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Clinical and histopathological features of myofibrillar myopathy in Warmblood horses

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Summary

Background—To report a novel exertional myopathy, myofibrillar myopathy (MFM) in Warmblood (WB) horses.

Objectives—To 1) describe the distinctive clinical and myopathic features of MFM in Warmblood horses and 2) investigate the potential inheritance of MFM in a Warmblood family.

Study design—Retrospective selection of MFM cases and prospective evaluation of a Warmblood family.

Methods—Retrospectively, muscle biopsies were selected from Warmblood horses diagnosed with MFM and clinical histories obtained (n = 10). Prospectively, muscle biopsies were obtained from controls (n = 8) and a three generation WB family (n = 11). Samples were assessed for histopathology [scored 0–3], fibre types, cytoskeletal and Z disc protein aggregates, electron microscopic alterations (EM) and muscle glycogen concentrations.

Results—Myofibrillar myopathy-affected cases experienced exercise intolerance, reluctance to go forward, stiffness and poorly localised lameness. Abnormal aggregates of the cytoskeletal

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Authors' declaration of interests

S. Valberg and her colleagues license the PSSM1 genetic test mentioned in this paper and she receives royalties from the test.

Ethical animal research

This study was approved by the Animal Use and Care Committees of the University of Minnesota, St Paul, Minnesota and Michigan State University where muscle samples were obtained. Owners gave informed consent for their horses' inclusion in the study.

Authorship

S.J. Valberg, S.S. Lewis and A.M. Nicholson developed the study design. S.J. Valberg contributed to methods development and implementation. S.J. Valberg, C.J. Finno, R. Reardon and A.N. Nicholson collected prospective muscle biopsies. S.S. Lewis, R. Reardon, A.M. Nicholson performed data collation. S.J. Valberg, S.S. Lewis, C.J. Finno and A.M. Nicholson contributed to data interpretation and manuscript preparation. All authors provided final approval for the submitted version of the manuscript.

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protein desmin were found in up to 120 type 2a and a few type 2x myofibres of MFM cases. Desmin positive fibres did not stain for developmental myosin, α actinin or dystrophin. Scores for internalised myonuclei (score MFM 0.83 ± 0.67, controls 0.22 ± 0.45), anguloid atrophy (MFM 0.95 ± 0.55, controls 0.31 ± 0.37) and total myopathic scores (MFM 5.85 ± 2.10, controls 1.41 ± 2.17) were significantly higher in MFM cases vs. controls. Focal Z disc degeneration, myofibrillar disruption and accumulation of irregular granular material was evident in MFM cases. Muscle glycogen concentrations were similar between MFM cases and controls. In the Warmblood family, desmin positive aggregates were found in myofibres of the founding dam and in horses from two subsequent generations.

Main limitations—Restricted sample size due to limited availability of well phenotyped cases.

Conclusions—A distinctive and potentially heritable form of MFM exists in Warmblood horses that present with exercise intolerance and abnormal hindlimb gait. Muscle tissue is characterised by ectopic accumulation of desmin and Z disc and myofibrillar degeneration.

Keywords

horse; desmin; myofilaments; intermediate filaments; exertional myopathy

Introduction

Back problems in dressage and jumping horses often manifest as resistance to saddling, pain on palpation, exercise intolerance, resistance to collection and rounding over fences and intermittent hindlimb gait abnormalities [1–3]. A specific diagnosis of the problem can be elusive and, in the absence of sacroiliac or spinous pathology, veterinarians often attribute back pain to secondary muscular strain induced by a primary orthopaedic lameness, poor saddle fit, or poor riding/training [1].

Another potential explanation for perceived back problems is a primary myopathy [3–5]. An early study identified polysaccharide storage myopathy (PSSM), affecting lumbar and gluteal muscles, as a primary cause of back soreness, poor jumping and poor dressage performance in Warmblood horses [3]. Subsequently, the molecular basis for PSSM was identified and, based on this genetic test, the condition split into type 1 PSSM (PSSM1) caused by a glycogen synthase 1 mutation and type 2 PSSM (PSSM2) of unknown cause [6,7]. Both PSSM1 and PSSM2 have been identified in Warmbloods; however, the majority of cases of PSSM in Warmbloods fall into the category of PSSM2 [8]. Unfortunately, since there is no known basis for PSSM2, it remains a histopathological description of abnormal aggregates of periodic acid Schiff's positive material in skeletal muscle fibres, rather than a specific disease process.

Recently, a subset of Arabian horses originally diagnosed with PSSM2 was found to have a newly identified muscle disorder termed myofibrillar myopathy (MFM) [9,10]. This myopathy was identified in athletic Arabian or Arabian cross horses that competed in 100 mile (161 km) endurance rides and intermittently developed signs of muscle pain and stiffness after exercise. No metabolic abnormalities were identified in the Arabian horses, but muscle biopsies revealed structural disruption of the myofibrils and ectopic

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accumulation of the cytoskeletal protein desmin [9,10]. The number and alignment of myofilaments is essential to muscle strength [11] and a subtle disorder affecting the number or alignment of contractile proteins could have a major impact on performance in Warmbloods competing in dressage, hunters and showjumping. Because Arabian bloodlines have played a part in the development of Warmblood breeds we began to incorporate immunohistochemical staining for cytoplasmic aggregates of desmin into the diagnostic investigation of muscle biopsies of Warmblood horses with poor performance. The objectives of the present study were to describe the clinical and histopathological characteristics of cases of MFM in Warmblood horses identified retrospectively and prospectively investigate the potential familial occurrence of MFM in a three-generation Warmblood family.

Materials and methods

Horses

MFM cases—The database of the Neuromuscular Diagnostic Laboratory at Michigan State University (NMDL) was searched between December 2014 and October 2016 to identify muscle biopsy submissions from Warmblood horses. MFM cases were selected for inclusion in the study if 6 muscle fibres contained abnormal aggregates of desmin [9] and if horses had been examined by one of the coauthors or had medical records available for evaluation. Signalment, history, physica examination findings, serum creatine kinase (CK) and aspartate transaminase (AST) activities were obtained from submission information and a follow-up questionnaire answered by referring veterinarians (Supplementary Item 1). Written recommendations were provided with muscle biopsy results and included providing maximal daily turnout, feeding a balanced diet with a nonstructural carbohydrate content for both hay and concentrate/ration balancer of <12% while providing fat supplementation if needed for maintenance of weight. Feeding the amount of an amino acid supplement (Topline Xtreme[a] or SuperSport[b]) recommended by the manufacturer in the feed (approximately 225 g) within 45 min prior to, or after, exercise was also advised. In addition, a 15 min programme of lungeing in a long and low frame prior to regular daily exercise was recommended. Follow-up assessment of the horses' response to recommendations (followed recommendations with no improvement, followed recommendations with improvement, did not follow recommendations) was conducted by email in between October and December of 2016.

Control horses—Gluteal muscle biopsy samples were obtained from eight healthy Warmblood horses housed on one of the farms that housed two MFM cases, as well as on two farms in proximity to an MFM case. Horses had competed in dressage or jumping and had no reported clinical signs of muscle pain or stiffness. The group consisted of one Dutch Thoroughbred cross, two Hanoverians, one Holsteiner, one Westphalian, one Swedish Warmblood, one Oldenburg and one Belgian crossbred. There were three mares, four geldings and one stallion with a mean age of 14.6 ± 5.5 years (range 7–24 years).

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[b]Purina Mills, St Louis, Missouri, USA.

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Warmblood family—Percutaneous *gluteus medius* needle biopsy samples were obtained from a family of Warmblood horses on a farm that had previously produced horses diagnosed with PSSM2. Inclusion criteria were: age 6 years; descendant of an unregistered dam that founded the herd.

Analyses

Muscle biopsy procedure

MFM cases—Samples had previously been obtained by open surgical biopsy of the semimembranosus muscle (n = 5) or by percutaneous needle biopsies of the *gluteus medius* muscle (n = 5) and shipped to the laboratory as previously described [9,12]. For biochemistry, a portion of each fresh sample was either frozen directly in liquid nitrogen upon arriva in the laboratory (n = 5), or frozen on site immediately after biopsy (n = 5). Frozen samples were stored at −80°C. Formalin-fixed samples were also submitted in five cases and paraffin embedded within 48 h of receipt.

Controls and Warmblood family—Muscle biopsies were obtained from the *gluteus medius* muscle by percutaneous needle biopsy at a standardised site [12]. Samples were divided into three aliquots oriented on cork and frozen in methylbutane suspended in liquid nitrogen within 12–24 h of sampling, snap frozen in liquid nitrogen, placed in formalin and paraffin embedded within 48 h. Frozen samples were stored at −80°C.

Muscle histopathology—Samples were sectioned and stained with haematoxylin and eosin (HE), modified Gomori trichrome (GT), periodic acid Schiff (PAS), amylase-PAS, oil red O and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), as previously described [9,13]. Sections were scored for the presence of internalised myonuclei, anguloid atrophy, macrophages, basophilic regenerative fibres with large internalised nuclei, inclusions in GT, subsarcolemmal glycogen, cytoplasmic PAS positive aggregates and amylase-resistant polysaccharide. Nicotinamide adenine dinucleotide tetrazolium reductase stains were examined for abnormalities in oxidative staining. A scoring system was used at 20× magnification to grade the above features based on; 0 = not present, 1 = present in approximately 10% of fibres in the biopsy, 2 = present in approximately 11–25% of fibres in the sample, 3 = present in more than 25% of fibres in the sample.

Desmin immunohistochemistry—Immunohistochemical (IHC) staining for desmin was performed on frozen and, where available, formalin fixed sections. For seven MFM cases, five controls and the Warmblood family, desmin staining was performed as previously described [9]. Due to relocation to another laboratory, a slight modification of desmin staining was used for three MFM cases and three controls (Supplementary Item 2). The number of muscle fibres in an entire muscle section that contained at least two aggregates of cytoplasmic desmin and that were at least 50% of the size of myonuclei were counted. The number of fields at 4× magnification that encompassed the sample were also counted as a reference for the size of the muscle biopsy. Desmin was scored as 0 = 1 desmin positive fibres, 1 = 2–10 positive fibres, 2 = 11–20 positive fibres, 3 = >20 positive fibres.

Immunohistochemical staining was also performed for a actinin, αβ crystallin, nebulin and dystrophin on serial frozen sections from five horses with MFM in the Warmblood family as previously described [9].

Muscle fibre typing—Serial frozen sections of muscle from the 10 MFM cases were stained for desmin, myosin ATPase activity (preincubation pH 4.6) [14] and for developmental myosin heavy chain (MyHCd) (Supplementary Item 2) [13].

Electron microscopy—Cubes of approximately 5 mm^2 were dissected from fresh muscle samples of two MFM cases (desmin scores 2 and 3), two control horses and one MFM horse in the Warmblood family with a history of exercise intolerance (desmin score 1). Samples were placed in 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, oriented longitudinally and processed as previously described [9].

Muscle glycogen concentrations—Muscle glycogen concentrations were determined in approximately 4 mg of muscle from MFM cases (five snap frozen and five shipped muscle biopsy specimens), the MFM family (snap frozen) and controls (snap frozen). Glycogen concentration was assayed fluorometrically as previously described [9,15].

Data analysis

Scores for anguloid atrophy, internalised nuclei, macrophages, basophilic regenerative fibres, inclusions in GT, subsarcolemmal glycogen, cytoplasmic glycogen aggregates, amylase resistant polysaccharide and desmin were compared between MFM cases and controls and between Warmblood family horses with desmin score 0 vs.   1 using a Mann Whitney *U* test. Muscle glycogen concentrations were normally distributed (Kolmogorov–Smirnov test) and therefore compared between MFM cases and controls and MFM family groups using an unpaired *t* test. Data are presented as mean ± s.d. Graph Pad Prism 5 statistical software was used for analysis. Significance was set at a $P<0.05$.

Results

MFM cases

Out of 103 muscle biopsy submissions to the NMDL from Warmbloods during the study period, there were 24 Warmblood cases diagnosed with MFM, 10 of which fit the inclusion criteria for the study. Six of the 10 horses had been examined by one of the coauthors and four by referring veterinarians. Horses were used either for dressage, or as hunters and comprised a variety of Warmblood types with a mean age of 11.4 ± 3.8 years (Supplementary Item 3). Serum CK and AST activities had been assessed in 5/10 horses at the time of muscle biopsy and all five were within the normal range (reference CK 194–346 u/L, AST 127–412 u/L) (Supplementary Item 3). The primary presenting complaint was an insidious onset of exercise intolerance characterised by a lack of stamina, unwillingness to go forward, inability to collect, abnormal canter transitions and inability to sustain a normal canter. These signs had a duration of at least a year or more. Lameness, stiffness, muscle pain and, in one case, a single episode of exertional rhabdomyolysis were reported (Supplementary Item 3). A lack of a specific orthopaedic disease, or a lack of response to

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treatments such as rest and intra-articular hock, stifle or sacroiliac joint injections, were the most common reason for performing a muscle biopsy. One horse had a concurrent suspensory desmitis and one a superficial digital flexor strain; however, lameness did not completely resolve after treatment.

Of the 10 MFM cases, five horses had a substantial improvement and one a modest improvement in willingness and tolerance to exercise after instituting the diet and exercise programme. Two horses did not improve with the recommendations provided and were retired due to extreme exercise intolerance. One had recurring suspensory desmitis and the other had a full medical and orthopaedic evaluation, with full body nuclear scintigraphy and cardiac ultrasound, without identifying any other abnormality apart from a myopathy. Two owners did not institute the written recommendations provided and one of the horses was lost to follow-up.

Desmin immunohistochemistry—Cytoplasmic aggregates of desmin were not present in six control horses (Fig 1a), whereas two control horses had one muscle fibre with a few desmin positive aggregates. The mean number of 4× fields examined for control samples was 7.1 ± 3.2. Based on inclusion criteria, all MFM cases had scattered muscle fibres with numerous desmin positive aggregates (Fig 1b). The mean number of desmin positive fibres in MFM horses was 33.2 ± 34.1, with a range of 6–120. The mean number of 4× fields examined for MFM samples was 6.9 ± 4.0. Scores for desmin and the number of desmin positive fibres per 4× field were significantly higher in MFM vs. control horses (Table 1).

Histopathology—Active myodegeneration or regeneration was not evident in muscle samples from control and MFM horses. Scores for internalised myonuclei (Supplementary Item 4), anguloid atrophy, cytoplasmic glycogen aggregates (Supplementary Item 4) and total myopathic scores were significantly higher in MFM cases compared with controls (Table 1). Three MFM cases had a few fibres with rod-like or stellate inclusions in the GT stain; however, most desmin positive fibres had no abnormalities in HE or GT stains (Supplementary Item 4). Nicotinamide adenine dinucleotide tetrazolium reductase staining for mitochondria was normal in all horses. Glycogen was amylase sensitive in all control horses and 9/10 MFM horses. One MFM case had three myofibres with amylase-resistant polysaccharide that had corresponding strong staining for desmin (Fig 1b, c).

Muscle fibre types—Desmin aggregates were present in type 2a muscle fibres in all MFM horses and in a few type 2× muscle fibres in two MFM cases (Supplementary Item 5). Muscle fibres with desmin positive aggregates did not stain positively for MyHCd myosin (Supplementary Item 5), whereas regenerating muscle fibres stained positively for desmin and MyHCd in the adult disease control muscle sample and MyHcd staining was present in a proportion of fetal muscle fibres (Supplementary Item 5).

Electron microscopy—Many regions of muscle fibres with properly aligned myofibrils were apparent in MFM and control horses (Supplementary Item 6). In MFM cases, some segments of muscle fibres had mild disruption of myofilament alignment (Fig 2a) and Z-disc streaming (Fig 2b) and other regions had complete loss of sarcomeres with altered alignment of the sarcoplasmic reticulum terminal cisternae (Supplementary Item 6). Both normal

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appearing mitochondria and focal areas with degenerate mitochondria and bizarre cristae were present in the MFM cases (Fig 2a). Sarcomere disruption could be severe with accumulation of degenerating myofilaments, flag like protrusion of Z disc material (Supplementary Item 6) and rounded dense accumulations of Z disc remnants (Fig 2c). In focal areas, irregular granular material accumulated to the extent that it replaced myofibrils (Fig 2d).

Warmblood family

Horses—There were ten descendants 6 years of age of the founding dam available for evaluation. The dam was unregistered and did not have a pedigree available. The family consisted of five full and one half sibling (11–19-years-old) in the first generation and three half siblings (7–10-year-old geldings) in the second generation with their sire (Fig 3). Five horses were ridden under saddle at the time of evaluation with one horse (Horse 4) reported to have consistent exercise intolerance, muscle pain and stiffness with exercise. One horse demonstrated a grade 2 general proprioceptive ataxia of the pelvic limbs [16].

Histopathology—The founding dam (desmin score = 2), three first generation offspring (desmin scores = 1) and one second generation offspring (desmin score = 3) had muscle fibres with abnormal desmin positive aggregates (Fig 3). Scores for anguloid atrophy and total myopathic scores were significantly higher in horses with desmin score 1 compared with horses with scores of 0 (Table 1).

Immunohistochemistry—A small number of desmin positive fibres had increased staining for αβ crystallin, but abnormal staining for a actinin, nebulin or dystrophin was not observed in myofibres with desmin aggregates.

Electron microscopy—Performed on Horse 4 (desmin score 1) electron microscopy revealed a few scattered regions of complete Z disc and sarcomere disruption (Fig 3).

Muscle glycogen concentrations

Muscle glycogen concentrations were not significantly different between MFM cases and control horses (Table 1), or among Warmblood family horses. The one MFM case with amylase-resistant polysaccharide had the lowest measured muscle glycogen concentration.

Discussion

The distinguishing myopathic features of muscle biopsies from Warmblood horses with exercise intolerance in the present study were internalised myonuclei, mild to moderate myofibre atrophy, aggregates of the cytoskeletal protein desmin and myofibrillar disarray [17,18]. Acute myodegeneration or over-exertion was eliminated as a cause of histopathology and myofibrillar disarray because horses had normal serum CK and AST activities, no preceding strenuous exercise and no reported episodes of rhabdomyolysis for years prior to the muscle biopsy procedure. Furthermore, a rapid widespread loss of desmin immunostaining occurs with over-exertion rather than focal desmin aggregation [19,20]. The histopathological features of Warmbloods in the present study are very similar to those

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described in Arabian horses with a suspected MFM which were internalised myonuclei, anguloid atrophy and distinctive desmin positive aggregates [9,10]. One potential explanation for desmin staining of myofibres is muscle regeneration [18]. Regeneration was excluded as a cause of increased desmin staining in the present study because desmin positive fibres did not contain developmental myosin (MyHCd). Rather, fibres with desmin aggregates were largely fast twitch type 2a fibres. The morphological term MFM was applied to this novel myopathy because it accurately describes the sarcoplasmic aggregates of desmin, as well as the myofibrillar disarray found in Warmblood horses with exercise intolerance.

In human medicine, the term MFM comprises both a morphological description of pathological findings, as well as a distinct group of muscle disorder caused by mutations in one of at least eight genes [21,22]. These genes encode proteins that are part of the cytoskeleton or Z disc of the sarcomere including desmin, filamin C, myotilin, LIM proteins and αβ-crystallin [21,22]. The histopathology of MFM in man is similar to, but more pronounced than, that described in the Warmblood horses in the present study. In MFM cases in man, ectopic protein accumulation is more apparent in HE and GT stains and there is often focal loss of mitochondrial staining. In addition, in MFM cases in man, IHC stains reveal a variety of protein aggregates including αβ-crystallin, filamin, dystrophin, myotilin and others in addition to desmin [23]. In contrast, in MFM horses, GT and NADH-TR stains were relatively normal and aggregates of proteins such as dystrophin, nebulin and α actinin did not co-localise with abnormal desmin. Electron microscopic findings of focal myofibrillar disarray, partial to complete Z disc rupture, accumulation of dense Z disc material and large areas of irregular granular material in Warmblood horses did, however, support a defect in myofibrillar alignment as the basis for this novel equine myopathy [21,24]. Light and electron microscopic findings in Warmbloods closely resembled those of Arabian horses previously diagnosed with MFM [9]. Thus, based on the results of the present study and a previous study of Arabian horses, we propose that horses have a form of MFM that results in milder ectopic protein aggregation than MFM in man.

It is interesting to note that mice with a knock-in heterozygous mutation in filamin C, which causes MFM in man, have no observable lesions in HE and GT stains and little ectopic protein accumulation [25]. When mutant mice are exercised, however, they develop significant disruption of sarcomeres and show significantly less spontaneous movement in their cages following exercise compared with wild type mice [25]. Thus, similar to this mouse model, MFM in horses could have a milder histological phenotype, but routine athletic activity in horses could trigger recurrent myofibrillar disruption with associated pain and exercise intolerance as a result of an inherent underlying weakness in sarcomeres with the myofibrils.

The milder histopathological phenotype of MFM in horses is mirrored by a milder clinical phenotype. Myofibrillar myopathy in man usually has a late onset in the fourth decade of life and progresses with gradual loss of muscle strength and muscle mass leading to difficulty ambulating and potentially breathing [21,22]. Cardiomyopathy and arrhythmias may be apparent. There is considerable heterogeneity in clinical signs in man with various forms of MFM [22]. It is interesting that early signs of MFM in man can be quite similar to horses,

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with myalgia of abdominal, back and neck muscles and exercise intolerance manifested as difficulty climbing stairs and a waddling gait [26]. Whether the predominant feature of reluctance to exercise in horses is due to pain, loss of strength, impaired respiratory function or a combination of these is unclear at this point. Mutations in desmin and filamin C have been shown to perturb not only mitochondrial morphology but also mitochondrial respiratory function [25,26]. A full understanding of the basis for clinical signs in horses with MFM awaits a more complete physiological, biochemical and molecular definition of this disorder.

One weakness of the present study was that a full evaluation of all causes of exercise intolerance in horses was not performed in more than one horse. Thus, it is difficult to conclude that the muscle pathology was solely responsible for the presenting clinical signs. Discerning the cause of exercise intolerance, irregular pelvic limb gaits and back issues in horses is onerous and a clear diagnosis is often not obtained [2]. Numerous factors including behaviour, training, rider skill, tack as well as various medical and orthopaedic disorders contribute to exercise intolerance and performance limitations in horses [1]. The results of the present study provide an additional diagnostic tool for investigating back issues and exercise intolerance in Warmblood horses; desmin staining of muscle biopsies. With the complexity of equine performance, muscle biopsy would not replace, but rather augment a work-up that includes a complete knowledge of the athletic discipline; an effective rider, medical and lameness evaluations and advanced diagnostic imaging [1].

A noninvasive genetic test would be an ideal tool for diagnosing MFM in horses. Our evaluation of a Warmblood family revealed that desmin positive aggregates were present in the founding dam, three offspring and a second generation offspring. Thus, the potential exists for a heritable form of MFM in Warmblood horses. Development of a DNA based test for MFM in Warmblood and in Arabian horses is appealing because the sensitivity of desmin positive aggregates in muscle biopsies could well vary depending upon the age of the horse, size of the muscle biopsy, muscle sampled and degree of biopsy preservation prior to performing IHC. This is similar to the scenario for type 1 PSSM prior to development of a genetic test where abnormal polysaccharide did not develop in muscle until horses were >18 months of age and a diagnosis could easily be missed in small muscle biopsy samples [27]. False positive diagnosis of MFM could also occur if care is not taken to assess samples for concurrent regeneration, degeneration or the type of desmin aggregates that occur with type 1 PSSM [28].

The approach recommended to manage horses with MFM in the present study was to enhance muscle strength and synthesis of contractile proteins using a consistent graded exercise programme that focused on strengthening the back and core muscles. In addition, a balanced diet with an amino acid supplement given around the time of exercise was recommended to potentially enhance protein synthesis and muscle mass [29,30]. We selected this approach based on previously demonstrated increases in muscle mass with whey-based amino acid supplements in horses [28] and a mouse model of nemaline myopathy in which 10 days of tyrosine supplementation improved the mice's mobility deficit and increased muscle weight, forearm strength and the pathological appearance of muscle after 4 weeks [31]. There was a wide range in severity of clinical signs in horses

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diagnosed with MFM and a range of responses to the recommendations provided. With dedication and time, it was possible for some MFM horses to continue to work under saddle, but often not at the level of performance owners originally envisioned. The recommendations provided were preliminary, based on previous work focused on increasing muscle mass and signaling of protein synthesis and there has not been time or case numbers yet to evaluate their efficacy [29,30]. A thorough investigation into potential benefits of dietary and exercise modifications is needed for MFM horses.

In conclusion, the results of the current study introduce MFM as a cause of decreased exercise tolerance, unwillingness to go forward and poor performance in Warmblood horses with a potential familial basis. Myofibrillar myopathy is characterised by abnormal aggregates of the cytoskeletal protein desmin and Z disc instability and myofibrillar disarray in skeletal muscle biopsies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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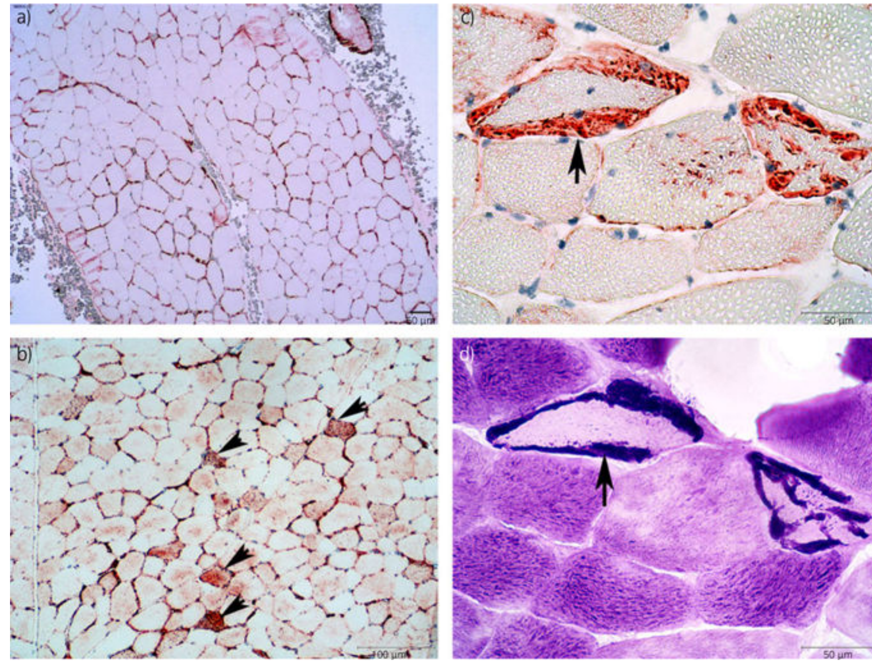

Fig 1.

a) Cross-section of semimembranosus muscle from a control horse with a normal pattern of desmin staining (desmin 10×). b) Scattered fibres containing desmin positive sarcoplasmic aggregates (arrows) in semimembranosus muscle from an MFM case (desmin 10×). c) Desmin positive aggregates in three myofibres of an MFM horse (arrows) (desmin 40×). d). Serial section to c) demonstrating that the desmin positive aggregates are PAS-positive (arrow). These PAS positive aggregates were resistant to amylase digestion (PAS 40×).

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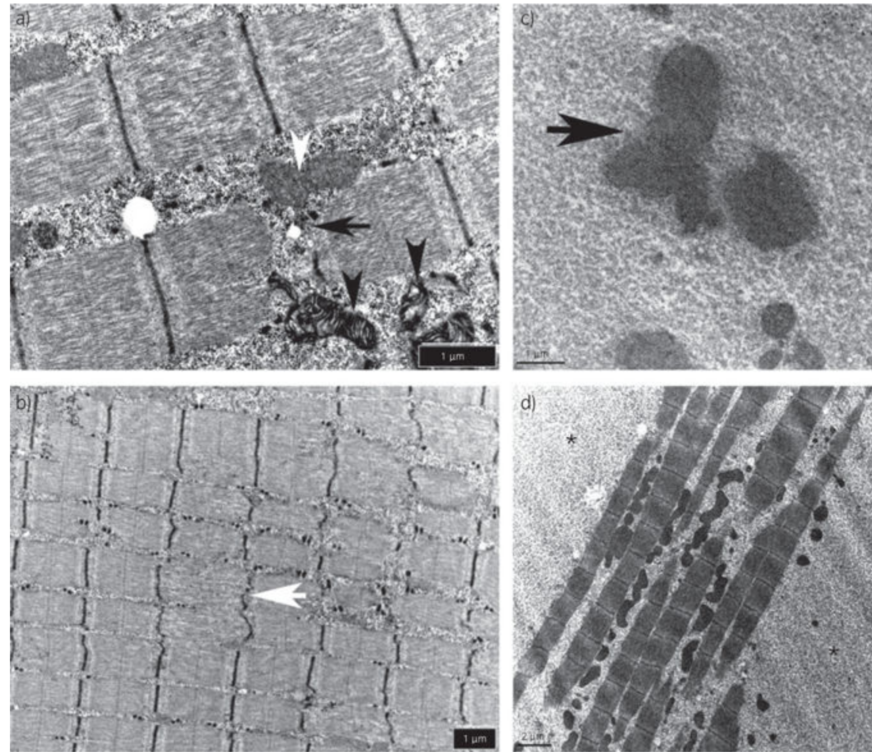

Fig 2.

Electron microscopy of gluteal muscle from two MFM cases. a) Sarcomeres have disrupted alignment of myofilaments, disruption of the Z disc (horizontal arrow) and degenerating mitochondria (vertical black arrow). Normal mitochondria are also present (white arrow) (20,000×). b) Sarcomeres with Z discs streaming (white arrow) and altered alignment of myofilaments (20,000×). c) Large accumulation of electron dense Z disc remnants (arrow) surrounded by irregular granular material (10,000×). d.) Low power view of disrupted myofibrils and accumulation of a large amount of irregular granular material (*) (4000×).

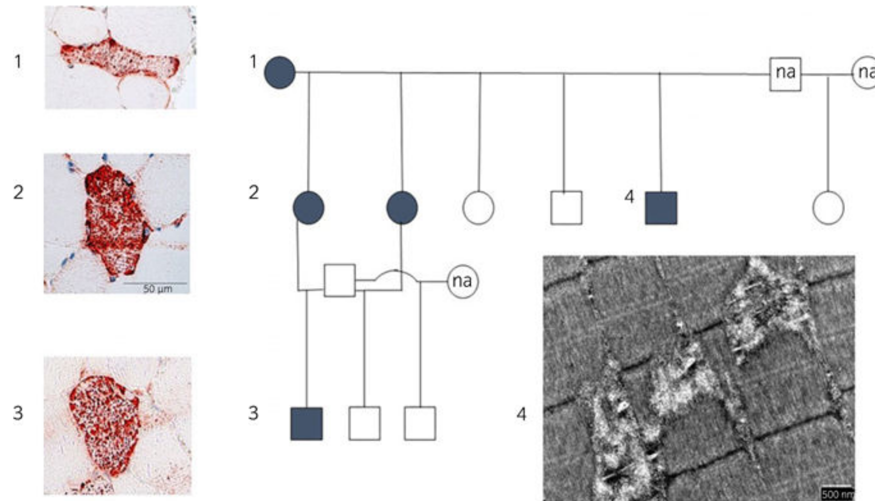

Fig 3.
Partial pedigree of the Warmblood family showing the presence of horses with myofibres containing desmin-positive aggregates in all three generations. Solid symbols have desmin-positive aggregates in myofibres, open symbols had no desmin aggregates. Note the presence of desmin-positive fibres typical of MFM in the founding dam (1) her colt (4) and two fillies (2) and a colt in the third generation (3). Clinical signs of exercise intolerance were reported in Horse 4. Electron microscopy sections of gluteal muscle from Horse 4 revealed disruptions in sarcomeres and Z disc degeneration (25,000×). Circles = female, squares = male, na = not available for muscle biopsy.

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TABLE 1

Mean scores (s.d.) for muscle histopathology and muscle glycogen concentrations in cases diagnosed with a myofibrillar myopathy (MFM) and healthy controls and between Warmblood family (WBF) horses with and without MFM. Scores ranged from 0 to 3 for severity for each feature

	Central nuclei	Anguloid atrophy	Subsarc. glycogen	Cytoplasmic glycogen	GT inclusions	Desmin	Desmin fibres/hpf	Total score	Glycogen concentration
Cases MFM n = 10	0.83 ± 0.67 *	0.95 ± 0.55 *	0.28 ± 0.38	1.13 ± 0.68 *	0.53 ± 0.73	2.15 ± 0.88 ***	8.58 ± 7.61 ***	5.64 ± 2.11 ***	135.0 ± 38.1
Controls n = 8	0.22 ± 0.45	0.31 ± 0.37	0.25 ± 0.38	0.44 ± 0.57	0.06 ± 0.18	0.13 ± 0.23	0.02 ± 0.04	1.41 ± 2.17	148.2 ± 35.2
WBF MFM n = 5	0.45 ± 0.46	1.40 ± 0.22£	0.00 ± 0.00	0.40 ± 0.38	0.00 ± 0.00	1.60 ± 0.89££	1.45 ± 0.63££	3.95 ± 0.91£	110.2 ± 41.5
WBF normal n = 6	0.33 ± 0.41	0.46 ± 0.51	0.33 ± 0.41	0.29 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.21	1.58 ± 1.37	115.3 ± 21.5

Subsarc, subsarcolemmal; GT, modified Gomori trichrome; hpf, 4× field of magnification.

* P<0.05,

** P<0.01,

*** P<0.001 differences between MFM Cases and controls,

£ P<0.05,

££ P<0.01 differences between WBF MFM and WBF normal.