Diagnosis and Management of Myofibrillar Myopathy in Warmblood Performance Horses

Stephanie J. Valberg, DVM, PhD, DACVIM, DACVSMR

Author’s address: Mary Anne McPhail Dressage Chair in Equine Sports Medicine, Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI 48824; e-mail: valbergs@msu.edu. © 2021 AAEP.

1. Introduction

Exertional muscle disorders cause muscle pain and/or weakness that impair athletic performance. One subset of exertional myopathies, exertional rhabdomyolysis, is characterized by muscle degeneration and elevations in serum creatine kinase (CK) and aspartate transaminase (AST) activities. Another subset of exertional myopathies that includes type 2 polysaccharide storage myopathy (PSSM2) and myofibrillar myopathy (MFM) in Warmbloods is characterized by exercise intolerance without elevations in serum CK and AST activities.1,2 The lack of a readily available blood test to screen these horses for a myopathy and the overlap in clinical signs with other disorders necessitate a detailed diagnostic work up to rule out other more common causes of exercise intolerance before proceeding to a muscle biopsy.

2. What is MFM?

Myofibrils contain myofilaments aligned within contractile units called sarcomeres that are bordered by Z discs. Z discs provide structural support as well as mechanosignaling whereby tension during muscle contractions sends Z disc proteins to the nucleus that activate genes which initiate training adaptations.3 Desmin is a cytoskeletal protein located at the Z disc that aligns adjacent sarcomeres. Horses with MFM have disrupted myofibrillar alignment within the sarcomere, Z disc disruption, and ectopic accumulation of desmin in select type 2A (fast-twitch oxidative) muscle fibers.2 Muscle samples from MFM horses may be characterized by cytoplasmic aggregates of glycogen, likely because pools of glycogen form within disrupted myofibrils.2 Transcriptomic and proteomic studies of muscle from MFM Warmbloods suggest that the basis for MFM could be aberrant gene and protein expression that impact molecular signaling, the alignment of contractile proteins, mitochondrial function, and oxidative stress.4 This hypothesis, however, requires further investigation.

3. Are PSSM2 and MFM the Same Disease in Warmbloods?

Type 1 polysaccharide storage myopathy (PSSM1) refers to horses with excessive muscle glycogen concentrations, abnormal amylase-resistant polysaccharide in muscle histology, and a mutation in the glycogen synthase 1 gene (GYS1).5,6 The term PSSM2 is applied to horses with abnormal appearing glycogen aggregates in muscle biopsies that do not possess the GYS1 mutation.7 PSSM2 in essence represents a
histologic description of glycogen aggregates in horses with exercise intolerance rather than a specific disease. To determine if PSSM2, like PSSM1, is associated with excessive glycogen storage, glycogen concentrations recently have been measured in a variety of breeds diagnosed with PSSM2. Mean glycogen concentrations in Quarter Horses diagnosed with PSSM2 (n = 67, 130 ± 60 mmol/kg) were found to be lower than those of PSSM1 Quarter Horses (n = 20, 175 ± 39 mmol/kg) and significantly higher than control Quarter Horses (n = 185, 80 ± 27 mmol/kg).

Glycogen concentrations in Warmblood and Arabian horses with PSSM2, however, were found to be similar to those in control horses. Thus, the term PSSM2 is a histologic descriptor that may encompass different diseases in different breeds. Further research into PSSM2 found that some PSSM2 Warmbloods have aggregates of desmin, myofibrillar disarray, and Z disc streaming resembling a condition described in humans as MFM. Because clinical signs of exercise intolerance are very similar between Warmblood horses with PSSM2 and MFM, and horses with MFM can have glycogen aggregates resembling PSSM2, it is suspected that PSSM2 in Warmbloods potentially represents an earlier stage of MFM. However, more research is required to state this definitively. The aggregates of amylase-sensitive glycogen used to diagnose PSSM2 in Warmbloods could occur because of pooling of glycogen in breaks within the myofibrils seen in electron microscopy of MFM horses.

4. Clinical Signs of PSSM2 and MFM
Warmblood horses diagnosed with PSSM2 and MFM have an insidious onset of exercise intolerance notable by 6 to 8 years of age characterized by a lack of stamina, unwillingness to go forward, inability to collect, abnormal canter transitions, and inability to sustain a normal canter. Stiffness, muscle pain, and rarely an episode of exertional rhabdomyolysis are reported with PSSM2 and MFM as well as a hindlimb lameness that remains unresolved after a thorough orthopedic assessment. Serum CK and AST activities are usually within normal limits unless samples are taken in conjunction with rare episodes of exertional rhabdomyolysis.

5. Diagnosis
A physical examination that includes a full lameness and neurologic examination is strongly recommended prior to pursuing a diagnosis of PSSM2 or MFM (Fig. 1). This is because other causes of poor performance are much more common than PSSM2 and MFM and because the sensitivity and specificity of muscle
biopsy histopathology leave room for false-positive and false-negative diagnoses. Tack and saddle fit should be inspected, and the horse should be observed under-saddle to determine if there are potential rider, training, or behavioral issues that are primary contributors to poor performance. Other potential diagnostics include endoscopy to rule out gastric ulcers, diagnostic joint/nerve blocks for associated lameness, radiography, ultrasonography, and scintigraphy. In the absence of other causes of reluctance to go forward and engage the hind quarters, a treatment trial of management changes or a muscle biopsy are reasonable next steps (Fig. 1). One potential complication of a treatment trial is that, unlike PSSM1, aggregates of polysaccharide and desmin appear to decrease with the prescribed management, leading to a potential false-negative diagnosis if biopsies are pursued after initiating appropriate management.举止

Muscle Biopsy

Either the gluteal muscle or the semimembranosus/tendinosus muscles are sampled to diagnose PSSM2 and MFM. A sample that provides a 2-cm square of tissue in cross-section is recommended in order to provide enough muscle fibers to identify the limited number of type 2A fibers that contain glycogen or desmin aggregates. It is recommended that both fresh and formalin-fixed samples be evaluated with a minimum of hematoxylin-eosin, periodic acid Schiffs (PAS), amylase-PAS, and desmin stains. Handling of muscle tissue is extremely important. It is recommended that 1) the sample be handled with forceps at only one end, 2) the sample is not squeezed with forceps, 3) the samples be placed in slightly damp gauze in a hard container (urine cup) packed with enough gauze to prevent movement during shipping, 4) the samples be kept chilled on ice packs until shipping, and 5) the samples should be shipped overnight to arrive in the laboratory within 24 hours. Squeezing of muscle creates abnormal glycogen (false positives) and delays in chilling or shipping result in glycogen and desmin depletion (false negatives) as well as artifactual cracks within muscle cells that stain positively with PAS (false positives). Prior to placing samples in formalin, best practices suggest the sample be allowed to sit in air for 3 to 5 minutes to fully contract. The diagnostic criteria for PSSM2 include the presence of aggregates of cytoplasmic glycogen in PAS stains and a negative GYS1 test. The aggregates of glycogen are usually amylase sensitive (similar to normal glycogen), in contrast to amylase-resistant aggregates seen in PSSM1. Because amylase-sensitive glycogen is a normal feature of muscle histology, the interpretation of the appearance of glycogen is a subjective diagnosis. The diagnostic criterion for MFM is the presence of aggregates of cytoplasmic desmin that are at least one-half the width of myonuclei in size and found in mature (not regenerating) muscle fibers. It is the author’s clinical impression that these desmin aggregates are not concurrent with the initial clinical signs of exercise intolerance, and thus it is possible to have a false-negative diagnosis of MFM in early stages.

Genetic Testing

Mutations in 16 genes are known to cause MFM in humans. A comparison of the sequences of these 16 genes (including MYOT and FLNC) between horses diagnosed with MFM by muscle biopsy (n = 8) and healthy Warmblood horses (N = 8) did not identify any mutations associated with MFM in the horses studied. A commercial company offers genetic testing for variants (single nucleotide polymorphisms) in the genes myotilin MYOT (P2), filamin C FLNC (P3), and myozien MYOZ3 (P4) and purports that they are causative of PSSM2 and MFM (http://equiseq.com/buy_pssm2). The prevalence of these variants in Warmblood horses with a muscle biopsy diagnosis of PSSM2 (n = 54) and MFM (n = 68) and no histopathology (n = 54) as well as in 205 horses in publicly available genetic databases has recently been determined. No statistical association was found between any of the P variants, or combinations of P variants, and the presence of either PSSM2 or MFM considered as one diagnosis, or when PSSM2 or MFM were considered separately. Only 38% of Warmbloods with MFM possessed a P variant and 29% of Warmbloods appear to have at least one P variant regardless of whether they have a muscle disease or not. Thus, there does not appear to be evidence to support that the P variants are themselves causative or diagnostic of a muscle disease in the horse.

6. Management

Management of PSSM2 and MFM requires both altering diet and exercise regimes and is based largely on retrospective studies or clinical impressions rather than controlled diet trials. More information needs to be collected from owners of horses who use the management approaches described below to determine their efficacy.

Diet

A low nonstructural carbohydrate (NSC), high-fat diet was previously recommended for PSSM2 based on the assumption that PSSM2 was a glycogen storage disorder similar to PSSM1. In Warmbloods, however, it is now clear there is not excessive glycogen storage in skeletal muscle based on biochemical analysis of glycogen concentrations. In addition, a survey of owners found that 80% of PSSM2 Warmbloods improved on the low NSC, high-fat diet; however, 53% of Warmbloods did not advance as expected in training, with reluctance to go forward and lack of collection persisting in approximately one-third of horses. A revised diet was developed based on transcriptomic and proteomic analyses of muscle from horses with MFM and based on controlled diet trials in healthy Thoroughbreds. The revised diet has not been evaluated in controlled diet trials of MFM horses.
Supplement designed for MFM horses that contains antioxidants. Amino acids such as lysine, methionine, and threonine are important for muscle repair and generation of cysteine-based antioxidants. Leucine stimulates protein synthesis in the muscle postexercise, which would be beneficial to MFM horses.

Forage. MFM horses will typically consume 1.5 to 2% of body weight per day of hay. Good quality grass or grass-legume mixed hays (55-65% neutral detergent fiber [NDF], 10-12% crude protein [CP], and 10-17% NSC) are preferable. Concentrates. Recommended concentrates for PSSM2 and MFM Warmbloods are those with moderate levels of NSC (20-30%) and fat (4-8%) and higher levels of protein (12-14% CP) containing high-quality amino acids. Amino acids such as lysine, methionine, and threonine are important for muscle repair and generation of cysteine-based antioxidants. Leucine stimulates protein synthesis in the muscle postexercise, which would be beneficial to MFM horses.

Supplements

Amino acids. Whey-based proteins or supplements containing N-acetyl cysteine are recommended for horses with MFM because they are rich in cysteine. Cysteine is a key component of many antioxidants. MFM horses may have an increased cysteine requirement based on alterations in genes involved in conversion of methionine to cysteine found in Arabian horses with MFM following exercise. A supplement designed for MFM horses that contains N-acetyl cysteine and branched chain amino acids has recently become commercially available.

Antioxidants. Coenzyme Q10 (CoQ10) is a key component of the first step in the mitochondrial electron transport chain, and Warmblood horses with MFM have a decreased expression of proteins involved in the first two steps in electron transport. When fed to healthy horses, N-acetyl cysteine and coenzyme Q10 were found to increase mitochondrial proteins. CoQ10 is now being trialed as a supplement for MFM horses.

Exercise

Exercise Schedule

An equally important part of the management of PSSM2 and MFM is an exercise regime. PSSM2 horses were previously recommended to exercise every day; however, a recent survey of owners suggests that PSSM2 and MFM Warmbloods require days off training during the week to recover. The number of days per week to ride varies with each horse. Many owners of PSSM2 and MFM horses have found that 3 days of work followed by 2 days off works best. For some horses, exercise more frequently than 3 days in a row or rest for more than 2 days in a row results in a stiff horse in the subsequent ride.

Warm-Up

A warm-up on the lunge-line in both directions in a long, low frame for 10 to 15 minutes is reported by owners to improve ridden work. During the warm-up, engagement of the hindquarters is not the aim, but rather, muscle relaxation, releasing at the base of the neck, and rounding and lifting of the back are desirable. Aids that help create a long, low frame on the lunge-line include Vienna reins, Pessoa lunging system, or neck-stretchers. Initially, exercise is performed at a walk and trot. Strides at a canter can gradually be added looking for the horse to release at the base of the neck. When transitioning down to trot, the horse should begin to move forward with more impulsion.

Riding

A long and low warm-up at the trot and canter with adequate stretching is recommended before any collected work. Rest periods that allow the horse to relax and stretch their muscles between 2- to 5-minute periods of collection under saddle may be of benefit. Total ride times in horses with MFM appear to be most productive if kept between 30 and 45 minutes, with horses just starting out having rides between 15 and 20 minutes (including walking).

Strengthening Exercises

Hill work, cavaletti, and small jumps can be used to build strength. Aids and core strengthening exercises such as those found in Activating Your Horse’s Core could also be beneficial.

Turnout

As much daily turnout as possible in an area where the horse is encouraged to move about is recommended.

Professional Help

Some Warmblood horses have strong, reactive temperaments and can develop behavioral issues such as refusing to go forward under saddle and bucking even after painful conditions have been resolved. In these cases, groundwork that reestablishes leadership and the help of a professional trainer experienced with difficult horses may be required to safely ensure “forward” means forward without protestation.

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Declaration of Ethics
The Author has adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest
The Author has research collaborations with Kentucky Equine Research, Versailles, KY, and receives financial support for research projects from the company. The Author receives royalties from the PSSM1 genetic test. The Author runs the Neuromuscular Diagnostic Laboratory at Michigan State University. She does not receive any personal remuneration from the Diagnostic Laboratory.

References and Footnotes

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