Muscle activity and aging affect myosin structural distribution and force generation in rat fibers

Dawn A. Lowe,1,2 Gordon L. Warren,5 LeAnn M. Snow,2,3 LaDora V. Thompson,2,4 and David D. Thomas1,2

1Department of Biochemistry, Molecular Biology, and Biophysics, 2Center on Aging, 3School of Kinesiology, and 4Department of Physical Medicine and Rehabilitation, University of Minnesota, Minneapolis, Minnesota 55455; and 5Department of Physical Therapy, Georgia State University, Atlanta, Georgia 30303

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SKELETAL MUSCLE CONTRACTILITY is attenuated with age. Much of the attenuation is a consequence of muscle atrophy that occurs with aging and decreased activity. However, if muscle atrophy is accounted for by normalization to muscle size, force generation is still lower in muscles of aged animals. For example, force generation relative to muscle mass, or muscle quality, is diminished in arm and leg muscles of elderly men and women compared with younger subjects (8, 19, 31). Force generation in individual muscles or muscle fibers is typically decreased in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: D. A. Lowe, Univ. of Minnesota, BMBB Dept. 6-155 Jackson Hall, 321 Church St., Minneapolis, MN 55455 (E-mail: dl@ddt.biochem.umn.edu).

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sized that increased muscle activity, by 8–10 wk of electrical stimulation, would reverse the detrimental aging effects by improving myosin structure during contraction and, thus, force generation (study 1). Conversely, we hypothesized that decreased muscle activity, achieved by denervation, would induce detrimental alterations in myosin structure and function in muscles from young animals (study 2).

We used EPR spectroscopy to test the hypotheses regarding myosin structure. EPR spectroscopy of an iodoacetamide spin label (IASL) attached to Cys707 has been shown to determine quantitatively the distribution between the two principal structural states of the myosin catalytic domain in muscle (21, 28). Subsequent use of the term “myosin structure” refers to this aspect of myosin structure. We also use the term “myosin function” in reference to one of the major functions of the myosin molecule, i.e., force generation.

METHODS

Animals

Fischer 344 × Brown Norway F1 hybrid male rats were obtained from the aging colony maintained by the National Institute on Aging. A total of 8 young and 14 aged animals were used in study 1 (increased activity by electrical stimulation), and 10 young animals were used in study 2 (decreased activity by denervation). Body masses (mean ± SD) of the young and aged animals used in study 1 were 404 ± 41 and 520 ± 43 g, respectively. Body mass of the animals used in study 2 was 429 ± 41 g. All young animals were 8–10 mo old and all aged animals were 31–32 mo old at the time of surgery. Animals received food and water ad libitum throughout the study. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee and met the guidelines set by the American Physiological Society.

Nerve Cuff Implantation for Study 1

Increased muscle activity was accomplished by electrical stimulation of the left semimembranosus muscle via a chronically implanted nerve cuff placed on the tibial branch of the ischiatic nerve. The semimembranosus muscle was selected for these studies because 1) it is composed primarily of type II fibers and, in general, muscles composed of type II fibers show greater age-related functional changes than muscles composed of type I fibers (4, 2) techniques for spin labeling and EPR spectroscopy have been established using this muscle (16), and 3) age-related changes in myosin structure and function have been shown in this hamstring muscle (16). Nerve cuffs were constructed as described by Warren and coworkers (35) with the following modifications. Each nerve cuff was constructed using two 21-mm Teflon-coated, multistranded 90% Pt-10% Ir wires (Medwire-Sigmund Cohn, Mt. Vernon, NY). Deinsulated ends of the wires were inserted through a 4-mm-long piece of silicone tubing (1.57 mm ID) in a direction perpendicular to the tubing’s longitudinal axis; the insertion points were separated by ~3 mm. The portions of the wires within the tubing were bent to conform to the tubing’s inner wall, such that the wires covered ~75% of the inner circumference. The ends of the wires protruding out of the cuff (~0.5 mm) were splayed and electrically insulated with epoxy glue. An ~1-mm-wide groove was then cut longitudinally through the tubing. This produced a trough through the ~25% of the inner circumference that was not lined by the deinsulated wires. This trough was wide enough for the nerve to be placed during the surgery. Nerve cuffs were sterilized by autoclave.

Nerve cuff implantation was done as described previously (35) with the following exceptions. Animals were anesthetized using 1.75% isoﬂurane mixed with oxygen, delivered at a rate of 200 ml/min. Body temperature was maintained by placement of the animal on a 37°C recirculating heating pad. The left hip and dorsal cervical areas were shaved and aseptically prepared. The tibial branch of the ischiatic nerve, the single nerve innervating the semimembranosus muscle (10), was first isolated by making an ~2-cm incision through the skin over the ischiatic notch. The incision was extended through the biceps femoris muscle in a direction parallel to the femur. The ischiatic nerve was located, and then, by proximal dissection, the tibial branch of the ischiatic nerve was located. Approximately 4 mm of this nerve was carefully freed of surrounding fat, connective tissue, and vasculature before the nerve cuff was implanted. The nerve was placed in the trough of the cuff, and the cuff was pinched so that it almost completely enclosed the nerve. The wires of the cuff were passed subcutaneously through a metal tube out to the dorsal cervical area as described in detail previously (35). The cuff was secured by 6-0 silk suture at both ends of the cuff’s strip of silicone tubing and at the wires ~10 mm proximal to the cuff’s tubing. A 9-mm wound clip was used to close the skin at the dorsal cervical area. Best results were obtained when the protected ends of the electrode wires were clamped along with the skin. After nerve cuff implantation, the incision through the biceps femoris muscle was closed using 6-0 silk suture, the overlying skin incision was stapled, and the skin wound was treated with hydrogen peroxide. Each animal was given 0.015 mg of buprenorphine intraperitoneally ~5 min after the isoflurane anesthesia was withdrawn. The surgical procedure was exactly the same for animals that underwent the sham-stimulation protocol. Animals were mobile within 30 min after surgery, and gait appeared normal by 3 days after surgery. Average body weight change 2 wk after surgery was ~1 ± 4 and ~4 ± 5% for young and aged animals, respectively.

Stimulation Protocol for Study 1

The following protocol began 2 wk after nerve cuff implantation. Animals were immobilized under light isoflurane anesthesia (0.5–0.8% isoflurane, 200 ml/min). The viability of the nerve cuff was checked in all animals, including those that were to be sham stimulated, at the beginning of the first session by exposing the electrode wires at the dorsal cervical area and attaching them to a stimulator (model S48, Grass-Telefactor, West Warwick, RI) with a stimulus isolation unit (model SIU5). After this verification, the animal was placed supine, and the torso, both thighs, and the left lower leg and foot were strapped to maintain isometric conditions for subsequent stimulations. Special attention was paid to the positioning of the left thigh, such that the hamstring muscles were fully extended, emulating in vivo resting muscle length. The tibial branch of the ischiatic nerve was stimulated through the nerve cuff at 154 Hz with a 200-ms train (0.1-ms pulse duration) and 5 V to produce maximal contractions. As described below, these stimulation parameters were those determined to be optimal. Five sets of contractions, 15 s between contractions and 3 min between sets, were done twice per week, for a total duration of 4, 8, or 10 wk. Six contractions per set were done in the 1st wk, 8 contractions per set in the 2nd wk, and 10 contractions per set thereafter. Stimulation protocols were automated using TestPoint 4.0 software (Keithley Instruments, Cleveland, OH). The average time from anesthesia induction to arousal from the 50-contraction protocol was ~50 min. The duration and depth of anesthesia were kept to minimums in an attempt to limit potential stress to the animals due to this twice-weekly regimen. A subset of animals completed sham-stimulation protocols. These animals were anesthetized, immobilized, and attached to the stimulator in the same manner and for the same length of time per session as the animals that completed the stimulation protocol. Two young and two aged animals completed the sham protocol for 8 wk, and two aged animals completed the sham protocol for 4 wk. Muscles from two aged and two young cage-control animals were also studied. These animals did not undergo any surgery and did not receive twice-weekly anesthesia.
An additional four young and two aged animals that had successful nerve cuff implantations completed <4 wk of the stimulation protocol and were eliminated from the study. The aged animals were eliminated because of health problems unrelated to our interventions. The main reason for the loss of the young animals was difficulty in maintaining the externalized electrodes in the dorsal cervical area. Muscles from these animals were not included in this study.

The stimulation protocol and parameters were optimized in preliminary experiments by intramuscular electromyographic (EMG) measurements. For these experiments, semimembranosus muscle left hindlimbs were exposed in nerve cuff-implanted animals, and two fine-wire EMG electrodes were inserted into the muscle belly such that the electrodes were parallel to each other and perpendicular to the fibers. Animals were stabilized at the knee, ankle, foot, tail, and torso to minimize movement during contractions. First, the stimulation parameters were optimized on the basis of maximal EMG root-mean-square (RMS) measurements (36). Voltage (1–8 V, 1-V intervals), pulse duration (0.1–0.3 ms, 0.05-ms intervals), and frequency (5–10 ms pulse interval, 0.5-ms intervals) were studied to determine the combination yielding the highest EMG RMS. Second, the optimal time between contractions was that determined to result in minimal EMG RMS reductions during a set. The number of contractions was based on human strength training studies that showed increased muscle strength (30).

**Denervation Procedure for Study 2**

Muscle activity was decreased by denervation of the semimembranosus muscle of one leg in young animals. The muscle was denervated, first, by isolation and exposure of ~3 mm of the tibial branch of the sciatic nerve in the left leg as described for the nerve cuff implantation. The most proximal and distal exposed points of the nerve were ligated using 6-0 silk sutures, and the nerve was severed in the middle of the two ligation points. The nerve ends were then retracted in opposite directions and sutured to adjacent muscles to avoid any possibility of the severed nerve ends rejoining and/or reinnervating the semimembranosus muscle (34). Postoperative procedures were the same as those described for the nerve cuff implantation, and animals recovered from this surgery similarly. Denervation of the semimembranosus muscle did not cause any noticeable change in animal gait or cage activity, presumably because the synergistic hamstring muscles were intact. Change in body weight was minimal 2 wk (n = 5) and 4 wk (n = 5) after denervation (~2 ± 2 and 0 ± 3%, respectively, relative to body weight before surgery).

**Tissue Preparation**

Two days after the last stimulation protocol or 2 or 4 wk after denervation, animals were anesthetized with pentobarbital sodium (55 mg/kg ip). All animals that had a nerve cuff implanted (including the sham-stimulated animals) were stimulated one time to test the functional status of the nerve cuff. The leg of these animals was not secured, so that the contraction was done against no load. Semimembranosus muscles from each leg were then carefully dissected, blotted, and weighed. All contralateral semimembranosus muscles were studied as in vivo control muscles. Animals were euthanized with an overdose of pentobarbital sodium.

About 10% of each muscle (alternating between proximal and distal muscle ends) was immediately frozen in liquid nitrogen and stored at ~80°C for mitochondrial enzyme assays. A small portion of some muscles was also taken and mounted for histology. The remaining muscle was submerged in a relaxing buffer [in mM: 7 EGTA, 0.016 CaCl₂, 5.6 MgCl₂, 80 KCl, 20 imidazole (pH 7.0), 14.5 creatine phosphate, and 4.8 ATP] on ice. Bundles of semimembranosus muscle fibers ~25 mm long and ~2 mm diameter were dissected free, tied onto capillary tubes, and permeabilized as described previously for the EPR experiments (16). Final storage of these fiber bundles was at ~20°C in fresh storage buffer containing 0.1 mM DTT. Bundles of fibers ~8 mm long and ~1 mm diameter were also formed, tied to pieces of capillary tubes, and stored in a 50% glycerol-50% relaxing solution for up to 5 wk at ~20°C for the single-fiber contractile measurements (16, 29).

**Single-Fiber Specific Tension**

Individual fiber segments (~2 mm long) from permeabilized bundles were isolated and studied at 22°C as described in detail previously (29). Fiber segments were mounted in relaxing buffer and lengthened to an average sarcomere length of 2.5 μm. Fiber diameters were measured at three places along the length of the fiber, and then maximal isometric force was determined by submersion of the fiber into a pCa 3.8 activating solution. Specific tension was calculated from the maximal force generated, and fiber CSA was determined by diameter measurements and the assumption that the fiber was circular in cross section. Nine to 15 fibers from each semimembranosus muscle were studied. After force measurements, each fiber was solubilized and stored at ~80°C until myosin heavy chain (MHC) isoform expression was determined by gel electrophoresis and silver staining (27).

**EPR Spectroscopy**

Fiber bundles were further dissected into 0.25- to 0.5-mm-diameter bundles and prepared for EPR experiments by spin labeling with 0.5 mM 4-(2-iodoacetamido)-2,2,6,6-tetramethyl-1-piperidinoxylo spin label (IASL; Sigma) specifically at Cys707 (SH1) in the catalytic domain of the myosin head (16, 21). Myosin ATPase activities of myofibrils were measured in high salt to determine the extent of myosin SH1 labeling by IASL in the denervated muscles compared with the contralateral control muscles (16, 24). IASL-labeled fiber bundles were further prepared for spectroscopy as described in detail previously (16). A capillary tube containing the labeled fibers was fixed in a TE102 cavity (model 4102ST/8838, Bruker Instruments, Billerica, MA) perpendicular to the magnetic field, with one end of the bundle attached to a force transducer (SensoNor Ackers 801 strain gauge, Aksjelskapet, Norway) and the other end stabilized to hold the fibers isometrically (6, 16). Buffers were flowed over fibers at a rate of 2 ml/min at 22°C; at this flow rate for ~0.5-mm-diameter bundles, there is adequate diffusion of substances to fibers in the core of the bundle (16). For each fiber bundle, EPR spectra were collected under conditions of rigor, relaxation, and contraction on a spectrometer (E500 EleXsys, Bruker Instruments). The following parameters were used to collect the low-field portion of the EPR spectrum: central peak = 3,425 gauss, sweep width = 38 gauss, peak-to-peak modulation amplitude = 5.0 gauss, and microwave power = 16 mW (see Fig. 1 for representative spectra). Spectra were analyzed to determine the fraction of myosin heads in the strong-binding structural state during contraction as described previously (16, 21). Two to three bundles from each semimembranosus muscle were studied and analyzed, and their values were averaged to represent myosin structural distribution during contraction for that muscle.

**Histological Analyses (Study 1 Only)**

Both semimembranosus muscles from three young and three aged 8- to 10-wk-stimulated rats were assessed histologically to determine whether the stimulation protocol produced hypertrophy or induced shifts in MHC expression. Cross sections (10 μm) were cut in a cryostat and stained for myofibrillar ATPase activities (3). Images of the stained muscle sections were acquired, and CSAs of ~150 fibers in each muscle were measured (Scion Imaging System, Frederick, MD). MHC type II fibers were randomly chosen, but because there are relatively few type I fibers in semimembranosus muscle, all type I fibers that were observed were measured. It was difficult to distinguish between type IIA and IIB fibers histologically, perhaps because of the high occurrence of type IIX and hybrid fibers (determined by gel
of cytochrome cenate was determined in triplicate by following the rate of oxidation. Student's t-tests were used. Animals were compared with those of the young animals, Student's results of electrically stimulated or denervated muscles with those of control muscles. Student's t-tests were used. Mitochondrial enzyme activities were assessed to determine whether the electrical stimulation or denervation protocols had an effect on muscle oxidative capacity. Piezoelectric enzyme activities were assessed to determine whether the electrical stimulation or denervation protocols had an effect on muscle oxidative capacity. Pieces of frozen muscles were individually weighed and homogenized in 30 vol of 33 mM phosphate buffer (pH 7.0) on ice. Cytochrome c oxidase activity of the homogenate was determined in triplicate by following the rate of oxidation of cytochrome c at 550 nm and 25°C (23). The remaining homogenate was subjected to three freeze-thaw cycles, and citrate synthase activity was determined. This assay was performed in duplicate as described by Srere (26), except the assays were done at 25°C and only one-half as much acetyl CoA was used in each assay.

Statistical Analyses

Values are means ± SE. Paired t-tests were used to compare the results of electrically stimulated or denervated muscles with those from the contralateral control muscles. When results of the aged animals were compared with those of the young animals, Student’s t-tests were used. P < 0.05 was considered significant.

RESULTS

All fiber segments that were analyzed for specific tension were subsequently assessed for MHC isoform expression. For studies 1 and 2, within an experimental group we found no differences in specific tension (or diameter) between fibers that expressed only type IIB MHC (58% of all fibers) and fibers that expressed other isoforms of MHC (P = 0.42). Thus data from all fibers within an experimental group, irrespective of MHC expression, were analyzed together in each study. Study 1: Increased Muscle Activity via Electrical Stimulation

Cage-control animals and animals that were sham stimulated were included in this study to control for possible confounding effects of the surgery and the twice-weekly anesthesia. In young and aged animals, we found no differences in specific tension or myosin structural distribution during contraction among fibers from 1) sham-stimulated, nerve cuff-implanted muscles, 2) sham-stimulated contralateral control muscles, 3) muscles from cage-control animals, and 4) contralateral muscles of 4- and 8- to 10-wk-stimulated animals (P > 0.32). Thus the effects can be attributed to the electrical stimulation of the muscle and not to anesthesia or surgery. All comparisons are made between muscles that were stimulated and muscles from contralateral limbs, unless otherwise stated. There were no differences in any of the variables measured between muscles that were stimulated for 8 wk and those that were stimulated for 10 wk (P ≥ 0.21), so data from those muscles were combined.

Single-fiber specific tension. For both age groups analyzed together, specific tension was lower in fibers after 4 wk of the increased-activity protocol, i.e., electrical stimulation, relative to fibers from contralateral control muscles (P < 0.01; Fig. 2A). However, after 8–10 wk of increased activity, fibers from aged animals generated 4% more specific tension than the contralateral control fibers (P = 0.02; Fig. 2C). Fibers from young animals that were stimulated for 8–10 wk were not different from fibers from the contralateral muscles (P = 0.58; Fig. 2B). Specific tension for all fibers from young animals (except those from the 4-wk-stimulated muscle) was 171 ± 4 kN/m² (n = 121). Among aged animals, specific tension for all fibers from contralateral control muscles was 156 ± 4 kN/m² (n = 99). The specific tension generated by fibers from aged animals after 4 and 8–10 wk of increased activity was 132 ± 5 kN/m² (n = 30) and 164 ± 6 kN/m² (n = 69), respectively. Despite the improvement in fibers from aged animals after 8–10 wk of stimulation, specific tension was still ∼4% lower in those fibers than in fibers from young animals (P ≤ 0.01). Myosin structural distribution. EPR data showed that the fraction of myosin heads in the strong-binding structure during contraction was lower in fibers after 4 wk of increased muscle activity (0.241 ± 0.020) than in fibers from contralateral muscles (0.269 ± 0.018, P = 0.046; Fig. 3) for both groups analyzed together. Relative to contralateral controls, there were no differences in myosin structural distribution during contraction after 8–10 wk of stimulation in fibers from young or aged animals (P ≥ 0.22). The fraction of strong-binding myosin heads during contraction in fibers of all muscles from young

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\text{Fraction strong-binding (A/B)} = 0.334 ± 0.002 \\
\text{Fraction weak-binding (1-(A/B))} = 0.666 ± 0.002
\]

\[
\text{Control} \\
\text{Denervated}
\]

Fig. 1. Representative low-field electron paramagnetic resonance (EPR) spectra of fibers labeled with iodoacetamide spin label (IASL) at myosin SH1 from contralateral control (left) and 4-wk-denervated (right) semimembranosus muscles. Note lower fraction of myosin heads in the strong-binding (force-generating) structural state during maximal isometric contraction in fibers from denervated muscle (21.8%) than in fibers from contralateral control muscle (33.4%). Contraction spectrum is closer to relaxation spectrum in denervated muscle than in the control example, showing that fewer myosin heads are in the strong-binding structure and, conversely, more are in the weak-binding structure during contraction after denervation.

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and aged animals (except those stimulated for 4 wk) was 0.326 ± 0.014 (n = 14) and 0.266 ± 0.008 (n = 32), respectively. This difference in myosin structure between fibers from the young and aged animals was significant (P < 0.01).

Additional outcomes of increased muscle activity. Semimembranosus muscle mass, fiber size, fiber MHC isoform expression, citrate synthase activity, and cytochrome c oxidase activity were measured to determine training effects of the increased-activity protocols.

The increased-activity protocol for 4 or 8–10 wk did not significantly affect semimembranosus muscle mass in young or aged animals relative to that of contralateral control muscles (P ≥ 0.14), although there was a trend for the 8- to 10-wk-stimulated muscles to be ~10% larger. Semimembranosus muscle mass of all young animals was 0.933 ±0.053 g (n = 16) and 0.650 ± 0.034 g (n = 28) for all aged animals.

Fiber diameter was measured on all single-fiber segments studied for specific tension. Stimulation for 4 or 8–10 wk did not significantly affect diameter of the fibers studied among aged or young animals (P ≥ 0.07). Diameter of all fibers from the young and aged animals in the increased-activity protocol was 81.7 ± 1.6 μm (n = 140) and 73.5 ± 1.1 μm (n = 269), respectively.

The mean CSA of type II fibers increased in three of three young and one of three aged animals that underwent the increased-activity protocol for 8–10 wk and were studied histologically (P ≤ 0.02). There were no differences between the stimulated and contralateral fiber CSAs from the two nonrespondent aged animals (P ≥ 0.28). The average increase in type II fiber CSA was 17% in the three stimulated muscles from young animals and 18% in the single aged rat that responded. Between 0 and 66 type I fibers were found and analyzed per muscle. One aged animal showed a 27% increase in type I fiber CSA with stimulation (P < 0.01).

In summary, a mild fiber hypertrophy was induced by the longer increased-activity protocol, i.e., 8–10 wk of electrical stimulation, as determined by the histological analyses. The lack of significant hypertrophy based on the muscle mass and single-fiber diameter measurements is most likely due to the small number of samples studied relative to >150 fibers analyzed per muscle for CSA.

The frequency of electrical stimulation was selected with the goal of not inducing changes in MHC isoform expression. However, the stimulation protocol produced a greater fraction of hybrid fibers (i.e., fibers that expressed multiple MHC isoforms) determined from gel electrophoresis, among those that were studied for specific tension. The fraction of hybrid fibers increased with stimulation from 14 to 41% in muscles from young animals and from 52 to 65% in muscles from aged animals. Of the 138 hybrid fibers studied, 103 expressed IIX/IIB MHC and 17 expressed IIX/IIB MHC.

The increased-activity protocol did not affect muscle oxidative capacity as measured by the two mitochondrial enzyme activities (P ≥ 0.20).

Study 2: Decreased Muscle Activity via Denervation

Single-fiber specific tension. Two and 4 wk of denervation of semimembranosus muscles in young animals resulted in reductions in fiber specific tension relative to that of contralateral control muscles (P < 0.01; Fig. 4, top). Specific tension of muscle fibers between stimulated and contralateral control muscles was 81.7 ± 1.6 g cm (n = 140) and 73.5 ± 1.1 g cm (n = 269), respectively.

The mean CSA of type II fibers was 0.42 ± 0.07 mm² (n = 140) and 0.36 ± 0.06 mm² (n = 269), respectively. There were no differences between the stimulated and contralateral fiber CSAs from the two nonrespondent aged animals (P ≥ 0.28). The average increase in type II fiber CSA was 17% in the three stimulated muscles from young animals and 16% in the single aged rat that responded. Between 0 and 66 type I fibers were found and analyzed per muscle. One aged animal showed a 27% increase in type I fiber CSA with stimulation (P < 0.01).

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control fibers was 163 ± 4 kN/m² (n = 114), and 2 and 4 wk of denervation reduced specific tension to 125 ± 5 kN/m² (n = 58) and 107 ± 5 kN/m² (n = 55), respectively.

Myosin structural distribution. Two and 4 wk of denervation resulted in a reduction of strong-binding myosin during contraction relative to that in contralateral control muscles (P ≤ 0.01; Fig. 4, bottom). The fraction of myosin heads strongly bound to actin during contraction in control fibers was 0.326 ± 0.010 (n = 20), and 2 and 4 wk of denervation reduced that fraction to 0.274 ± 0.014 (n = 10) and 0.213 ± 0.010 (n = 12), respectively. There was no difference in the extent of myosin SH1 labeling by IASL in the denervated muscles compared with the contralateral control muscles (80 ± 4 vs. 85 ± 5% of the SH1 sites in fibers were labeled, respectively, P = 0.54).

Additional outcomes of decreased muscle activity. Two and 4 wk of decreased muscle activity via denervation resulted in muscle mass losses of 35 and 64%, respectively: 1.04 ± 0.065 g (n = 10) for contralateral muscles, 0.698 ± 0.017 g (n = 5) at 2 wk, and 0.350 ± 0.036 g (n = 5) at 4 wk (P < 0.01). Denervation for 2 and 4 wk resulted in 15 and 35% reductions in fiber diameter, respectively [73.0 ± 2.1 μm (n = 58) and 51.8 ± 1.5 μm (n = 55)] relative to fibers from contralateral muscles (78.1 ± 1.9 μm, n = 114, P ≤ 0.01).

Decreased activity for 2 wk resulted in a low citrate synthase activity relative to contralateral muscles: 4.11 ± 0.75 vs. 6.40 ± 1.08 μmol-min⁻¹·g wet wt⁻¹ (P = 0.04). After 4 wk, citrate synthase and cytochrome c oxidase activities were reduced: 4.09 ± 0.48 vs. 7.72 ± 1.15 μmol·min⁻¹·g wet wt⁻¹ (P = 0.049) for citrate synthase and 6.92 ± 0.61 vs. 11.45 ± 1.37 μmol·min⁻¹·g wet wt⁻¹ (P = 0.04) for cytochrome c oxidase.

DISCUSSION

Study 1: Increased Muscle Activity

The goal of the stimulation protocol was to increase muscle activity. It is well known that a change in activity can affect muscle function metabolically or mechanically, depending on the type of muscular activity (2). Pertinent to this study, improvements in muscle strength can occur after an increase in an activity such as resistance training. Strength gains often result from muscle hypertrophy; however, changes in neural factors and factors intrinsic to muscle fibers also have the potential to affect muscle strength. Recently, the term “muscle quality” has been used to describe these features that are independent of “muscle quantity” (14, 19, 31). For example, Ivey et al. (14) showed that 9 wk of resistance training resulted in increased muscle quality in quadriceps muscles of young and aged men and women. They showed that muscle volume determined by magnetic resonance imaging increased, but to a lesser extent than the increase in leg strength. In other words, factors in addition to muscle hypertrophy contributed to the strength gains. We propose that one of these factors is alteration in myosin structure and function; this has been proposed by others as well (11–13). Therefore, in the present study, we were attempting to manipulate myosin structure by increasing muscular activity, which we predicted would then affect myosin function, i.e., specific force generation or strength. Because we previously demonstrated that aging affects myosin structure and function in a detrimental way (16), the goals of the present studies were to 1) reverse those molecular changes by increasing activity in muscles of aged animals and 2) induce changes in muscles of young, healthy animals by decreasing muscle activity.

We found that increasing the activity of semimembranosus muscles in aged animals via unilateral electrical stimulation for 4 wk initially was deleterious, as shown by a 15% decrement in the ability of permeabilized fibers to generate specific tension. This should not be surprising, because maximal activation of all motor units in a muscle can be considered an intense event not typically encountered by muscles. However, those muscles from young and aged animals recovered as the stimulation protocol continued for a total of 8–10 wk. Muscles that were assessed histologically after 8–10 wk of stimulation did not show signs of damage; i.e., no inflammation, necrosis, or central nuclei were observed, and fiber force generation recovered. In fact, the specific force-generating capacity was 4% greater in fibers from stimulated muscles of aged animals than from contralateral muscles. This 4% increase in “strength” was not enough to counter the effects of age, but, taken together with the prior 15% decrement, i.e., the decrement at 4 wk, it demonstrates that in vivo myosin structure and function may change by as much as 20%, indicating that it could be a molecular intrinsic factor affecting muscle strength.

We are aware of no data in the literature on the effects of increased muscular activity on myosin structure and function in
rodent animal models, but four studies have recently been published describing the effects of resistant training on fiber contractility in men and women (25, 32, 33, 37). Widrick and coworkers (37) showed that 12 wk of resistance exercise training by previously sedentary young men resulted in fiber hypertrophy and proportional increases in the force-generating capacity of permeabilized fibers. In a similar study, they showed that long-term resistance training (>7 yr) also had no effect on the intrinsic ability of fibers to generate force (25). That is, no change in muscle quality was found with short- or long-term resistance training in young men. We observed comparable results after 8–10 wk of training in our young animals; i.e., there were no changes in specific tension or myosin structural distribution during contraction. Trappe and coworkers (32, 33) studied permeabilized fibers from muscle biopsies of 74 ± 2-yr-old men and women before and after 12 wk of resistance training. Fiber size, absolute force generation, and contractile velocity improved with training in men, and specific tension was 7% greater in MHC type I fibers after training (33). These data suggest that the intrinsic capacity of fibers to generate force can be improved in muscles of aged men. We propose that this occurs via an increase in the fraction of myosin heads in the strong-binding structural state during contraction. Indeed, we found that changes in myosin structure followed patterns similar to the changes in specific tension in aged animals. The strong-binding structure of myosin was reduced by ~20% after 4 wk of increased activity but returned to normal after 8–10 wk, indicating that structural alterations in myosin can also be modulated in vivo.

Electrical stimulation of the motor nerve at a relatively high frequency maximally activates all motor units and, therefore, would be expected to have a greater impact on myosin structure and, thus, force generation than resistance training, which involves voluntary muscle contractions. We initially hypothesized that electrical stimulation of the tibial branch of the sciatic nerve would increase semimembranosus muscle activity optimally, because it is a controlled, quantifiable, and reproducible method for maximally activating all motor units in those muscles. As a result of the electrical stimulation protocol, specific tension did increase by 4% in fibers from aged animals. It is possible that greater effects would have been observed had we trained the semimembranosus muscles 1) dynamically instead of isometrically, 2) more than twice per week, or 3) for >10 wk. The twice-weekly regimen was designed for the aged animals, inasmuch as strengthening exercises two to three times per week for elderly individuals is recommended (20) and because we thought that three times a week would have been too stressful for the animal (anesthesia and externalization of the electrodes) and the stimulated muscles. We found it difficult to train the animals, especially the young animals, for much longer than 8 wk, primarily because of problems maintaining the electrodes at the dorsal cervical area. After 8–10 wk we detected a slight but nonsignificant increase in muscle oxidative capacity and a modest hypertrophy, similar to other reports of rodent muscles subjected to electrical stimulation (1, 7, 38). A critical point to keep in mind, however, is that the specific tension and myosin structural measurements employed in the present study are independent of fiber size and, thus, truly are reflective of muscle quality. Overall, our data indicate that muscle quality, in terms of myosin structure and function, improved modestly in muscles of aged animals following our increased-activity protocol.

**Study 2: Decreased Muscle Activity**

More pronounced effects on myosin structure and function were observed in muscles after 2 or 4 wk of decreased muscular activity. The intent of the denervation protocol was to detrimentally alter myosin in muscles of young animals in a way that mimicked the effect that we previously observed with aging. Age-related reductions in specific tension of 10–20% are typical in whole muscles from rodents (4, 22) and in permeabilized fibers from rodents and humans (8, 29), although not all studies on fibers have shown this (5). Specifically, for semimembranosus muscle fibers from rats, 16–27% age-related specific tension decrements have been reported (16, 17). In the present study, 2 and 4 wk of decreased activity resulted in specific tension decrements of 23 and 34%, respectively. Thus, by denervating the semimembranosus muscle for 2 or 4 wk, it was possible to affect myosin force generation to an extent similar to that during aging. The detrimental changes in myosin structure of 16 and 35% from 2 and 4 wk of denervation are comparable in magnitude to the 30% age-induced myosin structural changes reported previously as well (16). These similarities suggest that changes in muscle activity, specifically decreased activity via denervation, may affect muscle function by at least one molecular mechanism similar to that induced by aging.

Denervation is an interesting model to use for decreasing muscular activity, because muscle fiber denervation increases with age. Proximal nerves normally reinnervate denervated muscle fibers, but with age, motor neuron sprouting (reinnervation) becomes inefficient (15, 18, 34). However, completely severing the nerve to an entire muscle, such as we did in the present study, is relatively extreme and may not precisely reflect mechanisms of muscle weakness that occur with aging. Nonetheless, in vivo muscle strength is obviously affected by denervation, because the fibers cannot be activated. However, when denervated fibers are isolated and analyzed in vitro for contractile capacity by direct exposure of the contractile apparatus to Ca^{2+}. i.e., bypassing the nerve, there is still a decrement in force generation. For example, permeabilized fibers from 2-wk-denervated diaphragm muscles showed 20–40% reductions in specific tension (9). Even with normalization for the loss of MHC content per half-sarcomere, force generation was lower after denervation. The fraction of myosin heads in the strong-binding structure during contraction was estimated in that study on the basis of stiffness measurements, and no differences were detected between fibers from control and denervated muscles (9). The authors therefore suggested that denervation caused a decrease in the force per myosin head. However, measurement of stiffness is an indirect method for estimating myosin heads bound to actin, whereas EPR spectroscopy paired with a spin label on the catalytic domain of myosin is a direct determinant (28). Additionally, the myosin strong-binding structural alterations in the present study were similar in magnitude to the loss of specific tension in those fibers. Specific tension generated by fibers from control, denervated, and stimulated semimembranosus muscles from...
young adult and aged animals was positively correlated with the fraction of myosin heads in the strong-binding state during contraction in those muscles ($r = 0.58, P < 0.01$). The significant correlation between specific tension and myosin structure suggests that an alteration in the strong-binding structure of myosin is a molecular mechanism underlying changes in muscle function. This is a conclusion similar to that reached for aged muscle (16).

In summary, we have shown that muscle fiber quality, i.e., force generation per unit muscle, is mutable by manipulating muscle activity. Increasing activity of muscles in aged animals by electrical stimulation initially induced a decline in specific tension, but by 8–10 wk fibers recovered and fibers from aged animals showed a slight increase in specific tension relative to fibers from contralateral control muscles. Decreasing activity of muscles in young animals by surgical denervation induced a decline in specific tension similar in magnitude to that found with aging. EPR spectroscopy showed that myosin structural alterations followed patterns very similar to the changes in specific tension, indicating that an underlying molecular mechanism behind the age-related and denervation-induced specific tension decrements, and perhaps reversal of those decrements, may be myosin structure. More specifically, during contraction, there was a lower fraction of the strong-binding (force-generating) myosin structural state in fibers from 4-wk-stimulated muscles, denervated muscles, and muscles from old animals, coinciding with decrements in specific tension relative to control fibers from young animals.

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