Downhill treadmill running trains the rat spinotrapezius muscle


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Hahn SA, Ferreira LF, Williams JB, Jansson KP, Behnke BJ, Musch TI, Poole DC. Downhill treadmill running trains the rat spinotrapezius muscle. J Appl Physiol 102: 412–416, 2007.—There are currently no models of exercise that recruit and train muscles, such as the rat spinotrapezius, that are suitable for transmission intravital microscopic investigation of the microcirculation. Recent experimental evidence supports the concept that running downhill on a motorized treadmill recruits the spinotrapezius muscle of the rat. Based on these results, we tested the hypothesis that 6 wk of downhill running (−14° grade) for 1 h/day, 5 days/wk, at a speed of up to 35 m/min, would (1) increase whole body peak oxygen uptake (V\text{O}_2\text{peak}), (2) increase spinotrapezius citrate synthase activity, and (3) reduce the fatigability of the spinotrapezius during electrically induced 1-Hz submaximal tetanic contractions. Trained rats (n = 6) elicited a 24% higher V\text{O}_2\text{peak} (in ml min^{-1} kg^{-1}; sedentary 58.5 ± 2.0, trained 72.7 ± 2.0; P < 0.001) and a 41% greater spinotrapezius citrate synthase activity (in μmol min^{-1} g^{-1}; sedentary 14.1 ± 0.7, trained 19.9 ± 0.9; P < 0.001) compared with sedentary controls (n = 6). In addition, at the end of 15 min of electrical stimulation, trained rats sustained a greater percentage of the initial tension than their sedentary counterparts (control 34.3 ± 3.1%, trained 59.0 ± 7.2%; P < 0.05). These results demonstrate that downhill running is successful in promoting training adaptations in the spinotrapezius muscle, including increased oxidative capacity and resistance to fatigue. Since the spinotrapezius muscle is commonly used in studies using intravital microscopy to examine microcirculatory function at rest and during contractions, our results suggest that downhill running is an effective training paradigm that can be used to investigate the mechanisms for improved microcirculatory function following exercise training in health and disease.

intravital microscopy; microcirculation; oxidative capacity; citrate synthase activity; muscle fatigue

THE MICROCIRCULATION WITHIN skeletal muscle constitutes a prodigious surface area for O_2 and substrate exchange. Understanding muscle microcirculatory control and capillary red blood cell (RBC) hemodynamics is key to resolving those mechanisms by which blood-muscle exchange increases up to 100-fold during exercise in health and why this process malfunctions in pandemic diseases such as diabetes (5, 25, 44) and chronic heart failure (CHF) (18, 23, 45). Moreover, as exercise training has become established as a primary or adjunct therapy for many patient populations, including diabetic and/or CHF (40), it is important to have some knowledge of the effects of exercise training on muscle microcirculatory function.

Unfortunately, selection of skeletal muscles for transmission intravital microscopy requires that they be accessible to exteriorization without disruption of their neural innervation or vascular bed and sufficiently thin to permit light penetration and visualization of the microcirculation. The muscles that fulfill these criteria and those most commonly selected for these studies include the rat spinotrapezius (24, 26) and cremaster (39) and the hamster cheek pouch retractor (50). To date, however, none of these preparations has facilitated investigation of training adaptations where the paradigm selected actually recruits and trains the muscle of interest. Specifically, running on the level or inclined treadmill has been implemented in the rat to study microcirculatory adaptations in the spinotrapezius muscle (28, 29, 58). Because this exercise paradigm does not increase the spinotrapezius blood flow (38) or citrate synthase activity (29, 27), adaptations documented in the vascular bed are likely "crossover" effects from those muscles that were actually recruited or systemic adaptations. The same may be true for similar studies in the cremaster muscle (e.g., Ref. 39).

The rat spinotrapezius muscle is an excellent analog of human muscle from the perspective that it has a mixed fiber-type composition [41% type I, 7% type IIa, and 17% type IIb (8)] and oxidative capacity similar to that of the human quadriceps (32). This muscle functions to stabilize the scapula, and the additional stresses that impact the forelimbs during downhill running recruit this muscle, as evidenced by increased exercising blood flow (21) and eccentric contraction-induced muscle and vascular damage (22).

The purpose of the present investigation was to determine whether a training program using downhill running could effectively promote a "classic" endurance-training effect (19, 20, 49). Specifically, we tested the hypothesis that 6 wk of training on the declined treadmill would effectively increase whole body peak oxygen uptake (V\text{O}_2\text{peak}) and spinotrapezius citrate synthase activity (marker of muscle oxidative capacity) and reduce spinotrapezius fatigability. The results validated this hypothesis and indicate that downhill running constitutes a viable paradigm for investigating structural and functional microcirculatory adaptations within the rat spinotrapezius muscle.

METHODS

Animal selection and care. Twelve female Sprague-Dawley rats (2 mo old) were used in this investigation. Rats were kept on a 12:12-h light-dark cycle and were given food and water ad libitum. All handling of animals and experiments were performed according to the National Institutes of Health guidelines and approved by the Kansas State University’s Institutional Animal Care and Use Committee.

Experimental protocol. In the first 2 wk of the study, all rats were familiarized with treadmill running by exercising on a motor-driven treadmill (5 days/wk, 5 min/day, at 20–25 m/min and 0% grade). Postfamiliarization rats were then divided at random into trained and sedentary control groups. Trained rats ran 5 days/wk for 6 wk on a motor-driven declined treadmill (−14° grade), while sedentary control rats were confined to cage activities. At the beginning of the
training protocol, rats ran for ~10 min at 25 m/min. Treadmill speed and running duration were increased progressively until the rats were able to run for 1 h at 35 m/min. This final level of exercise intensity was maintained for at least 2–3 wk.

\( \dot{V}O_2 \) peak measurements. After the training program was completed, \( \dot{V}O_2 \) peak was measured in trained and sedentary rats during a treadmill-running test performed in a metabolic chamber placed on the motor-driven treadmill (35). After ~2 min of running at low speed (16 m/min, ~14° grade), the speed of the treadmill was increased progressively (~10 m/min) every 2 min until the rat could no longer keep up with the treadmill speed. \( \dot{V}O_2 \) peak was defined as the highest oxygen uptake \( (\dot{V}O_2) \) measured during the test. Due to specificity of training, we opted for measuring \( \dot{V}O_2 \) peak using the same exercise mode applied in the training session.

\( \dot{V}O_2 \) measurements were made by drawing ambient air through the metabolic chamber at a rate of ~5 l/min (STPD). A vacuum pump (Neptune-Dyna, model 4K) withdrew gas from the chamber through drierite (anhydrous CaSO\(_4\)) and then sequentially through a flowmeter (Fischer-Porter model 10A1378). Subsequently, this “dry” gas was delivered to the CO\(_2\) (Applied Electrochemistry model P 61-B) and \( O_2 \) (Applied Electrochemistry model N-22M) analyzers. These analyzers were calibrated before and after each run using precision-mixed gases that spanned the expected range of gas concentrations based on previous investigations (35).

Measurement of muscle tension. Animals were anesthetized with pentobarbital sodium (40 mg/kg ip to effect), and the left spinotrapezius was surgically exposed and exteriorized as described previously (3, 24). Briefly, the skin and overlying fascia that was not connected intimately to the muscle fibers were removed gently from the dorsal aspect of the spinotrapezius. The muscle was freed from connective tissue attachments to the underlying musculature and also from spinal connections, and any small distal feed arteries were ligated where necessary. The muscle was then tunneled to a lightweight horseshoe wire at five equidistant points and attached via a swivel and wire to a force transducer (model FT 0.03C, Grass Instruments, Quincy, MA) coupled to a pen deflection recorder (Gould Electronics, model 11–1202-26) to determine muscle tension. The force transducer was calibrated using a certified 50-g calibration weight. The scapula was stabilized, and the force transducer was aligned with the predominant longitudinal axis of the spinotrapezius muscle fibers. The muscle was preloaded to 4 g, which was near the length that developed the highest tetanic tension (optimal length) in this preparation using the protocol described below (S. A. Hahn, L. F. Ferreira, J. B. Williams, K. P. Jansson, T. I. Musch, and P. C. Poole, unpublished observation). Stainless steel electrodes were sutured to the muscle proximal to the motor point (cathode) and along the caudal periphery (anode), thereby facilitating contractions of the whole muscle. The exposed tissue was moistened with isotonic saline (38°C) and covered with Saran Wrap (Dow Brands, Racine, WI). Two stimulation protocols of 15-min duration were evaluated: protocol A, 1-Hz twitch contraction (0.2-ms pulses at 15 Hz, \( n \) = 2 sedentary and 2 trained); and protocol B, 1-Hz tetanic contractions (200 ms trains of 0.2 ms pulses at 50 Hz, 15 V, \( n \) = 4 sedentary and 4 trained). Twitch contractions did not elicit detectable muscle fatigue within 15 min of stimulation. Therefore, the tetanic contraction protocol (protocol B), which induced substantial fatigue (see RESULTS), was used in the majority of the animals, and those results are reported subsequently. Initially, protocol B yielded ~70% of maximal tetanic tension, as assessed through progressive increases in stimulation voltage after ~30 min of recovery from the fatigue protocol. Muscle tension was recorded continuously during the contraction protocol and assessed as percentage of initial (i.e., first contraction) force production over the time interval from minutes 0 to 15.

Citrate synthase activity measurement. Following the procedures described above, the right spinotrapezius and plantaris muscle were removed, dissected free of connective tissue, and weighed. Measurements of citrate synthase activity were made in duplicate from spinotrapezius and plantaris muscle homogenates by a modification of the method described by Serre (52). Citrate synthase activity was measured spectrophotometrically using a Spectramax 190 microplate (Molecular Devices, Sunnyvale, CA) in 300-μl aliquots at 30°C.

Data analysis. The unpaired \( t \)-test was used to compare two means of trained and control rats. Two-way ANOVA (within factor: time; and between factor: group) was used to compare muscle force production in the fatigue tests, while post hoc analyses were performed with the Student-Newman-Keuls test. Significance was accepted when \( P \leq 0.05 \). Values are presented as means ± SE.

RESULTS

All six animals in the training group completed the study. Body weight was not significantly different between trained (341 ± 9.4 g) and sedentary control rats (321 ± 6.3 g) after the training program was completed.

Exercise training produced adaptations at the whole body and isolated muscle levels, as indicated by significantly greater: 1) whole body \( \dot{V}O_2 \) peak (\( \dot{V}O_2 \) peak; \( P = 0.001 \), Fig. 1), 2) citrate synthase activity in the plantaris (\( \dot{V}O_2 \) peak; \( P = 0.001 \), Fig. 2) muscles, and 3) fatigue resistance in trained compared with sedentary rats (Fig. 3). Specifically, following 15 min of contractions, trained muscles produced >70% more force than their control counterparts.
4) rats. For clarity, standard error bars are shown only at 15 min of contraction.

DISCUSSION

The principal original finding of the present investigation is that a training protocol of downhill treadmill running induced classic whole body and spinotrapezius muscle-specific adaptations. Specifically, trained animals demonstrated greater whole body VO$_2$ peak, with the relative improvement dependent on intensity, duration, and mode of training, as well as the initial fitness of the subjects (1, 41, 47). Our finding of an ~25% higher VO$_2$ peak in trained animals is consistent with that found in previous studies in the rat (17, 36, 37, 46). We also found an ~40% increase in citrate synthase activity of the spinotrapezius muscle, which is within the 30–50% increase in oxidative capacity (fast- and slow-twitch muscles) induced by exercise training protocols employing level or inclined treadmill running of similar speed and duration as in the present investigation (9, 46). Another classic adaptation seen with exercise training is an improvement in contractile function that increases resistance to fatigue (48). Accordingly, downhill training resulted in ~70% greater force production (i.e., 25% less fatigue, Fig. 3) by the spinotrapezius muscle of trained compared with sedentary animals after 15 min of submaximal tetanic contractions. This finding is in agreement with previous studies (48, 54, 55), suggesting adaptations of structural and functional mechanisms affecting oxygen delivery [increased vasodilatory capacity (33) and capillarity (4, 49)], substrate utilization [increased oxidative enzyme capacity (9, 46), increased fat utilization (10, 53), decreased glycogen depletion (12, 14)], and excitation-contraction coupling (15, 56), all of which have been proposed to participate in the enhanced muscle contractile performance after training (16, 20, 49).

Relationship with existing literature. A plethora of studies have described the cardiovascular and metabolic effects of endurance exercise training (e.g., Refs. 9, 19, 49). Typically, a period of 6–12 wk yields an increase of 10–40% in both whole body as well as muscle VO$_2$ peak, with the relative improvement dependent on intensity, duration, and mode of training, as well as the initial fitness of the subjects (1, 41, 47). Our finding of an ~25% higher VO$_2$ peak in trained animals is consistent with that found in previous studies in the rat (17, 36, 37, 46). We also found an ~40% increase in citrate synthase activity of the spinotrapezius muscle, which is within the 30–50% increase in oxidative capacity (fast- and slow-twitch muscles) induced by exercise training protocols employing level or inclined treadmill running of similar speed and duration as in the present investigation (9, 46).

Exercise training intensity has a direct effect on the muscle blood flow adaptations. During exercise, flow is distributed preferentially toward highly oxidative muscles and muscle portions (e.g., Ref. 2). Moderate-intensity training fine tunes this redistribution without changes in total hindlimb blood flow (2), whereas high-intensity training selectively improves vascular function (assessed by the endothelium-dependent vasodilation) in the low-oxidative white portion of the gastrocnemius muscle (31). Therefore, the volume and intensity of training should be considered when examining microcirculatory adaptations in the mixed fiber-type spinotrapezius muscle using the methods introduced in the present study.

As mentioned above, the incidence of injuries is a concern with activities that elicit eccentric contractions (22, 42, 43). A high incidence of injuries would pose a limitation to the use of downhill running as a training method. In the present study, we selected a relatively slow running speed to minimize muscle and toenail injuries during the training program. Minor injuries sustained during the training program (e.g., damage to the toenails) were not substantially greater than those seen during level/uphill running (34). Therefore, we consider that the training paradigm and intensity selected were successful in facilitating training adaptations while incurring minimal injuries.

Eccentric exercise poses an increased risk of injury in the human hamstring muscle following a single bout of eccentric exercise (43). Moreover, prior eccentric training could lead to an adaptation that would limit the risk of injury, which is particularly useful for those individuals in competitive sports (43).

Implications of present findings. Skeletal muscle microcirculatory function has been investigated in healthy individuals, and derangements of the microcirculation have been demonstrated in chronic diseases such as diabetes (5, 25) and CHF (23, 45). However, the effects of exercise training, a standard treatment for chronic diseases, on the muscle microvascular function remain poorly described. Importantly, based on theoretical models of blood-muscle exchange of O$_2$ (e.g., Ref. 11), it appears that an extensive array of training-induced improvements in muscle metabolic and vascular function may depend on microcirculatory adaptations that have only been partially elucidated by postmortem and/or muscle biopsy studies (51).
For example, the increase in muscle VO$_2$ at pulmonary VO$_2$peak found after exercise training requires a greater muscle O$_2$ diffusion capacity (47). According to Federspiel and Popel (11), as well as Roca and colleagues (47), muscle O$_2$ diffusion capacity is largely determined by the flux and distribution (capillary hematocrit) of RBCs within the capillary bed as they determine the number of RBCs adjacent to each contracting muscle fiber. Each of these variables can be measured directly by intravital microscopy at rest and during contractions in the spinotrapezius (24). Thus the spinotrapezius model of exercise training described herein provides an invaluable opportunity to test the theoretical predictions posited in the Federspiel and Popel (11) and Roca et al. (47) models.

In conclusion, the present investigation provides evidence that downhill running constitutes an effective model for training the rat spinotrapezius muscle. This is supported by the greater citrate synthase activity and resistance to fatigue found in the spinotrapezius muscle of trained compared with sedentary animals. Since the spinotrapezius is suitable for intravital transmission microscopy, the ability to induce training adaptations in this muscle presents new and powerful opportunities to advance our understanding of muscle microvascular function in health and disease (e.g., Type 2 diabetes, hypertension, and CHF) and to evaluate the mechanistic bases for, and efficacy of, therapeutic exercise intervention.

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