5600 patients seen by a large primary care ambulatory equine exclusive practice was performed to identify the number of equine patients with the disease, their demographics, testing used to diagnose them, treatment plan and to identify concurrent non-PPID disease. At the time of this analysis, 2% of patients (n=118), in this practice were being managed for a PPID diagnosis. Diagnosis of PPID was made through elevated ACTH levels and/or a positive response to the TRH stimulation test. During a one year period the practice performed 38 thyroid releasing hormone (TRH) stimulation tests and 86 resting adrenocorticotropic hormone (ACTH) tests from two laboratories

The average age of PPID patients was 23 years with a range of 11 – 37 years of age. The percentage of affected animals increased as their age increased. There were less than1% of the 10-14 year old horses affected with PPID while the $\geq$ 30 year age group was over represented with 15% of animal affected. Morgan horses and ponies, in particularly Welsh ponies represented the highest breed predominance each at 10% of the breed. 115/118 affected horses were treated with Prescend with 60% receiving 1 mg/day and 5% of affected animals being treated with Prescend and cyproheptadine. The medical history of the PPID horses was reviewed for treatment of comorbid diseases. 40/118 animals (38%) of the PPID horses were being treated for insulin resistance with levothyroxin, 3 of which were also treated with metformin. 36/118 (36%) of the animals were treated for osteoarthritis or navicular disease. 22/118 (19%) of the PPID horses had a history of laminitis.

The reality of practice dictates that this is probably an underestimation of the real occurrence of PPID. This record system does not have any way to find or count the patients that have a classic end stage clinical appearance and have never been tested or treated.

Alterations in Small Intestinal Morphology and Carbohydrate Absorptive Capacity in Equids with Pituitary Pars Intermedia Dysfunction

J. L. De Vries, H.C. Schott II, R.J. Geor, J. Lubitz, D.P. Chamberlin, and N.L. Trottier, Michigan State University

Aims: Some, but not all, PPID-affected equids have insulin resistance (IR) and are predisposed to laminitis. Potential differences in gastrointestinal mucosal morphology and function that could contribute to alterations in glucose and insulin dynamics in aged PPID-affected horses have not been investigated.

Methods: 12 aged equids (20-31 years) were studied (November 2012 to January 2013). Eight horses (body condition score [BCS] 2-7) had clinical signs of PPID, elevated plasma adrenocorticotropin (ACTH) concentrations (82 ± 65 pg/mL, range 36-201 pg/mL), supportive overnight dexamethasone suppression test (ODST) results, and pars intermedia (PI) histological scores of 4 or 5. Four horses (BCS 3-7) were clinically normal, had normal ACTH concentrations (23 ± 7 pg/mL, range 15-32 pg/mL), non-supportive ODST results, and PI histological scores of 2 or 3. After endocrine testing and performance of an oral sugar test (OST) and a frequently sampled insulin-modified glucose tolerance test (FSIGT), horses were euthanized and multiple samples of small intestine were collected. Morphometric histological examination was performed (ten well-defined villi and associated crypts were measured for villus height [VH] and crypt depth [CD]), and mucosal scrapings were collected to prepare enterocyte vesicles for determination of maximal glucose uptake (Vmax).

Results: Morphometric examination revealed a lower VH in the duodenum and a greater CD in the jejunum of PPID-affected horses, as compared to aged normal horses. Further, Vmax for glucose uptake by enterocyte vesicles prepared from PPID-affected horses was nearly 50% lower than those prepared from normal aged horses. Neither baseline glucose nor insulin concentrations (prior to the OST) were different between groups. Across all 12 horses both baseline and peak OST insulin concentrations were strongly correlated with BCS ($r>0.8$ and $p<0.01$ for both). Although not a significant finding ($p=0.09$), the increase in glucose concentration during the OST tended to be greater in aged normal horses. There were no differences in the acute insulin response to glucose or insulin sensitivity between groups as assessed by the FSIGT. Of interest, strong inverse correlations were found between ACTH concentration and peak glucose concentration ($r=-0.77$, $p<0.01$) and the change in glucose concentration ($r=-0.63$, $p<0.03$) across all 12 horses.

Conclusions: PPID-affected horses have altered villus morphology and may have decreased absorptive capacity for glucose, as compared to normal aged horses. Whether these changes could increase the risk of spillover of soluble carbohydrates to the hindgut and increase the risk of laminitis in PPID-affected horses remains unknown. The strong inverse correlations between glucose dynamics during the OST and plasma ACTH concentrations suggest a potential link between altered PI hormone secretion and intestinal morphology and function in PPID-affected horses.

Acknowledgements: Supported by the Morris Animal Foundation.

Prior presentation: The data has been not been previously presented.

Thyroid Hormone and Thyrotropin Concentrations And Responses to TRH in Horses with PPID Compared to Age-Matched Normal Horses

Babetta A. Breuhaus, DVM PhD DACVIM North Carolina State University, Raleigh, NC

Glucocorticoids exert inhibitory action on the hypothalamic-pituitary-thyroid axis, resulting in diminished thyrotropin releasing hormone (TRH) and thyrotropin (TSH) release from the hypothalamus and pituitary, respectively. Glucocorticoids have been proposed to play a role in the pathogenesis of non-thyroidal illness syndrome and may alter thyroid function in Cushing's syndrome. To test the hypothesis that horses with pituitary pars intermedia dysfunction (PPID) exhibit central hypothyroidism, resting thyroid hormones (THs) and TSH, and responses to TRH stimulation, were measured in 13 horses with PPID and 9 age-matched normal horses. Baseline concentrations of THs tended to be lower in horses with PPID compared to normal horses, but only reached statistical significance (t-test) for free thyroxine by equilibrium dialysis (fT4D) and free tri-iodothyronine (fT3) (see table below, * = $p < 0.05$). Although the difference was not statistically significant, baseline TSH concentrations in PPID horses were lower than age-matched normal horses, which is the opposite of what would be expected, given that free THs were decreased. TSH and TH responses to TRH administration were not different between PPID and normal horses when tested by repeated measures analysis of variance. PPID horses may exhibit mild central hypothyroidism.

	TT4 nmol/L	fT4D pmol/L	TT3 nmol/L	fT3 pmol/L	TSH ng/ml
Normal Horses >16 yrs old	18.4 ± 9.9	25.4 ± 5.0	0.9 ± 0.3	2.7 ± 1.5	0.45 ± 0.31
PPID Horses	14.2 ± 10.1	17.5 ± 9.3*	0.6 ± 0.5	1.6 ± 1.1*	0.32 ± 0.19

Does Equine Pituitary Pars Intermedia Dysfunction (PPID) Affect Immune Responses to Vaccination?

AA Adams[1*], MH Siard[1], SE Reedy[1], J.P. Little[2] S Grubbs[3], M.P. Little[2]. 1. Gluck Equine Research Center, University of Kentucky, Lexington, KY. 2. Paris, KY. 3. Boehringer Ingelheim Vetmedica Saint Joseph, MO.

Over the past decade the aged horse population has expanded significantly with 20-30% of the equine population comprised of geriatric horses (≥ 20 years), and of these horses approximately 30% are affected by pituitary pars intermedia dysfunction (PPID), a progressive and debilitating endocrine disease. Along with advancing age, there is a decline in immune function, but currently no specific recommendations regarding vaccination of older horses, nor those affected by PPID, are available. Therefore, it remains to be determined if PPID affects immune responses to vaccination. Thus, the objective of this study was to determine if horses with PPID compared to non-PPID aged match controls would respond immunologically different to vaccination.

A total of 33 aged horses of mixed sex and breeds were used in this study. All horses were pre-screened to determine EIV antibody titers and PPID status based on responses to a dexamethasone suppression test (DST) and a thyrotropin releasing hormone (TRH) stimulation test. Further, all horses were assigned a numeric value for severity of clinical signs (hair coat, muscle condition, etc.) of PPID by two blinded DVMs, via an established scoring system. Non-PPID horses were characterized by cortisol levels (≤0.2 ug/dL) at 19 hrs post-DST and ACTH levels (≤35 pg/mL) pre-TRH stim and ACTH levels (≤110 pg/mL) at 10 min post-TRH stim test. PPID horses were further characterized by either double positive (DP) [DST no suppression & pre-TRH ACTH (≥35 pg/mL) and 10 mins post-TRH (≥110 pg/mL)] or single positive (SP) [DST suppression but Pre-TRH ACTH (≥35 pg/mL) and 10 mins post TRH (≥110 pg/mL)] responders. Treatment groups were randomly blocked according to these measures, along with age to the following vaccinate groups: (Group 1) Non-PPID horses, vaccinated i.m. (Vetera ®Gold, Boehringer Ingelheim Vetmedica) (n=12); (Group 2) Non-PPID controls, receiving a sterile saline vaccination (n=3); (Group 3) PPID horses (DP, n=7; SP, n=7) vaccinated (Vetera® Gold); and (Group 4) (n=6) PPID horses receiving sterile saline vaccine (DP, n=2; SP, n=2). Peripheral blood for antibody titer measures to EIV, EHV-1 and WNV were collected prior to the first vaccination (week 0) and again at week 2 and 4 post vaccination. A second Vetera® Gold vaccine or saline control was given at week 4 and blood collected at week 6 and week 8 post vaccination.

Overall, results showed that all vaccinated (Vetera Gold) horses compared to saline controls had significant ($P<0.05$) increases in HI antibody titers, EHV-1 antibody titers, and WNV titers post vaccination at two weeks post the first vaccination. Further, administration of a second vaccine dose at week 4 did not significantly ($P>0.05$) increase the HI antibody responses however it did significantly ($P>0.05$) increase the EHV-1 and WNV titers. Results are described below for two categories of comparisons for each antigen of interest (EIV, EHV and WNV): (1) for comparing immune responses to vaccination of combined PPID horses (both SP and DP) vs. non-PPID horses, and (2) for separating out and comparing sub-classes of PPID (SP vs. DP) to non-PPID horses. Results for immune responses to the EIV component of the vaccine are the following. There were no significant interactions between all PPID horses vs non-PPID horses by treatment or by time ($P>0.05$). Further, when separating out the different categories of PPID status (SP and DP) compared to non-PPID vaccinated horses there were no significant ($P>0.05$) interactions over time. Results for immune responses to the EHV-1 component of the vaccine are the following. There were no significant interactions between the PPID vs non-PPID horses by treatment or by time ($P>0.05$). However, when separating out the different categories of PPID status (SP and DP) of horses compared to the non-PPID vaccinated horses, SP PPID horses had an overall significantly ($P<0.05$) higher EHV-1 antibody titers compared to DP PPID and non-PPID horses. Results for immune responses to the WNV component of the vaccine are the following. There were no significant interactions between the PPID vs non-PPID horses by treatment or by time ($P>0.05$). However, when comparing the different categories of PPID status (SP and DP) to non-PPID vaccinated horse responses, the DP PPID horses had reduced ($P<0.10$) levels of WNV titers at week 4 compared to

SP and non-PPID horses. Thus, vaccination in the face of PPID does have an impact on how these horses respond immunologically. Further characterization of the duration of immunity and vaccine efficacy of PPID horses compared to non-PPID horses is warranted.

Single and Paired Measurements of Adrenocorticotropic Hormone for the Diagnosis of Pituitary Pars Intermedia Dysfunction

D.I. Rendle[1], M. Duz[2], J. Beech[3], T. Parkin[2] and A.E. Durham[1]

[1]Liphook Equine Hospital, Forest Mere, Liphook, Hampshire, GU30 7JG
[2]School of Veterinary Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, 464 Bearsden Road, Glasgow, G61 1QH
[3]Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348

Email: daverendle@me.com

Paired measurement of adrenocorticotropic hormone (ACTH) concentration has been suggested as a means of overcoming fluctuations in plasma ACTH levels. This study aimed to determine whether the mean of two measurements of ACTH concentration is more reliable in assessing pituitary pars intermedia dysfunction (PPID) than relying on a single measurement. Paired ACTH measurements were performed on (i) 148 occasions from 124 horses being investigated for PPID, (ii) 90 occasions from 76 horses with PPID that were receiving treatment with pergolide and (iii) 63 occasions from 50 horses in which there was no clinical suspicion of PPID. Histological examination of the pars intermedia was performed in 67 of the untreated horses. Outcome of testing using single and the mean of paired samples was compared directly using Bland-Altman analysis and receiver operating characteristic (ROC) analysis and both methods were compared against histology. Paired ACTH measurement altered binary classification as healthy or diseased in six out of 211 cases, all off which had equivocal initial ACTH concentrations between 20 and 39 pg/ml. Using histology as the gold-standard, optimal sensitivity and specificity for diagnosing PPID were 69.4% and 80.9%, respectively for single measurement and 72.2% and 76.2%, respectively for paired measurements. The area under the ROC curve was 0.72 and 0.73 for single and paired measurements compared to histopathologic diagnosis, respectively. Paired measurement of ACTH concentration offers no advantage over a single measurement. However, if borderline results are obtained using a single measurement of ACTH concentration further testing should be performed.

Further Observations of Seasonality of *Pars Intermedia* Secretory Function in 30,000 Horses and Ponies

Andy E Durham, Liphook Equine Hospital, Hampshire, UK. andy@theleh.co.uk

Background: Since the initial observations by Donaldson *et al* (2004), it is now well known that equine *pars intermedia* (PI) secretory function has a circannual rhythmicity and, furthermore, that this is retained in the presence of pituitary *pars intermedia* dysfunction (PPID) (Copas and Durham 2012).

Reasons for performing study: The timing of altered PI function has been investigated previously but all studies have been limited by case numbers inevitably leading to broad and imprecise time periods in order to maximise group sizes to facilitate statistical power. Copas and Durham (2012) published 2 reference intervals for plasma ACTH in horses applicable to different times of year (Nov 1st – July 31st <29 pg/mL; Aug 1st – Oct 31st <47 pg/mL), although it is biologically implausible that these 2 reference intervals would transition abruptly leading to interpretive difficulties especially during the periods of July/August and October/November. This study aimed to involve very large numbers of cases enabling greater precision for determination of changes in PI secretory activity with additional examination of insulin and glucose data and possible identification of seasonal cues.

Main findings: Data was examined from >30,000 submissions for plasma ACTH measurement including horses with and without PPID and divided into 1 week categories. A steady increase in ACTH concentrations occurred over approximately 15 weeks commencing at the end of June/early July and continuing until the end of September/early October (weeks 26-40). A decrease in ACTH concentrations then followed, at a more rapid rate than the prior increase, over an approximate 7 week period, ending in late November (weeks 41-47). ACTH concentrations were reasonably stable during the months of December to June inclusive with a possible nadir in late April/early May (Figure 1). Examination of serum insulin and plasma glucose concentrations showed a less marked circannual rhythmicity with possibly higher values during the winter months of December to March inclusive.

Examination of day-length data alongside ACTH concentrations indicated that a decreasing day-length (commencing at the end of June) was coincident with the start of the annual increase in PI secretory function and that this was curtailed when the rate of decrease in day-length began to decrease in early October (Figure 1).

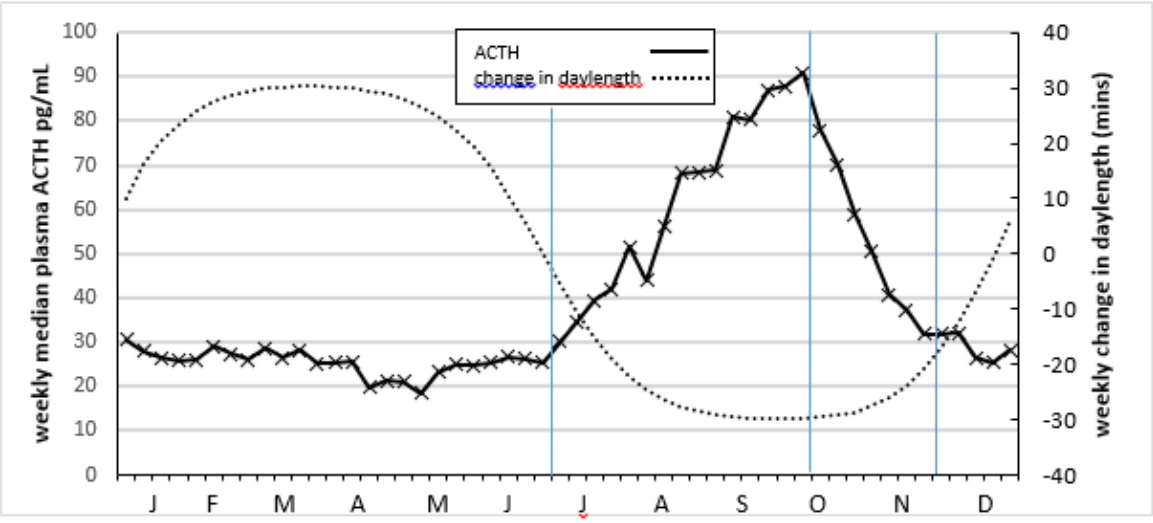


Figure 1. Weekly median ACTH concentrations and weekly change in day-length through the year. Vertical lines represent key time-points denoting the commencement of increase in plasma ACTH, the commencement of decrease and the re-establishment of stable (lower) plasma ACTH concentrations.

Conclusions: The period of increased pituitary activity detected by basal ACTH concentrations appears to span July to November inclusive, a longer period than previously reported. The summer solstice (June 21st) appears to coincide with the start of increasing pituitary activity, presumably as a result of perceived decreasing day-length. The autumn equinox (September 25th) appears to coincide with the end of this stimulatory process with rapidly decreasing pituitary activity thereafter, possibly as a result of perceived decreasing rate of day-length shortening or relatively greater darkness versus daylight hours.

Insulin and glucose measurements were not positively associated with plasma ACTH and, in fact, a possible negative association was evident with generally higher insulin and glucose concentrations from December to March inclusive.

Relevance: Extension of diagnostic reference intervals may be needed into the months of July and November. This study might offer a basis for further investigation of the influence, and possible manipulation, of ambient light-effects on PI function.

Comparison of Resting and Dynamic Adrenocorticotropic Hormone Following Administration of Thyrotropin-Releasing Hormone During Autumn and Non-Autumn Seasons in Horses

J. C. Haffner, R. M. Hoffman, H. S. Spooner, S.G. Grubbs
Middle Tennessee State University

Circannual variation in equine adrenocorticotropic hormone (ACTH) creates challenges in predicting early signs of Pituitary Pars Intermedia Dysfunction (PPID) without established autumn reference values. The goal was to establish predictive ACTH concentrations for early signs of PPID during Autumn based on established Non-Autumn predictors using a thyrotropin-releasing hormone (TRH) test. ACTH concentrations in 32 horses aged 1 to 27 yrs were evaluated before and after administration of TRH during June (Non-Autumn) and October (Autumn). Venous samples were collected into EDTA-treated tubes before (PRE) 1 mg TRH administered i.v. and 10 (T10) and 30 (T30) min post-TRH. Blood was chilled and centrifuged within 2 h, and plasma stored at -80ºC pending ACTH analysis using a sequential immunometric assay. Horses were scored for muscle wasting, hirsutism, sweating and abnormal fat deposits. Frequency histograms of the Autumn ACTH data indicated two populations at each sample time, and confidence intervals were used to designate the break between these populations. The 95% confidence breakpoint indicated PPID "Positive" at ACTH greater than 80, 620 and 230 pg/mL, and "Negative" at ACTH less than 60, 340 and 190 pg/mL, at PRE, T10 and T30 respectively. Chi-square measures of association indicated no difference in Non-Autumn versus Autumn predictions of PPID at PRE, T10, or T30 ($P>0.27$). Age positively correlated with PRE ($R^2=0.37$; $P=0.04$) and T10 ($R^2=0.43$; $P=0.01$). Muscle wasting scores positively correlated with age ($R^2=0.50$; $P=0.004$) and PRE ($R^2=0.63$; $P<0.001$). Hirsutism scores positively correlated with T10 ($R^2=0.46$; $P=0.01$), T30 ($R^2=0.42$; $P=0.015$), and sweating scores ($R^2=0.46$; $P=0.01$).

Evaluation of Seasonal Variation in Adrenocorticotropin Concentration in Response to Thyrotropin-Releasing Hormone in Ponies.

A. Chapman[1,3], K. Odom[2], J. Fletcher[3], M. Keowan[1] M. Kearney[4]

[1]Equine Health Studies Program, [2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[3]Department of Veterinary Clinical Sciences, [2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[4]Department of Pathobiological Sciences, [2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA

Objective: Pituitary pars intermedia dysfunction (PPID) is the most common endocrine disorder of geriatric horses and ponies. Some research suggests that pony breeds may be at increased risk for the development of PPID. A number of studies have detected a seasonal variation in endogenous adrenocorticotropin (ACTH) concentrations in normal horses and ponies that correlates to annual decline in daylight hours. Seasonally adjusted endocrine hormone reference ranges have been proposed to avoid falsely classifying normal horses as having PPID. The purpose of this study is to evaluate seasonal change in ACTH response to administration of thyrotropin releasing hormone (TRH) in normal adult ponies and ponies with PPID.

Methods: Clinically normal ponies between the ages of 4-14 years of age underwent an overnight dexamethasone suppression test (ODST) to evaluate for the presence of PPID in May 2014. Serum cortisol sample was obtained before and 19 hours after intramuscular administration of 40 µg/kg dexamethasone. Ponies with a normal ODST are undergoing TRH response test in 30-day intervals from June through October 2014. Plasma samples are collected at 0, 5, 10, 15 and 30 minutes after administration of TRH (1 mg IV) and analyzed for endogenous ACTH concentration using a validated chemiluminescent assay. In the second phase of this study, mature ponies with clinical signs of PPID will undergo ODST in May 2015. Ponies with an abnormal ODST will be examined for seasonal variations in TRH response test as described above.

Preliminary Results: Twelve clinically normal ponies (2 stallions, 2 geldings, 8 mares) with a mean age of 6.6 years had normal cortisol suppression on ODST. Plasma ACTH concentration peaked at 5 minutes after administration of TRH in 11 of 12 normal ponies in June and July. Plasma ACTH concentration peaked at 10 min in one normal pony (Pony #23) in June and July. Mean plasma ACTH concentrations in June were 33.7 ± 16.0 pg/mL at $T = 0$, 93.0 ± 41.6 pg/ml at $T = 5$, 58.1 ± 29.1 pg/mL at $T = 10$, 42.0 ± 16.1 pg/mL at $T = 15$, and 34.1 ± 12.4 pg/mL at $T = 30$. In July mean plasma ACTH concentrations were 58.5 ± 26.1 pg/mL at $T = 0$, 385.0 ± 320.4 pg/mL at $T = 5$, 170.0 ± 149.2 pg/mL at $T = 10$, 122.0 ± 81.84 pg/mL at $T = 15$, and 69.7 ± 52.8 pg/mL at $T = 30$. Mean endogenous plasma ACTH concentrations in July were significantly higher than concentrations in June at 5 minutes ($P < 0.001$) and 10 minutes ($P = 0.008$) post TRH administration. Using the reference intervals for ACTH concentrations after TRH administration recommended by the Equine Endocrine Society, plasma ACTH concentrations in June were above the upper limit in 4 normal ponies at $T = 0$ (> 35 pg/mL), and in one normal pony at $T = 10$ (> 110 pg/mL). In July, plasma ACTH concentrations were above the upper limit 9 normal ponies at $T = 0$ (> 35 pg/mL), in 5 normal ponies at $T = 10$ (> 110 pg/mL), and in 4 normal ponies at $T = 30$ (> 65 pg/mL). Sample collection of normal ponies is ongoing during August, September and October 2014. Identification of study cohort of PPID ponies is in process for summer of 2015.

How Do We Interpret High Adrenocorticotropic Hormone Concentrations in Young Horses?

Rendle, D.I.[1] and Turnbull, C.[2]

[1] Liphook Equine Hospital, Forest Mere, Liphook, Hampshire, UK, EX16 6AU
[2] Boehringer-Ingelheim Ltd, Ellesfield Avenue, Bracknell, Berkshire. UK, RG12 8YS

Pituitary *pars intermedia* dysfunction (PPID) is a degenerative condition that is associated with ageing. However, the disease is being diagnosed increasingly in young horses and the purpose of this study was to gather further data on horses with high adrenocorticotropic hormone (ACTH) concentrations aged ≤ 7 years. Through a laminitis disease awareness campaign performed in the United Kingdom, 12,214 horses were tested for PPID in 2013. Two-hundred-and-seventy-eight (2.3%) were aged ≤ 7 years of which 82/278 (29%) tested positive for PPID using a seasonally adjusted reference range. Corroborating and follow-up data were obtained for 48/82 cases. Geldings comprised 33/48 cases and both Shetland and Welsh pony breeds were over-represented, accounting for 16 and 12 cases respectively. A range of clinical signs were reported with laminitis being the most frequent (27/48). Follow-up testing prior to treatment was performed in 18 cases; 14 had thyrotropin releasing hormone stimulation tests performed, 5 were positive, 9 negative. Four had repeat ACTH concentration measurements performed; 1 was positive, 3 negative. Twenty-two horses were treated with pergolide; 5 were ultimately euthanased as a result of laminitis, the remaining 17 were all reported to have improved clinically. Sixteen of the 22 treated horses were re-tested whilst on treatment; 10 had high and 6 had normal ACTH concentrations. These findings suggest that horses aged ≤ 7 years may be affected by pituitary dysfunction. However, the condition was diagnosed infrequently and false positive results were common. Further testing is recommended for such cases before instigating treatment.

Treatment of Pituitary Pars Intermedia Dysfunctiion

Harold C. Schott II, DVM, PhD, DACVIM
East Lansing, MI

Introduction
Management of equids with pituitary pars intermedia dysfunction (PPID) consists of improved husbandry, including proper nutrition and limiting competition for feed, body-clipping, preventive health care, dentistry, and appropriate treatment of concurrent medical problems. In addition, specific treatment with the dopamine agonist pergolide can improve quality of life and reverse many clinical signs of PPID. Combination treatment with both pergolide and cyproheptadine, in the author's experience, may also prove beneficial in more advanced cases. Assessment for concurrent glucose and insulin dysregulation, especially in patients with laminitis, is also warranted. Management of these latter conditions requires appropriate feeding and exercise programs, proper hoof care, judicious use of analgesic medications, and in some cases use of additional medications (metformin and levothyroxine) may be considered. Finally, due to the costs of lifelong management and medication(s), the extent of treatment of PPID-affected equids should be made on a case-by-case basis, in consideration of the client's goals.

Husbandry and Nutritional Considerations
In the earlier stages of PPID, when hypertrichosis may be the primary complaint, body-clipping may be the only treatment required. Since many affected animals are aged, routine oral care and correction of dental abnormalities cannot be overemphasized. In addition, assessment of diet and incorporation of pelleted feeds designed specifically for older equids (e.g., senior diets) should be considered. Sweet feeds and other concentrates high in soluble carbohydrate are best avoided (unless that is all that they will eat), especially with concurrent glucose and insulin dysregulation. Affected equids may also need to be separated from the herd if they are not getting adequate access to feed. Unfortunately, because the ventral abdomen may become somewhat pendulous, weight loss and muscle wasting in more severely affected animals may not be well-recognized by owners. Consequently, regular measurement of body weight, or estimation with a weight tape, and assessment of body condition score are important parameters to monitor during treatment.

Whether or not it is "safe" to allow PPID-affected equids to graze pasture remains a controversial question. Pasture, especially lush spring and fall pasture, should be considered similar to feeding concentrates high in soluble carbohydrates and many veterinarians recommend that PPID-affected equids not be turned out on pasture. In this author's opinion, it is important to assess the overall condition of the patient. If the equid is overweight, has abnormal fat deposits, or a history of laminitis (all supportive of concurrent insulin dysregulation), pasture turn out would not be recommended. Instead, feeding grass hay at 1.5% of the body weight daily would be the preferred forage diet and animals that are overweight clearly do not need an additional "low starch" concentrate feed, although a "ration balancer" vitamin and mineral supplement designed to complement a forage diet is recommended. However, if body condition is poor, pasture grazing can be a useful way to increase caloric intake and produce weight gain. Again, caution is advised and access to lush spring or fall pasture should be limited to one or more shorter periods per day, preferably during the early morning hours. Horses that are thin can also be fed senior feeds and provided a fat supplement (vegetable oil at up to a cup twice a day).

Since the major musculoskeletal complication of PPID is chronic laminitis, regular hoof care is essential. It is important to emphasize to clients that medical treatment for PPID (pergolide) is not a direct medical treatment for laminitis, as the painful gait and hoof abscesses that can accompany chronic laminitis may persist, due to damage to the laminar bed that has already been sustained. Thus, intermittent use of non-steroidal anti-inflammatory drugs may be necessary. Although flare-ups of chronic laminitis remain a reason for euthanasia in PPID-affected equids, it also warrants emphasis that medical treatment of PPID combined with regular hoof care and management of glucose and insulin dysregulation can lead to

substantial improvement in this complication. Finally, because many PPID affected patients may have secondary infections (e.g., sinusitis, dermatitis, and bronchopneumonia), intermittent or long-term administration of antibiotics, typically a potentiated sulfonamide, may be necessary in some cases.

Horses with PPID have also been shown to have a more rapid rise in fecal egg counts following anthelmintic administration, in comparison to normal, aged horses. Thus, attention to parasite burden by monitoring fecal egg counts and implementing appropriate anthelmintic practices should warrant greater attention in PPID-affected equids.

Medications for Treatment of PPID

Medications that have been used to treat equids with PPID include serotonin antagonists (cyproheptadine), dopamine agonists (bromocriptine and pergolide), and trilostane, an inhibitor of adrenal steroidogenesis. Cyproheptadine was one of the initial drugs used because serotonin had been shown to be a secretagogue of ACTH in isolated rat pars intermedia tissue and because the medication was available at a reasonable cost. Early reports that cyproheptadine (0.5-1.0 mg/kg, PO, q 24 h) resulted in clinical improvement and normalization of laboratory data within 1-2 months have been disputed as similar clinical improvement has been achieved with improved nutrition, preventive care, and management alone. Further, two studies comparing both clinical improvement and endocrine test results have clearly shown that pergolide is more effective than cyproheptadine as monotherapy for treatment of PPID.

Because loss of hypothalamic dopaminergic innervation appears to be an important mechanism for development of PPID, treatment with dopaminergic agonists is a logical approach to therapy. Pergolide, a first generation dopamine agonist used for treatment of Parkinson's disease (PD), was found to acutely lower plasma concentrations of immunoreactive adrenocorticotropin (ACTH) and other pro-opiomelanocortin peptides in an early report. Subsequently, pergolide treatment produced clinical improvement in 23 of 25 PPID-affected equids; however, the dosage was quite high (6-10 µg/kg, PO, q 24 h [3-5 mg to a 500 kg horse]) and the expense of treatment precluded routine use of the drug. When Peters and colleagues (1995 AAEP Convention) later reported that using a lower dose of pergolide (2 µg/kg, PO, q 24 h [1 mg/day for a 500 kg horse]) was clinically effective in a series of horses and ponies with PPID, use of pergolide became more widespread as cost was no longer prohibitive. Reported adverse effects of pergolide include anorexia, diarrhea, and colic; however, the latter problems are more often associated with higher doses of the drug. Usually, only transient anorexia is recognized during the initial few weeks of "low dose" pergolide treatment and can be overcome by stopping treatment for a few days and starting back at half the dose, slowly increasing to the desired dose. Although pregnant mares have been treated with the drug, safety of pergolide use during pregnancy has not been studied in equids. Many pregnant mares treated with pergolide have been anecdotally reported to deliver healthy, term foals and produce adequate milk. Consequently, it does not appear that discontinuation of pergolide treatment prior to foaling is warranted unless udder development does not appear to be progressing as expected prior to the expected parturition date.

Limited information exists on pharmacokinetics of pergolide although the initial study performed reported plasma concentrations of the drug that were 4-10 times greater in horses than in humans after administration of a similar oral dose (10 µg/kg), suggesting greater bioavailability in equids. The authors of that study concluded that a daily dose of 1-2 µg/kg (0.5-1 mg to a 500 kg horse) would be expected to produce drug levels in equids similar to therapeutic plasma concentrations in humans with PD, yet twice daily administration would more likely provide sustained plasma concentrations of the drug. More recent pharmacological studies have yielded conflicting results with pergolide half lives of 5.6 and 24 h reported. Another recent study found no difference in the decrease in plasma ACTH concentration between once or twice daily pergolide dosing. Consequently, the question of the "best" dosing interval remains unresolved at present.

In humans with pituitary gland macroadenomas (notably prolactinomas), dopaminergic agonist therapy has goals of both reducing excessive hormone secretion as well as decreasing tumor size to correct

visual and other neurological deficits caused by the mass effect of the tumor. In a group of horses that had pituitary gland size determined by computed tomography (CT) before and after 6 months of pergolide treatment, no decrease in pituitary gland size was found. However, this study was potentially confounded by effect of season as the post-treatment CT scans were performed in August and September when activity of the hypothalamic-pituitary axis and pars intermedia and total pituitary gland size naturally increase with hormonal activity in preparation for winter.

Until 2007, pergolide had been available in tablet form (0.25-1.0 mg, Permax™, Eli Lilly) with this formulation costing $75-100/month for a treatment dose of 1 mg/day. Because this was not an inconsequential expense for many retired family pets, several compounding pharmacies started to market pergolide products as suspensions, granules, or even in treats, often at a cost that was less than half that of Permax™. In 2002, reports started to appear describing development of valvular heart disease with significant regurgitation in human patients that had been receiving long-term pergolide for treatment of PD. This complication ultimately led to voluntary withdrawal of Permax™ from the marketplace in 2007 and left compounding pharmacies as the only source of pergolide for PPID-affected equids. Unfortunately, there is limited regulatory oversight of compounding pharmacies and independent analysis of pergolide products from several compounding pharmacies revealed considerable variation in potency as well as degradation, especially of water based drug suspensions, after as little as 2 weeks of storage. Of interest, considerable variation in pergolide content was even found between different scoops of a compounded granular formulation taken from the same container.

A final medication that has been used to treat PPID-affected equids is trilostane, a competitive inhibitor of 3-β-hydroxysteroid dehydrogenase that is being increasingly used for treatment of pituitary-dependent hyperadrenocorticism in dogs. In one clinical study, trilostane (0.4-1.0 mg/kg, PO, q 24 h in feed) was reported to be effective in reversing both clinical signs (primarily laminitis) and abnormal endocrinologic test results in a group of PPID-affected horses and ponies. However, horses and ponies in that study received additional management for laminitis and the “improvement” in endocrinologic test results was not overly convincing. As with many chronic diseases in the horse, specific nutrient supplementation and complementary or alternative therapies, including acupuncture and herb mixtures, have been advocated for equids with PPID. A product made from chasteberry has been one of the more popular herbs used; however, a small field study demonstrated that this product was an ineffective treatment for PPID.

Prasacend™ Field Efficacy and Extemded Use Studies

Because there was both need and demand for a consistent, high quality pergolide product, Elanco Animal Health, the veterinary subsidiary of Eli Lilly, designed an open field clinical efficacy study with the goal of having pergolide approved by the FDA for treatment of equids with PPID. In the midst of the study, Boehringer-Ingelheim Vetmedica secured the rights to the drug and continued with development of Prascend™, a 1 mg scored pergolide mesylate tablet, that was approved by the FDA for treatment of PPID in equids in the fall of 2011. The open field clinical efficacy study enrolled 122 equids (59 male, 63 female, 10-35 years, 137-623 kg, and 16 breeds) at eight sites. Equids were enrolled between 11/1/08 and 1/31/09 based on clinical examination and endocrine testing results. Animals were scored (0-3) for hypertrichosis, hyperhidrosis, polyuria-polydipsia, abnormal fat distribution, and muscle wasting. Inclusion criteria were a hypertrichosis score $\geq$1 and either a plasma ACTH concentration $\geq$50 pg/ml (radioimmunoassay) or failure of endogenous cortisol concentration to suppress (<1.0 µg/dl) 19 h after dexamethasone administration (40 µg/kg, IM). Treatment with pergolide mesylate (2 µg/kg, PO, q 24 h) was started within 7 days of initial evaluation. Animals were re-evaluated (clinical exam and endocrine testing) after 90 (n=113) and 180 days (n=111) of treatment. When endocrine test results remained abnormal at 90 days (n=47), the dose was increased to 4 µg/kg, PO, q 24 h. Treatment success after 180 days was defined as either normalization of dexamethasone suppression test results (<1.0 ug/dl) or a decrease in plasma ACTH concentration by 50% (or to <50 pg/ml when initial value was <100 pg/ml) and improvement by a score of $\geq$1 in at least one clinical sign. Treatment was also considered successful when the sum of clinical scores decreased by $\geq$3, regardless of endocrine test results. Treatment compliance and minor adverse events were reported by reviewing daily written entries in a standardized

diary at 90 and 180 day evaluations. Adverse events requiring veterinary evaluation during interim periods were reported to study investigators within 24 h and further investigated to determine seriousness.

In all, 76% (86/113) equids were classified as treatment successes (two horses withdrawn by their owners between 90 and 180 days were categorized treatment failures). The remaining nine animals died (n=8) or were euthanized (n=1) due to worsening of pre-existing conditions (laminitis and dental disorders) or colic. After 90 days of treatment, 58% (66/113) had normal endocrine test results and clinical improvement. Both median scores of clinical signs and mean concentrations of ACTH and cortisol (following dexamethasone administration) decreased during the study period. Transient inappetance was the most common adverse event observed in 40/122 (33%) equids, mostly during the initial 30 days of treatment. Other adverse events reported included lethargy, colic, diarrhea, lameness, and weight loss in less than 10% of cases and it was unclear whether or not these events were related to use of the drug. There were 10 reports of laminitis during the study: seven were considered flare-ups of chronic laminitis and three were apparently new cases of laminitis. The results of the open field efficacy study clearly demonstrated that pergolide was effective in improving clinical signs of PPID; however, not all clinical problems may be corrected, specifically laminitis.

A group of 30 equids (28 horses and two ponies) that completed the clinical efficacy study at Michigan State University were subsequently enrolled in an extended use study in August, 2009. Most enrolled horses were re-examined after 24 and 36 months of pergolide treatment and these equids had continued to receive the same dose (either 2 or 4 µg/kg, PO, q 24 h) that they had been receiving at the 6 month end point of the initial study. After 2.5 years of treatment (spring, 2011), all owners reported ongoing clinical improvement and overnight dexamethasone suppression test (ODST) results remained normal in 79% of tested equids (19 of 24 equids). Four horses were not tested at this time and two horses were lost due to causes unrelated to PPID (one sudden death and one euthanasia for neurological disease). Of interest, six horses with abnormal ODST results after 3 and 6 months of treatment had normal ODST results after 2.5 years of treatment with no increase in pergolide dose. After more than 5 years of treatment (May, 2014) owners were satisfied with the remaining equids attitude and condition, although patients clearly aged during the extended use study. Of the 12 remaining equids tested in spring 2014, plasma ACTH concentration was <50 pg/mL in 9 animals (75%) and ODST results also remained normal in 75% of horses (9 of 12 equids). At present (fall 2014) 10 equids remain in the extended use study and of the 20 that died, 18 died or were euthanized for age-related disorders and only two were euthanized for recurrent laminitis, a problem attributable to PPID. To date, the results of the extended use study support that long-term treatment of equids with PPID with pergolide results in clinical improvement, normalization of endocrine test results, and owner satisfaction in a high percentage of cases. Further, equids with PPID can be maintained for several years on pergolide therapy without a need for a progressive increase in drug dosage.

Current Treatment Recommendations

At present, it is the author's recommendation that the initial medical treatment for equids with PPID should be pergolide at a dose of 2 µg/kg, PO, q 24 hours (1 mg/day for a 500 kg horse). If no improvement is noted within 30 days (depending on season as hair coat changes will vary with the time of year that treatment is initiated), the daily dose can be increased by 1-2 µg/kg (to 1.5-2 mg/day for a 500 kg horse) with reassessment after 30 days. The author typically increases pergolide to a total dose of 6 µg/kg (3 mg/day for a 500 kg horse). If only a limited response is observed at this dose of pergolide and endocrine test results remain abnormal, addition of cyproheptadine (0.5 mg/kg, PO, q 24 hours) to pergolide therapy has been effective in a limited number of cases treated by the author.

It is important to recognize that the rate of clinical improvement is higher than that for normalization of endocrine test results. For example, in a treatment study performed by the author, 13 of 20 pergolide treated horses were reported to have improved clinically while only seven of 20 had normalization of endocrine test results. Thus, it is prudent to regularly measure blood glucose concentration and perform follow-up endocrine testing when managing equids with PPID. The author currently recommends

measuring plasma ACTH concentration or performing an ODST at least yearly (between December and June in horses in the northern hemisphere) in horses that appear to be stable and 30 days after a change in medication dose or addition of cyproheptadine.

Prognosis

Once present, PPID is a lifelong condition. Thus, the prognosis for correction of the disorder is poor. However, PPID can be effectively treated with a combination of management changes and medications. Thus, the prognosis for life is guarded to fair. Following the Michigan State University cohort of horses described above, survival after 3 years of treatment with pergolide was 78% declining to 40% after 5 years. Finally, the author has followed a couple of horses treated with pergolide for nearly a decade and has become convinced that the drug improves the quality of life but that does not necessarily equate to prolonging life.

Challenges in Treatment Recommendations

Greater recognition of the prevalence and variable clinical syndromes of PPID, better understanding of endocrine tests, and FDA approval of Prascend™ for treatment of PPID have been major advances in our ability to manage PPID. Nevertheless, challenges remain for clinicians working with PPID-affected equids and researchers investigating the disease.

For clinicians, current endocrine tests have a limited ability to provide supportive evidence for "early" PPID when clinical complaints and signs are nonspecific. Consequently, a decision for starting medical treatment with pergolide often relies on clinical experience and judgment, rather than conclusive test results. As a result, "trial and error" therapy ensues, often with unclear goals or endpoints. This approach can lead to both overtreatment and undertreatment, depending on a clinician's clinical judgment and philosophical approach to treatment. Specifically, clinicians are faced with the challenge of deciding whether or not to treat suspect horses (e.g., middle-aged to older equids with vague clinical signs and/or laminitis that have normal plasma ACTH concentrations or clinically normal older horses with mildly elevated plasma ACTH concentrations) with pergolide. Comparisons could be made with use of statins in people with elevated cholesterol or low dose aspirin therapy in hopes of preventing heart disease: should we recommend pergolide treatment for "normal" horses with elevated plasma ACTH concentrations? Clinicians also remain faced with the challenge of defining treatment goals: is improvement in clinical signs adequate or should the goal of treatment also be normalization of endocrine test results? Finally, the ideal dosing interval and whether or not seasonal (fall) treatment can be an approach in some animals are unresolved questions.

For researchers investigating PPID, challenges include better understanding of pathophysiology of the varied clinical syndromes of PPID. Specifically, might there be varying hormonal profiles in equids that are afflicted with diabetes or laminitis as compared to hypertrichosis and muscle wasting? Additional questions include: 1) why do some horses develop signs of PPID at a relatively early age; and 2) what is the role of insulin dysregulation in PPID? Next, although PPID affects many breeds, it has also been anecdotally recognized in several generations within certain families of equids. Consequently, the genetic basis for development of PPID needs to be explored. Finally, might environmental risk factors may also play a role in development of PPID? Answers for many of these questions will likely require longitudinal, multi-center studies through which progression of PPID can be studied over a number of years.

Short-term Responses to Pergolide and Effects of Sample Timing in Horses with PPID

D.I. Rendle[1], S.Gough, K.J. Hughes and J.L. Ireland[2]

School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia. [2]Animal Health Trust, Newmarket, Suffolk, UK.
[1]Present address: Liphook Equine Hospital, Liphook, Hampshire, UK

Email: daverendle@me.com

Aims: i) To investigate short-term effects of pergolide on adrenocorticotropic hormone (ACTH) concentrations and results of thyrotropin releasing hormone (TRH) stimulation tests. ii) to investigate potential effects of sample timing in relation to pergolide dosing on ACTH concentrations.

Methods: Six horses with clinical evidence of PPID and positive responses to a TRH stimulation test received treatment with pergolide (4 µg/kg PO SID) for 18 days. Pergolide mixed with water was administered directly into the mouth using a dosing syringe immediately prior to feeding. Adrenocorticotropic hormone concentrations were measured immediately prior to the administration of pergolide and exactly 2 and 12 hours after the administration of each dose. On the day prior and on days 8 and 18 of the study, TRH stimulation tests were performed. Friedman's tests, with post hoc Wilcoxon signed-rank tests, were used to compare median ACTH values for sample time points on each of the study days.

Results: A statistically significant reduction in ACTH concentration occurred within 12 hours of the administration of the first dose of pergolide. On subsequent days (2-18), the timing of sampling in relation to pergolide dosing did not have a significant effect on ACTH concentration. Increases in ACTH concentration in response to TRH were reduced on both days 8 and 18 of treatment compared to the responses that occurred pre-treatment.

Conclusions: Pergolide resulted in a significant reduction in ACTH concentration within 12 hours. Thereafter, ACTH levels remained stable and the timing of sampling in relation to dosing did not have a significant effect on results. Changes in TRH response were noted within 8 days of pergolide treatment.

Effects of Pergolide Mesylate on Plasma Adrenocorticotropic Hormone Concentration in Horses with Pituitary Pars Intermedia Dysfunction

D.I. Rendle, E. Taylor, A.E. Durham, T.D. Parkin* and M. Duz*

Liphook Equine Hospital, Forest Mere, Liphook, Hampshire, UK GU30 7JG; *School of Veterinary Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, 464 Bearsden Road, Glasgow, G61 1QH, Scotland, UK.
Email: daverendle@me.com

Aims: Published evidence of the efficacy of pergolide is limited to small case series and anecdotal reports. This study evaluated adrenocorticotropic hormone (ACTH) responses in horses treated with pergolide and investigated factors that may influence response to treatment.

Methods: A retrospective review of submissions to The Liphook Equine Hospital Laboratory was performed from January 2007 to December 2012 and cases in which ACTH concentration was measured in the same season before and after instigation of pergolide treatment were identified. Data were analysed using Rv.2.15 software (R Development Core Team). Improvement was defined as a reduction of ACTH concentration of ≥ 50%, or a return of ACTH concentration to within seasonally adjusted reference intervals.

Results: 1664 cases satisfied the inclusion criteria. 361 of the 1664 cases, (21.7%) had no improvement in any of the re-check samples available. 1303 (78.3%) had improved in at least 1 re-check sample. 1135 (68.2%) had improved at the by the first re-check. Of the 168 that did not improve at the first re-check sample, 144 (a further 11%) had improved by the second re-check and a further 24 (1.4%) improved by the third re-check. Of the 1303 that improved, 456 (35.0%) had at least one further sample obtained. Of these, 361 (79.2% of the 456) remained improved while 95 (20.8%) had deteriorated.

Conclusions: Pergolide is an effective means of reducing ACTH concentration in the majority of horses with PPID; however, responses are difficult to predict in individual horses and regular monitoring is therefore indicated.

Evaluation of Resting ACTH Concentration as a Screening Test for Pituitary Pars Intermedia Dysfunction (PPID) in 23 Louisiana Horses

J. Fletcher[1], A. Chapman[2], P. Camacho[2], F. Andrews[2]; [1]Louisiana Animal Disease Diagnostic Laboratory Veterinary Endocrinology Laboratory and [2]Equine Health Studies Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA.

Aims: Pituitary pars intermedia dysfunction (PPID) is common in older horses (≥15 years of age). Diagnosis is based on clinical signs and endocrine testing. Recently, resting adrenocorticotropic hormone (ACTH) and increased ACTH in response to thyrotropin-releasing hormone (TRH) have been shown to accurately diagnose horses with PPID. It has also been suggested that a normal resting ACTH concentration can be used to rule out PPID. The purpose of this study was to evaluate resting ACTH concentration as an indicator of disease in horses suspected of having PPID in Louisiana and the surrounding region.

Methods: Resting ACTH and ACTH concentrations after the administration of TRH were obtained from TRH stimulation test samples submitted to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) Veterinary Endocrinology Laboratory at Louisiana State University. The results were evaluated to determine the sensitivity and specificity of an increased resting ACTH concentration. In addition, the ability of a normal resting ACTH concentration to reliably rule out PPID was evaluated by determining the percentage of horses that had TRH stimulation test results consistent with PPID with a normal resting ACTH concentration. The upper limits of the reference interval for resting ACTH and ACTH 10 minutes and 30 minutes after TRH stimulation were based on recommendations made by the Equine Endocrine Society, and were 35.0 pg/ml, 110 pg/ml, and 65 pg/ml respectively.

Results: Thyrotropin-releasing hormone stimulation test results from twenty-three horses were obtained from the LADDL Veterinary Endocrinology Laboratory database. Fifteen horses had resting ACTH concentrations below the upper limit (mean ± SD; 22.2 pg/ml ± 7.3 [range 11.3 – 34.3 pg/ml]) and 8 horses had resting ACTH above the upper limit (116.8 pg/ml ± 94.1 [range 47.4 – 318 pg/ml]). All horses (n=8) with a resting ACTH concentration above the upper limit had TRH stimulation tests consistent with PPID. Using the TRH stimulation test as an indicator of disease (n=23), a resting ACTH concentration > 35 pg/ml had a sensitivity of 44% and a specificity of 100%. Of the 15 horses with normal resting ACTH concentrations, 5 horses had normal TRH stimulation tests and 10 horses had abnormal TRH stimulation tests. Over 55% of the horses that had TRH stimulation test results consistent with PPID had a normal resting ACTH concentration.

Conclusions: Based on this small sample size of horses suspected to have PPID, a resting ACTH concentration ≤ 35 pg/ml could not be used to definitively rule out PPID, and horses with an increased resting ACTH concentration were likely to have an ACTH concentration above the upper limit at 10 minutes and 30 minutes after the administration of TRH. A TRH stimulation test is recommended when screening horses for PPID.

Pharmacokinetics and Pharmacodynamics of Pergolide Mesylate after Chronic Oral Administration in Horses with PPID.

D McFarlane, HE Banse, F Yang, LK Maxwell. Oklahoma State University, Center for Veterinary Health Sciences, Stillwater, OK.

Equine pituitary pars intermedia dysfunction (PPID) is a common, debilitating condition of aged horses. Pergolide mesylate is the drug of choice for treatment of PPID. Previous pharmacologic research has focused on the kinetic properties of pergolide following a single oral dose to young, healthy horses. However, long term administration of pergolide to x aged horses with PPID is what is typical in practice. Therefore, we investigated the pharmacokinetic and pharmacodynamic properties of pergolide mesylate in horses with PPID after 6 months of oral administration. Six horses with confirmed PPID were administered pergolide from July through January at 0.002 µg/kg po q24hr for 2 months, followed by 0.004 µg/kg po q24hr for an additional 4 months. Pergolide concentrations were assessed using liquid chromatography/mass spectrometry and pharmacokinetic analysis was performed by non compartmental analysis on samples drawn over 4 weeks after the last dose was administered. Pharmacodynamic properties of pergolide were assessed by measuring plasma ACTH. ACTH concentration was determined by chemiluminescent immunoassay. Pergolide was rapidly absorbed (T_{max}=0.5 ± 0.3 h) with a C_{max} of 0.6 ± 0.8 ng/ml, and a terminal elimination half-life of 24 ± 10 h. Pergolide was detectable in the serum for a maximum of 14 days (range: 5-14 d). Plasma ACTH concentration remained unchanged for 6 days compared to ACTH concentration on the last day of treatment. Plasma pergolide concentration and ACTH suppressing effects persist for several days after cessation of long-term pergolide administration in horses with PPID.

Neurologic Signs, Endocrine Testing and Rapid-Slice Contrast Computed Tomography in an 8 Year Old Arabian

N. Rademacher[1], L. Gaschen[1], R. Madrigal[2], D. Duplantis[3], F. Andrews[2]
[1]Section of Diagnostic Imaging and [2]Equine Health Studies Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA and [3]Equine Health Maintenance, Equine Mobile Veterinary Services, Bueche, LA, USA.

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

History and clinical signs were recorded. Complete blood count (CBC) and biochemical panel were performed in house. *Sarcocystis neurona* (SN, EPM) testing was performed at Equine Diagnostic Solutions, Lexington, KY, USA. Overnight dexamethasone suppression (DST) and a Thyroid Releasing Hormone (TRH) stimulation tests were performed one week apart. Plasma cortisol and ACTH were measured at the LADDL Endocrinology Laboratory, Baton Rouge, LA. A CT scan (GE lightspeed 16, GE Healthcare, Milwaukee, WI, USA) was performed under general anesthesia with the horse in dorsal recumbency. Contiguous 5 mm thick slices in soft tissue and bone window algorithms were taken before and after administration of iodinated contrast agent (200 ml, 240 mg/ml, IV: Iopomide Ultravist, Bayer Healthcare, Wayne, NJ, USA). History of progressive (3 months) onset of lethargy, ataxia, head pressing and loss of muscle mass was reported. Clinical signs of low body condition score (4/9), reduced top line, hypertrichosis, ataxia and depression were noted on presentation. CBC was unremarkable and the biochemical panel showed mild hyperglycemia (108 mg/dL). Serology for SN was negative (1:500) for EPM. Baseline cortisol and cortisol 18 hour after administration of dexamethasone (40 µg/kg, IV) was 35.0 ng/mL and 33.1 ng/mL, respectively. ACTH concentration increased from 318 pg/mL at rest to >1,250 pg/mL, 10 and 30 minutes after TRH administration. On rapid-slice CT scan, a rounded, well-defined, smoothly marginated, broad based mass, hyperattenuating (HU 54.3) to surrounding brain parenchyma (HU 32) was noted dorsal to the hypophyseal fossa, measuring 4.6 x 4.6 x 3.8 cm in size. There was focal dorsal displacement of the hypothalamus and focal compression of the third ventricle. There was uniform, moderate enhancement of the mass after contrast administration (**Figure 1)**. An extra-axial pituitary mass consistent with a pituitary macroadenoma was diagnosed.

This is an unusual presentation of a young horse with neurologic signs attributed to a large pituitary adenoma. A rapid-slice CT scan with contrast can help determine the size and extent of the lesion and takes less than 30 minutes to obtain. A large pituitary mass in a young horse with neurologic signs might carry a poor prognosis, as current treatment does not reduce tumor size.

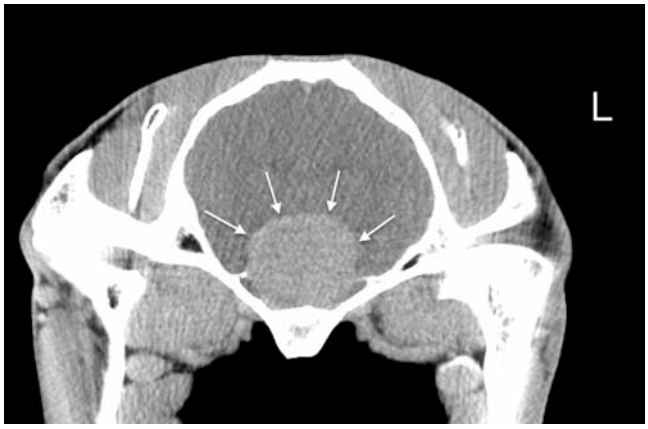


Figure 1. Transverse post contrast computed tomography image at the level of the temporomandibular joints. Note the broad based hyperattenuating, well defined round mass (outlined by white arrows) dorsal to the hypophyseal fossa consistent with pituitary macroadenoma.

Comparison of Magnetic Resonance Imaging and Histological Scores for Assessing Pituitary Pars Intermedia Enlargement in Horses with Pituitary Pars Intermedia Dysfunction

H.C. Schott II,[1] A.P. Pease,[1] J.S. Patterson,[1] D. McFarlane,[2] H. van der Kolk[3]. 1. Michigan State University, 2. Oklahoma State University, 3. Euregio Laboratory Services

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

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

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

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

Acknowledgements: Supported by the MSU Equine Endowment Fund and Boehringer-Ingelheim, Vetmedica, Inc.

Prior presentation: The data has been presented, in part, at the 2nd European Endocrinology Summit (May, 2014) and as a poster presentation at the 2014 ACVIM Forum in Nashville, Tennessee in June, 2014. A manuscript is in preparation for submission to JVIM in the near future.

Factors Associated with PPID in Donkeys Identified During a Nationwide PPID Awareness Initiative

Ireland, J.L.[1], Rendle, D.I.[2], Durham, A.E.[2], Turnbull, C.[3] and Barrett, E.J.[3]
[1]Animal Health Trust, Newmarket, Suffolk, UK; [2]Liphook Equine Hospital, Liphook, Hampshire, UK;
[3]Boehringer Ingelheim Vetmedica, Bracknell, Berkshire, UK

Aims: The aims of this study were to describe the presenting clinical signs and to determine factors associated with PPID in donkeys, during a nationwide laminitis disease awareness campaign in the UK.

Methods: Retrospective analysis of laboratory submissions during a nationwide disease awareness initiative ("Talk about Laminitis") was undertaken. As treatment status was not available for all animals, only the first laboratory assay was included for donkeys with multiple samples. PPID was defined based on ACTH concentration above seasonally adjusted reference ranges (>29pg/ml in non-autumn months and >47pg/ml during autumn). Factors associated with PPID were assessed using Pearson Chi-squared or Fisher's exact tests as appropriate.

Results: In total, 95 donkeys were sampled between 30th July 2012 and 26th November 2013, with a median age of 22.5 years (IQ 17-28 years). Overall, 73.7% of donkeys (95% CI 64.8-82.5%) were PPID-positive, and there was no significant difference in the age of these animals (median 22 years; IQ 17-29 years) compared to PPID-negative animals (median 23 years; IQ 17-26.5 years) (p=0.63). There was a significant association between season of submission and proportion of samples testing positive for PPID based on elevated ACTH: spring 48.5%, summer 66.7%, autumn 96.0% and winter 88.0% (p<0.001).

Median ACTH concentration was 64.4 pg/ml (IQ 29.5-156pg/ml), and there was no significant association between the number of clinical signs consistent with PPID reported and ACTH concentration (p=0.12). Overall, 78.9% of donkeys were reported to exhibit ≥1 clinical sign (median 1; IQ 1-2; range 0-7 signs), with current and/or historical laminitis (40.0%), hypertrichosis (33.7%) and muscle wastage (28.4%) being the most prevalent clinical signs.

No clinical signs, either in isolation or combination, were found to be significantly predictive of PPID based on elevated ACTH. ACTH concentration was greater for donkeys reported to have current and/or historical laminitis (median 87.7pg.ml; IQ 36.2-397.3pg/ml) compared to those with no reported history of laminitis (median 58.1pg.ml; IQ 28.0-108.6pg/ml), however this difference was not statistically significant (p=0.06). Median ACTH did not differ significantly between donkeys reported to exhibit any of the other clinical signs and those without. There were no significant associations between age and the presence or absence of individual clinical signs, however donkeys reported to exhibit both laminitis and hypertrichosis were significantly older (median age 27 years; IQ 22.5-34 years) compared to those without these signs (median age 22 years; IQ 17-27.5 years) (p=0.01).

Conclusions: Three-quarters of donkeys sampled during a 2-year disease awareness initiative were PPID-positive. While the most prevalent clinical signs reported are consistent with presenting signs of PPID in horses and ponies, no clinical signs were identified that were predictive for PPID in donkeys. This suggests that further evaluation of the clinical presentations and use of ACTH in the diagnosis of PPID in donkeys is warranted.

Acknowledgements: This study was funded by Boehringer Ingelheim. We gratefully acknowledge all participating veterinary practices.

Effects of Fasting and the Oral Sugar Test on Thyrotropin-Releasing Hormone Stimulation Test Results in Horses.

M Restifo[1], P Hermida[1], A. Sanchez[2], N Frank[1]. 1. Department of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA. 2. Department of Environmental and Population Health, Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

It is often necessary to test for pituitary pars intermedia dysfunction (PPID) and insulin dysregulation in the same horse. We hypothesized that results of the thyrotropin-releasing hormone (TRH) stimulation test would not be affected by feeding conditions or the oral sugar test (OST).

Thirty adult horses (aged 6 to 21 years of age; 8 mares and 22 geldings) from the same facility were tested on 4 occasions across a 6-week period. All horses underwent a TRH stimulation test under fed (day 1) and then fasted (day 14) conditions, an OST alone (day 28), and then a combined OST/TRH stimulation test (day 42). Test results were compared using the Wilcoxon rank-sum test or paired student's t-test, and agreement was assessed by Bland-Altman analysis.

Fasting or feeding did not affect plasma adrenocorticotropin hormone (ACTH) concentrations at 0 (P= 0.968) or 10 min (P= 0.598) in the TRH stimulation test. Baseline (time 0) ACTH concentrations did not differ (P= 0.167) when TRH stimulation tests were performed alone (fasted) or in combination with the OST. Plasma ACTH concentrations increased after TRH administration in all tests, but were lower at 10 min (P= 0.004) in the combined test, compared to the TRH stimulation test (fasted); with a mean bias of 26 pg/mL (95% confidence interval; -60 to 111 pg/mL) detected.

Feeding conditions did not impact TRH stimulation test results, but ACTH concentrations increased by a lower magnitude when testing was combined with the OST. Combination testing is not recommended at this time.

Understanding Metabolic Variation and Metabolic Syndrome in Horses

Molly McCue[1], DVM, MS, PhD, DACVIM (LAIM), Raymond J. Geor[2], BVSc, PhD, DACVIM (LAIM), DACVSMR; Nichol Schultz[1], DVM

[1]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN, 55108: email: mccu0173@umn.edu , mccar556@umn.edu [2]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, 736 Wilson Road, East Lansing, MI 48824; e-mail: geor@cvm.msu.edu

Defining EMS

Equine Metabolic Syndrome (EMS) refers to a cluster of clinical abnormalities associated with an increased risk of laminitis[1;2]. Johnson[1] recognized that primary features of a laminitis-prone phenotype (i.e. obesity, insulin resistance) were analogous to those described for the metabolic syndrome (MetS) in humans which is a constellation of abnormalities, including obesity, dyslipidemia, glucose intolerance and hypertension, associated with increased risk of cardiovascular disease and perhaps also type 2 diabetes mellitus[3]. The 2010 American College of Veterinary Internal Medicine (ACVIM) large animal consensus statement[2] listed several criteria for EMS based on research data available at the time; however, the features that define EMS are a subject of ongoing debate in the equine veterinary community. Descriptions of the metabolic phenotype of laminitis-prone horses and ponies have varied among published studies[4-6;8], making a unifying phenotypic definition difficult. The lack of consensus between reports may result from unmeasured explanatory variables, insufficient sample size to detect significant effects in the presence of confounding variables, and differences in experimental design including differences in test cohort, breed, time of sample collection (time of day and or season). Lack of consensus also likely reflects the complexity of the 'EMS' phenotype with multiple factors at both individual and environmental levels likely contributing to variation in metabolic traits.

In an effort to address some of the apparent discrepancies between previous reports we have recently completed phenotyping of a large across-breed cohort of horses and ponies representing a range of metabolic phenotypes. We have collected phenotypic, epidemiologic and environmental data from 610 horses/ponies from 166 farms. The predominant breeds sampled were Morgan horses, Tennessee Walking Horses (TWH), Arabs, Quarter Horses (QH) and Welsh ponies. Phenotypes include: measures of generalized and localized obesity (neck- and girth- to height ratio, body condition score), fasting blood glucose (GLU), insulin (INS), ACTH, triglyceride, non-esterified fatty acid, leptin and adiponectin (APN) concentrations, and post oral-sugar test insulin (INS_OST) and glucose. Additional data include medical history, laminitis status, age, breed, and sex, as well as environmental variables including month of sampling, latitude, housing, diet (hay, pasture grain and supplement samples all submitted for complete dietary analysis), exercise, and other management practices.

Multivariate, multilevel, multiple regression analysis was used to evaluate the 11 phenotypic responses and 16 individual (i.e., age, breed, etc) and environmental (i.e., diet, management, exercise) explanatory variables. Although complex, our statistical model allows relationships between explanatory and response variables to be evaluated at 3 levels. Level 1 is the multivariate responses within individual: for example normal physiologic control of glucose homeostasis is a complex system with many regulatory/counter-regulatory factors. Multivariate analysis allows for interpretation of the biochemical traits (i.e., insulin, glucose, triglycerides, adiponectin, etc) together, estimation of the correlation between traits, and identification of perturbations of the normal physiologic response. Level 2 is analogous to a multiple regression for differences between individuals: this level allows for elucidation of the effect of individual predictors (such as age, gender or breed) on metabolic traits. Level 3 accounts for similarities between individuals on the 166 farms: correlations between individuals at the farm level suggest that phenotypes are influenced by the shared environment. This statistical modeling also allows for partitioning of the phenotypic variability that is explained or not explained by the 16 measured predictors into unmeasured individual factors and environmental (farm) factors. From this analysis we have been able to asses several key aspects of EMS including:

1) How measured phenotypes and biochemical traits influence each other, and how these relationships differ in horses that are obese, have a history of laminitis or both;
2) How individual physiologic variables such as age, breed and gender affect trait measures;

3) The influence of environmental variables such as dietary adaptation, housing and exercise influence trait measures;
4) The relative importance of each of these confounding factors; and
5) How the measures differ in horses when pared into clinical groups (ie non-obese/non-laminitic (NO-NL), non-obese/ lamintic (NO-L), obese/non-lamnitic (O-NL), and obese/laminitic (O-L).

We believe that these data will allow for a more comprehensive understanding of all the factors that are in play in the measured metabolic phenotype in EMS 'suspect' horses; and further help define the key measures that are consistently different in horse with a previous history of laminitis in relation to those without. Our data indicate that elevated fasting insulin and triglycerides, elevated insulin 75 minutes post oral sugar challenge (OST insulin) and low serum adiponectin are the four measurements most consistently present in individuals with a history of laminitis, regardless of obesity status, age, gender or breed. Fasting insulin, OST insulin and triglycerides measurements are also higher in obese individuals without a history of laminitis, albeit not at the levels seen in previously laminitic horses.

In addition to differences in obese and/or laminitic horses, we have also identified significant differences in the relevant biochemical measurements between breeds regardless of clinical phenotype, and a large amount of variation between individual horses in phenotypic measures such as resting ACTH even after correction for age, season and other factors. Analysis of this large and comprehensive dataset in a cross-sectional cohort of horses and ponies has highlighted the complexity of the relationships and interactions between measured phenotypes in EMS and the wide range of factors that affect these measures. Despite a comprehensive consensus statement defining EMS, interpretation of phenotypic measures and the assignment of the EMS diagnosis are confounded by multiple factors including obesity (BCS), gender, breed and age as well as environmental factors.

Model of EMS Risk

Environmental factors including excessive nutrition, often from pasture, have been linked to EMS, obesity, insulin resistance and laminitis. Clinical experience with EMS animals suggests that episodes of laminitis often occur in pastured animals coincident with an increase in the pasture forage nonstructural carbohydrate (NSC, which includes sugars, fructans and starches) content, i.e. rapidly growing pastures in the spring, after summer and fall rains, or after frost. Episodes of laminitis in pastured ponies have been associated with exacerbation of hyperinsulinemia[9;10], likely due to the increase in NSC intake although an effect of season per se on insulin sensitivity and dynamics is also possible. However, the high planes of nutrition and/or changes in the pasture do not result in EMS in all horses. Recent studies indicate this individual variability may be due to an underlying genetic predisposition[9;11,12]. We propose that the risk for EMS and laminitis is a complex disease where the risk of developing the disease is a result of an individual's unique combination of genetic alleles, in concert with environmental influences are responsible for disease risk. Precedent for this model also comes from work in other species. Although regulation of insulin, glucose and lipid metabolism in health and disease is complex and not completely understood, work in humans and rodent models[13;14] has identified multiple primary genetic risk alleles that result in alterations in insulin, glucose and/or lipid metabolism. These genes interact in large common pathways and a related group of metabolic phenotypes (obesity, IR, dyslipidemia) that increase the risk of cardiovascular disease and type 2 diabetes mellitus, with the relative importance of a given risk allele varying between populations and ethnic groups.

Anecdotally, certain horse breeds such as Morgans, Arabians, Paso Finos, Tennessee Walking Horses, and most ponies, including the Welsh Pony, are more susceptible to EMS and subsequent laminitis. Other breeds such as the Quarter Horse and Thoroughbred appear to be less susceptible, though objective data in most of these breeds is lacking[1,6,15]. Affected horses and ponies appear to have high metabolic efficiency, meaning they require fewer calories for maintenance of bodyweight when compared to unaffected animals (i.e. they are "easy keepers"). Ponies in general have lower insulin sensitivity[12] and a higher prevalence of hyperinsulinemia when compared to horses[16;17]. Our preliminary data indicate that breeds of horses can share key metabolic features of EMS, such as hyperinsulinemia, exaggerated response to oral glucose, and elevation in serum triglycerides, but breeds can differ in magnitude of these responses or other features, such as fasting insulin, triglyceride, non-esterified fatty acid and adipokine levels. We propose that major genetic risk factors leading to EMS and laminitis susceptibility are shared across breeds, while differences in the severity and secondary features of the

EMS phenotype between breeds, or between individuals within a breed, result from modifying genetic risk factors with variable frequencies.

Identifying the genes and alleles underlying individual variability in the measured hormonal and biochemical measures of EMS susceptibility, and unraveling the complexity of gene by environmental interactions and variable phenotypic expression between breeds, requires a combination of genetic approaches. We have recently completed a genome wide association analysis in 286 Morgan horses for which 11 phenotypic responses and 16 individual (i.e., age, breed, etc) and environmental (i.e., diet, management, exercise) explanatory variables were collected. This genome wide association analysis has identified several genomic regions underlying traits including fasting and post-challenge insulin concentrations, and leptin, adiponectin and triglyceride concentrations.

References

1. Johnson P (2002), The equine metabolic syndrome: peripheral Cushing's syndrome, *Vet.Clin.North Am Equine Pract.* 18: 271-293
2. Frank N, Geor RJ, Bailey SR, Durham AE, Johnson PJ (2010), Equine metabolic syndrome, *J.Vet.Intern.Med.* 24: 467-475
3. Reaven GM (2011), Insulin Resistance: the Link between Obesity and Cardiovascular Disease, *Medical Clinics of North America* 95: 875-892
4. Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB (2006), Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies, *J.Am.Vet.Med.Assoc.* 228: 1538-1545
5. Carter RA, Treiber KH, Geor RJ, Douglass L, Harris PA (2009), Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies, *Equine Vet.J.* 41: 171-178
6. Frank N, Elliott SB, Brandt LE, Keisler DH (2006), Physical characteristics, blood hormone concentrations, and plasma lipid concentrations in obese horses with insulin resistance, *J.Am.Vet.Med.Assoc.* 228: 1383-1390
7. Cartmill JA, Thompson DL, Jr., Storer WA, Gentry LR, Huff NK (2003), Endocrine responses in mares and geldings with high body condition scores grouped by high vs. low resting leptin concentrations, *Journal of Animal Science* 81: 2311-2321
8. Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ (2008), Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis, *Am.J.Vet.Res.* 69: 122-129
9. Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB (2006), Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies, *J.Am.Vet.Med.Assoc.* 228: 1538-1545
10. Carter RA, Treiber KH, Geor RJ, Douglass L, Harris PA (2009), Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies, *Equine Vet.J.* 41: 171-178
11. Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ (2008), Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis, *Am.J.Vet.Res.* 69: 122-129
12. Treiber KH, Kronfeld DS, Geor RJ (2006), Insulin Resistance in Equids: Possible Role in Laminitis, *Journal of Nutrition* 136: 2094S-22098
13. Teran-Garcia M, Bouchard C (2007), Genetics of the metabolic syndrome, *Appl.Physiol Nutr.Metab* 32: 89-114
14. Benyamin B, Sorensen TI, Schousboe K, Fenger M, Visscher PM, Kyvik KO (2007), Are there common genetic and environmental factors behind the endophenotypes associated with the metabolic syndrome?, *Diabetologia* 50: 1880-1888
15. Johnson P, Messer N, Slight S, Wiedmeyer C, Buff P, Ganjam V (2004), Endocrinopathic laminitis in the horse, *Clinical Techniques in Equine Practice* 3: 45-56
16. Pleasant RS, Suagee JK, Thatcher CD, Elvinger F, Geor RJ (2013), Adiposity, Plasma Insulin, Leptin, Lipids, and Oxidative Stress in Mature Light Breed Horses, *Journal of Veterinary Internal Medicine* 27: 576-
17. McGowan CM, Geor RJ, McGowan T. The prevelance and incidence of hyperinsulinemia in ponies. Journal of Veterinary Internal Medicine 22, 734. 2008.

The Effect of Fasting Length on Baseline Blood Glucose, Insulin, Glucose-Insulin Ratio, Oral Sugar Test, and Intravenous Insulin Response Test Values in Horses.

Francois Bertin DVM, Sandra Taylor DVM, PhD, Janice Sojka-Kritchevsky VMD, MS, Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN. 47907

Published descriptions of the oral sugar test (OST) and insulin response tests (IRT) have been inconsistent when specifying the protocol for fasting horses before they are performed. The purpose of this study was to examine the effect of fasting length on blood glucose, insulin, glucose/insulin ratio, OST, and IRT values.

Both OST and IRT were performed on 10 healthy adult horses after fasting for 0, 3, 6, and 12 hours. Thus 8 tests were performed per horse in a randomized order. Blood collected at the initial time point of the OST was analysed for both insulin and glucose so that baseline values and the glucose/insulin ratio could also be determined. Unless being fasted, horses had free-choice access to grass hay. They ate only hay and were not offered concentrate.

There was no effect of fasting times on glucose, insulin, or glucose/insulin ratio. The OST in horses with access to hay was significantly different from those after any length fast. Response to insulin in the IRT was decreased in fasted horses. The effect increased with fasting length, with the least change in blood glucose concentration post insulin administration following a 12-hour fast.

The reason why fasting decreases response to insulin is not known. These data highlight that insulin sensitivity is not a fixed trait in any horse. How long a horse is fasted and the specific test used to examine insulin dynamics will affect the assessment of that animal's ability to metabolize carbohydrates.

Blood Glucose Homeostasis in Donkeys: Intravenous Glucose and Combine Glucose-Insulin Testing.

Francisco J. Mendoza DVM, PhD, Dip. ECEIM; Ramiro E. Toribio* DVM, PhD, Dip. ACVIM; Carlos A. Gonzalez-De Cara DVM; Raul Aguilera-Aguilera DVM; Jose C. Estepa DVM, PhD, Dip. ECEIM; Alejandro Perez-Ecija DVM, PhD.

Department of Animal Medicine and Surgery, University of Cordoba, Campus Rabanales, Ctra. Madrid-Cadiz km 396, 14104, Cordoba, Spain.
[*]Department of Veterinary Clinical Sciences, The Ohio State University, 601 Vernon Tharp Street, 43210, Columbus, OH, USA.

Corresponding author: fjmendoza@uco.es

Similar to horses, donkeys also develop disorders of energy dysregulation. The intravenous glucose tolerance test (IGTT) and combined glucose-insulin test (CGIT) have not been evaluated in donkeys. Whether these dynamic tests could be interpreted in a similar way in donkeys is unknown. The aim was to characterize the IGTT and CGIT in adult donkeys. Ten healthy donkeys (7.1±0.8 yo) were fasted overnight and the CGIT and IGTT were carried out as described for horses. Parameters calculated included positive phase duration (PPD), positive phase glucose clearance rate (PGCR), time to nadir (TN), negative phase duration (NPD), negative phase glucose clearance rate (NGCR), glucose area under curve (AUCg) and insulin area under curve (AUCi). Insulin concentrations were determined by radioimmunoassay. Compared to horses, the CGIT and IGTT were right-shifted; PPD, TN, NPD, AUCg, and AUCi were higher, while PGCR and NGCR were lower in donkeys. Results of this study indicate that donkeys have unique glucose/insulin dynamics that appear to be different from horses. Therefore, caution should be taken when extrapolating data from horses to donkeys. This is the first study to investigate glucose homeostasis via CGIT and IGTT in donkeys.

Lipid Profiling in Laminitic, Obese, and Healthy Horses

M Coleman, N Cohen, R Walzem, J Sucholdolski, T Minamoto, A Kieffer, J Mawyer, J Belknap*, L Bramlage*, S Eades*, B Fraley*, H Galantino-Homer*, R Geor*, R Hunt*, M McCue*, W McIlwraith*, R Moore*, J Peroni*, H Townsend*, N White*

Lipoproteins are water-miscible macromolecules enabling transport of lipid in blood. In humans, altered proportions of lipoprotein classes are used to detect and classify various metabolic diseases. Metabolic disorders of insulin dysregulation and obesity are recognized in horses and are particularly concerning because of their putative association with laminitis. The objective of this pilot study was to compare lipoprotein profiles between horses with laminitis (N=9) and non-laminitic horses (N=11). Blood was collected from laminitis horses within 4 weeks of the first known episode of laminitis; control horses were healthy horses from different ranches/farms/stables with no history of laminitis. Body condition score was recorded for all horses. Lipoprotein profiling, including evaluation of triglyceride-rich lipoprotein (TRL), low density lipoproteins (LDL), and high density lipoproteins (HDL) was performed using a bismuth sodium ethylenediaminetetraacetic acid density gradient ultracentrifugation method. A significant ($p < 0.05$) difference was observed between laminitic and non-laminitic horses for HDL and total lipoprotein fractions. All but 1 laminitic horses was obese whereas only 2 non-laminitic horses were obese, and the HDL and total lipoprotein were significantly ($p < 0.05$) greater for obese horses than for non-obese horses. Based on this pilot study, lipid profiling for approximately 200 horses is currently underway in which 4 groups of horses will be considered: 1) non-obese, non-laminitis (NO-NL); 2) non-obese, laminitis (NO-L); 3) obese, non-laminitic (O-NL); and, 4) obese, laminitic (O-L). Results of this larger study will be available at the time of presentation. (239 words)

What We Learned from the First Havemeyer Geriatric Workshop

Mary Rose Paradis DVM, MS Diplomate ACVIM(LAIM)

The first Dorothy R Havemeyer Foundation Geriatric Workshop took place in Cambridge Massachusetts October 24 – 27, 2010. It was conceived as a chance to gather clinicians and scientists who had an interest in the health and welfare of the geriatric horse. Topics that were presented included a spectrum of talks on how aging may affect different body systems – endocrine, immune, respiratory, cardiovascular, and nutrition. The demographics and diseases of older horses were explored and compared from Australia, the United Kingdom and the United States.

In the wrap up session, discussions centered on defining "aged" in the horse, what criteria do you use to define the healthy horse vs diseased horses in equine aging studies? How do you separate normal aging changes from disease symptoms? What recommendations can we make to the horse owner for health management (preventive and therapeutic) of the older horse? What are the gaps in our knowledge?

In trying to define "aged" in the horse, many ideas were put forth. Owners generally use a combination of chronological age and phenotypical age to classify their animals as old. In a survey of owners, a horse was considered old at 22 year of age but signs of aging of their horses began around 23 years. Aged can also be define through a decline in physiologic functions. Walker, Arent, McKeever demonstrated that maximal aerobic capacity in the horse declined significantly between 18-20 years of age in the horse. McKeever et al also found that an older group of horses (mean age 26 years of age) had a compromised ability to handle exercise and thermoregulation in part due to decreased pre-exercise plasma volume. Christmann found that ageing in the horse resulted in decreased surfactant phospholipid in BAL fluid. Immunosenescence was discussed by Horohov, Adams and McFarlane as a physiologic change as horses aged.

McGowan and Ireland reported that there was a high prevalence of health problems in the older horse and these conditions increased with advancing age. It was proposed by the attendees that perhaps the use of chronologic age combined with a "Is your horse aging well?" score card would be a way to define old age in the horse. Several suggestions of parameters to look at on a "score card " included full clinical assessment including cardiac murmurs, osteoarthritis, PPID, activity level, body condition scoring, medical history, preventive care and current medications. Practitioners could use this to identify developing problems early in the aging process. This type of ongoing assessment may also be helpful in the geriatric research arena especially when trying to define what a healthy geriatric horse control is.

Finally - Gaps in our knowledge of the older horse were identified. Here are a few. The items in bold were reported on at this meeting.

- Nutrition
- **PPID diagnostics**
- **Appropriate dose of pergolide**
- vaccination protocols
- Look for early diagnostic markers of aging or disease prediction. Inflammatory cytokine production from serum samples
- Identify early clinical markers for "unsuccessful aging"
- Epidemiologic studies to compare horses that "age well" versus "unsuccessful aging". Look at management changes in a horse's early life that may predict or prevent unsuccessful aging.
- Development of the aging score card that can be used by practitioners and researchers alike.

The question for the group is, "What are our next steps?"

Interleukin-6: A Predictor of Metabolic Health Status in Geriatric Horses?

A.A. Adams[1], J. L. Brogie[1*], L. Costa[2], D.W. Horohov[1], D. McFarlane [3]& M.R. Paradis[2]. [1]Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY. [2]Dept of Clinical Sciences, Large Animal Medicine, Tufts University, Cummings School of Veterinary Medicine, North Grafton, MA. [3] Dept of Physiological Sciences, CVHS-OK State

Horses over 20 years of age constitute about 15% or more of the equine population, and yet like the elderly population this number is ever increasing. Many of these aged horses remain actively involved in equestrian sports and reproductive capacities as stallions and brood mares. Advanced age in horses, as with other species, is eventually associated with a decline in body condition, muscle tone and immune function. A hallmark characteristic of the aged immune system is an increased production of pro-inflammatory cytokines, termed "inflamm-aging". In fact, older humans have several-fold increases in plasma/serum levels of inflammatory cytokines, in particular interleukin-6 (IL-6). This increase in inflammatory mediators predicts both increased mortality and morbidity in a variety of chronic diseases in humans.

We have shown that horses 20 years of age and older exhibit similar "inflamm-aging" characteristics, however it remains to be determined if there is an age-related increase in serum levels of pro-inflammatory cytokine IL-6. Further, it remains to be determined if increased IL-6 protein levels correlate with age-related health conditions of the horse such as arthritis, equine Cushing's disease, hyperinsulinemia, etc.

Therefore, the purpose of this study was to determine if serum IL-6 protein levels increase with age of the horse and/or age-related health conditions of the horse. Blood (serum) samples were collected from the field setting from 109 horses of mixed breed and sex, ranging in age from 6-36 years (6-10 yrs; n=19), (11-19; n=28), and (20-36; n=62). IL-6 protein levels in the serum were measured using an equine-specific, enzyme-linked immunosorbent assay (ELISA). Blood was also analyzed for metabolic parameters including MSH, ACTH, fasting glucose and insulin. Health status data was collected from a survey (age, breed, sex, nutrition, exercise, body scoring, weight tape, medical history) and farm visit (body scoring, ultrasound fat thickness over various body regions, neck adiposity scoring, measurements for weight calculation, feed measurement, hay analysis). However for the purposes of this study we focused on BCS.

Data were analyzed using SigmaPlot10.0. Data not normally distributed were log10 transformed. Multiple linear regressions followed by backward stepwise simple linear regression were performed to assess the effect of age and serum IL-6 on BCS and endocrine parameters of a-MSH, ACTH, fasting insulin and glucose. A t-test was performed to determine the effect of age on serum IL-6. A 'P' value of less than or equal to 0.05 was considered significant. Results indicated that there is a significant ($P=0.030$) increase of serum IL-6 concentrations in geriatric horses (>20 yrs) compared to young horses (<10 yrs). However, within the group of geriatric horses (>20 yrs) there was not a significant linear relationship between increasing age and serum levels of IL-6 ($R=0.0781$, $P=0.546$). There were no significant ($P>0.05$) relationships between age (6-36 yrs) and serum IL-6, a-MSH, ACTH, fasting insulin or glucose. BCS was the only significant relationship found with age in this study resulting in decreasing body condition with old age ($R=0.324$, $P=0.002$). Further, results from this study indicated that there were no significant ($P>0.05$) relationships between serum IL-6 concentrations with a-MSH, ACTH, BCS, fasting insulin or glucose. Thus, conclusions from this study indicate IL-6 plays a role in characterizing the inflamm-aging response in geriatric horses however serum IL-6 does not appear to play a role in predicting endocrine dysregulations in the geriatric horse.

Comparison of Inflammation, Nutritional Status, Muscle Mass, Pituitary Function, and Age in Geriatric Horses.

MH Siard[1], A Betancourt[1], PA Harris[2], AD Moffett[3], KE McMurry[1], SE Reedy[1], DW Horohov[1], AA Adams[1]. [1]Gluck Equine Research Center, University of Kentucky, Lexington, KY. [2]WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Melton Mowbray, LE14 4RT, UK. [3] MARS Horsecare US, Inc., Dalton, OH.

Pituitary pars intermedia dysfunction (PPID, also known as equine Cushing's disease) is an endocrinopathy commonly associated with aging in the equine population. Inflamm-aging (systemic low-grade chronic inflammation) also occurs with aging. Little is currently known about whether the inflammation or nutritional status of geriatric horses may be associated with the occurrence of PPID. Sarcopenia frequently accompanies PPID, thus muscle mass is also of interest. To determine whether inflamm-aging, nutritional status, muscle mass, and PPID status may be correlated, various measures of these parameters were compared in geriatric horses.

Forty-three old horses (mean 24.4±3.0 yrs) were used to measure immune, endocrine, muscle, vitamin and mineral, as well as fatty acid parameters. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood, purified, antibody-stained intracellularly, and analyzed via flow cytometry to determine lymphocyte production of interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα). RNA was also isolated from PBMCs and underwent polymerase chain reactions to determine real quotient (RQ) values for various inflammatory cytokines. Serum interleukin-6, C-reactive protein, and TNFα levels were determined via enzyme-linked immunosorbent assays (ELISAs). Serum vitamin, fatty acid, and mineral levels were also measured. Muscle and fat mass were determined via ultrasound. Thryotropin releasing hormone (TRH) stimulation testing was performed to determine PPID status, in which adrenocorticotropin hormone (ACTH) levels were measured in plasma pre and 10 minutes post (T-10) intravenous administration of TRH (1mg/mL saline/horse).

Pearson correlation testing was performed to determine correlations between various parameters. Baseline ACTH and T-10 ($R=0.631$; $p<0.001$) yielded a strongly significant relationship. Age and T-10 ACTH ($R=0.256$; $p=0.0973$) exhibited a trend, while age and basal ACTH ($R=0.389$; $p=0.0109$) were significantly correlated. ACTH was not significantly correlated with muscle, fatty acid, vitamin, or mineral measures. Inflammatory markers also did not appear correlated with ACTH, thus PPID and inflamm-aging may not be associated. Age, however, exhibited a positive correlation with fatty acids C18:2n6c, C20:4n6c, and C24:1n9c, as well as with lymphocyte production of IFNγ and TNFα, and serum selenium and vitamin E ($p<0.05$). Age showed a negative correlation with fatty acids C16:0 and C20:1n9c, fat free mass, fat weight, and muscle score ($p<0.05$). The correlation of age with various measured parameters indicates that age plays a role in regulating inflammatory and metabolic function of the horse.

HSP70 and HSP90 in Whole Blood and Skeletal Muscle in Young and Aged Standardbred Mares

R. C. Avenatti[1], K. Malinowski[1], D.W. Horohov[2] and K. H. McKeever[1]
[1]Rutgers Equine Science Center, Rutgers, the State University of New Jersey and [2]Gluck Equine Research Center, University of Kentucky

Approximately 15% of the equine population in the United States is over the age of 20 years and many horses continue to participate in athletic activities. Understanding the molecular mechanisms behind the adaptive response to exercise will aid in the development of strategies to preserve the health and well-being of this socio-economically important species. Heat shock proteins (HSPs) are important mediators of cellular response to disturbances in homeostasis, but little work has been done to investigate HSPs in horses. We hypothesized that *HSP* expression in whole blood and skeletal muscle are altered by age and acute exercise. Young (n=6; 5.5 ± 2.8 years) and aged (n=6; 22.6 ± 2.25 years) Standardbred mares underwent an acute submaximal exercise test until fatigue. Whole blood and gluteus medius biopsy samples were collected and analyzed for *HSP70* and *HSP90* expression via RT-PCR. Young and aged horses had increased *HSP70* expression in whole blood following acute exercise, with young horses exhibiting 3-fold greater *HSP70* expression than aged mares at 2 hours post-exercise. *HSP90* expression in whole blood was increased only in young horses. Both young and aged horses had increased *HSP70* and *HSP90* expression in skeletal muscle following exercise, but there was no difference due to age. In conclusion, the magnitude and timing of the HSP expression following acute submaximal exercise is altered by age in horses. Quantification of HSP expression in whole blood may be a useful biomarker, with implications for cellular adaptation and survival in aged horses.

Key Words: Horses, Aging, Exercise

Transcription Regulation of Skeletal Muscle Proteolysis in Underweight Aged Horses

HE Banse, D McFarlane

Loss of muscle mass is commonly observed in aged horses. Furthermore, equine aging is associated with decreased aerobic capacity and a shift in myofiber type. Despite the implications of these changes in skeletal muscle metabolism to performance, there are limited investigations into mechanisms of loss of muscle mass in the aged horse. Our hypothesis was that aged underweight horses had increased skeletal muscle proteolysis compared to aged control horses. For this study, our specific aim was to evaluate transcriptional regulation of protein clearance and protein synthesis.

Fourteen aged horses (≥18 years) were included. Horses were grouped by body condition score (underweight, BCS≤3; ideal, BCS 4-6). Expression of genes controlling protein clearance, including regulators of the autophagy-lysosomal pathway (microtubule-associated light chain-3 [LC3], Bcl2/adenovirus E1B 19kD-interacting protein 3 [Bnip3]) and regulators of the ubiquitin proteasome pathway (atrogin 1, Muscle RING Finger 1), were evaluated. In addition, expression of myostatin was assessed as an inhibitor of protein synthesis. Following log transformation of gene expression results, data were normally distributed. Groups were compared using a t-test. Among the markers of proteolysis, increased expression of atrogin-1 (underweight, 3214±2187 units; ideal, 270±233, p=0.0021) muscle RING finger 1 (2005±1628; ideal, 234±222, p=0.009), and LC3 (393±288, ideal 50±38, p=0.02) was identified. No significant differences in Bnip3 or myostatin expression were observed.

These findings suggest that increased proteolysis may contribute to loss of skeletal muscle in underweight aged horses. Analysis of the enzymatic degradation pathway of proteolysis and activity of regulators of autophagy and ubiquitin-mediated protein clearance are ongoing.

Activation of the Signaling Pathways Regulating Muscle Protein Synthesis and Degradation in Aged Horses

K.L. Urschel[1], A.A. Adams[2], L.M. Mastro[1]

Muscle mass is largely determined by the balance between rates of muscle protein synthesis and degradation. The objective of this research was to determine the effects of feeding and insulin infusion on the activation and/or abundance of proteins in the signaling pathways regulating muscle protein synthesis (Akt, AMPK, 4E-BP1, rpS6) and degradation (FoxO, atrogin-1 and MuRF1) in two groups of aged horses: those with pars pituitary intermedia dysfunction (PPID: n = 6, 25.0 ± 2.5 y) and healthy, age matched controls (Control: n = 6, 25.7 ± 2.0 y). Three gluteus medius muscle biopsies were collected: 90 min after eating, at baseline insulin concentrations (Basal) and following a 2-h isoglycemic, hyperinsulinemic clamp (INS). The abundance and/or activation of protein factors of interest were studied using Western immunoblot analysis. There were no differences in the activation or abundance of any of the protein factors studied between the Control and PPID in response to feeding or insulin infusion ($P > 0.05$); however, in both groups, there was a significant increase in the activation of Akt, rpS6 and 4E-BP1 in the INS state compared to basal ($P < 0.05$). Therefore, in the group of aged horses studied, regardless of PPID status, there was an increase in the activation of the positive regulators of muscle protein synthesis in response to insulin infusion. Additional research using younger horses, in addition to aged horses, is needed to determine whether there is an age related decline in the responsiveness of these pathways to insulin and feeding.

Funding: partially funded by the Morris Animal Foundation First Award Grant (D09EQ-310)

Age-Effects on Blood Gas, Spirometry, Airway Reactivity, and Bronchoalveolar Lavage Fluid Cytology in Clinically Healthy Horses.

Ana P. Pacheco, Mary R. Paradis, Andy M. Hoffman, Pilar Hermida, Alfredo Sanchez, Jenifer A. Nadeau, Marc Tufts, and Melissa R. Mazan

Background: Despite the increasing number of geriatric horses attended by veterinarians, there is a lack of understanding of the aging-related changes on the respiratory system of horses.

Objective: To identify aging-related changes on the respiratory function and on the bronchoalveolar lavage fluid (BALF) cytology of horses.

Animals: 15 healthy young adult (2-11 years) and 16 healthy aged (≥ 20 years) horses.

Methods: The respiratory system was examined by measurement of arterial blood gases (ABG), use of respiratory inductive plethysmography (RIP) for assessment of breathing pattern and ventilatory parameters, histamine bronchoprovocation and BALF cytology.

Results: No significant differences were detected with in regards to the values obtained by ABG or bronchoprovocation of young adult and aged healthy horses. In aged horses, there were significant differences in mean ± SD of the following parameters when compared to young horses: prolonged expiratory time (Te) measured by RIP (3.9 ± 1.5 s vs 3.0 ± 0.6 s), a decreased percentage of alveolar macrophages (40.6 ± 11.3% vs. 53.5 ± 9.6%) and an increased percentage of lymphocytes (53.4 ± 9.5% vs. 43.9 ± 11.0%). No correlations between airway reactivity and ventilatory parameters, ABG or BALF cytology were found in this asymptomatic population.

Conclusions: These results suggest that aging does not cause a changes in the results obtained by ABG, most RIP-derived variables and bronchoprovocation in the horse. A decreased percentage of macrophage and an increased percentage of lymphocytes in the BALF cytology may be expected in the asymptomatic geriatric horse and may be a result of aging.

Prevalence of and Risk Factors for Recurrent Airway Obstruction in Geriatric Horses and Ponies

Ireland, J.L.[1], Christley, R.M.[2], McGowan, C.M.[2], Clegg, P.D.[2], Newton, J.R.[1], and Pinchbeck, G.L.[2]

[1]Animal Health Trust, Lanwades Park, Kentford, Newmarket, CB8 7UU.
[2]School of Veterinary Science, University of Liverpool, Leahurst Campus, Neston, Wirral, CH64 7TE.

Presenting Author Contact details:
Telephone Number: 01638 751000
Fax Number: 01638 555659
Email Address: jo.ireland@aht.org.uk

Reasons for performing study: Data from our previous Leahurst Equine Geriatric Health Study indicates that respiratory disease is a common cause of morbidity in geriatric horses, with a high prevalence of respiratory clinical signs, which increases with increasing horse age. However, there is often no known cause of these owner-reported signs as they are frequently not attributed to disease and owners may fail to seek veterinary attention.

Aims: To estimate the prevalence of recurrent airway obstruction (RAO) in the British geriatric equine population using a risk-screening questionnaire (RSQ) and to identify factors associated with RAO.

Methods: Owners of horses/ponies enrolled in a previous geriatric cohort study were sent a postal questionnaire, which combined a previously validated RSQ with additional questions regarding management, preventive healthcare, and owner-reported respiratory-specific clinical signs. An overall RSQ score of >0.87 was the positive cut-off for RAO.

Results: A useable response rate of 43.1% was obtained, providing data for 285 horses/ponies (median age 23.3 years). Coughing, at any time in the past (50.5%), or within the preceding year (27.0%), was the most prevalent owner-reported clinical sign. Among the horses/ponies included in the study, the apparent prevalence of RAO using the RSQ was 20.7%. While 10.5% of horses/ponies were reported to have veterinary-diagnosed RAO (median age at diagnosis 13 years), only 2.5% were reported to have RAO as a current known disease/disorder. Of the 30 animals with veterinary-diagnosed RAO, 33.3% had a positive RSQ score, and 16.7% had received some form of veterinary-prescribed medication for the condition within the previous year. Age was not significantly different between RAO-positive (median 22.8 years) and RAO-negative (median 23.3 years) animals based on RSQ ($p=0.24$). Significantly greater proportions of animals with a positive RSQ score exhibited respiratory clinical signs in November – March ($p<0.001$). A veterinary diagnosis of summer pasture-associated obstructive pulmonary disease was reported in 2.8% of animals (median age at diagnosis 15 years), of which 62.5% were also reported to have RAO.

Conclusions: The prevalence of RAO as determined by RSQ score was considerably higher than the proportion of animals previously diagnosed with RAO. Together with the high prevalence of owner-reported respiratory clinical signs, this suggests there are under-diagnosed respiratory problems in the geriatric population, much of which may be unrecognised or undiagnosed RAO.

Ethical Considerations: This study was granted institutional ethical approval from the University of Liverpool and the Animal Health Trust.

Acknowledgements: The original cohort was generously funded by the Horse Trust and this study was funded by the Animal Health Trust.

Comparison of Anesthetic and Recovery Risk in Geriatric Versus Non-Geriatric Equine Patients (2004-2010).

Kirstin A Bubeck, Mary-Rose Paradis, and Lois A Wetmore
Department of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University.

To compare anesthetic risk of geriatric with non-geriatric horses, records of geriatric patients (20 years and older) were matched with records of horses younger than 17 years old with similar disease and similar anesthesia duration. The following parameter were analyzed: anesthesia complications, interventions during anesthesia, survival to recovery, recovery quality, recovery time post general anesthesia, and survival to discharge.

Records of a total of 194 horses were evaluated. Group 1 consisted of 99 geriatric horses with an average age of 22.66 ± 2.3 years and Group 2 consisted of 95 non-geriatric horses with an average age of 9 ± 3.65 years. 60 geriatric horses had celiotomy, and 38 horses survived to recovery. 63 non-geriatric horses had celiotomy, and 40 survived to recovery. In addition, 39 geriatric and 32 non-geriatric horses had routine surgical procedures. Anesthetic complication were hypotension and hypoxemia. Average recovery time for geriatric and non-geriatric patients was 76 ± 36 min and 69 ± 25 min respectively. There was no significant difference ($p=0.22$) between the duration of recovery between the geriatric and the non-geriatric group. Recovery was considered smooth in 46/77 geriatric and 48/72 non-geriatric patients. Complications during recovery were upper airway obstructions in three geriatric horses. All horses undergoing general anesthesia for routine surgery survived to discharge. 34/38 geriatric and 38/40 non-geriatric horses after colic surgery did survive to discharge from the hospital.

Thyroid Hormone Concentrations in Aging Horses

Babetta A. Breuhaus, DVM PhD DACVIM North Carolina State University, Raleigh, NC

Changes in circulating thyroid hormone (TH) concentrations with aging have been noted in other species. THs decrease with age in healthy dogs and cats, although they tend to remain within established reference ranges. In healthy elderly people thyrotropin (TSH) is increased, with or without mild alterations in THs. Use of an age-based reference range for TSH concentrations in people has been suggested to prevent erroneous diagnosis of subclinical hypothyroidism. Neonatal horses are born with very high circulating concentrations of TH that gradually decrease to the adult reference range over the first few months of life. However, little attention has been given to TH concentrations in geriatric horses. Data from 71 normal, healthy horses that had participated in various prior research projects were examined, and found to contain 42 horses 3-10 years of age and 10 horses $\geq$ 20 years. Resting THs were not significantly different between the two groups when compared with the Mann Whitney Rank Sum test. However, TSH concentration was significantly higher in older horses (median 0.52 ng/ml) compared to younger horses (0.33 ng/ml), $p=0.012$. TRH stimulation tests had been performed in 19 of the younger horses and 7 of the older horses. Two-way repeated measures ANOVA on ranks revealed no significant differences in TH responses to TRH. The TSH response to TRH appeared to be slightly greater in the older horses over the first hour, but the overall p value for the entire response was 0.058. Thyroid function in aged horses may be similar to elderly humans.

Aging Alters Thermoregulation and Cardiovascular Function in Standardbred Horses

K.H. McKeever, PhD, FACSM, R.C. Avenatti, PhD, and K. Malinowski, PhD

Equine Science Center, Department of Animal Sciences, Rutgers - the State University of New Jersey, New Brunswick, NJ 08901

Older horses have an increased risk for hyperthermia due to impaired cardiovascular function. While many studies have investigated thermoregulation in horses during exercise, only a few performed at Rutgers have investigated the effects of aging. This presentation focuses on data from a series of studies that were performed to test the hypothesis that aging alters thermoregulation, cardiovascular function and plasma volume during acute exercise and following exercise training.

In the first experiment it was demonstrated that aging compromises the ability of the cardiovascular system to handle the combined demand of exercise and thermoregulation. Paradoxically, young and old horses had the same percentage decrease in plasma volume and total body water. We hypothesized that the old horses started exercise with a lower reserve of fluid and performed a second experiment that demonstrated that much of the decline in thermoregulatory and cardiovascular functional capacity in the older horse was associated with a substantially smaller absolute pre-exercise plasma volume. Such a decline would affect cardiovascular stability as well as thermoregulatory stability during acute exertion.

A third experiment was performed to examine if aging affects the adaptive response to exercise training. More specifically, the study examined the effect of aging and training on resting, maximal, and intrinsic heart rate as well as the adaptive hypervolemia associated with exercise training. Thirteen, healthy, unfit Standardbred mares (young = 12 $\pm$ 1 yr; mean $\pm$ SE, n=8) and old (old = 22 $\pm$ 1 yr, n=5) were used to test the hypothesis that there would be age and training related differences in resting heart rate (RHR), intrinsic heart rate (IHR), maximal heart rate (HR_{max}) and plasma volume (PV). Training resulted in an expansion of PV in young horses but not the old horses. This lack of an adaptive training induced hypervolemia may be due to previously reported aging related differences in the endocrine response to acute exercise in horses. The decrease in RHR and IHR in the young horses following training appears to be related to enhanced pre-load associated with a training–induced hypervolemia as well as changes in autonomic function. The lack of a change in either in the old horses may have functional significance when it comes to the management of the older equine athlete.

Evaluation of Hypercoagulability in Horses with Pituitary Pars Intermedia Dysfunction (PPID)

J Mahon, MR Paradis, D McFarlane, A Sanchez[3], LL Price, E Rozanski, D Meola, and AM de Laforcade

Pituitary Pars Intermedia Dysfunction (PPID) is the most common endocrine disorder in geriatric horses, and share the characteristic of increased ACTH with human and canine pituitary dependent hyperadrenocorticism (PDH). PDH and aging (in humans) has been associated with evidence of systemic hypercoagulability. The goal of this study was to determine if coagulation status differed between normal young horses, and geriatric horses with and without PPID.

Healthy young (<10 years of age) and geriatric (> 20 years of age) horses and PPID geriatric horses were recruited between May 15th and June 15th in 2010 and 2011. Horses were categorized as PPID by the presence of hypertrichosis, and if ACTH > 50 pg/mL and α-MSH >35 pmol/dL. Horses were classified as normal based upon phenotype and an ACTH <35 pg/ml and α-MSH of <20 pmol/dl. Kaolin-activated thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen (Fib C) were performed on all horses. T-tests were used to compare normally distributed data and Mann-Whitney for non-normally distributed data, with a $p < 0.05$ considered significant.

Forty-four horses were screened and 12 were categorized as normal young horses, 8 as normal geriatric horses, and 13 as geriatric PPID horses. Eleven horses had equivocal results and were not further evaluated. There were no significant differences in any TEG variable, fibrinogen or aPTT between young and geriatric horses, or healthy and PPID geriatric horses. The PT in PPID horses was longer than healthy geriatrics (18 ± 1.6 seconds versus 16.7 ±0.7 seconds; $p=0.03$) but not clinically significant. By study design, ACTH and α-MSH were different between groups ($p<0.001$). PPID geriatrics were older ($p=0.02$) than healthy geriatric horses. Older horses and horses with PPID do not appear to develop systemic evidence of hypercoagulability as evaluated by laboratory testing.

Tear Cortisol Concentration in Horses and Ponies with Pituitary Pars Intermedia Dysfunction

KM Kitchings 1, KE Myrna 1a, NA Norton 1b, KA Hart 1b) College of Veterinary Medicine, University of Georgia; 1; Department of Small Animal Medicine and Surgery 1a; Department of Large Animal Medicine and Surgery1b

Purpose: To compare tear cortisol concentrations in horses with Pituitary Pars Intermedia Dysfunction (PPID) and unaffected animals.

Methods: 11 client-owned horses with PPID and 11 healthy control horses were used. No animals had historical or active ophthalmic disease. Tears were collected from the medial conjunctival fornix with a glass capillary tube and blood was collected via jugular venipuncture. Serum total cortisol concentration (serum cortisol) was measured with a previously validated chemiluminescent immunoassay and tear cortisol concentration (tear cortisol) determined using an ELISA assay validated in equine tears for the purpose of this study. Serum and tear cortisol were compared between PPID and control horses with Mann-Whitney tests, and tear and serum cortisol correlated using Spearman rank correlation analysis.

Results: Peak serum cortisol in PPID horses was within laboratory reference ranges and tended to be lower than in control horses (P=0.07). Peak and median tear cortisol in PPID horses were significantly higher than in control horses (P=0.022, P=0.049 respectively). Tear cortisol did not correlate significantly with serum cortisol in PPID horses (r=0.281, P=0.118), but approached significant correlation in control horses (r=0.606, P=0.052). Tear cortisol was not significantly different between horses being treated for PPID with pergolide (n=6) and untreated PPID horses (n=5, P=0.13).

Conclusion: Horses with naturally occurring PPID have higher tear cortisol than unaffected horses, despite comparable or lower serum cortisol. Further study is needed to determine if increased endogenous cortisol in the tear film contributes to delayed corneal wound healing in horses with PPID.