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Moderate Aging Does Not Modulate Morphological Responsiveness of the Neuromuscular System to Chronic Overload in Fischer 344 Rats

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Abstract

The purpose of this investigation was to determine the effect of aging on neuromuscular adaptations to chronic overload. Eight young adult (8 mo old) and eight aged (22 mo old) Fischer 344 rats underwent unilateral synergist ablation to overload the plantaris and soleus muscles of that hindlimb and to provide control muscles from the contralateral hindlimb. Cytofluorescent staining and confocal microscopy were used to quantify pre- and post-synaptic features of neuromuscular junctions (NMJs). Histochemical staining and light microscopy were used to assess adaptations of myofibers to chronic overload. Results demonstrate that NMJs of young adult and aged muscles did not undergo morphological remodeling as a result of four weeks of chronic overload. In contrast, myofibers of young and aged rats displayed significant ($P < 0.05$), but similar hypertrophy (~18%) following that four week intervention. In both age groups, however, this hypertrophy was detected in the plantaris, but not the soleus. These data indicate that moderate aging (the equivalent of 65 years in human lifetime) does not modify the sensitivity of the neuromuscular system to chronic overload.

Keywords

myofibers; neuromuscular junction; synapse; hypertrophy; exercise; resistance

The neuromuscular system exhibits impressive sensitivity to alterations in its pattern of usage. For example, numerous studies have shown that myofibers can either increase their size in response to overload (Rosenblatt et al., 1994; Allen et al., 1995; Kano et al., 1997), or experience atrophy as a result of unloading (Riley et al., 1990; Deschenes et al., 2001; Hurst and Fitts, 2003). Similarly, the neuromuscular junction (NMJ) – the synapse that permits communication from the motor nervous system to skeletal muscle – also can be remodeled as a result of changes in neuromuscular activity. Although most reports document such remodeling following inactivity (Brown and Ironton, 1977; Fahim and Robbins, 1986; Fahuim, 1989; Pachter and Spielholz, 1990; Deschenes and Wilson, 2003), a few have characterized

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NMJ adaptations to increased activity in the form of exercise training, i.e., treadmill running (Andonian and Fahim, 1988; Deschenes et al., 1993; Fahim, 1997).

However, unlike the effects of unloading which, whether presented as microgravity (Riley et al., 1990; D'Amelio et al., 1992; Deschenes et al., 2005) or hindlimb suspension (Pierotti et al., 1990; Fahim, 1997; Huckstorf et al., 2000; Deschenes et al., 2003), have been examined previously, the capacity of chronic overload to remodel the neuromuscular system – myofibers and NMJs – is not well defined. Since it has been demonstrated that during natural development expansion of the NMJ is concomitant with the growth of muscle mass and increased size of the myofibers on which those synapses reside (Balice-Gordon and Lichtman, 1990) it was postulated that the myofiber hypertrophy stimulated by chronic overload would also result in increased NMJ size. To extend the scope of the project, the effect that aging may have on neuromuscular responsiveness to chronic overload was also explored. Therefore, the aim of the present investigation was twofold: 1) to determine the adaptations of the neuromuscular system to the stimulus of chronic overload, and 2) to assess whether those overload induced adaptations were age specific.

EXPERIMENTAL PROCEDURES

Subjects

Eight young adult (8 mo old) and eight aged (22 mo old) male Fischer 344 rats were purchased from the National Institute on Aging Colonies. The average lifespan of male Fischer 344 rats is 25.5 months (Turturro et al., 1999). Thus, at 22 months of age the rats used in the present study had lived 86% of their life expectancy. Relative to the average lifespan of men in the United States (75.2 years; Arias, 2006), these rats would be the equivalent of 65 years old, or the age at which humans in the U.S are first considered to be aged (Older Americans Update 2006).

Animals were provided with standard rat chow and water ad libitum, and were housed in a 21–22°C environment with a 12:12 light/dark cycle. All procedures utilized were approved beforehand by the institutional animal care and use committee, which operates in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as revised in 1996. Throughout the study, all efforts were made to minimize the number of animals used and their suffering.

Chronic overload intervention

Unilateral synergist ablation was used to implement a state of chronic overload on the neuromuscular system. In this procedure, the distal two-thirds of the gastrocnemius muscle of a single hindlimb was surgically removed, thus overloading the soleus and plantaris muscles of that hindlimb. To serve as an internal control, the animal's contralateral hindlimb underwent a sham operation in which an incision was made in the skin and the gastrocnemius was exposed, but otherwise left intact. This resulted in normal loading demands of the plantaris and soleus muscles of the sham operated hindlimb. Surgical procedures were performed under aseptic conditions after rats were anesthetized with 2–3% isoflurane gas supplemented with oxygen. Following the ablation and sham operation procedures, wounds were closed with stainless steel suture clips and animals were administered a subcutaneous injection of analgesic (Buprenex, 0.03 mg/kg body mass).

Following the unilateral synergist ablation procedure, animals were returned to their cages and allowed to resume normal weight bearing and ambulatory activity for four weeks. Daily observation revealed that within 2–3 days, animals were no longer favoring one leg over the other, suggesting that weight bearing demands were similar for both hindlimbs. At the end of

the four week intervention period, rats were anesthetized with ketamine and xylazine (90 and 10 mg/kg body mass, respectively) and decapitated. Immediately following death, plantaris and soleus muscles from both hindlimbs were surgically removed, separated from each other, cleared of fat and connective tissue, and quickly frozen in isopentane chilled with liquid nitrogen. Whole muscles were then stored at -80°C until analysis. During the surgical removal of the plantaris and soleus muscles, it was visually confirmed that re-attachment of the ablated gastrocnemius to its tendon had not occurred.

Cytofluorescent 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 (Pearson and Sabarra, 1974). Sections were washed 4×15 min 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), diluted 1:20 in PBS with 1% BSA. The RT97 antibody reacts with non-myelinated constituents of pre-synaptic nerve terminals (Anderton et al., 1982). The next day, sections were washed 4×15 min in PBS with 1% BSA before incubating for 2 h at room temperature in fluorescein isothiocyanate (FITC) conjugated secondary immunoglobulin (Sigma Chemical, St. Louis, MO) that was diluted 1:150 in PBS with 1% BSA. Sections were then washed 4×15 min in PBS with 1% BSA. Following this, sections were incubated in a humidified chamber overnight at 4°C in a solution containing rhodamine conjugated α -bungarotoxin (BTX; Molecular Probes, Eugene, OR) diluted 1:600 in PBS along with either anti-slow (soleus) or anti-fast (plantaris) myosin heavy chain ascites fluid (Sigma Chemical, St. Louis, MO) diluted 1:40. BTX recognizes post-synaptic acetylcholine (ACh) receptors, while the anti-slow immunogen enabled us to determine whether the endplate resided on a fast- or slow-twitch myofiber. The next day, sections were washed 4×15 min in PBS with 1% BSA before incubating them for 1 h at room temperature in AlexaFluor 647 (Molecular Probes, Eugene, OR) labeled secondary antibody to bind with the anti-slow primary antibody. Sections were given a final wash (4×15 min) before being lightly coated with Pro Long (Molecular probes, Eugene, OR) and having cover slips applied. Slides were then coded with respect to treatment group to allow for blinded evaluation of NMJ morphology and then stored at -20°C until analysis. Pre-synaptic variables of NMJs assessed included: 1) number of branches identified at the nerve terminal, 2) the total length of those branches, 3) average length per branch, and 4) branching complexity which, as described by Tomas et al., (1987) is derived by multiplying the number of branches by the total length of those branches and dividing that figure by 100. Postsynaptic variables of interest included: 1) total perimeter, or the length encompassing the entire endplate comprised of stained receptor clusters and non-stained regions interspersed within those clusters, 2) stained perimeter, or the composite length of tracings around individual receptor clusters, 3) total area, which includes stained receptors along with non-stained regions interspersed among receptor clusters, 4) stained area, or the cumulative areas 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, pre- to post-synaptic coupling was quantified by dividing the NMJ's post-synaptic stained area by its total length of nerve terminal branching.

Histochemical staining

To quantify myofiber profiles, 10 μm thick transverse sections were obtained from the midbelly of the muscle using a cryostat set at -20°C . Sections were stained for myofibrillar ATPase activity following pre-incubation at a pH of either 4.55 (soleus) or 4.40 (plantaris) according to Nemeth and Pette (1981). To be consistent with our staining of NMJs, in which underlying

myofibers were identified as either slow- or fast-twitch, myofibers following histochemical staining were categorized as either Type I (slow-twitch) or Type II (fast-twitch). The procedures employed in the present study, however, did not enable the detection of hybrid myofibers that co-express more than a single myosin heavy chain isoform. Methodologies allowing greater resolution of myosin heavy chain expression have revealed that, indeed, chronic overload increases the expression of these hybrid fibers in the affected muscle (Roy et al., 1997). Slides were coded so that measurements could be conducted in a blinded fashion regarding treatment group.

Microscopy

An Olympus Fluoview FV 300 confocal system featuring three lasers and an Olympus BX60 fluorescent microscope (Olympus America, Melville, NY) was used to collect images of NMJs and to identify whether they were located on slow or fast-twitch myofibers. Using a 100X 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 1 μ m thick increments. To ascertain the myofiber type on which the NMJ resided, a single scan was collected of the fiber using the appropriate wavelength to detect AlexaFluor 647. Digitized, two-dimensional images of NMJs were stored on the system's hard drive and later quantified with the Image Pro-Plus software (Media Cybernetics, Silver Spring, MD). For each fiber type within the muscle, 10–12 NMJs were imaged and measurements averaged to represent NMJ structure for fast- and slow-twitch fibers in that muscle.

An Olympus BX41 phase contrast microscope was used to assess myofiber profiles with a 40X objective. Myofiber cross-sectional areas were quantified with the Image Pro-Plus software. 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

Data are reported as means $\pm$ SE. Pre- to post-intervention body mass data were compared with dependent t-tests. In addition, independent t-tests were used to compare body mass of young vs. aged animals both before, and after the four week intervention. All other variables of interest were assessed with analysis of variance. In the event of a significant F-ratio, a Tukey post-hoc test was performed to identify pair-wise differences. In all cases, statistical significance was established at $P \leq 0.05$.

RESULTS

Body mass

Our analysis of body weight changes during the 4 week experimental period indicates that differences occurred in both young adult and aged rats. However, while younger rats exhibited a significant, but moderate (~ 4%) increase in body mass over those four weeks, aged rats experienced a significant decrease in body mass of 6.3%. Accordingly, the significant age related difference in body mass (aged > young adult) observed prior to the overload intervention was no longer evident following it. It is important to note that all data regarding NMJ and myofiber morphology were collected subsequent to the treatment period, thus potential differences in those variables cannot be attributed to differences in whole animal size. Table 1 presents our findings on body mass.

NMJ morphology

Soleus—Data collected from NMJs found on slow-twitch myofibers of the soleus failed to reveal any significant between group differences in their pre- or post-synaptic characteristics. Moreover, those synapses located on the few fast-twitch myofibers in this primarily slow-twitch, weight bearing muscle also displayed similar structural features regardless of experimental group. And again, this absence of treatment effects – overload or aging – was detected both among pre-synaptic nerve terminal branches, and post-synaptic ACh receptors. In short, these findings indicate that neither aging by itself, nor the intervention of chronic overload significantly altered the architecture of either slow- or fast-twitch NMJs, respectively. Further, both young adult and aged, as well as slow- and fast-twitch synapses (Table 2 and Table 3, respectively) proved resilient to the overload stimulus.

Plantaris—Our examination of NMJs in the principally fast-twitch, locomotor plantaris muscle also failed to identify any significant between group differences in synaptic morphology. And as with the soleus, this lack of remodeling was apparent both in pre- and post-synaptic features, and in NMJs associated both with fast-twitch and slow-twitch myofibers. Again, neither aging nor chronic overload presented stimuli sufficiently potent to elicit NMJ remodeling. Results from our analysis of fast-twitch and slow-twitch NMJs residing within the plantaris are presented in Table 4 and Table 5, respectively.

Myofiber morphology

Soleus—When examined irrespective of fiber type, i.e., data from Type I and Type II fibers combined, our results indicate that no significant differences in fiber cross-sectional area existed between the four treatment groups. Similarly, when the predominantly expressed Type I myofibers were compared among the four groups, no significant effects of aging or overload were manifested. But when Type II fibers, exclusively, were analyzed, several age-related differences were observed. The size of Type II fibers in aged control soleus muscles were significantly smaller than those expressed in young control and young overloaded muscle, while Type II fibers in aged overloaded solei were significantly smaller than those of young overloaded muscles. But in neither young adult, nor aged soleus muscles did chronic overload result in significant hypertrophy of Type II myofibers.

Regarding fiber type composition of the soleus, it was demonstrated that Type I fibers were primarily – and similarly – expressed in each of the four treatment groups. Myofiber profile data from soleus muscles are displayed in Table 6.

Plantaris—Unlike the soleus, the myofibers of the principally fast-twitch (Type II) locomotor plantaris muscle experienced significant hypertrophy when exposed to 4 weeks of chronic overload. This was apparent in both young adult and aged animals, and whether fiber size was quantified irrespective of fiber type, or when only the predominantly expressed Type II fibers were analyzed. And although not statistically significant, Type I fibers of both age groups exhibited a trend ($0.05 < P < 0.10$) toward overload-induced hypertrophy. Our results also indicate that age had no effect on the size of myofibers in the plantaris. That is, the cross-sectional of myofibers – whether Type I or Type II – in control muscles of aged and young adult rats were not found to be different, and rats of both age groups manifested similar rates of hypertrophy upon exposure to chronic overload.

As with the soleus, the fiber type composition of the plantaris did not differ among the four experimental conditions imposed on that muscle. Myofiber profiles of the plantaris can be found in Table 7.

DISCUSSION

Because of its accessibility and relatively simple design, the NMJ is commonly used as a model to study synaptic plasticity (Sanes and Lichtman, 1999; Walsh et al., 2000). While even under normal conditions the mature NMJ displays constant, yet moderate remodeling (Wigston, 1989; Hill et al., 1991), alterations in neuromuscular activity, whether increased (exercise training) or decreased (paralysis), markedly exacerbate that remodeling (Brown and Ironton, 1977; Andonian and Fahim, 1988; Tsujimoto and Kuno, 1988; Deschenes et al., 1993). More recently, an interest in the capacity of aging to modulate activity related NMJ adaptability has been pursued. Indeed, our laboratory recently demonstrated that the neuromuscular adaptations to four weeks of partial disuse as imposed by muscle unloading were influenced by aging. It was determined that not only was NMJ remodeling apparent in aged (22 mo old), but not young adult (8 mo old) rats (Deschenes and Wilson, 2003), but also that the myofiber atrophy elicited by unloading was significantly more severe among aged animals compared to young adult ones (Deschenes et al., 2001).

To further our investigation of age-related sensitivity to altered levels of neuromuscular activity, we subjected young adult and aged rats of the same ages used previously (8 mo and 22 mo old, respectively) to chronic overload of the neuromuscular system for a period of four weeks. Combined with results from our earlier study, the data presented here suggest that the effects of aging are manifested differently in response to either decreased (muscle unloading) or increased (muscle overload) use of the neuromuscular system.

NMJ adaptations

The results gathered from the present study clearly indicate that the stimulus of chronic overload is insufficient to promote synaptic remodeling within the neuromuscular system. Our data showed that neither pre- nor post-synaptic structure was different in overloaded muscles, regardless of whether the muscles examined ordinarily function principally in a weight bearing (soleus) or ambulatory (plantaris) capacity, or even whether NMJs were located on slow- or fast-twitch myofibers within those muscles. Moreover, it is evident that this resistance to overload induced synaptic remodeling is unaffected by aging. That is, NMJs of both young adult and aged rats were unaffected – morphologically – to four weeks of chronic overload. These findings differ from those of our earlier work investigating the impact of aging on four weeks of muscle unloading. In that study (Deschenes and Wilson, 2003), it was determined that although the NMJs of young adult rats did not demonstrate unloading-induced remodeling, those residing in aged muscles did. Combined, data from these two experiments using rats of the same age and interventions of the same duration, suggest that aging amplified the sensitivity of NMJs to chronic muscle unloading, but not to chronic muscle overload. The mechanism(s) accounting for this divergence in age-related responsiveness to muscle unloading vs. muscle overload are not known at this time, but certainly warrant further investigation.

Somewhat unexpected among the results of the current study was our finding that aging, by itself, did not modify NMJ structure. Previously, it has been documented that the NMJs of rats were morphologically distinct from those of young adult animals (Courtney and Steinbach, 1981; Smith and Rosenheimer, 1982). But in those earlier studies, the aged rats examined were considerably older than the ones observed here (28–30 mo vs. 22 mo). It would appear, then, that the aging process itself must be quite advanced before it is associated with remodeling of the NMJ, as the 22 month old rats studied here would be considered only moderately aged given the fact that the average lifespan of male Fischer 344 rats is 25.5 months (Turturro et al., 1999).

Myofibers

Data from our analyses of myofiber profiles indicate that, unlike their synapses, fibers of the plantaris muscle adapted to the stimulus of chronic overload. When examining them irrespective of their type, i.e., fiber types pooled together, it was established that both young adult and aged fibers were hypertrophied following four weeks of overload. Interestingly, the degree of hypertrophy documented (~ 18%) was similar in aged and young adult plantaris muscles, confirming that aging did not diminish the capacity of myofibers to grow when exposed to chronic overload. These results are consistent with previous work which demonstrated that even after eight weeks of chronic overload – twice the intervention period used here – aged and young adult rats experienced similar levels of myofiber hypertrophy in the plantaris (Linderman and Blough, 2002).

Reflecting our results with fibers types collapsed together, it was determined that the predominantly expressed Type II fibers (~ 85%) of aged and young adult plantaris muscles also demonstrated significant, and similar (~ 16%) overload-induced hypertrophy. Although statistical analyses revealed only a trend ($0.05 < P < 0.10$) for Type I fibers of the plantaris to enlarge following chronic overload, the degree of that growth was at least as impressive (~ 20%) as that noted among Type II fibers. Again, this was true in both aged and young adult rats. Much like our failure to identify age-related differences in NMJ structure, the similarity in the degree of overload-induced hypertrophy of plantaris fibers observed here is probably rooted in our use of moderately aged animals. Blough and Linderman (2000) have recently reported that compared to young adult rats, the plantaris muscles of very old rats (36 mo) demonstrate a blunted hypertrophic response to the stimulus of chronic overload. But those same authors have also shown that the strain of rats used in that study – the Fischer 344 X Brown Norway cross – is particularly susceptible to age-related decline in muscle mass, unlike the inbred Fischer 344 which was found to be resilient to the detrimental effects of aging on skeletal muscle (Rice et al., 2005). Perhaps then, it is the strain of the rats utilized in our investigation - the inbred Fischer 344 - along with their moderate degree of aging that accounts for the similar degree of hypertrophy detected in the myofibers of young adult and aged overloaded plantaris muscles.

In contrast to the plantaris, myofibers comprising the soleus muscle did not exhibit significant hypertrophy in response to the chronic overload intervention. This was evident whether data from type I and II fibers were combined for analysis, or whether fiber types were assessed individually. In attempting to explain this between-muscle difference, it is unlikely that the absence of cellular hypertrophy within the soleus is related to the fact that – unlike the plantaris – it is primarily comprised of Type I fibers (> 90%). Recall that Type I fibers residing in the plantaris experienced impressive hypertrophy following chronic overload. A more plausible explanation for the disparate overload-induced responses of the soleus and plantaris muscles relates to their different functions. Under normal conditions, the soleus acts as the primary weight bearing muscle in the rat (Roy et al., 1991). Although surgically removing the gastrocnemius increases weight bearing demands upon the soleus, its principal function does not change; it remains the animal's main postural muscle. Conversely, the primary role of the plantaris – under normal conditions – is to serve as a locomotor muscle (Laughlin and Armstrong, 1982). Only upon the ablation of the gastrocnemius does the plantaris significantly participate in weight bearing activity. Thus, its function is dramatically altered following the synergist ablation procedure employed here. As a result, the stimulus presented to the plantaris is more robust than the one imparted on the soleus following removal of the gastrocnemius, and probably accounts for the myofiber hypertrophy displayed in the plantaris, but not the soleus.

In the data reported here, the only significant age-related difference occurred in the myofibers of the soleus. More specifically, it was observed that Type II fibers from aged control muscles

were smaller than the Type II fibers in young control solei. This was not surprising because several studies have previously demonstrated that aging-related atrophy, or sarcopenia, selectively affects Type II, or fast-twitch myofibers (Lexell et al., 1988; Holloszy et al., 1991). The data presented here only confirm those earlier reports.

As with our NMJ findings, results from myofiber profile analyses suggest differences in the effect of aging on neuromuscular unloading vs. chronic overload. Although the data presented here show that 8 mo and 22 mo old rats adapt likewise – fiber hypertrophy within the plantaris, but not the soleus – our previous work showed that four weeks of unloading elicited a significantly greater degree of atrophy in 22 mo old rats than in 8 mo old ones (Deschenes et al., 2001). This again implies that aging has a greater influence on neuromuscular responses to muscle unloading than to muscle overload. And again, mechanism(s) explaining this difference will require further investigation.

In closing, the results of this study indicate that: 1) the NMJs of aged and young adult muscles are equally resistant to chronic overload-induced remodeling, and 2) among both aged and young adult rats, the removal of the gastrocnemius elicits myofiber hypertrophy within the plantaris, but not the soleus. Combined, these two findings suggest that aging renders the neuromuscular system no more, or less, adaptable to the stimulus of chronic overload. These results may also be clinically important since they demonstrate that the neuromuscular systems of aged and young individuals can be expected to respond similarly to a post-injury program of physical therapy that features overload, e.g. resistance exercise.

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REFERENCES

- Allen DL, Monke SR, Talmadge RJ, Roy RR, Edgerton VR. Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibers. *J Appl Physiol* 1995;78:1969–1976. [PubMed: 7649936]
- Anderton BH, Breinburg D, Downes MJ, Green PJ, Tomlinson BE, Ulrich J, Wood JN, Kahn J. Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share antigenic determinants. *Nature* 1982;298:84–86. [PubMed: 6178036]
- Andonian MH, Fahim MA. Endurance exercise alters the morphology of fast- and slow-twitch rat neuromuscular junctions. *Int J Sports Med* 1988;9:218–223. [PubMed: 3410628]
- Arias, E. National vital statistics reports. Hyattsville, MD: National Center for Health Statistics; 2006. United States life tables, 2003.
- Balace-Gordon RJ, Lichtman JW. In vivo visualization of the growth of pre- and postsynaptic elements of the neuromuscular junction in the mouse. *J Neurosci* 1990;10:894–908. [PubMed: 2156964]
- Blough ER, Linderman JK. Lack of skeletal muscle hypertrophy in very aged male Fischer 344 x Brown Norway rats. *J Appl Physiol* 2000;88:1265–1270. [PubMed: 10749817]
- Brown MC, Ironton R. Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. *Nature* 1977;265:459–461. [PubMed: 834299]
- Courtney J, Steinbach JH. Age changes in neuromuscular junction morphology and acetylcholine receptor distribution on rat skeletal muscle fibres. *J Physiol* 1981;320:435–437. [PubMed: 7320945]
- D'Amelio F, Danton NG. Effects of spaceflight in the adductor longus muscle of rats flown in the Soviet Biosatellite COSMOS 2044. A study employing neural cell adhesion molecule (N-CAM) immunocytochemistry and conventional morphological techniques (light and electron microscopy). *J Neuropathol Exp Neurol* 1992;51:415–431. [PubMed: 1619441]
- Deschenes MR, Britt AA, Chandler WC. A comparison of the effects of unloading in young adult and aged skeletal muscle. *Med Sci Sports Exerc* 2001;33:1477–1483. [PubMed: 11528335]

- Deschenes MR, Maresh CM, Crivello JF, Armstrong LE, Kraemer WJ, Covault J. The effects of exercise training of different intensities on neuromuscular junction morphology. *J Neurocytol* 1993;22:603–615. [PubMed: 8229087]
- Deschenes MR, Wilson MH. Age-related differences in synaptic plasticity following muscle unloading. *J Neurobiol* 2003;57:246–256. [PubMed: 14608661]
- Deschenes MR, Wilson MH, Kraemer WJ. Neuromuscular adaptations to spaceflight are specific to postural muscles. *Muscle Nerve* 2005;31:468–474. [PubMed: 15685624]
- Fahim MA. Rapid neuromuscular remodeling following limb immobilization. *Anat Rec* 1989;224:102–109. [PubMed: 2729612]
- Fahim MA. Endurance exercise modulates neuromuscular junction of C57BL/6NNia aging mice. *J Appl Physiol* 1997;83:59–66. [PubMed: 9216945]
- Fahim MA, Robbins N. Remodelling of the neuromuscular junction after subtotal disuse. *Brain Res* 1986;383:353–356. [PubMed: 3768700]
- Hill RR, Robbins N, Fang ZP. Plasticity of presynaptic and postsynaptic elements of neuromuscular junctions repeatedly observed in living adult mice. *J Neurocytol* 1991;20:165–182. [PubMed: 1903804]
- Huckstorf BL, Slocum GR, Bain JL, Reiser PM, Sedlak FR, Wong-Riley MT, Riley DA. Effects of hindlimb unloading on neuromuscular development of neonatal rats. *Brain Res Dev Brain Res* 2000;119:169–178.
- Hurst JE, Fitts RH. Hindlimb unloading-induced muscle atrophy and loss of function: protective effect of isometric exercise. *J Appl Physiol* 2003;95:1405–1417. [PubMed: 12819219]
- Kano Y, Shimegi S, Masuda K, Ohmori H, Katsuta S. Morphological adaptation of capillary network in compensatory hypertrophied rat plantaris muscle. *Eur J Appl Physiol Occup Physiol* 1997;75:97–101. [PubMed: 9118989]
- Linderman JK, Blough ER. Aging does not attenuate plantaris muscle hypertrophy in male Fischer 344 rats. *Med Sci Sports Exerc* 2002;34:1115–1119. [PubMed: 12131250]
- Nemeth P, Pette D. Succinate dehydrogenase activity in fibres classified by myosin ATPase in three hind limb muscles of rat. *J Physiol (Lond)* 1981;320:73–80. [PubMed: 6459451]
- Older Americans Update 2006: Key Indicators of Well-Being. Hyattsville, MD: Federal Interagency Forum on Aging-Related Statistics;
- Pachter BR, Spielholz NI. Tenotomy-induced motor endplate alterations in rat soleus muscle. *Anat Rec* 1990;228:104–108. [PubMed: 2240598]
- Pearson J, Sabarra A. A method for obtaining longitudinal cryostat sections of living muscle without contraction artifacts. *Stain Technol* 1974;49:143–146. [PubMed: 4135792]
- Pierotti DJ, Roy RR, Flores V, Edgerton VR. Influence of 7 days of hindlimb suspension and intermittent weight support on rat muscle mechanical properties. *Aviat Space Environ Med* 1990;61:205–210. [PubMed: 2317173]
- Rice KM, Linderman JK, Kinnard RS, Blough ER. The Fischer 344/NniaHSd X Brown Norway/BiNia is a better model of sarcopenia than the Fischer 344/NniaHSd: a comparative analysis of muscle mass and contractile properties in aging male rat models. *Biogerontology* 2005;6:335–343. [PubMed: 16463110]
- Riley DA, Ilyina-Kakeuva EI, Ellis S, Bain JL, Slocum GR, Sedlak FR. Skeletal muscle fiber, nerve, and blood vessel breakdown in space-flown rats. *FASEB J* 1990;4:84–91. [PubMed: 2153085]
- Riley DA, Slocum GR, Bain JL, Sedlak FR, Sowa TE, Mellender JW. Rat hindlimb unloading: soleus histochemistry, ultrastructure, and electromyography. *J Appl Physiol* 1990;69:58–66. [PubMed: 2144272]
- Rosenblatt JD, Yong D, Parry DJ. Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* 1994;17:608–613. [PubMed: 8196703]
- Roy RR, Talmadge RJ, Fox K, Lee M, Ishihara A, Edgerton VR. Modulation of MHC isoforms in functionally overloaded and exercised rat plantaris fibers. *J Appl Physiol* 1997;83:280–290. [PubMed: 9216974]
- Sanes JR, Lichtman JW. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci* 1999;22:389–442. [PubMed: 10202544]

- Smith DO, Rosenheimer JL. Decreased sprouting and degeneration of nerve terminals of active muscles in aged rats. *J Neurophysiol* 1982;48:100–109. [PubMed: 7119839]
- Tomas J, Ferre J, Mayoya E, Fenoll R, Brunet R. Morphometric study of neuromuscular synapses in the adult rat with special reference to the modeling concept. *Biol Cell* 1987;60:133–144. [PubMed: 2961385]
- Tsujimoto T, Kuno M. Calcitonin gene-related peptide prevents disuse-induced sprouting of rat motor nerve terminals. *J Neurosci* 1988;8:3951–3957. [PubMed: 2903917]
- Walsh FS, Hobbs C, Wells DJ, Slater CR, Fazeli S. Ectopic expression of NCAM in skeletal muscle of transgenic mice results in terminal sprouting at the neuromuscular junction and altered structure but not function. *Mol Cell Neurosci* 2000;15:244–261. [PubMed: 10736202]
- Wigston DJ. Remodeling of neuromuscular junctions in adult mouse soleus. *J Neurosci* 1989;9:639–647. [PubMed: 2465393]

Abbreviations

ACh, acetylcholine; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; NMJ, neuromuscular junction; PBS, phosphate buffered saline; R-BTX, rhodamine labeled α -bungarotoxin.

Table 1

Body mass (grams) of young adult and aged rats before and after 4 weeks of chronic overload

Group	Pre-intervention	Post-intervention
Young adult	374.1 ± 11.7 [*]	388.5 ± 9.4 [†]
Aged	420.6 ± 12.1	394.0 ± 8.9 [†]

Values are means ± SE.

* indicates significant (P<0.05) difference from Aged rats at Pre-intervention.

† indicates significant (P<0.05) Pre- to Post-intervention difference within that age group.

Table 2
Effects of chronic overload on slow-twitch neuromuscular junctions in young adult and aged soleus muscles.

Variable	Pre-synaptic nerve terminal branching			
	Young Control	Young Overload	Aged Control	Aged Overload
Branch number	5.6 ± 0.4	5.1 ± 0.2	5.4 ± 0.2	5.3 ± 0.2
Total length of branching (µm)	177.8 ± 15.4	165.2 ± 7.0	177.1 ± 10.3	171.5 ± 10.3
Average length/branch (µm)	31.2 ± 1.1	29.3 ± 0.6	28.7 ± 1.1	29.8 ± 1.2
Branching complexity	15.9 ± 2.4	13.4 ± 1.0	14.4 ± 1.4	13.6 ± 1.4
Post-synaptic endplate structure				
Variable	Young Control	Young Overload	Aged Control	Aged Overload
Total perimeter (µm)	171.5 ± 12.7	173.9 ± 11.0	187.6 ± 11.6	180.8 ± 9.3
Stained perimeter (µm)	258.2 ± 31.5	235.2 ± 16.9	244.0 ± 18.9	253.8 ± 10.6
Total area (µm²)	609.7 ± 120.1	536.0 ± 98.5	539.8 ± 95.5	577.6 ± 89.0
Stained area (µm²)	479.2 ± 60.6	397.2 ± 29.3	402.8 ± 26.8	423.6 ± 28.0
Dispersion (%)	66.2 ± 2.3	73.0 ± 1.2	76.2 ± 1.6	70.5 ± 2.0
Pre- to post-synaptic coupling	2.8 ± 0.2	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.2

Values are means ± SE.

Branching complexity = branch number × total branch length/100.

Dispersion = stained area/total area

Pre- to post-synaptic coupling = endplate's stained area/total nerve terminal branch length.

Table 3
Effects of chronic overload on fast-twitch neuromuscular junctions in young adult and aged soleus muscles.

Variable	Pre-synaptic nerve terminal branching			
	Young Control	Young Overload	Aged Control	Aged Overload
Branch number	5.8 ± 0.5	5.4 ± 0.4	5.8 ± 0.4	4.7 ± 0.6
Total length of branching (µm)	164.5 ± 14.4	158.9 ± 15.3	163.1 ± 14.1	142.1 ± 30.1
Average length/branch (µm)	28.8 ± 1.8	29.1 ± 2.8	28.7 ± 1.2	30.0 ± 2.4
Branching complexity	14.3 ± 2.7	12.4 ± 1.8	14.0 ± 2.0	11.1 ± 3.7
Post-synaptic endplate structure				
Variable	Young Control	Young Overload	Aged Control	Aged Overload
Total perimeter (µm)	168.1 ± 16.1	175.4 ± 20.7	150.9 ± 10.3	176.8 ± 27.5
Stained perimeter (µm)	198.4 ± 16.6	208.7 ± 28.9	203.6 ± 18.9	189.1 ± 23.8
Total area (µm²)	461.4 ± 54.0	523.4 ± 79.0	479.2 ± 64.2	451.8 ± 83.0
Stained area (µm²)	347.8 ± 40.2	431.2 ± 66.0	356.5 ± 55.2	387.2 ± 55.9
Dispersion (%)	76.3 ± 1.9	81.4 ± 2.5	73.1 ± 4.5	76.0 ± 8.3
Pre- to post-synaptic coupling	2.2 ± 0.2	2.6 ± 0.3	2.3 ± 0.4	2.7 ± 0.6

Values are means ± SE.

Branching complexity = branch number × total branch length/100.

Dispersion = stained area/total area

Pre- to post-synaptic coupling = endplate's stained area/total nerve terminal branch length.

Table 4
Effects of chronic overload on fast-twitch neuromuscular junctions in young adult and aged plantaris muscles.

Variable	Pre-synaptic nerve terminal branching				P value
	Young Control	Young Overload	Aged Control	Aged Overload	
Branch number	7.8 ± 0.4	7.7 ± 0.4	7.6 ± 0.4	7.8 ± 0.5	0.98
Total length of branching (µm)	287.0 ± 20.3	290.7 ± 15.6	282.0 ± 23.6	298.3 ± 21.2	0.95
Average length/branch (µm)	41.1 ± 1.5	37.7 ± 2.4	37.1 ± 2.1	38.7 ± 1.8	0.93
Branching complexity	25.6 ± 3.1	26.0 ± 2.7	24.4 ± 2.9	26.3 ± 3.0	0.97
Post-synaptic endplate structure					
Variable	Young Control	Young Overload	Aged Control	Aged Overload	P value
Total perimeter (µm)	133.2 ± 28.1	142.0 ± 27.3	139.8 ± 38.1	186.1 ± 43.5	0.68
Stained perimeter (µm)	254.7 ± 9.6	259.7 ± 10.5	260.2 ± 15.3	272.6 ± 16.9	0.80
Total area (µm²)	660.7 ± 46.1	689.7 ± 64.5	671.7 ± 62.1	782.1 ± 97.5	0.60
Stained area (µm²)	528.1 ± 15.1	551.7 ± 15.2	528.4 ± 15.9	615.6 ± 22.3	0.72
Dispersion (%)	78.2 ± 2.3	81.4 ± 1.4	73.9 ± 3.2	70.0 ± 2.6	0.44
Pre- to post-synaptic coupling	2.0 ± 0.8	1.9 ± 0.5	1.9 ± 0.9	2.1 ± 0.8	0.99

Values are means ± SE.

Branching complexity = branch number × total branch length/100.

Dispersion = stained area/total area

Pre- to post-synaptic coupling = endplate's stained area/total nerve terminal branch length.

Table 5
Effects of chronic overload on slow-twitch neuromuscular junctions in young adult and aged plantaris muscles.

Variable	Pre-synaptic nerve terminal branching				P value
	Young Control	Young Overload	Aged Control	Aged Overload	
Branch number	6.3 ± 0.6	6.7 ± 1.0	6.5 ± 1.1	7.0 ± 0.9	0.95
Total length of branching (µm)	240.3 ± 33.1	242.3 ± 31.7	220.2 ± 35.3	296.8 ± 58.4	0.60
Average length/branch (µm)	38.9 ± 2.5	36.6 ± 1.9	34.0 ± 5.6	41.7 ± 5.1	0.82
Branching complexity	15.2 ± 3.5	16.2 ± 3.8	14.3 ± 4.2	21.1 ± 5.8	0.70
Post-synaptic endplate structure					
Variable	Young Control	Young Overload	Aged Control	Aged Overload	P value
Total perimeter (µm)	144.3 ± 62.5	120.7 ± 40.9	105.3 ± 56.7	190.8 ± 52.4	0.16
Stained perimeter (µm)	283.0 ± 34.9	269.2 ± 26.7	269.0 ± 13.7	306.7 ± 16.9	0.65
Total area (µm²)	725.3 ± 130.6	648.4 ± 76.5	738.9 ± 74.9	801.1 ± 79.9	0.42
Stained area (µm²)	480.1 ± 56.9	470.9 ± 47.0	454.7 ± 34.2	581.7 ± 67.3	0.57
Dispersion (%)	67.8 ± 4.5	76.5 ± 4.7	62.0 ± 5.1	72.4 ± 5.4	0.51
Pre- to post-synaptic coupling	2.3 ± 0.9	2.2 ± 0.7	2.6 ± 0.8	2.7 ± 1.0	0.92

Values are means ± SE.

Branching complexity = branch number × total branch length/100.

Dispersion = stained area/total area

Pre- to post-synaptic coupling = endplate's stained area/total nerve terminal branch length.

Table 6
Effects of chronic overload on myofiber profiles of young adult and aged soleus muscles.

Variable	Young Control	Young Overload	Aged Control	Aged Overload	P value
Cross-sectional area (μm^2)					
Fiber types combined	2720 $\pm$ 97	3096 $\pm$ 170	2560 $\pm$ 109	2606 $\pm$ 258	0.12
Type I	2760 $\pm$ 99	3138 $\pm$ 174	2619 $\pm$ 107	2621 $\pm$ 263	0.12
Type II	2325 $\pm$ 114	2627 $\pm$ 199	1673 $\pm$ 138*	2011 $\pm$ 257†	0.003
Fiber type composition (%)					
Type I	91.5 $\pm$ 1.7	91.3 $\pm$ 1.5	92.7 $\pm$ 1.6	95.2 $\pm$ 3.0	0.15
Type II	8.5 $\pm$ 0.6	8.7 $\pm$ 0.4	7.3 $\pm$ 0.6	4.8 $\pm$ 0.3	0.17

Values are means $\pm$ SE.

* indicates significant ($P < 0.05$) difference from Young Control and Young Overload groups.

† indicates significant ($P < 0.05$) difference from Young Overload group.

Table 7
Effects of chronic overload on myofiber profiles of young adult and aged plantaris muscles.

Variable	Young Control	Young Overload	Aged Control	Aged Overload	P value
Cross-sectional area (μm^2)					
Fiber types combined	1966 $\pm$ 120	2306 $\pm$ 78*	1809 $\pm$ 95	2146 $\pm$ 70 [†]	0.005
Type I	1756 $\pm$ 104	2097 $\pm$ 142 ^{†‡}	1554 $\pm$ 52	1858 $\pm$ 164 [‡]	0.03
Type II	1997 $\pm$ 126	2343 $\pm$ 76*	1861 $\pm$ 108	2188 $\pm$ 75 [†]	0.009
Fiber type composition (%)					
Type I	13.4 $\pm$ 0.4	17.6 $\pm$ 0.4	15.4 $\pm$ 0.3	15.0 $\pm$ 0.5	0.38
Type II	86.6 $\pm$ 1.1	82.4 $\pm$ 1.4	84.6 $\pm$ 2.2	85.0 $\pm$ 2.4	0.40

Values are means $\pm$ SE.

- * indicates significant ($P < 0.05$) difference from Young Control and Aged Control groups.
- [†] indicates significant ($P < 0.05$) difference from Aged Control group.
- [‡] indicates trend ($0.05 < P < 0.10$) toward difference from controls of same age group.