A one-health approach to identifying and mitigating the impact of endocrine disorders on human and equine athletes

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doi.org/10.2460/ajvr.22.11.0194

ABSTRACT
Endocrinopathies affect multiple species in ever-increasing percentages of their populations, creating an opportunity to apply one-health approaches to determining creative preventative measures and therapies in athletes. Obesity and alterations in insulin and glucose dynamics are medical concerns that play a role in whole-body health and homeostasis in both horses and humans. The role and impact of endocrine disorders on the musculoskeletal, cardiovascular, and reproductive systems are of particular interest to the athlete. Elucidation of both physiologic and pathophysiologic mechanisms involved in disease processes, starting in utero, is important for development of prevention and treatment strategies for the health and well-being of all species. This review focuses on the unrecognized effects of endocrine disorders associated with the origins of metabolic disease; inflammation at the intersection of endocrine disease and related diseases in the musculoskeletal, cardiovascular, and reproductive systems; novel interventions; and diagnostics that are informed via multiomic and one-health approaches. Readers interested in further details on specific equine performance conditions associated with endocrine disease are invited to read the companion Currents in One Health by Manfredi et al, JAVMA, February 2023.

Endocrinopathies, with their various systems interconnections, have the ability to greatly affect athletic performance in multiple species. Recognizing the similarities between human and equine endocrine disorders (Figure 1) allows us to take a one-health approach to investigate the potential impact of endocrinopathies on the musculoskeletal, cardiovascular, and reproductive systems in athletes. Human metabolic syndrome (MetS) is a consortium of disorders that affects 25% of the United States population and is a major risk factor for development of obesity, diabetes, cardiovascular disease, and orthopedic disease.1-3 Endocrine disease is also prevalent in the horse population, with 21% of horses over 15 years old diagnosed with pituitary pars intermedia dysfunction (PPID) and an estimated 20 to 40% of the equine population being at risk for the development of equine metabolic syndrome (EMS).4 Details of the impact of PPID, EMS, and the insulin dysregulation (ID) that can accompany them in body systems and conditions other than laminitis are limited. However, the unrecognized effects of endocrine disorders, which will be the focus of this article, include suspensory ligament degeneration, lameness, osteoarthritis (OA), developmental orthopedic disease, muscle atrophy, cardiovascular disease, placental dysfunction, and infertility.5–12 Future research collating phenotype to multiomic analyses (genomics, metabolomics, transcriptomics, proteomics, and microbiomics; Figure 2) and examination of the developmental origin of metabolic disease to evaluate the links between endocrinopathies and athletic performance can contribute to our understanding of the pathophysiology, while shedding light on novel biomarkers and treatments.

Current Understanding of the Pathophysiology of Insulin Dysregulation

ID, defined as disruptions in the interconnected relationships between insulin, glucose, and lipid metabolism, plays an important role in metabolic syndrome and contributes to the pathology associated with other athletic performance-limiting conditions.13 In normal conditions, insulin is released by β-cells from pancreatic islets of Langerhans primarily...
in response to hyperglycemia, but other macronutrients, hormones, and neurotransmitters can also stimulate insulin release. Glucose readily crosses the β-cell membrane via high-capacity, low-affinity glucose transporters (GLUT). Although GLUT2 is the primary β-cell transporter in the mouse, GLUT1 and GLUT3 appear to be more important in humans and the horse β-cell transporter has yet to be identified. In rodent models and humans, insulin secretion is biphasic with an initial rapid release followed by a more prolonged but less concentrated release. However, it has not been determined as to whether insulin release is mono or biphasic in horses.

In the periphery, insulin mediates its effects by binding to insulin receptors located on its main target tissues: adipocytes, skeletal muscle, and liver. This binding activates intracellular insulin-responsive substrates (IRS), which are responsible for insulin’s metabolic, vascular, and mitogenic effects. For example, IRS activation of the downstream effector pathway phosphatidylinositol 3-kinase (PI3K) promotes insulin’s metabolic effects including the translocation of intracellular glucose transporter proteins (GLUT4) to the cell membrane, stimulating lipid and protein synthesis, and inhibiting glycogenolysis and

Figure 1—Shared systemic effects of endocrine disease in the horse and human. Created with BioRender.com.

Figure 2—A combined approach to assessing phenotype in light of multiomic analyses (genomics, metabolomics, transcriptomics, proteomics, and microbiomics) to understand the effects of endocrine disorders on performance. Created with BioRender.com.
hepatic gluconeogenesis. Once the glucose transporter is translocated to the plasma membrane, glucose is transported into the cell where it is phosphorylated to be stored as glycogen or utilized for adenosine triphosphate (ATP) production.

Hyperinsulinemia and insulin resistance are the mainstays of MetS. With insulin resistance, as blood glucose levels fail to return to normoglycemia, the pancreatic β-cells hyperfunction by increasing insulin secretion via β-cell hypertrophy and decreasing β-cell expression of glucokinase while increasing expression of hexokinase, shifting the insulin-glucose response curve to the right. In addition, in normal conditions, the first portal passage through the liver is responsible for 50 to 60% of insulin clearance in humans and up to 70% in horses; however, insulin insensitivity markedly reduces hepatic insulin clearance across species, contributing to hyperinsulinemia.

**Incretin response and oral therapies**

Postprandial stimulation of insulin release via the enteroinsular axis has been found to result in a greater insulin response versus intravenous or intraperitoneal glucose administration. This has been deemed the incretin response based on the effects of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). Breed differences in GLP-1 concentrations were identified in ponies and Andalusians, 2 breeds considered high risk for EMS, who had higher GLP-1 and insulin concentrations compared to Standardbred horses, potentially indicating a risk factor for ID. Ponies with ID had hyperinsulinemia and an exaggerated incretin response 4 hours after consumption of pasture compared to controls. In contrast, other studies found that both active GLP-1 and total GLP-1 levels were decreased in horses with ID compared to controls.

Drugs that specifically target incretins are becoming more prominent. In humans, synthetic GLP-1 and drugs targeted at inhibiting dipeptidyl peptidase 4, a serine protease that inactivates GIP and GLP-1, have been shown to increase glycemic control in humans with MetS while also decreasing body weight. In a small cohort of horses with mild ID, a single dose of exenatide, a synthetic GLP-1 receptor agonist (also known as an incretin mimetic), resulted in a significant decrease in area under the curve of plasma insulin concentrations and improved insulin sensitivity after an oral sugar test. This seemingly contradictory result was postulated to be due to slower gastric emptying, thus decreasing the rate of glucose absorption or other independent actions that achieved increased insulin sensitivity.

Traditional dietary medications and supplements for treating EMS (above and beyond exercise and dietary change) include metformin, levothyroxine, and a resveratrol + amino acid blend, with the first and last also being used for humans with MetS. However, a new promising class of oral treatment options, sodium-glucose cotransporter 2 inhibitors (ertugliflozin and canagliflozin), which modulate the sodium and glucose transport in the kidney by increasing urinary glucose excretion, have been described. These drugs have been shown to have efficacy in treating refractive cases of EMS/ID, ones where the typical diet, exercise, and supplementation with metformin or levothyroxine has failed; limit insulin peaks after eating; and improve Obel scores. Further investigation is required to determine if these new potential therapeutics are successful in a larger number of clinical cases and compared to other treatments.

**Dyslipidemia**

Dyslipidemia is a central component of the pathophysiology behind metabolic syndrome. Direct inhibition of insulin action by elevated fatty acids eliminates the negative feedback on hormone-sensitive lipase, leading to further accumulation of triglycerides in the liver and ectopic depositions of fat in muscle (intramyocellular lipid), reducing the cellular uptake of glucose, promoting hyperglycemia, and contributing to insulin resistance.

In horses, elevated nonesterified fatty acids (NEFAs) and triglycerides have been associated with EMS, although not consistently across studies. Increased triglyceride levels were found in ponies with EMS, and hypertriglyceridemia was identified as a risk factor for the development of laminitis. Another study identified a seasonal elevation in triglyceride levels in ponies with EMS. In a cohort of large-breed horses, a significant elevation in NEFA concentrations was noted; however, triglyceride concentrations were not different. These studies reflect possible differences between ponies and horses with EMS. In a small cohort of obese large-breed horses with insulin resistance compared with metabolically healthy horses, the plasma concentration of very low-density lipoproteins and high-density lipoproteins (HDL) were found to be increased in horses with insulin resistance. The elevation in HDL is opposite to what is characteristically seen in humans with MetS and may reflect the absence of plasma cholesterol ester transfer protein in equids. Using continuous lipid profile measurements, subfractions of HDL, specifically HDL3a, were found to be significantly lower in healthy horses compared to horses with obesity, laminitis, or both despite there being no difference in total HDL concentrations, possibly indicating a novel method of lipoprotein profiling. Unfortunately, this study did not evaluate metabolic status and further studies need to be performed to determine the utility of EMS.

**Inflammatory cytokines**

Evidence supports that a large contribution to the pathophysiology of metabolic syndrome is related to the role of adipose tissue in promoting chronic, low-grade inflammation. Adipose tissue is a biologically active endocrine organ that secretes a myriad of substances including cytokines, eicosanoids, complement proteins, binding proteins, vasoactive factors, and regulators of lipid metabolism that are collectively known as adipokines. The
exact mechanism behind adipose-induced inflammation in metabolic syndrome is unknown, but it is proposed that "sick fat" is a result of adipose hypertrophy and hyperplasia secondary to excess nutrition leading to endoplasmic reticulum stress or hypoxia from an insufficient blood supply,\textsuperscript{53,54} which results in macrophage and mast cell infiltration and the production of inflammatory mediators.\textsuperscript{54,55} In humans, this theory is supported as certain adipose tissue depots (the subcutaneous and visceral abdominal depots) more commonly associated with MetS contain hypertrophied adipocytes, which undergo lipoapoptosis releasing inflammatory mediators.\textsuperscript{56} Additional hypotheses have proposed that endothelial stress and hyperactive platelets are the primary etiology behind adipose tissue inflammation as well as the low-level inflammation observed in other tissues.\textsuperscript{57} Regardless, in humans, the increased production of inflammatory mediators is a risk factor for type II diabetes mellitus\textsuperscript{58} and is associated with higher fasting insulin levels and dyslipidemia.

The interrelationship of inflammatory mediators in obesity and ID has also been investigated in horses with EMS. Similarly to humans, histological examination of adipose tissue in obese, hyperinsulinemic ponies revealed a marked degree of hypertrophy\textsuperscript{59,60} and macrophage infiltration\textsuperscript{59} compared to the adipose tissue of non-obese ponies, indicating adipose dysfunction. Other early support for adipose tissue inflammation in driving disease processes is the clinical improvement of a severely insulin-resistant animal that improved greatly after systemic injection with autologous 5-azacytidine-treated and resveratrol-treated (an anti-inflammatory agent)\textsuperscript{61} adipose stem cells.\textsuperscript{62} Studies\textsuperscript{56,60,61}–\textsuperscript{65} evaluating inflammatory cytokine mRNA or protein expression levels have identified significant differences between nuchal ligament, abdominal, and subcutaneous fat, suggesting that adipose tissue depots have unique biological behavior as also seen in humans. Horses with EMS were found to have marked increases in TNFa, interleukin-1β (IL-1β), and CCL2 in both perirenal and retroperitoneal fat depots,\textsuperscript{66} and IL-6 was significantly increased in the subcutaneous fat.\textsuperscript{59} When comparing insulin sensitive versus insulin-insensitive horses, 1 study\textsuperscript{64} concluded that the nuchal ligament adipose tissue contributed the most to the EMS pro-inflammatory profile, with an increased expression of IL-1β and IL6 in insulin-resistant horses despite no difference in other adipose tissue depots or systemic inflammatory mediators.

Differences in serum cytokine levels have also identified statistically significant differences between control and obese and/or EMS horses but with conflicting results. In a group of 110 light-breed horses, serum amyloid A concentrations were correlated with body condition score (BCS) and baseline insulin concentrations after correcting for age and sex, although correlations were not identified for TNFa or IL-6.\textsuperscript{66} Decreased concentrations of TNFa, IL-6, and IL-1 were identified in obese horses; however, endogenous cytokine levels were not associated with insulin levels.\textsuperscript{57} Serum IL-6 and activin A did positively correlate to insulin levels in another study\textsuperscript{68} but did not differ between EMS and control horses. These findings contrasted with several studies that identified (i) higher concentrations of TNFa in previously laminitic ponies versus nonlaminitic ponies;\textsuperscript{69} (ii) higher concentrations of TNFa and IL-6 in a group of obese, hyperinsulinemic ponies versus obese ponies;\textsuperscript{70} and (iii) an inverse association between TNFa and insulin sensitivity in 60 mares after adjusting for BCS and percent fat.\textsuperscript{70} Interestingly, neutrophil oxidative burst activity was found to be markedly increased in obese, hyperinsulinemic horses. However, in the same study\textsuperscript{72} peripheral cytokine gene expression was lower for IL-1 and IL-6 and there was no difference between groups in cytokine response after an inflammatory challenge, potentially reflecting a lack of a direct inflammatory response due to obesity and hyperinsulinemia in horses. However, EMS horses that had an oral sugar test performed with stevioside had an exaggerated inflammatory response systemically as compared to metabolically normal controls.\textsuperscript{71} Thus, the role of adipose tissue and inflammatory mediators in ID is still under investigation, but the presence of low-grade systemic inflammation could contribute to a metabolic OA phenotype, reproductive disease, and cardiovascular disease.

**Myokines and adipokines**

The roles of myokines and adipokines in horses are in the early stages of understanding, with an imbalance between the 2 being investigated as a contributing cause of metabolic syndrome. Myokines can enhance insulin sensitivity and myostatin inhibition also reduces inflammatory cytokines in muscle and adipose, while stimulating fatty acid oxidation.\textsuperscript{72} Local myostatin inhibitors can also result in increased skeletal muscle glucose dispersal, ameliorating insulin resistance while increasing growth rate and lean muscle mass.\textsuperscript{73} and a single nucleotide polymorphism (SNP) intron 2 of myostatin (MSTN)\textsuperscript{74,75} has been implicated as a powerful predictor of racing performance. This intronic SNP has been shown to be a marker of the true functional variant in a short interspersed element in the MSTN promoter that disrupts MSTN gene expression.\textsuperscript{76} Decreased MSTN gene expression has also been linked to increased insulin sensitivity in bulls, which lends support to this occurring in horses as well.\textsuperscript{77}

Studies\textsuperscript{50,78–81} have demonstrated that low serum high-molecular-weight (HMW) adiponectin is a consistent feature of the EMS phenotype, with adiponectin concentrations being inversely proportional to body mass. Adiponectin is considered insulin sensitizing, which makes it a biomarker of interest for a possible screening test for EMS versus leptin, which is more a marker of obesity in the horse.\textsuperscript{82} Adiponectin is released from adipose tissue in both low-molecular-weight trimers and HMW multimers. There is also a globular proteolytic fraction of the protein.\textsuperscript{83} The HMW form of adiponectin is considered the most metabolically active with the overall decrease in adiponectin concentration seen
in obese human patients due to a decrease of the HMW form.34 Low levels of adiponectin have been associated with ID in the horse35 and are considered predictors of future laminitic events in ponies.36 The globular form of adiponectin also promotes glucose uptake and lipid oxidation while enhancing insulin signaling and myogenesis in humans but has an unknown role in the equine.37

Endocrine Effects on Musculoskeletal Health

Overtraining and muscle atrophy
Muscle itself is an endocrine organ, as addressed above with its production of myokines, and it plays a role in the athletic concern of overtraining. This condition is the result of a combination of excessive training and poor recovery. Clinical signs in the horse include decreased performance, weight loss, and behavior changes.38–40 Possible endocrine-related causes behind this condition could include chronic glycogen depletion and increased reactive oxygen species and thus inflammatory pathways,41 and in both horses and humans, a dysregulation of the hypothalamus-pituitary-adrenal axis.42 As basal testing is not informative, stimulation tests of growth hormone and adrenocorticotropic hormone appear to offer the most promise as diagnostic tests for this condition, as humans affected by overtraining showed blunted responses.43

Muscle atrophy is classically associated with PPID in horses,9 but systemic inflammation in humans with MetS is thought to also result in sarcopenia.44 Past work in horses affected by EMS that evaluated selected inflammatory markers within the muscle did not find signs of inflammation present, but a more extensive evaluation of skeletal muscle of ID versus metabolically healthy horses would be beneficial.45 Of clinical interest, pergolide treatment by itself did not appear to ameliorate muscle atrophy in PPID-affected horses, suggesting that other muscle-building exercises, such as dynamic mobilization exercises, are needed.63

Lameness and obesity
Obesity is a common feature of EMS and has links to lameness, as heavier horses were more likely to not complete an endurance race due to lameness.46 In horses fed a high-energy diet to induce obesity, more gait asymmetry was seen and blinded judges gave them reduced performance scores.47 Obese humans have several reported negative effects on gait biomechanics such as development of angular limb deformities and increased joint stress and pain,45,46,48 and weight loss by itself has been shown to improve gait kinematics and alleviate pain.49 Obese horses with abdominal obesity (a component of MetS) and dynapenia (age-associated loss of muscle strength) have a faster decline in walking speed with age than healthy controls,50 which may have ramifications on performance if this holds true in our older equine athletes. Another musculoskeletal condition that has links to endocrine disease is osteochondrosis dessicans (OCD). Increased frequency of OCD and insulin resistance was seen in foals and yearlings born of obese mares, which is discussed further below (see endocrine effects on reproductive health). Readers interested in further details on specific equine performance conditions such as laminitis (a critically important and commonly recognized sequela of equine metabolic disorders) are invited to read the companion Currents in One Health by Manfredi et al, JAVMA, February 2023. Endocrine disorders in humans have also been associated with a metabolic OA subtype,100 as well as tendon and ligament disease, for which we will now go into more detail.100–108

Metabolic osteoarthritis
Previous research efforts have indicated the appropriateness of using the equine as a model for human OA because the forces exerted with exercise on a human joint are similar to those observed in the horse, so it is reasonable to take a one-health approach considering shared pathophysiology.109–111 Type II diabetes, which can be associated with MetS, has been linked to development of severe OA in humans.107 Furthermore, individuals with OA of which metabolic OA is now considered a subtype,2 have annual healthcare costs ~$11,000 greater than non-OA-affected individuals.112 The precise role that MetS plays in the development of metabolic OA has yet to be fully elucidated but could be involved via several mechanisms.104 One possibility is that the hyperglycemia and low-grade systemic inflammation that occurs during MetS can diffuse into the joint from systemic circulation creating a toxic environment.111,113 Dyslipidemia could also result in ectopic lipid deposition in the chondrocytes, and hypertension could cause ischemia of the subchondral bone.2 While previous work was not able to distinguish between obesity or MetS as the cause of the OA, recent work has shown that MetS by itself can contribute to the development of excessive knee OA pain, with studies102,103 suggesting that gut dysbiosis may play a role in this syndrome. The idea that gut dysbiosis can contribute to joint pain is supported by the fact that oral resveratrol supplementation has been shown to alleviate equine tarsal OA pain and improve insulin regulation,39,114 while also having a beneficial effect on the gut microbiome in rat and mice models of MetS.116,117 Given that obesity has been reported in 30 to 45% of horses,100,111 and that some riding disciplines seem to favor the overweight phenotype (dressage, hunters, ponies), there is a concern that metabolic OA could be more of a present and future problem in horses than is currently recognized.120

Biomarkers for metabolic OA
In humans, the adipokines adiponectin, leptin, and resistin have been found to be potential biomarkers of metabolic OA.108 Systemically, adiponectin has contradicting information about its role, as some studies105,121 found that it causes metabolic OA while others claim it could have a protective effect.
Another study demonstrated that increased levels of leptin can contribute to metabolic OA systemically. While there are currently no studies in horses looking at the possibility of adipokines as synovial fluid biomarkers in metabolic horses with OA, 2 human studies looked at adipokine expression in synovial fluid and serum that have OA in patients with and without MetS. Both studies found that humans with metabolic OA had higher serum leptin concentrations and lower serum adiponectin concentrations compared to humans without metabolic OA. The studies also looked at concentrations in the synovial fluid and the protein expression of the infrapatellar fat pad and found greater expression and concentrations of leptin and less expression and concentrations of adiponectin in humans with metabolic OA. These findings support leptin and adiponectin as both biomarkers for and possible therapeutic targets in patients with metabolic OA. Another study evaluated resistin concentrations in serum or synovial fluid and did not find a difference between MetS or non-MetS patients; however, there was a positive correlation between resistin serum and synovial fluid concentrations and OA grade, as well as a negative correlation between adiponectin serum and synovial fluid concentration and OA grade. In horses with ID, resistin was elevated in plasma, which was attributed to the inflammatory nature of severe ID, and evaluation of its presence in equine synovial fluid and synovium should be examined. While other traditional biomarkers for OA should be evaluated in ID and metabolically healthy horses, future research for novel biomarkers should also include assessment with the multicomponent approaches as discussed below.

**Tendon and ligament injury**

Individuals with type II diabetes have a 3 times higher risk of tendon injury or rupture than control individuals, which can be due to the weight of the individual, inflammatory cytokines, or hyperglycemia and hypercholesterolemia with subsequent production of advanced glycation end-products. EMS has not been identified as a risk factor for tendon and ligament injury in horses, but given the findings in humans, more research should be pursued. However, PPID in horses has been identified as a risk factor for suspensory ligament degeneration, potentially due to excess circulating cortisol. This is similar in humans where Cushing’s syndrome has been implicated in Achilles tendon rupture.

**Exercise to mitigate the effects of endocrine disease**

In humans, there is some indication that an active lifestyle and diet modification have beneficial effects on prevention of MetS and can relieve the symptoms associated with OA. Similar findings have been seen in horses, and exercise and dietary changes are the primary management strategies employed when treating animals with EMS or ID. A novel type of exercise investigated in humans, which has been gaining popularity in horses, that could be an effective therapeutic intervention, is resistance band training. In recent studies, resistance band training and a restricted calorie diet ameliorated many of the symptoms of MetS, as did just 1 hour a day of resistance band training alone. Resistance band training in the horse has been shown to increase thoracolumbar stability, although it was not shown to activate and strengthen the multifidus muscle while walking over caudalitis. Resistance band training is attractive as a therapy as it can be performed at the walk, allowing previously laminic ponies and horses to still exercise. More work should be done to concurrently assess endocrine status changes and muscle development with resistance band training.

**Endocrine Effects on Cardiovascular Health**

Football linemen, while athletes, have higher percentages of cardiovascular death and MetS than nonlinemen and the general public. The effects of EMS on cardiovascular health have received less attention, as cardiovascular disease-related death is uncommon in equines in general. However, EMS horses during a euglycemic-hyperinsulinemic clamp procedure demonstrated maintenance of systolic, diastolic, and mean arterial pressure over time as compared to control horses where blood pressure decreased, suggesting cardiac dysfunction in the EMS group. Ponies with EMS have also been found to have myocardial hypertrophy correlated to insulin responses during dynamic testing as compared to controls but no difference in blood pressure. Shetland ponies, a high-risk breed for EMS, when placed on a high-energy diet, developed increased blood pressure and ultimately cardiac hypertrophy, albeit no arrhythmias, when compared to ponies on a control diet. This suggests that our equine athletes that are considered high-risk breeds should not be fed the high-energy diets that are often given to performance horses. Performance horses with EMS should also have more attention paid to clinical signs that could indicate early cardiac dysfunction.

**Endocrine Effects on Reproductive Health**

**Reproductive health**

Obesity and MetS are linked to infertility in humans. In mares with EMS, the intrafollicular environment, ie, the follicle, the oocyte, and the uterus, is affected, and in the latter case inflamed, which could have an impact on breeding success. Other work has shown the negative impact of endocrine disease (both EMS and PPID) on reproductive seasonality and ovulation, with subsequent increased incidences of pregnancy complications. Selection of desirable traits for an equine athlete during breeding comes with both risk and reward; however, optimization of whole body health is important for short-term and long-term breeding and athletic success. There are also costs (more time...
away from sport, financial ramifications) associated with a missed breeding season due to an endocrine disorder. Athletic mares with endocrine disease that are having issues with infertility or maintaining a pregnancy may require more aggressive therapeutic management and possibly need assisted reproduction techniques such as embryo transfer.

### Developmental origin of metabolic disease and placental function

The concept that the maternal environment can lead to permanent fetal adaptations that manifest at different stages of the offspring’s life is a well-recognized theory known as the Developmental Origin of Health and Disease. Data in the horse are relatively scarce but have highlighted the role of the maternal environment on postnatal body conformation, sexual maturity, immune response, metabolism, osteoarticular state, and cardiovascular function of the foal. A specific area of interest has been the evaluation of the effect of maternal overnutrition and obesity on foal outcomes. Overfeeding of mares during pregnancy to induce late-gestation obesity resulted in foals with delayed testicular maturation and insulin resistance up to 2 years of age. Maternal overnutrition was also found to reduce colostrum IgG concentrations, resulting in lower foal serum IgG levels at 12 and 18 hours of age, although none of the foals were diagnosed with failure of passive transfer. Further, foals born to mares that were obese at the time of insemination and throughout their pregnancy had higher serum amyloid A concentrations, insulin resistance at 6 and 18 months of age, and an increased incidence of OCD at 1 year of age. Embryos from obese mares had increased expression of inflammatory genes, lipid homeostasis, and oxidative stress. These studies indicate that maternal obesity can have a deleterious affect on offspring health, reproduction, metabolism, and athletic performance. Importantly, although the obese mares present in these studies had metabolic values higher than nonobese mares, values were not consistent with a diagnosis of EMS or impaired insulin sensitivity only occurred at certain points during gestation. Therefore, the effect of EMS on foal health remains an area to be investigated.

The link between maternal ID and adverse health outcomes in their offspring is well established in diabetic pregnant women and has been linked to placental pathology. Increased placental weight and thickness have been correlated with higher fetal birth weights and shoulder dystocia, doubling the risk of a non elective cesarean section in diabetic women. In addition, placental histological changes include an increased incidence of ischemia, infarction, fibrinoid necrosis, villous immaturity, edema, choriangiosis, and fetal vessel thrombosis. These lesions result in altered fetoplacental circulation and impaired oxygen transport and have been independently associated with stillbirths and fetal morbidity, confirming significant placental pathology in women with gestational diabetes.

Compared to other species, the equine fetus relies more heavily on placental transfer of glucose and nutrients due to the limited degree of deamination and gluconeogenesis, which could indicate an even more critical role of the placenta in EMS-induced fetal pathologies. In addition, this early fetal programming may lead to long-term insulin resistance in the offspring, increasing the risk of juvenile and adult obesity and EMS as seen in humans. Obesity has been shown to limit a horse’s athletic career through a reduced physiological exercise response and athletic performance and increased joint strain in adults, as well as having a higher incidence of developmental orthopedic problems in growing horses. Further, having an increased risk of EMS represents a vicious cycle that will continue to perpetuate this disease, increasing its economic impact on the industry.

### Multiomic Insights into Pathophysiology, Novel Diagnostic Tests, and Biomarkers of Disease

#### Genomics

The primary goal of genetic discovery is to gain insight into the pathogenesis of a trait for the development of genetic tests, which can be used to identify affected individuals and to help guide breeding decisions. This is of particular importance for EMS and PPID as the current diagnostic tests are imperfect and often miss early onset individuals. Therefore, there is a need to be able to identify high-risk individuals before they develop clinical signs to implement proper environmental or pharmaceutical intervention thus securing longer athletic life spans while improving horse welfare.

Unlike Mendelian (simple) traits where a single genetic mutation leads to a phenotype, endocrine disorders are often complex genetic diseases with dozens to hundreds of genetic risk alleles contributing to their phenotypic expression. Further, these genetic risk alleles can be unique to specific groups or have variable allele frequencies, variant effect size (amount of phenotypic variation explained by the variant), and penetrance across groups. Therefore, a single genetic test would provide minimal information about an individual’s genetic risk for an endocrine disorder and instead requires a panel of well-validated shared and group-specific genetic risk alleles in conjunction with their variant effect size (Table 1). Interpretation of these results would not be as straightforward as Mendelian diseases and would benefit from the consultation of a geneticist or genetic counselor, particularly when including environmental risk factors to determine an individual’s overall risk of developing disease or when evaluating the impact of specific genetic alleles on the panel that have been associated with muscle or reduced athletic function.

Toward these goals, advances have been made in the genetic discovery of EMS. There is concrete evidence that EMS has a strong genetic contribution with hundreds of shared and breed-specific regions of the genome contributing to its development.
Table 1—Hypothetical example for an EMS genetic risk panel.

<table>
<thead>
<tr>
<th>Genetic Risk</th>
<th>Breed</th>
<th>Affect Size</th>
<th>Allele relative risk</th>
<th>Horse 1</th>
<th>Horse 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 1</td>
<td>All</td>
<td>Large</td>
<td>0.7</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>Allele 2</td>
<td>All</td>
<td>Large</td>
<td>0.6</td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>Allele 3</td>
<td>All</td>
<td>Small</td>
<td>0.2</td>
<td>POS</td>
<td>POS</td>
</tr>
<tr>
<td>Allele 4</td>
<td>Morgan</td>
<td>Moderate</td>
<td>0.5</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>Allele 5</td>
<td>Morgan</td>
<td>Moderate</td>
<td>0.5</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>Allele 7</td>
<td>Ponies</td>
<td>Large</td>
<td>0.9</td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>Allele ...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<tr>
<td>Genetic RR</td>
<td>...</td>
<td>...</td>
<td>1.7</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

A genetic panel that will accurately assess genetic risk for a complex genetic disease would need to include both shared and breed specific risk alleles and their estimated allelic affect size and relative risk. For example, if the genotypes for a hypothetical pony (horse 1) include 2 across-breed alleles of large and small affect size and 1 pony-specific allele of large effect, then that individual would have a hypothetical EMS genetic relative risk of 1.7. If we compare that to the genotypes for a hypothetical Morgan horse (horse 2) who has 1 across breed allele of small effect and 2 breed specific risk alleles of moderate affect, then this individual’s hypothetical EMS genetic relative risk would be less than horse 1.

NEG = Negative. POS = Positive. RR = Relative risk. (...) = indicates the continuation of the number of alleles that would be on the panel since it will be more than seven.

However, despite the identification of multiple associated genomic regions, only 2 genetic variants have been proposed,<sup>158,159</sup>1 of which was not replicated in follow-up studies,<sup>160,161</sup> Therefore, it is imperative to continue to explore the specific genes and genetic risk factors for EMS. However, given the complexity of the EMS phenotype and the high percentage of associated SNPs located in noncoding regions of the genome,<sup>162</sup> an integrated multiomics approach (Figure 2) must be implemented to aid in this genetic discovery. Most previous studies of EMS have focused on the individual effects of the genome,<sup>157,158,162</sup> transcriptome,<sup>163</sup> or metabolome,<sup>164,165</sup> and while each type of omics data provided a list of differences associated with EMS, it only represented 1 layer of the genetic effect that limited the identification of specific risk alleles. In contrast, the rationale for a multiomics approach is that phenotypic effects may be expressed through alterations in gene expression or regulation, or protein abundance, function, or regulation, which manifest through alterations in metabolic pathways and functions, aiding in the genetic discovery of causative alleles. This approach, used successfully for genomic discovery in humans with complex genetic diseases, identified four associated genomic regions for EMS in Arabians<sup>166</sup> and was considered a priority in the USDA Blueprint for Animal Genome Research toward bringing genome to phenotype.<sup>167</sup>

Of particular interest is the inclusion of the epigenome, a layer of omics data that has yet to be explored in horses with EMS. Epigenetics are cell-specific changes in gene expression that are independent of DNA sequences. Epigenetic modifications are initially established in utero and can be affected by maternal health and nutrition; however, many epigenetic changes can be altered throughout the offspring’s life secondary to environmental exposures. In humans, a link between gestational diabetes and risk of metabolic disorders in their offspring has been well established, and these changes have been associated with alterations in the placental or offspring epigenome.<sup>168</sup> Another study<sup>169</sup> identified differential DNA methylation levels near the leptin genes, which were found to mediate the effects of maternal blood glucose concentrations on neonatal leptin concentrations. Therefore, the effect of the mare’s EMS status may have a significant impact on her offspring’s risk of ID and warrants further exploration.

The contribution of genetics to PPID has not yet been proven. Therefore, the first step is to confirm a genetic etiology, which will require using big data in well-phenotyped individuals. Whole-genome sequencing or SNP data could be used to estimate the heritability of PPID and to perform a genome-wide association analysis for the identification of putative functional alleles. Alternatively, a candidate gene approach would determine if horses with PPID suffer from the same causal variants as Parkinson disease, a human disease also caused by dopaminergic neurodegeneration in aged individuals. This approach would be particularly useful in horses with suspected familial PPID (high prevalence of PPID within a line) as these cases are more likely to contain few genes of a larger impact compared to sporadic cases. Finally, a multiomics approach could identify biological pathways responsible for PPID. Thus, these genetic discoveries will be essential to help elucidate the underlying pathophysiology of PPID and to determine an appropriate genetic test to accurately identify high-risk individuals.

Metabolomics

Metabolomics, the study of molecules involved in cellular metabolism, refers to the global interrogation of the biochemical components in a biological sample such as serum, plasma, urine, feces, saliva, or cerebrospinal fluid. Small molecule metabolites such as lipids, amino acids, peptides, nucleic acids, organic acids, fatty acids, vitamins, carbohydrates, hormones, and steroids are the end products of cellular regulatory processes. As the ultimate response of biological systems to genetic or environmental changes, the comprehensive measurement of metabolites reflects perturbations in metabolism,
thus providing insight into biological mechanisms and pathogenesis of disease through an understanding of molecular pathways. In addition, metabolomics defines metabolic changes in both the physiological and pathological states. On the most basic level, metabolomics can differentiate between healthy individuals and diseased individuals.170,171

Studies172–176 in horses have identified characteristic metabolic signatures of type II diabetes mellitus, obesity, and fatty liver disease. A consistent pattern of reduced glycine and acylcarnitines177–178 with increased concentrations of valine, isoleucine, and α-hydroxybutyrate179,180 has been associated with both basal and dynamic measures of insulin resistance.177,178 The metabolic perturbations that characterize and contribute to type II diabetes mellitus are evident years before disease onset, and comprehensive metabolomic profiles have elucidated alterations in novel metabolic pathways implicated in disease development.

In horses, examination of the serum metabolome in a cohort of Welsh ponies demonstrated significant differences in metabolites involved in the tricarboxylic acid (TCA) cycle, fatty acid metabolism, and branched-chain amino acid metabolism when comparing animals with and without the EMS phenotype.180 In addition, evaluation of the fecal metabolome of obese horses and nonobese horses noted significant differences in metabolites of the TCA.181

Metabolomics in human sport and exercise has revealed insights into muscle energetics, and it is just starting to be of interest in the horse.182 In a paper183 on acute fatigue and training in normal horses, increases in lipids were noted in response to training. This increased abundance of lipids, if present after training in horses with ID and lipid dysregulation, could be of concern, especially as this response was seen in humans with MetS after high-intensity interval training.184 In that study,183 the lipid increase was more pronounced after afternoon training sessions. In a second study,185 humans with MetS saw greater improvements in insulin sensitivity after a morning workout session. If these findings hold true for ID horses, morning training sessions may be the best time to perform exercise for our EMS-affected individuals. Further, these approaches allow for better connection into prevention and treatment, while as shown with the human exercise work, it may guide other management strategies.

Transcriptomics

Transcriptomics, or analysis of RNA transcripts made by a genotype at a certain time, promises to be a powerful way to assess genes that affect both metabolic health and performance.186,187 In Arabians, a breed “at risk” for EMS, there was an upregulation in the PI3K-Akt signaling pathways in trained endurance horses as compared to untrained horses, suggesting a more efficient glucose uptake, which can explain the insulin-sensitizing effect of exercise.188 Transcriptomic analysis also has the ability to analyze gene coexpression networks and reveal their biological functions, in different tissues from the same individuals, assessing “cross-talk” as has been described in muscle and adipose tissue in horses.185 A human paper189 taking this approach compared metabolically healthy and unhealthy obese individuals and found IL-6 and IL-1β were differentially coexpressed genes, and similar studies could better reveal the pathophysiology of endocrine disorders in the horse.

Proteomics

Proteomics are also an up-and-coming research tool to assess therapeutic interventions and explore pathophysiology of disease. In a paper190 investigating dietary omega-3 supplementation in ID mares, proteomics of the uterine fluid found inflammatory biomarkers decreased when compared to controls and ID mares not receiving the supplement. Proteomic approaches have also shown utility in assessing treatment and biomarkers for equine muscle diseases191 and biomarkers for human OA.192 In humans with type II diabetes mellitus, GLUT4 protein expression increased with exercise, helping to increase their insulin sensitivity.193

Microbiomics

The gastrointestinal tract of humans and animals is composed of numerous microorganisms that play a role in overall health. In humans, the gastrointestinal microbiome has been shown to be an important factor in the development of obesity and type II diabetes and it is postulated to play a role in the development of metabolic OA.194,195 The complex microbial population of the equine intestinal tract plays a key role in health and disease, and the diverse, variable composition and complexity of this population are starting to be revealed, now that methods to characterize the genetic makeup of the microbiota have been developed.196–198 In a small cohort of horses with EMS, there was a decrease in microbiota diversity compared to healthy controls.200 In addition, horses with chronic laminitis were noted to have differences in microbiota diversity compared to healthy controls. In mildly ID ponies, the introduction of pasture was able to perturb their microbiome more significantly than metabolically healthy ponies.21 In addition, ID horses on metformin, a common treatment for EMS, experienced a microbiota change similar to that seen in humans on that drug.22 The availability of next-generation sequencing and bioinformatics methods offers an opportunity to improve understanding of the role of the gastrointestinal microbiome in the pathogenesis of metabolic disorders and ID.

In summary, the effects of endocrine disease on musculoskeletal, cardiovascular, and reproductive health have repercussions on athletic performance in both humans and horses. Multimomic approaches provide information about molecular and cellular processes in endogenous and exogenous pathways and insight into physiology and pathophysiology of an individual. Further, these approaches allow for better connection of genotype and phenotype leading to identification of biomarkers that can provide insight for
novel diagnostic and therapeutic targets and strategies. Formulation of a management plan for individuals with metabolic disorders should focus on interventions that improve overall health and athletic performance.

Acknowledgments

None of the authors have any financial or personal relationships that could bias the content of the manuscript.

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