Equine Metabolic Syndrome: Challenges and Advances

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Defining and Diagnosing EMS

Equine metabolic syndrome (EMS) was first recognized as a problematic health condition of the obese, easy-keeper or good-doer horse in 2002 (Johnson). A major advance came in 2010 when a Consensus Statement of the American College of Veterinary Internal Medicine (ACVIM) panel of experts provided the veterinary community with information characterizing and defining the EMS phenotype and formally recognizing it as a clinical syndrome of the horse. The challenge remains in continuing to educate horse owners about the seriousness of EMS and to make all aware that the fat horse is not happy but suffering from a condition called EMS.

While statistics disclosing the percentage of horses suffering from EMS are scarce, one needs only to peruse the popular equine press to locate a myriad of articles on the subject of obesity and/or EMS to find ample evidence that EMS is a major concern for the horse population. Unfortunately, EMS is a complex disorder with many unknowns. To date, there are no epidemiology studies of EMS, although anecdotal evidence suggests that typically middle-aged horses (5 to 14 years old) are mostly affected and certain breeds may be predisposed to EMS such as some pony breeds (Welsh and Shetland) and horse breeds (Morgans, Paso Finos, Spanish Mustangs, Quarter Horses, and Arabians).

The EMS phenotype manifestations include increased regional or general adiposity, insulin resistance, and a predisposition toward developing laminitis (Frank, 2009). Other phenotypes of EMS include dyslipidemia, hyperleptinemia, arterial hypertension, and increased system inflammation (Frank, 2009). While physical characteristics (regional adiposity, neck crest, and increased body condition score of >7) aid in diagnosing a horse with EMS, laboratory tests are critical in confirming EMS and perhaps the severity of EMS. Diagnostics remain a challenging area, not only to improve diagnostics for EMS but to provide an easier, more confirmatory test for horse owners and for the horses being tested.

Resting insulin concentrations remain to be the standard test for diagnosis of EMS, though the results can be confounded by many variables such as stress and owner compliance to feed restrict the horse the night before the blood sample is collected. Hyperinsulinemia (>20μU/mL) is found in EMS horses when a blood sample is collected during the morning hours (8-10 a.m.) after approximately six hours of grain withholding and minimal amounts of hay provided (Frank, 2009). If increased insulin is not the result obtained in a horse with outward physical characteristics of EMS, a dynamic test should be performed. Typically, an IV glucose tolerance test is used to raise blood glucose and insulin levels to determine how long it takes for these measures to return to baseline. Recent advances for this diagnostic have been developed by Dr. Nick Frank with an oral glucose test, which uses the same concept as the IV tolerance test, except this test uses oral glucose and is less labor intensive for both the human and horse. While EMS diagnostic tests have historically relied on insulin and glucose to be modified and measured, other physiological parameters should be considered for diagnostic measurements given their potential role in EMS. A test panel that does not require fasting that could provide a more comprehensive characterization of the EMS horse would be an advancement. Measurement of inflammation might perhaps be part of this diagnostic panel, given the role inflammation plays in obesity, as discussed below.

What Do We Know About the Pathophysiology of EMS?

Unfortunately, we have limited understanding of the pathophysiology of EMS due to its complexity involving both alterations in metabolism and inflammation (Frank, 2009). EMS often results from overnutrition and lack of exercise, especially in the easy keeper, which eventually leads to obesity or increased adiposity (Frank, 2009). Chronic obesity can develop in horses when they reach maturity and may continue for more than 20 years. Obesity in horses is defined using a standardized 1 (poor) to 9 (obese) body condition scoring system (Henneke et al., 1983) or by using morphometric measurements (Carter et al., 2009). More precise measurements of body fat mass can be obtained by measuring rump fat thickness with B-mode ultrasound to determine percent body fat (Powell et al., 2000).

Increased adiposity, or obesity, is also associated with increased inflammation. Adipose tissue is an endocrine organ, capable of producing hormones and inflammation-related cytokines (Ruan and Lodish, 2003). In fact, we have shown that tumor necrosis factor-alpha (TNF-alpha), a pro-inflammatory cytokine, is produced by adipocytes derived from adipose tissue from an obese horse (Vick et al., 2008). In addition, it has recently been shown that different adipose depots from the horse secrete many different types of pro-inflammatory cytokines including IL-1beta, IL-6, and TNF-alpha (Burns et al., 2010). In a study performed in collaboration with our laboratory, we showed that obesity and insulin resistance were associated with elevated plasma TNF-alpha concentrations (Vick et al., 2007). In addition, others have shown that ponies affected with EMS have higher plasma TNF-alpha concentrations (Treiber et al., 2009). TNF-alpha in other species has been directly implicated in the development of insulin resistance. In fact, administration of exogenous TNF-alpha results in insulin resistance in cattle and dogs (Lang et al., 1992; Kushibiki et al., 2001), and TNF-alpha has been shown to directly interfere with insulin signaling (Hotamisligil et al., 1996). The mechanisms of insulin resistance are not completely characterized in the horse; however, it is implicated that inflammatory cytokines play a role in this process, as stated above (Vick et al., 2007).

Insulin resistance or decreased insulin sensitivity can be simply defined as a failure of insulin to stimulate glucose uptake by metabolically active tissues (muscle, adipose, and liver) when nutrients are abundant after feeding (Kahn, 1978). After the intake of nutrients, glucose stimulates the production of insulin by the pancreas. In the skeletal muscle, adipose, and liver tissues, insulin binds to its receptors on the surface of plasma membranes, which triggers a series of intracellular events that ultimately leads to the movement of glucose transporter (GLUT4) proteins to the plasma membrane and facilitates rapid glucose uptake (Kitamura and Accili, 2004). Normally, binding of insulin stimulates the intrinsic tyrosine kinase of the insulin receptor, resulting in autophosphorylation of the beta subunits on tyrosine residues and subsequent phosphorylation of insulin receptor substrate-1 (IRS-1). TNF-alpha markedly decreases autophosphorylation of the insulin receptor and subsequent tyrosine phosphorylation of IRS-1, which causes this receptor to be less active and thus decreases the GLUT4 uptake of glucose in metabolic active tissues, muscle, and adipose (Hotamisligil et al., 1996). In humans, skeletal muscle insulin resistance is a cardinal feature involved in metabolic syndrome (Benton et al., 2008). In addition to inhibition of insulin receptor signaling, TNF-alpha also increases lipid storage and decreases lipid breakdown, which is involved with insulin resistance (Ruan and Lodish, 2003). Furthermore, TNF-alpha-mediated activation of the transcription factor NF-kappa beta further suppresses a number of adipoctye-abundant genes involved in the proper uptake and storage of free fatty acids and glucose in adipocytes. Thus, there is a causative relationship between inflammation and obesity-related insulin resistance in the horse. Moreover, increased inflammation contributes to higher risk of laminitis in obese, insulin resistant equids

(Geor and Frank, 2009). Therefore, a treatment option that not only targets insulin metabolism but inflammation is critical for horses with EMS.

What Can We Learn From Caloric Restriction?

A review of the literature would lead one to believe that the treatment for EMS is simple: induce weight loss by decreasing dietary intake, thereby decreasing adiposity in the horse and improving insulin resistance. Indeed, insulin sensitivity significantly increases as body fat mass decreases when weight loss is induced in obese ponies through dietary energy restriction (Van Weyenberg et al., 2008). It has also been shown that insulin sensitivity decreases as body fat mass increases when horses are deliberately overfed to increase body fat mass (Carter et al., 2009). Dietary manipulation of body fat mass is therefore an effective method of raising or lowering insulin sensitivity in horses. Decreasing body fat has not only been shown to improve insulin sensitivity but has also been shown to modulate inflammation. We have recently shown that decreasing adiposity by restricting caloric intake in the obese horse results in significantly reduced levels of inflammatory cytokine production, particularly serum levels of TNF-alpha protein (Adams et al., 2009). Decreasing fat mass not only improves insulin sensitivity but dampens inflammatory cytokine production, both components of EMS. However, the effectiveness of any weight-loss program depends on the willingness of the owner to not only implement the process but to continue these dietary changes over the lifespan of the horse; this is why it is critical to find an alternative treatment that modulates both inflammatory cytokine production and insulin sensitivity similar to dietary restriction in the EMS horse.

Caloric restriction, in humans and rodents, has provided insight to mechanisms that modulate insulin sensitivity and inflammation in obese subjects. Caloric restriction in both obese rodents and humans has been shown to improve endpoint measures, similar to that in the obese horse, including improved insulin sensitivity and decreased inflammation (Picard and Auwerx, 2002; Clement et al., 2004; Heilbronn et al., 2006; Zheng et al., 2009; Crisostomo et al., 2010; Ye and Keller, 2010); moreover, caloric restriction has provided insights to the mechanisms that have been shown to improve insulin sensitivity and decreased inflammation, that involve the activation of a "metabolic master switch," SIRT1 (Leibiger and Berggren, 2006).

SIRT1 is the mammalian sirtuin (silent information regulator 2 homolog) 1, which was named after the *Saccharomyces cerevisiae* gene Sir2 because of its homology (Dali-Youcef et al., 2007; Canto and Auwerx, 2009; Yu and Auwerx, 2009). There are seven sirtuin families of proteins, SIRT1-SIRT7 (Dali-Youcef et al., 2007). All sirtuins are NAD+-dependent protein deacetylases that catalyze the removal of acetyl groups from lysine residues in substrate proteins (Dali-Youcef et al., 2007; Yu and Auwerx, 2009). Sir2 was first identified in caloric-restricted yeast and was found to be involved in chromatin remodeling and gene silencing, which caused the prolongation of the lifespan of yeast. More importantly, it has been shown that caloric restriction of *Drosophila*, increases Sir2 activation (Rogina et al., 2002). Caloric restriction in humans and rodents upregulates SIRT1 expression in many metabolically active tissues, including muscle, adipose, and lymphocytes (Cohen et al., 2004; Rodgers et al., 2005; Gerhart-Hines et al., 2007; Chen et al., 2008; Crujeiras et al., 2008). Further, SIRT1-transgenic mice show phenotypes resembling caloric-restricted mice (Bordone et al., 2007). Activation of SIRT1 in mice fed a high-caloric diet protects them from insulin resistance (Pfluger et al., 2008). In humans, it has recently been reported that certain genetic variations of SIRT1 gene influence the survival of subjects with metabolic syndrome (Zillikens et al., 2009a,b). In the horse, we have recently been able to show that serum from dietary-restricted horses increases SIRT1 expression measured by the "in vitro assay of inducible SIRT1 expression" (Le Couteur et al., 2011),

developed at the National Institutes of Aging (NIA) (data not shown). Increasing SIRT1 expression and activity is important because this protein regulates a variety of transcription factors and transcriptional co-regulators in metabolic and inflammatory processes (Imai, 2010).

In the muscle, SIRT1 increases the expression of one of the most versatile metabolic coactivators, peroxisome proliferator-activated receptor-coactivator-alpha (PGC-1gamma) (Benton et al., 2008; Amat et al., 2009). PGC-1gamma is a master regulator of mitochondrial biogenesis and has been shown to activate genes that are upregulated during exercise and caloric restriction, including those that regulate fatty acid oxidation and insulin signaling in the muscle. In fact, PGC-1gamma activation has been shown to increase insulin sensitivity in the muscle and reductions in PGC-1gamma expression have been shown in insulin-resistant muscles (Benton et al., 2008). During dietary restriction and exercise, SIRT1 activates PGC-1gamma in muscle cells and mediates the switch from using glucose to free fatty acids, thereby modulating glucose homeostasis and improving insulin sensitivity (Rodgers, et al., 2005; Gerhart-Hines et al., 2007).

In white adipose tissue (WAT), SIRT1 inhibits a transcription factor peroxisome proliferator-activated receptor-gamma (PPAR-gamma). PPAR-gamma is known as the "fat regulator" and when upregulated is responsible for fat storage and adipogenesis, maturation of pre-adipocytes to adipocytes capable of producing TNF-alpha. During caloric restriction, SIRT1 represses PPAR-gamma gene expression in adipocytes, thereby increasing lipolysis or fat mobilization, measured by increased hydrolysis of triglycerides or decreased triglyceride levels (Picard and Auwerx, 2002; Hayashida et al., 2010).

In white blood cells, SIRT1 expression is increased by caloric restriction and has been shown to decrease inflammatory cytokine production via decreasing the activity of transcription factor, nuclear factor-kappa beta (NF-kappa beta), which is responsible for activating genes responsible for inflammatory cytokine production (Crujeiras et al., 2008; Csiszar et al., 2009; Yoshizaki et al., 2010). Taken together, activation of SIRT1 and related pathways, induced by caloric restriction, are key to identifying a targeted treatment for insulin resistance.

SIRT1 Activator, Resveratrol, a Caloric Restriction Mimetic for the Treatment of EMS?

Currently, there are limited available medical treatments for EMS horses that will improve insulin resistance and decrease inflammation to minimize the risk of these horses developing laminitis. The common recommended treatment and management of EMS is decreasing caloric intake and increasing exercise (Frank, 2009). While caloric restriction in some horses can improve EMS by increasing insulin sensitivity and decreasing inflammation, it can take months to achieve this improvement and is not always successful. Moreover, some horses are unable to exercise due to debilitating effects of laminitis or other conditions. Therefore, alternative approaches may be required.

Only two pharmaceuticals, metformin (decreases hepatic glucose production) and levothyroxine sodium (synthetic thyroxine), have been investigated for off-label use as treatment for insulin resistance in the horse (Durham et al., 2008; Frank et al., 2008a,b). The mechanism of action and targets of these two drugs are not known, and published results have provided conflicting data. Safety concerns remain regarding the prolonged used of these compounds (Vick et al., 2006; Durham et al., 2008; Frank et al., 2008a,b). Therefore, a safe and effective means of treating both the insulin resistance and inflammatory component of EMS is needed.

The ability to mimic the effects of caloric restriction without undergoing the physical means of decreasing adiposity without significant weight loss is key to implementing a therapeutic treatment for metabolic syndrome. Resveratrol has been identified as a key SIRT1 activator causing

beneficial downstream signaling events that mimic protective mechanisms induced by caloric restriction (Barger et al., 2008; Chung et al., 2010). Resveratrol, a polyphenolic phytoalexin found in grapes, red wine, and various berries, has undergone considerable evaluation by the academic and biopharmaceutical research communities over the last 10 years (Pervaiz and Holme, 2009). Resveratrol was first used as a traditional Chinese and Japanese medicine for treatment of human inflammatory, allergic, hypertensive, and lipid diseases (Juan et al., 2002). Furthermore, this compound is thought to play a major role in the phenomenon known as the "French Paradox" (Renaud and Delorgeril, 1992); the surprising relationship between frequent consumption of red wine and reduced mortality due to heart disease. Resveratrol has been shown to safely improve insulin resistance in diabetic rats by decreasing glucose and increasing insulin via altering activities of key enzymes involved in metabolism (Palsamy and Subramanian 2008, 2009). In fact, the compound is currently in human clinical trials as a potential treatment for Type II diabetes, which has similarities to EMS (Frank, 2009; Geor and Frank, 2009). Most important, resveratrol has been shown to stimulate protective signaling pathways otherwise triggered by caloric restriction (Smith et al., 2009). In fact, resveratrol has been shown to activate SIRT1 and PGC-1gamma in muscle tissue, thereby improving insulin sensitivity in mice (Lagouge et al., 2006). Resveratrol has also been shown to upregulate SIRT1 activity and downregulate PPAR-gamma in adipocytes (Costa et al., 2010). Many studies have documented the anti-inflammatory activity of resveratrol using various models of inflammation (de la Lastra and Villegas, 2005). These studies have shown that resveratrol inhibits NF-kappa beta, in turn decreasing the gene expression of pro-inflammatory cytokines, including TNF-alpha (de la Lastra and Villegas, 2005; Chaudhary and Pfluger, 2009). We have recently designed PCR primers to detect the gene expression of equine SIRT1, PPAR-gamma, and PGC-alpha in different tissues including adipose, muscle, and lymphocytes and will determine the expression of these genes in EMS resveratrol-treated horses. In addition, we have recently been able to show resveratrol can decrease equine inflammatory cytokine production in vitro and in vivo in an "inflamm-ageing" geriatric horse model.

In short, insulin resistance and increased body fat or obesity are considered key risk factors for EMS (Geor and Frank, 2009; Geor and Harris, 2009). It has been reported that obesity affects 19% of horses in the United States (Geor and Harris, 2009). One of the factors that make EMS a devastating condition for the horse is its association with increased laminitis risk (Frank et al., 2006; Treiber et al., 2006; Bailey et al., 2008). Laminitis is a degenerative inflammatory condition of the hoof that often leads to euthanasia (Johnson, 2002; Johnson et al., 2004). Improved, comprehensive, and less labor-intensive diagnostics for both the horse owner and horse to diagnosis EMS are warranted. Currently there are limited available medical treatments for EMS horses that will improve insulin resistance and decrease inflammation to minimize the risk of these horses developing laminitis. Thus, a safe and effective means of treating both the insulin resistance and inflammatory component of EMS is needed. The goal for veterinarians and horse owners is to prevent EMS or treat the condition before it manifests as laminitis.

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