Effects of training at a walk on conventional and underwater treadmills on fiber properties and metabolic responses of superficial digital flexor and gluteal muscles to high-speed exercise in horses

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Received July 16, 2014.
Accepted February 19, 2015.

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OBJECTIVE
To compare effects of training on conventional and underwater treadmills on fiber properties and metabolic responses of the superficial digital flexor (SDF) and gluteal muscles to high-speed exercise in horses.

SAMPLE
6 unconditioned Quarter Horse–type horses.

PROCEDURES
6 horses were walked on underwater and conventional treadmills for 5 d/wk (maximum, 40 min/d) for 8 weeks in a randomized crossover design (60-day detraining period). Horses underwent a standardized exercise test (SET) at high speed before and after training. Analyte concentrations and fiber characteristics were measured in muscle biopsy specimens obtained from horses before and after each SET.

RESULTS
Lactate concentration increased 2- to 3-fold in SDF and gluteal muscle after SETs. No training effect was identified on muscle fiber type composition, type II fiber diameter, muscle analyte concentrations, blood lactate concentration, or heart rate responses. Maximum diameters of type I fibers decreased significantly in gluteal muscle with conventional treadmill training and decreased in SDF muscle with both types of training, with maximum diameters greater for horses after underwater versus conventional treadmill training. No change was identified in minimum fiber diameters.

CONCLUSIONS AND CLINICAL RELEVANCE
SETs involving near-maximal exertion resulted in an anaerobic response in SDF and gluteal muscles of horses. Eight weeks of conventional or underwater treadmill training resulted in minor changes in type I muscle fiber sizes, with no effect on muscle metabolic or heart rate responses to SETs. After rehabilitation involving underwater treadmills, training at progressing speeds is recommended for horses to develop the required fitness for speed work. (*Am J Vet Res* 2015;76:1058–1065)

Injury to the SDF tendon is one of the most common musculoskeletal injuries in racehorses.[1] Progressive microdamage to collagen fibrils occurs when a digital load of up to 1 metric ton is applied to the relatively small cross-sectional area of the SDF tendon in the metacarpophalangeal region during racing or jumping.[2] Sudden failure of the SDF tendon appears to occur when a critical stress point is reached; however, injury to the tendon may not be due to weakness in the tendon alone.[2] Recognition is increasing that the entire SDF muscle-tendon complex contributes to tendon injury, with fatigue of the SDF muscle contributing to an inability to stabilize the metacarpophalangeal or metatarsophalangeal (fetlock) joint at high speeds.[3] To date, no studies have been performed to evaluate the metabolic response of SDF muscles to high-speed exercise in horses. One study[4] involving metabolic properties of the SDF muscle at rest revealed low concentrations of glycogen and ATP relative to concentrations in gluteal muscle, and investigators suggested that this characteristic could predispose the SDF muscle to fatigue with maximal exertion.

ABBREVIATIONS

SDF	Superficial digital flexor
SET	Standardized exercise test

Underwater treadmill training provides a potential mechanism to enhance fatigue resistance and strengthen SDF muscles by creating workload through the drag that limbs encounter as they move through water.[5] Other forms of resistance training in horses have been shown to increase muscle oxidative capacity in a manner that resembles adaptations to training at higher speeds.[6] Underwater treadmill training is used in the rehabilitation of horses with tendon injuries, but few data exist regarding the impact of this method on SDF muscles and their resistance to fatigue. The purpose of the study reported here was to evaluate the metabolic response of SDF muscle to high-speed exercise and to determine whether underwater treadmill training, compared with conventional treadmill training, would have a different impact on the properties and metabolic response of gluteal and SDF muscles to high-speed exercise.

Materials and Methods

Animals

Six sound unconditioned horses of Quarter Horse–related breeds (3 geldings and 3 mares), with a mean ± SD age of 7 ± 2 years (range, 5 to 10 years), were used for the training portion of the study. Horses had no forced exercise for at least 90 days prior to the start of the study. All were housed in a quarter-acre drylot and fed grass hay on a free-choice basis. Two weeks before the study began, the 6 horses were introduced to exercise on a conventional treadmill[a] and underwater treadmill[b] for 2 sessions of < 20 minutes.

In addition, biopsy specimens of SDF muscle were obtained from 1 Thoroughbred and 3 other horses of Quarter Horse–related breeds to represent SDF muscle in horses at rest (ie, at-rest specimens). These comparison horses consisted of 1 gelding and 3 mares between 2 and 10 years of age, and additional data regarding them have been published.[4] The decision to use different horses as a source of specimens of SDF muscle at rest was made because the small size of the SDF muscle, the need to tranquilize horses to perform the biopsy, and the swelling and pain associated with the biopsy would have interfered with results of exercise testing of the training horses. The study protocol was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Study protocol

A randomized 3 X 2 crossover design was used, consisting of two 6-week training periods involving a conventional, land-based treadmill (3 horses/period) or underwater treadmill (3 horses/period), separated by a 60-day detraining period. Horses were randomly assigned to treadmill order by selecting names out of a hat. The 60-day detraining period consisted of housing in the quarter-acre drylot, with no forced exercise and free-choice feeding of grass hay. Standardized exercise tests were performed, blood samples were collected, and muscle biopsy specimens were obtained at various points before, during, and after the training periods.

Treadmill training

During each training period, horses were exercised on the assigned type of treadmill for 5 d/wk at a speed of 1.5 m/s, with slight variation allowed for each horse's walk stride. Durations of daily training sessions were the same for both treadmills and increased by 1 min/d as follows: week 1, 16 to 20 minutes; week 2, 21 to 25 minutes; week 3, 26 to 30 minutes; week 4, 31 to 35 minutes; week 5, 36 to 40 minutes; and weeks 6 to 8, 40 minutes.

For underwater treadmill training, filling of the treadmill tank with water began at time 0, when the treadmill belt was started, and with the horse walking continuously during filling. Filling continued until the water level reached the level of the olecranon for each horse (approx 5 minutes). Draining of water from the tank was initiated in the last 60 seconds of each exercise session, with the horse walking continuously.

SETs

Each horse underwent an SET 2 weeks before the first training period began and again after each of the 2 treadmill training periods concluded for a total of 3 tests. Acclimation to the conventional treadmill and underwater treadmill occurred just prior to the first SET. To prepare each horse for the SET, a catheter was placed in a jugular vein and heart rate was recorded with a wireless heart rate monitor.[c] Each SET required horses to walk on a flat conventional treadmill for 4 minutes at 1.5 m/s, then for 2 minutes each at 3.2, 8.9, and 10.5 m/s. For the first SET only, each horse was also humanely encouraged to complete 2 minutes of exercise at 11.2 m/s, but if it could no longer keep pace with the treadmill such that it moved back from the front bar of the treadmill > 12 inches, the test was stopped.

Heparinized blood samples for measurement of blood lactate concentration and PCV were collected from each horse just before each SET began, during the last 30 seconds at each speed, and 5 minutes after the SET. Whole blood lactate concentration was analyzed with a handheld device.[d]

Muscle biopsy

One muscle biopsy specimen was obtained before each of the 3 SETs (from gluteus medius [middle gluteal] muscle) and within 5 minutes after each SET (from the middle gluteal and SDF muscles). Because biopsy specimens of SDF muscle could not be collected prior to the SET without potentially influencing the horse's performance, biopsy specimens representing SDF muscle tissue at rest were obtained from the 4 comparison horses and were used to represent pre-SET values for SDF muscles. The biopsy site for the middle gluteal muscle was 17 cm along a line running from the dorsal aspect of the selected tuber coxae to the tail head at a depth of 6 cm.[7] The skin at that site was aseptically prepared and desensitized with an SC

injection of 2% lidocaine solution, and a 2-cm incision was made. A percutaneous modified 6-mm Bergstrom biopsy needle was inserted through the incision. Immediately after the SET, horses were placed in nearby stocks and sedated with xylazine hydrochloride and a second gluteal tissue specimen was obtained via the same skin incision but at a different angle of insertion. An additional middle gluteal muscle biopsy specimen was obtained with horses at rest at the end of the detraining period. Biopsy sites alternated between left and right sides with each SET and were within 2 cm of the previous specimen collection site for subsequently obtained specimens.

The site chosen for SDF muscle biopsy was on the caudal aspect of the proximal portion of the forelimb, a third of the distance between the olecranon and accessory carpal bone. The same collection technique was used for the training and comparison horses. Skin over the region was aseptically prepared, 2% lidocaine solution was injected SC, a 2-cm was incision made through the skin and fascia, and a Bergstrom biopsy needle biopsy was inserted to a depth of 10 mm.[4] Fascia and skin were closed with sutures. Biopsy sites alternated between the left and right forelimbs with each SET. To minimize swelling after biopsy specimen collection, the forelimb was bandaged for 24 hours, 1 g of phenylbutazone was administered PO, and horses were allowed to rest for 48 hours in a stall prior to return to the paddock.

All biopsy specimens for histochemical analysis were oriented in cross section, mounted on cork, and frozen in isopentane suspended in liquid nitrogen. All specimens for biochemical analysis were immediately plunged into liquid nitrogen and stored at −80°C until analyzed.

Muscle histochemical analysis

Muscle biopsy specimens for histochemical detection of muscle fiber types were preincubated at a pH of 4.6[8] and sliced in a cryostat at −13°C into 10-µm-thick sections. Slides were then prepared by staining for ATPase activity as previously described.[9] Percentage of fibers with staining for type I, IIA, and IIB fibers was calculated by counting at least 200 fibers. Computer software[e] was used to measure the minimum and maximum diameter of 25 fibers of each fiber type on digital images of muscle tissue specimens obtained with horses at rest (gluteal muscle) and after the SET (SDF muscle).[10] One investigator performed all measurements.

Muscle biochemical analysis

Frozen muscle specimens were freeze-dried and dissected free of blood, fat, and connective tissue. One- to 2-mg portions of muscle tissue were boiled for 2 hours in 1N hydrochloric acid, and glycogen content was measured fluorometrically as glucose residues remaining.[11] Separate 4-mg portions of muscle were homogenized by crushing with a glass rod in 1.5M perchloric acid and cold centrifugation for 10 minutes at 9,300 × *g*. The supernatant was neutralized with $KHCO_3$ and centrifuged again, and the remaining supernatant was used for measurement of lactate and ATP concentrations by use of fluorometric techniques.[11]

Statistical analysis

Muscle fiber type composition and minimum and maximum fiber diameters as well as muscle glycogen, lactate, and ATP concentrations were compared among measurement points (before training, after conventional and underwater treadmill training, and after the detraining period) by means of general linear model ANOVA. Fiber type, training type, and measurement point (ie, before or after SET) were used as factors, and Bonferroni multiple comparison post hoc testing was performed. A 1-tailed *t* test was used to compare substrate and metabolite concentrations in SDF muscle of the 6 training horses after the SET, with resting values established by use of SDF biopsy specimens from the 4 comparison horses. One-way ANOVA was used to compare substrate and metabolite concentrations in SDF muscle after the SET before training with concentrations after conventional and underwater treadmill training.

Heart rate during the last 15 minutes of each treadmill speed setting was plotted against treadmill speed to determine the work speed at a heart rate of 180 beats/min. The study design required that horses have no treadmill training prior to the study. As a result, horses could not comfortably canter on the treadmill at speeds between 6 and 8 m/s, and the work speed at which blood lactate concentration is 4 mmol/L could not be calculated. Therefore, rather than use work speed at which blood lactate concentration is 4 mmol/L, blood lactate concentration at 10.5 m/s was selected as the variable for comparison because this was the highest speed that all horses completed successfully. One-way ANOVA and post hoc testing via the Bonferroni multiple comparison method were used to compare speed at a heart rate of 180 beats/min and blood lactate concentration at 10.5 m/s among the 3 SETs. Values of $P < 0.05$ were considered significant. Data are reported as mean ± SD.

Results

Animals

All 6 horses completed both training periods (one on a conventional treadmill and the other on an underwater treadmill). All but 1 horse completed all 3 SETs (the first SET was performed 2 weeks before the first training period began, and the second and third SETs were performed after each of the 2 treadmill training periods). That 1 horse was unable to perform the first SET because of a laceration of the distal aspect of the limb that precluded high-speed exercise at that time. Mean ± SD body weight of the horses did not differ significantly among measurement points (before training, 526.9 ± 63.3 kg; after conventional treadmill train-

ing, 529.6 ± 43.7 kg; and after underwater treadmill training, 527.9 ± 29.4 kg).

SETs

Before training, a maximal speed of 11.2 m/s was sustained during the SET for 2 minutes by 1 horse, 1 minute by 1 horse, and 30 seconds by 3 horses (mean duration, 0.9 ± 0.7 seconds). None of the horses completed 2 minutes of exercise at 11.2 m/s during the subsequent SETs after conventional and underwater treadmill training. Mean duration at 11.2 m/s was 0.3 ± 0.4 minutes after conventional treadmill training and 0.4 ± 0.4 minutes after underwater treadmill training.

Middle gluteal muscle

Percentages of type I, IIA, and IIB fibers did not differ among gluteal tissue specimens obtained from horses before training, after conventional and underwater treadmill training, and after the detraining period **(Table 1)**. Mean minimum diameters for all fibers or muscle fiber types did not differ among specimens obtained before training, after conventional and underwater treadmill training, and after the detraining period **(Figure 1)**. Mean maximum fiber diameters for all fiber types combined decreased significantly ($P < 0.001$) with underwater treadmill (59.5 ± 34.4 μm) and conventional treadmill (58.5 ± 34.8 μm) training from before training (70.2 ± 41.5 μm) and remained smaller during the detraining period (61.9 ± 36.6 μm). Maximum diameters of type IIA and IIB muscle fibers did not differ significantly among specimens obtained before training, after conventional or underwater treadmill training, and after the detraining period. Maximum diameters of type I muscle fibers were significantly ($P < 0.001$) smaller after conventional treadmill training, compared with before training, with no difference among maximum diameters in type I fiber before training, after underwater treadmill training, or after the detraining period.

Glycogen concentrations in specimens of middle gluteal muscle decreased after the SETs, albeit not significantly ($P = 0.053$). Muscle glycogen concentrations did not differ significantly among specimens obtained before training, after conventional treadmill training, and after underwater treadmill training, compared with concentrations in specimens obtained before and after the SET **(Table 2)**. Muscle lactate concentration increased significantly ($P < 0.001$) during the SET in a similar manner with each treatment, with large interindividual variation in values obtained for the first SET. Muscle ATP concentration did not decrease significantly from pre-SET values after the SET, and no significant differences were identified among specimens obtained before training, after conventional treadmill training, and after underwater treadmill training.

SDF muscle

Percentages of type I and IIA fibers did not change significantly in specimens of SDF muscle tissue with either type of treadmill training. Type IIB fibers were lacking in specimens. Mean minimum diameters of all muscle fibers or muscle fiber types did not differ among specimens obtained from horses before training, after conventional treadmill training, and after underwater treadmill training (Figure 1). Mean maximum fiber diameters for all fiber types combined decreased significantly with underwater treadmill (116.4 ± 30.2 μm) and conventional treadmill (108.7 ± 34.2 μm) training from before training (124.4 ± 37.3 μm), with mean maximum fiber diameters after underwater treadmill training significantly ($P < 0.001$) larger than after conventional treadmill training. The interaction between fiber type and treatment was significant ($P < 0.007$), with the maximum diameter of type I fibers decreasing with conventional and underwater treadmill training and diameters significantly larger after underwater treadmill training than after conventional treadmill training. Maximum diameters of type IIA muscle fibers did not differ significantly among specimens obtained before training, after conventional treadmill training, and after underwater treadmill training.

Table 1—Mean ± SD percentages of various types of muscle fibers in biopsy specimens of middle gluteal and SDF muscles obtained from 6 previously unconditioned horses at various points during 8 weeks of training (walking 5 d/wk for a maximum of 40 min/d) on conventional and underwater treadmills.*

Variable	Type I	Type IIA	Type IIB
Middle gluteal muscle			
Before training	25.6 ± 9.6	25.31 ± 6.5	49.1 ± 7.7
After conventional treadmill training	20.9 ± 7.8	32.0 ± 10.0	47.1 ± 9.6
After underwater treadmill training	20.6 ± 3.6	31.0 ± 10.2	48.4 ± 10.1
After detraining	17.2 ± 8.1	26.3 ± 6.2	56.5 ± 10.9
SDF muscle			
Before training	52.3 ± 11.2	47.7 ± 11.2	0
After underwater treadmill training	44.7 ± 10.3	55.3 ± 10.3	0
After conventional treadmill training	45.4 ± 12.5	54.6 ± 12.5	0

*A randomized 3 X 2 crossover design was used, consisting of two 6-week training periods involving a conventional treadmill (3 horses/period) or underwater treadmill (3 horses/period), separated by a 60-day detraining period.

No significant differences in muscle fiber percentages were identified among measurement points for either muscle type.

Muscle glycogen concentrations were significantly ($P < 0.04$) lower in SDF muscle specimens obtained after the first SET (before training) than in specimens obtained from the 4 comparison horses at rest (ie, at-rest specimens). However, concentrations did not differ between at-rest specimens and specimens obtained after underwater treadmill training (Table 2). Compared with values for specimens obtained after the first SET, glycogen concentrations did not differ significantly for specimens obtained after conventional or underwater treadmill training. Compared with values for at-rest specimens, muscle lactate concentrations were significantly higher for specimens obtained after the first SET ($P < 0.03$), after the conventional treadmill training SET ($P < 0.01$), and after the underwater treadmill training SET ($P < 0.05$); however, no differences in values were identified among treatment groups. High interindividual variation was identified in muscle lactate concentrations after the first SET. Muscle ATP concentrations did not differ significantly among at-rest specimens and specimens obtained after any SET.

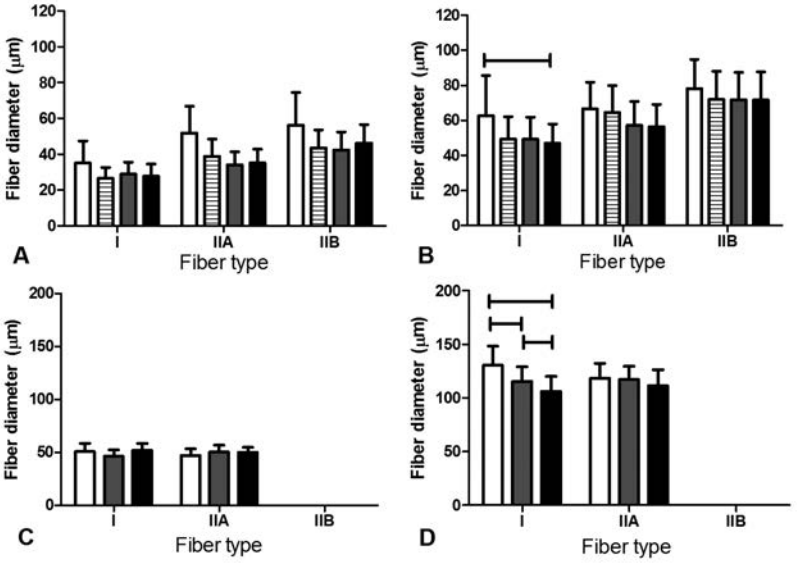

Figure 1—Mean ± SD minimum (A and C) and maximum (B and D) muscle fiber diameters in biopsy specimens obtained from the middle gluteal (A and B) and SDF (C and D) muscles of 6 horses before treadmill training (white bars), after 8 weeks of training (walking 5 d/wk for a maximum of 40 min/d) on conventional (black bars) and underwater (gray bars) treadmills, and after a 60-day detraining period that separated the 2 training periods (striped bars). A randomized 3 X 2 crossover design was used. Significant ($P < 0.05$) differences in fiber diameter within muscle fiber types are indicated by the origin and endpoint of horizontal bars.

Cardiocirculatory response to SET

No significant difference in mean work speed at a heart rate of 180 beats/min was evident among SETs performed before training (8.2 ± 2.2 m/s), after conventional treadmill training (9.3 ± 1.6 m/s), and after underwater treadmill training (9.0 ± 1.0 m/s). At a speed of 10.5 m/s, there was also no significant difference in blood lactate concentration among SETs performed before training (11.0 ± 5.1 mmol/L), after conventional treadmill training (8.8 ± 1.8 mmol/L), and after underwater treadmill training (8.6 ± 1.9 mmol/L; **Figure 2)**.

Discussion

The SDF muscle-tendon complex requires strength to stabilize the digit and prevent fetlock joint hyperextension during locomotion.[12] Results of electromyography suggest that the deep digital flexor muscle may fatigue before the SDF muscle does, leaving the suspensory ligament and SDF

Table 2—Mean ± SD muscle substrate and lactate concentrations (mmol/kg of dry weight) in biopsy specimens of middle gluteal and SDF muscle obtained from the 6 horses in Table 1 before and within 5 minutes after each of 3 SETs was performed.

	Before training		After underwater treadmill training		After conventional treadmill training	
Variable	**Before SET**	**After SET**	**Before SET**	**After SET**	**Before SET**	**After SET**
Glycogen						
Gluteal	436.7 ± 102.8	388.9 ± 113.3	507.7 ± 82.6	429.0 ± 47.1	504.6 ± 67.8	458.3 ± 79.2
SDF	265.5 ± 47.3*	177.0 ± 75.6‡	—	213.6 ± 46.6	—	225.4 ± 58.5
ATP						
Gluteal	24.3 ± 4.3	24.6 ± 5.3	26.4 ± 5.7	22.6 ± 5.6	27.4 ± 1.6	25.0 ± 1.9
SDF	13.5 ± 2.7 *	16.6 ± 5.9	—	14.2 ± 5.8	—	16.5 ± 5.0
Lactate						
Gluteal	33.2 ± 11.7	76.3 ± 32.6	25.4 ± 5.0	54.9 ± 18.1†	30.5 ± 6.4	61.6 ± 18.2†
SDF	17.3 ± 6.7*	54.6 ± 31.7‡	—	29.6 ± 11.6‡	—	40.9 ± 15.0‡

*Resting values were established by use of biopsy specimens of SDF muscle tissue obtained from 4 other comparison horses. †Difference between values obtained before and after the SET is significant ($P < 0.05$). ‡Difference between resting value for comparison horses and post-SET value for training horses is significant ($P < 0.05$).

— = Not measured.

The SETs were performed 2 weeks before the first training period began and again after each of the 2 treadmill training sessions. No significant differences were identified in values measured before or after SET at any point.

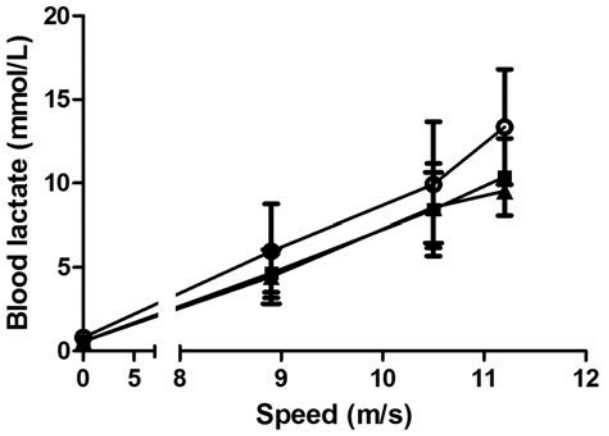

Figure 2—Mean ± SD blood lactate concentrations in the horses in Figure 1 undergoing an SET at a treadmill speed of 10.5 m/s before treadmill training (circles), after training on a conventional treadmill (squares), and after training on an underwater treadmill (triangles). Although all 6 horses were trained on both treadmills, 4 horses refused to gallop at 11.2 m/s during the SET performed after conventional and underwater treadmill training; therefore, data from the 10.5 m/s speed were used. No significant differences in concentrations were identified among the 3 SETs. Note that the x-axis is not to scale and that 1 horse was unable to perform the first pretraining SET because of a laceration of the distal aspect of the limb that precluded high-speed exercise at that time.

muscle and tendon as the final line of defense against excessive tendon strain.[f] Therefore, the ability of the SDF muscle to sustain isometric or eccentric contractions with loads of up to 1 metric ton is important to prevent injury.[3] Remarkably, despite the importance of the SDF muscle in resisting fatigue, to the authors' knowledge, the present study was the first in which the metabolic response of that muscle to exercise was characterized. Results suggested the potential for SDF muscle to fatigue during maximal exertion, given that post-SET lactate concentrations in SDF muscle specimens from 6 training horses were 2- to 3-fold as high as the values for specimens obtained from 4 resting comparison horses (ie, resting values), and this increase in lactate concentration was similar to that identified in specimens of gluteal muscle obtained after SETs. This apparent increase in muscle lactate concentration with near-maximal exertion was particularly striking given the high proportion of slow-twitch type I fibers and absence of fast-twitch type IIB fibers in SDF muscle.[4]

A weakness in our study design was that biopsy specimens of SDF muscle tissue could not be obtained when horses were at rest before each SET because of the small size of the SDF muscle and the discomfort and swelling inherent to the biopsy procedure. For this reason, specimens of SDF muscle tissue were obtained from a different group of resting horses (ie, at-rest specimens) by means of the same biopsy technique that was used to collect post-SET specimens from training horses. Substrate and metabolite concentrations in at-rest specimens were anticipated to be comparable with those that would have existed in training horses when at rest, before the SET, considering that the substrate and metabolite concentrations measured in gluteal tissue specimens obtained from the training horses at rest, before the SET, were similar to those measured in specimens obtained from control Quarter Horses in a previous study.[13] Results of the present study suggested that SDF muscles engaged in anaerobic glycolysis to a considerable extent in horses during maximal exertion, indicating that this muscle could readily fatigue without proper training of horses.

In the present study, 8 weeks of walking on an underwater or conventional treadmill yielded no evidence of adaptations within SDF or gluteal muscles to training. Muscle metabolic responses to SETs were similar for underwater and conventional treadmill training. This finding is in agreement with that of a previous study[4] in which no change in SDF or gluteal muscle oxidative capacity was identified in horses after 4 weeks of underwater treadmill training. Furthermore, the present study did not reveal differences in the treadmill speed required for horses to achieve a heart rate of 180 beats/min or in the blood lactate concentration attained at 10.5 m/s (the highest speed achieved by all horses) between conventional and underwater treadmill training. Therefore, no clear differences in cardiocirculatory or metabolic findings could be discerned between underwater or conventional treadmill training.

Difficulties with study design precluded a full comparison between SETs performed before training and after training. The SET performed before training was not identical to the SET performed after underwater or conventional treadmill training because horses were not willing to gallop at the top speed of 11.2 m/s for ≥ 1 minute after training. One would expect that if a training effect on SDF and gluteal muscle had existed, then horses would have galloped longer at top speeds. We also believe that the horses in the present study became accustomed to their handlers in their new environment during training and less responsive to encouragement to increase their speed during the SETs. Their apparent willingness and aptitude for galloping at high speed were relatively low, compared with maximal speeds of > 11 m/s at a 6% slope that were attained by Thoroughbreds previously trained on a conventional treadmill in other studies.[14,15] However, the study design allowed us to accurately assess any differences between effects of underwater and conventional treadmill training because the SETs performed after conventional and underwater treadmill training were practically identical. Ideally, work speed at which blood lactate concentration reaches 4 mmol/L would have been assessed in the present study; however, the horses were so unaccustomed to the treadmill initially that they could not perform the slow canter speed necessary to reproducibly measure the onset of anaerobic metabolism (velocity at a blood lactate concentration of 4 mmol or anaerobic thresh-

old). For this reason, blood lactate concentration at 10.5 m/s (the highest speed achieved by all horses) was used to assess anaerobic glycolysis. Clearly, in agreement with findings of a previous 4-week study[4] of horses training on an underwater treadmill, training of horses at a walk on an underwater or conventional treadmill for 8 weeks was not strenuous enough to induce a measureable training effect on muscle metabolic response or systemic indicators of fitness. Therefore, it would appear prudent to allow horses to progressively develop cardiovascular and muscular fitness at gradually increasing speed after they have completed a period of rehabilitation on an underwater treadmill.

Exercise conditioning of horses involves optimizing the muscle's ability to sustain muscle contractions over the desired performance distance while minimally impacting a decrease in muscle force that occurs with a reduction in muscle fiber diameter. Many equine training studies[16–20] have revealed that cross-sectional areas or diameters of middle gluteal muscle fibers decrease with aerobic training. The metabolic advantage of smaller-diameter, fast-twitch muscle fibers versus larger-diameter, slow-twitch muscle fibers is believed to be the higher density of capillaries around these smaller muscle fibers, thus reducing distances for lactate efflux and glucose and free fatty acid influx. In the present study, a decrease in maximum fiber diameters of type I gluteal muscle occurred with conventional treadmill training. Type I fibers are recruited at walking speeds; therefore, this finding appears consistent with the previously reported response of gluteal muscle to horses training at a walk.[21]

The SDF muscle had much larger diameters of muscle fibers than did the gluteal muscle in the study reported here. Large fiber diameters and a multipennate shape maximize force production through an increase in functional cross-sectional area, which is proportional to force production.[21] In the present study, muscle fiber size was assessed by measuring minimum and maximum fiber diameters. Maximum fiber diameters were measured to detect any potential hypertrophy of muscle fibers with underwater treadmill training; however, unlike minimum fiber diameters, maximum fiber diameters are influenced by fiber obliquity in tissue specimens.[10] We made every effort to ensure that the fibers evaluated were in actual cross sections of tissue specimens to minimize this potential error. No differences were apparent in minimum fiber diameters of SDF muscle with training, whereas maximum fiber diameters of type I SDF muscle decreased with training and were larger after underwater treadmill, compared with after conventional treadmill training.

The larger maximum type I fiber diameters after underwater treadmill versus conventional treadmill training could have indicated less recruitment of muscle fibers during underwater treadmill training owing to the water's buoyancy effect. Walking of horses in water at a level above the carpus decreases the degree of lower limb joint extension, decreases stride frequency, and increases stride length,[5,22] which could have influenced the extent to which these fibers were recruited and thus their training adaptation in the present study. A lesser degree of recruitment of SDF type I muscle fibers with underwater treadmill training would be beneficial during rehabilitation of horses involving underwater treadmills, in which a decrease in the extent of loading of the digit and SDF tendon is desirable. An alternative explanation for a lesser decrease in type I fiber maximum diameters with underwater versus conventional treadmill training is that the resistance created by drag of the lower limb through water counteracted the typical training-induced decrease in fiber size. A potential beneficial effect of underwater treadmill training for muscles such as the SDF muscle, which requires strength to stabilize the fetlock joint, could be preservation of the cross-sectional area of SDF muscle and force production during training. However, the differences in maximum fiber size between underwater versus conventional treadmill training in the present study were fairly small and could have been affected by methodological error such as evaluation of obliquely aligned muscle fibers. Given the findings regarding both minimum and maximum fiber diameters and with acknowledgment of the potential for methodological error, we identified a negligible to relatively small differential effect on muscle fiber sizes with underwater versus conventional treadmill training.

Despite the high proportion of type I muscle fibers in SDF muscle specimens obtained from horses trained on 2 types of treadmills, a substantial amount of lactate was detected in blood samples collected after the high-speed exercise associated with the SETs, indicating a significant anaerobic response of the muscle to near-maximal exertion and the potential for that muscle to fatigue. Eight weeks of water or conventional treadmill training did not result in a cardiocirculatory or metabolic training response in SDF or gluteal muscles; therefore, a gradual introduction of speed training is likely necessary after water treadmill rehabilitation to protect the SDF muscle from fatigue and tendon injury in racehorses.

Acknowledgments

Supported by the University of Minnesota Equine Center with funds provided by the Minnesota Racing Commission, University of Minnesota Agricultural Experiment Station, and private donors.

Presented in poster form at the International Conference on Equine Exercise Physiology, Chester, Chesire, England, June 2014.

The authors thank Michelle Lucio for technical assistance and Drs. Dionne Benson, Alex Bianco, Liz Collar, Hannah Daigle, Marta Davis Tetrault Powers, Lauren Haverstock, Jennifer Heesen, Jaime Horner, Shantel Julius, Claire Kamrath, Jess Kirk, Anna Lembcke, Angela Lockhart, Grace Meyer, Jonathan Nevins, Laura Pritzker, Danielle Renstrom, Daylin Taylor, Emily Van Arsdale, and Sarah Wahlert for assistance performing the trial.

Footnotes

a. SÄTO AB, Knivsta, Sweden.
b. Aquapacer equine underwater treadmill, Hudson Aquatic Systems LLC, Angola, Ind.
c. Polar equine heart rate monitor, Polar Electro Inc, Lake Success, NY.

d. VetScan i-STAT handheld analyzer, Abaxis North America, Union City, Calif.
e. IMT i-solution lite software, Focus Precision Instruments, Victoria, Minn.
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