Equine endocrine diseases are commonly encountered in the primary care setting, yet they can be challenging to diagnose and manage. The most common endocrinopathies are Equine Metabolic Syndrome (EMS) and pituitary pars intermedia dysfunction (PPID). Both conditions are likely to increase in prevalence over time as the companion horse population ages and obesity rates continue to rise. Along with compromising the horse’s overall health, a potentially life-threatening complication of both conditions is laminitis.

Frequently, concern arises about thyroid dysfunction in horses with phenotypical characteristics of EMS and PPID. Because a multitude of factors affect the complex hypothalamic-pituitary-thyroid axis, thyroid hormone concentrations are often low in horses with other endocrinopathies or systemic illnesses. The challenge for veterinarians therefore becomes differentiating animals with true thyroid pathology from those in which thyroid dysfunction is secondary to another underlying condition. Furthermore, the decision to administer levothyroxine is complicated by the fact that obese equids and those with insulin dysregulation may benefit from receiving supraphysiologic doses of levothyroxine in the absence of primary thyroid pathology; this helps facilitate weight loss and improves insulin sensitivity.

Primary hypercalcemic and hypocalcemic disorders are occasionally encountered. Here, assessment of calcium metabolism can help distinguish between true parathyroid gland pathology and secondary derangements of calcium metabolism. Likewise, uncommon diseases such as endocrine neoplasias and pancreatitis warrant consideration in certain cases.

Research into equine endocrine disorders is ongoing; consequently, diagnostic testing strategies continually evolve as understanding of disease pathophysiology improves and new tests are developed. The aim of this presentation is to familiarize practitioners with available laboratory tests and discuss how best to utilize these diagnostic tools.

ENDOCRINE TESTING STRATEGIES FOR EQUINE METABOLIC SYNDROME AND PITUITARY PARS INTERMEDIA DYSFUNCTION

For details on testing protocols and interpretation of results, refer to the Equine Endocrine Society consensus statement (http://sites.tufts.edu/equineendogroup/)

For detailed instructions on sample collection and submission, refer to the Michigan State University Diagnostic Center for Population and Animal Health website (https://dcpah.msu.edu/Sections/Endocrinology/)
**Diagnosing EMS** – The triad of generalized and/or regional adiposity, insulin dysregulation, and either clinical or subclinical laminitis defines EMS. This complex disease is a culmination of genetic predispositions towards abnormal glucose and insulin metabolism, combined with exacerbating environmental and physiologic factors such as obesity, carbohydrate-rich diets, and concurrent illnesses. High sugar diets and the low-grade systemic inflammatory state that accompanies obesity promote peripheral insulin resistance. Resting hyperinsulinemia may develop as pancreatic β-cells compensate by overproducing insulin and hepatic insulin clearance declines. However, some affected horses are normoglycemic and only demonstrate exaggerated hyperinsulinemic responses when faced with an oral or intravenous glucose challenge. Additionally, abnormal enteroinsular axis incretin responses may exacerbate postprandial hyperinsulinemia in some affected animals. Hyperinsulinemia is a major risk factor for laminitis; while the exact mechanism remains unclear, it likely involves a combination of vascular endothelial dysfunction, altered vascular tone, and harmful effects on laminar epidermal cells. With the prevalence of overweight and obesity reaching nearly 50% in some horse populations, screening for insulin dysregulation is important to identify animals at risk of developing laminitis while it is still subclinical. Cumulative laminar damage may be ongoing for years prior to the first recognized painful episode. Once clinical laminitis develops, many animals experience recurrent episodes, underscoring the importance of managing EMS before the onset of laminar pain. Testing is also important to identify the rare EMS case where pancreatic β-cell exhaustion has occurred, and compensated insulin resistance has progressed to type 2 diabetes mellitus.

Resting serum insulin and glucose concentrations represent the simplest method of assessing insulin regulation. While easy to perform, sensitivity is poor; however, specificity is high, and these assessments are therefore useful in identifying severely insulin resistant individuals. Glucose and insulin dynamics are affected by many factors, although pain, stress, and systemic illness generally must be severe before they falsely increase resting serum insulin concentrations. A myriad of dynamic tests have been developed to more comprehensively assess glucose and insulin metabolism. The combined glucose-insulin test (CGIT) is an intravenous glucose challenge that is simple enough for field use, and has shown reasonable sensitivity and specificity for identifying whole-body insulin resistance. Oral glucose challenges such as the oral sugar test (OST) quantify postprandial hyperinsulinemia (an estimate of insulin resistance) and are intuitively more physiological than intravenous challenges, as they incorporate enteroinsular axis responses to feeding. These tests have also been touted as less laborious alternatives to the CGIT. Although not yet commercially available, measurement of glucagon-like peptide-1 and glucose-dependent insulinotropic peptide (incretin hormones) shows promise in diagnosing equine insulin dysregulation.

All current field tests of insulin sensitivity are fraught with limitations. When compared, tests often show poor agreement in classifying horses as insulin-resistant or insulin-sensitive. In general, dynamic tests such as the CGIT and OST are more sensitive than resting insulin and glucose concentrations, however some field dynamic tests perform better than others. While easily performed dynamic tests such as the OST are gaining in popularity, it remains to be determined whether or not their sensitivities for identifying insulin-resistant horses are adequate. Housing, diet, and season affect OST results in normal horses; this has not been evaluated in insulin-resistant animals. The CGIT appears to be less affected by factors such as season. Additionally, recommended fasting length prior to insulin sensitivity testing has not been
critically evaluated, and there is some evidence to suggest that fasting duration may alter the performance of certain tests.

Clearly, additional research is needed to identify a highly sensitive and specific field test for insulin resistance and to standardize testing protocols. Until such time, the current recommendations state that it is acceptable to measure resting insulin and glucose concentrations for screening purposes; if abnormal, insulin dysregulation is confirmed. If normal, a dynamic test such as the OST or CGIT should be performed. If feasible, dynamic testing is recommended for initial screening as well.

Diagnosing PPID – Pituitary pars intermedia dysfunction occurs in over 20% of aged horses and ponies. As hypothalamic dopaminergic neurons degenerate due to oxidative stress, tonic inhibition of pars intermedia melanotropes is lost; this gives rise to the hyperplasia, microadenomas, and macroadenomas that characterize the disease. The abnormal pars intermedia therefore produces excess pro-opiomelanocortin (POMC), from which several peptides such as ACTH and α-melanocyte-stimulating hormone (α-MSH) are derived. Excesses of these various peptides are believed to cause clinical signs of the disease.

Measurement of endogenous plasma ACTH concentration is the most commonly used test to diagnose PPID. Blood α-MSU elevations generally parallel those of ACTH, but to a greater magnitude, and may offer improved sensitivity and specificity under certain conditions. However, assays are currently not available commercially and the diagnostic advantage of measuring α-MSU over ACTH has not been conclusively demonstrated. Endogenous ACTH has entirely supplanted measurement of endogenous cortisol in the diagnosis of PPID. Adrenocorticotropic hormone and ACTH-like peptides derived from the abnormal pars intermedia represent the majority of the immunoreactive ACTH detected in the circulation of PPID-affected horses, and are less bioactive with regard to stimulating cortisol secretion than pars distalis-derived ACTH. Commercially available assays detect both normal ACTH and biologically inert ACTH-like peptides; as a result, endogenous blood ACTH and cortisol concentrations are dissociated, rendering cortisol measurement invalid for assessing pituitary dysfunction. Because assays used by different diagnostic laboratories appear to detect abnormal ACTH-like peptides to varying degrees, lab-specific reference ranges should be used to diagnose PPID and, when monitoring treatment efficacy, serial ACTH concentrations should only be compared if measured with the same assay.

Also common is the overnight dexamethasone suppression test (ODST). In normal horses, 98% of the endogenous ACTH in circulation is produced by the pars distalis, thus administration of exogenous steroids suppresses both blood ACTH and cortisol concentrations. However, the pars intermedia is not subject to negative feedback by glucocorticoids, thus steroid administration to horses with PPID fails to suppress cortisol secretion. This test is falling out of favor, as it does not show improved diagnostic performance over endogenous ACTH concentration, is more laborious (the test spans 2 consecutive days), and, although not proven, is perceived by some to carry a small risk of precipitating a laminitic episode.

The sensitivities of endogenous ACTH and the ODST are reasonably high in severely affected horses with advanced PPID, but are considerably lower in early and subclinical cases. Specificity is reasonably high for both tests, but false positives can occur in horses with severe concurrent illness, stress, or pain; feeding status, exercise, and transportation can also confound test results. Dynamic testing has been investigated as a means to improve sensitivity in
early/mild PPID cases. Currently, the thyrotropin-releasing hormone (TSH) stimulation test (measuring ACTH response) is recommended; only ACTH responses are valid, and this represents a change from the original test protocol that measured cortisol responses. This test works on the premise that TRH administration elicits ACTH release from both pars intermedia melanotropes and pars distalis corticotropes (due to the presence of TRH receptors on these cell types); normal horses exhibit a small rise in plasma ACTH concentration following TRH administration, but the rise is of a much greater magnitude in PPID-affected horses due to their pars intermedia pathology. As with measurement of endogenous ACTH, illness, stress, and pain may confound TRH stimulation test results; in contrast, the TRH stimulation test does not appear to be affected by fed versus fasting state. However, TRH stimulation testing should not be performed immediately after the OST, as the latter has been shown to blunt pituitary ACTH responses to exogenous TRH through an as of yet unknown mechanism.

Hypothalamic and pituitary activity is upregulated during fall months (mid-July to mid-November in the temperate Northern hemisphere), with high concentrations of ACTH, α-MSU, and other POMC-derived peptides effecting metabolic changes and increasing fat stores in preparation for winter. We now know that PPID-affected horses retain this normal circannual cycle; furthermore, the magnitude of increase in blood concentrations of POMC-derived peptides is often much greater than in normal horses. In a paradigm shift from early recommendations to avoid autumn testing, we now exploit this phenomenon of natural stimulation to increases the sensitivity of diagnostic tests during the fall months. Currently, seasonal reference ranges are only available for endogenous ACTH testing, and not all diagnostic laboratories have established their own geographically-appropriate seasonal values. Seasonally adjusted reference ranges are not yet available for TRH-stimulation testing, although this research is ongoing. No seasonal ranges have been reported for the ODST.

**Instituting and monitoring pergolide therapy in PPID** – Advanced cases presenting with pathognomonic hypertrichosis, epaxial muscle wastage, a pendulous abdomen, abnormal regional adiposity, sweating, polyuria/polydipsia, laminitis, and other classic signs are easily recognized. The challenge lies in identifying and treating early cases before the development of subclinical cumulative laminar damage that often precedes overtly recognizable laminitis. Ambiguous results are common in early disease; hormone concentrations may be normal in horses with clinical signs of PPID or may be abnormally high in horses that appear normal.

**In horses with obvious clinical signs of PPID, the currently recommended diagnostic approach is to measure endogenous ACTH concentration.** If normal, a TRH stimulation test should be performed; until seasonal reference ranges are established, this test should only be performed from mid-November to mid-July. Alternatively, a therapeutic trial of pergolide may be considered. In advanced disease, the main value of testing is to establish a baseline from which to monitor therapeutic efficacy in the future. **In horses with early/mild PPID and subtle clinical signs, the TRH stimulation test is preferred over either endogenous ACTH or the ODST.** If positive, pergolide therapy should be instituted. If negative, the horse should be retested in 3-6 months; ideally reevaluation (by endogenous ACTH measurement using seasonally adjusted reference ranges) should occur during the fall to maximize test sensitivity.

Many horses with a favorable clinical response to pergolide therapy will show a decrease in endogenous ACTH concentration, and most will eventual normalize over a period of 1-3 months. Current recommendations state that endogenous ACTH concentration (or TRH
stimulation, if used for initial diagnosis of PPID) be measured 1-2 months after starting pergolide therapy. If the dose is adequate, endogenous ACTH is expected to be within or near the normal range for the season and clinical improvement should be seen. If laboratory tests are abnormal and clinical signs persist, the pergolide dose should be increased and the horse reassessed monthly until an appropriate pergolide dose is determined or the maximum dose has been reached. The same approach should be taken in situations where laboratory test results are normal, yet the clinical response remains poor. Dilemmas arise when laboratory tests are abnormal, yet the patient is responding well clinically to pergolide therapy. In such cases, the author generally advocates remaining on the current pergolide dose and reassessing the horse in 3-6 months. However, abnormal laboratory values imply ongoing pituitary dysfunction. We do not yet know which pathophysiologic abnormality confers the greatest risk of developing laminitis, and it is conceivable that the laminitis risk does not decrease if pituitary function remains abnormal, even when other signs such as hypertrichosis improve. For this reason, some experts recommend increasing the pergolide dose until laboratory tests normalize. Once the proper pergolide dose has been established and the clinical signs are stable, laboratory tests should be repeated every 6 months to monitor therapy, with one test performed during fall season (mid-July to mid-November). It should also be noted that, in some horses that initially fail to respond to pergolide therapy, prolonged administration over several months to years may eventually result in normalization of laboratory tests and clinical improvement.

Assessing insulin dysregulation in PPID – Although glucose and insulin metabolism can be normal in PPID-affected horses, insulin dysregulation and resting hyperinsulinemia are very common findings and are likely associated with a poorer long-term prognosis. The exact relationship between PPID and laminitis has yet to be fully elucidated, but we are beginning to recognize that laminitis in these animals appears highly correlated with concurrent hyperinsulinemia, suggesting a shared pathophysiology of endocrinopathic laminitis in EMS and PPID. As such, it is imperative to identify the subset of PPID-affected horses with insulin dysregulation, as improving insulin sensitivity could reduce the risk of laminitis in these animals. **Assessment of glucose and insulin homeostasis is recommended in all PPID cases, as metabolically deranged animals require strict dietary nonstructural carbohydrate restriction, more intensive husbandry, and occasional pharmacologic strategies to improve insulin sensitivity.** Horses with PPID also occasionally develop type 2 diabetes mellitus, necessitating more intensive therapy.