PPID and Equine Metabolic Syndrome Review & Literature Update

Toby Pinn-Woodcock, DVM, DACVIM
Veterinary Support Services
Cornell Animal Health Diagnostic Center
Large Animal Internal Medicine Clinician
Cornell College of Veterinary Medicine
Pituitary Pars Intermedia Dysfunction (PPID)
PPID Anatomy and Physiology

- Melanotropes of pars intermedia produce the precursor protein proopiomelanocortin (POMC)

- Pars intermedia converts POMC to:
  - ACTH (also produced by corticotropes in pars distalis)
  - α-MSH
  - CLIP
  - β-end

Function of POMC-derived peptides

- ACTH
  - Stimulates cortisol release by adrenal gland
- α-MSH
  - Coat/skin pigmentation
  - Regulator of energy homeostasis
  - Anti-inflammatory by regulating cytokine response
- β-end
  - Endogenous opioid providing analgesia and behavioral modification
- CLIP
  - Not well understood

PPID Anatomy and Physiology

• Melanotropes are stimulated to produce POMCs by thyrotropin-releasing hormone (TRH) originating from the hypothalamus

• Periventricular neuron end terminals that originate from the hypothalamus transmit inhibitory dopamine to the pars intermedia, regulating the production of POMCs

PPID Pathophysiology

1. Degradation of functional dopaminergic periventricular neurons due to oxidative damage
2. Decreased dopamine inhibition to melanotropes in pars intermedia
3. Hypertrophy, hyperplasia and micro- and macroadenoma formation of pars intermedia
4. Compression of nearby pituitary lobes and hypothalamus with loss of function
5. Pars intermedia loss of regulation results in increased POMC peptide secretion


PPID Clinical Signs

- **Laminitis due to insulin dysregulation**
- Hypertrichosis (hirsuitism)
- Muscle atrophy
- Regional fat accumulation
- Polydipsia and Polyuria
- Sweating dysregulation
- Secondary infection
- Lethargy
- Infertility
- Persistent Lactation
- Exercise Intolerance

PPID Testing Strategies

**Baseline ACTH**
- Sensitivity 70%, Specificity 80%

- Seasonal rise in ACTH occurs in fall (August-October) in North America

- Seasonal rise reference intervals for United States by latitudinal zone do not exist...are the needed?

  - **Objective**: The goal of this study was to determine whether seasonal variation in plasma ACTH concentrations of healthy horses is affected by latitude in the United States.
Effects of Latitude, Age and Season on Equine Adrenocorticotropic Hormone Concentrations in the United States

Study Population
- Healthy horses >5 years of age
- Location: United States between latitude 25°-45°N

Data Collection
- Study conducted between June, 2019 and May, 2020
- EDTA plasma
  - Collected once monthly during non-fall (December – June)
  - Collected twice monthly during fall (July – November)

<table>
<thead>
<tr>
<th>Location</th>
<th>Initial study population (n)</th>
<th>Horses remaining after outlier analysis (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Latitude</td>
<td>57</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>New York</td>
<td>31</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>13</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Minnesota</td>
<td>13</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Central Latitude</td>
<td>102</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>California</td>
<td>35</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Colorado</td>
<td>24</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>43</td>
<td>33</td>
<td>78</td>
</tr>
<tr>
<td>Southern Latitude</td>
<td>38</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Florida</td>
<td>27</td>
<td>22</td>
<td>82</td>
</tr>
<tr>
<td>North Carolina</td>
<td>8</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>Arizona</td>
<td>52</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1. (left). Line plot representing ACTH upper reference limits of apparently healthy horses at three different latitude locations. The upper error bar value for August 1−15 in the southern latitude is 626.5 pg/mL.

Figure 2. (left) Line plot representing the photoperiods (minutes of daylight per day) during the course of the study from June 2019 to May 2020 for northern, central and southern latitudes in the United States. Data were obtained from NOAA’s Global Monitoring Laboratory.
Effects of Latitude, Age and Season on Equine Adrenocorticotropic Hormone Concentrations in the United States

Figure 3. (Above) Line plots representing ACTH upper reference limits generated from Box-Cox-transformed data of apparently healthy horses from two age categories using the bootstrapped robust parametric method

Summary and Conclusions
• Fall rise in ACTH occurred in all latitude groups (Fig. 1).
• The fall rise in ACTH corresponds to the rapidly decreasing photoperiod, which is most pronounced between late July and early October (Fig. 2).
• Healthy horses >15 years of age with no clinical signs of PPID have higher ACTH in fall than apparently healthy younger horses (Fig. 3).

Individual horses in this study demonstrated considerable variability in ACTH during both fall and non-fall seasons, which suggests a single baseline ACTH determination at any time of year has limited diagnostic sensitivity for PPID.

Storage of ACTH Samples

• Spin down and separate EDTA plasma within 4hrs of collection
  • If left on cells >24hrs → ACTH drops ~25%

• Once plasma is separated:
  • Ship overnight with icepacks
  • If delay occurs (weather/holiday/weekend/brainfart):
    • Ideally, freeze plasma to preserve ACTH
    • Ok for 1 week in the frig – ACTH decreases by ~2% daily

Separated EDTA plasma
Plasma
Buffy Coat (WBC and Platelets)
RBC
UK study at similar latitude of Northeastern US

A significant difference in ACTH between PPID and non-PPID horses occurred during the seasonal rise.

PPID Testing Strategies

- Dynamic Tests?
- Previously the Dexamethasone Suppression Test (DST) was gold standard
  - PPID horses fail to suppress cortisol following dexamethasone injection due to continued ACTH production from the pars intermedia
  - DST fails to diagnose early-stage PPID cases
  - DST is sensitive in the diagnosis of end stage PPID
PPID Testing Strategies

Thyrotropin-Releasing Hormone (TRH) Stimulation Test
• Sensitivity 94%, specificity 78%
• PPID horses with lack of dopaminergic inhibition will respond with increased ACTH levels

Withhold grain 12hr prior to test
Collect Baseline ACTH
Administer TRH Intravenously
Horse >250 Kg: 1mg
Pony <250 Kg: 0.5 mg
10min after TRH administration, draw post-TRH blood sample

PPID Testing Strategies
TRH Stimulation Test – Normal Horse with intact dopaminergic inhibition of pars intermedia

Hypothalamus
Exogenous TRH Injection
Pituitary
Normal Dopaminergic Inhibition
Adrenal Cortex
Cortisol
**PPID Testing Strategies**

**TRH Stimulation Test** – PPID horse with loss of dopaminergic inhibition responds with excessive release of ACTH

- The aim of this study was to determine if trailering affects ACTH concentrations 10 min after a TRH-stimulation test (T10ACTH).
  - 10 horses rotated through 5 trailer positions
  - TRH-stimulation was performed at 0, 15, 30, 60, and 120-min post-trailering

- A 40-min trailer ride caused false positive PPID diagnosis using baseline ACTH for up to 30min post-trailer

- T10 after TRH-stimulation was not elevated by trailer stress in all but 1 horse at time 0
Baseline ACTH and TRH Response Test Reference Intervals

- Reference intervals in fall months for post TRH response test are not yet well established in North America

<table>
<thead>
<tr>
<th>Non-fall months: mid-November to mid-July</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference intervals</td>
</tr>
<tr>
<td>Interpretation of results³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>basal ACTH or time 0</th>
<th>&lt;30 pg/mL</th>
<th>30-50 pg/mL</th>
<th>&gt;50 pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min after TRH</td>
<td>&lt;110 pg/mL</td>
<td>110-200 pg/mL</td>
<td>&gt;200 pg/mL**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fall months: mid-July to mid-November³³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference intervals</td>
</tr>
<tr>
<td>Interpretation of results³</td>
</tr>
</tbody>
</table>

| basal ACTH | <50 pg/mL | 50-100 pg/mL | >100 pg/mL |

PPID Treatment

- Pergolide (Prascend)
  - Dopamine agonist
  - Reassess ACTH level 1 month after treatment initiated, and q6-12mo after that

ACTH AFTER TRH STIMULATION IN PPID PATIENTS TREATED WITH PEROGLIDE FOR SIX TO EIGHT WEEKS

Christiane Schorn¹, Klaus Failing², Kerstin Fey³

EEG Summit 2020

N=11
- Horses with normal baseline ACTH after pergolide treatment continued to have abnormal TRH stimulation tests.
- More research needed. Base decisions regarding pergolide dosing on:
  - Compare serial tests in same horse to establish trend
  - Trend of clinical signs in response to treatment
PPID Treatment

Cyproheptidine

- Mechanism – anti-serotonergic
  - May decrease ACTH release from corticotropes in pars distalis of pituitary

- When to use:
  - Adjunct therapy used when horses don’t respond to pergolide
  - Alternative treatment in horses that experience significant pergolide side effects

- Side effects – associated with seizures in rodents, do not use in horses with previous history of seizure.

Additional PPID Management Considerations

- Dietary management
  - Horses with concurrent insulin dysregulation require low starch diet

- Routine fecal egg counts to assess parasite load

- Body clip when necessary to manage hypertrichosis and excessive sweating

- Monitor for infections
Equine Metabolic Syndrome

EMS – Definition of Terms

Hyperinsulinemia-Associated Laminitis (HAL)
- Mild to moderate laminitis due to sustained undetected hyperinsulinemia which leads to laminar hoof damage.
  - Horses with HAL can appear lame or have subclinical laminitis with no obvious lameness
  - Horses with EMS and 30% of horses with PPID develop HAL

- Pasture-Associated Laminitis is an example of HAL

• Mechanism? Two theories:
  1. Hyperinsulinemia induces inappropriate stimulation of insulin-like growth factor-1 receptors on laminar epidermal cells
  2. Decreased lamellar perfusion with altered lamellar energy regulation
How does hyperinsulinemia cause laminitis?

- Popular working theory:
  - Through inappropriate stimulation of insulin-like growth factor 1 receptors on lamellar epidermal cells
  - Mitosis of lamellar cells → stretching and failure

EMS – Definition of Terms

**Insulin Resistance (IR)**
- Impaired response of the body to insulin, resulting in elevated blood glucose

**Insulin Dysregulation (ID)**
- Excessive insulin response to sugars, resting and postprandial hyperinsulinemia and insulin resistance
- Associated with EMS, may also occur secondary to PPID, systemic illness, stress, pregnancy and starvation

**Nonstructural Carbohydrates (NSC) – Dry Matter Basis**
- Starches and sugars in feed
- NSC = WSC + Starch

| Sugar (WSC) | 10.45 |
| Starch     | 4.42  |
| NSC        | 14.87 |
Equine Metabolic Syndrome (EMS)

- EMS is defined as the risk factors associated with HAL development:
  - Insulin dysregulation
  - Increased adiposity (generalized or regional)
  - Hypertriglyceridemia
  - Hypertension

EMS Risk Factors

- Genetics
  - High genetic risk animals develop EMS with low environmental influences
    - British native pony breeds

- Environment
  - Diet - Feeds with high NSC (grain, grass)
  - Lack of exercise
EMS Clinical Signs

- Obese ‘easy keeper’ with thick neck crest or other regional adiposity
  - BCS>7 (where 1 is emaciated, 5 is ideal and 9 is obese)

- Evidence of subclinical laminitis
  - Divergent hoof capsule growth rings
  - Widening of white line

- Laminitis

EMS Diagnostic Testing

Pros and Cons of EMS Diagnostics used in the field:

1. Baseline Insulin
   - Complete fasting is not required
   - No need to remove hay or pasture, but must wait 4hr after high carb grain meal
   - Low sensitivity for diagnosis of EMS

2. Oral Sugar Test (OST)
   - Dynamic test that assesses insulin response following sugar ingestion (corn syrup)
   - Requires fasting for 3-6 hrs
   - Improved sensitivity over insulin baseline
   - Makes some owners nervous

3. Insulin Tolerance Test
   - Dynamic test
   - Fasting not required
   - Baseline glucose → 0.1 IU/kg regular insulin → collect 2nd glucose 30min after insulin, then feed meal to prevent hypoglycemia
Influence of Feed on Insulin Baseline (RIA)

- Reference ranges created in the context of 2 feeding scenarios:
  - Horses on hay and pasture: 10-40 uIU/ml
  - Horses fasted overnight: <20 uIU/ml

- If grain has been fed, wait 4 hours before sampling insulin baseline
  - Most normal horse’s insulin will return to reference range within 2hr of meal
Validation and method comparison for a point-of-care lateral flow assay measuring equine whole blood insulin concentrations

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

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

• Evaluated the Wellness Ready Equine Insulin Test (WRT)
  • Stall-side lateral flow assay that measures insulin in whole blood
  • Compared to radioimmunoassay

• WRT insulin concentrations averaged 10% higher than the RIA
  • Sensitivity ranged 87-95%
  • Specificity ranged 92-96%

• Conclusion – good association with RIA

Practice Tip: Stall-side/point-of-care insulin analyzers have been recently developed and are now commercially available but have not been critically evaluated at this time. The EEG recommends that referral laboratories be used until further independent research is performed.

OST Steps

Fast for 3-6 hours (no hay/grain)

Administer corn syrup with oral dosing syringe
  • Karo Syrup Lite

Draw blood for insulin at 60min and 90min (EDTA or red Top Tube)
Oral Sugar Test for Diagnosis of EMS (RIA)

- New research suggests that using a higher dose of corn syrup (Karo Lite) is more sensitive for the diagnosis of EMS
  - 0.45 ml/Kg rather than 0.15 ml/Kg


<table>
<thead>
<tr>
<th>Corn Syrup Dose</th>
<th>Post-OST Insulin Ref. Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Dose 0.15 ml/Kg</td>
<td>&lt;45 IU/ml – no evidence  &gt;45 IU/ml – evidence of ID</td>
</tr>
<tr>
<td>High Dose 0.45 ml/Kg</td>
<td>&lt;65 IU/ml no evidence  &gt;65 IU/ml supportive of EMS</td>
</tr>
</tbody>
</table>

*Novel unexpected finding - Insulin was higher in the winter months in the Northern Hemisphere*
Seasonal Effects of Insulin and ACTH
Andy Durham, Liphook Equine Hospital, UK

- 14,737 submissions with baseline insulin and ACTH
- 1605 submissions with insulin following OST and baseline ACTH

- Seasonal variation of insulin occurred
  - Higher in winter, lowest in summer and autumn

- ACTH concentrations inversely correlated with insulin

- Why? Evolution?
  - Grazing season typified by relative insulin sensitivity, promoting energy stores
  - Winter season may have relative insulin resistance to facilitate mobilization of energy stores

---

OST is more sensitive for EMS diagnosis
Case example

**Signalment:** 10yo Morgan mare

**History:** Apparently healthy, BCS 7/9, pleasure horse in moderate work turned out on lush pasture

<table>
<thead>
<tr>
<th>Oral Sugar Test: Insulin</th>
<th>Pre: 19.85 uIU/ml</th>
<th>0 - 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post: 134.08 uIU/ml</td>
<td>0 - 45</td>
</tr>
<tr>
<td></td>
<td>Post: 67.63 uIU/ml</td>
<td>0 - 45</td>
</tr>
</tbody>
</table>
Coming soon? Glycemic pellets for OST

- New commercial product to replace kar0 syrup in OST - DysChEq™

- Standardized, *fairly* palatable glycemic pellet to stimulate insulin secretion
  - Horse is fed standard volume per BW
  - Given 10min to consume pellets
  - Blood sample collected 2hr later for glucose and insulin measurements

EMS Diagnostic Testing

**Leptin**

- Serum or EDTA plasma, fasting not required

- Function – ‘the satiety hormone’
  - Inhibits hunger when the body does not need energy
  - Released from adipose cells, rises with increased body condition

- Objective measurement to correlate with increased adiposity, particularly *intra-abdominal* fat
EMS Management

1. Low NSC diet of <10%
   • Hay Analysis of hay core samples
   • Restrict grass and grain
   • Use ration balancer to supplement essential vitamins and minerals
   • Slow feeder nibble nets to simulate grazing and small frequent meals

2. Exercise if not laminitic

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

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

**Figure 2.** Postprandial insulin concentrations for non-insulin dysregulated (NID) and insulin dysregulated (ID) horses in Study A. Mean insulin concentrations ± SEM for postprandial insulin (FINS) for five dietary treatments of cracked corn with molasses (CC), oat groats (OG), low NSC pelleted feed (L-NSC), ration balancer with high protein (BB-HP) and straw flaked corn (SFC). All ID horses T60 responses were different than NID horses (p < .001). Significance is denoted by difference of superscript.
EMS Management

Can some grazing/grain ever be re-introduced?

• Always remain cautious, but restricted access to grass may be considered if:
  1. Resolve obesity, return to ideal BCS of 5
  2. Demonstrate normal insulin regulation through OST results
  3. Perform grazing trial – allow 1-2hr grazing, then measure insulin 1-2hrs later.
     • Unsafe if insulin >200 uIU/ml (this is not a precise test)

EMS Treatment
When Diet and Exercise Fail

Levothyroxine sodium (Thyro-L)

• High Dose: 0.1mg/kg PO SID for ≤ 6 months
  • Side Effects – weight loss, tachycardia, hyperexcitability, arrhythmia, collapse

• Treat until reaching ideal body condition or when 6 month period ends
  • Gradually weaning off Thyro-L over 1 month period

• Long-term administration is not recommended
EMS Treatment
Persistent Hyperinsulinemia Management

**Metformin**
- Mechanism: may decreases intestinal absorption of glucose
- Poor oral bioavailability in horses (7%)
- Dose range: 15-30 mg/kg PO BID to TID given 30-60min prior to feeding
  - Oral ulcers seen with high dose
  - Individual horse variability in bioavailability

**Sodium-glucose co-transporter 2 inhibitors (SGLT2)**
- Mechanism: Inhibit the reuptake of glucose from the renal glomerular filtrate. Glucose is lost in urine → decreases blood glucose and insulin concentrations
- Brand available for people in United States is Canagliflozin (Invokana)
- More research now available in safety and efficacy
  - Expensive and Novel

---

**EMS Treatment**
Persistent Hyperinsulinemia Management

**Sodium-glucose co-transporter 2 inhibitors (SGLT2)**
- Recommended use:
  - Short-term management of acute carbohydrate overload in EMS patient (escapes into grass paddock or grain bin)
  - Contraindications – avoid in horses with liver disease
  - Side Effect – Stimulates lipid mobilization resulting in hypertriglyceridemia

---

**Research Article**

The sodium-glucose co-transporter 2 inhibitor velagliflozin reduces hyperinsulinemia and prevents laminitis in insulin-dysregulated ponies

Alexandra Meier¹, Dania Reiche², Melody de Las¹, Christopher Poff³, Donald Walsh², Martin Silsence²

All ponies enrolled had insulin dysregulation (ID)
The sodium-glucose cotransporter-2 inhibitor velagliflozin decreases basal plasma insulin concentrations in horses with moderate-severe insulin dysregulation

K. Thane¹, R. Voth², R. Klee³, T. Wannen⁴, N. Frank⁵

• Investigate the ability of SGLT2 inhibitor velagliflozin to decrease insulin concentrations in horses with ID

• Privately owned horses with moderate to severe insulin dysregulation
  • OST insulin >75 uIU/mL

• Design:
  • n=19 placebo
  • n=18 active drug for first 20 weeks
  • Then all horses received active drug for 20 weeks

• Velagliflozen significantly decreased basal plasma insulin in ID horses

• All horses experienced increase in serum triglycerides with no apparent clinical abnormalities (lethargy, anorexia)
**Figure 4 - Algorithm for management of challenging cases (June 2022)**

**Horse requiring long-term management of insulin dysregulation (ID)**

**Routine cases**
- Obesity improves
- No further weight gain
- PPI/EMS present
- Resists treatment

- Consider treatment with 6.1 mg/kg levothyroxine for 2-4 months
- Repeat testing every 6 months during weight loss

**Management challenges**
- Obesity persists
- Compliance with low NSC diet decreases
- PPI develops or worsens
- Laminitis occurs or reoccurs

- Consult internal medicine specialist
- Consider treatment with the 12-8 combination

**PPID vs EMS**

**Obese EMS; no PPI**
- Decrease body fat mass
- Limit caloric intake
- Low NSC diet
- Restricted or no access to grass
- Exercise (if feet have stabilized)

- Remains obese
- Levothyroxine

**Obese EMS + PPI**
- Maintain body condition
- Lower NSC, higher fat, good quality fiber diet
- Restricted access to grass
- Exercise (if feet have stabilized)

**Non-obese EMS; no PPI**

**Obese EMS + PPI**
- Pergolide and diet to manage ID and obesity
- (see boxes to the left)

**Non-obese EMS + PPI**
- Pergolide and diet to manage ID and maintain body condition
- (see boxes to the left)

- Horses > 10 years; refer to EEG Recommendations on PPI
- Re-test insulin status to assess response. Consider other medical treatments for refractory cases.
Diagnostic evaluation of insulin and glucose dynamics in light-breed horses receiving dexamethasone  CVJ, June, 2022
Kathryn J. Timko, Laura D. Hostnik, Mauria R. Watts, Chiaming Chen, Adam Bercz, Ramiro E. Toribio, James K. Belknap, Teresa A. Burns

- Glucocorticoids can exacerbate insulin dysregulation
- 14 horses – administered dexamethasone 0.08 mg/kg PO SID for 7 days

- Take caution using glucocorticoids in ‘at-risk’ horses:
  - Obese
  - Subclinical laminitis or history of laminitis
  - PPID
  - Breed predisposition

- Sometimes the benefits outweigh the risks – requires careful client communication

Questions?