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Effect of Resistance Training on Neuromuscular Junctions of Young and Aged Muscles Featuring Different Recruitment Patterns

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To examine the effects of aging on neuromuscular adaptations to resistance training (i.e., weight lifting), young (9 months of age) and aged (20 months of age) male rats either participated in a 7-week ladder climbing protocol with additional weight attached to their tails or served as controls (n = 10/group). At the conclusion, rats were euthanized and hindlimb muscles were quickly removed and frozen for later analysis. Longitudinal sections of the soleus and plantaris muscles were collected, and pre- and postsynaptic features of neuromuscular junctions (NMJs) were visualized with immunofluorescence staining procedures. Cross-sections of the same muscles were histochemically stained to determine myofiber profiles (fiber type and size). Statistical analysis was by two-way ANOVA (main effects of age and treatment) with significance set at $P \leq 0.05$. Results revealed that training-induced remodeling of NMJs was evident only at the postsynaptic endplate region of soleus fast-twitch myofibers. In contrast, aging was associated with pre- and postsynaptic remodeling in fast- and slow-twitch myofibers of the plantaris. Although both the soleus and the plantaris muscles failed to display either training or aging-related alterations in myofiber size, aged plantaris muscles exhibited an increased expression of type I (slow-twitch) myofibers in conjunction with a reduced percentage of type II (fast-twitch) myofibers, suggesting early stages of sarcopenia. These data demonstrate the high degree of specificity of synaptic modifications made in response to exercise and aging and that the sparsely recruited plantaris is more vulnerable to the effects of aging than the more frequently recruited soleus muscle.

Key words: synapse; acetylcholine; bungarotoxin; nerve terminal; exercise

Important national and international health organizations, such as the Centers for Disease Control and Prevention, the American College of Sports Medicine, and the World Health Organization, have released recommendations for adults and older individuals to participate in a regular program of exercise training (Pate et al., 1995; Chodzko-Zajko et al., 2009; World Health Organization, 2010). These statements and position stands laud the ability of exercise training to prevent or manage effectively a host of noncommunicable maladies, including cardiovascular disease, stroke, type II diabetes, obesity, and arthritis.

In addition to endurance, i.e., aerobic-style, exercise, it is strongly recommended that health-related exercise programs include resistance training, i.e., weight lifting. Resistance training is especially valuable among the aged because this mode of exercise has been found to be successful in treating or preventing chronic health conditions associated with senescence, including sarcopenia (age-related loss of muscle mass), osteoporosis, insulin resistance, incidence of accidental falls, bone fracture, and even cognitive decline (Engelke et al., 2006; Liu-Ambrose and Donaldson, 2009; Visser, 2011; Westcott, 2012; Anton et al., 2013; Cederholm et al., 2013; Gregory et al., 2013). Resistance training typically results in positive adaptations of the neuromuscular system, including myofibers and neuromuscular junctions (NMJs) that link the motor nervous system with skeletal muscles that are activated by those motor neurons (Deschenes et al., 2000; Folland and Williams, 2007; Andersen and Aagaard, 2010). Clearly, there are distinct advantages to adding resistance training to the exercise regimens performed by

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the aged. Less clear, however, is whether the neuromuscular systems of older individuals respond to the stimulus of resistance training in a manner similar to the neurons and myofibers of younger individuals. This is a legitimate concern because it has recently been reported that adaptations of the NMJ to endurance training (treadmill running) were different among young and aged animals (Deschenes et al., 2011). The present investigation seeks to determine whether aging affects adaptations of the NMJ and associated myofibers to resistance training.

MATERIALS AND METHODS

Subjects

Twenty young adult (9 months of age) and 20 aged (20 months of age) male Fisher 344 rats were purchased from the National Institute on Aging colonies and randomly assigned to either resistance-trained (RT) or control (CTL) treatment groups, resulting in a total of four groups, with n = 10/group. The average life expectancy of male Fisher 344 rats is 25.5 months (Turturro et al., 1999); thus, at 20 months of age the older rats used in this study had lived 78.5% of their life span. Relative to the average life span of men in the United States, which currently is 76 years (United States Census Bureau, 2012), these older rats were the equivalent of 60 years old, the age considered to be the onset of senescence by some health organizations and experts (Forman et al., 1992; Bloom et al., 2011).

Animals were provided standard rat chow and water ad libitum and were housed at a constant temperature of 21–22°C under a 12-hour light/dark cycle. All procedures were approved beforehand by the institutional animal care and use committee operating in accordance with the NIH *Guide for the care and use of laboratory animals*.

Resistance Training

The resistance training protocol that was used consisted of three sessions per week for 7 weeks. Animals climbed a ladder that was 1 meter long and set at an 85° angle, with additional weights attached to their tails with Velcro strips. Each training session featured eight repetitions of ladder climbing, and added resistance was initially set at 50% of body mass with 30 g increments added weekly. When necessary, rats were motivated to climb the ladder via a cool-water spray. This animal model of resistance training has been shown to recruit the hindlimb extensor muscles (i.e., gastrocnemius, plantaris, soleus) and forelimb muscles (Linderman et al., 1994; Kim et al., 2012) effectively. Animals assigned to control conditions simply remained in their tubs throughout the 7-week intervention. At the end of the intervention period, all animals were euthanized, and hindlimb muscles were surgically removed, cleared of fat and connective tissue, and quickly frozen at resting length in isopentane chilled with liquid nitrogen. Muscles were then stored at −80° C until analysis. The soleus and plantaris muscles were selected for analysis because, although both are ankle extensors, they have vastly different myofiber-type distributions and recruitment patterns. More specifically, the soleus consists mainly of slow-twitch, or type I, fibers and as a postural muscle displays a high duty cycle, whereas the plantaris principally expresses fast-twitch, or type II, myofibers and is extensively recruited only during locomotor activity (Laughlin and Armstrong, 1982; Delp and Duan, 1996).

Cytofluorescence Staining

To visualize NMJs, 50-μm-thick longitudinal sections of the middle one-third of the muscle and along its most superficial region were obtained at −20°C on a cryostat (Cryocut 1800; Reichert-Jung, Nußloch, Germany). To prevent contraction of sections, microscope slides were pretreated in a 3% EDTA solution as previously described by Pearson and Sabarra (1974). Sections were washed four times for 15 min each in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). Sections were then incubated in a humidified chamber overnight at 4°C in supernatant of the primary antibody RT97 (Developmental Studies Hybridoma Bank, University of Iowa) and diluted 1:20 in PBS with 1% BSA. The RT97 antibody reacts with nonmyelinated constituents of presynaptic nerve terminals (Anderton et al., 1982). On the next day, sections were washed four times for 15 each in PBS with 1% BSA before they were incubated for 2 hr at room temperature in fluorescein isothiocyanate-conjugated secondary immunoglobulin (Sigma, St. Louis, MO) that was diluted 1:150 in PBS with 1% BSA. Sections were then washed four times for 15 min each in PBS with 1% BSA. After this, sections were incubated in a humidified chamber overnight at 4°C in a solution containing rhodamine-conjugated α-bungarotoxin (Invitrogen, Grand Island, NY) diluted 1:600 in PBS with either antislow (soleus) or antifast (plantaris) myosin heavy chain ascites fluid (Sigma) diluted 1:40. Bungarotoxin recognizes postsynaptic acetylcholine (ACh) receptors, whereas the antislow and antifast immunogen allowed us to determine whether the endplate resided on a fast- or slow-twitch myofiber. On the next day, sections were washed four times for 15 min each in PBS with 1% BSA before incubating them for 1 hr at room temperature in Alexa-Fluor 647 (Invitrogen)-labeled secondary antibody to bind with the antislow or antifast primary antibody. Sections were given a final wash (four times for 15 min each) before being lightly coated with ProLong (Invitrogen) and having coverslips applied. Slides were then coded with respect to treatment group to allow for blinded evaluation of NMJ morphology and stored at −20°C in the dark until analysis. An example of this cytofluorescence staining of pre- and postsynaptic components of the NMJ is displayed in Figure 1.

Presynaptic variables of NMJs that were assessed included 1) the number of branches identified at the nerve terminal; 2) the total length of those branches; 3) the average length per branch; and 4) the branching complexity, which, as described previously by Tomas et al. (1990), is derived by multiplying the number of branches by the total length of those branches and dividing that number by 100. Postsynaptic variables of interest included 1) total perimeter, or the length encompassing the entire endplate comprising stained ACh receptor clusters and nonstained regions interspersed within those clusters; 2) stained perimeter, or the composite length of tracings around individual ACh receptor clusters; 3) total area, which includes the stained receptors along with the nonstained regions interspersed among receptor clusters; 4) stained area, or the cumulative areas

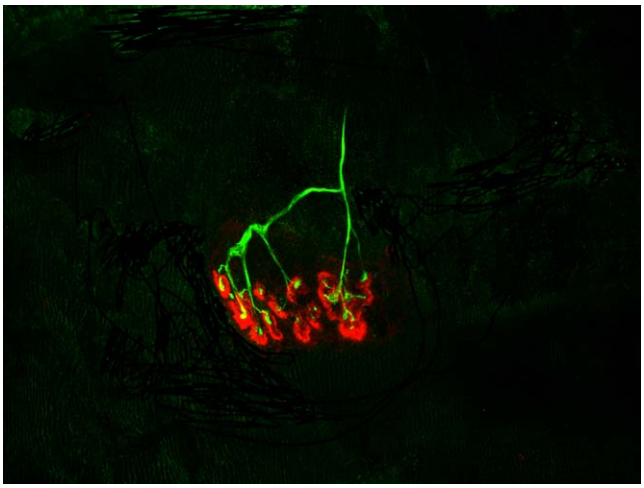

Fig. 1. Representative image of fluorescently stained neuromuscular junction at ×1,000. Presynaptic nerve terminal branches are stained in green, and postsynaptic ACh receptors are stained in red. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

occupied by ACh receptor clusters; and 5) dispersion of endplates, which was assessed by dividing the endplate's stained area by its total area and multiplying by 100. In this study, presynaptic-to-postsynaptic coupling was quantified by dividing the NMJ's postsynaptic stained area by its total length of presynaptic nerve terminal branching. Figure 2 displays how measurements were made of presynaptic branching as well as an example of how line tracings were either made manually (total) or generated by software (stained) around postsynaptic ACh receptors.

Histochemical Staining

To quantify myofiber profiles, 10-μm-thick transverse sections were obtained from the midbelly of the muscle with a cryostat set at −20°C. Sections were stained for myofibrillar ATPase activity following preincubation at a pH of either 4.55 (soleus) or 4.40 (plantaris) as described by Nemeth and Pette (1981). It should be noted that this staining technique allows identification of the three fiber types found in the soleus (types I, IIA, and IIX). However, the fourth fiber type included in the plantaris (type IIB) cannot be distinguished from type IIX fibers. A representative sample of myofibers stained in this way can be seen in Figure 3. Slides were coded so that measurements could be conducted in a blinded fashion regarding treatment group.

Microscopy

A Fluoview FV 300 (Olympus America, Melville, NY) confocal system featuring three lasers and a BX60 (Olympus) fluorescent microscope were used to collect images of NMJs and to identify whether they were located on slow- or fast-twitch myofibers. By using a ×100 oil immersion objective, it was initially established that the entire NMJ was within the longitudinal borders of the myofiber and that the area of interest was not damaged during sectioning. A detailed image of the entire NMJ was constructed from a z-series of scans taken at

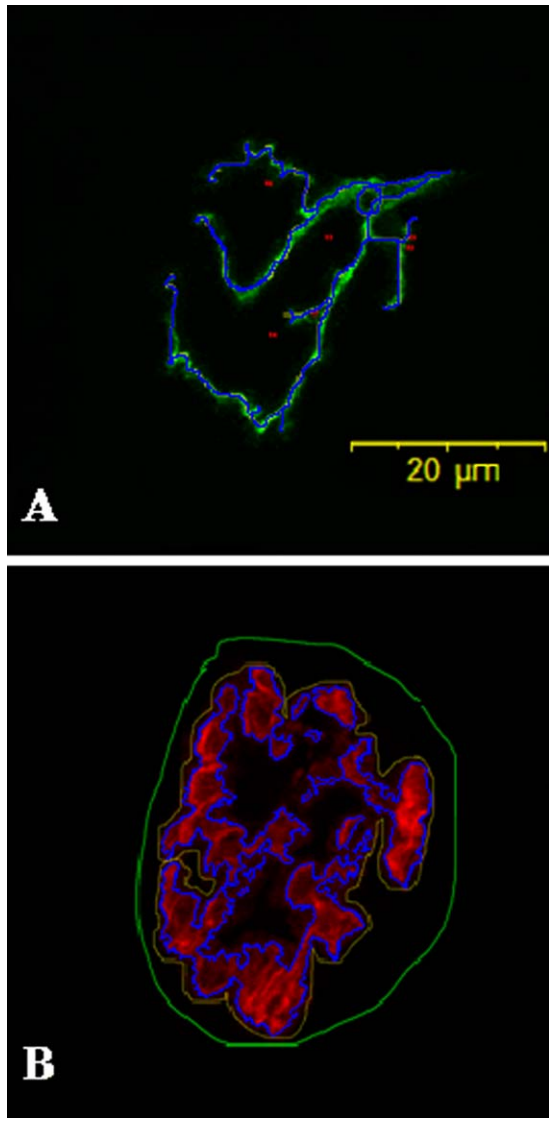

Fig. 2. Representative image of tracings used to quantify morphological aspects of the neuromuscular junction. **A:** Tracings used to quantify presynaptic nerve terminal branches. **B:** Tracings (both manually drawn and generated by software) used to quantify postsynaptic ACh receptors. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

1-μm-thick increments. To ascertain the myofiber type on which the NMJ resided, a single scan of the fiber was collected by using the appropriate wavelength to detect AlexaFluor 647. Digitized, two-dimensional images of NMJs were stored on the system's hard drive and later were quantified in Image Pro-Plus (Media Cybernetics, Silver Spring, MD). In the majority fiber type within each muscle, 10–12 NMJs were imaged, and measurements were averaged to represent NMJ structure, but, because of paucity, a minimum of five NMJs on the minority myofiber type were used to determine average NMJ structural characteristics for that fiber type.

A BX41 (Olympus) phase-contrast microscope was used to assess myofiber profiles with a ×40 objective. Myofiber

cross-sectional areas were quantified in Image Pro-Plus. A random sample of 125–150 myofibers from each muscle was analyzed to determine average myofiber size (i.e., cross-sectional area) and fiber-type composition for that muscle.

Statistical Analysis

All data are reported as mean ± SE. For each variable of interest, a two-way ANOVA with main effects of age and treatment was conducted. In the event of a significant main or interactive effect, a Fisher PLSD post hoc test was performed to identify significant pairwise differences. In all analyses, statistical significance was set at $P \leq 0.05$.

RESULTS

Body Mass

Prior to the start of the 7-week intervention program, aged animals weighed significantly more than young animals, but in neither age category were there differences in body mass between rats assigned to resistance training and those assigned to CTL groups. When animals were weighed again at the conclusion of the experimental period, it was once again noted that aged rats weighed significantly more than young rats. Unlike results at preintervention, the postintervention ANOVA results indicated a significant main effect for treatment when the CTL animals weighed more than the RT group. However, when post hoc procedures were performed, it was only among the young rats that the effect of treatment category was found to be significant; i.e., RT rats weighed less than CTL animals.

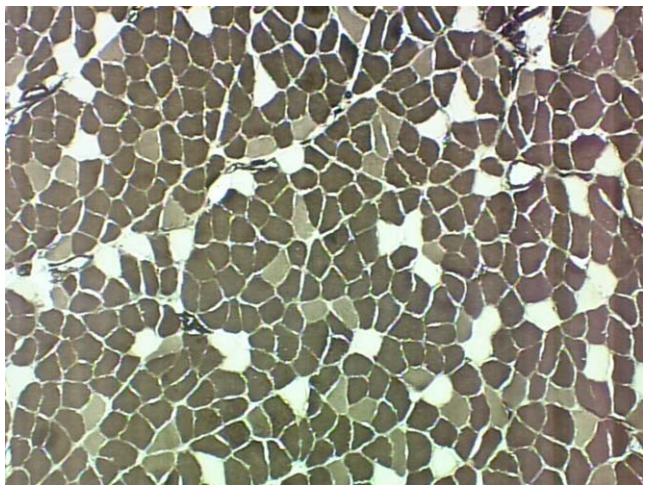

Fig. 3. Sample of myofibers stained for myosin ATPase activity following acidic preincubation at ×100. Darkly stained myofibers are type I, lightly stained fibers are type IIA, and intermediately stained fibers are type IIX/B. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Whole-Muscle Wet Weight

Upon euthanization at postintervention, the soleus and plantaris muscles were surgically removed and weighed before they were frozen. In the highly recruited soleus, a significant main effect of age was found (aged > young), although no effect for treatment was identified. Post hoc results indicated that, in both treatment groups, aged soleus muscles displayed greater mass than young ones. Conversely, data collected on the wet weight of plantaris muscles demonstrated a significant main effect for treatment (RT > CTL) but not for age. Post hoc analyses showed that, although resistance training resulted in heavier plantaris muscles in aged rats, this was not apparent in young animals. Data regarding body mass and whole-muscle wet weight can be found in Table I.

NMJ Morphology

Soleus (slow twitch). When synapses found on the predominant myofiber type of the soleus (slow twitch) were examined, it was ascertained that neither resistance training nor age impacted presynaptic structure. This was found to be true for each of the variables quantified regarding nerve terminal branching. However, with respect to presynaptic-to-postsynaptic coupling, results indicated a significant effect of aging in which aged NMJs showed a greater endplate area per total nerve terminal branch length. More specifically, this greater postsynaptic-to-presynaptic ratio was established in aged RT rats compared with young CTL and RT animals.

When postsynaptic endplate dimensions among slow-twitch NMJs were examined, observation confirmed that cumulative or total perimeter length of tracings surrounding stained clusters of ACh receptors in aged CTL animals exceeded those lengths in young RT rats. When endplate area was quantified, both total area (which included stained receptor clusters and unstained areas interspersed among clusters) and stained area (aggregate area of stained clusters only), it was revealed that aging had resulted in expanded areas under both CTL and

TABLE I. Effects of Resistance Training and Aging on Body Mass and Whole-Muscle Wet Weight★

	Young control	Young RT	Aged control	Aged RT
Body mass (g)	352.7 ± 11.1†	315.4 ± 6.7†	450.9 ± 11.9	442.5 ± 8.1
Soleus wet weight (mg)	120.6 ± 5.7‡	111.2 ± 4.4†	133.9 ± 6.7	136.8 ± 3.7
Plantaris wet weight (mg)	286.0 ± 18.0	299.0 ± 21.1	284.1 ± 6.9	336.8 ± 15.2§

★Values are mean ± SE.
†$P \leq 0.05$ indicates significant difference from all other groups.
‡$P \leq 0.05$ indicates significant difference from aged RT.
§$P \leq 0.05$ indicates significant difference from young control and aged control.

TABLE II. Effects of Resistance Training and Aging on Slow- and Fast-Twitch NMJs of the Soleus Muscle★

	Young control	Young RT	Aged control	Aged RT
Presynaptic (slow twitch)				
Branch number	6.6 ± 0.6	6.4 ± 0.4	5.9 ± 0.6	5.8 ± 0.4
Total branch length (μm)	99.3 ± 3.1	109.6 ± 6.5	94.4 ± 13.8	104.5 ± 7.8
Average branch length (μm)	16.7 ± 1.0	15.4 ± 1.5	19.5 ± 0.9	16.5 ± 1.8
Branching complexity	7.7 ± 0.9	7.9 ± 0.9	7.8 ± 1.6	7.2 ± 1.0
Presynaptic-to-postsynaptic coupling	2.5 ± 0.3†	2.1 ± 0.2‡	2.9 ± 0.2	3.4 ± 0.4
Postsynaptic (slow twitch)				
Total endplate perimeter (μm)	135.9 ± 10.7	120.1 ± 6.0	137.4 ± 9.3	132.3 ± 6.4
Stained endplate perimeter (μm)	223.3 ± 14.0	213.6 ± 15.8§	264.9 ± 19.0	240.8 ± 23.2
Total endplate area (μm^2)	410.2 ± 16.0‡	383.1 ± 40.5‡	564.3 ± 53.7	546.7 ± 52.8
Stained endplate area (μm^2)	222.6 ± 15.8‡	230.0 ± 27.6‡	323.4 ± 32.9	356.0 ± 38.3
Endplate dispersion (%)	50.0 ± 2.6#	65.6 ± 3.4	61.0 ± 1.0	62.0 ± 2.5
Presynaptic (fast twitch)				
Branch number	6.2 ± 0.7	6.8 ± 0.8	5.8 ± 0.9	7.1 ± 0.5
Total branch length (μm)	106.9 ± 10.9	109.0 ± 13.6	101.4 ± 20.9	132.7 ± 12.4
Average branch length (μm)	16.5 ± 0.5	17.2 ± 1.2	17.4 ± 1.4	19.5 ± 1.5
Branching complexity	10.7 ± 1.4	8.4 ± 1.7	7.3 ± 2.8	12.4 ± 3.4
Presynaptic-to-postsynaptic coupling	2.4 ± 0.1	3.1 ± 0.8	2.3 ± 0.6	2.7 ± 0.5
Postsynaptic (fast twitch)				
Total endplate perimeter (μm)	119.4 ± 8.2	125.6 ± 10.3	123.9 ± 28.0	131.2 ± 10.8
Stained endplate perimeter (μm)	243.0 ± 23.9	246.4 ± 9.7	186.2 ± 61.5	286.9 ± 38.9
Total endplate area (μm^2)	315.0 ± 48.8¶	504.2 ± 69.7	386.5 ± 166.9¶	557.5 ± 74.0
Stained endplate area (μm^2)	240.5 ± 23.0¶	326.1 ± 82.7	248.8 ± 110.6¶	352.4 ± 58.7
Endplate dispersion (%)	61.3 ± 1.5	62.5 ± 7.5	68.0 ± 4.9	58.8 ± 4.8§

★Values are mean ± SE; branching complexity = branch number × total branch length/100; presynaptic-to-postsynaptic coupling = endplate stained area/total terminal branch length; dispersion = stained endplate area/total endplate area × 100.
†$P \leq 0.05$ indicates significant difference from aged RT.
‡$P \leq 0.05$ indicates significant difference from aged control and aged RT.
§$P \leq 0.05$ indicates significant difference from aged control.
#$P \leq 0.05$ indicates significant difference from all other groups.
¶$P \leq 0.05$ indicates significant difference from young RT and aged RT.

RT conditions. When data from total endplate area and stained endplate area were used to determine dispersion of ACh receptors, a treatment effect was identified indicating that resistance training resulted in a more compact (i.e., receptors occupying a greater proportion of total endplate area), or less dispersed, distribution of postsynaptic receptors among young but not aged synapses.

Soleus (fast twitch). Similar to what was detected among NMJs of the predominant slow-twitch myofibers, synapses among the minority fast-twitch myofibers of the soleus failed to display significant effects of age or treatment in nerve terminal branching parameters. Although the presynaptic-to-postsynaptic coupling ratio was significantly influenced by age in slow-twitch NMJs, no such effect was discerned among NMJs residing on fast-twitch myofibers.

When perimeter lengths surrounding postsynaptic fast-twitch endplate regions were quantified, neither aging nor training had modified either total or stained lengths. However, when total and stained endplate areas were quantified, there was a significant effect of treatment (RT > CTL) in both young and aged animals. This differed from what was found in solei slow-twitch NMJs, in which there was an effect of aging (aged > young) but no training effect. Finally, with respect to ACh receptor dispersion in fast-twitch NMJs, it was noted that resistance training resulted in more dispersed receptor clusters but only among aged endplates. This, too, differed from what was observed in slow-twitch endplates, in which training was associated with more compact (i.e., higher percentage of total endplate area occupied by ACh receptors), or less dispersed, NMJs but only among young and not aged animals. All data on soleus NMJ morphology are presented in Table II.

Plantaris (fast twitch). In the plantaris, it is fast-twitch myofibers that are predominantly expressed. When NMJs residing on those fast-twitch myofibers were analyzed, a significant main effect for aging was detected for each presynaptic variable assessed. That is, aged synapses featured greater numbers of nerve terminal branches, total branch length, average branch length, and branching complexity relative to young NMJs. In contrast, results indicated that resistance training failed to alter presynaptic morphology. Moreover, neither age nor training altered presynaptic-to-postsynaptic coupling.

When postsynaptic endplates on these fast-twitch NMJs were examined, statistical analysis again revealed a main effect of aging in which aged endplates were found to have significantly longer perimeter lengths, in both total and stained-only measurements, as well as greater

TABLE III. Effects of Resistance Training and Aging on Slow- and Fast-Twitch NMJs of the Plantaris Muscle*

	Young control	Young RT	Aged control	Aged RT
Presynaptic (fast twitch)				
Branch number	5.8 ± 0.4	5.3 ± 0.3†	6.7 ± 0.3	6.6 ± 0.4
Total branch length (μm)	130.0 ± 8.0†	128.7 ± 5.3†	172.5 ± 7.4	168.3 ± 8.8
Average branch length (μm)	23.1 ± 0.7†	24.2 ± 0.7†	26.4 ± 0.6	26.8 ± 1.0
Branching complexity	7.5 ± 1.0†	6.5 ± 0.6†	11.9 ± 1.1	11.5 ± 1.4
Presynaptic-to-postsynaptic coupling	2.9 ± 0.1	3.3 ± 0.2	3.3 ± 0.1	3.2 ± 0.2
Postsynaptic (fast twitch)				
Total endplate perimeter (μm)	156.1 ± 5.5†	158.8 ± 9.5†	200.4 ± 7.8	191.4 ± 7.6
Stained endplate perimeter (μm)	292.4 ± 22.7†	287.1 ± 29.9†	434.4 ± 28.9	391.0 ± 29.4
Total endplate area (μm^2)	452.4 ± 35.1†	457.7 ± 48.3†	666.2 ± 50.7	615.5 ± 51.6
Stained endplate area (μm^2)	385.3 ± 26.0†	398.7 ± 38.7†	559.5 ± 42.9	525.1 ± 49.8
Endplate dispersion (%)	71.0 ± 2.4	73.3 ± 1.7	70.2 ± 1.7	70.0 ± 2.0
Presynaptic (slow twitch)				
Branch number	5.5 ± 0.5	5.1 ± 0.6†	6.9 ± 0.3	6.8 ± 0.4
Total branch length (μm)	129.1 ± 10.1†	111.4 ± 11.4†	167.7 ± 7.4	169.5 ± 11.6
Average branch length (μm)	25.4 ± 4.1	23.3 ± 1.2	25.2 ± 1.2	25.5 ± 1.3
Branching complexity	7.5 ± 1.3†	6.5 ± 1.7	12.3 ± 1.0	12.6 ± 1.6
Presynaptic-to-postsynaptic coupling	3.8 ± 0.3	3.1 ± 0.4	2.6 ± 0.4	2.5 ± 0.4
Postsynaptic (slow twitch)				
Total endplate perimeter (μm)	163.2 ± 12.8‡	144.0 ± 15.0†	203.0 ± 9.2	191.1 ± 10.1
Stained endplate perimeter (μm)	327.5 ± 42.7‡	290.1 ± 28.4†	440.2 ± 37.2	377.2 ± 29.5
Total endplate area (μm^2)	492.2 ± 65.8	399.3 ± 35.0‡	629.8 ± 55.2	525.4 ± 53.1
Stained endplate area (μm^2)	413.9 ± 48.4	341.6 ± 29.1‡	533.4 ± 56.6	429.6 ± 46.8
Endplate dispersion (%)	68.5 ± 2.7	70.6 ± 2.3	68.0 ± 2.0	66.2 ± 3.6

*Values are mean ± SE; branching complexity = branch number × total branch length/100; presynaptic-to-postsynaptic coupling = endplate stained area/total nerve terminal branch length; endplate dispersion = stained endplate area/total endplate area × 100.
†$P \leq 0.05$ indicates significant difference from aged control and aged RT.
‡$P \leq 0.05$ indicates significant difference from aged control.

endplate areas, again, in both total and stained-only measurements, compared with young animals. However, no effect of training was identified in endplate morphology. Finally, dispersion of postsynaptic ACh receptors was impervious to both resistance training and aging.

Plantaris (slow twitch). In slow-twitch NMJs of the plantaris, it was once again revealed that aging was coupled with significantly larger presynaptic nerve terminal branching patterns. This closely paralleled what was identified in the predominant fast-twitch NMJs. The sole exception was that average branch length in slow-twitch NMJs was unaffected by aging, whereas average branch length in aged fast-twitch NMJs was longer than that in young fast-twitch synapses. As was discovered among plantaris fast-twitch NMJs, neither age nor resistance training altered presynaptic to postsynaptic coupling in slow-twitch NMJs of the plantaris.

Again, our analysis of postsynaptic slow-twitch plantaris NMJs yielded results that mimicked what was found in endplates of the predominant fast-twitch NMJs of that muscle. More specifically, aging resulted in significant enhancement of total and stained perimeter lengths and significantly expanded total and stained endplate areas. Resistance training, on the other hand, had no effect on postsynaptic morphology. As with fast-twitch endplates, neither age nor training altered the dispersion of ACh receptors in postsynaptic endplates of slow-twitch myofibers of the plantaris. All data regarding NMJ morphology of plantaris muscles can be examined in Table III.

Myofiber Morphology

Soleus. Statistical results from analysis of the heavily recruited, postural soleus muscle indicate that neither age nor treatment (i.e., resistance training) affected myofiber size when data from all fiber types were pooled. This was also the case when examining predominant type I (slow-twitch) fibers exclusively as well as when type IIA and IIX fibers were quantified by themselves. Similarly, neither age nor training significantly changed the fiber-type composition of the soleus.

Plantaris. In contrast to the soleus, the plantaris, although also an ankle flexor, is mainly comprised of type II (fast-twitch) myofibers and is only sparsely recruited under resting conditions, inasmuch as its main function is to serve in locomotor activity. With data from fiber types collapsed together, plantaris myofiber size was unaffected by age or by resistance training. This same resilience to alterations in size was also observed when myofibers were quantified by individual fiber type (i.e., types I, IIA, or IIX/B). However, when fiber-type composition of the plantaris was assessed, it was found that, although resistance training failed to alter fiber-type distribution, aging did result in a significant modification. Specifically, it was found that aging resulted in a significant decrease in the

TABLE IV. Effects of Resistance Training and Aging on Myofiber Profiles of Soleus and Plantaris Muscles*

	Young control	Young RT	Aged control	Aged RT
Soleus (cross-sectional area; μm^2)				
Types combined	2,159 ± 101	2,051 ± 107	2,243 ± 126	2,226 ± 69
Type I	2,217 ± 110	2,086 ± 109	2,285 ± 126	2,242 ± 75
Type II (A, X)	1,879 ± 102	1,815 ± 116	2,022 ± 180	2,152 ± 84
Type IIA	1,939 ± 111	1,908 ± 118	2,164 ± 193	2,189 ± 105
Type IIX	1,667 ± 135	1,497 ± 77	1,796 ± 195	1,832 ± 167
Soleus (fiber type composition; %)				
Type I	85.2 ± 2.1	88.2 ± 1.2	86.0 ± 1.1	84.3 ± 1.1
Type II (A, X)	14.8 ± 7.5	11.8 ± 1.2	14.0 ± 1.1	15.7 ± 1.1
Type IIA	11.6 ± 2.2	8.0 ± 1.0	9.0 ± 1.2	11.7 ± 1.0
Type IIX	3.2 ± 0.7	3.8 ± 1.1	5.0 ± 0.8	4.0 ± 0.7
Plantaris (cross-sectional area; μm^2)				
Types combined	2,218 ± 119	2,100 ± 114	2,017 ± 93	2,106 ± 110
Type I	1,359 ± 117	1,467 ± 202	1,292 ± 46	1,427 ± 80
Type II (A, X)	2,394 ± 119	2,256 ± 111	2,209 ± 109	2,312 ± 120
Type IIA	2,632 ± 173	2,543 ± 157	2,369 ± 112	2,588 ± 117
Type IIX/B	1,303 ± 159	1,513 ± 188	1,249 ± 86	1,417 ± 99
Plantaris (fiber-type composition; %)				
Type I	16.5 ± 1.3	20.1 ± 3.0	22.3 ± 1.8†	25.7 ± 3.2‡
Type II (A, X/B)	83.5 ± 1.3	79.9 ± 3.0	77.7 ± 1.8†	74.3 ± 3.2‡
Type IIA	67.8 ± 6.1	65.9 ± 3.7	66.6 ± 2.3	64.5 ± 6.3
Type IIX/B	15.7 ± 5.4	14.0 ± 3.7	11.1 ± 1.5†	9.8 ± 1.8‡

*Values are mean ± SE
†$0.10 < P < 0.05$ indicates trend for difference from young control.
‡$P \leq 0.05$ indicates significant difference from young control.

expression of type II fibers that was accompanied by a similar increase in the percentage of type I fibers in the plantaris. This finding was explained mainly by a decline in the content of type X/B fibers because no appreciable variation in the percentage of type IIA fibers was associated with aging. Myofiber profile results are presented in Table IV.

DISCUSSION

With the aging segment of populations in virtually all industrialized nations showing increasing growth, a major public health effort has been put forth to develop interventions to maintain good health so that health care costs associated with aging might be prevented from spiraling out of control (Pandya et al., 2013; Charness, 2014). Convincing evidence has accrued demonstrating the success of exercise training as a cost-effective method of maintaining or improving health among senescent individuals (Allen and Morelli, 2011; Fleg, 2012; Desveaux et al., 2014). Included among exercise regimens prescribed for the aged is resistance training, or weight lifting. This mode of exercise might confer benefits, such as greater strength and muscle mass, improved skeletal health, and better control of blood glucose levels (Chodzko-Zajko et al., 2009; Gasiorowski and Dutkiewicz, 2012). An important component of the neuromuscular system that is responsive to resistance exercise is the NMJ, which functionally and anatomically connects the motor nervous system to skeletal muscle fibers (myofibers). It is known that aging results in remodeling of the NMJ (Fahim et al., 1983; Anis and Robbins, 1987; Andonian and Fahim, 1989; Jang and Van Remmen, 2011) and that it also influences NMJ adaptability to endurance training (Fahim, 1997; Deschenes et al., 2011). The present investigation sought to determine whether aging also impacts the ability of the NMJ to respond to resistance training. We examined this in two muscles with vastly different myofiber-type compositions and principal functions. The soleus is composed mainly of slow-twitch myofibers, and it is characterized by a high duty cycle, meaning that it is regularly recruited because of its function as the main postural muscle. In turn, the plantaris is primarily comprised of type II (fast-twitch) myofibers and functions mainly as a locomotor muscle and thus is recruited far less than the soleus (Laughlin and Armstrong, 1982). One of the unexpected findings of this study was that, among young rats, RT soleus muscles weighed less than the soleus muscles of untrained CTLs. This result mirrored what was found in body mass; that is, young RT rats weighed significantly less than young CTLs. It is possible that the training regimen sufficiently increased physical activity levels such that declines in body mass were observed. Alternatively, it is possible that regular exercise curtailed the appetite of young rats such that they consumed fewer calories during the intervention period, thus adding on less body mass than those subjected to control conditions.

More germane to the focus of the current investigation, our results show that, among the muscles examined, it was the soleus and not the plantaris that displayed NMJ adaptations to resistance training, but those adaptations

were evident only among NMJs that resided on the sparingly expressed fast-twitch myofibers and were assessed as increased postsynaptic endplate area noted among both young and aged rats as well as greater ACh receptor dispersion among aged NMJs only. Also noted was a main effect of aging in presynaptic-to-postsynaptic coupling of NMJs residing on slow-twitch muscle fibers. This could be explained by expansions of postsynaptic endplate size among the aged animals without a concomitant increase in nerve terminal branch length. Furthermore, no presynaptic training-induced adaptations were identified in either young or aged animals. This was true for nerve terminal branching quantified in both fast- and slow-twitch myofibers.

In contrast to the effect of resistance training, which was apparent exclusively on postsynaptic measures of fast-twitch NMJs, the influence of aging was conferred solely upon the postsynaptic endplates of the predominant slow-twitch NMJs of the soleus. As has been documented previously (Fahim, 1997; Deschenes et al., 2011), aging resulted in endplate expansion on slow-twitch myofibers of the soleus.

Unlike postsynaptic parameters, not a single main effect of aging was detected for any presynaptic variable measured in either the predominant slow-twitch NMJs or the minority fast-twitch NMJs found in the soleus. This absence of an aging effect might well be explained by the high recruitment patterns exhibited by that postural muscle.

Conversely, the plantaris, composed mainly of fast-twitch myofibers and acting mainly as a locomotor muscle, almost uniformly evinced a main effect of aging. This effect was observed as expansions of all features of presynaptic nerve terminal branching as well as postsynaptic endplate regions. Such age-related increases in NMJ parameters were observed in both fast-twitch and slow-twitch synapses of the plantaris. Because of the pre- and postsynaptic uniformity of this age-related morphological growth, presynaptic to postsynaptic coupling was maintained in the face of aging, as was the dispersion of ACh receptors within the endplate region. Given its role as an ambulatory muscle, it is surprising that the NMJ of the plantaris remained wholly devoid of training-induced adaptations. This might be related to the well-known difficulty in emulating the intensity and volume of resistance training typified in human weight lifters when using an animal model (Timson, 1990; Cholewa et al., 2014). Indeed, myofiber profiles of both the soleus and the plantaris did not reveal any of the hypertrophy that would be expected with an effective resistance training program. This lack of myofiber hypertrophy is not supported by whole-muscle wet-weight data showing that plantaris muscles of trained aged rats were significantly heavier than those of young animals. We believe that this result likely was due mainly to inconsistencies during the surgical removal of those muscles. Unlike the soleus, which has easily identified points of origin and insertion that are easily accessible when extracting the muscle, the plantaris does not so readily present obvious cutting sites during surgical removal; it is also embedded in the gastrocnemius muscle. As a result, sometimes smaller or larger sections of what are intended to be whole muscles are removed that are then later weighed.

In contrast to myofiber cross-sectional area, myofiber-type composition was affected by aging, at least in the plantaris, in that aged rats displayed a higher percentage of type I (slow-twitch) myofibers that was concomitant with a decreased percentage of type II (fast-twitch) myofibers. This pattern of fiber-type conversion (II→I) is a cardinal feature of the sarcopenia observed in aged muscle. These data suggest that the first sign of sarcopenia might be fiber-type conversion rather than fiber atrophy and that sparsely recruited muscles (plantaris) might be more vulnerable to the effects of sarcopenia than highly recruited ones (soleus). A caveat might be in order here, however. It has recently been reported that various staining procedures used to determine myofiber-type composition in muscles might yield inaccurate results resulting from misclassification, especially among aged muscles (Purves-Smith et al., 2014). Among the numerous staining procedures used to assess fiber-type composition, the one employed here, ATPase histochemistry, is particularly vulnerable to such error.

The combination of using different age groups (young vs. aged) to study the effects of resistance training on disparate muscles (soleus vs. plantaris) along with different NMJs (fast vs. slow twitch) provided important new findings regarding the inherent synaptic plasticity of the peripheral nervous system. A vital finding was that it was among the NMJs of the highly recruited soleus but not the lightly recruited plantaris that morphological adaptations to the resistance training stimulus were apparent. Again, the resistance training paradigm used might have lacked adequate rigor to recruit the type-II-predominant, high-threshold plantaris.

It is important to note that these training-induced NMJ adaptations in the soleus were detected among both young and older rats, suggesting that aging, at least early-onset aging, did not impact the sensitivity of synapses to an exercise stimulus. This bodes well for older people trying to derive the many health benefits associated with weight training. However, the training-provoked enhancement of postsynaptic endplate dimensions, i.e., perimeter length and area, of the soleus was noted in fast-twitch but not slow-twitch NMJs. This likely can be attributed to the fact that under normal conditions fast-twitch myofibers of the soleus are infrequently recruited, but during the weight training sessions they would be expected to be activated. In contrast, significant interactive effects among aging and training were observed in both fast-twitch and slow-twitch NMJs of the soleus. More specifically, it was determined that resistance training increased dispersion of ACh receptors of aged NMJs, whereas that same stimulus brought about a more compact receptor distribution within the endplates of young NMJs. Clearly, there is a nuanced, complex relationship between the influences of exercise training and aging with respect to NMJ remodeling.

Unlike the effects of resistance training, which triggered NMJ adaptations only among the small number (~15%) of fast-twitch myofibers that make up the soleus, the effects of aging were identified in the more commonly expressed slow-twitch myofibers of that muscle. It was notable that adaptations to both aging and training occurred only at postsynaptic regions of NMJs identified in the soleus. However, perhaps the most noteworthy outcome of this investigation was the fact that age-related synapse reconfiguration was uniformly evident in the plantaris. That is, the effects of aging were manifested in both the predominant fast-twitch myofibers and the sparingly expressed slow-twitch myofibers of the plantaris. Moreover, this remodeling was revealed both in presynaptic nerve terminal branching and in the postsynaptic endplate region of the myofiber's sarcolemma. It appears that a very pervasive series of NMJ adaptations occurs during even the earliest stages of aging (recall that the aged rats used here are the equivalent of 60-year-old humans, with that age serving as the threshold for the "youngest" of the aged population [Forman et al., 1992]), at least among lightly recruited muscles such as the plantaris.

Altogether, the data gathered in the present investigation indicate that lightly recruited muscles are more sensitive to aging, even at the very earliest stages of aging, than those muscles that demonstrate a higher duty cycle. There is also evidence that the effects of aging first appear in the postsynaptic region, suggesting that this process might begin with the myofiber before progressing in a retrograde fashion up to presynaptic nerve terminals. Additionally, among muscles such as the plantaris that are particularly sensitive to the effects of aging because of their modest habitual activity levels, aging-induced synaptic remodeling is apparent in the NMJs of both slow- and fast-twitch myofibers. In all cases, aging resulted in an expansion of pre- and postsynaptic features even without changes in the size of the underlying myofibers that are coupled with NMJ size increments noted during natural growth and development (Balice-Gordon and Lichtman, 1990).

Although the current study focused mainly on NMJs, an interesting finding is that sarcopenia might begin not with myofiber atrophy but rather with myofiber conversion (type II→I). Finally, although the resistance training protocol employed here was not sufficient to produce myofiber hypertrophy, the NMJs affected by that training regimen displayed expansions that have also been exhibited by endurance-trained rats (Deschenes et al., 1993, 2011; Fahim, 1997). Apparently, physical training of any kind elicits a sequence of molecular responses that results in larger pre- and postsynaptic components of the NMJ. Presumably, these exercise-induced adaptations result in improved physiological functioning of the neuromuscular system inasmuch as previous reports (Fahim, 1997) indicate that morphological changes of the NMJ are linked with positive physiological adaptations, whereas age-related NMJ structural remodeling is associated with declines in neuromuscular function (Banker et al., 1983).

REFERENCES

Allen J, Morelli V. 2011. Aging and exercise. Clin Geriatr Med 27:661–671.

Andersen JL, Aagaard P. 2010. Effects of strength training on muscle fiber types and size; consequences for athletes training for high-intensity sport. Scand J Med Sci Sports 20(Suppl 2):32–38.

Anderton BH, Breinburg D, Downes MJ, Green PJ, Tomlinson BE, Ulrich J, Wood JN, Kahn J. 1982. Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share antigenic determinants. Nature 298:84–86.

Andonian MH, Fahim MA. 1989. Nerve terminal morphology in C57BL/6NNia mice at different ages. J Gerontol 44:B43–B51.

Anis NA, Robbins N. 1987. General and strain-specific age changes at mouse limb neuromuscular junctions. Neurobiol Aging 8:309–318.

Anton SD, Karabetian C, Naugle K, Buford TW. 2013. Obesity and diabetes as accelerators of functional decline: can lifestyle interventions maintain functional status in high-risk older adults? Exp Gerontol 48: 888–897.

Balice-Gordon RJ, Lichtman JW. 1990. In vivo visualization of the growth of pre- and postsynaptic elements of neuromuscular junctions in the mouse. J Neurosci 10:894–908.

Banker BQ, Kelly SS, Robbins N. 1983. Neuromuscular transmission and correlative morphology in young and old mice. J Physiol 339:355–377.

Bloom DE, Boersch-Supan A, McGee P, Seike A. 2011. Population aging: facts, challenges, and responses. Program on the Global Demography of Aging at Harvard University, working paper No. 71 http://www.hsph.harvard.edu/program-on-the-global-demography-of-aging/WorkingPapers/2011/PGDA_WP_71.pdf.

Cederholm T, Cruz-Jentoft AJ, Maggi S. 2013. Sarcopenia and fragility fractures. Eur J Phys Rehabil Med 49:111–117.

Charness N. 2014. Utilizing technology to improve older adult health. Occup Ther Health Care 28:21–30.

Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, Skinner JS. 2009. American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerc 41:1510–1530.

Cholewa J, Guimaraes-Ferreira L, da Silva Teixeira T, Naimo MA, Zhi X, de Sa RB, Lodetti A, Cardozo MQ, Zanchi NE. 2014. Basic models modeling resistance training: an update for basic scientists interested in study skeletal muscle hypertrophy. J Cell Physiol 229:1148–1156.

Delp MD, Duan C. 1996. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. J Appl Physiol 80:261–270.

Deschenes MR, Maresh CM, Crivello JF, Armstrong LE, Kraemer WJ, Covault J. 1993. The effects of exercise training of different intensities on neuromuscular junction morphology. J Neurocytol 22:603–615.

Deschenes MR, Judelson DA, Kraemer WJ, Meskaitis VJ, Volek JS, Nindl BC, Harman FS, Deaver DR. 2000. Effects of resistance training on neuromuscular junction morphology. Muscle Nerve 23:1576–1581.

Deschenes MR, Roby MA, Glass EK. 2011. Aging influences adaptations of the neuromuscular junction to endurance training. Neuroscience 190:56–66.

Desveaux L, Beauchamp M, Goldstein R, Brooks D. 2014. Community-based exercise programs as a strategy to optimize function in chronic disease: a systematic review. Med Care 52:216–226.

Engelke K, Kemmler W, Lauber D, Beeskow C, Pintag R, Kalender WA. 2006. Exercise maintains bone density at spine and hip EFOPS: a 3-year longitudinal study in early postmenopausal women. Osteoporos Int 17:133–142.

Fahim MA. 1997. Endurance exercise modulates neuromuscular junction of C57BL/6NNia aging mice. J Appl Physiol 83:59–66.

Fahim MA, Holley JA, Robbins N. 1983. Scanning and light microscopic study of age changes at a neuromuscular junction in the mouse. J Neurocytol 12:13–25.

Fleg JL. 2012. Aerobic exercise in the elderly: a key to successful aging. Discov Med 13:223–228.

Folland JP, Williams AG. 2007. The adaptations to strength training: morphological and neurological contributions to increased strength. Sports Med 37:145–168.

Forman DE, Berman AD, McCabe CH, Baim DS, Wei JY. 1992. PTCA in the elderly: the "young-old" versus the "old-old". J Am Geriatr Soc 40:19–22.

Gasiorowski A, Dutkiewicz J. 2012. Weight training and appropriate nutrient supplementation as an alternative method to pharmacological treatment in rehabilitation of postmyocardial infarction patients. Ann Agric Environ Med 19:333–338.

Gregory MA, Gill DP, Petrella RJ. 2013. Brain health and exercise in older adults. Curr Sports Med Rep 12:256–271.

Jang YC, Van Remmen H. 2011. Age-associated alterations of the neuromuscular junction. Exp Gerontol 46:193–198.

Laughlin MH, Armstrong RB. 1982. Muscular blood flow distribution patterns as a function of running speed in rats. Am J Physiol Heart Circ Physiol 243:H296–H306.

Liu-Ambrose T, Donaldson MG. 2009. Exercise and cognition in older adults: is there a role for resistance training programmes? Br J Sports Med 43:25–27.

Nemeth P, Pette D. 1981. Succinate dehydrogenase activity in fibres classified by myosin ATPase in three hind limb muscles of rat. J Physiol 320:73–80.

Pandya A, Gaziano TA, Weinstein MC, Cutler D. 2013. More Americans living longer with cardiovascular disease will increase costs while lowering quality of life. Health Aff 32:1706–1714.

Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC. 1995. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 273:402–407.

Pearson J, Sabarra A. 1974. A method for obtaining longitudinal cryostat sections of living muscle without contraction artifacts. Stain Technol 49:143–146.

Purves-Smith FM, Sgarioto N, Hepple RT. 2014. Fiber typing in aging muscle. Exerc Sport Sci Rev 42:45–52.

Timson BF. 1990. Evaluation of animal models for the study of exercise-induced muscle enlargement. J Appl Physiol 69:1935–1945.

Tomas J, Fenoll R, Mayayo E, Santafe M. 1990. Branching pattern of motor nerve endings in a skeletal muscle of the adult rat. J Anat 168: 123–135.

Turturro A, Witt WW, Lewis S, Hass BS, Lipman RD, Hart RW. 1999. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J Gerontol A Biol Sci Med Sci 54: B492–B501.

United States Census Bureau. 2012. Births, deaths, marriages, and divorces: life expectancy. The 2012 Statistical Abstract, the National Data Book.

Visser M. 2011. Obesity, sarcopenia, and their functional consequences in old age. Proc Nutr Soc 70:114–118.

Westcott WL. 2012. Resistance training is medicine: effects of strength training on health. Curr Sports Med Rep 11:209–216.

World Health Organization. 2010. Global recommendations on physical activity for health. Global strategy on diet, physical activity, and health. Geneva: WHO.