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Differences in the metabolic properties of *gluteus medius* and superficial digital flexor muscles and the effect of water treadmill training in the horse

L. A. BORGIA*, **S. J. VALBERG** and **B. ESSEN-GUSTAVSSON**†

College of Veterinary Medicine, University of Minnesota, USA; and †Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agriculture Sciences, Uppsala, Sweden.

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Summary

Reasons for performing study: **Flexor tendon injury may be due to flexor muscle fatigue, contributing to fetlock joint hyperextension and tendon damage. A water treadmill provides resistance training on flexor tendon muscles, which might reduce the risk of tendon injury.**

Objective: **To determine the effect of water treadmill training on the properties of the gluteal and superficial digital flexor (SDF) muscles and on cardiocirculatory response to a standardised exercise test.**

Methods: **Five healthy unfit horses were trained on a water treadmill for 5 days/week for 4 weeks, starting with 5 min/day increasing to 20 min/day. Before and after the water treadmill training, an incremental SET was performed on a land treadmill to determine velocity at a heart rate 200 beats/min (V_{200}) and resting gluteal and SDF muscle biopsies were obtained for biochemical analyses.**

Results: **There was no measurable difference in resting concentrations of gluteal or SDF muscle glycogen, lactate, ATP or glucose-6-phosphate (G6P), or activities of citrate synthase (CS), 3-hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) after training and no change in V_{200}, Lactate, glycogen, G6P and ATP concentrations were 50% lower and *type 1* fibres 30% higher in SDF compared to gluteal muscles. CS and HAD activities were similar between SDF and gluteal, while LDH was lower in the SDF muscle.**

Conclusions: **A more strenuous water treadmill conditioning protocol may be needed to induce a training effect in gluteal and SDF muscle and heart rate response. The low substrate concentrations and oxidative capacity of SDF may predispose this muscle to catastrophic fatigue during maximal exercise.**

Introduction

Injury to the superficial digital flexor (SDF) tendon generates substantial loss within the horse racing industry (Jeffcott *et al*. 1982; Rossdale *et al*. 1985; Wilsher *et al*. 2006) and as many as 10% of Thoroughbred racehorses, as well as other sport horses, suffer such injury (Rossdale *et al*. 1985; Goodship *et al*. 1994; Palmer *et al*. 1994). Williams *et al*. (2001) reported that 46% of limb injuries per 1000 British race starts over 3 years involved flexor and suspensory tendons. Biomechanical modelling suggests that the SDF tendon is exposed to considerable mechanical stress as it acts to stabilise fetlock and pastern joints during the stance phase, potentially flex the limb during the swing phase and store and utilise elastic strain energy during running (Anderson and Pandy 1993; Brown *et al*. 2003; Zarucco *et al*. 2004). This stress and the relatively small cross-sectional area of the superficial digital flexor tendon in the metacarpal region (Kerr 1988) combine to create a higher frequency of tendon breakdown than is observed in the deep digital flexor tendon (Hermanson and Cobb 1992).

The ability of the SDF muscles to maintain a forceful contraction over a range of muscle fibre lengthening and shortening and properties of the collagen fibrils within the tendon, may influence the degree of strain placed on the SDF muscle and tendon during the stance phase (Zajac 1989; Brown *et al*. 2003; Swanstrom *et al*. 2004). Furthermore, the ability of the flexor muscles to resist fatigue while shortening may play a key role in injury to the flexor tendon following maximal exercise (Butcher *et al*. 2007).

The combined effects of low oxidative capacity, lactic acidosis and depletion of myofibre ATP and deamination with subsequent production of IMP contribute to fatigue in equine skeletal muscle following maximal exertion (Valberg *et al*. 1988; Essen-Gustavsson *et al*. 1997; Harris *et al*. 1997). However, there are no studies of the metabolic properties of the SDF muscle at present, whereas there are numerous studies that describe the morphological and biomechanical properties of the SDF muscle tendon complex (Hermanson and Cobb 1992; Biewener 1998; Kasashima *et al*. 2002; Brown *et al*. 2003; Zarucco *et al*. 2003, 2004; Firth *et al*. 2004; Swanstrom *et al*. 2004, 2005; Dowling and Dart 2005; Lin *et al*. 2005a,b; Firth 2006).

It is well understood that the metabolic properties of skeletal muscle can be modulated with as little as 10 days of training, thereby delaying the onset of fatigue (Geor *et al*. 1999). Resistance training in particular increases the workload on the muscle without

*Corresponding author email: lisa_b50@yahoo.com
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increasing exercise speed and may in so doing provide a means to quickly enhance muscle oxidative capacity (Gottlieb *et al*. 1989). Further, resistance training was shown to increase the percentage of *type I* fibres (Gottlieb *et al*. 1989), and increase electromyographic activity in *brachiocephalicus* muscle (Tokuriki *et al*. 1999). One method that uses resistance training is exercising horses using water treadmills. These treadmills are currently popular and are being used to train horses; however, while some studies explored biomechanics and heart rate (Tokuriki *et al*. 1999; Lindner *et al*. 2003) there are no studies of muscle metabolic properties to indicate this modality's effectiveness (Voss *et al*. 2002; Nankervis and Williams 2006). One study suggested that heart rate and blood lactate levels remain unchanged by water treadmill training (Lindner *et al*. 2003) but any training effect on specific muscle groups or cardiocirculatory effects remains unclear.

The purpose of this study was to determine if exercising horses using a water treadmill and the manufacturer recommended protocol altered the velocity at which heart rate during maximal exercise reached 200 beats/min or altered SDF and gluteal muscle oxidative and glycolytic capacity or the metabolite and substrate concentrations of those muscles. In addition, this study compared markers of muscle oxidative and glycolytic capacity, as well as substrate and metabolite concentrations, between the SDF and gluteal muscles.

Materials and methods

Horses

Five healthy horses, one Arabian stallion, one Quarter Horse Arabian cross gelding, 3 mares (one Paint, one Thoroughbred, and one Quarter Horse-Arabian cross) owned by the University of Minnesota were used. Horses were unfit and had had no forced exercise for at least 12 months prior the start of the study. Mean age was 6 ± 3.8 years. Horses were weighed and examined by a clinician prior to the commencement of any acclimation or training. During the study, all horses were cared for in accordance with principles outlined by the University of Minnesota Animal Use and Care Committee and housed in accredited facility.

Acclimation

All horses were acclimated to the high-speed (land) treadmill[1] and water treadmill[2] for 2 days.

Standardised exercise test

Prior to starting the water treadmill training regime and at the completion of training, horses performed a standardised exercise test (SET) on the high-speed treadmill at 0% slope preceded by 24 h of rest. The SET consisted of a 4 min walk at 1.9 m/s, followed by 2 min intervals of trot (3.0–3.8 m/s), canter (8.5–9 m/s) and gallop (10–11 m/s). The horses performed until a heart rate of 200 beats/min was attained. Heart rate was recorded by a Polar[3] equine heart monitor for the last 15 s at each speed and plotted against treadmill speed. The treadmill velocity at a heart rate of 200 beats/min was considered the V_{200}. A catheter was placed in the jugular vein prior to the SET and heparinised venous blood samples from each speed were spun down in a microcentrifuge and packed cell volume (PCV) measured. Whole blood lactate concentrations were measured with a hand held lactate meter; however, values proved to be inaccurate and were not included in analysis. Horses repeated the identical SET protocol after their training period.

Water treadmill training

The training regime recommended for bowed tendon rehabilitation by the water treadmill (Aquapacer) manufacturer was used. The water treadmill training protocol, performed daily Monday–Friday, started with 5 min during Week 1, progressed to 10 min during Week 2 and 15 min during Week 3 and finished with 20 min during Week 4. The water treadmill was filled over a period of 5 min until the water reached the level of the ventral abdomen while the horse walked continuously. Once the proper water level was attained, timing of the training interval commenced. After the proscribed training interval, the water level was lowered over a period of 5 min, while the horse walked continuously. The treadmill pace was 2.0 m/s, with slight variation for each horse's stride and ability to maintain pace. No horse exhibited discomfort or lameness; nor was any session terminated early in this study. Venous blood samples were collected 4 h post exercise for measurement of plasma CK by use of an automated chemistry analyser, once per week[4].

Muscle biopsy

Biopsy specimens were obtained at rest 24 h after the SET from gluteal and SDF muscle in the same approximate site but in the opposite limb, for the first and second SET. Biopsy specimens were obtained with a 6 mm diameter Bergstrom biopsy needle by use of SC local anaesthetic and incision over the right or left gluteal or SDF muscle at a depth of 60 mm for the gluteal and 10 mm for the SDF. Gluteal specimens were obtained 17 cm along a line running from the most dorsal part of *tuber coxae* to the head of the tail. SDF specimens were sampled caudally on the forearm, at a third of the distance distal from the olecranon between olecranon and carpus. Biopsy specimens were immediately frozen in liquid nitrogen and stored at −80°C until biochemical analysis was performed. A portion of muscle was rolled in talc and frozen until histochemical analysis was performed.

Muscle fibre typing

Pretraining frozen muscle specimens were mounted in cross section in optimal cutting temperature (OCT) medium and sectioned 10 μm thick. Muscle fibre types were determined by staining for myosin adenosine triphosphatase activity after preincubation at pH 4.6 (Brooke and Kaiser 1970; Pestronk *et al*. 1992). A minimum of 250 muscle fibres were typed per biopsy to calculate fibre type proportions.

Muscle biochemistry

Frozen muscle specimens were lyophilised; dissected free of blood, fat and connective tissue; and then weighed. Glycogen was assayed fluorometrically in muscle biopsy specimens as glucose residues remaining after portions (1–2 mg) of muscle tissue were boiled for 2 h in 1 mol/l HCl (Lowry 1972). A separate portion (4 mg) of muscle was homogenised by crushing with a glass rod in 1.5 mol/l perchloric acid and then was cold centrifuged for 10 min at 9300 ***g***. The supernatant was neutralised with $KHCO_3$, centrifuged again and the remaining supernatant used for analysis of lactate, glucose-

TABLE 1: Mean ± s.d. heart rates (beats/min) during a standardised exercise test (SET) before and after a 4 week period of underwater treadmill training for 5 horses recorded during the last 15 s of 2 min intervals at different speeds on a land treadmill. One horse reached V_{200} at 9 m/s, 2 reached V_{200} at 10 m/s and 2 reached V_{200} at 11 m/s

Speed (m/s)	Before training	After training
0	44 ± 8	41 ± 6
1.9	98 ± 13	101 ± 12
3.4	131 ± 14	129 ± 11
9	195 ± 8	192 ± 9
10	204 ± 6	203 ± 8
11	209 ± 3	208 ± 1
5 min after exercise	111 ± 6	108 ± 7

6-phosphate (G6P) and ATP concentrations via fluorometric techniques (Lowry 1972). Citrate synthase (CS), 3-hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) activities were determined from muscle tissue homogenised in phosphate buffer using fluorometric techniques (Essen-Gustavsson and Lindholm 1985).

Statistical analysis

Muscle lactate, glycogen and ATP concentrations and CS, HAD and LDH enzymatic activities were compared before and after training using 2-way ANOVA blocked for muscle and training state. Muscle lactate, glycogen, G6P and ATP concentrations and CS, HAD and LDH enzymatic activities were compared between muscle groups using 2-way ANOVA. Fibre type proportions were compared using 2-way ANOVA blocked for muscle group and fibre type. The V_{200} responses to the SET were compared before and after training using paired *t* tests. Significance was set at $P<0.05$. Results were presented as mean ± s.d.

Results

Plasma CK activities were found to be within the normal range for all horses during the entire training session (272.4 ± 52.8 u/l). Horses' weight did not change (mean weight 438.2 ± 67.3 kg before training, 444.5 ± 55.0 kg after training) during the water treadmill training period.

Cardiocirculatory effects

There was no significant difference in PCV (maximum 44.4 ± 4.1% first SET and 42.9 ± 4.7% second SET) and V_{200} between the first and the second SET. The mean pretraining V_{200} was 9.0 ± 1.0 m/s and post training V_{200} was 9.3 ± 2.4 m/s. Four horses attained V_{200} at a slightly higher velocity (2 at 10 m/s, 2 at 11 m/s), while one attained V_{200} at a slower velocity (9 m/s) (Table 1).

Training effect on skeletal muscle

The concentrations of glycogen, ATP, G-6-P and lactate as well as CS and HAD enzymatic activity, were not significantly different before and after training in either gluteal or SDF muscle biopsy specimens (Fig 1).

Characteristics of SDF and gluteal muscle

The SDF (Fig 2A) muscle tissue had a higher proportion of *type 1* muscle fibres and lower proportion of *type 2B* muscle fibres than the gluteal muscle (Fig 2B). The mean percentage of *type 1* fibres found in the SDF biopsy specimens was 45.2 ± 10.5% compared to 14.8 ± 7.7% in gluteal specimens. The mean percentage of *type 2A* fibres in the SDF was not different (52.7 ± 12.4%) compared to the percentage found in gluteal biopsy specimens (30.8 ± 12.9%) and the mean of *type 2B* fibres in the SDF was 2.1 ± 4.2% compared to 54.4 ± 17.8% in the gluteal muscle.

The SDF muscle had significantly lower concentrations of glycogen, ATP, G6P and lactate than gluteal muscle biopsy specimens (Table 2). CS and HAD activity were not different between SDF and gluteal muscle specimens; whereas LDH activity was significantly lower in the SDF muscle as compared with the gluteal biopsy specimens (Fig 1).

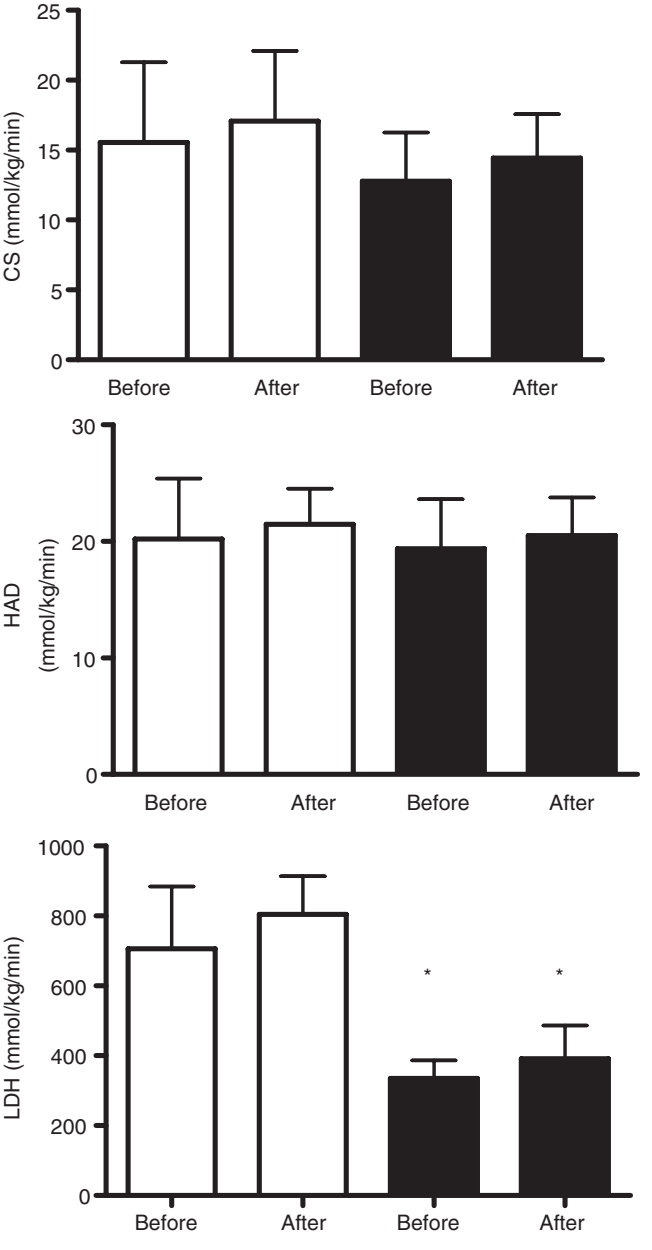

Fig 1: Mean ± s.d. citrate synthase (CS), 3-hydroxyacyl CoA dehydrogenase (HAD) and lactate dehydrogenase (LDH) activities from gluteal (white) and superficial digital flexor (black) muscle biopsies taken from 5 horses before and after water treadmill training. LDH activity was significantly lower in SDF muscle than gluteal muscle. CS, HAD and LDH activities did not change with training.

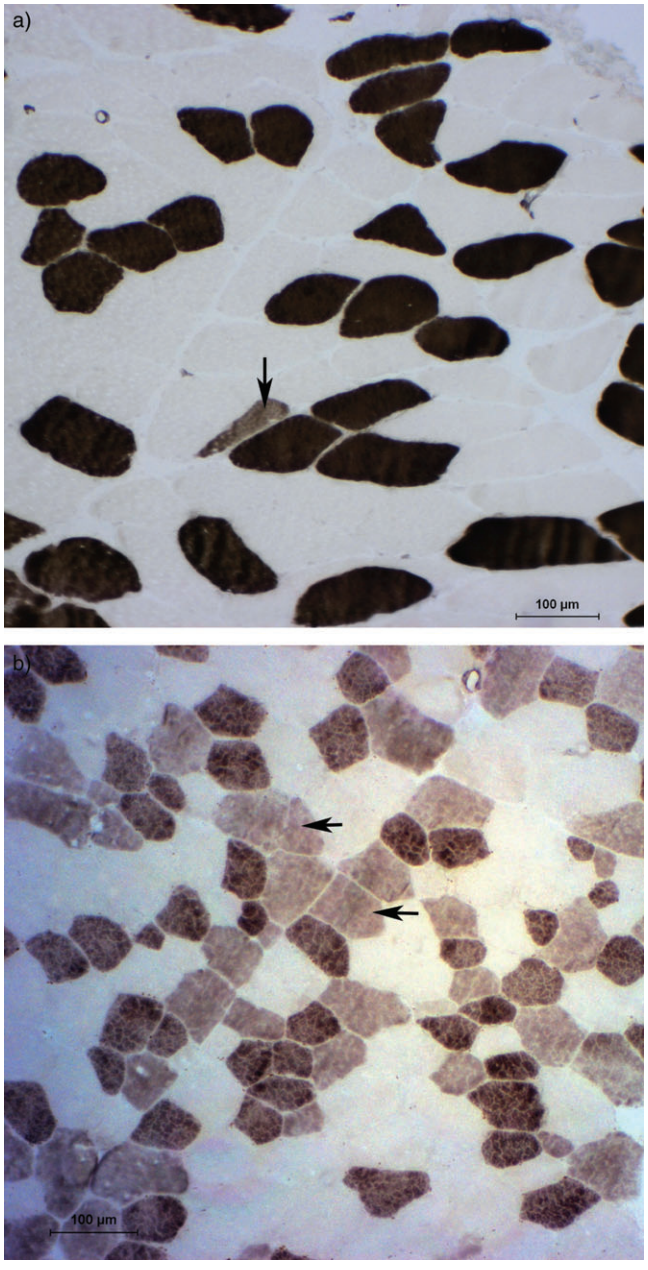

Fig 2: Muscle fibre type composition of the SDF (A) and gluteus medius *muscle (B). Note the higher proportion of* type 1 *muscle fibres (black) than* type 2 B *muscle fibres (arrow) in the SDF compared to* gluteus medius *muscle.*

TABLE 2: Concentration of substrates and metabolites at rest in the *gluteus medius* and superficial digital flexor (SDF) muscles before and after training

Substrate or metabolite (mmol/kg)	*Gluteus medius*		SDF	
	Before	After	Before	After
Glycogen	517 ± 92§	582 ± 130§	254 ± 48	271 ± 29
ATP	23 ± 6§	24 ± 4§	12 ± 4	14 ± 2
Lactate	20 ± 9	17 ± 7	8 ± 4	8 ± 3
G-6-P	2.3 ± 0.6§	2.4 ± 0.4§	1.2 ± 0.4	1.4 ± 0.2

§Significant difference between *gluteus medius* and SDF muscle biopsy specimens for corresponding time point. No difference was detected between values for biopsy specimens obtained before and after training within the same muscle.

Discussion

The results of this study show that no demonstrable cardiocirculatory or skeletal muscle training effect occurred with 4 weeks of water treadmill exercise using the protocol recommended by the consultant for the water treadmill manufacturer. SET V_{200} is often used as used as a measure of cardiocirculatory fitness (Persson 1997) and has been shown to increase with training in horses (Vermeulen and Evans 2006). No significant change in V_{200} occurred in the present study after water treadmill training in agreement with results seen by Lindner *et al*. (2003) in their study of water treadmill conditioning. This may not be surprising, since heart rates of approximately 78 beats/min have been previously reported during water treadmill exercise at a walk (Voss *et al*. 2002; Nankervis and Williams 2006) and walking produces little change in blood lactate concentrations (Weber *et al*. 1987).

Furthermore, no change in oxidative capacity occurred in either the SDF or *gluteus medius* muscle in the present study. Using weights for resistance training, Standardbreds performing 3 to 5 intermittent bouts of 2 min trot (7 m/s) on a treadmill while pulling weights 3 times per week showed no change in V_{200} during a SET after 4 weeks of training; however, there was an increase in gluteal muscle CS activity after 2 weeks of exercise (Gottlieb-Vedi *et al*. 1996). Based on the lack of change in SET V_{200} and muscle oxidative enzyme activities, it would appear that in order to induce a training effect with a water treadmill, a protocol involving more prolonged exercise sessions, greater water resistance and/or velocity of exercise will be necessary.

The muscle fibre type composition of the biopsy specimens of SDF muscle tissue in this study was similar to those identified in a previous study of Standardbreds, Thoroughbreds and Quarter Horses (Hermanson and Cobb 1992). In the previous study, fibre type composition of the midbelly of the SDF muscle was *type 1* 54 ± 6%, *type 2A* 45 ± 5%, and *type 2B* 1 ± 1%. Past research has shown that fibre type composition of gluteal muscle can vary by breed (Snow and Guy 1980; Rivero and Diz 1992) and by sample depth (Rivero *et al*. 1993). Less is known about variability in fibre type distribution in SDF muscle by breed. It is possible that variability in the fibre type composition in the present study is due to the study horses being of different breeds.

Based on previous findings of fibre type composition in SDF and *gluteus medius* muscle specimens, the expectation was that SDF muscle would have a higher oxidative and lower glycolytic capacity than gluteal muscle, which has a high proportion of *type 2B* fibres. It is well known that *type 1* fibres have high oxidative and low glycolytic capacity relative to *type 2B* fibres (Valberg *et al*. 1988). While glycolytic capacity was lower in the SDF than was found in gluteal biopsy specimens, surprisingly no difference was detected in the activities of oxidative enzymes CS and HAD between the 2, despite a 3-fold higher percentage of *type 1* fibres found in the SDF specimens. This confirms previous reports, which determined that contractile fibre types in horses do not always correspond to their expected metabolic properties (Valberg *et al*. 1988; Karlstrom *et al*. 1994).

The apparently low oxidative capacity of the SDF muscle specimens might suggest a greater reliance on anaerobic glycolysis during muscle contraction. However, the SDF does not appear highly suited for anaerobic metabolism, as indicated by low LDH activity and low resting concentrations of glycogen and ATP found in the specimens. The resting ATP concentrations in SDF muscle tissue samples were 50% lower than resting gluteal muscle levels

and only slightly higher than concentrations measured in fatiguing gluteal muscle after maximal exercise (Valberg 1987; Schuback and Essen-Gustavsson 1998). Rat *soleus* muscle, which like the SDF is predominantly slow twitch fibres, has a resting ATP concentration of 18 mmol/kg dry weight (Meyer and Terjung 1979). These past findings make the 12–14 mmol/kg measured in the equine SDF muscle in the present study surprising. It is possible that the small number of biopsies collected were not a comprehensive representation of the mean *type 1* fibres, as one study showed that SDF muscle has a unique and uneven distribution of *type 1* fibres (Hermanson and Cobb 1992). While SDF muscle, by its slow contractile nature, requires less ATP, the combination of low oxidative and glycolytic capacities and low substrate concentrations in the SDF muscle could potentially predispose this muscle to early onset of fatigue during maximal exercise.

Depletion of ATP and adenine nucleotide is believed to be a strong contributing factor to fatigue at maximal exercise intensity (Schuback and Essen-Gustavsson 1998; Essen-Gustavsson *et al*. 1999; Essen-Gustavsson and Jensen-Waern 2002). With the very low resting ATP concentrations found in the SDF muscle specimens in this study, any further decline in ATP concentrations with maximal anaerobic exercise could contribute to the inability to prevent hyperextension of the fetlock, with subsequent damage to tendon collagen fibrils.

Another potential interpretation of why the metabolic capacity found in SDF muscle varies from what is expected based on fibre composition relates to the function of the muscle-tendon complex. Previous studies demonstrate that the SDF muscle functions in a passive manner as a support to the fetlock joint and does not actively flex the forelimb (Swanstrom *et al*. 2005). The muscle-tendon complex stiffens as the fetlock hyper-extends (Hermanson and Cobb 1992) and dampens high-frequency oscillations during loading (Wilson *et al*. 2001). Swanstrom *et al*. (2004) demonstrated that the SDF muscle-tendon complex has a relatively small active force component, suggesting a primary function in storing energy during loading. In a related study (Zarucco *et al*. 2004), it was determined that the SDF contribute to predominately tendinous support with little muscle fascicular shortening during stance at rest and in locomotion. In comparison, the gluteal muscle is well characterised as actively generating propulsion and locomotion (Essen *et al*. 1980). It is therefore possible that the metabolic properties of the SDF tissue relative to gluteal tissue reflect a more passive role during locomotion.

In conclusion, the water treadmill protocol used in this study did not produce an increase in fitness as measured by V_{200} and by muscle oxidative enzyme activities. If increased fitness was the purpose for water treadmill use, then a more strenuous protocol would be needed to provide intended results. Furthermore, the finding of approximately 50% lower oxidative, glycolytic and substrate concentrations in the SDF than gluteal muscle at rest suggest this muscle is either passive during locomotion or could be precariously predisposed to fatigue during maximal exercise.

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Conflicts of interest

SV receives royalty payments from Kentucky Equine Research and the patent for type 1 PSSM. The remaining authors have no potential conflicts to declare.

Manufacturers' addresses

[1]SÄTO AB, Knivsta, Sweden.
[2]FernoVeterinary Systems, Wilmington, Ohio, USA.
[3]HRM USA Inc., Warminster, Pennsylvania, USA.
[4]Marshfield Laboratories, Marshfield, Wisconsin, USA.

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